MAIZE GENETICS COOPERATION NEWSLETTER

84

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and
Division of Plant Sciences
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NOTE: The 53rd Maize Meeting was held at St. Charles, IL USA March 17-20, 2011.
The 54th Maize Meeting will be held at Portland, OR USA March 15-18, 2012.

Check MaizeGDB for more details.

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I. FOREWORD

The Maize Genetics Cooperation Newsletter exists for the benefit of the maize community as an informal vehicle for communication. Its inception and continuation has been to foster cooperation among those interested in investigating maize. This cooperation has distinguished our field from others and as a consequence has moved it forward at a pace greater than would have occurred otherwise. Your submissions are encouraged to disseminate knowledge about our field that might otherwise go unrecorded. We encourage the community to carry studies of general scientific interest to the formal literature. However, there is a great need to share technical tips, protocols, mutant descriptions, map information, ideas and other isolated information useful in the lab and field.

Because maize is both a commercial species and a genetic model system, the danger exists that the sharing of research materials might be diminished. It is imperative for us to work together to prevent this from occurring. Certainly, basic findings should be transferred to the industrial sector and basic advances in industry should be shared with the academic community for the benefit of both. Published materials must be shared for research purposes with the only restriction being against commercial use.

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes can be submitted at any time and are posted without editing at the staging site, which has access from MaizeGDB, www.mgdb.org/mnl.php. When the print copy is finalized, the staging site copies are updated and moved to MaizeGDB. We set an arbitrary cutoff of March 31, 2012 for print copy of volume 86. Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions must require minimal editing to be accepted. Please check the staging site about any delays in processing or mailing of the print copy.

This year we have spent substantial effort updating the mailing list to accommodate the precise addressing standard required for electronic mail readers, and in updating our address listings for persons that are unable to regularly attend the Maize Genetics Conference, and have moved. We apologize to our international subscribers, where there was a significant delay in the mailing of Vol 83. It would be most helpful if you know of persons who do not receive a copy, that they email the editors: MaizeNewsletter@missouri.edu.

As in the past, Shirley Kowalewski has been responsible for final redaction and layout of the Newsletter copy. She has performed this task with precision and a great sense of humor. The maize community owes her much gratitude for her continued service in this capacity.

Mary Schaeffer (Polacco)
James A. Birchler
Co-editors
Ed Coe
Distinguished editor

BEIJING, CHINA
Institute of Genetics, Chinese Academy of Sciences

Application of mutant types induced by space flight in maize (*Zea mays L.*)

--Zeng, M; Zeng, Z

In our previous papers we have described a significant influence on progeny of maize seeds subjected to space flight where various traits were observed (MNL.74:2-3,75:4, 77:3-4, 79:3, 80:1, 81:1; Chinese Space Sci. Technol. 18(6):63-67,1998, 23(6):64-68, 2003, 29(6):60-64, 2009). Mut5 (v8112), Mut2 (vi 01-4-1), Mut8 (Me141), and Mut7 (U8112) were used with 2-5 of the heterotic groups to develop new germplasm, Me12, XH3, Mv02 and Met88 with highly effective biotic and abiotic factor-resistant genes.

BERGAMO, ITALY
Unità di Ricerca per la Maiscoltura - CRA

Constitution and characterization of maize lines with down-regulation of nfc102 gene expression

--Altana, A; Battaglia, R; Michelotti, V; Locatelli, S; Lauria, M; Rossi, V

The *nfc102* gene encodes a WD-repeat protein belonging to the *Multicopy suppressor of IRA* (*MSI*) family, originally identified in yeast (Ruggieri et al., Proc. Natl. Acad. Sci. USA 86:8778-8782, 1989). In maize, five genes of the *MSI* family have been identified and named *nfc* by the Plant Chromatin DB USA initiative (http://www.chromdb.org), because they display homology with a NURF complex component. NURF, or Nucleosome Remodeling Factor, is a multi-protein complex that regulates transcription by catalyzing nucleosome sliding (Kwon and Wagner, Trends Genet. 23:403-412, 2007). On the basis of sequence homology with the five *Arabidopsis MSI* sequences, the maize *nfc* genes can be subdivided into two separate groups (http://www.chromdb.org). The first group, *nfc103*, *nfc104*, and *nfc108*, are the putative orthologs of three *Arabidopsis MSI1*-like genes involved in the epigenetic control of several aspects of reproductive development (Hennig et al., Trends Cell Biol. 15:295-302, 2005; Jullien et al., PLoS Biol. 6:e194, 2008). Maize *nfc101* and *nfc102* genes are closely related to the *Arabidopsis FVE* gene, which is a component of an autonomous pathway regulating flowering time (Ausin et al., Nature Genet. 36:162-166, 2004; Kim et al., Nature Genet. 36:167-171, 2004). Recently it has been shown that *FVE*, together with other genes of the autonomous pathway, has a more general role and acts in regulating epigenome stability (Baurle and Dean, PLoS ONE 3:e2733, 2008).

In maize, the *nfc102* gene was previously named *ZmRbAp1* because, similar to the mammalian Retinoblastoma associated proteins RbAp46/48, it associates with the maize homolog of Retinoblastoma protein (ZmRBR; Rossi et al., Plant Mol. Biol. 51:401-413, 2003). The ZmRbAp1/NFC102 protein associates with histones (Rossi et al., 2001 Mol. Genet. Genomics 265:576-584, 2001) and cooperates with ZmRBR and with maize Rpd3-type histone deacetylases (HDAs) to repress gene transcription (Rossi et al., Plant Mol. Biol. 51:401-413, 2003; Varotto et al., Plant Physiol. 133:606-617, 2003). These findings suggest a role for the ZmRBR/ZmRbAp1/HDA complex in controlling G1/S progression in maize, directing changes in histone modification of specific targets to modulate their chromatin structure and transcription.

To functionally characterize the *nfc102* gene, we have generated transgenic maize plants that constitutively express the antisense *nfc102* transcript (AS plants). The *nfc102* antisense transcription is driven by the constitutive maize ubiquitin promoter (Christensen et al., Plant Mol. Biol. 18:675-689, 1992). The polyadenylation domain of the *nos* gene was inserted opposite to the ubiquitin promoter. The resulting cassette was cloned into a vector expressing the *phosphinothricin acetyl transferase* (*PAT*) gene, which permits selection for resistance to the herbicide gluphosinate. The final construct was used to transform protoplasts from maize suspension cells as described by Rossi et al. (Plant Cell 19:1145-1162, 2007). Transgenic clones were subcultured in the presence of gluphosinate, and callus lines were further screened for the presence of the transgene by PCR with specific primer combinations. A total of 30 independent T0 transformants were obtained, and on the basis of preliminary quantitative RT-PCR (qRT-PCR) findings of *nfc102* down-regulation, seven plants were selected for further characterization. In addition, a maize RNAi *nfc102* line (RNAi plant), with constitutive expression of the *nfc102* RNAi transgene, was obtained from the Maize Genetic Stock Center (Plant Chromatin DB USA initiative (http://www.chromdb.org; stock number: 3480.04). Regenerated T0 plants were crossed sequentially to the B73 recurrent parent to minimize mixed genetic background influence (T1 and T2 generations). After crosses to B73, plants heterozygous for the transgene were self-pollinated to obtain the T3 generation. T3 plants showing resistance to gluphosinate were further self-pollinated, and 35 seeds from individual T4 ears were germinated and screened for gluphosinate resistance and PCR evidence for presence of the transgene. The T4 plants homozygous for the transgene were selfed to produce the T5 homozygous plants for molecular analysis. DNA gel blot analysis showed that *nfc102* mutant plants contained one to four independent insertions of the transgene within the genome and that six AS and the RNAi mutant plants contain at least one intact transgene cassette. The mRNA level of *nfc102* and of its closest homolog, *nfc101*, was measured in leaves sampled at the V2/V3 developmental stage and in tissue enriched in the shoot apical meristems collected at the V6 stage by manual dissection. Total RNA was extracted from both tissues, and the transcript level was assessed by means of real-time PCR analysis (for details of the method see Rossi et al., Plant Cell 19:1145-1162, 2007). The results showed statistically significant reductions of both *nfc102* and *nfc101* transcript in three AS and in the RNAi plants with respect to the wild-type B73 line. Reduction in transcript levels ranged from two to four in AS plants, to a ten-fold reduction in the RNAi line. For phenotypic analysis, the T4 heterozygous plants were selfed to produce T5 segregating plants, which were subsequently screened to identify ears with homozygous and wild-type seeds. At least 5 different homozygous and 5 different wild-type ears were used to prepare two pools of 200 seeds each, and planted for phenotypic observations. Preliminary phenotypic analysis indicates that down-regulation of *nfc102* and *nfc101* is associated with developmental defects such as delayed germination and flowering, reductions of seed size and plant height, etc. A more detailed analysis of various quantitative and morphological

traits is planned. Experiments for genome-wide identification of *nfc102* targets and for investigation of the effect of *nfc102* mutation on the epigenome are in currently progress.

Neonicotinoid insecticide seed coatings for the protection of corn kernels and seedlings, and for plant yield

--Balconi, C; Mazzinelli, G; Motto, M

Planted corn seeds and corn seedlings can be attacked by a variety of insect pests, including beetles adults and/or larvae): billbugs, wireworms, corn rootworms, seed-corn beetle, white grubs and flea beetles. Other pests include caterpillars, fly larvae, aphids and thrips. When abundant, these pests injure germinating seeds or attack the small emerging corn seedlings, resulting in low-producing fields. The yield and/or the vigor of an agronomic plant can be increased or improved in locations with high levels of insect infestation by treating seed with a neonicotinoid compound. This new family of insecticides, which includes such agents as thiamethoxam, imidacloprid, clothianidin, and fipronil, is effective in protecting important agronomic crops from insect damage (Bai et al., Pestic. Sci. 33:197-204, 1998; Nauen et al., Pestic. Sci. 51:52-56, 1998). The use of these insecticides as seed treatments, rather than as field-applied formulations, is believed to reduce the exposure and odor of the pesticide, and to reduce the amount of post-planting cultivation and application (Elbert et al., Insecticides with novel modes of action: mechanism and application, 50-74, 1998). However, in Italy and in other European countries, one of the risk factors for honeybee health and Colony Collapse Disorder (CCD) is considered to be agrochemical treatments, because of the release of active seed coating ingredients through the fan drain of pneumatic seed drills during corn sowing operations (Greatti et al., Bull. Insectol. 56:69-72, 2003; 59:99-103, 2006). For this reason, the use of all four neonicotinoid active ingredients registered for seed dressing mentioned above was suspended in Italy during 2008-2009. To explore the possible causes of colony losses and high bee mortalities reported in recent years and to evaluate the efficacy of the cited suspension of insecticides used for seed dressing, the Italian Ministry of Agriculture financed a national research project, Apenet (Bortolotti et al., APOidea 6:2-21, 2009).

The aims of our research in the frame of the Apenet project are devoted: i) to comparing, in 20 locations of Northern-Central Italy, the yield and agronomic traits of a commercial hybrid grown with and without any insecticide treatment, using the four neonicotinoid insecticide seed coatings (thiamethoxam, imidacloprid, clothianidin, fipronil); ii) to detecting the presence of these four neonicotinoid active ingredients in leaves and other corn plant tissues periodically collected from the emerging seedling to the flowering stage.

*Research under the project "APENET: monitoring and research in apiculture", funded by the Italian Ministry of Agricultural Food and Forestry Policies".

BIORES* PROJECT: The use of bioactive proteins in plant protection against pathogens

--Lanzanova, C; Hartings, H; Berardo, N; Motto, M; Balconi, C

Plants respond to attack by pathogenic fungi with a complex network of active responses that may involve the production and accumulation of proteins such as RIP (Ribosome Inactivating Protein) which are toxic or inhibitory to pathogens. In maize endosperm, a cytosolic albumin termed b-32 is synthesized in temporal and quantitative coordination with the deposition of storage proteins. In past years, b-32 was shown i) to enzymatically inactivate ribosomes by modifying rRNA and inhibiting protein synthesis in vitro (Maddaloni et al., Genet. Breed. 45:377-380, 1991); and ii) to inhibit the growth of *Rhizoctonia solani* mycelia in an in vitro bioassay and in plant assays (Maddaloni et al., Transgen. Res. 6:393-402, 1997). We have recently shown that maize b-32 is effective in wheat transgenic lines as an antifungal protein, reducing *Fusarium culmorum* head blight (FHB) (Balconi et al., Eur. Plant Pathol. 117:129-140, 2007), and in maize transgenic lines, reducing *Fusarium verticillioides* symptoms in leaf tissue assays (Lanzanova et al., Eur. J. Plant Pathol. 124:471-482, 2009). Similar to other RIPs, maize RIP accumulates in seed as an inactive precursor, which is converted into an active form by proteolytic processing to remove peptide segments from the N- (residues 1-16 of pro-RIP) and C- (residues 295-301) termini, and also from the center of the polypeptide (residues 162-186; Hey et al., Plant Physiol. 107:1323-1332, 1995).

The BIORES* project is devoted to deepening our knowledge about relationships between structure and substrate specificity of b-32 protein in order to clarify the role of segments of the processed *b-32* gene on the ability of maize RIP to inhibit fungal growth. A series of genetic constructions was made deleting the N-terminal, C-terminal or internal linker domain, regions of the b-32 gene that are apparently responsible for suppressing enzymatic activity in the precursor. The constructs will be expressed in *Escherichia coli* to produce sufficient quantities of modified proteins. To assess the role of bioactive b-32 modified protein protection against fungal pathogens (*F. verticillioides, Aspergillus flavus),* a series of in vitro bioassays will be performed that measure their effect on fungal growth and on mycotoxin accumulation

*Research developed in the “BIORES - Use of bioactive proteins in plant protection against pathogens - Utilizzo di proteine bioattive nella protezione contro patogeni in pianta." CRA - Consiglio per la Ricerca e Sperimentazione in Agricoltura-funded project.

Analyses of *o2*, *o7*, and *o2o7* mutations on amino acid metabolism in maize endosperm by transcript profiling*

--Hartings, H; Pirona, R; Rossi, V; Motto, M

Our long-term goal is comprehensive dissection of the molecular mechanisms underlying endosperm development and metabolism in maize kernels. In this context, genetics has played an important role by discovering a series of opaque endosperm mutants and demonstrating their effects on genes mediating zein deposition (see Motto et al., Maydica 54:321-342, 2009). For example, the recessive mutations *opaque2* (*o2*) and *opaque7* (*o7*) induce specific decreases in the accumulation of 22- and 19-kDa α-zeins, respectively.

The *o2* mutation has been widely studied at the genetic, biochemical, and molecular levels. It was shown that *O2* encodes a basic leucine zipper (bZIP) transcriptional regulator that is specifically expressed in the endosperm; it also activates the expression of 22-kDa α-zein and 15-kDa β-zein genes by interacting with the TC-CACGT(a/c)R(a/t) and GATGYRRTGG sequences of their

promoters, displaying a broad binding specificity and recognizing a variety of target sites in several distinct genes (Gavazzi et al., Plant Physiol. 145:933-945, 2007). *O2* also regulates directly or indirectly a number of other non-storage protein genes, including b-32, encoding a type I ribosome-inactivating protein; *cyPPDK1*, one of the two cytosolic isoforms of the pyruvate orthophosphate dikinase gene; and b-70, encoding a heat shock protein 70 analogue, possibly acting as a chaperonin during protein body formation (Motto et al., 2009). *O2*, furthermore, regulates the levels of lysine-ketoglutarate reductase (Brochetto-Braga et al., Plant Physiol. 98:1139-1147, 1992) and aspartate kinase1 (Azevedo et al., Phytochemistry 46:395-419, 1997). These broad effects suggest that *O2* plays an important role in the developing grain as a coordinator of the expression of genes controlling storage protein, and N and C metabolism. Although the molecular basis of *o7* mutation is yet unknown, evidence indicates that this mutation, in addition to repressing the lower molecular weight α-zeins, drastically affects the development of maize endosperm due to a reduction in starch content. Moreover, the high content in *o7* endosperms of non-protein N has suggested the existence in *o7* of a block in the synthetic route leading to proteins similar to that observed for the starch modifying gene shrunken4 (Motto et al., 2009).

To advance our understanding of the nature of the *o2* and *o7* mutations in affecting amino acid metabolism, we used genome-wide analyses of gene expression profiles during kernel development. Specifically, for this study we have collected mRNA transcripts from endosperms at 14 days after pollination for A69Y+, nearly isogenic *o2* and *o7* mutants, and the double mutant *o2o7*, collected. Additional, endosperm transcripts profiles were obtained with the Zeastar Unigene set, representing f >7,200 maize genes, mainly derived from maize endosperm and covering a wide range of metabolic pathways and cellular and physiological processes Our study indicate that several ESTs homologous to enzymes involved in amino acid synthesis were differentially expressed in the *o2*, *o7*, and *o2o7* endosperms. In particular, tryptophan synthase (EC 4.2.1.20) homologues showed a significant reduction of expression in *o2* endosperms, while anthranilate phosphoribosyl transferase (EC 2.4.2.18) and anthranilate synthase (EC 4.1.3.27) homologous were differentially expressed in all three mutant backgrounds. The former showed a significant reduction, while the latter appeared up-regulated by 50%. ESTs homologous to phosphoglycerate dehydrogenase (EC 1.1.195), cysteine synthase (EC 2.5.1.47), methionine synthase (EC 2.1.1.14), S-adenosylmethionine synthetase (EC 2.5.1.6), and a methyl transferase (EC 2.1.1.37), all enzymes involved in the Ser, Gly, Cys, and Met pathways were negatively affected in the *o2* endosperm. However, neither of these showed a significantly altered expression level in the *o7* and *o2o7* endosperms. Finally, the Ile, Val and Leu pathways were affected in all three lines. ESTs homologous to acetolactate synthase (EC 2.2.1.6) and ketolacid reductoisomerase (EC 1.1.1.86), and involved in the biosynthesis of these amino acids were significantly reduced in expression in all three backgrounds, while leucine dehydrogenase (EC 1.4.1.9) was significantly different from wild-type only in the *o7* endosperms.

In conclusion, the current study indicates that transcription levels of various genes encoding key enzymes involved in amino acid synthesis are significantly affected in the *o2* mutant. *O7* regulates the expression of some genes of the amino acids biosynthesis, but in only a few cases arethe affected mRNAs up- or down-regulated similar to the *o2* mutant, suggesting that the *O2* and *O7* factors act on specific target genes. Among the pathways affected by both *o2* and *o7* mutants are those leading to the synthesis of the aromatic (Phe, Trp, and Tyr), Asp-derived, and branched chain amino acids (BCAA). These pathways are deeply interconnected both in C precursor supply and allosteric interactions (Curien et al., Plant Physiol. Biochem. 46:325-339, 2008). A complex interplay of regulators is known to control the metabolic flow through the aromatic, Asp and BCAA-pathways (Galili and Höfgen, Metabolic Eng. 4:3-11, 2002). Alterations in enzymes affecting amino acid metabolism have pleiotropic effects on free amino acid levels in plant tissues. For example, a feedback-insensitive aspartate kinase mutant in tobacco, not only has a higher level of amino acids that derive from the Asp pathway, but also of amino acids from other pathways (Frankard et al., Plant Physiol. 99:1285-1293, 1992). In addition, it has been reported that the alteration of Trp and Tyr levels in transgenic tobacco leaves affects levels of the aliphatic amino acids Met, Val, and Leu (Guillet et al., Plant Physiol. 122:933-943, 2000).

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Evaluation of maize genetic resources

--Losa, A; Chittò, A; Balconi, C; Motto, M; Redaelli, R

The continuous and significant loss of genetic variability in crops has become a main concern in many countries and much research is underway to maintain the existing variability and to identify new sources to be exploited. In Italy, over the past few years, the Ministry of Agriculture in cooperation with CRA has funded a project (Risorse Genetiche Vegetali, RGV) that is focused on the existing genetic resources for most plant species (cereals, fruits, flowers, forest trees, forage species and so on). The aims of this project were: i) to maintain and to regenerate the germplasm present in the CRA, ii) to describe these materials from several points of view (morphological, biochemical, genomic, and so on), and iii) to evaluate the potential to enhance the available genetic variability towards the development of new products with innovative characteristics. The genetic variability observed in Italian maize germplasm was found to be quite large, and represents a good source of favourable alleles (Chittò et al., Maydica 45:257-266, 2000; Hartings et al., Theor. Appl. Genet. 117:831-842, 2008; Berardo et al., J. Agric. Food Chem. 57:2378-2384, 2009). In the framework of the RGV project, the maize work has focused on the characterization of a set of inbreds, collected and stored at the CRA-Maize Research Unit since the 1960s. These materials had never been evaluated for agronomic performance, morphological parameters and chemical characteristics of the grain. In 2008, about 600 inbreds, both from Italy and foreign countries, were grown in the field, and the main descriptive parameters were recorded: GDD [growing degree days <editor>] for male and female flowering, plant height and structure, number of ears/plant and the height of their insertion point, leaf orientation, and tassel structure.

On the basis of these observations, a group of 53 inbreds were considered interesting for being introduced in breeding programs.

In 2009, the selected materials were selfed, and crossed to a tester line with good agronomic value. In parallel, a preliminary evaluation of their susceptibility to fungal pathogens was carried out by kernel infection with *Fusarium verticilloides*. After harvest, the ears' parameters were also recorded. Future work on these materials will include their chemical characterisation by NIRS, the dosage of mycotoxins in the infected ears, and agronomic trials with the hybrid seed combinations.

Biofuels such as bio-ethanol are becoming an interesting alternative to fossil fuels (Ragauskas et al., Science 311:484-489, 2006). The use of agricultural biomass for the production of biofuel has drawn interest in many science and engineering disciplines. As one of the major crops, maize offers promise in this regard: in fact, the rapidly expanding information from genomics and genetics, combined with improved genetic engineering technologies, offer a wide range of possibilities for enhanced bioethanol production from maize (Torney et al., Curr. Opin. Biotechnol. 18:193-199, 2007). Two key parts of maize plants can be converted into bio-ethanol: the kernel, which is mainly starch (Jobling, Curr. Opin. Plant Biol. 7:210-218, 2004), and the stover, which is predominantly lignin and cellulosic components (cell wall) (Grabber, Crop Sci 45:820-831, 2005). In 2009, 98 Italian and American maize inbreds were sown in the field, and random crosses were made among them towards increasing the genetic variability. About 278 hybrid combinations were selected; they will be tested in a set of agronomic trials for kernel yield and plant biomass, towards exploitation for bio-ethanol production.

BLACKSBURG, VIRGINIA
Virginia Polytechnic Institute and State University

A small gene family in maize encodes a family of β-glucosidase aggregating factor (BGAF)-like proteins, and the product of the *bgaf2* gene also aggregates β-glucosidase

--Kittur, FS; Bevan, DR; Esen, A

In certain maize genotypes called "null", β-glucosidase, a major defense related enzyme, forms large insoluble aggregates when tissue integrity is compromised (Biochem. Genet. 28:31-36, 1990). We have shown that a protein called β-glucosidase aggregating factor (BGAF) is responsible for β-glucosidase aggregation and hence the β-glucosidase null-phenotype (Plant Physiol. 122:563-572, 2000). BGAF is a modular protein containing an N-terminal dirigent domain and a C-terminal jacalin-related lectin (JRL) domain (J. Biol. Chem. 282:7299-7311, 2007). BGAF is a lectin; it shows high preference for galactose but binds other carbohydrates as well. BGAF specifically interacts with maize β-glucosidases (isozymes Glu1 and Glu2), forming large insoluble complexes (J. Biol. Chem. 282:7299-7311, 2007). Aggregation of β-glucosidase by BGAF does not affect enzyme activity (unpublished results) nor does bound β-glucosidase interfere with the ability of BGAF to bind carbohydrates.

Proteins sharing sequence similarity and modular architecture with BGAF are also reported from wheat, rice, barley, sorghum and creeping bentgrass (*Agrostis stolonifera*). In wheat, rice, and barley, a small family of genes encodes BGAF-like proteins, four genes each in wheat and rice, and three in barley. The products of these genes from wheat (Plant Physiol. Biochem. 43:185-192, 2005; Plant Physiol. 147:1412-1426, 2008) and one of the genes from rice (Toxicon. 47:133-139, 2006) are shown to be lectins with monosaccharide preference for mannose. No protein-aggregating activity was, however, reported for these proteins. Recently, we showed that an ortholog of BGAF from sorghum is a GalNAc-specific lectin, but it lacks protein-aggregating activity (Glycobiol. 19:277-287, 2009). Although the above observations suggest that the β-glucosidase aggregating activity is unique to maize BGAF alone, the occurrence of other BGAF-like proteins in maize with protein aggregating activity cannot be ruled out. We predicted that multiple genes encoding BGAF-like proteins must be present in maize also, since its close relatives wheat, rice, and barley each have a small family of genes encoding BGAF-like proteins.

Searching the maize genome database (www.maizeGDB.org) using the maize BGAF cDNA sequence as query led to identification of at least six genes. The gene encoding BGAF, which we described earlier (J. Biol. Chem. 282:7299-7311, 2007) is located on chromosome 7 and was designated as *bgaf1*. The remaining genes were denoted as *bgaf2*, *bgaf3*, *bgaf4*, *bgaf5* and *bgaf6*, where the order reflects their divergence distance in sequence from *bgaf1*. One gene (*bgaf* 7) predicted from EST sequences was not found in the maize genome sequence. The remaining genes are located on chromosomes 2 (*bgaf2*), 6 (*bgaf3*, *bgaf4*, and *bgaf5*) and 8 (*bgaf6*), respectively.

The predicted protein products of genes *bgaf2*, *bgaf3*, *bgaf4*, *bgaf5* and *bgaf6* share 69%, 44%, 45%, 43%, and 41% sequence identity, respectively, with BGAF1, and vary in size from 32 to 35 kD. Maize EST database searches identified EST clones AY105022, AY104689, AY103569, BT016225 and BT042436 whose sequences matched with the sequences of *bagf2*, *bgaf3*, *bgaf4*, *bgaf5*, and *bgaf6* genes, respectively. Moreover, we were able to construct complete cDNA coding sequences corresponding to seven different *bgaf* genes including *bgaf7* using sequence overlaps among EST clones in the database. To investigate if the products of these genes (other than *bgaf1*) participate in protein-protein interactions, an EST clone (AY105022) corresponding to the *bgaf2* gene was selected. Complete sequencing of EST AY105022 indicated that it contained a 948 bp open-reading frame encoding a 315 amino acid long polypeptide, consisting of a predicted N-terminal dirigent domain and a C-terminal JRL domain. The predicted protein was identical to the product of *bgaf2* gene located on chromosome 2.

Both full-length BGAF2 protein and its JRL domain (produced in *E. coli*) showed binding to maize Glu1. In the pull-down assay, precipitable complexes of maize Glu1 were obtained with full-length protein, whereas no such complexes were observed with its JRL domain. In the gel-shift assay, in the presence of full-length protein, the Glu1 activity zone showed smearing, extending from the sample well to the boundary between stacking and resolving gel (Fig. 1, lane 4), indicating formation of large aggregates. The JRL domain also showed a distinct activity band of mobility slower than Glu1, suggesting formation of a smaller, soluble JRL-Glu1 complex (Fig. 1, lane 5). The above results clearly indicate that

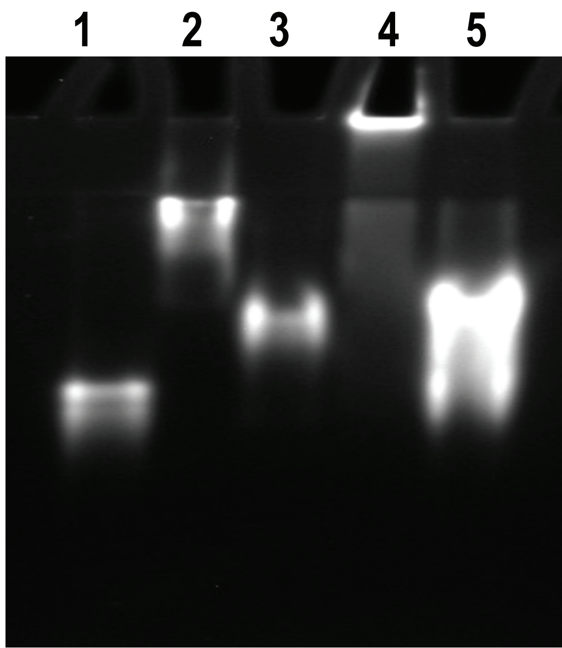

Figure 1. Gel-shift assay to detect binding of recombinant BGAF2 and its JRL domain to maize Glu1. Maize Glu1 (125 nM) was mixed with BGAF1 (150 nM), its JRL domain (500 nM), BGAF2 (400 nM), and its JRL domain (10 μM), and incubated at room temperature for 30 min. Following incubation, 40 μl of reaction mixtures were mixed separately with 20 μl of sample buffer and electrophoresed on a 8% native gel. β-Glucosidase activity was detected by incubating gel with 4-methylumbelliferyl-β-D-glucopyranoside. *Lane 1*, Glu1; *lane 2*, Glu1+BGAF1; *lane 3*, Glu1+JRL domain of BGAF1; *lane 4*, Glu1+BGAF2; lane *5*, Glu1+the JRL domain of BGAF2.

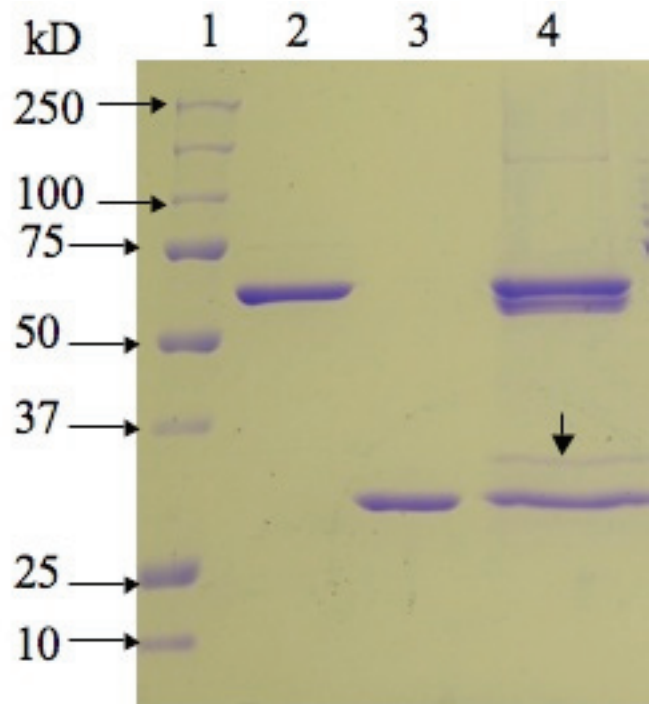

Figure 2. SDS-PAGE profile of complexes of maize β-glucosidase with BGAF1 and BGAF-like protein isolated from maize null-line H95. *Lane 1*, Molecular weight markers; *lane 2*, recombinant maize Glu1 expressed with His-tag; *lane 3*, recombinant BGAF1; *lane 4*, β-glucosidase-BGAF complexes isolated from maize null-line H95. Note that there are four bands in lane 4, two of which are native (60 kD, lower band) and recombinant (62 kD, upper band) Glu1, respectively. The protein band of size similar to rBGAF1 is native BGAF1. The band (34 kD) immediately above native BGAF1, which was immuno-reactive with BGAF2 specific antibody, was excised and subjected to LC-MS/MS analysis to establish its identity.

the product of the *bgaf2* gene is also a β-glucosidase aggregating factor, which we designated as BGAF2.

To investigate whether BGAF2 is expressed in maize, null-line H95 shoots were extracted with PBS (pH 7.4) and the BGAF2-β-glucosidase complexes were isolated after passing through a column of Nickel with immobilized rGlu1 on it, followed by affinity chromatography on lactosyl-agarose. SDS-PAGE of fractions obtained from the lactosyl-agarose column showed the presence of four bands of sizes 62, 60, 34 and 32 kD, respectively (Fig. 2, lane 4). The 60 and 62 kD bands are native and recombinant maize Glu1, respectively. The latter (rGlu1) is larger than native Glu1 because of the presence of a His-tag. The 32 kD band is native BGAF1. The ~34 kD protein band was found to be immuno-reactive with antisera raised against a BGAF2 specific peptide, suggesting that the 34 kD protein is in fact a BGAF-like protein, namely BGAF2. To establish the identity of the 34 kD protein band unequivocally, it was excised from the gel and subjected to LC-MS/MS analysis. Three peptides of sequence ANQAAILESK, FSGSTLEVR and VGPWGGSGGPMELTETETPMR were identified from LC-MS/MS analysis. When these were used as query to search the current (Nov 2008) release of the NR (NCBI) database, the product of the *bgaf2* gene received the highest hits, indicating that the 34 kD protein band is in fact BGAF2.

The β-glucosidase null phenotype in maize (H95 null-line) is not due to BGAF1 alone. BGAF2 is also responsible for β-glucosidase aggregation although its contribution is minor compared to BGAF1 because it is less abundant than BGAF1. There are at least eleven maize null-lines in which we have observed that BGAF1 is a major factor responsible for β-glucosidase aggregation (unpublished data). It is not known at this time whether *bgaf3, bgaf4, bgaf5* and *bagf6* genes are expressed in maize at the protein level, and if so, whether their products participate in protein-protein interaction. The fact there are cDNAs corresponding to each of the seven predicted *bgaf* genes in the maize EST database indicates that these genes are transcribed and show both temporal and spatial expression patterns. For example, *bgaf1* and *bgaf2* are expressed in aerial organs, whereas *bgaf4*, bgaf5, and *bgaf6* are expressed in embryo and endosperm, respectively (http://www.ncbi.nlm.nih.gov/unigene).

The precise physiological role of β-glucosidase-BGAF interactions is not well understood at this time. We speculate that these interactions have a key function in plant defense responses. There is good reason to believe that these lectins deter the insect larvae from feeding onto plants by lodging the enzymes in the oral cavity or the midgut (by binding to glycoproteins) and causing a localized burst of toxic chemicals (aglycones and their break-down products) in these cavities. In fact, the protein product of the *Hfr-1* gene, a BGAF homolog from wheat, has been shown to deter Hessian fly larvae from feeding on resistant plants by binding to sensory receptors (Plant Physiol. 147:1412-1426, 2008). It is now becoming clear that there are at least two chimeric lectins in maize that specifically interacts with β-glucosidases. We postulate they help the plant to launch a powerful defense response to attack by pests.

BROOKINGS, SOUTH DAKOTA
South Dakota State University

Scoring *Pr* vs. *pr* aleurone color

--Whalen, RH; Auger, DL

Most offices and labs have fluorescent lighting, and it is often difficult to accurately distinguish *Pr* (purple) from *pr* (red) aleurone color under fluorescent light. The best way to score *Pr* vs. *pr* kernels indoors is by viewing them under pure incandescent light rather than fluorescent light or a mixture of the two. The closer one holds the kernels to the incandescent bulb, the easier it is to accurately distinguish *Pr* from *pr*. Even under incandescent light, a very small proportion of kernels that appear to be purple are found to be red when held much closer to the light bulb, presumably due to segregation of modifying factors. These observations have been confirmed by several people.

BUENOS AIRES, ARGENTINA
Universidad de Buenos Aires

Karyotype comparison between *Zea luxurians* and maize Amarillo Chico through DAPI-banding and FISH of knob sequence

--González, GE; Fourastié, MF; Poggio, L

The variation in DNA content in *Zea* has been attributed to differences in the heterochromatin amounts that form distal blocks named knobs (Laurie and Bennett, Heredity 55:307-313, 1985; Poggio et al., Ann. Bot. 82:115-117, 1998). These knobs may vary in quantity and size among the different races of maize and its wild relatives (Kato, Agric. Exp. Stn. Bull. 635: 1-185, 1976; McClintock et al., Col. Postgrad., Chapingo, México, 1981). *Zea luxurians* possesses the highest genome size of the 2n=20 *Zea* species (2C= 8.83 pg), while the DNA content of maize varies between 2C= 4.92 and 6.79 pg (Tito et al., Theor. Appl. Genet. 83:58-64, 1991). The heterochromatic knobs from maize and *Zea luxurians* correspond to C- and DAPI-positive bands on mitotic metaphase chromosomes (Tito et al., 1991; Poggio et al., 1998). Knobs are composed principally by a 180-bp *tandem* repeat sequence (Peacock et al., Proc. Natl. Acad. Sci. USA 78:4490-4494, 1981).

The materials used in this study were the *Zea luxurians* cv. 9478 (Guatemalan) Leg. CIMMYT and the Argentinean race of maize, Amarillo Chico (VAV 6451), from NOA Leg. by Vavilov Laboratory, University of Buenos Aires (UBA). The materials were cultivated in the greenhouse of Facultad de Agronomía, UBA. DAPI banding and FISH performed according to Summer (Chromosome banding. Unwin Hyman, London, 1990) and González et al. (Chrom. Res. 14:629-635, 2006), respectively, were carried out on mitotic metaphases from *Zea luxurians* and maize. For FISH, the knob-180bp sequence was obtained and used as probe. Slides were examined with a Carl Zeiss Axiophot epifluorescence microscope and the photographs were taken using a Leica CCD digital camera.

Chromosomal parameters were measured in at least 10 metaphases for each species, using the freeware program MicroMeasure 3.3 (http://www.colostate.edu/depts/biology/micromeasure). For karyotyping the relative chromosome length, arm ratio and centromeric index were calculated. The chromosomal morphology was described according to Levan et al. (Hereditas 52:201-220, 1964). Intra- and inter-chromosomal asymmetry indexes, A1 and A2 were calculated as described by Romero Zarco (Taxon 35:526-530, 1986). These indexes, in addition to the formulae and karyotypic parameters from *Zea luxurians* and the Amarillo Chico maize are shown in Table 1.

The cytogenetic studies presented here reveal important karyotypic and chromosomal differences between *Zea luxurians* and maize. The differences in inter-chromosomal asymmetry are due to the number, size and distribution of heterochromatic knobs on both chromosome arms.

Table 1.

	Maize Amarillo Chico	*Zea luxurians*
2n=4x	20	20
DNA content 2C (X +/- Standart Error)*	5.63 pg (+/- 0.05)	8.83 pg (+/- 0.08)
Karyotypic Formula	6 m + 4 sm	5 m + 4 sm + 1 sm-st
TCL	135um	173um
TCL without knobs	124um	168um
% of heterochromatin †	7.65	21.16
Range of knob sizes	1.62um - 0.54um	2.74um - 0.8um
Media of knob sizes	1.04um	1.75um
A1	0.36	0.39
A2	0.27	0.15

Ref.: *From Tito et al., 1991. **TCL**: Total Chromosome Length. **†**: Calculated as a percent of TCL. **A1**: intra-chromosomal asymmetry index. **A2**: inter-chromosomal asymmetry index. **m**: metacentric. **sm**: sub-metacentric. **st**: sub-telocentric.

The analysis of the karyotype parameters allow us to elaborate the idiograms from the taxa analyzed (Fig. 1). It is important to note that the knobs 180bp sequence showed FISH-positive signals on all heterochromatic DAPI-positive bands, as is indicated in Figure 1.

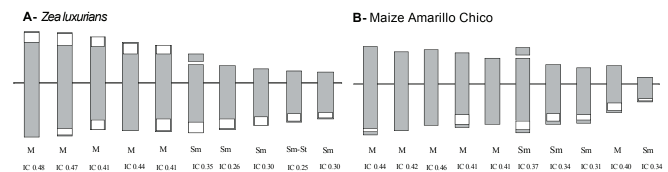

Figure 1. Idiograms of *Zea luxurians* (A) and maize race Amarillo Chico (B). The white blocks represent the coincident DAPI-positive bands and the 180bp sequence FISH signals. **M**: metacentric. **Sm**: sub-metacentric. **St**: sub-telocentric. **IC**: Centromeric index.

Zea luxurians has 14% more knob-heterochromatin than maize, and indeed, knobs of *Zea luxurians* are about 40% bigger than those of maize (Fig. 1). Moreover, we observed that the TCL of *Zea luxurians* is 28.6 % higher than maize, and approximately 26% higher if knobs are not considered. We also find that the total chromosome volume of *Zea luxurians* is about 28% higher than maize. Therefore, the differences in DNA content and the chromosome sizes between both species can account for both their dissimilar number and size of knobs, and the different amounts of interspersed DNA.

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Academia Sinica
LONDON, ONTARIO
University of Western Ontario

Stomata on maize anthers

--Tsou, C-h; Cheng, P-c; Walden, DB

Stomata are commonly present on floral parts and can function primarily in nectar/water secretion rather than gas exchange (Varassin et al., Ann. Bot. 102(6):899-909, 2008). Kenda (Phyton 4:83-96, 1952) investigated the stomata on the anthers of many species and found they were distributed on the connective, but generally absent in the filament. We examined anthers at anthesis of maize cultivars Gaspé Flint and Ohio 43, using cryo SEM and conventional SEM, and found stomata on adaxial and abaxial surfaces of the connective (Fig. 1), but not on filament nor the surfaces of the four microsporangia. Full opening of stomata as observed under cryo SEM (Fig. 2) indicated they were functioning. Ten anthers from a single Ohio 43 plant were counted for the number of stomata, the numbers were 22, 24, 19, 20, 18, 22, 21, 31, 19, and 28 on one side of the connective, which gives an average of 42, with a range from 36 to 62, stomata per anther. The biological significance of stomata on the connective in maize plants is under investigation.

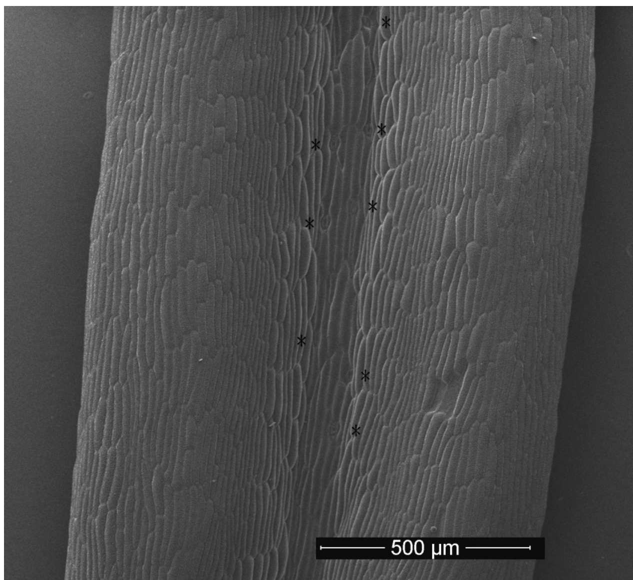

Figure 1. Distribution of stomata (*) on the adaxial surface of connective of an anther of Ohio 43.

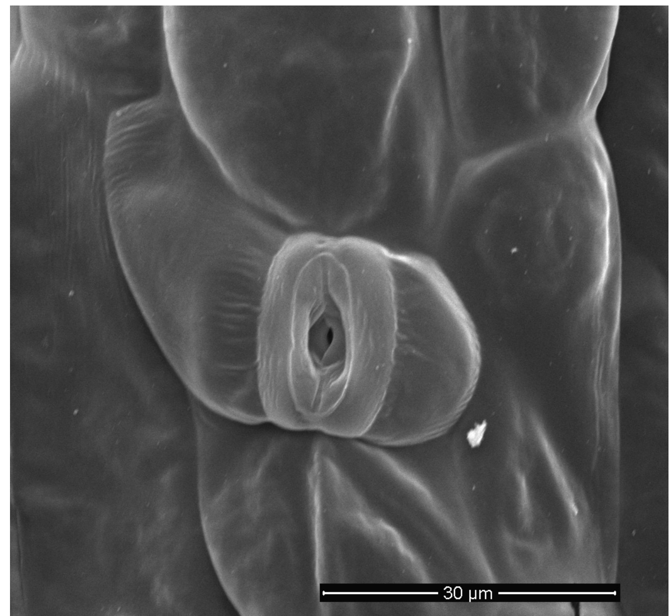

Figure 2. Higher magnification of a stoma on the connective.

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Yield evaluation trial of specialty maize inbreds

--Corcuera, VR; Kandus, M; Salerno, JC; Moreno-Ferrero, V

A completely randomized block design yield trial was sown by the beginning of November 2009 in a plot generously loaned by the School of Agriculture-Universidad Nacional de Lomas de Zamora within their experimental campus at Virrey del Pino (Province of Buenos Aires, Argentina). High temperatures and drought characterized the growing season in 2008/09. Forty-four inbreds, including the six termed CIG8, CIG12, CIG29, CIG30, CIG18 and CIG39, which are in process at the National Register of Cultivar Property (R.P.N.C.), were evaluated for grain yield, as follows. The ears were hand harvested, threshed and the grain weighed. Kernel humidity was determined using a portable humidimeter *Protimeter Grainmaster 900* and weight was corrected to the commercial standard humidity (15%). The *average yield per plant* (AYP) is expressed as grams per plant. Potential yield (PY), expressed as kilograms of grain per hectare, was estimated multiplying the AYP by 71,500 (plant density at harvest). The inbreds tested can be grouped according to the type of grain as follows: 1) Modified starch, high amylopectin content maize (waxy); 2) High-quality protein maize (HQP); 3) High-quality protein and modified starch maizes (double mutants or DR); 4) Soft and starchy endosperm (SE); 5) Hard and vitreous endosperm maize (VE).

The data for AYP and PY calculated for each inbred tested are provided in the online supplemental data. The overall average was 52.3 ± 10.6 grams of kernels/plant (range= 31.4 to 72.9

grams.) and 3739.9 ± 759.6 kilograms of kernels/hectare (range= 2245.1 to 5212.4 Kg. kernels/hectare), of which the minimum values belong to the waxy inbred CIG8 and the highest to the double mutant *wx o2* CIG29.

The AYP, average PY and yield range for each group of inbreds are as follows:

1) waxy 50.3 ± 10.1 gr. kernels/plant and 3597.0 ± 724.1 Kg. kernels/ha
(31.4 to 64.5 gr. kernels/plant to 2245.1 to 4611.8 Kg. kernels/ha)
2) HQP 45.4 ± 10.3 gr. kernels/plant and 3244.2 ± 740.4 Kg. kernels/ha
(32.5 to 69.4 gr. kernels/plant and 2323.8 to 4962.1 Kg. kernels/ha)
3) DR 55.7 ± 8.5 gr. kernels/plant and 3983.6 ± 607.0 Kg. kernels/ha
(44.1 to 72.9 gr. kernels/plant and 3153.2 to 5212.4 Kg. kernels/ha)
4) SE 57.6 ± 6.1 gr. kernels/plant and 4118.4 ± 439.9 Kg. kernels/ha
(50.1 a 64.1 gr. kernel/plant and 3582.2 to 4583.2 Kg. kernel/ha)
5) VE 69.0 ± 3.8 gr. kernels/plant and 4933.5 ± 273.0 Kg. kernels/ha
(66.3 to 71.7 gr. kernels/plant and 4740.5 to 5126.6 Kg. kernels/ha)

Inbreds with hard and starchy endosperm (VE and SE) showed the shortest dispersion of values for AYP (CV%= 5.5 and 10.7, respectively), whilst the HQP inbreds showed the greatest divergence for this descriptor (CV%= 22.7*)*. On average, the quality protein materials have lower yields. When the averages were compared applying Student´s t test, highly significant differences were found amongst the *waxy*, HQP and DR inbreds in relation to the VE group, as well as significant differences detected between HQP and SE or HQP and DR inbreds (see Table).

Table. Comparison of groups AYP by means of Student´s t test.

	HQP	DR	SE	VE
waxy	1.17 ns	1.47 ns	1.75 ns	4.79 **
HQP		2.64 *	2.79 *	5.73 **
DR			0.49 ns	3.71 **
SE				2.78 ns

ns = *non-significant differences*; *= *significant at p: 0.05 level*; **= *significant at p: 0.01 level*

Of note, the yield of the inbreds termed CIG1 to CIG32 decreased approximately 15% in relation to the values calculated previously at the location of Llavallol (Province of Buenos Aires) during 2002/03 to 2004/05.

Near-infrared analysis (NIRT) of kernels of value-enhanced maize inbreds

--Corcuera, VR; Fernández, G; Salerno, JC; Salmoral, EM; Moreno-Ferrero, V

Our study included forty-four inbreds of which five were testers. A complete randomized block design field trial with three replicates was grown in 2009 at Virrey del Pino, a province of Buenos Aires (34º49´57´´S, 58º43´23´´W). Plant density at harvest was 71,500 plants/ha. The materials generically termed CIG can be grouped based on their endosperm attributes as follows: 1) Modified starch, high amylopectin content maize (waxy); 2) High-quality protein maize (HQP); 3) High-quality protein and modified starch maize (double mutants or DR); 4) Soft and starchy endosperm (SE); 5) Hard and vitreous endosperm maize (VE). Only kernels obtained by hand pollination (selfings or sib´s) were analyzed to prevent xenia, particularly with respect to oil content. On harvest, kernels were kept in a cold room until analysis. Protein content (%), starch content (%) and oil content (%) were determined through a non-destructive manner using an infrared spectrophotometer model Foss Infratec 1241 Grain Analyzer. Two 60 g samples of each genotype were analyzed, and the results and simple correlation coefficient (Pearson) estimated. See the online supplemental data table.

The oil content of the forty-four inbreds varied from 4.4% to 8.2%, and 29.5% (13/44) of the genotypes analyzed may be considered high oil maize (HOC) using the 6% criterion of the U.S. Grain Council (1999), the ILSI (*Crop Composition Database version 2.0; www.cropcomposition.org*) and MAIZAR (Argentine Maize Association). The inbred CIG18 had the highest oil content (8.34%) followed by CIG52 (7.3%) and CIG30 (7.1%).

Maize kernel protein content usually varies from 8.0% to 11.0% according to FAO reports. ILSI Argentina estimated an average protein content of about 9.5%, based on 109 commercial hybrids sampled in the provinces of Buenos Aires and Córdoba between 1999 and 2001. This value is consistent with others published in the Argenfoods database (Universidad Nacional de Lujan, 2002). We found an average protein content ranging from 8.9% to 13.3%, with 61.4% of the inbreds having values ranging from 11% to 13.3%, surpassing the upper limit for protein content reported by FAO. The highest kernel protein content was shown by the waxy inbred CIG1 (13.3%), followed by the double mutant CIG38 (13.2%), the hard endosperm inbred CIG32 (13.1%) and the waxy inbred CIG8 (12.8%).

Kernel starch content ranged from 65.5% to 72.9% for the CIG inbreds. The highest kernel starch content belongs to the waxy inbred CIG8 (72.9%), followed by the starchy inbred CIG42 (72.5%) and the double mutant CIG42 (72.2%). The average starch content for inbreds in this study is consistent with published reports for Argentine maize varieties (INTA Pergamino Technical report 320, 1999; National Food Office in 2007; Borrás et al., Crop Sci. 42:781-790, 2002) .

Pearson´s correlation coefficients for oil, starch and protein content were calculated. The results suggest once more, as in previous MNL reports, that there is not a significant association between oil and protein content (r= -0.03; ± Student´s t= 0.17), although negative and highly significant associations (*p: 0.01*) were found between oil and starch content (r= -0.64; ± Student´s t= 5.34) as also reported by Wassom et al. (Crop Sci. 48:243-252, 2008). Negative and highly significant associations were also found between protein and starch content (r= -0.43; ± Student´s t= 3.07).

The results obtained clearly demonstrate that many CIG inbreds are improved with regard to the average protein, starch and oil content reported for maize by several authors and organizations, both nationally and internationally. Those inbreds yielding more than 6.0% oil and considered as HOC genotypes could be

used as male progenitors in future crossings. Similarly, inbreds with 12.0% or more protein content are suitable to be used as females in future breeding according to the protein inheritance model proposed by Corcuera and Naranjo in 1995 (Proc. of the III Reunión Latinoamericana and XVI Reunión de la Zona Andina de Investigadores en Maíz, Tomo II, pp. 855-864, Cochabamba-S. Crúz de la Sierra, Bolivia).

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Institute of Plant Genetics and Physiology

Callus genesis, somatic embryogenesis and plant regeneration in F1 hybrids of maize

--Climenco, OA; Kravchenko, AN; Jacota, AG

The purpose of this study was to determine heritability and correlation of characters such as callusogenesis, somatic embryogenesis and plant regeneration. Twenty F1 hybrids (Co125 x P502, Co125 x MK390, Co125 x MK159, Co125 x MK01, Co125 x P101, 092 x P502, 092 x MK390, 092 x MK159, 092 x MK01, 092 x P101, A239 x P502, A239 x MK390, A239 x MK159, A239 x MK01, A239 x P101, 459 x P502, 459 x MK390, 459 x MK159, 459 x MK01, 459 x P101) were used as experimental material. Tissue cultures were initiated from 12- to 13-day-old immature embryos. The data were processed by analysis of variance (Statgraphics Plus 5.1), and coefficients of heritability (h^2) for the characters under study were calculated. For the comparison of the relationships between the processes of callusogenesis, somatic embryogenesis and plant regeneration correlation coefficients were determined among parental inbred lines as well as among hybrid combinations.

Highly significant differences in frequency of embryogenic callus formation and plant regeneration process among F1 hybrids were found. Such combinations as A239 x P502 and A239 x MK159 proved to be the best for somatic embryogenesis (83.58% and 86.89%, respectively). However, A239 x MK159 had a much lower frequency of plant regeneration than A239 x P502 (34.96% and 76.54%, respectively). It was also found that 092 x P502 had a rather high frequency of all processes under study (somatic embryogenesis – 73.39% and plant regeneration – 67.78%). In 4 hybrids, somatic embryogenesis and plant regeneration were not observed.

Correlation analysis showed a positive correlation ($r = 0.69$, $P<0.05$) between the somatic embryogenesis and plant regeneration processes for all the inbreds used as parents. At the same time, a positive relationship ($r = 0.44$, $P<0.05$) between callus formation and somatic embryogenesis was determined for F1 hybrids. A similar relationship exists between callus-genesis and plant regeneration ($r = 0.48$, $P<0.05$). However, the correlation between somatic embryogenesis frequency and plant regeneration frequency was much stronger ($r = 0.95$, $P<0.001$) than that of parental lines. Among F1 hybrids a strong negative correlation ($r = -0.87$, $P<0.001$) was observed between embryogenic callus formation and non-embryogenic callus formation. It should be noted that somatic embryogenesis and plant regeneration also negatively correlated with non-embryogenic callus formation ($r = -0.43$, $P<0.05$ and $r = -0.56$, $P<0.01$, respectively). Embryogenic callus formation was positively correlated ($r = 0.66$, $P<0.001$) with plant regeneration.

Heritability for the processes under study was estimated by two-factor analysis of variance. Coefficients of heritability are presented in Table 1. It was shown that male parent and interaction of both parents had the highest values of heritability for callusgenesis, somatic embryogenesis and plant regeneration. Female parents had the lowest heritability value and this coefficient was significant only for non-embryogenic callus formation. Taking into account the relatively high heritability values of male parents, heritability coefficients of male parents for each hybrid combination were calculated on the basis of single-factor analysis of variance (Table 2). It should be noted that the highest values of male parent heritability were observed when inbred line A239 was the female parent. In contrast, the lowest coefficients of heritability for the processes under study were observed when inbred line 092 was used as a female parent. The lowest value of male parent heritability for embryogenic callus formation was noted when 459 inbred line was used as a female parent. Thus, these results can be used in the elaboration of new and improved methods of obtaining maize regenerants.

Table 1. Coefficients of heritability for the characters under study.

Character	h^2 for female parent	h^2 for male parent	h^2 for interaction of both parents
Embryogenic callus formation	-	0.47***	0.32*
Non-embryogenic callus formation	0.26***	0.35***	0.31***
Plant regeneration	-	0.47***	0.41***

* - P<0.05 ** - P<0.01 *** - P<0.001

Table 2. Heritability corfficients (h^2) of male parent in F1 hybrids.

FEMALE PARENT	MALE PARENT	CHARACTERS		
		Embryogenic callus formation	Non-embryogenic callus formation	Plant regeneration
Co125	P502 MK390 MK159 MK01 P101	0.692*	0.665*	0.731*
092	P502 MK390 MK159 MK01 P101	0.396*	0.366*	0.438*
A239	P502 MK390 MK159 MK01 P101	0.773**	0.823**	0.919**
459	P502 MK390 MK159 MK01 P101	0.293*	0.538*	0.888*

* - P<0.05 ** - P<0.01

COLLEGE STATION, TEXAS
Texas A&M University
LUBBOCK, TEXAS
Texas AgriLife Research

Identifying maize germplasm with extremely late flowering and photoperiod sensitivity in Texas

--Murray, SC; Xu, W; Mayfield, KL

Maize cultivars that are extremely late to flower or fail to flower in the temperate/ sub-tropical summer are of interest: 1) to develop tall dedicated cellulosic feedstocks (non-grain), 2) to better understand genetic mechanisms of flowering, 3) to create genetic mapping populations for flowering time and other traits of interest, and 4) to identify genetic diversity in linkage with extreme flowering time genes that may otherwise be inadvertently selected against. We are also very interested in these tropical lines as an untapped source of aflatoxin resistance but flowering time confounds this analysis. We planted 55 accessions obtained from GRIN (USDA-ARS) and up to three checks (B73, Mo17, CML254), in three Texas locations, Weslaco (planted 2/18/2009), College Station (planted 3/20/2009), and Lubbock (planted 5/8/2009) if enough seed was available. Accessions of interest were identified based on previous MGNL notes, data found in GRIN and suggestions found on blogs. Plants were managed with normal agronomic practices for a breeding nursery but stress was still evident from a record breaking hot and dry summer across Texas. Flowering dates (anthesis) were taken every few days in College Station and Lubbock, but estimated in Weslaco on May 15th by KLM and SCM. Because many of these accessions were heterogenous landraces, some within accession segregation was observed. For nearly all accessions, segregation was often no more than a week and plants appeared otherwise uniform. To increase seed and develop homozygous lines for future experiments, individuals were self- or sibling-pollinated in College Station. Many of the accessions that flowered very late failed to produce seed or had excessive ear rot due to late season stress. Asynchronous flowering was observed (always with silking after anthesis) within some accessions, but attempts to sib-pollinate were occasionally successful. The wild species *Z. diploperennis* and *Z. huehuetenangensis* were the latest to flower, while among domesticated accessions the latest were from Ecuador and Columbia.

Late flowering accessions were then also planted in a winter nursery in Weslaco, TX on August 10, 2009 to produce more seed for evaluation. Under winter nursery conditions (going into shorter days, moderate day temperatures, cool nights) most of the accessions flowered around the same time or slightly later than normal breeding material. This suggests that the delayed flowering in summer was caused primarily by photoperiod response rather than by high temperatures. This confirms that the challenge of producing and evaluating genotypes that do not flower in the summer can be overcome by making crosses and producing seed in a winter nursery under a short-day length.

Ultimately, we wish to develop extremely late flowering inbred lines out of landrace material that can be used for a variety of experiments, tests, and breeding objectives. It is clear that given the heat of Texas summers the inbreeding of landrace material is likely to be quite challenging and some crossing to elite adapted material may be necessary. We hope that crosses to elite material can also be utilized, in coordination with the Germplasm Enhancement of Maize (GEM) program, as sources of genetic diversity for continued maize improvement in areas such as aflatoxin resistance. See also online supplemental data.

COLUMBIA, MISSOURI
University of Missouri

Chromosome breaking *Ds* sites in maize, revisited. Part I: Background, Methods, Description

--Neuffer, MG

This past year I received some supplemental funding (via NSF grant 0743804, with many thanks to Carolyn Lawrence and Mary Schaeffer for their help in this process) to update and revise information on my maize mutant collection that I had submitted to the Stock Center and to MaizeGDB back in 1995. A second objective was to provide information and stocks on a new dominant mutant collection I have generated in the past few years, with the generous support of Sarah Hake. In the process of organizing the data I decided it would be a good time to revisit a collection of *Ds* marker stocks that I had generated. The purpose of this Newsletter article is to combine published information (Neuffer, Pp. 258-262 in The Maize Handbook, M. Freeling and V. Walbot, eds., Springer-Verlag, New York, 1994; Maydica 40:99-116, 1995) about the *Ds* markers with the images and case descriptions already posted at MaizeGDB (http://www.maizegdb.org/), and to provide a general review of the uses and expression of these stocks. This text portion will be supplemented by information in Part II, where we use photographs of the stocks to illustrate our discussions of the proven cases. We plan to post Part II at Maize Gene Review (http://maizegenereview.org/) concurrently with this article, and the original data at MaizeGDB will be updated at the same time. I wish to thank Lou Butler for assistance in gathering the data, editing the text, preparing the artwork, and formatting this material for posting online. Thanks also are extended to Kelly Dawe and Hugo Dooner for reading the material and providing many helpful suggestions.

Background of Marker Stock Development: Loss of the normal allele in a heterozygote to produce a hemizygote with the recessive phenotype can be very useful in genetic studies. This may be produced in a number of ways, including x-radiation, marked ring chromosomes, and B-A chromosome translocations. The chromosome-breaking *Ds* elements are especially useful in studying the expression of lethal mutant tissue in chimeras produced by loss of the normal allele in a heterozygote. Our purpose in this project was to generate chromosome breakage in heterozygous, lethal embryo, defective kernel (*dek*) whole kernel maize mutants on all chromosome arms. Plant chimeras of hemizygous mutant whole kernel and seedling *-/dek* tissue, sustained by adjoining normal *+/dek* tissue, could thus be observed and compared. By taking advantage of its chromosome breaking properties, we were able to produce *Ds* markers on many genetically marked chromosome arms (Table 1).

The transposable element *Ds*, discovered and analyzed in detail by McClintock (Cold Spring Harbor Symp. Quant. Biol. 16:13-47, 1951), is the responding element of the *Ac Ds* system. It

Table 1. Transposition sites for chromosome breaking *Ds* stocks.

Symbol	Marker	Position
Ds-1S1	*Dek1*	distal
Ds-1S2	*Dek1*	probably distal
Ds-1S3	*Dek1*	distal
Ds-1S4	*Dek1*	proximal
Ds-1L1	*Bz2*	proximal
Ds-1L2	*Bz2*	at the *Bz2* (*bz2-m3*) locus
Ds-1L3	*Bz2*	at the *Bz2* (*bz2-m3*) locus
Ds-1L6	*Bz2*	at the *Bz2* (*bz2-m*) locus
Ds-2S1	*B1:Peru*	distal
Ds-2S2	*B1:Peru*	unknown
Ds-2S3	*B1:Peru*	at the *B1:Peru* (*b1-m1*) locus
Ds-2S4	*B1:Peru*	at the *B1:Peru* (*b1-md2*) locus
Ds-2L1	*W3*	unknown
Ds-3L1	*A1 Sh2*	proximal
Ds-3L2	*A1 Sh2*	proximal
Ds-4S1	*Bt2*	unknown
Ds-4S2	*Bt2*	unknown
Ds-4L1	*C2*	distal
Ds-4L3	*C2*	at the *C2* locus
Ds-4L4	*C2*	distal
Ds-4L5	*C2*	distal
Ds-4L6	*C2*	distal
Ds-4L7	*C2*	distal
Ds-5S1	*A2*	proximal
Ds-5S2	*A2*	proximal
Ds-5L1	*Bt1*	distal to *Bt1* and proximal to *Pr1*
Ds-7L1	*O5*	distal
Ds-7L2	*O5*	proximal?
Ds-7L3	*O5*	proximal
Ds-8L1	*Pro1*	unknown
Ds-9S1	*C1*-I	proximal?
Ds-9L2	*Dek13*	unknown
Ds-10L2	*R1-sc*	proximal
Ds-10L4	*R1-sc*	proximal
Ds-10L5	*R1-sc*	*Ac* at the *R1* locus
Ds-10L6	*R1-sc*	*Ac* at the *R1* locus

has the unique property of being able to move about (transpose) in the genome when *Ac* is present. Thus, sites throughout the genome can be selected for further genetic analysis. At the resident site *Ds* can also suppress the function of an associated active gene and/or cause a break in the chromosome at that site, initiating the breakage-fusion bridge-cycle described by McClintock (Cold Spring Harbor Symp. Quant. Biol. 9:72-81, 1941; for diagram see http://profiles.nlm.nih.gov/LL/B/B/R/S/) of sequential chromosome breaks with associated losses and consequent gain of genetic material in the daughter cells of a mitotic or meiotic division. Mutations induced by *Ds* at the gene site are observable when they interfere with the gene's function, producing a recessive null phenotype. This loss of genetic function in a heterozygote with *Ds* on the homolog with the dominant allele allows a recessive allele to be uncovered as a chimera of recessive tissue. In some cases *Ds* acts as both breaking and suppressing. If the breakage feature is associated with suppression of the resident gene, this can affect observation of the genes used to mark chromosome loss.

It is complicated to interpret results from *Ds* experiments. The state of activity, variations in *Ac* dosage, and other genetic modifiers can all lead to variation at the insertion or neighboring site. Moreover, the characteristics of each variation depend on the marker used, the relative position of *Ds* and the marker on the chromosome arm, and on other types of genetic modifiers. Originally we intended to find a site between the marker and the centromere, so that the marker would be lost as an early *Ds* event. It became clear, however, that the size, frequency, and characteristics of sectors shown at each new *Ds* site were related to the position on the chromosome arm relative to the marker used. In fact, expression was related to the position of three components: *Ds*, the marker, and the centromere. This position could be determined by the type of loss pattern in the kernel. Location on the distal side of the arm from the centromere was observable as frequent, small sectors, associated with twin duplicate-deficiency spots. Proximal location was characterized by frequent large sectors along with normal tissue in single dots, or dots in chains or clusters within the large deficient sector. Location at the gene site was usually accompanied by suppression of the gene function to produce the equivalent of the recessive or null allele that was exceptionally unstable when *Ac* was present, producing frequent reversions to some level of mutant gene expression. These appeared as sectors, usually dotted, of normal tissue on the null kernel background or elongated dominant streaks of red on the pericarp and anthocyanin on normal green plant leaves.

Methods and stock preparation: The original *Ds1* site described by McClintock (1951) was located proximal to *Wx1* on the short arm of chromosome 9. My stock of this original *Ds1* material has been termed *Ds-9S1*. Dr. Jerry Kermicle generously provided two additional stocks that were presumed to carry the *Ds1* site on the long arm of chromosome 10 near the *g1* locus; these were used to generate the remaining *Ds* stocks. One stock (*P1-vv, Ds-10L2 R1-sc* with *Ds* 10 cM proximal to *g1*) carried *Ds* on the long arm of chromosome 10, proximal to the centromere from the *R1-sc* allele at the *R1* locus and the other stock was *P1-vv*, *Ds-10L4 R1-sc* with *Ds* in the same general region. The pollen source was *P1-vv*/*P1-wr*, *Ds-10L2 R1-sc*/*Ds-10L2 R1-sc* or *R1-r* and homozygous dominant for all the markers in the tester stocks used except chromosome 2S (see below). Heterozygous *P1-vv* was used in order to retain the early large sector properties of 1 dose of *Ac*, thus increasing the likelihood of gametic events. The probability of capturing duplicate events from a single large tassel sector was minimal since cases were used from trials in two or more seasons and by selecting for visually different characteristics. All the genes used as marker stocks were present as dominant alleles in the pollen stock, except for chromosome 2S, where a special pollen stock was prepared using the dominant aleurone color allele *B1:Peru*. Since *B1:Peru* and *R1-sc* are duplicate factors, we used a *b1 r1* tester. Changes of aleurone color *B* function would appear as a color change on ears with purple kernels.

The *Ac* used was the one associated with *P1-vv* and in many of the *Ds* cases *P1-vv* can be clearly seen as red streaks on the colorless kernels. This is an expression of *P1-vv* in the maternal parent and is caused when *Ac* is inserted in or near the red pericarp locus. The streaks are caused whenever *Ac* moves away from the locus. *Ac* has most of the same properties as *Ds* and can act an autonomous element.

Vigorous testers homozygous for the appropriate recessive aleurone, endosperm, and seedling markers were prepared. If the

tester mutant was lethal (*dek1*, *w3*, *o5*) normal kernels from a segregating F2 were used. Of these, 2/3 would be heterozygous (1/2 correct gametes), and 1/3 would be discarded as homozygous normal. For each tester, 100 or more plants were used as female parent. These were grown in an isolated, open-pollinated, detasseled plot according to the method of Stadler (Genetics 31:377-394, 1946). The ears were marked for each of the 16 chromsome arms that carried a usable marker. They were examined for two types of single kernel events: (1) Transposition of *Ds* near the marker and the centromere. Depending on the particular tester stock, mosaicism or sectoring for purple vs. colorless or bronze aleurone, and normal vs. shrunken, brittle or collapsed endosperm would reflect chromosome breaking activity in the endosperm, and many would be expected to have the same activity in the embryo thereby transmitting it to the next generation. (2) Transposition of *Ds* to the marker site which would suppress the dominant allele. This would result in a recessive mutant case with dominant revertant sectors due to the presence of *Ac*. Those without dots and revertant sectors are due to the absence of *Ac* because of chance segregation in gamete formation. They were considered potentially valid recessive mutable cases that could potentially show their mutability when recombined with *Ac*.

Single kernel cases were planted and observed for any variations from normal plant phenotype. Any kind of sectoring was especially noted as potentially indicating *Ds* activity in the plant. Plants were selfed and backcrossed to their respective tester for confirmation of *Ds* activity. Stable recessive cases were crossed to an *Ac* stock to test revertability.

Seedling markers were used in cases where endosperm markers were not available for that chromosome. All kernels were planted in sand benches and examined for seedlings of two kinds: 1) whole seedling mutant cases with multiple recessive sectors (*Ac*) and without sectors (no *Ac*); and 2) normal green seedlings with chimeras of recessive tissue for the marker used. Observed cases were transplanted to pots and were grown to maturity. The mutant seedlings for lethal phenotypes died, except when there was adequate revertant tissue to support plant growth. Those seedlings that survived to maturity were selfed and backcrossed to the recessive tester to confirm.

Many putative cases were observed in most of the tester stocks included in the crosses. Any that survived were grown to maturity and tested. The precision of observations varied greatly due to variation in the mutant. The aleurone color stocks gave excellent mosaic kernels with colorless or bronze sectors on part of the endosperm or aleurone layer (which is a part of the endosperm), and those with shrunken or brittle endosperm were also fairly good. However, the collapsed and opaque endosperm cases were difficult to recognize and many ambiguous cases were tested, most of which failed confirmation. Losses of other kernel cases to normal field conditions forced us to abandon any efforts at quantitative measurement of the frequency of events involving markers on individual arms.

The Non-transmitting cases: There were 79 kernel cases which failed to transmit; these were thought to be misinterpretations or nontransmission. However, recent reconsideration of these cases led to another interpretation. The non-transmitting cases should have been included, because we now know that losses occur as a consequence of nuclear division separating unequal products. We originally looked only for those cases where losses were apparent or where the product was associated with a mutant phenotype. The other product of the event would be a duplication or some other variation that often did not have an immediate phenotype. These cases should have been examined for unusual phenomenon and/or delayed expression. Photographs were taken of all of these cases and data from these apparent non-corresponding cases will be revisited to consider what evidence we still have and what that evidence points to. This information will be presented in a later publication.

Observations: Usually *Ds* showed a chromosome breaking property, but cases of suppression, either with or without breakage, were also observed. *Ds* losses appeared to occur much sooner in the kernel (larger sectors) than in the seedling (smaller sectors). This observation, however, could merely reflect the observed relative maturity of the respective tissues. Determining whether a mutable allele due to suppression was also a chromosome breaker related largely to the properties of the marker gene. For example, identifying twin spots required different dosage levels of gene expression. As we accumulated chromosome breaking *Ds* cases for arms using aleurone color markers we observed two major types of aleurone color mosaics: large mutant sectors indicated early breakage events, while smaller sectors indicated later events. These were distinct from timing changes in *Ds* events resulting from changes in *Ac* dosage, because our material usually had only one dose of *Ac* from the male parent and therefore should have had only large sectors. This was not always true, as other factors such as genetic modifiers and an unidentified independently segregating *Ac*, which occasionally appeared in the stocks used.

Several types of *Ds* cases were observed. There was a high frequency of large sectors. According to a personal communication from Dr. Jerry Kermicle, the original *Ds-10L2 R1-sc* case similarly displayed a high number of large sectors. The kernels appear more colorless than colored due to the large amount of tissue with lost gene function. The *Ds* site was shown to be proximal (between the marker and the centromere). We also observed an unusual mosaic pattern, which we originally thought was cases of parental colored tissue with repeated subsequent loss of color. However, these were actually found to be chains, clusters and/or islands of colored tissue (Figures 1 and 2) which we interpreted to be retention of the functional gene in acentric fragments. These fragments were carried along in daughter cells as clones from the initial break. The clusters, chains and islands were as a rule more intensely pigmented for those markers having a dilution dosage effect (i.e., *C2*, *R1-scm*, *B1:Peru*), to be expected because the acentric fragment from a proximal break would carry duplications for the gene being followed. These observed islands of normal tissue within a sector of mutant tissue were similar to those seen many years ago by L.J. Stadler and also by me, his student. We were performing experiments involving radiation-induced color losses and were unable to interpret the meaning of the spots. Stadler called these islands "recovery spots" (Figure 2). We observed that the frequency and size of these recovery spots depended on the chromosome arm and the proximity of the marker, and therefore the *Ds* site to the centromere. It appears that short acentric fragments are not retained or "recovered" as frequently as long ones.

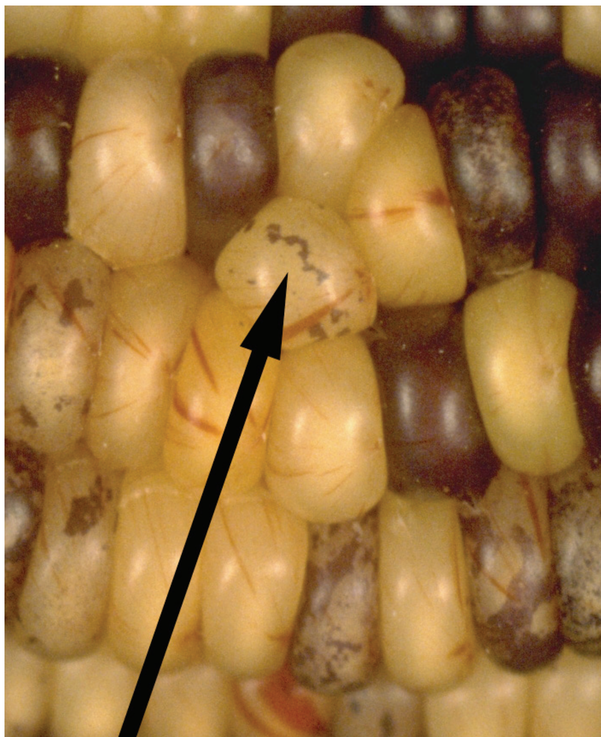


Figure 1. A good example of chains of dots.

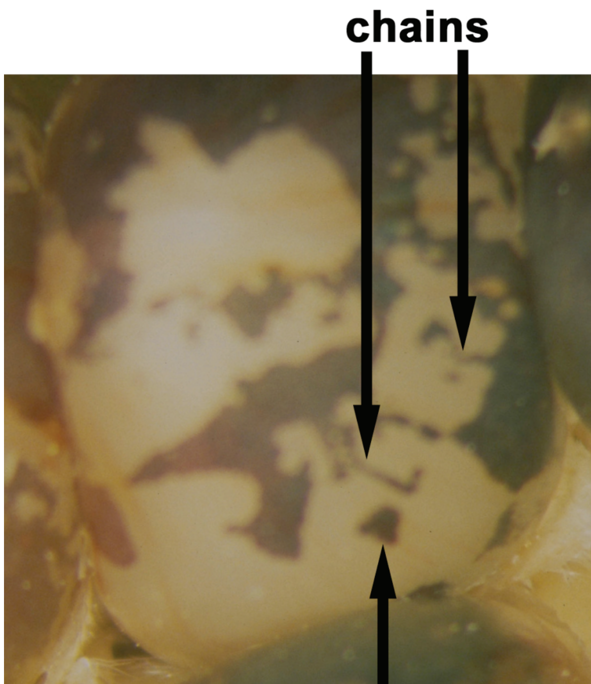


Figure 2. Two examples of chains and an example of a recovery spot.

Other *Ds* cases displayed a relatively high frequency of medium and small sectors, and a low frequency of large sectors. For genes with a dosage effect on aleurone expression (for example, *Ds-4L6* with a *C2* marker, and *Ds-1S3*), the mosaic kernels were dilute (depending on the dosage threshold for gene expression of the gene studied) with a few large sectors and many small colorless sectors or patches scattered randomly over the aleurone layer. Considering that only one *Ac* was present, these were rather late events. The small patches ranged from about 1/32 of the kernel surface to those composed of only a few aleurone cells. Large sectors could cover from 1/4 to 1/3 of the aleurone surface, but these were rare.

Frequently, the colorless patches were angular and sharply outlined, and were edged by a smaller and intensely pigmented sector. The smaller sector was clearly on the dilute side of the border rather than on the colorless side. These spots of colorless and intense pigmentation arising from a dilute background were termed twin spots (Figure 3). McClintock (1941) explained these

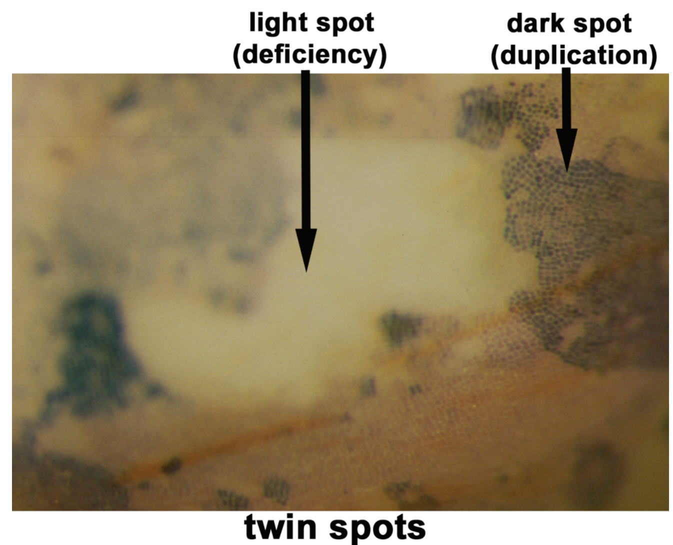


Figure 3. Typical expression of twin spots.

spots to represent a deficiency and duplication for the marker gene which occurred as a result from a break in the chromosome distal to the marker. The broken ends then rejoined, forming a bridge at mitotic anaphase with two copies of this marker. Then, a second non-median break occurred at telophase such that both copies of the gene went to one daughter cell and none to the other.

CRAIOVA, ROMANIA
Agricultural Research and Development Station - Simnic

The influence of climate on maize production in the centre of Oltenia

--Urechean, V; Bonea, D; Borleanu, IC

In recent years, the climate in our country has had wide variations of temperature, rainfall and other weather factors which negatively influenced the level and stability of production in the field. We describe here climatic variations that have influenced the

production capacity of 10 maize hybrids cultured for 3 years at SCDA Simnic, Romania (2004, 2005, 2006) In all cases, grain yield was determined at 15.5% humidity. The processing and interpretation of the results were made by variation analysis and the variation coefficient (VC%). All tables are presented in online supplemental data. Over the growth period, we registered 305.0 mm of rainfall. This was 99.9% of the normal value, and a satisfactory quantity for the maize needs. We find that even if the sowing is in May, the water accumulated in the soil during April was very important for the maize in the germination period and in the beginning of the vegetative growth. The moisture deficit from the 2nd and the 3rd period of June and even into the first half of July, when the water consumption is maximal, created unfavorable conditions for the last phases of growth, silking and tasseling, when the elements of grain production are forming, leading to serious production losses. A little rainfall from the 2nd and then lack over the 3rd periods of August have created a major water deficit, in the period of nutrient depositing in the kernels, leading to shriveling and decline of production.

In 2005 (Table 1, online supplemental data) the rainfall during the growth period exceeded the normal by 271.0%, making 2005 a very favorable year for the maize crop. The abundant rainfall from July and August as well as those equal or above the normal from the first period of vegetation positively influenced the growing rhythm and the flowering, silking, pollination, and grain filling physiological processes

In 2006 (Table 1, online supplemental data), the rainfall from the vegetation period represented 160.0% of the average, creating favorable conditions for maize culture. Unfortunately, the distribution of the rainfall was not in accordance with maize needs from the first period of vegetation. Thus, 23.7 l/ mp moisture deficit in May negatively influenced the germination of kernels and the growing rhythm of the plants in the first period of vegetative growth, with implication on the initiation of flowering, leading to diminished grain yield.

The air temperature over the three years of experiments surpassed the normal (Table 2, online supplemental data). The drought was most intensive in July 2004 when we had 12 days with temperatures above 34°C and 6 tropical nights with temperatures above 20°C. In 2005, this phenomenon was less intense with 8 days of drought and in 2006 we registered only 2 days of drought.

The productions realized by the 10 maize hybrids (Table 3, online supplemental data) reflect the different climate conditions from the 3 years of experiments, the greatest yield being registered in 2005. The high values of the production variation coefficients (Table 3) show that during the period of experiments (2004, 2005, 2006) the changes suffered from one year to another, but also those which took place during the same year determined a big fluctuation in the productivity.

In 2004, only 20% of the total rainfall accumulated during the flowering and silking period and severely diminished grain yield (Table 4, online supplemental data). The most balanced distribution of rainfall took place in 2005 when 60% accumulated in the period of vegetative growth and the forming of production elements and 40% during the periods of silking, flowering and grain filling. In 2006 the difference between the two periods of vegetation was of only 1% and the difference between the medium production of 2005 and 2006 years was almost 3000kg/ha.

However, rainfall is just one of the many biotic and abiotic factors which have an influence on the forming of production. Therefore, only an analysis of the influence of rainfall from sowing to physiological maturity on the production is not adequate. The mean productions of the 10 maize hybrids studied in the 3 years was analyzed only by the rainfall from each month (April – September). In 2004 we find that the drastically diminished production is in a large measure because of lack rainfall in April and August (Table 5, online supplemental data). The rainfall from June-August 2005 determined a substantial increase in production. The pluses and minuses from 2006 are almost equal excepting those from August.

Although it is believed that the intensity of the physiological processes decreases after reaching physiological maturity, it seems that the rainfall from August had a decisive role in defining the production capacity in the draughty years as well as in the favorable years.

Tables referred to in this note are available in the online copy, initially at the staging site, and eventually at MaizeGDB.

DEFIANCE OHIO
Defiance College

Environmental control of *R*-paramutation
--Mikula, BC; Studer, A

Over the past 50 years our reports to the MNL on *R* paramutation develop the evidence for photo thermal (PT) control of heritable changes in a Mendelian gene. Since the environment was dismissed as Lamarckian in the 20th Century, efforts were directed at a specific Mendelian gene. In 1956 *R* paramutation provided an example in which every *R* allele from the *R/Rst* heterozygote was heritably silenced (reduced in level of pigmentation), thus violating the first law of Mendelian genetics. Because *R* silencing occurred in 100% of the *R* alleles segregating from the heterozygote, there was a high probability that a phenotypic change could be detected if environment perturbed the paramutation process. A further advantage of *R* paramutation was the continuum of expression-states available for monitoring changes in *R* on a testcross ear, where as many as 200 kernels could exhibit changes in expression-states. Another advantage was that paramutated *R* alleles showed incremental change ("memory") from year to year, which meant small changes in expression one year could be amplified the following year. The continuum of expression-states in testcrosses of paramutated *R* alleles suggested that progression of epigenetic variation in pollen from different tassel branches should be examined carefully. Thus conditions were available to test whether environmental conditions (photo thermal, PT) could influence paramutation, and cause heritable change in Mendelian gene expression.

Plants grown under field conditions show no clear variation between pollen tested from the upper and lower tassel branches, so efforts was directed to the time when tassels are formed in the W22 inbred. The Kettering Foundation provided controlled environment chambers where maize seedlings were germinated under defined conditions of light and temperature. Under continuous

light (LL), seedlings germinated and developed quickly during the first week, after which necrotic lesions were observed on leaf tips. The appearance of necrotic lesions indicated that seedlings were ready for tassel induction by a light/dark (LD) cycle. When LD conditions were applied, seedlings continued developing, necrotic tissues were reduced. Under LL, seedlings continued to initiate leaf nodes until LD conditions are applied.

At maturity, pollen from upper tassel branches of plants, subjected as seedlings to various controlled conditions, showed more silencing than pollen testcrossed from lower branches of the same plant. Results shown in the Table were reported to the 2003 Genetics Congress in Australia. The pooled means, columns three and four, represent several plants and obscure epigenetic changes in individual plants. Early pollen from upper branches showed less pigment (more silencing) than later pollen from lower branches among seedlings started at LL 22° (continuous light, 22° C). The epigenetic dynamics in the tassel pollen of individual plants is also obscured in the pooled mean scores. The dynamics of paramutation responses, in single plants, to PT conditions becomes more apparent in the spreadsheet display (Figures 1A, 1B, 1C) of changes in pigment profiles for the 75 individual plants whose pigment expression was presented as pooled means in Table 1. The ovals in the three figures show how the pigment scores were biased by the early conditions experienced by the seedlings.

Table 1. Effect of photo-thermal environments on *R* paramutation. Multiple pollen testcrosses were made from upper (early) and lower (later) tassel branches of each plant over a period of eight days. Pigment scores were determined by visually matching individual kernels against a set of standard kernels that ranged from 1 (near colorless) to 20 (full pigment). The range of pigment scores from single plants is presented in the figure for each of the 75 plants sampled.

		Pollinations		Growth Chamber Environment	
		Early	Late		
Line[a]	n[b]	Pooled Means[c]		Day 1-15	Day 16-21
48	8	8.0 ±3.4	9.3 ±5.0	LL 22°	2 LD 22°-4 LL32°
47	7	10.3 ±2.0	13.5 ±3.2	LL 22°	2 LD 22°-4 LL 22°
46	6	8.7 ±3.1	10.9 ±2.9	LL 22°	6 LL 22°
49	9	9.3 ±1.7	12.5 ±1.1	LL 22°	4 LD 22°-2 LL 22°
50	6	9.9 ±4.0	14.4 ±3.3	LL 22°	4 LD 22°-2 LL 32°
45	8	9.8 ±2.6	13.4 ±2.1	LL 22°	6 LD 22°
				Day 1-10	Day 11-15
30	6	3.3 ±.6	3.2 ±1.5	LL 32°	5 LL 32°
31	7	8.2 ±2.3	9.6 ±3.8	LL 32°	5 LL 22°
26	5	8.7 ±3.5	9.2 ±3.2	LL 32°	5 LD 32°
27	7	9.3 ±3.2	10.7 ±2.5	LL 32°	5 LD 22°
28	7	9.2 ±3.8	9.2 ±3.6	LL 32°	2 LD 32°-3 LL 22°
29	4	8.8 ±1.2	10.8 ±3.2	LL 32°	2 LD 22°-3 LL 22°

Chamber environments: LL, continuous light; LD light dark cycle. Temperatures are in degrees centigrade. The number preceding each environment is the number of cycles (days) for the environment specified. Thus 5LL22 represents 5 days continuous light and 22° C.
[a] line identifier; [b] number of sibling plants tested for each line; [c] mean pigmentation.

Pigment scores were determined by visually matching individual kernels against a set of standard kernels that ranged from 1 (near colorless) to 20 (full pigment). In all 75 plants, the earliest pollen sampled from the upper tassel branches showed the greatest silencing expressions when compared with late pollen from the same plant.

Figure 1A represents plants that as seedlings received mostly 32° conditions with pigment scores falling largely in the lower half of the pigment scoring range (most silencing). Figure 1B shows plants that received combinations of 32° and 22° for various cycles over a six-day period show scores that range across the upper and lower half of the pigment range. Figure 1C shows plants that received 22° conditions as seedlings gave pigment scores represented in the lower half of the scoring range (least silencing).

Within the second and third week a critical period of development was identified by pigment responses from pollen testcrosses of the mature plants exposed to early temperature and LD cycles. In Figure 1B temperature shifts for as few as two cycles into and out of 22° and 32° can cause differences in the level of *R* silencing. The variation in the profiles of the three figures helps to understand why the continuum of pigment expression was difficult to interpret from field grown material when, for 50 years, only single pollinations from single plants were examined. The variation in a single tassel was interpreted as stochastic when reported as pooled means from single pollen samples from single plants.

The superscript numbers over each profile indicate the interval in days between the early and late pollen samples from the same plant. The expression gradient between upper and lower tassel

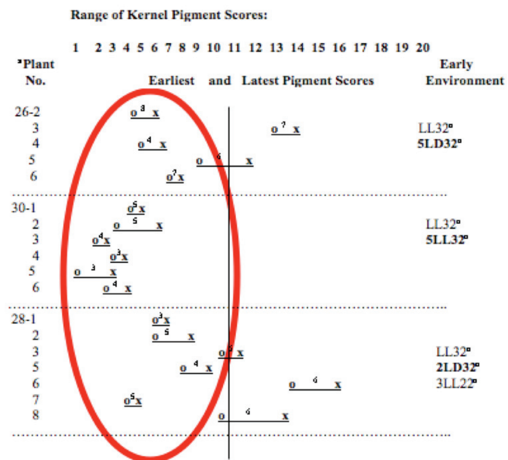


Figure 1A.

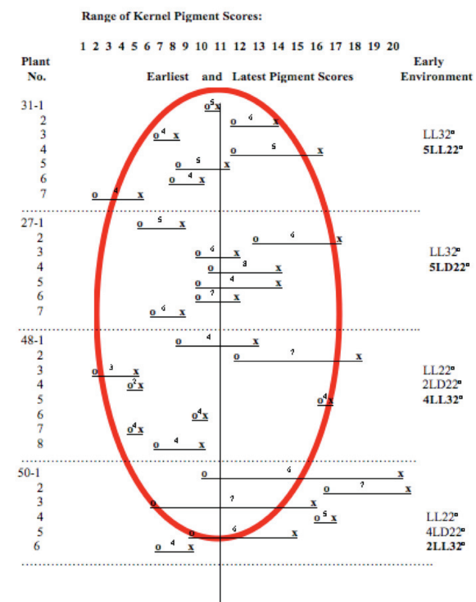


Figure 1B

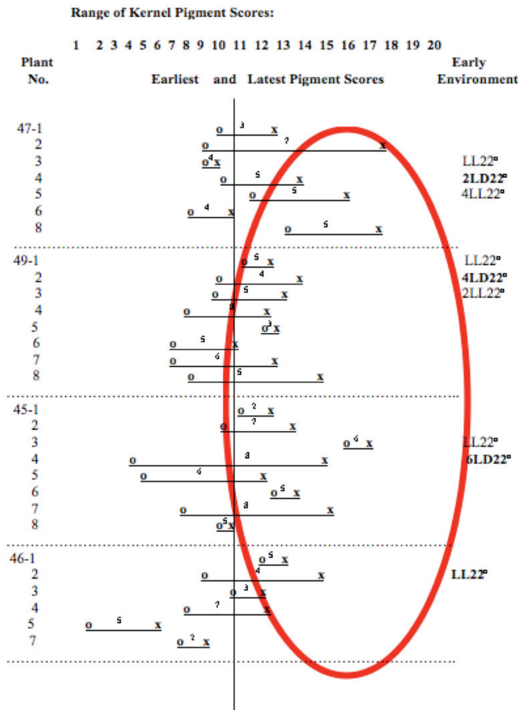


Figure 1C

Figure 1. Range of scores for individual plants. Symbols o and x represent means of 50 kernels from early and late pollinations, respectively; the superscript indicates the number of days between early and late pollen collections. The conditions during the critical period are in bold-face. Abbreviations for growth conditions are provided in the table which presents the pooled data for these plants.

A. Three lines of sibling plants, 26, 30 and 28 were started at 32° C, continuous light (LL) conditions, days 1-10; then shifted to 5 days light/dark cycle conditions at 32° C (early condition 5LD32°).

B. Lines 31 and 27 were started at continuous light (LL) and 32° C for days 1-10, then shifted to 22° and the light-dark (LD) conditions for days 11-15 (5LD22°). Lines 48 and 50 were started at **continuous light, 22°** C conditions. Line 48: on days 16-17, line 48 received light-dark cycles at 22°, then was transferred four days to continuous light at 32°. Line 50: on days 16-19 received 4LD22o cycles then was transferred to 2LL32° cycles, days 20-21.

C. Lines 47, 49, 45, and 46, were started and grown days 1-15 under 22° and continuous light (LL) conditions. During the critical period, days 16-21, seedlings were shifted to the conditions and numbers of cycles listed under column Early Environment.

branches from plant to plant could be quite variable and suggests that the rate of silencing in the tassel was determined by early conditions but is variable from plant to plant.

The three figures represent 7500 epialleles, 100 from each plant, 50 from early pollen and 50 from late, with only two days of pollen sampling represented in each profile. Since pollen is shed over a period of eight days, there is at least six times more variation available from the 75 plants sampled. Thus under the conditions of paramutation, an immense amount and range of *R* variation is available for natural selection during a single generation. The epigenetic nature of silencing means that the expression-states can be incrementally augmented each generation, using either lower temperature or one - two LD cycles at higher temperature. This means that temperature variation early in plant development can have a significant role in determining variable heritable phenotypes. What is remarkable is that the variation is PT regulated. This means that pigment will be more intense at the lower temperature and more UV will be filtered by the red pigment. Seventy-five profiles of epigenetic *R* expression-states (epialleles) are determined by PT conditions early in seedling development but expressed at maturity in pollen testcrosses following gametogenesis. It is remarkable that the silencing is orchestrated by a TE fragment in the promoter of an *R* inverted repeat and amplified by *R* duplications on the homologous chromosome in the heterozygote *R/Rst*.

The TE silencing mechanism has taken on the role of modulating gene expression in response to PT (environmental conditions) at a critical stage of development. The heritable epigenetic nature of the change in expression permits the inference that TEs can have a role providing diverse expression-state for natural selection.

References from several other laboratories in support of our interpretation are provided in the accompanying note.

We are grateful to the Charles F. Kettering Foundation for providing growth chambers and to former students Beth Besaw and Tammie Rettig for technical assistance.

_R_ paramutation primer (_R_ paramutation made simple): How transposable elements (TEs) may explain _R_ paramutation

--Mikula, BC; Studer, A

TEs have occupied genomes for millions of years (Fedoroff, PNAS 97:7002-7007, 2000; Feschotte and Pritham, Rev. Genet. 41:331-368, 2007). Silencing machinery was required to maintain TE silencing (Slotkin et al., Nature Rev. Genet. 8:272-285, 2007). TEs have increased gene redundancies throughout the genome - gene duplications like TEs had to be silenced. Haplotypes of *R1* and *R1-st* contain *R* duplications (Kermicle, in Russo et al., eds., Epigenetic Mechanisms of Gene Regulation, Cold Spring Harbor Press, 1996; Walker et al., EMBO J. 14:2350-2363, 1995). *R* duplications are attributable to TEs. The inverted repeat duplications of *R* are weakly silenced by *doppia* in S2 promoter. Weakly silenced *R* was known as *R* "mottle" (Brink, Genetics 41:872-889, 1956; Brink, Quart. Rev. Biol. 35:120-137, 1960). The silencing of *R* mottle was amplified by the *R1-st* haplotype, and amplified silencing of *R* "mottle" was recognized as paramutation. Paramutation engages the silencing machinery of TEs (Sidorenko and Chandler, Genetics 180:1983-1993, 2008; Chandler and Stam, Genetics 5:534-543, 2004).

The silencing machinery is photo thermally sensitive (Hashida et al., Plant Cell 18:104-118, 2006; Szittya et al., EMBO J. 22:633-640, 2003; Mikula, Genetics 65:733-742, 1967; Mikula, Genetics 1140:1379-1387, 1995). Photo thermal conditions also induce transition to flowering (Lin et al., Science 318:1302-1305, 2007). These same photo thermal conditions modulate paramutation. Transition to flowering is induced by phytochrome, and phytochromes are photo thermally sensitive (Lin et al., 2007). Phytochrome is dependent on transposases from MULE TEs (*Mu*-like elements). TEs have provided *R* duplications, silencing machinery, and photo thermal modulation of epigenetic change, i.e., paramutation! Paramutation results in heritable chromatin marks, and heritable chromatin marks provide an epigenetically heritable system (Jablonka and Lamb, Epigenetic Inheritance and Evolution, Oxford Univ. Press, 1995).

DICKINSON, NORTH DAKOTA
North Dakota State University

Release of populations carrying *Ga1-S*
--Kutka, FJ

Seeds of two new maize populations have been released into the public domain via a donation to the USDA National Plant Germplasm System in spring 2009. This donation was made to preserve the long known public use of the *Ga1-S* allele for preventing unwanted outcrossing and to move forward research and breeding with this allele. The populations should serve as bases for further development of inbreds, populations, and/or hybrids (single crosses, three-way crosses, double crosses, top crosses, and population crosses) carrying *Ga1-S* that have white, yellow or orange endosperm; that have colored and/or uncolored aleurone and/or pericarp; that have white capped and/or non-capped kernels; that have flint, dent, flour, opaque, waxy, shrunken, and/or sweet kernels; and/or are bred to exhibit any other obviously useful traits or morphological markers already selected for by maize breeders, including but not limited to high yield, vigorous emergence, lodging resistance, drought tolerance, cold tolerance, heat tolerance, low fertility tolerance, low pH tolerance, disease tolerance or resistance, insect tolerance or resistance, fast drydown, and/or morphological and chemical traits expressed by alleles known and maintained by the Maize Genetic Cooperation Stock Center.

The first population was submitted as "Non-Stiff Stalk *Ga1-S*." Mo508w, a white endosperm, non-elite inbred, was the source of the *Ga1-S* allele. It was also used as the female parent in an old backcrossing procedure described by Walter Thomas in 1955. This procedure should render the population mostly homozygous for *Ga1-S*. The other parents were non-elite, public non-stiff stalk inbreds first formed into two single crosses, N199/N152 and B97/Mo42. The population is 50% or less Mo508w by pedigree. It is a dent corn with white and yellow endosperm and is not inbred.

The second population was submitted as "Stiff Stalk *Ga1-S*." Mo501w, a white endosperm, non-elite inbred, was the source of the *Ga1-S* allele. It was also used as the female parent in the same backcrossing procedure described above. The other parents used were non-elite, stiff stalk inbreds, PHG80 and N28, and a B73/Cateto population from the GEM program. The population is 50% or less Mo501w by pedigree. It is a dent corn with white, yellow and orange endosperm and is not inbred. It also carries the *Ga1-S* allele and should be mostly homozygous based on pedigree and the Thomas procedure.

Breeders, researchers, farmers, and others interested in using these populations should contact Mark Millard, the maize curator, at the USDA Plant Introduction Station in Ames, Iowa, USA. Access should become available via the USDA National Plant Germplasm System Genetic Resources Information Network sometime soon.

DNIEPROPETROVSK, UKRAINE
Institute of Grain Farming

Inheritance of matroclinal haploidy in diallel crosses
--Satarova, TN; Cherchel, Vyu

Gynogenesis is an apomictic pathway of reproduction from an unfertilized egg cell and produces a matroclinal haploid. S. S. Chase reported the first matroclinal haploidy in maize (Genetics 34:328-332, 1949). E. H. Coe (Am. Nat. 93:381-382, 1959) and J. L. Kermicle (Science 166:1422-1424, 1969) created marker lines to use as pollinators to identify haploids, thereby enhancing the ability to produce haploids. In current maize breeding practice, diploidization of matroclinal haploids is widely used for rapid production of homozygous lines. Genetic control of matroclinal haploidy is important to the characterization of apomixis in flowering plants and for designing synthetic populations as the initial material for selection of new lines. Inheritable peculiarities of this phenomenon should be taken into consideration also in breeding lines with high haploid production for use as testers in selection of marker genotypes.

To analyse the inheritance of the ability to produce matroclinal haploids in maize, we conducted full diallel crosses in accordance with the methods of B. I. Hayman (Biometrics 16:369 - 381, 1960). The diallel scheme included five lines, DK276-1, DK247, DK293, DK303/427, DK205/710, and their reciprocal hybrids, resulting in 25 genotypes. For production of matroclinal haploids, 4 individuals of each genotype were pollinated with pollen of Zarodyshevij marker krasnodarskij 1 (ZMK-1). This marker, selected by E. R. Zabirova et al. (Kukuruza i sorgo 4:17-19, 1996), conditions a dominant purple coloration of plumule and endosperm. Haploids were identified as caryopses with colourless embryos and coloured endosperm. Caryopses with haploid embryos were sown the following year in soil and grown to anthesis to verify their haploid status. The frequency of matroclinal haploidy is computed from the ratio of the number of caryopses with haploid embryos to the total number of caryopses with coloured endosperm. According to our observations, pollination with ZMK-1 greatly reduces the number of kernels per ear in comparison with open pollination (Fig. 1).

Average values of the frequencies of matroclinal haploidy and decreased seed set are shown in the online supplemental data, Table 1. The frequency of matroclinal haploidy fluctuated from 0.59% to 11.12%. Seed set after pollination with ZMK-1 was reduced to 12.22-50.12%. This reduction could be related to the to induction of matroclinal haploidy except that the coefficients of pair correlations were not significant between the frequency of matroclinal haploidy and the degree of seed set, either in total for the experiment, or separately for hybrids or lines. Where parents were inbred lines, multiple allelism is improbable and data on irregularity of meiotic chromosome disjunction are absent. Because there were few differences between reciprocal hybrids, their mean values were used in genetic analysis. For the frequency of matroclinal haploidy, the coefficient of regression *Wr/Vr* was b=0.88±0.11

Figure 1. Maize ears after pollination with marker genotype ZMK-1 show decreased seed set.

(t_b=7.70, $t_{1\text{-}b}$=1.03, $t_{0,05}$=3.20); and for seed set it was b = 0.98 ± 0.14 (t_b=6.83; $t_{1\text{-}b}$=0.16; $t_{0,05}$=3.20). The significance of regression coefficients for both traits and their non-significant deviation from 1 testifies to the absence of effects of nonallelic interaction and dependent gene distribution in parents. Therefore, an additive and dominant genetic system determines the two characters.

The analysis of variance of a half of diallel table is represented in the online supplemental data (Table 2). For both traits the significance of mean squares *a* and *b* testifies to the effect of additivity and dominance. The significance of b_1 proves that the effects of dominance are mainly concentrated in the same direction. The significance of b_2 shows that dominant alleles are not dispersed among lines identically. Mean square *a* includes not only additive variance, but also a part of variance that is connected with dominant effects. Mean square b_3 is not significant for frequency of matroclinal haploidy, so dominant effects specific for every cross which are not connected with b_1 and b_2 are not established. Non-significant *R* means a weak effect of the environment on the development of the characters.

On the diagram of regression *Wr/Vr* for frequency of matroclinal haploidy, the regression line intersects the positive part of axis *Wr* (Fig. 2). Therefore, the middle degree of dominance for all the loci are incomplete, H1/D < 1. Values for inbreds DK276-1, DK247, DK293 and DK303/427 are located nearer to the start of the regression line and so they contain mainly dominant alleles (from 75% to 100%) that decrease the frequency of matroclinal haploidy. For inbred DK205/710, the ratio of dominant and recessive genes reaches the level 25:75 and so this inbred includes mostly recessive alleles for increasing matroclinal haploidy.

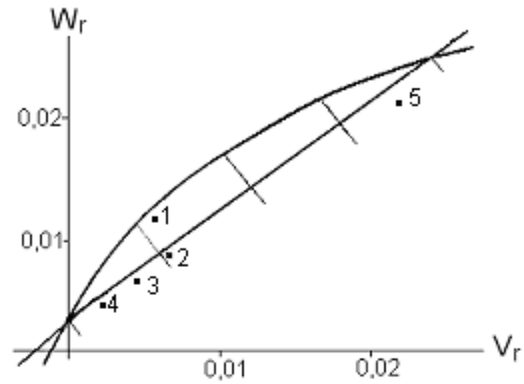


Figure 2. Regression W_r/V_r for frequency of matroclinal haploidy in maize (1 – DK276-1, 2 – DK247, 3 – DK293, 4 – DK303/427, 5 – DK205/710).

For seed set the regression line (Fig. 3) passes through the negative part of axis *Wr,* so superdominance plays the significant role in the manifestation of this character, $H_1/D > 1$. Dominant alleles are responsible for decreasing seed set, and recessive ones for the increasing of seed set. The distribution of dominant and recessive alleles is 75% and 25%, respectively, for inbreds DK293 and DK303/427; for inbred DK276-1 it is near to 50%-50%; and for inbreds DK205/710 and DK247, this distribution approaches 25% and 75%.

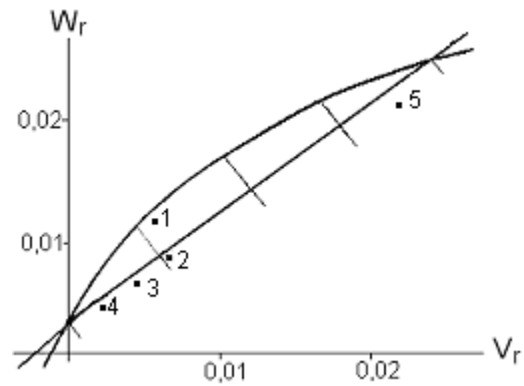


Figure 3. Regression W_r/V_r for seed set in maize (1 – DK276-1, 2 – DK247, 3 – DK293, 4 – DK303/427, 5 – DK205/710).

The estimates of genetic parameters are represented in the online supplemental data (Table 3). For frequency of matroclinal haploidy there is incomplete dominance (H_1/D=0.54), including separate loci ($\sqrt{H_1 D}$=0.73). For seed set, superdominance is shown (H_1/D=5.4) with every locus ($\sqrt{H_2 D}$=2.3). For the studied traits, the estimate of ½F/√D(H1-H2) differs from 1 and the level of dominance varies in different loci. $H_1 \neq H_2$, so dominant and recessive alleles are distributed irregularly among parental inbreds. The positive estimate of F verifies an excess of the number and/or effects of dominant alleles over the recessive ones in the given set of lines and hybrids.

For the given characters, the high values of heritability in a wide sense prove the primary effect of genotypic variance on the characters. The significant value of heritability in the narrow sense

for frequency of matroclinal haploidy (0.67) confirms the significant role of additive gene effects and indicates a favourable prognosis in using phenotype selection for high frequency of matroclinal haploidy. This is important for the production of testers, which are necessary in breeding programs of new inducer genotypes. The definite role of dominant effects in seed set is indicated also by the considerable difference between the heritability in a wide sense (0.82) and the heritability in the narrow sense (0.28).

The development of isolated maize caryopses in vitro
--Satarova, TN; Lyapustina, OV

The development of different isolated generative structures grown to maturity in vitro is an important step in genetic transformation procedures with egg cells, sperms, zygotes, young embryos, in manipulations with single cells, and in transferring organelles to gametes and zygotes. In vitro culture of maize kernels attached to cob tissue has been used in investigations of carbohydrate and protein metabolism and phyto-hormonal regulation of kernel development (Misra and Oaks, Plant Physiol. 77:520-523, 1985; Cobb et al., Ann. Bot. 62:265-270, 1988; Singletary and Below, Plant Physiol. 89:341-346, 1989; Hole et al., Plant Physiol. 91: 105-107, 1989). The culture of maize caryopses isolated from the cob has not yet been reported. The objectives in the present study were to investigate effects of genotype and culture duration on the in vitro development of isolated maize caryopses and their embryos.

Caryopses from field donor plants of maize inbred DK366 and hybrid A22xDK307 were isolated from ear cobs at 5-7 days after pollination. They were sterilized in 70% alcohol for 1-2 seconds, then washed three times in sterile water and explanted, using petri dishes with nutrient medium NBM (Mól et al., Planta 189:213-217, 1993) containing 90 g/l sucrose and 1 mg/l 6-benzylaminopurine. Caryopses were incubated at 26° C in darkness. Assessments were made on the 20th and 50th days in culture. Starch in different parts of kernels was determined using Lugol's solution. Well-developed embryos were excised from cultured caryopses and transplanted for germination onto modified Murashige and Skoog medium containing a twice-reduced concentration of macro- and micro-salts. At the time of explanting, caryopses were 5 mm long, white coloured (Figure 1A), and without starch. Embryo sacs inside caryopses were 1.2-1.7 mm long, and each contained an embryo of about 0.1 mm at a transient stage (Figure 1B), with degenerating synergids, endosperm cells and antipodal complex. By the 20th day, cultured caryopses had increased longitudinally by 16% for DK366 and by 12% for A22xDK307. Caryopses did not enlarge significantly after this time (see online supplemental data, Table 1), similar to the in vivo situation. Under field conditions, caryopsis enlargement for the genotypes investigated arrests by the 27th day after pollination; this time corresponds to the 20th day in culture of 7-day-old caryopses. However, on a plant, caryopses were larger: 8.09±0.12 mm for DK366 and up to 8.82±0.15 mm for A22xDK307, 35.5% and 42.7% higher respectively, than in culture.

Genotype appears to be an important factor affecting in vitro culture of caryopses. Under in vitro conditions, accumulation of nutrient storage substances started before the 20th day and continued up to the 50th day. Starch at the 20th day in culture was

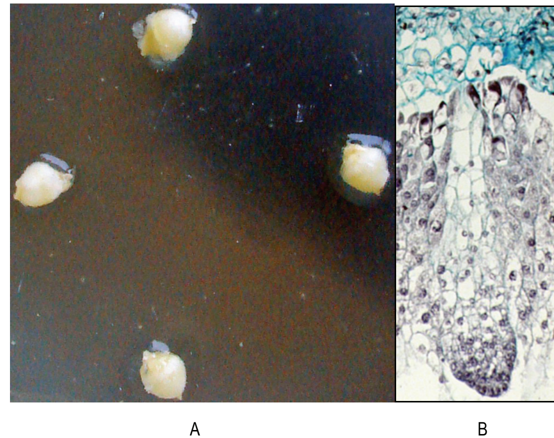


Figure 1. Caryopses (A) and an embryo (B) at the 5th-7th day after pollination.

observed in about 40% of DK366 caryopses and about 10% of A22xDK307 caryopses. Similar to the situation for caryopsis length, integuments accumulated starch only up to the 20th day (see online supplemental data, Table 1). During culture, some kernels changed colour, and some became swollen. By the 20th day in culture, 7% of DK366 caryopses and 3.5% of A22xDK307 caryopses had become yellow (Figure 2A), while others preserved their white colour. At the 50th day, 60.8% of DK366 caryopses had retained their initial white colour, with the remainder being brown. In hybrid A22xDK307, only 4.4% of caryopses retained their initial colour, but 50.7% and 45.0% had turned yellow and brown, respectively. All kernels of A22xDK307 remained swollen during the entire period of culture, whereas 14% of DK366 kernels, largely brown, had shrunk by the end of culture.

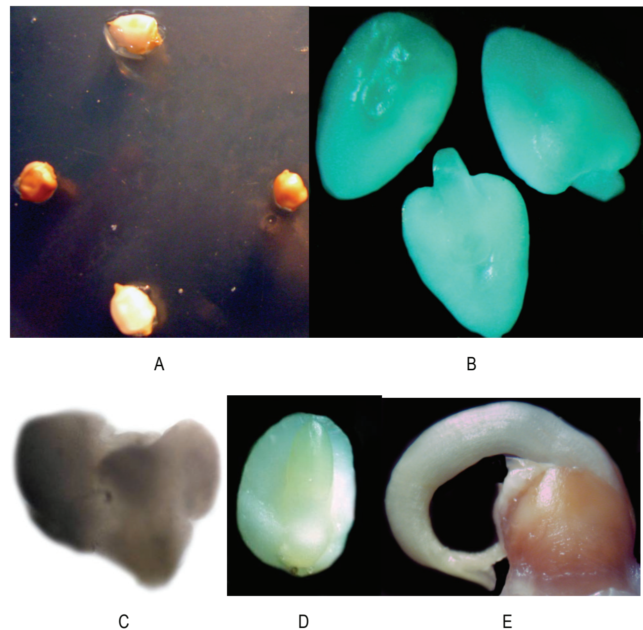
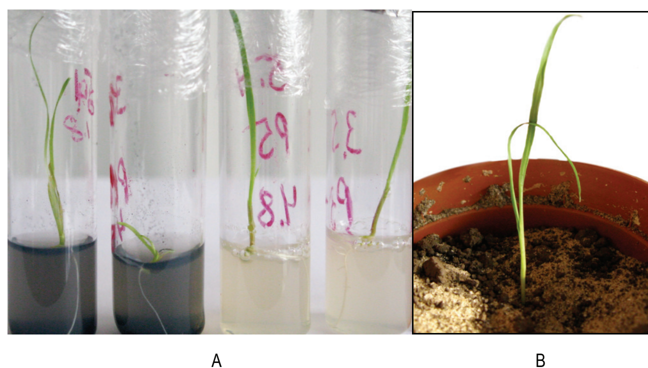


Figure 2. Kernels and embryos at the 20th day in culture. A – cultured caryopses; B –D - embryos isolated from cultured caryopses, B – embryos of regular structure, C – an embryo of irregular form, D – an embryo beginning to germinate inside a caryopsis; E – a caryopsis with a germinating embryo on NBM medium.

Fertilized embryo sacs inside cultured caryopses had differing extents of development, classified into 5 fractions. Some embryo sacs remained at the initial size – 1.2-1.7 mm long (fraction 1). Others enlarged to 1.75-3.05 mm, but without organogenesis (fraction 2). Fraction 3 caryopses had 3.1-5.0 mm long embryo sacs, with developed endosperm where one third had accumulated starch and one fourth had advanced embryos with differentiated organs. Fraction 4 caryopses include embryo sacs smaller than the initial 0.50-1.15 mm long, as a result of drying. In A22xDK307, but not the inbreds, there were some caryopses, dried or watery, without embryo sacs (fraction 5). Some caryopses of fraction 5 underwent callusogenesis. Ratios of different fractions for two genotypes are presented in online supplemental data, Table 2. For DK366, fraction 3 was a prevailing one, for A22xDK307, fractions 1 and 2 were more common. Correlation between the length of embryo sacs and the length of caryopses was not found in DK366; in A22xDK307, it was significant but very weak.

Caryopses with starchy endosperm belonged mainly to fraction 3. The effect of genotype on starch accumulation in the endosperm was discovered. Only a single caryopsis accumulated starch in A22xDK307, while 17% of cultured DK366 kernels accumulated starch (see online supplemental data, Table 1). If a caryopsis had starch in the endosperm, it also had starch in the integuments. The converse did not hold, and a considerable number of instances with starch in the integuments, but not the endoperm were found. This pattern in starch allocation was observed both at the 20th day and at the 50th day in culture. Thus, genotype makes a significant contribution to the expectation of starch accumulation in the endosperm in culture. Culture longer than 20 days did not significantly increase effective starch accumulation.

Embryos inside cultivated caryopses had been successfully grown from the initial transient stage to completely formed embryos with plumula, radicles closed in coleorhiza, and scutella (Figure 2B). In rare cases, embryos were of irregular form, with laciniate extension of scutellum (Figure 2C). Well-developed embryos from cultivated caryopses were isolated and transplanted, scutella down, onto nutrient medium. These formed plantlets, confirming the viability of embryos from caryopses grown in vitro (Figure 3). Sometimes embryos germinated inside caryopses in culture on NBM medium (Figure 2D, E). Genotype affected the frequency of caryopses with developed embryos (see online supplemental data, Table 3).



A B

Figure 3. Germination of embryos developed under caryopsis culture. A – plantlets on modified MS medium; B – a plantlet in soil.

In inbred DK366 11.4% of caryopses had fully developed embryos, with regular structure (70.6%), and mostly (64.3%) viable, producing normal green seedlings with good roots. In A22xDK307 only a single case of an embryo, not viable, was formed in a cultured caryopsis. The DK366 embryos were 1.76±0.39 mm long by the 20th day in culture, and 2.06±0.39 mm long by the 50th day. Significant differences in frequency of caryopses with developed embryos at the 20th and the 50th days in culture were not found. It may be assumed that the development of embryos is complete after 20 days in culture.

Our experimental data confirm that embryo development can occur in the culture of isolated maize caryopses and produce regularly developed green plantlets. Genotype significantly affected both growth and development of caryopses in culture, including starch accumulation in the endosperm and integuments, and the frequency of developed embryos. Embryo development and caryopsis growth were finished by the 20th day in culture, independently of genotype.

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Procera Agrochemicals

New inducers of maternal haploids in maize

--Rotarenco, V; Dicu, G; State, D; Fuia, S

On the basis of the first inducer of maternal haploids in maize (*Zea mays* L.), Stock 6 (Coe, 1959), a number of new inducer lines have been created (Tyrnov et al., 1984; Lasharmes et al., 1988; Sarkar et al., 1994; Shatskaya et al., 1994; Chalyk, 1999; Rober et al., 2005). The inducers possess dominant anthocyanin marker genes allowing haploids to be identified at different stages (dry kernel, seedling and mature plant), and their haploid-inducing rate is significantly increased in comparison with the initial inducer (Stock 6). Nevertheless, the existing inducers have some essential disadvantages. Due to a small plant size, most of the inducer lines cannot be used for the production of haploids by open pollination in the field. While hybrids between inducers are often utilized, the following problems may result: (1) reduced frequency of haploid induction and (2) altered expression of marker genes (unpublished). The *R1-nj* marker gene (purple scutellum and a purple crown of the aleurone) is widely used to screen for haploids in dry kernels. However, expression of this gene has a strong maternal influence, and can make the screening of haploids difficult, or even impossible, especially when there are inhibitor genes (*C1-I)* in females (common for flint maize). If the moisture of kernels at harvest is high, the screening of haploids can also be impossible, even in absence the of *R1-nj* inhibitors.

The aim of our work was to create new inducers with (1) improved plant traits, (2) good expression of marker genes, and (3) high rates of haploid induction. Two inducer lines were selected as initial material: MHI (Chalyk, 1999), as a source of favorable plant traits and high frequency of haploid induction; and Stock 6 (Maize Genetics Cooperation Stock Center), as a source of the *B1* and *Pl1* marker genes with sunlight-independent purple pigmentation in plant tissues. These allow identification of haploids by the lack of anthocyanin coloration in seedlings. During the F2 - F5 generations, selection for desirable characteristics, including resis-

tance to lodging and disease was carried out. Only those plants where the haploid-inducing rates were more than 10% were used for successive generations. From 92 lines in F5, 9 new inducers were selected and divided into four groups based on phenotype and other characteristics. These are named PHI – Procera Haploid Inducer (Figure 1).

Figure 1. Initial and PHI inducers, (from left to right) Stock 6, MHI, PHI-1, PHI-2, PHI-3 and PHI-4.

For the estimation of the haploid-inducing frequency, the following females were used: two inbred lines - A464 and A619; their hybrid (A464/A619); and a synthetic population - SP. These females have a very high accuracy in haploid screening, using the expression of the *R1-nj* gene. Five to ten plants of each female were crossed with each inducer. Progeny included about 1000 kernels for the hybrid and the synthetic population, and about 400 kernels for each inbred line. The main characteristics of the haploid inducers are presented in Table.

Table 1. Main characteristics of the inducers.

Inducer, Male	Planting-flowering, days	Plant height, cm.	Haploid-inducing frequency, %				
			Female				Mean
			A464 line	A619 line	A464/A619	SP pop.	
Stock 6	60	158	0.7	1,3	2.1	0.9	1.2
MHI	65	192	9.1	5.5	8.2	6.0	7.2
PHI-1	55	151	11.1	12.5	12.1	12.7	12.1
PHI-2	60	198	15.6	12.3	12.0	12.0	13.0
PHI-3	70	180	14.3	14.5	14.2	15.1	14.5
PHI-4	65	200	11.8	10.7	12.0	16.8	12.8

The lowest rate of haploid induction in all the PHI inducers was 10%. In some cases, haploid-inducing rates were almost two times higher in the PHI inducers in comparison with the initial line, MHI. The percentage of haploids may vary significantly due to different reasons (Rotarenco, 2002; Rotarenco and Eder, 2003; Rober et al., 2005; Rotarenco and Mihailov, 2007).

In addition to increased haploid induction frequency, both quantitative and qualitative traits of inducers can be improved (see Table 1). There was a two-week interval in the flowering time of the earliest inducer (PHI-1) and the latest one (PHI-3). Plant height in PHI-2 and PHI-4 was excessive. The PHI inducers had large tassels with good pollen production. After their self-pollination, a rather good seed set (for inducers) has been obtained (Figure 4). Selection for good expression of the *R1-nj* gene has been carried out among the PHI inducers, using B73 and Mo17 lines as females (Figure 2). The PHI inducers have been crossed with a carrier of the *C1-I* gene (inhibitor of the *R1-nj* gene). The expression of the *R1-nj* gene was completely suppressed; however, haploids were easily screened by the lack of purple pigmentation in 4-day seedlings (Figure 3).

Figure 2. B73 pollinated with (from left to right) Stock 6, MHI, PHI-2 and PHI-4.

Figure 3. Screening of haploids by the lack of purple pigmentation in roots.

Figure 4. Ears of the PHI-4 inducer.

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***Narrow Leaf3* (*nl3*) on chromosome 1**

--Brewbaker, JL

Narrow leaves occur in several maize mutants as pleiotropic effects (e.g., *Corngrass*, *Ragged*) but only two loci have been labeled "*narrow leaf*" and these are rather gross dwarfs. "*Narrow leaf3*" is proposed here to designate near-isogenic lines we've developed that have a reduced leaf width compared to parent Hi27 but are otherwise quite normal.

Our Hawaii Foundation Seeds assemblage of near-isogenic lines (NILs) based on tropical inbred Hi27 currently includes about 150 genotypes (MNL81:15). These are now summarized on our website www.ctahr.hawaii.edu/hfs. All genes have been accessed from temperate-adapted inbreds or hybrids of the Maize Genetics Coop. Our initial crosses of the temperate lines with Colombian flint-derived Hi27 resulted in very impressive heterosis for all quantitative traits we measured. We've also encountered impressive heterosis among our own 6-BC NILs (MNL81:16). We have thus sought evidence for quantitative trait loci (QTLs) that are linked to the mapped mutant loci we're transferring.

The narrow leaf trait was observed in conversions of the pericarp and cob color locus *P* (chromosome 1S-68.5). This locus is represented by allele *p1-ww* in parent Hi27 which has no color in pericarp or cob. The conversion of Hi27 to *p-rw* (red pericarp, white cob) began in 1967 with MGC stock 63-2656-3/2655-5 (*a1 Dt et1 lg2*), a bronze-pericarp stock that was found also to carry *p1-rw*. When selfing was initiated after 8 backcrosses (BCs) to produce homozygous red-pericarp lines, the narrow leaf trait segregated as a simple recessive.

Two NILs are now maintained, *nl3*^Hi27 and (*nl3 p1-rw*)^Hi27, the latter a double mutant with the red pericarp allele. Each conversion represents the 8 BCs and many sibs or selfs over more than 30 cycles of breeding. The narrow leaf NILs are otherwise indistinguishable from Hi27 in leaf length, plant height, maturity, ear traits or in yield. The *nl3* locus must be closely linked to the *P* locus. Also derived from this *P* locus stock were NILs of the double-cob mutant *dbcb1* we reported last year in MNL83, all of which have normal leaf widths.

The *nl3* trait is quite distinct from those described in the literature. Emerson's locus *nl1* on chromosome 10S was a very weak mutant with linear leaf stripes resembling the gene *lineate*, also on 10. The lineate stripes in our stocks *li*^Hi27 and (*g li*)^Hi27 are in leaves of normal width, and stripes occur only along the veins, resembling symptoms of Maize Mosaic Virus. Neuffer's *nl2* on 5S-62 was described also as weak and irregular (formerly called "*ragged*"), and the gene acted as a dominant. Similarly, the EMS-induced mutant *nl*-1517* was reported by Scanlon and Freeling (MNL70:15) to have shortened, narrow leaves with blades occasionally missing altogether and to be on chromosome 3. Neuffer also reported *nld* (narrow leaf dwarf), a small compact plant with narrow, rolled, chlorotic leaves. Among the 120+ genes represented in Hi27 NILs only *Cg* (corngrass) and *Rg* (ragged) have consistently narrower leaves than the parent. The leaves on tillers are often quite narrow, of course, as in our NILs for *gt1, tb1* and *tlr1* (cf. our photo in MNL81:15), and many diseases and abiotic stresses can affect leaf length and width. Most mutants with shortened leaves (*d1, na1, na2, py1*) have either normal or somewhat wider leaves.

The *nl3* phenotype was most obvious among young plants (30-45 days) that also have a somewhat erect habit, not unlike our NILs for *gs1* and *gs2*. The data in Table 1 were taken in the middle of first fully expanded leaves at 35 days after planting (about meiosis). Grand growth was just beginning (anthesis occurred 30 days later). The *nl3* plants had leaves reduced in width by 9 mm without change in vein numbers on each side of the midrib. Hi27 was almost identical in these traits to Marcus Zuber's classic inbred Mo17. Contrasting data are presented in the table for dwarf *d1*^Hi27 with its highly expanded leaf blades with 11 veins on each side.

Table 1. Seedling leaf widths (mm) of *nl3* mutants vs. Hi27 and *d1*.

Line	Width	CV	Veins
nl3	46.4	7.2%	8
Hi27	55.3	7.9%	8
d1	76.2	9.7%	11

Figure 1 shows Hi27 (center) and NILs *nl3*^Hi27 (left) and *d1*^Hi27 (right), a photo taken 43 days after planting and 23 days before anthesis.

Figure 1. Leaves of *nl3*^Hi27, Hi27 and *d1*^Hi27 (from left to right).

Complementary studies at anthesis included measurements over several years of the uppermost leaves and of leaves subtending the ear. These comparisons showed leaves of *nl3* to average about 10% narrower than normal throughout their length without variation in vein number. The data are summarized in Table 2, again representing mid-leaf section. The data were also characterized by very low variances (CVs of 5.6% for ear leaf and 9.1% for uppermost).

Table 2. Mature leaf widths (mm) of *nl3* mutants and inbred Hi27.

Line	Ear leaf	Uppermost leaf
nl3	76.7	54.4
Hi27	86.0	65.3

Conversions of Hi27 from other sources to alleles *p-vv*, *p1-wr* and *p1-rr* did not segregate narrow leaf. There has been no evidence in our (*nl3 p1-rw*)^Hi27 stock of mutability for the *p1* locus. Both lines have always been characterized by reddish anthers, as

contrasted with the bronze anthers of parent Hi27. Red anthers also occur in four of our NILs having red cobs due to allele *p1-wr*. However, neither of the narrow leaf NILs ever showed red cobs.

Variation in leaf length is a common pleiotropic trait with our dwarfing loci *d1, na1, na2* and *py*. However, only the *d1* locus (3S-30) has especially wide leaves with high vein number throughout development (Table 1, Figure 1). Our *d1*^Hi27 NIL also has a closely-linked locus conferring high susceptibility to southern rust, unique among Hi27 NILs that are routinely highly tolerant.

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Zea mays L. mitochondrial Mn-superoxide dismutase: evidence for a role in DNA protection

--Katyshev, AI; Subota, IY; Deineko, EV; Konstantinov, YM

A number of environmental stresses can lead to enhanced production of superoxide anion (O_2^-) radicals within maize plant tissues, and plants are believed to rely on the enzyme superoxide dismutase (SOD) to detoxify this reactive oxygen species (ROS). The SOD gene family in eukaryotes and prokaryotes consists of multiple genes encoding SOD isoforms. Plant cells contain almost all known SOD types. Based on the metal cofactors, SODs are classified into three groups: iron SOD (FeSOD), manganese SOD (MnSOD), and copper/zinc SOD (Cu/ZnSOD). FeSOD isoforms are located in chloroplasts, MnSOD isoforms in mitochondria and peroxisomes, and Cu/ZnSOD isoforms in chloroplasts, cytosol, and extracellular space. In this note we describe some biochemical properties of one MnSOD isoform in mitochondria. We suggest that this MnSOD isoform is involved in mitochondrial DNA protection from ROS.

Maize *MnSOD3.1* was cloned into bacterial expression vector pQE60 and recombinant protein isolated (Figure 1, online supplemental data). Total RNA isolation from 3-day-old etiolated hybrid VIR46MV seedlings was performed using a QIAGEN RNeasy Mini Kit, and cDNA prepared using the Promega Universal RiboClone cDNA Synthesis System. The translated region for the *MnSOD3.1* gene, GenBank Acc. number M33119 and flanked by NcoI and BglII sites, was amplified from cDNA using sequence-specific primers: 5'-CGACCAAAGCCATGGCTCT-3' as a forward and 5'-CCGTTAAGACAGATCTAGCAAGAACA-3' as a reverse primer. Primers were designed using the VectorNTI5 program (Bethesda, USA). PCR-products were eluted from agarose using QIAEXII kit (QIAGEN, USA), digested by NcoI and BglII enzymes and ligated into pQE60 expressing vector (QIAGEN, USA). Positive clones were confirmed by sequencing of PCR products on ABI PRISM 377. The expression of recombinant protein was analyzed in total protein extracts according to Garnik et al. (Russ. J. Plant Phys. 51:386-391, 2004).

According to Fridovich (J. Biol. Chem. 272:25071-25076, 1997) and Descheneau and Newton (Int. Congr. Plant Mitochondrial Biol., p. 23, 2005), MnSOD in bacteria and plants is DNA-associated and protects DNA against damage by reactive oxygen species. We hypothesized that to perform this function MnSOD should not possess DNA cleavage activity mediated through generation of hydroxyl radicals in the Fenton reaction. To test this suggestion, we compared DNA cleavage activity of commercial bovine Cu/ZnSOD (Sigma, USA) and recombinant maize MnSOD. Figure 2 shows that maize MnSOD does not possess DNA cleavage activity, in contrast to bovine Cu/ZnSOD. DNA cleavage activity of the recombinant protein was assayed as described by Chen et al. (Arch. Biochem. Biophys. 404:218-226, 2002) using pBlueScript KS II (+) plasmid DNA isolated by using a GeneJet Plasmid purification kit (Fermentas, Lithuania). In vitro phosphorylation of MnSOD was conducted as described by Subota et al. (Russ. J. Plant Phys. 57:37-44, 2010).

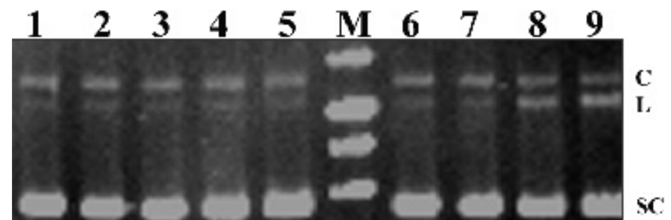

Figure 2. Analysis of DNA cleavage activity of SOD. 1- plasmid DNA, 2-5 – plasmid DNA + maize MnSOD (1, 2.5, 5 and 10 µg, respectively), 6-9 - plasmid DNA + bovine Cu/ZnSOD (1, 2.5, 5 and 10 µg, respectively). C – coiled DNA, L – linear DNA, SC – supercoiled DNA.

It is known that the mitochondrial electron transport chain is one of the main sources of ROS in the cell. Phosphorylation of MnSOD suggests that mitochondrial metabolism of ROS is regulated (Bykova et al., FEBS Lett. 540:141-146, 2003). Our analysis revealed two phosphorylated MnSOD3.1 forms – the native tetrameric and an unusual dimeric form. Both of these forms possess superoxide dismutase activity (Figure 3, online supplemental data).

We suggest that some unknown properties of mitochondrial MnSOD are responsible for the special role of this enzyme in mitochondrial DNA protection from reactive oxygen species. This work was financially supported in part by the Integration projects SB RAS 7, 83, 98 and RFBR projects 08-04-01426, 09-04-00992.

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Metaphase I pairing of B-chromosomes of *Zea mays* L. in the alien genetic background of *Avena sativa* L.

--Kynast, RG[1]; Joseph, JA[1]; Phillips, RL[2]; Rines, HW[2, 3]

Maize plants with native maize B-chromosomes (*B's*) in their complements prevail with different frequencies in tested populations by balancing the impact of selfish drive (i.e., microgametophytic chromatid non-disjunction associated with preferential ovum fertilization) with that of counteracting factors (i.e., meiotic loss through lagging univalents with micronuclei formation). Driving, as well as counteracting factors, for *B* presence in maize plants were assigned to the *B's* themselves and to the regular A-chromosomes (*A's*) of the host genome with complex interactions among them (reviewed in: Jones et al., Cytogenet. Genome Res. 120:265-280, 2008).

In order to study host species-specific influences on behavior and mode of inheritance of maize *Bs* in a very remotely alien genetic background, we transferred native *Bs* of maize (*Zea mays* L. ssp. *mays* cv. *Black Mexican Sweet*) into common oat (*Avena sativa* L. ssp. *sativa* cv. *Starter*) (Kynast et al., MNL 81:17-19, 2007) and proved their maternal and paternal transmission to successive offspring generations (Kynast et al., MNL 82:19-21, 2008).

Native maize *B's* are among the first-discovered (Kuwada, Bot. Mag. Tokyo 39:227-234, 1925) and presumably molecularly and cytogenetically best-described *Bs* in the plant realm (Jones and Diez, The B chromosome database, http://www.bchromosomes.org/bdb/, 2004). The extensive experimental data on behavior and mode of inheritance of maize *B's* in their native host species can serve as excellent references when evaluated and compared with those data on behavior and mode of inheritance of maize '*Bs* in an alien host species.

Native oat *B's* have not been reported to exist in wild and cultivated species of hexaploid oats. These species apparently have not been exposed to native *B's* during their evolution. Therefore, the oat genomes are not likely to have developed and/or to have been selected for genetic factors that control behavior and mode of inheritance of *B's*.

Hybridization experiments involving different hexaploid oat lines ($2n = 6x = 42$) and the maize line $B73^B$ ($2n = 2x + 6\ Bs = 20 + 6 = 26$) produced two fertile inter-species F1 plants (5811_1 and 5845_1) carrying maize *B's* in shoot and root tissues. PCR assays of their genomic DNAs using two maize *B*-specific markers and a selected set of maize *A*-specific SSR markers proved presence of maize *Bs* and absence of maize *A's* in the leaf tissues of both F1 plants. GISH assays using fluorophore-labeled genomic maize DNA as probe on root meristems proved that the F1 plant 5811_1 retained three maize *B's* along with the complete haploid set of 21 oat chromosomes ($2n_i - n_j = 3x + 3\ B's = 21 + 3 = 24$; *B's are named B.1*), and that one maize *B* was retained along with the complete haploid set of 21 oat chromosomes ($2n_i - n_j = 3x + 1\ B = 21 + 1 = 22$; *B is named B.2*) in the F1 plant 5845_1. Controlled self-pollination of the F1 plants 5811_1 and 5845_1 produced 59 and 73 seeds (F2 1188_1 … 1188_59, and F_2 1190_1 … 1190_73), respectively. Cytological and molecular analyses of 36 F2 offspring of the F1 plant 5811_1 revealed 27 F2 plants with different numbers of *B's* and nine F2 plants without a *B* (Figure 1), whereas in 36 F2 offspring of the F1 plant 5845_1 no F2 plant had a *B*, neither in its shoots nor in its roots based on PCR and GISH assays.

We addressed the question in our research objectives: **How do maize *B's* pair in microsporocytes during meiosis I after being transferred from maize into oats, i.e., after being converted from native *B's* to alien *B's*?**

Oat inbred lines *Starter-1*, *Sun II-1*, oat cultivar *Paul* and maize inbred line $B73^B$, were grown and crossed, hybrids cultivated in vitro and molecularly and cytologically tested, and consecutive offspring produced and analyzed as described elsewhere (Kynast et al., 2007, 2008). The oat inbred lines *Starter-1* and *Sun II-1* are single plant descendents from the oat cultivars *Starter* and *Sun II*, respectively; *Starter*, *Sun II* and *Paul* were from the oat stock collection at the University of Minnesota-Saint Paul. The line $B73^B$ is a dent corn inbred *B73* derivative that carries six *B's* of the sweet corn cultivar *Black Mexican Sweet* ($2n = 2x + 6\ Bs = 20 + 6 = 26$);

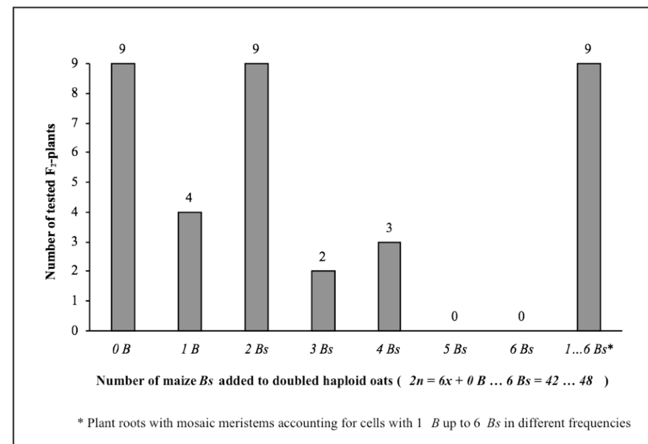

Figure 1. Numbers of 36 F2 offspring tested without and with maize *B* additions, reflecting the transmission frequencies of added maize *Bs* from F1 to F2 through doubled haploid formation by unreduced gametes of the aneuhaploid F_1-plant 5811_1 ($2n_i - n_j = 3x + 3\ Bs = 21 + 3 = 24$) after self-fertilization; presentation includes data from Kynast et al. (2007) (20 tested F2 offspring) and Kynast et al. (2008) (10 tested F2 offspring).

seed of $B73^B$ was generously provided by J. A. Birchler, University of Missouri-Columbia.

We selected oat genotypes with different numbers of added *B's* in their complements from 36 tested and cultivated F2 offspring of the F_1-plant 5811_1 based on estimated chromosome numbers and histological stability/instability of their root meristems by using the Feulgen-staining technique and GISH assay of ice water-pretreated and fixed root tips. The following four genotypes with stable karyotypes without any indication of mosaic meristems in their roots (F2 plants 1188_3, 1188_7, 1188_20 and 1188_8) were selected from a total of 27 maize *B*-positive F2 plants (Figure 1) in order to develop an oat-maize addition (OMA) series of alien maize *B* addition lines of hexaploid oats (*OMAmB.1*, *OMAdB.1*, *OMAtB.1,* and *OMAqB.1* for monosomic, disomic, trisomic, and tetrasomic addition, respectively) for seed production (Table 1, online supplemental data) and for the analyses of metaphase I pairing performance of the alien maize *B's* (Table 2, online supplemental data).

We germinated eight F3 seeds from each of the four offspring populations (F3 1374_10 … 1374_18, F_3 1378_1 … 1378_8, F_3 1391_1 … 1391_8, and F_3 1379_1 … 1379_8) of these four different F2 plants and verified chromosome numbers and histological stability/instability of their root meristems as described for the 36 F2 plants analyzed. F3 seed production was by controlled self-fertilization of individual F2 panicles (a-, b-, and c-panicles) from the physiologically first three F2 tillers (a-, b-, and c-tillers, correspondingly) with flag leaves, the genomic DNAs of which tested *B*-positive in PCR assays using two maize *B*-specific markers. For each of the four lines, we selected one out of the eight F3 plants with appropriate stable karyotypes for meiosis analyses. Because the panicles of the F3 plants' a-tillers were reserved for controlled F4 seed production, we used the b- and c-panicles for meiosis assays (Table 2).

In order to increase the conclusive force of our data generated from F3 plants, we also observed *B* pairing performance in plants of the successive offspring generation with appropriate stable karyotypes. We analyzed meioses from b- and c-panicles of one

out of six cultivated F4 plants for each line (F4 000769_1 … 000769_6, F4 000805_1 … 000805_6, F4 000837_1 … 000837_6, and F4 000853_1 … 000853_6) grown under environmental conditions equal to those used for F3 plant cultivation (Table 2).

Anthers of an appropriate physiological age to have the majority of microsporocytes at metaphase I (tested using aceto-carmine quick squash technique) were fixed in a mixture of 1.5 volume parts of methanol, 1.5 volume parts of ethanol and 1 volume part of glacial acetic acid at room temperature (RT) for two days. For slide preparation, fixed anthers were rinsed in $45\%_{v/v}$ acetic acid at RT for about 15 min. The two thecae of an anther were split and processed further separately. Theca ends were cut open and microsporocytes gently stroked out into a droplet of $45\%_{v/v}$ acetic acid onto a glass slide. Pressing a cover slip firmly upon the cell area, freezing the slide in liquid N_2, and flicking off the cover slip accomplished squash preparation. Slides – dried in absolute ethanol at RT for about 30 min followed by air-drying at RT for about 1 h – were processed through a regular GISH procedure at a stringency of ≥85% using fluorophore-labeled genomic maize DNA as hybridization probe. Microsporocytes were observed and pairing scored by the use of an epifluorescence microscope equipped with a CCD camera for picture documentation.

Table 3 (online supplemental data) summarizes the data of chromosome pairing performance for the different numbers of *B's* observed in microsporocytes at metaphase I of the four different addition lines *OMAmB.1* (monosomic *B* addition), *OMAdB.1* (disomic *B* addition), *OMAtB.1* (trisomic *B* addition), and *OMAqB.1* (tetrasomic *B* addition). We screened 30 microsporocytes for each line from four panicles of the selected F3 and F4 plants of the addition line. In the monosomic *B* addition line *OMAmB.1*, 30 sporocytes had one *B* univalent, and no pairing between a *B* and any of the oat *As*. In the disomic *B* addition line *OMAdB.1*, 18 sporocytes had two *B* univalents, and 12 sporocytes had one *B* bivalent. Again, no sporocytes showed pairing between a *B* and any of the oat *As*. In the trisomic *B* addition line *OMAtB.1*, 19 sporocytes had three *B* univalents, and 11 sporocytes had one *B* univalent plus one *B* bivalent. None of the 30 sporocytes had a *B* trivalent, and none of the 30 sporocytes showed pairing between a *B* and any of the oat *As*. In the tetrasomic *B* addition line *OMAqB.1*, 12 sporocytes had four *B* univalents, 13 sporocytes had two *B* univalents plus one *B* bivalent, and 5 sporocytes had two *B* bivalents. None of the 30 sporocytes had a *B* quadrivalent, and none of the 30 sporocytes had a *B* trivalent plus a *B* univalent configuration. Again, the line did not show pairing between a *B* and any of the oat *A's*. Taking all data together (Table 4, online supplemental data), there are four main results and conclusions from our experiments:

First, there were no configurations of pairing between maize *B's* and oat *A's* in any sporocytes, even when the maize *B* was a monosomic addition only, and thus, did not have a homolog to pair with as was the case, for instance, in F3 plant 1374_13 and F4 plant 000769_2 (monosomic *B* addition line *OMAmB.1*). There was no sporocyte with chiasmatic conjugation of any maize *B* with an oat *A*. Moreover, the maize *B* univalents did not show any spatial proximity indicative of secondary end-to-end, end-to-side or side-by-side alignments of achiasmatic association with oat *A's*. In contrast, maize *B's* in any configuration appeared physically well separated from oat *A's* even while being perfectly co-orientated with the complement of oat *A* bivalents across the equatorial plate (Figure 2).

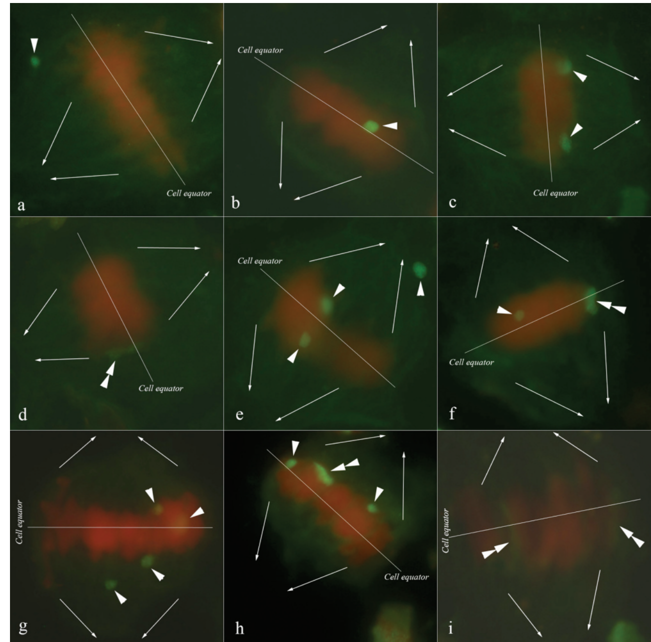

Figure 2. Metaphase I microsporocytes of *OMAxB.1* plants with different numbers (*x*) of added maize *Bs* labeled by GISH (green fluorescing, univalent marked with one arrow head, bivalent marked with one double arrow head, pairs of thin arrows indicate direction of chromosome migration towards the poles); (2a) *OMAmB.1*, one *B* univalent scattered across the spindle figure outside the cell equator; (2b) *OMAmB.1*, one *B* univalent well integrated into the cell equator; (2c) *OMAdB.1*, two *B* univalents scattered across the spindle figure outside the cell equator; (2d) *OMAdB.1*, one *B* bivalent well integrated into the cell equator; (2e) *OMAtB.1*, three *B* univalents scattered across the spindle figure outside the cell equator; (2f) *OMAtB.1*, one *B* univalent plus one *B* bivalent, both well integrated into the cell equator; (2g) *OMAqB.1*, four *B* univalents scattered across the spindle figure outside the cell equator; (2h) *OMAqB.1*, two *B* univalents scattered across the spindle figure outside the cell equator plus one *B* bivalent well integrated into the cell equator; (2i) *OMAqB.1*, two *B* bivalents well integrated into the cell equator

Second, univalents of maize *B's* frequently scattered across the spindle figure off the equator (Figure 2a). Nonetheless, every so often *B* univalents were remarkably well integrated into the heterotypic cell equator (Figure 2b). Although at an overall higher frequency (20/30 non-equatorial positions versus 10/30 equatorial positions), the variable positions were of no recognizable pattern for the distance to equator or pole, and therefore, more likely resulting from the impact of the methodology of squashing the 3D structure of the sporocyte to a flat plate by the slide preparation technique. True 3D analysis of the sporocytes by using, for instance, confocal microscopy will deliver data that are more reflective of the genuine position pattern of univalent *B's*. Further research experiments are in progress to investigate the spatial organization of alien maize *B* univalents and bivalents in oat sporocytes. Yet, our observations point to an amazing stability of *B* univalents in the alien oat background probably contributing to high frequencies of maternal as well as paternal transmission to offspring (Kynast et al., 2008). The overall harmonic performance is leaving us with the impression that the alien maize *B's* behave quite normally in the oat genome with a good fit into the oat timing for chromosome formation, pairing and orientation during prophase I and metaphase I.

Third, when a *B* had a homologous partner for potential pairing, bivalents were formed, notwithstanding univalent formation at a modest frequency, e.g., in the disomic *B* addition line *OMAdB.1* (F3 plant 1378_4 and F4 plant 000805_2). The significant number of sporocytes showing two *B* univalents instead of one *B* bivalent ($\bar{x}$ *per* sporocyte: 1.2^{I} *versus* 0.4^{II}) distinguishes pairing performance of alien maize *B's* from that of native maize *B's* (i.e., maize *B's* in maize) and of native *B's* in other plant species (e.g., rye) too (Figures 2c and 2d). Also, the alien maize *B* pairing differs from that of alien regular *A's*. In general, homologous *A's* in a disomic condition hosted by an alien genome almost always pair and, hence, form bivalents instead of two univalents setting aside major structural rearrangements between homologs by chromosome mutations and plant mutants with alleles for asynaptic behavior of individual chromosomes and whole genomes. However, we cannot exclude in our conclusions drawn from metaphase I pairing data alone that the enlarged number of sporocytes with *B* univalents could result from (during zygotene) perfectly synapsed, but (during diplotene) prematurely desynapsed chromosome pairs due to (during pachytene) achiasmatic conjugation caused by, for instance, failure or extreme reduction of crossing over. Further molecular cytogenetic analyses of *B's* in sporocytes at zygotene, pachytene, and diplotene are in progress and will address the question of whether the increased *B* univalent frequency in disomic *B* additions does result from asynapsis and/or premature desynapsis due to achiasmatic chromosome conjugation.

Fourth, in all observed sporocytes, the trisomic addition of homologous maize *B's* in the line *OMAtB.1* (F3 plant 1391_8 and F4 plant 000837_4) did not result in the formation of *B* trivalents (Figures 2e and 2f). Likewise, the sporocytes with tetrasomic addition of homologous maize *B's* in the line *OMAqB.1* (F3 plant 1379_4 and F4 plant 000853_3) did not form one *B* quadrivalent or one *B* trivalent plus one *B* univalent either (Figures 2g and 2h). All maize *B's* appeared to form univalents and bivalents only in an oat background with a modest preference for univalents ($\bar{x}$ *per* sporocyte: 2.3^{I} *versus* 0.4^{II} in *OMAtB.1* and 2.5^{I} *versus* 0.8^{II} in *OMAqB.1*, respectively), though more than two homologous pairing partners were present in the sporocytes of both genotypes. This obvious restraint on trivalents and quadrivalents seemed intriguing, particularly owing to the fact that all *B's* in the plant materials tested were descended from the same origin of maize germ plasm *Black Mexican Sweet* and go back to one defined (*Starter-one* oat × $B73^{B}$ maize) hybridization event (F1 plant 5811_1). Hence, all the maize *B's* represent, from a cytogenetic view, multiple copies of the same chromosome. It seems feasible to exclude significant structural differences among the individual *B's* as a cause for the multivalent suppression. In addition, *B* bivalent formation is not suppressed though modestly reduced. The restraint on multivalent formation causes an additional intriguing feature to the *B* behavior in metaphase I sporocytes. When the disomic, trisomic and tetrasomic addition lines are compared, the frequency distributions of sporocytes with *B* univalents are more polynomial than normal (Figure 3). The average bivalent frequencies ($\bar{x}$ *per* sporocyte: 0.4^{II} in *OMAdB.1*, 0.4^{II} *OMAtB.1*, and 0.8^{II} in *OMAqB.1*) appear remarkably constant (Table 4).

In order to track down potential genetic factors for the multivalent suppression that interact between the maize *B's* and the host

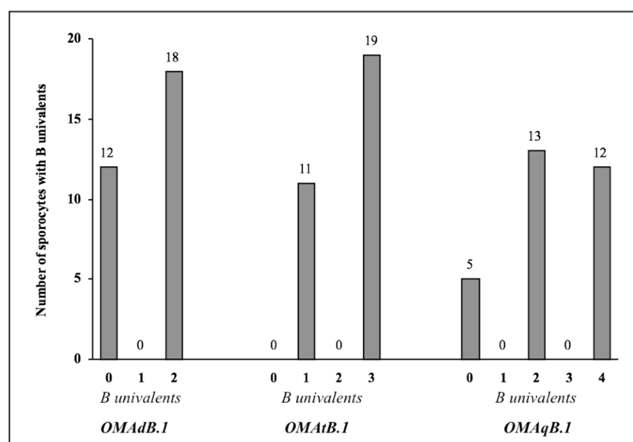

Figure 3. Polynomial frequency distributions of metaphase I sporocytes with *B* univalents in the addition lines *OMAdB.1*, *OMAtB.1*, and *OMAqB.1*

species, we are trying to transfer the *B's* from *Starter-I* oat into further different genetic backgrounds. We have recently started a backcross program of the two lines *OMAtB.1* and *OMAqB.1* to the hexaploid oats *A. fatua* L., *A. sterilis* L., and *A. sativa* L. subspp. *byzantina* and *nuda*.

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General and specific combining ability for traits associated to grain yield in crosses among Argentinean flint landraces and Reid and Lancaster inbred lines

--Aulicino, MB; Collado, MB; Molina, MC; Arturi, MJ

Argentine corn production is mainly based on hybrids generated from materials with different types of grain. The use of divergent heterotic patterns among Argentine flint landraces and North American Corn Belt inbred lines could assure the generation of new populations with high genetic variability and with good potential for traits associated to grain yield. The objective was to evaluate the combining ability and heterosis among them and to define breeding strategies. 10 flint early landraces obtained from the Germplasm bank at INTA Pergamino, Argentina, were evaluated: ARZM 1151, ARZM 6070, ARZM 12209, ARZM 14049, ARZM 16019, ARZM 17051, ARZM 18033, ARZM 18052, ARZM 18054 and ARZM 19007. They had differences in geographic origin and maturity (MNL 75:36-37, 2001). A top-cross was used. The landraces, as female parents, were crossed with the inbred lines B73, Mo17 and B68. The 30 genotypes (hybrids) and 3 commercial hybrids (checks) were evaluated during two growing seasons (2001-2002 and 2003-2004) at Lavallol, Buenos Aires (34°47'S, 58|27'W). The experiment designed was a randomized complete block with three replications. Plant traits evaluated were: EIH, ear insertion height (in cm); PH, plant height (in cm); and several characters that contribute to yield EL, ear length (cm); KNPR, kernel number per row; RNPE, row number per ear; and KDW, kernel dry weight per plant (g). Statistical analysis consisted primarily of a combined analysis of variance using the two experiments conducted in different environments (years). The Griffing genetic model (1956) was applied, in order to estimate the additive effects

or general combining ability (GCA) of the landraces (females) and the tester (males) and the epistatic interaction effects or specific combining ability (SCA). The decomposition of the mean squares can estimate the additive and dominant genetic effects as follows: $\sigma^2_m = CM_{males} - CM_{m*h})/ hr = Cov (H.S.) = ¼ \sigma^2_A$;$\sigma^2_f = (CM_{females} - CM_{m*h})/ mr = Cov (H.S.) = ¼ \sigma^2_A$ y $\sigma^2_m = CM_{m*f} - CM_E)/ r = Cov (F.S.) = 2 Cov (H.S.) = ¼ \sigma^2_D$. According to the combined analysis of variance, the environment and the interactions G x E varied significantly for all variables considered. The second trial (2003-2004 growing season) was conducted in a field with good edaphic characteristics and a rainy period during the development of the trial, which caused higher averages, both for vegetative and reproductive traits. Therefore, these results justified the use of ANOVAS separately for each trial.

Significant differences among genotypes were found in both trials, except for KDW in the first environment. The commercial hybrids differed significantly from experimental hybrids only for vegetative characters and for RNPE in the first trial. However, checks differed from genotypes for all the characters evaluated in the second trial. GCA of the testers was significant for all variables except for KDW in the first trial, and PH in the second trial. The GCA of the landraces was significant for all variables except EL and KDW in the first trial (see online supplemental data, Table 1). The SCA (Male x Female) was significant only for the variables EL and RNPE in the first trial. However, in the second trial, all the variables showed a significant interaction.

Landraces contributed genes towards increased vegetative variability, especially for ear insertion height and the reproductive RNPE. The environment affected the magnitude of additive genetic variance and produced a biased behavior for the HP and KDW. The variance of dominance appears to control the inheritance of EL and KDW in both environments, so do not wait for those variable genetic advances during the selection process. The KNPR trait presented a medium additive component, indicating that both parents as lines have favorable alleles for these, although it is also controlled by the effect of heterosis. We conclude that landraces had a high frequency of favorable alleles for vegetative variables and some reproductive traits, indicating good potential for the use of these landraces in recurrent selection programs. Otherwise, the best crosses could generate composites which would be used as a germplasm source in breeding programs.

Acknowledgement: The authors want to thank Dr. Marcelo Ferrer, Germplasm Bank at INTA Pergamino, Argentina, for supplying seeds of the Landrace evaluated.

The use of electrolyte leakage in the evaluation of salinity tolerance in maize seedlings (*Zea mays* L.)

--Collado, MB; Aulicino, MB; Molina, MC; Arturi, MJ

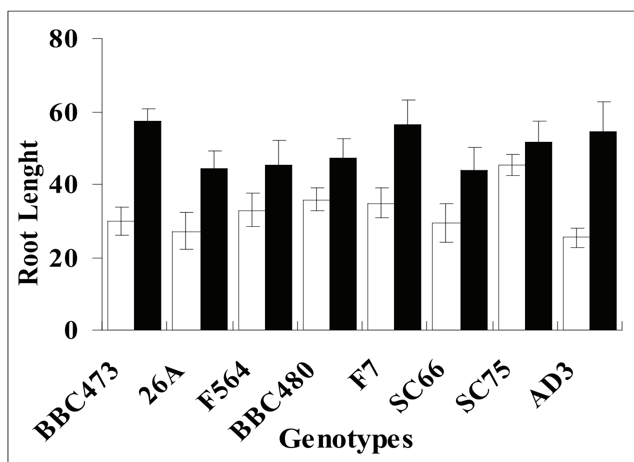
Maize is classified as a salt-sensitive crop plant (Maas and Hoffman, J. Irrig. Drain. Div. ASCE 103:15-34, 1977). The response of maize to salinity varies depending on the stage of development (Kaddah and Ghowail, Agron. J. 56:214-217 1964; Maas et al., Irrig. Sci. 4:45-57, 1983; Pasternak, Malach and Botovic, Agric. Water Manage. 10:47-60, 1985). Vegetative growth appears to be most sensitive to salinity, while plants at later stages are much less affected (Cramer, pp. 449-459 in Handbook of Plant and Crop Stress, M. Pessakli, ed., Marcel Dekker, New York, 1994). This paper examines the use of the electrolyte leakage (cell membrane stability) trait at the seedling stage as a screening tool for tolerance to salinity.

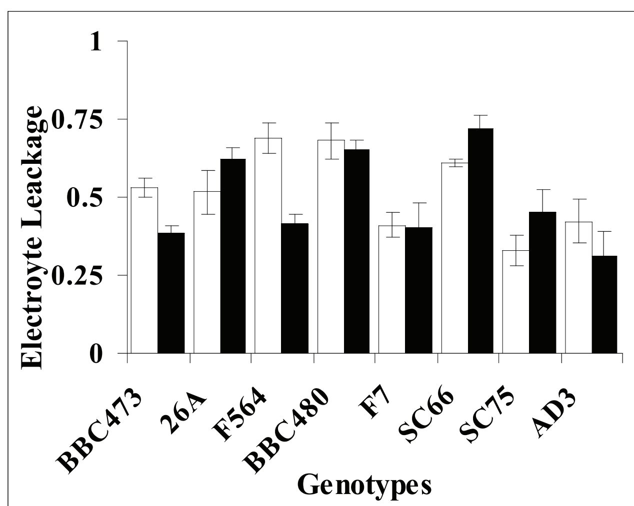
The eight accessions/lines used included five populations and three inbred lines. Seeds were surface sterilized in 1% sodium hypochlorite solution for 5 minutes, then rinsed with distilled water. Three seeds were planted in each pot containing perlite; these pots were put in trays with a nutrient solution. Two treatments were applied: control (cont.) where no NaCl solution was added and the other treatment receiving 100 mM NaCl (salt). The experiment was carried out in a controlled environmental room at 25ºC, with 16 h day length and with a relative humidity of 60%. After 14 days of each salt treatment, the seedlings were harvested. The length for shoot and radicle (SL and RL, respectively) were recorded. Shoot and radicle were separated and the samples were dried for two days until constant weight, for dry weight determination (DS and DR respectively).

The cell membrane stability was estimated on the third leaf. A piece of leaf was cut, weighed, and washed with distilled water to remove the solution from tissue. The samples were then immersed in 10 ml distilled water and incubated at 10º C for 24 h. After incubation, samples were equilibrated to room temperature and the electrical conductivity of the medium was recorded (EC1) with a portable EC meter (Consort C931). The samples were autoclaved for 15 min to disrupt all tissues, cooled to room temperature, and the conductivity of the solutions read again (EC2). Electrolyte leakage (%) was calculated as: $EL = (EC1/ EC2) \times 100$.

The data were subjected to an analysis of variance, and the means were compared by the least significance differences test (LSD) at a 5% level (Sokal and Rohlf, Biometry, 3rd ed., Freeman and Co., New York, 1995). The ANOVA indicated that although the genotypes tested have significant and highly significant differences for the traits measured, the salinity treatment had insignificant effects for most of the traits tested with the exception of RL, EC1 and EL (see online supplemental data, Table 1). Consequently, these traits would be extremely useful in salinity tolerance improvement programs, especially root length, which had a major reduction compared to the controls. Our data support the importance of Root Length in the identification of a tolerant response, as previously suggested (Rao and McNeilly, Euphytica 108:145-150, 1999; Khan, Rao and McNeilly, Breed. Sci. 55:321-325, 2003).

Measurement of membrane damage seems to be associated with salt sensitivity at the cellular level. Figure 1 shows that genotype F564 could be considered sensitive because it shows the highest level of EC1, while SC75 shows the lowest level of EC1 and therefore a lower electrolyte leakage indicative of a tolerant behavior. However, when growth parameters are analyzed, in particular RL, these two genotypes were the ones that suffered the least growth loss. Both genotypes appear to be tolerant to salinity but probably associated with different mechanisms. The SC75 line probably did not accumulate an excessive amount of Na^+ in the shoot during the period of exposure to salt, as this ion was accumulated in the root (sodium exclusion mechanism). F564 suffered significant damage in the membrane, which could be associated with an accumulation of sodium in the shoot (vacuole) without severely affecting the cellular metabolism (tissue tolerance to sodium).





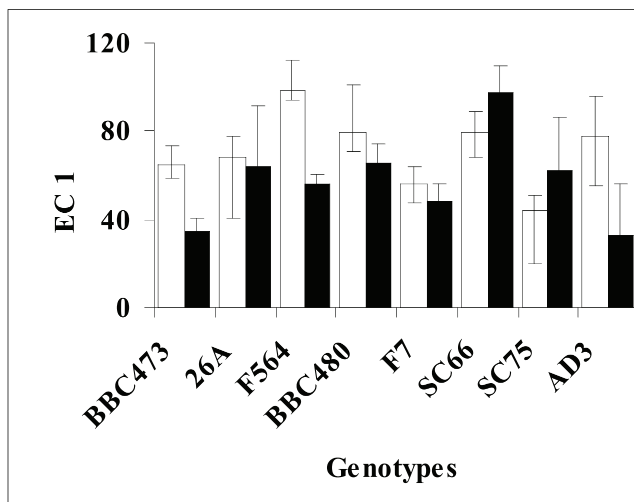


Figure 1. Average of each genotype for control (black bars) and salt (white) treatments of the traits: Root Length (RL), Electrolyte Leakage (EL) and Electrical Conductivity 1 (EC1). Controls are represented by black bars and salt treatment by white. Error bars are the S.D. of four replications.

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Density, tillering and yield of three *Tripsacum dactyloides* (L.) L. genotypes during the crop establishment period

--Huarte, RH; García, MD

The genus *Tripsacum* consists of perennial grasses that are widespread in the Western Hemisphere from Massachusetts, United States to Paraguay, South America (de Wet et al., Am. J. Bot. 70:1139-1146, 1983). *T. dactyloides (*Eastern gamagrass) is a relative of field corn *(*Zea mays*)* characterized as a highly productive and palatable warm-season, perennial forage grass, with good performance as a forage crop for grazing (Burns et al., J. Anim. Sci. 70:1251-1261, 1993; Aiken, J. Anim. Sci. 75:803-808, 1997), hay (Burns et al., Postharvest Biol. Tec. 7:261-269, 1996), and silage (Eun et al., J. Anim. Sci. 82:170-178, 2004). Recently, eastern gamagrass has also gained attention as a grass for growing vegetative hedges to control erosion or filter strips to reduce water pollution from agricultural runoff (Rankins et al., Weed Technol. 19:73–77, 2005; Kaspar et al., J. Environ. Qual. 36:1503–1511, 2007), and as a crop to ameliorate marginal (Gilker et al., Soil Sci. Soc. Am. J. 66:931–938, 2002) or contaminated soils (Euliss, Bioresource Technol. 99:1961–1971, 2008). Eastern gamagrass is especially tolerant to adverse subsoil conditions, such as extreme acidity and compaction (Ritchie et al., Field Crop. Res. 97:176–181, 2006; Foy et al., J. Plant Nutr. 22(10):1551-1566, 1999; Clark et al., Plant Soil 200:33-45, 1998), and has winter hardiness and high dry matter production (Faix et al., J. Range Manage. 33(5):388-390, 1980). Nevertheless, stand establishment can be difficult because of poor seed germination and slow seedling growth. Extended cold stratification of hydrated seeds was reported to overcome dormancy (Ahring and Frank, J. Range Manage. 21:27–30, 1968; Anderson, Bot. Gaz. 146:353–364, 1985), but planting dry seed in the fall has been more successful (Gibson et al., Crop Sci. 45:494–502, 2005, among others).

The aim of this study was to compare emergence rates, tillering and dry matter (DM) production of three genotypes of *T. dactyloides* during the crop establishment period and under 3 fertilizer regimes: unfertilized, addition of nitrogen, or nitrogen plus phosphorus. Genotypes used in this study were the diploid cultivars Iuka and Pete, kindly supplied by Dr. Maria Hyatt (Iowa State University), and a tetraploid genotype (GT) from CIMMYT (Mexico). The research site was located at the Instituto Fitotécnico de Santa Catalina, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Llavallol, Buenos Aires (34 º 48' S, 48 º 31' W). Planting was done on June 14, 2007, to permit a period of low temperature to alleviate caryopsis dormancy. Field trials were on a Typic Argiudoll soil, which at a 20 cm depth had 32 g kg^{-1} organic matter and pH = 6. Seeds were planted approximately 3-4 cm deep in rows 0.7 m apart, and spaced at 0.2 m in the row. Weeds were controlled pre-emergence with glyphosate [N-(phosphomethyl)glycine] applied at 2 L ha^{-1} and then mechanically post-emergence. From emergence (September 20, 2007) to har-

vest (March 3, 2008), the cumulative precipitation was 545.3 mm and the mean air temperature was 20.75°C. Trials were conducted in three replicates, using a randomized complete block design with a factorial arrangement of genotypes and fertilization treatments. Nitrogen was applied as NH_4NO_3 (92 kg ha^{-1}) and nitrogen/phosphate as $(NH_4)_2PO_4H$ (143 kg ha^{-1}). The two fertilizer treatments provided equal doses of nitrogen each. Each genotype was planted separately (plot size: 6.3 by 8 m), and fertilization treatments applied to subplots (plot size: 2.1 by 8 m). The data were subjected to analysis of variance, and significant differences among the means and treatments were compared by the Tuckey test at the 5% level, using the Statistix software package (Analytical software, 2003). Seedling emergence was similar for the 3 genotypes (P = 0.87), with values (Mean ± SEM) for Pete (49.3% ± 4.6), Iuka (46.6% ± 4.8) and GT (46.6% ± 2.4), recorded on October 4, 2007. The number of plants per hectare was 70,476 for cv. Pete and cv. Iuka and 66,667 for GT. The number of tillers per plant also did not differ among genotypes (P = 0.78) and the interaction between number of tillers X fertilization was not significant (P = 0.73). Dry matter was assessed for plants harvested on March 3, 2008. The GT genotype produced higher dry mass (DM) per area than Pete and Iuka cultivars (P = 0.002). Plots fertilized with $(NH_4)_2PO_4H$ showed a higher DM per area than the control (P = 0.042). Genotype X fertilization interaction was significant (P = 0.031) (Figure 1). The GT genotype fertilized with $(NH_4)_2PO_4H$ showed the highest DM production (2911.6 kg ha^{-1}). In a parallel study in the same environment, but without fertilization, in its first year of production, the same material showed a yield of 13,731.5 kg ha^{-1} DM. These results show the potential of the GT genotype for crop yield efficiency with appropriate fertilization.

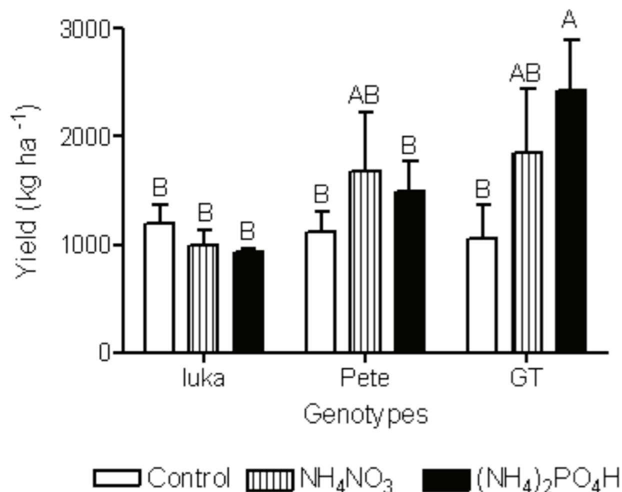

Figure 1. Total *Tripsacum dactyloides* dry matter production at the end of the crop establishment period. Fertilization treatments were: control (without fertilization), NH_4NO_3 (92 kg ha^{-1}) and $(NH_4)_2PO_4H$ (143 kg ha^{-1}). Vertical bars represent SEM. Different letters indicate significant differences between treatments.

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The *brachytic2* and *brachytic3* double mutant shows alterations in plant growth and embryo development

--Pilu, R; Villa, D; Cassani, E; Durante, M; Cerino Badone, F; Sirizzotti, A; Bucci A

In maize there are three brachytic mutants, *br1*, *br2* and *br3*, showing short stature and a gibberellin-insensitive phenotype. So far, only the *brachytic2* gene has been cloned. It encodes a putative protein of the Multidrug Resistant (MDR) class of P-glycoproteins (PGPs) possibly involved in polar movement of auxins. With the aim of elucidating the relationship between *brachytic2* and *brachytic3* mutations, we performed crosses to produce the *br2 br3* double mutant. We observed for the first time a strong additive effect regarding plant stature and architecture, and also an involvement of these two genes in embryo development. In fact, *br2 br3* plants, called "gnomes", showed shorter internodes compared to the single mutants, and had curled and wrinkled leaves (Fig. 1). Gnomes are 85% shorter than wild type plants. We

Figure 1. From left to right are shown wild type, *br2*, *br3* and gnome plants at maturity (A). Gnome phenotype in detail (B).

performed four cycles of selfing of these gnome plants to produce near-isogenic lines for use in the following studies. We noticed high sterility of these plants, with only a few seeds in the ears. While *br2* and *br3* seedlings do not show any obvious differences vs. wild type seedlings (data not shown), the offspring of these selfed gnomes, germinated on paper, exhibited aberrant seedling and plant morphology, roughly classified into four phenotypic classes: stunted plants, similar to a dwarf mutant (Fig. 2B), tube-seedlings, where the first leaf remains closed (Fig. 2C), seedlings with distorted growth (Fig. 2D) and seedlings without any shoot, called shootless (Fig. 2E). We also noticed a high level of ungerminated seeds. See Table 1 where we summarize the quantitative data regarding the phenotypic distribution. We also observed differing distributions of seedling abnormalities among the different selfed *br2/br2 br3/br3* near isogenic lines developed, ranging from entirely ungerminated seeds to almost entirely stunted seedlings (data not shown). Of course, the only *br2/br2 br3/br3* phenotypic class able to grow were the stunted seedlings that at maturity became gnome plants. These results suggest an important new role

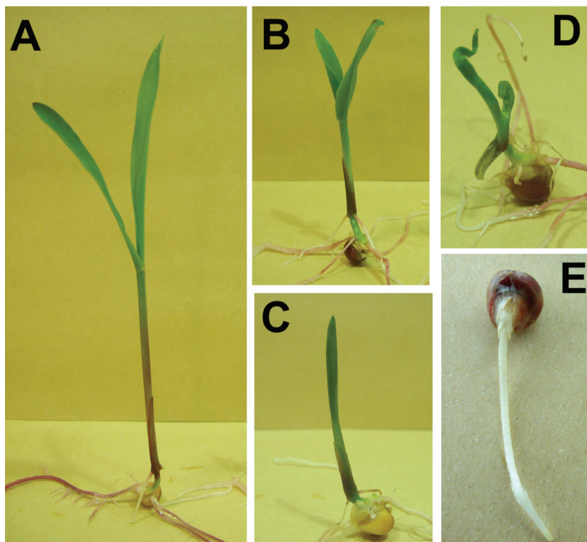

Figure 2. Range of abnormalities shown by *br2/br2 br3/br3* seedlings. (A) wild type seedling, (B) dwarf-like seedling that will became gnome at maturity, (C) "tube" seedling, (D) seedling with distorted growth, (E) shootless seedling.

Table 1. Segregation of mutant seedling phenotypes obtained by selfing the *br2/br2 br3/br3* gnome plants. The seeds were germinated on imbibed paper.

cross	segregation		
br2/br2 br3/br3 selfed	gnome	abnormal seedlings	ungerminated
	116 (38.66%)	49 (16.33%)	135 (45%)

of *br2* and *br3* genes in maize embryogenesis, which could contribute to answering several open questions regarding PAT and embryo development in maize and other cereals.

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Response of different *Ht* genes to Northern Corn Leaf Blight in Ottawa, Ontario

--Zhu, X; Reid, LM; Woldemariam, T; Voloaca, C; Wu, J

Twenty-nine inbred lines, with different *Ht* resistance genes for northern corn leaf blight [*Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs (teleomorph = *Setosphaeria turcica* (Luttrell) K. J. Leonard & E. G. Suggs; syn. = *Helminthosporium turcicum* Pass.], and four susceptible inbred lines (A619, Pa91, CO388, and CO442) were evaluated for the expression of resistance in our leaf blight breeding nursery at Agriculture and Agri-Food Canada (AAFC), Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Ontario in 2006 and 2007. Each genotype was planted in a five-row plot with single row plots of 15 plants. All plants were inoculated twice with 0.1 g of ground diseased leaf powder collected from the previous field season. The powder was placed into the whorl of each plant using a Bazooka (Sistrunk Inoculators, Starkville, MS 39759, USA) at 6-8-leafgrowth stages and again at the 10- to 12-leaf stages. If there was no rain after inoculation, the plots were irrigated for 10-15 minutes every day to provide suitable environment for disease to develop.

Resistance was observed and recorded first at 15-20 days after the first inoculation (about 12 leaf stage). Resistance was classified as: highly resistant (HR), resistant lesion (R), moderately resistant lesion (MR), moderately susceptible lesion (MS), and susceptible lesion (S). HR types have no typical lesions and the infection point changed color from yellow to brown, sometimes to purple, and had only limited extension. The R types have stripes or narrow elliptical greenish-yellow lesions. MR types have narrow, long, elliptical gray lesions with greenish-yellow or purple margins. MS lesions are long, elliptical, and gray lesion with greenish-yellow or purple margins. The S lesions are long, elliptical, and gray or tan in color. HR, R, and MR plants were selected to self for further resistance breeding to northern leaf blight.

At the soft dough stage (about 3 weeks after silk emergence), resistance data were recorded a second time, with classification as above, and scoring for general resistance. The disease rating scale for general resistance is based on disease severity and ranges from 1 to 7 where: 1= no symptoms; 2= < 1% of the leaves are symptomatic; 3= 1- 10% of the leaves are symptomatic; 4= 11-25% of the leaves are symptomatic; 5= > 50% of the lower leaves and < 25% of the mid and upper leaves are symptomatic; 6= lower leaves are dead, > 50% of the mid leaves and < 25% of the upper leaves are symptomatic; 7= plant is dead. Mid leaves are the four leaves near the primary ear. Hand-pollinated plants with HR, R, or MR, and disease rating ≤5 were selected for continued breeding. Plants with MS or S lesions, or disease rating ≥6, were discarded.

The screening results of inbred lines in 2006 and 2007 were shown in Table 1 (see supplemental data on line). In summary:

1. Three of the initial sources of blight resistant inbreds [A509N, A553N (orange), and A553N (red)] do not have uniform (pure) resistance since HR or R and S lesions were observed in the 2006 screening. However, self pollination of the HR plants resulted in all HR plants when screened the following year.
2. We observed distinct differences in the expression of the *Ht* genes at early stages (about 12 leaf stage) of plant growth and as expected, symptoms in susceptible genotypes increased as the plants matured. Some specific observations were:
   a. Lesions on *Ht* and *Ht1* plants were very similar. All *Ht* and *Ht1* inbreds were susceptible in Ottawa, Ontario, and are thus not suitable sources of resistance for our region.
   b. All *Ht2* and *Ht3* inbred lines had similar MR type resistance in 2006 and 2007. They all had long lesions with yellow or purple margins. During the later stages of plant development, some of these lesions became larger and developed a gray center thus becoming MS type lesions.
   c. All *Htm1* and *HtN* inbred lines had HR or R type resistance. As plants developed these either did not change or became slightly larger in size.

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Heterotic groups of Canadian maize inbred lines based on pedigree and SSR markers

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Corn breeders of Agriculture and Agri-Food Canada (AAFC) have collected maize genotypes from around the world and adapted them to the different short growing season regions of Canada. In many cases, the heterotic backgrounds of these genotypes were unknown, while others had been developed from mixing two or more heterotic groups. In the early years of inbred development at AAFC, testers of known heterotic backgrounds were not available with maturities suitable to Canadian climates. This sometimes resulted in the release of inbreds with little knowledge of their combining ability with the different heterotic groups. Over the past few years, we have been evaluating the inbred lines released from AAFC over the last 20 years and classifying them into known heterotic groups based on pedigree and Simple Sequence Repeat (SSR) markers.

A total of 129 inbred lines were classified into nine groups based on pedigree (Table 1, online supplemental data). Group I (BSSS group) includes eighteen inbred lines with five major sources: BSSS, B73, B14, B37, and BSTE; Group II (European flint group) includes 19 inbred lines with three major sources: INRA 258, Pfister 44 and Lethbridge Gene Pool; Group III (Lancaster group) includes 15 inbred lines with the major sources:Mo17, Oh43, LH83, W153 and H99; Group IV (Minnesota #13 group) consisted of seven inbred lines with the major sources Minnesota #13 and Pride 5; Group V (Early Butler group) consisted of seven inbred lines; Group VI (Iodent group) consisted of four inbred lines; Group VII (Pioneer 3990 group) consisted of 11 inbred lines; Group VIII (Pioneer 3994 group) consisted of 13 inbred lines; and 35 inbred lines with no major sources were classed as Group IX.

The 129 inbred lines analyzed with 105 SSR primers were distributed across the 10 maize chromosomes, with an average of 10.5 SSR per chromosome. A total of 380 alleles were detected, with 2 to 7 per locus, averaging of 3.62. Cluster analysis was based on the matrix of genetic similarities using the Unweighted Pair Group Method Using Arithmetic Average clustering algorithm from NTSYS-pc version 2.2 (Exeter Software New York, USA). All the inbred lines were classified into 13 groups (Table 1, online supplemental data).

Pedigree grouping and the SSR grouping were basically identical for the majority of the inbred lines (Table 1, online supplemental data). There were two to six SSR groups per pedigree grouping; pedigree group VII included two SSR groups and the group VIII included six groups.

Ordinations were conducted by using canonical discriminant analysis (Kshirsagar, Multivariate analysis. 1972) and classification probabilities by carrying out classificatory discriminant analyses (Figure 1). The analyses were performed using SAS (SAS Institute, Inc 1989) version 9.1.3 (2007). Comparing the groups based

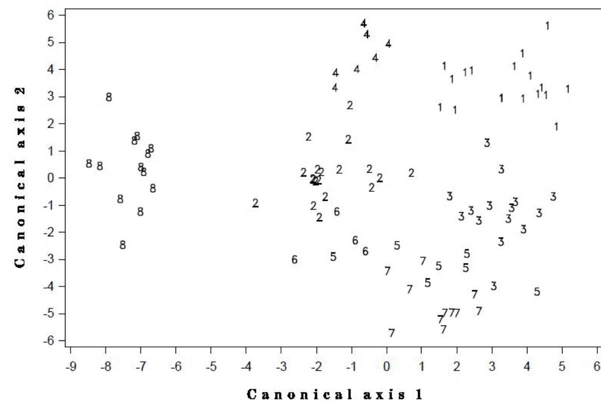

Figure 1. Canonical discriminant analyses of the SSR markers. 1 = BSSS group; 2 = European flint group; 3 = Lancaster group; 4 = Minnesota #13 group; 5 = Early Butler group; 6= Iodent group; 7= Pioneer3990 group; 8= Pioneer 3994 group.

on the discriminant analysis of SSR data with the pedigree groups, the inbred lines in the ninth pedigree group with unknown sources could be classified into one of the eight groups with known pedigree (see supplemental data, Table 1). Additionally, some inbred lines in the same pedigree group were classified into different SSR groups. This classification has 94.7% probability reliable, according to the cross-validation analyses,

We are currently conducting further classification analysis based on combining ability data of these inbreds with testers of known heterotic patterns.

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Frequency of reversion of *o2::rbg* alleles as a characteristic of specificity of their interaction with regulatory *Bg* elements[1]

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Earlier (Maydica 48:275-281, 2003), a specificity in interaction was reported between *Bg* elements and mutable *o2::rbg* alleles. Both the regulatory *Bg-hf* and *Bg-lf* elements, and the responsive *o2-hf* and *o2-lf* alleles were obtained under disruptive selection for whole endosperm revertants (WER; MNL 73:76-79, 1999). In two or three doses, the *Bg-lf* element determines low reversion frequency of the *o2-lf* allele and a high reversion frequency of the *o2-hf* allele. The presence of 2-3 doses of *Bg-hf* determines high reversion frequency of both the *o2-hf* and *o2-lf* alleles (Maydica 48:275-281, 2003).

Rather unexpectedly, we find very high reversion frequency of the *o2-hf* allele in the presence of the standard *Bg-Ref* element (this note). The percentage of WER (i.e., phenotypically normal kernels) on homozygous *o2-hf*, *Bg-Ref* ears was higher than that of the *o2-hf*, *Bg-hf* ears, and often did not differ significantly from the content of normal kernels observed on heterozygous *O2* ears. Most variegated kernels on homozygous *o2-hf, Bg-Ref* ears were characterized by small and very small opaque sectors surrounded by vitreous tissue (Figure 1).

The high content of phenotypically wildtype kernels in *o2-hf, Bg-Ref* genotypes could arise from: (i) action of modifiers; (ii) ear-

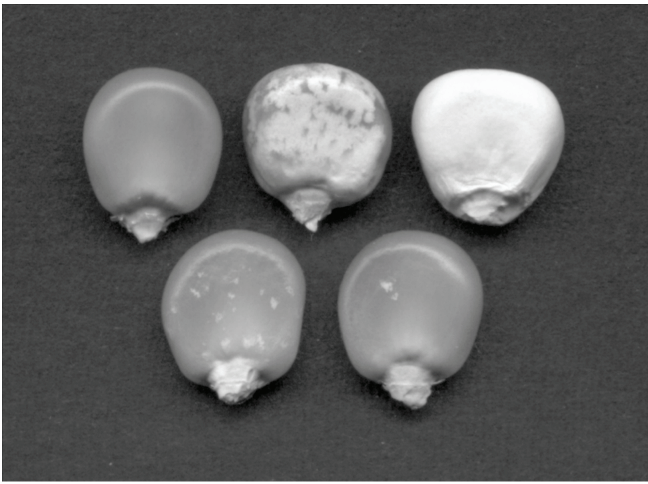

Figure 1. Kernel phenotypes observed at *o2::rbg* alleles. Upper row (from left to right): (1) phenotypically **wildtype** (whole endosperm revertant, WER) kernel formed as a result of *rbg* excision from *o2::rbg* before the first division of the primary endosperm nucleus; (2) variegated kernel (vitreous sectors in opaque background) arising from *rbg* excision during endosperm development and commonly observed for *o2::rbg* alleles; (3) opaque kernel phenotype conditioned by the *o2::rbg* allele in the absence of *Bg*. The two variegated kernels in the lower row depict the small and very small opaque sectors in vitreous background usually observed in *o2-hf*, *Bg-Ref* genotypes.

lier, pre-meiotic reversion of *o2-hf* where a significant amount of embryo revertants and their clustering on the ear would be observed; (iii) an interaction between the *o2-hf* allele and the *Bg-Ref* element. The involvement of modifiers is unlikely since sharply different revertant content in genotypes *o2-hf*, *Bg-Ref* and *o2-lf*, *Bg-Ref* have been obtained in the crosses of the same *o2-R*, *Bg-Ref* line with closely related strains *o2-hf*, $+^{Bg}$ and *o2-lf*, $+^{Bg}$. In these strains, differences in reversion frequencies of responsive were not caused by modifiers unlinked to the *o2* locus (Maydica 44:195-203, 1999; MNL 73:76-79, 1999). Most WER kernels on *o2-hf, Bg-Ref* ears are not embryo revertants, indicating that *Bg-Ref* does not condition a developmentally earlier reversion of the *o2-hf* allele. Of 67 progenies tested for an ear of the *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ genotype, ear 02-4574×4742p152, three were heterozygous *O2* plants. In 59 progenies of the second *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear studied, ear 02-4573×4742p131, no heterozygous *O2* plants were found. In the progeny of the selfed homozygous *o2-hf*, *Bg-Ref* ear, ear 02-4568p131,18 plants can be considered as embryo revertants (online supplemental data, Table 1).

Two plants homozygous for the wildtype *O2* allele, originating from WER kernels, were found in the progeny of a selfed *o2-hf*, *Bg-Ref* ear (supplemental data, Table 1). Two heterozygous *O2* plants, descendents of an *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear, and 12 homozygous *o2-hf*, *Bg-Ref* ears were found in progenies of 37 and 108 WER kernels, respectively, representing frequencies of 5.41% (2/37×100) and 5.56% (12/(2×108)×100)) respectively.

It is necessary to mention that the presence of variegated kernels in two out of three embryo revertant ears (i.e., in ears heterozygous for wildtype *O2* allele and non-mutable *o2-R* allele) found in the progeny of the *o2-hf/o2-R, Bg-Ref/*$+^{Bg}$ ear (Table 1A) is unexpected and needs to be explained (see below).

In 3 out of 16 plants (03-4225p4, 03-4411p4, 03-4432p4 in Table 1B) considered as *O2* heterozygotes (i.e., carrying wildtype *O2* and *o2-hf* alleles) in the progeny of a selfed *o2-hf, Bg-Ref* ear, the content of variegated kernels in crosses with the *o2-R*, $+^{Bg}$, tester (used as female parent), was significantly higher than expected. This excess of variegated kernels is apparently conditioned by the same causes as the phenomenon described above of the appearance of variegated kernels on heterozygous *O2/o2-R, Bg-Ref/*$+^{Bg}$ ears (see below). Significant deviations from expected ratios due to an excess of normal kernels which are mostly observed in the crosses of the *o2-hf*, *Bg-Ref* strains with the *o2-R*, $+^{Bg}$ tester used as male parent and on selfed ears (Table 1B) are conditioned by a high frequency of WER formation, especially in the presence of two or three doses of regulatory element.

The embryo revertants revealed did not belong to the same pairs of spikelets; however, five embryo revertants on homozygous *o2-hf, Bg-Ref* ear (supplemental data, Table 1) were in two clusters consisting of two and three contiguous kernels presented in adjacent pairs of spikelets (data not shown). Observed frequency of embryo revertants (5.41% and 5.56%) is approximately on the same level with this trait of the *o2-hf, Bg-hf* strains (1.82-8.18%) (Genetika (Moscow) 39:709-712; 2003) indicating that reversion of the *o2-hf* allele in the presence of the *Bg-Ref* element (as well as in that of the *Bg-hf*) occurs mostly at the period from fertilization to the first division of the primary endosperm nucleus. The size of embryo revertant clusters formed from the kernels (of two and three kernels) was also equal to that observed in homozygous *o2-hf*, *Bg-hf* genotypes (Genetika (Moscow) 39:709-712; 2003). Proceeding from the frequency of formation of heterozygous *O2* ears in the progeny of WER kernels obtained from the selfed homozygous *o2-hf*, *Bg-Ref* ear (the ear 02-4568p131) analyzed and the number of WER kernels on this ear (5.56% and 253, respectively) the appearance of two plants homozygous for the wildtype *O2* allele found in the progeny of the ear mentioned and the appearance of two kernel embryo revertant clusters, as a result of *o2-hf* reversion in gametes at postmeiotic stages of development is not excluded. However, frequency of formation of three kernel embryo revertant clusters ($1.7 \cdot 10^{-4}$ or 0.0556^3) on selfed *o2-hf*, *Bg-Ref* ears indicates that premeiotic reversion of *o2-hf* is more probable. The small size of this cluster indicates both the late stages of premeiotic development at which the reversion of *o2-hf* can rarely occur and confirms that the early reversion of this allele is not the main cause of high WER content in *o2-hf*, *Bg-Ref* genotypes.

Three features of *o2-hf, Bg-Ref* strains derivatives observed were: 1) kernel phenotype characterized by small and very small opaque sectors in a vitreous background (Figure 1); 2) the presence of variegated kernels on some embryo revertant *O2/o2-R, Bg-Ref/*$+^{Bg}$ ears (containing non-mutable *o2-R* allele, see Table 1); 3) an excess of variegated kernels in 3 out of 16 heterozygous *O2/o2-hf, Bg-Ref/*$+^{Bg}$ ears (see Table 1). These features can be explained by insertion of *Bg* or *rbg* elements in the wildtype *O2* allele leading to inactivation of this allele and to the appearance of opaque tissue in a vitreous background. In the case of *rbg* insertion, it can be classified as reinsertion since the wildtype *O2* alleles had previously been originated due to excision of the *rbg* element from the *o2-hf* allele.

The high frequency of reversion of the *o2-hf* allele in the presence of *Bg-Ref* is most probably a particular case of a specific interaction between Bg elements and o2::rbg alleles, being another example of the phenomenon observed for *o2-hf* and *o2-lf* alleles and *Bg-hf* and *Bg-lf* elements (Maydica 48:275-281, 2003).

The mechanism could involve participation of the *rbg* product, with the *Bg* encoded transposase in transposition complexes. There is sequence similarity between the *Bg* and *rbg* elements; *rbg* differs from *Bg* by small deletion and insertion events, and the two elements share more than 75% sequence homology (Hartings et al., Mol. Gen. Genet. 227:91-96, 1991). In this case, sharply different *o2-hf* and *o2-lf* alleles are a change in state of the initial *o2-m(r):3449* allele, under disruptive selection for WER content (Maydica 44:195-203, 1999) , conditioned by changes in the *rbg* elements that affect the ability of *rbg* products to interact with transposition complexes responsible for *rbg* excision. Accordingly, changes in the initial *Bg-3449* element, which conditioned formation of *Bg-hf* and *Bg-lf* elements under the same disruptive selection (Maydica 44:195-203, 1999), could lead to the changes in their encoded transposases that affected affinity of these transposases toward *rbg* element products.

The higher frequency of reversion of *o2-hf* in the presence of the *Bg-Ref* element than in the presence of *Bg-hf* is another indication that an effect of selection is the ability of Bg elements to control *rbg* excision. The changes in the *Bg-hf* conditioning certain upper levels of the *rbg* excisions could be determined by the method of disruptive selection for high WER content used (in which the *Bg-hf* element was obtained): the ears containing significantly more than 50% of WER were not selected for the next cycle of selection (Maydica 44:195-203, 1999), since in this case it was more difficult to distinguish the ears with high WER content from the ears heterozygous for the normal *O2* allele.

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[1]This note is a shortened version of a full size article which had been submitted for publication. However, due to termination of my work in Maize and Sorghum Research Institute its publication has not been finalized. Meanwhile, I would like to share the main results and conclusions with the maize community.

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Gene expression analysis in maize ears and silks after *Fusarium verticillioides* infection

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The genus *Fusarium* includes numerous plant pathogens that infect some of the world's most agriculturally important plant species, including maize. Of particular importance, *F. verticillioides* causes root, stalk and ear rots, blights and wilts, and can contaminate kernels with a family of closely related mycotoxins known as fumonisins. Plant defenses consist of physical barriers such as the cell wall and its modifications, as well as chemical defense mechanisms that are induced in response to external stimuli. In addition, plant defense involves expression of pathogenesis-related (PR) proteins (Kitajima and Sato, J. Biochem. 125:1-8, 1999). PR proteins are grouped into 17 independent families, and antimicrobial properties have been described for some of them. The PR-2 proteins display β-1,3-glucanase activity, whereas the PR-3 proteins (as well as PR-4, PR-8 and PR-11 proteins) show endochitinase activity (Campo et al., Proteomics 4:383-396, 2004).

In maize, few detailed studies are presently available on the response to *F. verticillioides* infection. Although differences in susceptibility to this pathogen attack have been described, resistant maize varieties have not yet been developed (Bluhm and Woloshuk, Mol. Plant-Microbe Interact. 18:1333-1339, 2005). Our objective is the identification of genes expressed in kernels and silks of maize tolerant and susceptible to *F. verticillioides* infection, using microarray technology.

The tolerant CO441 and susceptible CO354 lines were used. Their ears were infected with a fumonisin-producing strain of *F. verticillioides*, using the pin-bar technique, and harvested 48 hours after infection. RNA was extracted, reverse transcribed and labelled with fluorophor dyes, and then hybridized on the array slides. Venn Diagrams showed that only 60 genes were differentially expressed in CO441 and CO354 48h after infection, compared to uninfected ears. Similar functional categories of genes were involved in the response to infection in both tolerant and susceptible lines, and included: defense response proteins, oxidative burst-associated enzymes, enzymes involved in sugar metabolism and proteins involved in amino acid synthesis, folding and stabilization. The main *PR* genes identified in the array experiments were also tested in silks, infected and harvested 12, 24, 48 and 72h after infection, and from uninfected samples using Real-Time-PCR.

The presence and the activity of *F. verticillioides* in seeds and silks was quantified by assaying levels of a constitutive gene for β-tubulin and the target gene *FUM21*, a transcriptional regulator of the fumonisin metabolic pathway, of *F. verticillioides*. The results showed a higher copy number for both genes in the susceptible line CO354.

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Analysis of cell size and endopolyploidy level in the mutant *defective endosperm18* (*de18*) of maize

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The maize mutant *defective endosperm18* (*de18*) accumulates less dry matter in the endosperm tissue and shows a level of indole-acetic acid (IAA) at least 15 times lower than its normal counterpart. The addition of synthetic auxins to developing *de18* grains rescues the wild type phenotype (Torti et al., Theor. Appl. Genet. 72:602-605, 1986). The analysis of differentially expressed genes indicates that auxin metabolism is impaired in the *de18* mutant. The plant hormones auxins and cytokinins are involved in regulation of mitosis and endoreduplication, key cellular processes during seed development. In fact enlargement of the maize endosperm relies upon cell division and cell expansion, in turn linked to endoreduplication of nDNA (Kondorosi et al., Curr. Opin. Plant Biol. 3:448-492, 2000). Endoreduplication begins at 10 DAP (days after pollination) (Kowles and Phillips, Proc. Natl. Acad. Sci. USA 82:7010-7014, 1985; Kowles and Phillips, Int. Rev. Cytol. 112:97-136, 1988). Cells with highly endopolyploid nuclei occupy a major

part of the volume of the starchy endosperm (Vilhar et al., Plant Physiol. 129:23-30, 2002) and the highest nDNA amount, expressed as C value, is typically 96C to 192C as measured by nuclear volume (Tschermak-Woess and Enzenberg-Kunz, Planta 64:149-169, 1965), Feulgen cytophotometry, and flow cytometry (Kowles et al., Genome 35:68-77, 1992; Schweizer et al., Proc. Natl. Acad. Sci. USA 92:7070-7074, 1995; Larkins et al., Exp. Bot. 52:183-192, 2001; Settler and Flannigan, Exp. Bot. 52:1401-1408, 2001).

To investigate whether the reduced endosperm of *de18* is due to impaired cell division and endoreduplication, wild-type B37 and *de18* kernels were analyzed at 8, 12 and 16 DAP. The collected seeds were fixed, embedded in Paraplast and sectioned for microscopy analysis. Nuclear endoreduplication, the number and size of cells were measured with the optical miscroscope and computer image analysis using the 3D model developed for maize endosperm. Observations of cell distribution with different ploidy levels in both genotypes showed that at 8 DAP most of the cells in the endosperm were 3C and 6C cells, and they were mainly in the outermost layers. Endoreduplication began in the nuclei of the central starchy endosperm cells (12C) and proceeded basally and outward until 16 DAP, where 96C and 192C nuclei were localized in the central part of the endosperm. The most significant differences between *de18* and B37 were detected at 12 DAP, where the mutant showed deficiencies in ploidy level, number and volume of cells. These results suggest that the mutant is characterized by a defective cellular proliferation, and reduced cell volumes, contributing to a decreased endosperm development. The next step of this study will be to analyze the correlation between starch content and ploidy level in *de18*.

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Application of error correcting codes for heterotic group assignation

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Introduction of exotic maize (*Zea mays* L.) into breeding programs may enhance genetic variability and lead to greater progress from selection. However, the pool of available exotic germplasm is large and diverse, making choices of potential parents difficult. Two major heterotic group-classification methods are currently used widely across the world. The traditional method uses specific combining ability with some line-pedigree information and/or field hybrid-yield information (Hallauer and Miranda, Quantitative Genetics in Maize Breeding, 2nd ed., Iowa State Univ. Press, Ames, IA, 1988). The second method employs various molecular markers to compute genetic similarity (GS) or genetic distance (GD) (Mohammadi and Prasanna, Crop Sci. 43:1235–1248, 2003). However, the results of these associations are inconsistent (dos Santos Dias et al., Genet. Mol. Res. 3: 356-368, 2004).

We conjecture that the traditional distance-based methods currently available do not capture the non-linear relationship between parental molecular data and progeny performance, and that supervised learning algorithms can overcome that hindrance. Among them, support vector machines (SVMs) have shown high generalization abilities and have become very popular in the past few years (Rifkin and Klautau, JMLR 5:101-114, 2004). However, they are binary classifiers and a combination scheme is necessary to extend SVMs for problems with more than two classes (Rifkin and Klautau, JMLR 5:101-114, 2004). In this work, we explore the performance of the recently introduced class of ECOC-SVM (Error Correcting Output Coding-Support Vector Machine) classifiers in heterotic group assignation. This method is based on recursive error correcting codes of communication theory (Tapia et al., LNCS 3541:108–117, 2005). As a control, we used four Native multiclass classifiers: (1) Naive Bayes (John and Langley, 11th Conf. on *Uncertainty* in *A*, 338–345, 1995; (2) Bayes Network (Friedman et al., Mach Learn. 29:131–163, 1997); (3) Decision Tree J48 (Quinlan, C4.5: Programs for Machine Learning. Morgan Kaufmann Publishers, San Mateo, CA., 1993); and (4) Logistic Model Trees or just Simple Logistic (Landwehr et al., Mach Learn. 161–205, 2005). We also report the performance of the ensemble method using as a base classifier Naive Bayes and J48 (Witten and Frank, Data Mining: Practical Machine Learning Tools and Techniques, 2nd ed., Morgan Kaufmann, San Francisco , 2005).

Classifiers were evaluated with two datasets: the Morales dataset, involving 26 inbreds of temperate germplasm clustered into four heterotic groups by topcross (Eyherabide et al., Plant Breeding: The Arnel R. Hallauer International Symposium, Blackwell Publishing, pp. 352–379, 2006) and characterized by 42 attributes derived from 21 microsatellites (MNL 79:36-37, 2005); and the Xia dataset, comprising 73 inbreds of tropical germplasm clustered into 8 heterotic groups and characterized by 166 attributes derived from 83 microsatellites (Xia et al., Crop Sci. 4:2230-2237, 2004). The decomposition method of ECOC employs a binary matrix of order $k \times n$ to convert a k-multiclass problem into n binary tasks ($\log_2 k \leq n \leq 2^{k-1}-1$) (Dietterich and Bakiri, JAIR 2:263-286, 1995); this allows us to explore the performance of matrices with $n = [2,3,\ldots, 7]$ for the Morales Data, and $n = [3, 16, 29,\ldots , 120]$ for the Xia data. The predictive power of proposed algorithms was evaluated by means of Cohen's Kappa coefficient (Landis and Koch, Biometrics 33:159–174, 1977) across 30 Montecarlo runs of stratified 10-Fold Cross Validation (CV) experiments (Kirchner et al., Comput. Electron. Agric. 42:111–127.2004). The Kappa coefficient is a better measure of the agreement between binary inter-annotators than the traditional error rate, because it takes into account chance agreements and it is better suited for unequal class distribution datasets (Kirchner et al., 2004). Figures 1 and 2 show the performance of the 7 classifiers on the Morales and Xia datasets, respectively. Results of ECOC codes correspond to kappa values obtained with codes that, with the lowest number of columns, reached the best kappa value (n = 55 for the Xia Data and n = 7 for the Morales data).

Although a further statistical test is needed to define the significance of the performance of classifiers, visual inspection of both boxplots shows that ensembles (ECOC codes) outperform the performance of most native classifiers. The only exception is "simple logistic", which outperforms the ECOC-SMO ensemble for the Morales data. Optimizations of SVM parameters, not yet performed, are reported to significantly improve the final performance of the ensemble (Rifkin and Klautau, 2004). It has been reported

that the aim of the classifier ensembles is to take advantage of the individual classifiers' capabilities by selecting or weighting their individual decisions (Dietterich, LNCS 1857:1-15, 2000). It also can be seen from both boxplots that ensembles (ECOC codes) of Decision tree and Naïve Bayes did improve the performance of the single classifier (Figs. 1 and 2).

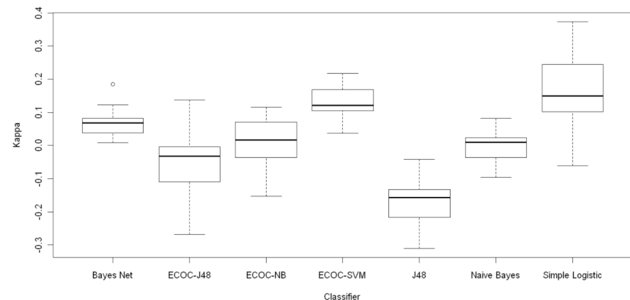

Figure 1. Morales data. Boxplots of Cohen's Kappa coefficient in 30 Montecarlo runs of 10-Fold CV experiments. ECOC - xx Error correcting output code + base classifier: NB - Naïve Bayes, SVM - Support vector machine, J48 -Decision Tree J48.

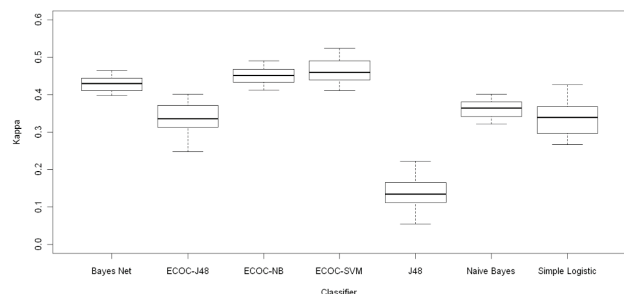

Figure 2. Xia data. Boxplots of Cohen's Kappa coefficient in 30 Montecarlo runs of 10-Fold CV experiments. ECOC - xx Error correcting output code + base classifier: NB - Naïve Bayes, SVM - Support vector machine, J48 -Decision Tree J48.

Finally, although results obtained do not allow molecular markers to replace field essays (top cross or diallel) in heterotic group assignment[1], there is strong evidence more training instances could generate successful classifiers. Moreover, the potential impact, in time and money, on crop sustainability makes our approach worth trying. Traditional genetic breeding requires expensive field testing and a time scale on the order of years for heterotic assignment. Our proposed framework significantly lowers costs and time required, with two weeks for growing a small plant, a week to obtain molecular data and a couple of days for computational analysis.

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[1]Kappa values ranging between 0.41-0.60 indicate a moderate agreement between observed and predicted data whereas values below 0.20 indicate only a slight agreement (Landis and Koch, 1977).

SAGAM, ANANTNAG, INDIA
High Altitude Maize Research Sub-Station, Sagam

Shalimar Maize Composite-3 - an opportunity to break the yield barrier under the high altitude conditions of the Kashmir valley

--Rather, AG; Najeeb, S; Sheikh, FA; Wani, SA; Wani, AA; Bhat, MA; Ahanger, MA

In the Kashmir valley, high altitudes (>6500 ft amsl) comprise 75 % of the total maize growing area (1 lakh hectare; 100,000 hectares). The average productivity of maize under high altitude conditions is very low (around 10q/ha), which is dismal when compared to national productivity (2.2 t /ha). Farmers in this region mainly grow traditional varieties and land races, which are not only low yielding but highly susceptible to biotic and abiotic stresses. To boost maize production, SKUAST-K has recently released a high yielding variety of maize (Shalimar Maize Composite-3) suitable for the growing conditions of the Kashmir valley. This variety was developed by pooling material from local land races and exotic collections (CIMMYT) selected on the basis of different potentialities but uniform in different morpho-agronomic traits. The variety has shown excellent performance in different locations of the state with an average yield of 39.9 kg/ha, which is 10.5 % over the standard check (C15) in the target environment. The benefit/cost ratio of this variety has been worked out as 1.52 versus 1.22 for C15. The specific area of adaptation for this variety includes high altitudes, between 1800-2250 meters, of rainfed Kashmir. A timely sown crop with adequate moisture at critical growth stages like tasseling, silking and grain filling, and with soils having a good quantity of organic manure and fertilizer, are required to exploit the maximum potential of this variety

Figure 1. Shalimar Maize Composite-3.

Figure 2. Shalimar Maize Composite-3.

Diseases, mainly *Turcicum* leaf blight and common rust, take a heavy toll on the crop under the high altitude conditions of the Kashmir valley. The variety under report shows moderate resistance to *Turcicum* leaf blight and common rust. Fewer of the Shalimar Maize Composite-3 plants were affected by *Turcicum* leaf blight (23.7% vs. 32.2% for the check, C15). Similarly, for common rust 23.7 % of Shalimar Maize Composite-3 plants were affected, compared to 36.4 % of the check. For affected plants, using a disease scale of 1-5, the Shalimar Maize Composite-3 scored 2 for leaf blight and common rust versus a score of 3 for the check, C15.

Shalimar Maize Hybrid-1: a high yielding maize hybrid under the high altitude conditions of the Kashmir valley

--Rather, AG; Najeeb, S; Sheikh, FA; Wani, SA; Wani, AA; Bhat, MA; Ahanger, MA

SKUAST-K has recently released a high yielding hybrid of maize (Shalimar Maize Hybrid-1) to boost maize production in the Kashmir valley. See the accompanying note for a brief description of the maize crop in this region. The hybrid exploits the hybrid vigour between two elite maize inbreds (W3 and W5), and was developed by selection and proper assessment over various locations and years. The hybrid has excellent performance in different locations of the state with an average yield of 40.4 kg/ha, representing a superiority of 26.6% over the standard check (C15) in the target environment. The benefit/cost ratio of this variety has been worked out as 2.4 as opposed to 1.22 for C15. The specific area of adaptation for this variety is the high altitudes, 1800-2250 meters (amsl), of rainfed Kashmir

Figure 1. Shalimar Maize Hybrid-1 along with parents (W3 and W5).

Figure 2. Shalimar Maize Hybrid-1.

The hybrid under report has moderate resistance to *Turcicum* leaf blight and common rust. Fewer plants were affected by *Turcicum* leaf blight: 18.1% vs. 33.2% for the check, C15. Similarly for common rust, 17.3 % of Shalimar Maize Hybrid-1 plants were affected, compared to 35.5 % of the check. For affected plants, using a disease scale of 1-5, the Shalimar Maize Hybrid-1 scored 2 for leaf blight and common rust, as opposed to a score of 3 for the check, C15.

SARATOV, RUSSIA
Saratov State University

Maize tetraploid pollen studies

--Lobanova, LP; Kolesova, AY

Maize tetraploids show a wide range of valuable selective features such as increased vegetative mass, increased yield, etc. At the same time polyploidy can lead to decreases of male and female fertility, seed production and germination. We have analyzed maize tetraploid pollen of the Krasnodar population 1(KrP-1). The results of this research show that frequency of morphologically normal pollen formation of different plants varies from 67.8% to 84.0%. In contrast to normal pollen grains, which are spherical, with one aperture, one vegetative cell and two sperm cells, defective pollen was characterized by numerous anomalous structures, plasmolyzed, and hollow pollen grains. Underdeveloped pollen (unicellular and bicellular) was observed, as well as normal size pollen grains, but with an additional vegetative nucleus and four or five additional sperm cells. A considerable portion of the defective pollen was represented by big pollen grains that were not spherical, but of oval, egg and dumbbell shapes, and which might have been formed as a result of cell wall formation failure during microsporogenesis. Big pollen grains usually contain one vegetative nucleus and two sperm cells, but also have constriction of cytoplasm and contain two apertures. Two pollen grains were observed that contained four sperm cells and two apertures. Pollen grains with three and four apertures have also been found. One of the dumbbell-shaped pollen grains had seven apertures, one of

the latter is typical and is on one part of the dumbbell, while the other six are underdeveloped and are on the other part. The frequency of plasmolyzed pollen grains of different plants varied from 2.3% to 12.9%, with a frequency of dead pollen of 5.7% to 9.4%.

This number of anomalous pollen grains of maize tetraploid pollen KrP-1 is increased if compared to earlier studied diploids and is apparently associated with disturbances in microsporogenesis and microgametogenesis of polyploids.

Mitotic activity stimulation of corn apical root meristems under the influence of a low-frequency magnetic field

--Belyachenko, JA; Usanov, AD; Tyrnov, VS; Usanov, DA

Mitotic activity (MA) of meristems is the important parameter defining plant proliferation intensity. This process substantially determines the morphological characters of plants and their physiological characteristics affecting rates of development and productivity. MA management represents independent value for biomass development for producers of pharmacological, alimentary, cosmetic and other matters; the regeneration of the whole plants from cells subjected to various bioengineering operations; a vegetative propagation of unique specimens; sporophyte production from gametes; generation of polyploids and mutants from single cells.

We have reported the stimulating effect of a low-frequency magnetic field (MF) on the MA of apical root meristems of various floral plants (in Russian: Belyachenko et al., Biomed. Technol. Radioelectr. 11:57-60, 2007; Belyachenko et al., The Bulletin Saratov University. Chemistry. Biology. Ecology. 8(2):84-88, 2008; Belyachenko et al., The Bulletin Saratov State Agrarian University 6:5-8, 2009). The MF parameters which had the greatest effects on the physical properties of water (in Russian: Usanov et al., Biomed. Technol. Radioelectr. 5-6:65-69, 2006) have been chosen to assess the effects of an alternating MF on apical root meristems MA of corn line Precocious Purple Tester.

Dry or water imbibed (less than18 hours) corn seeds were exposed to MF influence. Root tips 1-1.5 cm long were fixed for cytological analysis. The number of cells at different stages of a cellular cycle were estimated on temporary acetocarminic squash preparations. In each of three replicates mitotic index values were calculated for over 3000 cells.

A stimulating effect on corn meristems is observed at 1 and 3 Hz MF applied to corn seeds for 6 h, with the average level of MA stimulation being 8-10 %. The greatest (or significant) effect is typical of larger corn seeds. MA stimulation of germinating small seeds represents a special interest as the small seed size may cause delays in growth and development that could be overcome by MA increase of those germinating plants.

Two technologies for maize breeding on the basis of parthenogenesis

--Tyrnov, VS

There exist two different mechanisms of haploid formation. The first is connected with specific features of the male gametophyte and it leads to a unitary fertilization. Using this method, we have created haplo-inducing lines, which, as pollen parents, lead to haploids with frequencies up to 10 % and above. The second mechanism is associated with parthenogenesis and is controlled by nuclear factors of the maternal parent, demonstrated by genetic marking and cyto-embryological analysis (Tyrnov, 1983, 1994, 1997, 2000, 2002, 2007). The frequency of parthenogenesis, as a rule, reaches some dozens of percents, up to 100 percent. We consider it a good basis for work on synthesis of unreduced obligate and facultative apomixes. However, a great number of haploids produced in the field by heritable reduced parthenogenesis have limited possibilities for commercial use. We now know that parthenogenesis capacity can be transferred to progeny by egg and pollen and have discovered signs and conditions for effective selection of parthenogenesis and production of new lines. We have worked out a simple and reliable genetic syste to produce with high frequency (dozens of percents) matroclinic haploids, i.e., by homozygous line development. About 3-10% matroclinic diploids produce additively, and are spontaneous diploidized haploids. In addition, a system has been worked out whereby haploids from commercial hybrids are produced in the field with a frequency of about 0.3-1.0%. It is somewhat higher than the average frequency for maize, 0.1%. In the future, haploids can be the initial material for selection, descending from hybrids and commercial materials and undergoing hard selection under field conditions towards being the bearers of the new valuable genetic combinations.

SHALIMAR, INDIA
K. D. Research Station, Sher-e-Kashmir University of Agricultural Sciences & Technology Shalimar
PANTNAGAR, INDIA
G. B. Pant University of Agriculture & Technology

Studies on character association in winter maize under normal and excess soil moisture (ESM) conditions

--Lone, AA; Warsi, MZK; Nehvi, FA; Dar, SA

Excess soil moisture (ESM) is a problem for maize, with 15 percent of the total maize growing area affected by floods and water logging in Southeast Asia alone. In India, each year 25-30 percent loss of maize production occurs because of ESM stress (Directorate of Maize Research, 49th Annual Maize Workshop, Kanpur, India, April 5-9, 2001). A set of forty-five genotypes differing in their reaction to ESM conditions was used for estimating inter-character correlations between different morphological traits. The lines included five parents (three tolerant and two susceptible), their F1's, F2's and backcross generations. The experimental materials were planted during the winter season, Rabi 2005-06, at the Crop Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar. Plantings were in randomized block design with three replications, in two row plots of 5-meter length, a row-to-row spacing of 75 cm, and plant-to-plant distance of 25 cm. In the ESM trial, when plants were knee high they were subjected to continuous submergence to an average depth of ponding of about 5 cm for six days, followed by draining of water. Observations were recorded for the days to 50 percent tasseling, days to 50 percent silking, the anthesis silking interval (ASI), plant height, ear height, cob length, cob diameter, leaf temperature, SPAD value, transpiration rate and photosynthetically active radiation (PAR). The correlations between all possible pairs of characters

under study, including genotypic, phenotypic and environmental were worked out from the analysis of variance and covariance as suggested by Searle (1961). Data details are presented in online supplemental data (Table 1).

Under both normal and ESM conditions, the days to 50 percent tasseling and days to 50 percent silking were positively and significantly correlated. The anthesis silking interval (ASI) and number of nodes bearing adventitious roots were negatively and significantly correlated under ESM conditions at both genotypic (r_g=-0.409) and phenotypic levels (r_p=-0.250). This correlation under ESM conditions has practical implications, as a greater ASI indicates susceptibility to ESM and fewer nodes bearing adventitious roots will add to the problem. Under ESM conditions, ASI and yield were negatively and significantly correlated with each other at both genotypic (r_g =-0.175) and phenotypic levels (r_p=-0.116), in agreement with Zaidi et al. (European J. Agron. 19:383-399, 2003) and Lone and Warsi (Pantnagar J. Res. 4:61-64, 2006). Yield and 100 kernel weight were positively correlated under both normal (r_g = 0.237) and ESM conditions (r_g=0.322). Under ESM conditions, yield and number of nodes bearing adventitious roots had a positive correlation at both genotypic (r_g=0.190) and phenotypic level (r_p=0.130), as also observed by Rathore et al. (Biometrics 17:474-480, 1996) and Zaidi and Singh (J. Plant Biol. 28:61-67, 2001). An increased number of nodes bearing adventitious roots assists in avoiding lodging under ESM conditions, which in turn results in increasing the overall yields (Zaidi et al., Proc. 8th Asian Reg. Maize Workshop, Bangkok, Thailand, August 5-9, 2002). Yield, under both normal and ESM conditions, was positively correlated to SPAD values at both genotypic and phenotypic levels. Higher SPAD values correspond to enhanced greenness of leaves, which contributes to higher photosynthetic rates and yields. Also yield was positively correlated with cob length and cob diameter under both sets of conditions. Under ESM conditions, transpiration rates were reduced and positively correlated (r_g=0.179) with yield. Plant height was positively correlated with yield under both normal (r_g =0.606) and ESM conditions (r_g = 0.267) in confirmation of the findings of Lizaso and Riche (Agron. J. 89:125-134, 1997) and Ajaz and Warsi (2006). Generally, correlation coefficients at genotypic levels were similar in direction to phenotypic correlation coefficients, but with higher magnitude.

In any breeding programme directed to improve the yield under ESM conditions, due importance is to be given to the growth parameters ASI and the number of nodes bearing adventitious roots owing to their interrelationships to the yield.

TALLAHASSEE, FLORIDA
Florida State University

A recombinant C-terminal fragment of maize Initiator Binding Protein 2 (IBP2) binds to telomere-repeat DNA in vitro

--Moore, JM; Bass, HW

The maize (*Zea mays* L.) *initiator-binding protein2* (*IBP2*) gene is known to encode a protein, IBP2, that binds to the *initiator* (*inr*) element in the *Shrunken1* promoter (Lugert and Werr, Plant Mol Biol, 25:493–506, 1994). The duplicate genes *IBP1* and *IBP2* were initially identified in ligand-binding screens using cDNA expression libraries. The binding of IBP to the transcription start site *inr* element was confirmed by footprint and band-shift assays. We and others have observed that IBP1 and IBP2 also resemble telomere DNA-binding proteins from other plant species. We investigated therefore whether the IBP2 protein exhibits telomere DNA-binding activity.

Using Electrophoretic Mobility Shift Assay (EMSA) we found that a C-terminal subclone of the IBP2 protein (rIBP2 Myb-Q, AA 579-667, GenBank GU080214), binds telomeric repeat DNA *in vitro* (Fig. 1, arrows indicate band shifts). We also found that rIBP2 Myb-Q binds strongly to double stranded telomere repeat sequences with three or four tandem repeats, but only weakly to sequences with two (Fig. 2, arrows indicate band shifts). Furthermore, we found no evidence of binding with only one telomere repeat (5' TTTAGGG 3') sequence. Single point mutations (T1:A and G6:T) in the three-repeat sequence diminished, but did not entirely abolish, binding activity (via competition EMSA assays, data not shown).

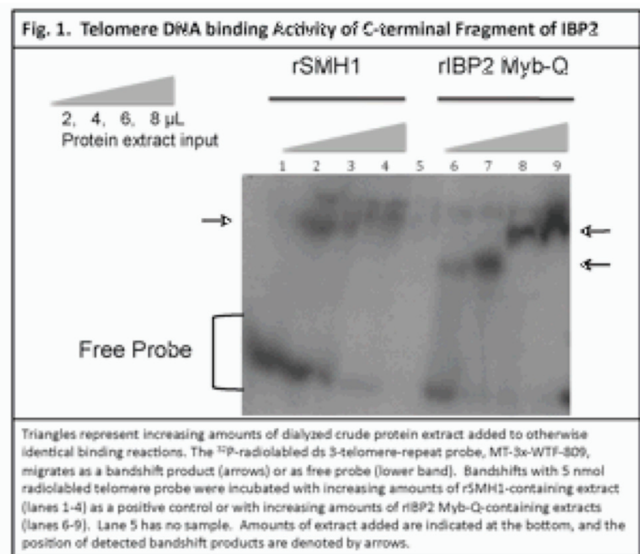

Fig. 1. Telomere DNA binding Activity of C-terminal Fragment of IBP2

Triangles represent increasing amounts of dialyzed crude protein extract added to otherwise identical binding reactions. The ^{32}P-radiolabled ds 3-telomere-repeat probe, MT-3x-WTF-809, migrates as a bandshift product (arrows) or as free probe (lower band). Bandshifts with 5 nmol radiolabled telomere probe were incubated with increasing amounts of rSMH1-containing extract (lanes 1-4) as a positive control or with increasing amounts of rIBP2 Myb-Q-containing extracts (lanes 6-9). Lane 5 has no sample. Amounts of extract added are indicated at the bottom, and the position of detected bandshift products are denoted by arrows.

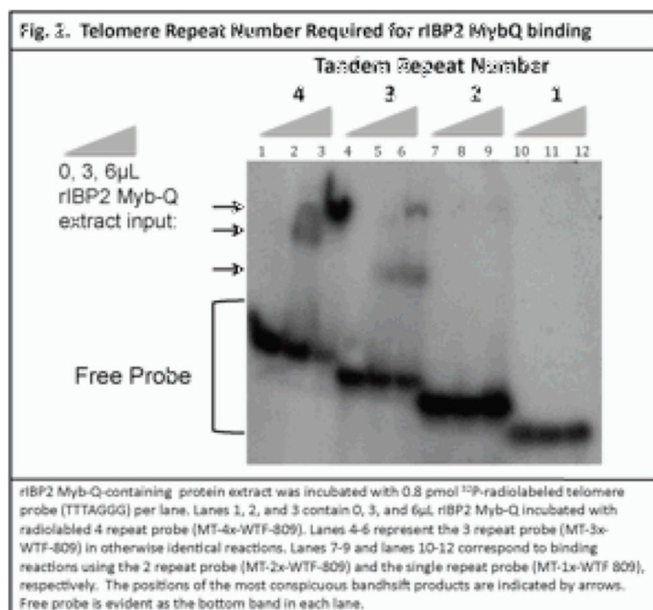

Fig. 2. Telomere Repeat Number Required for rIBP2 MybQ binding

rIBP2 Myb-Q-containing protein extract was incubated with 0.8 pmol ^{32}P-radiolabeled telomere probe (TTTAGGG) per lane. Lanes 1, 2, and 3 contain 0, 3, and 6µL rIBP2 Myb-Q incubated with radiolabled 4 repeat probe (MT-4x-WTF-809). Lanes 4-6 represent the 3 repeat probe (MT-3x-WTF-809) in otherwise identical reactions. Lanes 7-9 and lanes 10-12 correspond to binding reactions using the 2 repeat probe (MT-2x-WTF-809) and the single repeat probe (MT-1x-WTF 809), respectively. The positions of the most conspicuous bandhsift products are indicated by arrows. Free probe is evident as the bottom band in each lane.

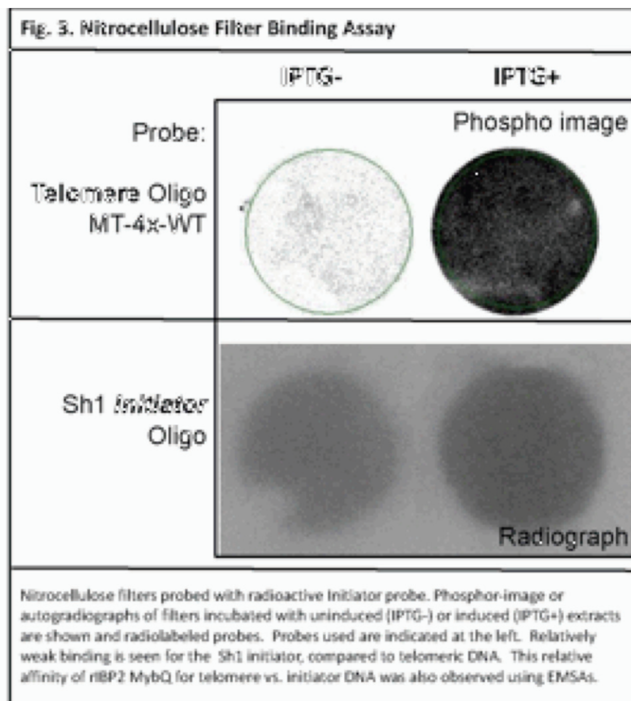

Fig. 3. Nitrocellulose Filter Binding Assay

Nitrocellulose filters probed with radioactive Initiator probe. Phosphor-image or autogradiographs of filters incubated with uninduced (IPTG-) or induced (IPTG+) extracts are shown and radiolabeled probes. Probes used are indicated at the left. Relatively weak binding is seen for the Sh1 initiator, compared to telomeric DNA. This relative affinity of rIBP2 MybQ for telomere vs. initiator DNA was also observed using EMSAs.

In order to compare the binding of rIBP2 Myb-Q to telomere vs. initiator DNA, we carried out filter binding assays. In this experiment, total protein extract from E coli cultures expressing rIBP2 Myb-Q (IPTG+) were incubated with nitrocellulose filters. These filters were then incubated with radiolabeled oligonucleotide probes corresponding to telomere DNA or *Sh1 initiator* DNA (Fig. 3). From these experiments, we found that the rIBP2 Myb-Q showed very weak binding to the *initiator* sequence of the *Shrunken*1 promoter. These findings confirm the prediction that the maize IBP protein (IBP1 or IBP2) has telomere-DNA-binding activity. Consequently, maize IBP1/2 may reside at maize telomeres, raising the intriguing possibility of dual functions, telomeric and transcriptional, for IBP.

TURDA, ROMANIA
Agricultural Research and Development Station

Characterization of TURDA, a Romanian maize germplasm, for the chemical composition of the grain

--Has, V; Has, I; Copandean, A

The aim of this research was to evaluate maize TURDA germplasm for grain quality components: protein, oil, fiber, ash and starch concentration. A total of 754 maize samples were evaluated, including: 265 local populations (landraces) collected in different regions of Romania (Transylvania and Moldavia); 59 synthetics/composites, including 30 synthetics created at ARDS-Turda and 29 synthetics acquired from different countries (Spain, Italy, Germany, University of Minnesota, University of Pennsylvania); and 430 TURDA inbred lines. The concentration of starch, protein, oil, fiber and ash in the ground (flour) samples was determined with a Dickey-John Instalab 600 near-infrared reflectance analyzer, and curve calibration. The coefficients of phenotypic variation were over 5% for most grain components (see online supplemental data, Table 1); they were higher for percentage of oil (12.3 to 21.2%), fiber (10.5 to 18.9%) and ash (51.1 to 88.2%). Among the samples, oil content ranged between 2.4-9.1%, protein between 10.8-15.6%, starch between 52.8-72.7%, fiber between 2.3-7.5%, and ash between .01-10.6%. Inbred lines were most divergent in grain starch concentration as compared to landraces and synthetics. The grain fiber and ash content showed high variability among all the genotypes.

About 100 genotypes have high starch content. Among "TURDA" inbred lines, a high level of starch content was found in: TC 384AcmsC (72.5%), TE 210 (72.1%), TC 378 (72.0%), TC 182 (71.9%), and TD 375 (71.9%). High levels of oil concentration were found in the local populations: Blaj (Veza)/01 (7.3%), Iclod/01 (7.0%), Salva/01 (7.1%), Sarmisegetuza/01 (7.1%), and Vanatori/01 (7.1%). High oil concentrations were found in the following synthetics: Tu Syn 1 (7.1%), Tu Syn 2 (7.0%) and Tu Syn 3 (7.3%). High oil local populations and synthetics have a large reduction in starchy endosperm and most are flint or semi-flint grain types. Pedigree selection has been used to develop some elite high oil lines from this germplasm (Smith, 1990). Inbred lines showed the highest mean value for oil percentage. Inbred lines with a high concentration of both oil and protein include: TC 344A (7.6% oil and 15.2% protein), TC 334 (7.5% oil and 15.1% protein), TC 106 (7.5% oil and 16.4% protein), and T 442 (7.2% oil and 15.6% protein). Local populations with both high protein and oil content in grain include: Carnesti/01 (15.5% protein and 6.9% oil), Ghiula/04 (15.2% protein and 6.7% oil), Iclod/01 (15.1% protein and 7.0% oil), Salva/01 (15.5% protein and 7.1 oil), and Satu Lung/01 (15.6% protein and 6.7% oil). Work at the University of Illinois has also shown that protein varies from 8-11% in maize (Smith, 1990).

The results of this study demonstrate a great variability in the 3 groups of genotypes, which represent only a small part of germplasm available in Turda, Romania. One can imagine the amount of phenotypic variability which could be used by breeders.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center

Additional new allele of *pink scutellum1* found in Maize COOP phenotype-only collection

--Jackson, JD

This report summarizes additional tests of viviparous stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection (Jackson, MNL 74:69-70, 2000; MNL 78:66, 2004; Jackson, MNL 81:32, 2007). This new allele was first noticed during routine propagation of viviparous stocks. Older stocks tend to lose the characteristic pink color of *ps1* as they age in storage.

Crosses were made as follows: *+/ps** X *+/ps1* or *+/ps1* X *+/ps** and ears were scored at maturity for the pink scutellum phenotype. New designations have been assigned to these alleles and they have been placed on our current stock list. It is expected that with further sorting and allelism testing of other stocks charac-

terized by phenotype only, additional *ps1* alleles will be discovered.

Previous designation	Allelism test with *ps1*	New designation	MGCSC: stock number
vp-8117*	4 positive	*ps1-8117*	526L

Linkage tests of *waxy1* marked reciprocal translocations obtained from the collection of Don Robertson

--Jackson, JD; Stinard, P

In the collection of A-A translocation stocks maintained at MGCSC is a series of *waxy1*-linked translocations that are used for mapping unplaced mutants. Over the last decade, new *wx1*-linked translocations have been introduced into this series and are in a conversion program to convert each translocation to the inbred backgrounds M14 and W23. These inbred conversions are then crossed together to produce vigorous F1's to fill seed requests. Below is a summary of the linkage results for some of these stocks. Additional translocation stocks will be tested as time allows.

We report here the positive results of two-point linkage tests with *f1* and *P-ww* for 6 of these new accessions: for *f1-wx1* T1-9's (Tables 1& 2) and for *P1-ww-wx1* T1-9's (Tables 3-6). The linkage tests were set up as modified backcrosses as indicated. These new *wx1* marked translocations have been converted and F1's are now available for distribution. A sixth translocation was found to show no linkage with its appropriate marker stock.

Table 1. ***wx1*** T1-9(8886) (1L.33; 9L.23)
wx29E

A) The Robertson source showed linkage of *wx1* with *f1.*

Two-point linkage data for *f1-wx1* T1-9(8886)
Testcross: [*F1 wx1* T1-9(8886) x *f1 Wx1* N] x *f1 wx1* N

source: Robertson 86-2247-3

Region	Phenotype	No.	Totals
0	Wx f	98	
	wx +	91	189
1	Wx+	10	
	wx f	10	20 / 209

% recombination *f1-wx1*= 9.6+ 2.0

Table 2. ***wx1*** T1-9(4398) (1L.51; 9S.19)
wx29F

A) The Robertson source showed linkage of *wx1* with *f1.*

Two-point linkage data for *f1-wx1* T1-9(4398)
Testcross: [*F1 wx1* T1-9(4398) x *f1 Wx1* N] x *f1 wx1* N

source: Robertson 67-5242-10

Region	Phenotype	No.	Totals
0	Wx f	535	
	wx +	582	1117
1	Wx+	29	
	wx f	26	55 / 1172

% recombination *f1-wx1*= 4.7+ 0.6

Table 3. ***wx1*** **T1-9(8460)** (1S.13; 9L.24)
wx29A

Two-point linkage data for *P1-ww-wx1* T1-9(8460)
Testcross: [*P1-ww Wx1* N x *P1-wr wx1* T1-9(8460)] x *P1-ww wx1* N

source: Robertson 68-7246-4

Region	Phenotype	No.	Totals
0	P1-wr wx	122	
	P1-ww Wx	115	237
1	P1-ww wx	39	
	P1-wr Wx	47	86 / 323

% recombination *P1-ww-wx1*=26.6+2.5

Table 4. ***wx1*** **T1-9(8919)** (1S.21; 9L.20)
wx29B

Two-point linkage data for *P1-ww-wx1* T1-9(8919)
Testcross: [*P1-ww Wx1* N x *P1-wr wx1* T1-9(8919)] x *P1-ww wx1* N

source: Robertson 89-3002-6

Region	Phenotype	No.	Totals
0	P1-wr wx	83	
	P1-ww Wx	75	158
1	P1-ww wx	16	
	P1-wr Wx	20	36 / 194

% recombination *P1-ww-wx1*=18.6+2.8

Table 5. ***wx1*** **T1-9(8129)** (1S.53; 9L.27)
wx29C

Two-point linkage data for *P1-ww-wx1* T1-9(8129)
Testcross: [*P1-ww Wx1* N x *P1-wr wx1* T1-9(8129)] x *P1-ww wx1* N

source: Robertson 67-5354-4

Region	Phenotype	No.	Totals
0	P1-wr wx	315	
	P1-ww Wx	288	603
1	P1-ww wx	13	
	P1-wr Wx	66	79 / 682

% recombination *P1-ww-wx1*=11.6+1.2

Table 6. ***wx1*** **T1-9(024-7)** (1S.71; 9L.13)
wx29D

Two-point linkage data for *P1-ww-wx1* T1-9(024-7)
Testcross: [*P1-ww Wx1* N x *P1-wr wx1* T1-9(024-7)] x *P1-ww wx1* N

source: Robertson 68-7256-1

Region	Phenotype	No.	Totals
0	P1-wr wx	276	
	P1-ww Wx	265	541
1	P1-ww wx	35	
	P1-wr Wx	42	77 / 618

% recombination *P1-ww-wx1*=12.5+1.3

wx1 T8-9(4643) (8S.37; 9L.11)

A) The Robertson sources showed no linkage of *wx1* with *v16.*

Additional new alleles of *pale yellow9* found in viviparous stocks in the Maize COOP phenotype-only collection

--Jackson, JD

This report summarizes allele testing of viviparous stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection (Jackson, MNL 81:32, 2007; Jackson, MNL 82:31, 2008). Here pale kernels linked to the viviparous trait characterized all stocks. They had previously given negative results in tests with *vp9*. Since *y9* is also characterized by pale endosperm and is slightly viviparous, allelism tests were next conducted with this stock. The *y9* stock also gives green to pale green seedlings and plants. This and zebra striping had been noticed previously in the phenotype-only stocks. Crosses were made as follows: [+/*vp**]@ X *y9* and +//+/*vp** X *y9*. Ears were scored for the segregation of pale kernels.

New designations have been assigned to these alleles and these have been placed into the main collection. It is expected that with further sorting and allelism testing of viviparous stocks characterized by phenotype only, additional alleles of *y9* will be discovered. Stocks with this same phenotype that were found to complement *y9* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	allelism test with *y9*	New designation	MGCSC: stock number
*l**-89-90-1552-10	2 positive	*y9*-89-90-1552-10	X07CE
*y1-l**-Funk-84-9	4 positive	*y9*-Funk-84-9	X07CF

Linkage tests of *waxy1* marked reciprocal translocations obtained from the collection of William Findley
--Jackson, JD; Stinard, P

In the collection of A-A translocation stocks maintained at MGCSC is a series of *waxy1*-linked translocations that are used for mapping unplaced mutants. Over the last decade, new *wx1*-linked translocations have been introduced into this series from various Cooperators.

We report here the positive results of two-point linkage tests for three of these new accessions. The linkage tests were set up as modified backcrosses as indicated. These new *wx1* marked translocations have been propagated and are now available for distribution. Additional Findley translocation stocks will be tested as time allows.

Table 1. *wx1* T4-9e (4S.53; 9L.26)

A) The Findley source showed linkage of *wx1* with *su1*.

Two-point linkage data for *su1*-*wx1* T4-9e
Testcross: [*Su1 wx1* T4-9e x *su1 Wx1* N] x *su1 wx1* N

source:2004-1216-3^Findley

Region	Phenotype	No.	Totals
0	+ wx	542	
	su Wx	475	1017
1	+ Wx	146	
	su wx	46	192 / 1209

% recombination *su1*-*wx1*= 15.9 $\pm$ 1.1

Table 2. *wx1* T4-9g (4S.27; 9L.27)

A) The Findley source showed linkage of *wx1* with *su1*.

Two-point linkage data for *su1*-*wx1* T4-9g
Testcross: [*Su1 wx1* T4-9g x *su1 Wx1* N] x *su1 wx1* N

source:2004-1217-3^Findley

Region	Phenotype	No.	Totals
0	+ wx	393	
	su Wx	328	721
1	+ Wx	128	
	su wx	90	218 / 939

% recombination *su1*-*wx1*= 23.2 + 1.4

Table 3. *wx1* T7-9(027-9) (7L.61; 9S.18)

The Findley Source showed linkage of *wx1* with *gl1*.

Two-point linkage data for *gl1*-*wx1* T7-9(027-9).
Testcross: [*Gl1 wx1* T7-9(027-9) x *gl1 Wx1* N] x *gl1 wx1* N

source:2004-1223-1^Findley

Region	Phenotype	No.	Totals
0	Wx gl	633	
	wx +	710	1343
1	Wx +	144	
	wx gl	122	266/1609

% recombination *gl1*-*wx1*= 16.5$\pm$ 0.9

New alleles of *chlorophyll1* found in lemon white endosperm stocks in the Maize COOP phenotype-only collection
--Jackson, JD

This report summarizes allele testing of lemon white endosperm stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here pale kernels linked to pale green or albino seedlings characterized all stocks. Many had previously given negative results in tests with *vp*9, *w3* and *y9*. The *cl1 Clm1-3* stock used in crosses here carries a dominant modifier of *cl1* that allows for viable green plants, making crosses with a homozygous stock possible. Crosses were made as follows: [+/*lw**]@ X *cl1 Clm1-3* or +//+/*lw** X *cl1 Clm1-3*. Ears were scored for the segregation of pale yellow kernels.

New designations have been assigned to these alleles and they have been placed in the main collection. Stocks with this same phenotype that were found to complement *cl1* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	allelism test with *w3*	New designation	MGCS stock number
5910E *lw*-1981-10*	4 positive	*cl1-1981-10*	306L
5912B *lw*-B73*	4 positive	*cl1-B73*	306M
5912F *y-pg*-1981-17*	3 positive	*cl1-1981-17*	306N

Cherry pericarp color confirmed in *R1-r(Ecuador1172) Lc1* stock
--Stinard, PS

Dominant alleles at the *Lc1* locus confer cherry pericarp color in the presence of sunlight or *Pl1* (Bray, MNL 38:134, 1964), a pattern of expression similar to that of the displaced *r1* genes *Sn1* and *Hopi1*. The Maize Genetics Stock Center has been maintaining a line of *Lc1* in coupling with *R1-r(Ecuador1172)* (stock number X19E). However, during the course of its propagation, this line was maintained by crossing to *R1-g* and re-extracting a homozygous *R1-r(Ecuador1172)* isolate. The presence of the linked *Lc1* allele was inferred, but never directly tested. Last winter, we crossed our *R1-r(Ecuador1172) Lc1* stock to an *r1-r B1 Pl1* line in order to test for cherry pericarp expression of *Lc1*. F1's were grown to maturity in our observation nursery last summer and all ears on these plants had deep cherry pericarp, confirming the presence of *Lc1* in the *R1-r(Ecuador1172)* line. We are in the process of checking other Stock Center *Lc1* lines by crossing with *Pl1*.

A new mutable allele of *vp1*
--Stinard, PS

A viviparous kernel mutant with large revertant sectors of aleurone color on a colorless background was found segregating on the selfed ear of a colored aleurone *Spm-w* line (genotype [M14/W22 ACR X *a1 Spm-w Sh2*; *wx1*] selfed). Colored nonviviparous kernels from this ear were planted, and the resulting plants selfed and outcrossed to *vp1-Mc* and *wx1-m8::Spm-I8* testers. This mutant was found to be allelic to *vp1*, and has been named *vp1-Pookie* for the person who identified it. All plants segregating for sectored viviparous kernels segregated for sectored kernels in outcrosses to the *vp1* tester; all such plants also happened to carry *wx1*. However, none of the outcrosses of these same plants to the *wx1-m8* tester segregated for waxy mutable kernels; all waxy kernels were stable, indicating that *Spm* is not responsible for the aleurone sectoring observed in *vp1-Pookie*. Tests will be conducted with receptor alleles for other transposable element systems in order to determine the cause of mutability.

Isolation and characterization of a dominant inhibitor of *Bn1*
--Stinard, PS

For several years, the Maize Genetics Stock Center grew a hybrid between Mo20W and 4Co63 as a *p1-ww* standard for purposes of re-extracting pericarp and cob color traits. Both inbreds have white endosperm due to the presence of *y1* and *Wc1*. How-

ever, we were surprised to observe segregation for a low frequency of dark pale yellow kernels on selfed ears of this standard. The pale yellow kernel trait was tested against *Bn1* (dominant brown aleurone color) and found to be allelic. Tests of the two inbred lines revealed that 4Co63 is *bn1* and Mo20W is *Bn1*, but Mo20W carries a dominant inhibitor of *Bn1*, which explains why Mo20W has white endosperm despite the presence of *Bn1* (in retrospect, Mo20W has kernels that are slightly off-white in comparison with 4Co63). This also explains the observed segregation for pale yellow kernels in the F2 between the two lines: the F1 is heterozygous for both *Bn1* and the inhibitor; *Bn1* expression is observed in the F2 kernels that carry *Bn1*, but do not carry the inhibitor due to independent segregation.

A second white endosperm *y1 Wc1* inbred line, K55, was also found to carry a dominant inhibitor of *Bn1* (although it carries a recessive allele at the *bn1* locus). The inhibitors from both Mo20W and K55 were converted to a B73 inbred background by crossing to a B73 *Ht1* conversion of *y1 Bn1* for seven generations, selecting white endosperm kernels carrying the inhibitor each generation. Both conversions were self-pollinated to homozygosity for the inhibitor and curiously, both homozygous conversions were also found to be homozygous for *Wc1*. This would indicate either that the inhibitor of *Bn1* is tightly linked to *Wc1* in both Mo20W and K55, or that the inhibitor is an allele of *Wc1*. Dominant alleles of *Wc1* are known to reduce the colored carotenoid content of *Y1* endosperms due to overexpression of a carotenoid cleavage dioxygenase (Vogel et al., J. Biol. Chem. 283:11364-11373, 2008; Tan et al., Maize Genetics Conference Abstracts 46:T14, 2004). The nature of the brown pigment that accumulates in the aleurones of *Bn1* kernels is unknown, other than that it is water soluble (Kulkarni, Mich. Acad. Sci. Arts Lett. Papers 6:253-273, 1927). It is conceivable that this pigment may provide a substrate that is degraded by carotenoid cleavage dioxygenase; however, if that is the case, then not all *Wc1* alleles inhibit *Bn1* expression since 4Co63 does not carry an inhibitor of *Bn1* although it carries a dominant *Wc1* allele. In the absence of data supporting the allelism of the inhibitor of *Bn1* with *Wc1*, we have named the locus corresponding to the inhibitor isolated from Mo20W *ibn1* (*inhibitor of Bn1*) and the inhibitor isolated from Mo20W has been named *Ibn1-Mo20W*. Tests of allelism will be made between *Ibn1-Mo20W* and the inhibitor isolated from K55. We are also testing two other isolates of *Wc1* (*Wc1-Wh* and a *Wc1* allele isolated from a PI accession of South American Caragua maize, possibly the source of the dominant white endosperm trait named *Wh* by O. White; Am. J. Bot. 4:396-406, 1917) for the presence of linked inhibitors of *Bn1*.

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Genetic diversity and combining ability of maize landraces from China's Sichuan basin

--Xiang, K; Yang, KC; Pan, GT; Reid, LM; Zhu, XY

Maize landraces are adapted to the specific environmental conditions of their habitats and selection by humans. It is important to systematically evaluate landraces for desired traits and to maintain this genetic diversity for future plant breeding. The objective of this research was to characterize the agronomic and quality traits of 22 maize landraces and select the landraces with important traits and the most potential for future breeding programs.

The 22 landraces selected, on the basis of their origin, agronomic performance and other important characteristics as determined by The Institute of Variety Resources Research, CAAS (1988), for this study included: Hanyuanhongbaogu, Baibaogu, Jinhuanghou, Wenchuanerbai, Rongzhaiyumi, Nuoyumi, Dadudu, Yuzuibaogu, Qiuzi, Daguangyuan, Lengfengwu, Dabaimaya, Junlianhongbaogu, Wuer, Xuesi, Dahuang, Xiaobai, Dababai, Dazhaibaogu, Dabanya, Ganzierbai, and Dazihuang. In the fall of 2006, the 22 landraces were planted in Yuanjiang, Yunnan. Data was recorded on plant height, ear height, total leaf number, ear length, barren-tip length number of rows per ear, kernels per row, kernel depth, grain yield, kernel weight, oil composition, protein and starch content. During flowering, 13 of these races with medium to late maturity ratings and five testers (478, Mo17, 48-2, 9636 and Huangzao4) were chosen to create a diallel cross to examine combining abilities. For each of the landraces, pollen was combined from 30 plants and used to pollinate the five testers in the diallel method of North Carolina Design II (NCII). In the spring of 2007, the resulting 65 F1 crosses were evaluated in Duoying, Ya'an, and grain yield per plant was recorded.

Analysis of variance (ANOVA) was carried out by the DPS2000 method (http://www.chinadps.net). Coefficient of variation (CV) was analyzed among populations by using Microsoft Excel (2003) to determine which had significantly different traits. The CV was computed as: CV = s/x (100%), where x was mean of a trait, 's' was standard deviation. General combining ability (GCA) and specific combining ability (SCA) of grain yield in the 13 landraces were calculated by using the incomplete diallel cross model (Ming et al., 1994; Rong et al., 2003). Heterosis was investigated by analyzing the SCA for grain yield per plant.

ANOVA revealed that significant genotypic variation existed among the 22 landraces for many of the agronomic and quality traits measured (Table 1, online supplemental data). CV of the 12 agronomic traits measured ranged from 5.59% to 32.42%, with an average of 15.78%. Grain yield per plant and traits directly related with grain yield, such as rows per ear, kernels per row, kernel depth, etc., had some of the highest CVs, indicating that the landraces differed more on these traits rather than others such as plant height, leaf number and flowering time. In contrast, the CV of the three quality traits (oil, protein and starch ratio) ranged from 0.91% to 5.64%, with an average of 4.06%, which suggested that variation for quality traits was lower than that for agronomic traits. For each trait, we determined which landrace had the most desired or optimum data for that trait (Table 1, online supplemental data). Three landraces, Dazhaibaogu, Dahuang and Yuzuibaogu, exhibited the best agronomic performance while another three, Nuoyumi, Rongzhaiyumi and Dadudu, had highest oil, protein and starch content, respectively.

Individual ANOVA for grain yield of 65 crosses between 13 landraces and five testers showed that differences in grain yield per plant were significant. The results of the combining ability analysis showed that GCA and SCA effects on grain yield per plant were highly significant among landraces as well as among crosses

(Table 2, online supplemental data). Five landraces (Hanyuan-hongbaogu, Dabaimaya, Xuesi, Dahuang and Dazhaibaogu) had the highest GCA effects (Table 2, supplemental data online), which suggested that these landraces had the greatest potential for future breeding. The CV of GCA effects on grain yield per plant was 48.85%, which indicated that there are more selecting options in future breeding. Several SCA effects on grain yield per plant were also significant (Table 2, supplemental data online). The SCA effects ranged from -18.21 (Dabanya×478) to 27.41 (Xuesi×9636).

In conclusion, Dazhaibaogu, Dahuang, Yuzuibaogu, Xuesi and Dabaimaya were the landraces determined to have the most promising characteristics for further use in maize breeding programs.

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III. AN INTERVIEW WITH EDWARD H. COE, JR.

On July 3, 2009, James Birchler sat down to interview two of his long-time colleagues and fellow maize geneticists, Edward H. Coe, Jr., and M. Gerald Neuffer. The interviews took place in room 219 of Curtis Hall, the building on the Columbia campus of the University of Missouri that has been research home to both Coe and Neuffer for over 55 years and has housed many leaders in plant genetics. These interviews are part of a larger, longer-term project of the Interdisciplinary Plant Group at MU to document some of the history of the diverse plant scientists who have worked, and continue to work, in Curtis Hall.

Edward H. Coe received his bachelor's and master's degrees in the Department of Agronomy and Plant Genetics at the University of Minnesota in 1949 and 1951, respectively. Under the mentorship of Professor John Laughnan, he received his Ph.D. degree in botany at the University of Illinois in 1954. Following a short postdoctoral position at California Institute of Technology with Ernest G. Anderson from 1954-1955, Coe joined the Plant Genetics Unit of the U.S. Department of Agriculture - Agricultural Research Service at MU, where he is currently Professor Emeritus of Plant Sciences.

Coe has had a tremendous influence on the history of maize genetics over the last half century. His research has contributed to our understanding of anthocyanin biosynthesis, gametophyte functions, non-Mendelian inheritance (paramutation at the B locus), and extrachromosomal inheritance (*ij* and *ncs*) (McCormick, 1995). He is also responsible for discovering male sterility in white pollen plants, for demonstrating that gametophytically expressed genes can disrupt precise developmental events during micosporogenesis, and Stock 6 (McCormick, 1995). Coe is author or co-author of 132 refereed journal articles as well as author and/or editor of 2 books. Perhaps most well known among his publications is *Mutants of Maize*, an authoritative reference book on mutant phenotypes in maize co-authored with Neuffer. Coe is also highly regarded for his ongoing service to the larger maize community, which includes twenty-six continuous years as editor of the *Maize Newsletter* (1974–2000). He also played a central role in the establishment of the Maize Genome Database and participated in the early planning meetings that led to sequencing of the first plant genome and, most recently, the maize genome. In recognition of his "lifetime of contributions to the field of genetics," Coe was awarded the distinguished Thomas Hunt Morgan Award by the Genetics Society of America in 1992.

Following are excerpts from Birchler's interview with Coe. Some of the questions and answers have been edited for conciseness and clarity, extraneous material omitted, and footnotes added for elaboration or clarification. Footnotes added by Coe are noted EHC. A future issue of the *Maize Newsletter* will include excerpts from Birchler's interview with Neuffer.

Early Years and Influences

BIRCHLER: I recently read this quote by Craig Venter: “Like so many people who have succeeded in life, I have had some great teachers who encouraged and inspired me, taking a real interest in my education.” Who might you equate to that who was an inspiration to you to get into genetics or maize genetics in your early days?

COE: Very indirectly, George Beadle, but that was because of learning about his work, not direct contact. However, my father-in-law [Albert Longley[1]] worked with maize, and I became acquainted with the interesting variability that he had in his materials and the fascination that he had with solving problems. He informed me of Beadle’s work while I was at Minnesota as an undergraduate student taking genetics[2]. This was in the Department of Agronomy and Plant Genetics, and it was a course taught with a modest genetics text, so nothing of the caliber of biochemical understanding that Beadle had been finding was involved in that course. I shared [Beadle's work] with the teacher. He was quite interested, and he encouraged me. During the time I was at Minnesota, I met Charles Burnham and, similar to my father-in-law, I picked up an interest and became his student as a master’s candidate.

BIRCHLER: I guess I didn’t realize that you knew your father-in-law, Dr. Longley, before you got into maize genetics.

COE: [My parents and I] were living in Virginia, a couple of blocks from [Dr. Longley’s] home.[3] We moved there, and [Mary and I] became acquainted in a personal way…before we were married, of course.

BIRCHLER: So, one might say you married into maize genetics?

COE: That’s right. The only reason I’m in the field is that I chose the right person. [Laughs]

BIRCHLER: How did you get on to Illinois and John Laughnan’s laboratory?

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[1] A. E. Longley, an early maize cytogeneticist who worked for a short time with Ernest G. Anderson at California Institute of Technology on A-bomb maize material (Peterson & Bianchi, 1999, p. 21). Longley and Anderson identified, characterized, and cataloged hundreds of reciprocal translocations in this material and developed the waxy-marked reciprocal translocations (Rhoades, 1984, p. 3).

[2] In the fall of 1947 (EHC).

[3] For more on Coe's early years, see (McCormick, 1995).

COE: After completing my master's, which I did on the pigments of the aleurone tissue (red and purple pigments), I don't recall walking into Burnham and saying "What should I do now?" and I don't recall his saying "You could continue here." He probably did; he was that kind of person — he was a very encouraging person. But [John] Laughnan at that time had just done a study identifying the order of function of three of the genes in the anthocyanin pathway. I thought I would find a sympathetic soul there to go on with what I was doing, which I did. What's interesting is that my first contact, though, was with Marcus Rhoades, who was at Illinois at that time. He hosted me as I went to visit and get acquainted. The [Botany] Department chairman at the time, Oswald Tippo, went with us to the cornfield, so I felt very much shepherded. Tippo, it turned out, even knew my father-in-law's thesis from Harvard, from "Ha'vad." I was really taken that this was a big community of people who were all acquainted and knew what was going on in diverse areas. A very good example of what one should understand as a graduate student.

BIRCHLER: What might you relate as interesting, fun interactions with John Laughnan in your graduate career, as he was your Ph.D. advisor?

COE:[4] Probably the most fun was climbing up on a ladder to pollinate or get pollen from some of the exotic strains that he went to seed banks for from Latin America. These, of course, grew very tall. He had to start them indoors, give them short days, and then transplant them to the field. I helped with that, but the really amusing thing was climbing these 12-13-foot tall plants to pull the tassel, bag it, and take pollen from it. Of course, what he was interested in was some of the exotic alleles of the *a* gene.

Laughnan's office was right next to his lab, where he would come in and out doing some things himself. But he stayed away from the flavonoid pigments, the anthocyanins and their relatives. The next office over was Ellen Dempsey's and then [Marcus] Rhoades. So it was all an interconnected group of labs and offices. In the lab, [Laughnan] worked quite hard doing the chemistry of the carbohydrates, and we had fun with that.

When John discovered the Super Sweet Shrunken-2, I had had the impression from him that he had put a [dry] seed in his mouth and found it was sweet. But after his death, at his funeral, his first wife, Lynette, told me that she was the one who first recognized the taste. But the memorable thing to me was the occasion when Rhoades and Laughnan brought together a group of people from the corn canning industry and were proposing to them that here was a new prospect. John came out of that meeting shaking his head saying, 'They want to know if it will fit in the can.' [Birchler and Coe laugh.]

As a mentor, he was very relaxed. He did not do much more than point out papers to me, asked my opinion on things, and very gentle. Rhoades was rather strict with questions and with feedback. That's an interpretation on my part, but that's the way I found him. Not that he wasn't a nice man. He was a top-notch person. Laughnan was very occupied with sports. He loved sports. And, of course, Rhoades also. John never looked like he was a protégé. That word has been used for him, of Rhoades. I think he learned a lot from Rhoades, but John was already an established scientist and had been teaching at Princeton before he came to Illinois. So protégé is a word that implies more "juniorness" than was true.

BIRCHLER: You continued on with the aleurone pathway in his lab?

COE: Actually it was plant colors that I was working on. And I kind of carried forward what [Laughnan] had been doing, but tried to do some chemistry with it. Even at the time, I was sort of hesitant about the quality of the chemistry. I took organic qualitative analysis and recognized how hard it was at that time to isolate a particular compound and get its melting point, its reaction products, and absorption spectrum and really make sure you know what it is when you've got a mixture to begin with. So I had hesitation at that time that I'm not sure John shared. [Laughs] He was very much excited at the work. What I did was kind of old-fashioned chemistry separations and chromatography. I had already done chromatography at Minnesota. It was only paper chromatography. I got one of these globes — a glass cylinder, maybe a foot across and two feet high, that you could use on the old-fashioned gasoline pump to fill up with gas — from a junkyard, and that made a nice enclosed chamber for the chromatography. I still have that globe in my basement. I used it for studies at Illinois, as well. The chemicals you had to use at that time were pretty interesting, things you wouldn't want to handle today without getting approval — phenol and metacresol, and so on. So I made quite a stink in that lab and was famous for it.

Coming to the University of Missouri

BIRCHLER: How did you transition to the University of Missouri?

COE: I'm not sure who wrote the key letters to Beadle [California Institute of Technology], but I had applied and somebody recommended me. I had a postdoc there starting in the fall of '54. It was to work in the Kerckhoff Laboratory, carrying forward whatever I wanted but also interacting with E. G. Anderson.[5] I was on kind of a schedule, as I recall it. Certain days I'd go out to Arcadia farm, work with Anderson, and other days I'd work indoors. Beadle would drop by the lab every now and then and ask me how things were going. I [also] had great fun at Cal Tech, visiting with and working in [Margaret] Mitchell's lab on *Neurospora*. I think I may have been stimulated to

[4] For more on Coe's memories of J. R. Laughnan and a retrospective of the latter's contributions to genetics, see (Coe, 1995).
[5] For a portrait of E. G. Anderson, see (Rhoades, 1973).

care by John Laughnan's interest in working on *Ustilago*. He'd tried to do some biochemical genetics with the smut fungus while I was at Illinois. Of course, *Neurospora* was the top microbial system at the time, and dissecting asci was the way to get genetics done with *Neurospora*. Margaret Mitchell taught me how to dissect asci. I sat down and I literally got a Mendelizing separation of pairs of ascospores. [E. G. Anderson] would sit me down and talk me over everything that I ought to know, but also we would go through sand bench after sand bench after sand bench of seedlings that he was screening for mutants. He would chuckle — he had this wonderful chuckle — and show me things like blue fluorescent. With the lights on, there was nothing; turn the lights off and a UV lamp [on] and here were these white-looking seedlings, blue-light seedlings. He was much excited with that and rightfully so, [as] it turned out to be a very important biochemical step. He and Howard Teas did the chemistry. After seven months at Cal Tech, I came here, having applied for half of [Lewis J. Stadler's] position, the USDA part.

BIRCHLER: How did that fall into the two positions of USDA and the University with you and Dr. [Gerald] Neuffer?

COE: While I was still at Illinois, Merle Jenkins from USDA talked with me about the possibility of a position here at Missouri. We had a very good conversation. It was middle summer. I was pointed out to him by Laughnan and/or Rhoades, so this was before I went to Cal Tech. Then along in late fall or early winter, probably December-ish, I had a call from Jenkins; I guess that must have been after I came here and interviewed in January. John Laughnan was here, having come to fill Stadler's shoes, and he hosted me. I interviewed, gave a seminar on anthocyanins. Nobody said, why in the world are you doing that? So that went all right. The offer was made. I came here May 1st with a month old baby. [Mary and I] drove across country. Made the mistake of shipping an old couch from Illinois to California and back from California to Missouri; an old beat-up couch. Dumb.

BIRCHLER: So then Dr. Laughnan left shortly thereafter?

COE: Yes, and much to my chagrin. I was hoping that he would stay.

BIRCHLER: Did he know that there was a possibility he might leave again when you interviewed?

COE: I don't recall that he said anything about it at that time. He might have, and I just have forgotten. They made him an offer that I guess he couldn't refuse, which was to chair Botany. As a chairman, following the model of Oswald Tippo, I could see him finding that attractive. Of course, he was very much a research-focused person -- really loved, loved the research and continued it long after, the rest of his life.

The Origins and Story of Stock 6

BIRCHLER: Once you got to Missouri you continued working on pigments, of course, and then found some other things.

COE: Actually I had found some of them before I came here, some of the most key things of my career, if I may judge them. And here's a case where Rhoades deserves some real credit. I had planted a whole lot of the strain I called Stock 6, purple-seeded that I had used for pigment work at Minnesota. Gotten it from Burnham.

BIRCHLER: What does Stock 6 mean?

COE: It's the 6th stock that I got from anyone. My 6th accession.

BIRCHLER: So Stock 6, which is now of course a well-known stock, got its name because it was the 6th stock that Ed Coe had in his collection?

COE: Yes. And it was. It was Burnham's number something or other and five digits that he traced back to when he first got it. I actually copied off his accession record.

So at Illinois, in maybe my second summer, I planted a lot of that stock and a lot of an F1 where it was a female parent and strangely saw a lot of runty plants -- small, short, and they turned out to be sterile. Not only in the Stock 6 planting, but among the plants in the crosses. I thought that was funny. I thought, 'Well, I wonder if those are haploids.' Rhoades said, 'Well, why don't you dig up some roots from them?' [Laughs] So I did. And lo and behold, they were haploid plants. So that stock, as you know, [has] become now the initial source of haploid-inducing capability.[6] Now, commercially, the major companies and a lot of minor ones are actually producing inbred lines very rapidly by using derivatives of Stock 6 and strains also from Eastern Europe that had had the same property and combining them.

[6] The discovery of the properties of Stock 6 was pure serendipity; there was no "intent-to-discover" in these plantings (EHC).

BIRCHLER: Did you have any advisory role in this development of the use of Stock 6 and its derivatives?

COE: No. There was a long lag in serious interest. Of course, I was never patent-oriented. Had I been contacted by companies early, I might have been a little hesitant, as a federal employee, to do much more than give public advice. This discovery was maybe in '53 or '54; '54, I bet. And there were a few contacts probably as early as the late 70s.[7] Actually Garst Seeds, later ICI Seeds, asked me to come up to Iowa and confer with them and see whether I saw any ideas of what to do. But they didn't get very far into it until Ming-Tang Chang joined that company. Ming-Tang picked up the ability to select for higher rates, and of course, used genetic tools to do it. I did have fun with the people that I tried to consult with, but it was always as a public consultant. I thought at the time that they were not even sure that they were going in that direction. I don't think they did for a long time. So it wasn't until maybe 1990, somewhere in there, I began to realize that in France they were producing inbred lines that way and had been doing it in Eastern Europe and the Soviet Union.

BIRCHLER: Do you care to speculate on how Stock 6 works?

COE: Speculate? Here, the expert is asking me the question. [Laughs] I've always thought it had to do with something unusual about the pollen grains, the individual pollen grains, but except for some hints out of the work of Chang, I'm not sure the evidence is clear. I know Akio Kato has done this and tried to understand it. If it's been solved, I'm not aware of it.

BIRCHLER: I'm not either, but I thought you might have thought about it since you discovered it. [Laughs]

COE: The reason for growing out those hybrids in particular was to try to use chemical mutagenesis to produce mutations. Rhoades laughed when he saw that I had tipped plants over in the greenhouse (the male parent), stripped the tassel back, and dumped the tassel into a formaldehyde solution. Formaldehyde had been studied in *Drosophila* as a mutagen. I took pollen that developed and made the crosses, looking for anthocyanin mutants.

BIRCHLER: Did you find any?

COE: No. Not in the treatment. I had controls, and the controls had two things. One of them was *c2*. This new anthocyanin factor in fact turned out to have a semi-duplicate[8]. I did find a mutation of *a2*, but it was confounded. The second thing was a curious plant color change. This was sun red material. A few plants suddenly were more pale than their siblings. Not many. Not a segregation. And from there I did crosses and discovered that this was a phenomenon of paramutation.

BIRCHLER: So that traces back to experiments that you did in Illinois?

COE: Yes. Almost all of my fun of my career goes back to graduate student times at Illinois.

BIRCHLER: The early stuff.

COE: The early stuff. That chemical mutagenesis was something I really thought had a prospect, and so it was quite exciting when Gerry Neuffer here got into that.

BIRCHLER: When you first found those plants that had reduced pigment, what was your first thought?

COE: Well, I guess I better cross these and do the Mendelian test.

BIRCHLER: To see if they were contaminants?

COE: Actually, they were so much like the siblings in form, in flowering time, and so forth, that I found it hard to believe they could be contaminants, but, yes, that would always be in the back of your mind as something that might have happened. But I also thought, well, maybe there is spontaneous change.

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[7] Sherret Chase had published in 1947 and subsequently, on haploid selection work, in DeKalb seed company. I communicated with him and, sent seed to him; he advised me on selective markers (EHC).

[8] Not only was the c2 mutation discovered serendipitously, but so was the semi-duplicate factor. This factor, *whp1* (white pollen), pre-existing as a recessive allele in certain lines, turned out to be responsible for the same step as *c2*, duplicate to it in biochemical function, chalcone synthetase, the first step in forming the 15-carbon skeleton of flavonoids. However, the two factors show markedly different regulation: activity of both is required for yellow pigmentation in the pollen and for limited anthocyanin formation in the margins of leaf sheaths, but activity of c2 is sufficient for anthocyanin formation in the aleurone tissue. The recessive *whp1* would not have been found except for a strategy to convert each of the anthocyanin recessives to uniform lines (in this case, the inbred K55). The purpose was to derive uniform stocks, and to prepare vigorous hybrids between these stocks (EHC).

BIRCHLER: So in that experiment, you were looking for some kind of change. And you were looking for loss of function of anthocyanin, right?

COE: That's right. Yeah[9].

BIRCHLER: So your mind was poised to find something. This undoubtedly has been in the materials of people going back to early days.

COE: I suppose. It's very curious. The strains of purple plant and sun red plant that [Barbara] McClintock worked with and others at Cornell worked with, those strains were never reported to be inconstant. I frankly have had a suspicion — and I've raised this with people working with it — that possibly there is a cause that is environmental. Maybe in hot conditions, there is a system by which there is a mechanism of protection—cut down on the amount of pigment. This is a winging, but since it had never happened out east where [the weather is] moderate and suddenly it began to happen. It ended up that I could hardly maintain the stock. I've been puzzling about how come [I was] having trouble and no one else did before. Karen Cone. thinks it's because what I did was cross them with a different allele of a modifier of paramutation, *mop1,* which resulted in the potential for a change. She's probably right.[10]

The *b* Gene

BIRCHLER: You continued working on the *b* gene when you came here to Missouri, and that was eventually published where?

COE: In *Proceedings of the National Academy*.

BIRCHLER: Was that where you initially submitted the paper?

COE: Oh, yes. And I asked Rhoades if he would communicate it, and he did, very graciously.

BIRCHLER: What was the reception to this conversion of a wild-type allele to a mutant effect?

COE: I thought people were very tolerant of an abnormality. [Laughs] I have a feeling that it could have been because [R. A.] Brink was already reporting similar phenomena in another gene.

BIRCHLER: At what point did you understand the relationship [between Brink's finding and your own], or did you think they were different at first?

COE: At first I thought this was one member of a spectrum of possibilities, so it was the same kind of phenomenon. I deliberately called it a conversion-type phenomenon. So, yes, I recognized [the relationship] right away, and unless I'm mistaken, I cited Brink's work. I think [Brink] treated the phenomenon as less within a spectrum than I did. But the differences were that the *b* system was final. Once it happened, it didn't change back, and it constantly changed in every new exposure, while the *r* phenomenon showed a drift back. There were some experiments that I set up at one time to do with *r*, but I had such trouble growing that particular line, W22, and getting good seed production that I finally gave it up. I wanted to see about this reversion back, whether it was something that could be considered to be predictable or not.

Clonal Analysis and Development

BIRCHLER: You also did a lot of stuff on clonal analysis and development. How did you get into those particular areas?

COE: I suppose that was driven by my interest in the paramutation phenomenon. I wanted to know whether the *b* gene was changed immediately on fertilization or over time during the growth of the plant. So I tried some experiments with that and was not very successful, although I satisfied myself that it was changed promptly. I argued at one time that it wasn't, but I'm not so sure about that now. I saw these sectors and it dawned on me, 'Oh, I'm treating pollen and I'm even getting sectors from pollen treatments.' Gerry [Neuffer] also knew this. So whatever mutation, whatever change is happening, is not consummated out of the haploid sperm until there's at least one division. If you treat seeds, you often get multiple sectors. Gerry and I analyzed data from an initial experiment, and I suddenly realized I could figure out how many cells there are and extend [Otto] Stein and [Dale] Steffensen's anatomical studies. Actually Steffensen had already done experiments of this kind, so I could expand on that. Happily, along came Scott Poethig, who conducted precise experiments as a postdoc. Even further study was done by Man Mohan Johri, who had done some physiological studies in Joe Varner's lab at Wash U,

[9] Serendipity redux (EHC).
[10] The fact that regulatory loci in maize, in contrast to structural loci, have been found to show paramutation is intriguing and may bear study (EHC).

and he came here on a visiting scientist arrangement. Although Gerry and I had already published the initial work on the structure of the meristem in the seed, he picked up and examined the effect of sectoring on mutants, in particular, but, at the same time, expanding on the proliferation of cell lineages. That was probably as much fun as anything I've ever, ever done—walking through a field and recognizing the implications of sectors and quantifying them so that you could then put a number on how often this happens versus that.

BIRCHLER: How did you get into re-examining, if you will, *iojap*?

COE: That was curious. I had crossed *iojap* to different lines to try to see if I could get a constant phenotype, because in the original lines, it was kind of irregular.

BIRCHLER: Was this at all connected with your interest in clonal analysis or was this independent?

COE: No. That came separately. I was motivated by the cytoplasmic inheritance side[11]. I think that's how I originally got started with *iojap* and discovered I wasn't getting the maternal white seedlings that had been reported. I contacted Earl Patterson at the stock center, and he said, 'Oh, you need to use Ohio 51 A as the male parent, the normal parent.' Well, that led me to think I had better get the female parent constant because it was in 'whatever' background. So I crossed it to four different lines. And the first generation I picked out the expressing seedlings and backcrossed them and pretty soon realized that I was getting four *entirely* different phenotypes. Some of them (Oh51A and Tr) showed sectors of reversion. I kind of gave up the idea of exploring further the number of chloroplasts — that was my motivation in trying to do the *iojap* [experiment], to find out from sectorial maternal seedlings. I gave up that idea and went instead into the sectoring. I had a very good graduate student at the time, Chang-deok Han. He started working with it [and then] went to Cold Spring Harbor and cloned the gene. It turned out there was a transposon in the gene. [Han] had already discovered what the defect was in his thesis — the ribosomes were lost in the *iojap* white tissues — and then he studied two other albino seedlings and did the analytical biochemistry on those.[12]

From Gene Lists to the Maize Database

BIRCHLER: Eventually you moved on to other things.

COE: I'm trying to remember what else was exciting. [Laughs] Well, I got deeply involved, especially working with Gerry Neuffer, on compiling gene lists and maps. The motivation of that is not clerical. The motivation is that we, including individuals like myself, won't really understand the system unless we have it systematized. Gerry [Neuffer] was already working pretty hard on compiling, and we partnered and just advanced it together.

BIRCHLER: In the *Maize Newsletter* in those days, you would always contribute a gene list. So that was the embodiment of this work that you initiated with Gerry?

COE: That's right.

BIRCHLER: And then that grew into taking over the *Maize Newsletter*?

COE: Yeah.

BIRCHLER: And that then grew into database analysis and the documentation of maize. And so then the whole Maize Database sort of grew out of documenting the gene list that you started?

COE: That's true. I think that justified it.[13] I have to say though that my motivation goes all the way back to the applied potential. I was not raised on a farm, so I don't have a good justification for feeling the need for improvement of the crop from the ground up. But I certainly have consciousness of a social need. I saw people, like Marcus [Zuber], working so hard to keep people together who were doing crop improvement, right here at Missouri and others. Zuber was really the "yeaster" of corn breeding, and I think he was recognized as [such] by the people in that community. But outside of that, they and geneticists and cytogeneticists were not interacting that much. So my motivation was to try to pull together what was available to both kinds of motivation, kinds of interest. For example, to pull together the report by someone on the resistance to some disease in a strain from Peru or what have you, to get that literature documented, and, if there was genetic information, to document that it had a name and description and perhaps chromosome location or reference attached to

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[11] Rhoades reported maternal progeny that were sectorial, which implied transmission of two or more plastid primordia through the egg, and could open a way to estimate the number of plastid primordia (EHC).

[12] The biochemical and molecular research was conducted in the laboratory of Mary (Polacco) Schaeffer, with her guidance (EHC).

[13] The compiled genetic data and references were readily migrated into the Maize Database, providing an early and efficient resource (EHC).

it. That was the thing that motivated me on the gene listing work and really motivated me deeply by the time the database came along in 1991. That had to become possible for quantitative trait characterization to be documented and the big next step of actual mapping of where one or more genes are responsible for the differential trait between different lines. [While that potential is still] a long way away, [there has to be] a database to provide the tools of access and the data compilations to make it possible for somebody who is really targeting on [a trait] to have all the information they need in front of them. Unfortunately, the literature compilation, the curation of literature, that was necessary to develop that had to stop. There weren't enough resources for it. There was not enough enthusiasm for doing it among people who might be surprised at how much they'd benefit. When it comes to the corn growers, that's what they keep telling corn research people: we need this capability to study traits, to find out how they work, what genes are controlling them. People are doing these studies, but they aren't being pulled together into a synthesis. And that [is what has] driven me from before 1991—to try to find ways to make that happen. It sounds grandiose, but it's still what motivates me.

Plant Genome Project

BIRCHLER: You also played a role in the start of the plant genome project. Can you tell us how that came about?

COE: As we got the database started, we had an opportunity or a request from an informatics group at [Washington University] to come over and tell us what they can do to help us make a database. Well, we already had a database, and we were actually online with Gopher before the human genome database was on. We went over there with the computer, and we were able to call up the database and walk them through all of the compiled information. They were quite impressed. In the back of the room was a tall fellow from the National Corn Growers, Bob Mustell. He said, after this presentation, the most important thing we could do was sequence the corn genome. That wasn't the only time that came up. [The USDA and National Science Foundation] had called together a meeting that involved a number of experts in the sequencing field. Ron Phillips was there and a number of other folks. Charles Cantor, a human geneticist who participated in the meeting, said 'You know, you people ought to sequence the corn genome.' The whole atmosphere was one of crop improvement and basic science combination. Soon after the Wash U meeting, Bob Mustell contacted me and said, 'Let's get together.' I may have been an initiator in that as well. Around the same time, someone had talked at the Plant and Animal Genome Meeting, and made it sound very simple and cheap to sequence a genome. And several of us from the corn community went up to talk to him after that, and he seemed to just wave his hands and sort of say it could be done. So all of this began to ferment, and pretty soon I was having visits probably every couple of weeks from one or more people from Corn Growers coming over from St. Louis, including Jim McLaren and Kellye Eversole. Of course, this was the National Corn Growers, not just the state. But it is true that Gary Marshall was, at that time, the president of the National Corn Growers Association, and he was a Missourian, so this helped. I called together a meeting in St. Louis that included mostly people with corn genetics or breeding interest – Ron Phillips, Charlie Stuber, seven or eight other people with interest. If there was someone from the Corn Growers there, I can't recall. I think Kathy [Newton] went. The Corn Growers began to work with Kit Bond to push the 'corn initiative.' Pretty soon, the plant physiologists were up in arms, and so were some of the other crop organizations. The next thing I knew, the National Corn Growers had gotten together all of the stakeholders for all other crops, except rice, and they all were on board. And [the American Society of Plant Biologists] was celebrating that they had achieved a miracle. [Laughs] And they had. It came into Congress and flew. The request for proposals came out before 1998. And a group of us applied. I was P.I., with Rod Wing, then at Clemson, and Andrew Paterson at Georgia. And we won the biggest research grant ever won at MU at the time.[14]

BIRCHLER: The maize genome sequence is to be published any day now I suppose. That must be gratifying.

COE: A lot is happening. It's exciting.

Important Questions or Problems in Genetics

BIRCHLER: What do you think are important questions in genetics — maize genetics — that need to be pursued or knotty problems that can be addressed that you would think would be interesting directions?

COE: If I had to put number one, it would be genetic control of productivity traits. That's a much, much more broad thing than the two words cover because it goes all the way from root expansion and root physiology to stalk strength to resistance to X and Y and Z and abiotic stresses, as well. All of these things are the complex of productivity. I'm not about to do what has tended to be done by people not familiar with plant breeding [and say,] 'Well, we saw this stress resistance thing and add it as a gene to the crop and everything will be fine.' The thing is that farmers know very well it may rain this year and it may not rain this year. There are just scores and scores of circumstances that farmers have to deal with, that the plants have to deal with. The word *stability* is often used for the problem. The crop has to be stable in production, not necessarily always the highest it could be in certain circumstance, but always reliable. On the other hand, you do want to get 400 bushels of corn if it's possible. That's number one on my list.

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[14] For a published chronology of these events, see (Coe and Schaeffer, 2005).

Number two involves a lot of the pathway biochemistry and regulation. It's really a shame that we know so much more about the biochemical pathways and their regulation in species like Arabidopsis. It's a shame we don't know every last step and every last enzyme that controls it and the alternative ways that the biochemical system works and is regulated. And we have to so often turn to work that's been done in everything from humans to rats to mice to Arabidopsis to *Neurospora* to *E. coli* and *Bacillus subtilis* in order to *suppose* that we've got a handle on it. One of the things that's quite interesting to me [is that] in order to draw conclusions, [to] say 'Yes, this controls this step, this function, this sequence,' it's been necessary not only to have evidence of an EST being produced, but the most ideal thing is to have a mutation in yeast or coli that this sequence from corn put into that species replaces a function that is missing in the other one. So it literally is made in the plant and it literally accomplishes the purpose in a counterpart system. I thought that's been a fascinating way of deciding [whether] this really is or is not really a function because lacking that kind of thing you can wave your hands, but otherwise you have to do it within the species. And that's great, but it, it's quicker and cleaner the other way.[15] But those two things are probably top of my concern — to get us traits and the biochemistry and physiology really in our hands.

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[15] Most recently, it has become more nearly routine, and even more definitive, to transform maize plants with the candidate gene from maize itself, or with its antagonist (EHC).

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13,185 seed samples have been supplied in response to 299 requests for 2009. These include 85 requests received from 24 foreign countries. Popular stock requests include the NAM RIL populations, Hi-II lines, *ig1* lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, transposable element lines (including the UniforMu sequence indexed stocks), and Maize Inflorescence Project EMS lines.

Approximately 5.7 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. A cool, wet spring necessitated the planting of our crossing nurseries about two weeks behind schedule. Smaller than normal stands were observed in our first crossing nursery due to soil compaction caused by heavy rains. Seedling predation by redwing blackbirds reduced the stands in our second crossing nursery. Despite the reduced stands, there were sufficient plants for an adequate increase in most instances. Seasonable to above normal rainfall patterns were established, and we had a normal pollination season without the need for supplemental irrigation. Moderate temperatures and low plant stress during and following pollination resulted in excellent yields.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of James Brewbaker (Hi27 near-isogenic mutant lines), Wayne Carlson (various Chromosome 9/B Chromosome rearrangements), Tom Brutnell (*bsd2-m1*), Jerry Kermicle (various *r1* alleles), Jane Langdale (various photosynthetic and developmental mutants), Gerry Neuffer (recent EMS-induced mutants), Marcus Rhoades (*DfK10(I)*), Peter Rogowsky (*bt2-H2328*), Pat Schnable (*rth3-3*), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.
2. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We identified additional alleles of *pink scutelum1, viviparous1, viviparous2, chlorophyll1*, and *pale yellow9*. In 2010, we plan to continue testing additional members of the viviparous and pale endosperm classes of mutants. In this manner, we hope to incorporate more stocks from our vast collection of unplaced uncharacterized (phenotype-only) mutants into the main collection.
3. Occasionally, requestors bring to our attention stocks that do not carry the traits they are purported to carry. We devote field space each year to analyzing these stocks, fixing or enhancing those we can, and soliciting replacements from researchers for those we can't. In those rare instances in which a particular variation or combination of variations cannot be recovered, we modify our catalog to reflect this.
4. We are continuing to characterize the *Enr* (*Fcu*) system of *r1* aleurone color enhancers as well as other factors that affect expression of *r1*. We are characterizing additional alleles of *Enr1* and other *r1* aleurone color enhancers.
5. Samples of 486 phenotype–only stocks were sent to the National Center for Genetic Resources Preservation in Fort Collins, Colorado for back up. 655 stocks from the main collection not yet backed up were also sent this year. Our new inventory system has made selecting ears to be sent and producing a packing list to accompany them a much more efficient procedure.
6. Despite reduced stands, fresh increases were made of many older A-A translocation stocks. The outcrosses will be grown out in 2010 Observation to confirm by seed set which ones actually carry the translocation. We continued checking translocations received from W. R. Findley and Don Robertson marked with *wx*1 to confirm the chromosome arms involved. For those where we found no linkage, all sources were discarded.
7. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0110189) were grown again this summer. Approximately 300 families of M2 materials that were produced in 2006 and 2007 were grown to increase seed supplies and recover previously observed mutations. Also, 1,642 families of 2007 and 2008 EMS seed increase materials were grown for adult plant observation; the materials observed include mutated B73 and Mo17 inbred lines and the B73xMo17 hybrid.

We did not have sufficient funds in our budget to grow a winter crop in Puerto Rico during the 2008/2009 growing season; however, this year we are growing a winter nursery of 0.5 acres at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico. This may be the last year we have sufficient funds for a winter nursery.

Our IT Specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our

collection. The tools are also used for our internal database (e.g., inventory, pedigrees and requests). A tool to find and correct parent family information in our pedigree data was written, tested and used successfully. Currently, a more advanced search tool is being written in order to allow more flexibility in locating specific items in our inventory. Improvements have been made to the pedigree input tool in order to fix some problems with certain types of entries. Maintenance continues on our web site (http://www.uiuc.edu/ph/www/maize).

The new greenhouse space in Urbana is being used for our fourth winter crop. The space has proven to be excellent for growing material that doesn't do well under our field conditions. Our new seed storage space presently has 990 seed storage drawers of the 1,584 the room will eventually hold (pending funding).

*NOTE: A list of new stocks available since MNL83 is at http://curation.maizegdb.org/cgi-bin/stockcatalog.cgi?limit=2010. The full list of new stocks available will be printed in MNL85.

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V. MAIZE GENOME DATABASE

www.maizegdb.org

Over the course of 2009 -10 we focused on implementing and improving tools and interfaces to represent the B73 reference genome assembly, which was published in November 2009 under the name B73 RefGen_v1 (Schnable et al., Science 326:1112-1115, 2009). Our efforts necessarily included the careful integration of genetic data with sequence. The centerpiece of our transition to represent the maize genome from a sequence-centric paradigm is the deployment of the MaizeGDB Genome Browser (Sen et al., Database (Oxford) 2009:bap020, 2009), which is available online at http://gbrowse.maizegdb.org and from links throughout the MaizeGDB website. In Nov 2009, two versions of the browser were available: a B73 BAC-based, pre-pseudomolecule view and the B73 RefGen_v1 browser. We encouraged maize researchers to align their data to the pseudomolecule, and provide data to MaizeGDB, so as to coincide with the formal release of the B73 RefGen_v1 assembly by the Maize Genome Sequencing Project (maizesequence.org) with the publication of their paper in November of 2009. Tracks on the B73 RefGen_v1 browser included: centromere regions and anit-CENH3 ChiP data from the Jiang/Presting groups; MAGIs aligned by the Schnable lab; ESTs, cDNAs and Ac/Ds insertions (of Vollbrecht/Brutnell) aligned by PlantGDB; and MIPS repeats and filtered gene sets from MaizeSequence.org. The quality of the filtered gene set from maizesequence.org is now shown as a color-coded track, served via DAS from PlantGDB, as is a track showing community annotations of gene structure. Over 2010, we added other community tracks: IBM 2008 Neighbors (Arizona Genome Institute AGI), ISU integrated IBM markers from the Schnable lab, UniformMu, and PLEXdb microarray probes (via PlantGDB). A third version, the B73 RefGen_v2, was released in March of 2010 by the Arizona Genomics Institute (AGI). RefGen_v2 was available via the MaizeGDB Genome Browser in May of 2010 and is the current default view of the genome at MaizeGDB. Tutorial videos outlining how to use the MaizeGDB Genome Browser and other items at MaizeGDB are available from the front page via links to "MaizeGDB Tutorials" and directly at http://outreach.maizegdb.org/.

The Locus Lookup Tool is key tool for integrating genetic data with the B73 reference genome sequence (Andorf et al. 2010), and has been upgraded to allow for more flexible searching. This tool finds pseudomolecule regions for genetically mapped loci, or pairs of loci, which may not yet be annotated to coordinates on the pseudomolecule. The tool works by (1) checking physical map coordinates to find out whether the locus is already placed. If so, the physically mapped locus is highlighted in red in the region returned within the browser window. If not, the tool (2) checks the locus record at MaizeGDB to find out if any BACs are known to detect the locus and that BAC is returned within its genomic context. If not, (3) genetically mapped probes that are nearest the input locus are identified, the tool checks whether those probes have known genomic coordinates (working outward until appropriate probes are identified) and finally the region of the genome contained by the identified probes is reported with bounding probes shown in red. The tool is an integral part of the MaizeGDB Genome Browser, and can be implemented for other GBrowse instances deployed at other sites and supportind data for other species. In addition we have implemented updates to several other interfaces including: BLAST, cDNA, EST, BAC, and Mo17 SNP records. The Mo17 SNP pages include a detailed view of sequence centered on a specified Mo17 SNP. This region shows any nearby insertions, deletions, or substitutions and the user can change the region size to 81, 241,401, or 561 base pairs as needed. An integrated tool also 'predicts' the Mo17 sequence for the specified region.

The POPcorn resource (http://popcorn.maizegdb.org) has continued to develop. We provide access to 104 projects and 132 resources, searchable by category (including, e.g., sequencing, mapping, mutation, bioinformatics, and breeding). Listings are regularly updated by MaizeGDB and POPcorn staff. In addition, project PI's can submit new projects and to correct project information directly. Participating projects that support BLAST are searchable by two utilities: POPcorn BLAST, which permits researchers to BLAST against maize sequence at multiple sites, and the Sequence-Indexed Data Search, which carries out multi-step searches of sequence-indexed data. Prototype versions of POPcorn BLAST and the Sequence-Indexed Data Search were released in February of 2010. Sites available to POPcorn BLAST include: GRASSIUS, MAGIs, MaizeGDB, PalomeroToluqueño.org, PlantGDB, PlexDB, and NCBI. Examples of queries supported by the Sequence-Indexed Data Search include mutant seed stocks from the Ac/Ds. PML, Mu insertions, TILLING, and UniformMu projects as well as more complex queries, such as phenotypes associated with sequences similar to the query. At this time, the latter query returns phenotypes, and various alleles/variations associated with each phenotype, often from more than one gene. To document provenance of the data and avoid poor interpretation of the results, POPcorn collects attribution and citation information, links to collaborators' sites, and displays short descriptions prominently on all POPcorn pages where collaborator data and project descriptions are made available.

Our focus on data curation has been reinvigorated with: (1) further development of the 'maize gene review', first released in 2009 as part of the MNL, and accessible online at http://www.maizegenereview.org; (2) the addition of two new personnel – Lou Butler, who entered much of the original data and images from the M. Gerry Neuffer collection into MaizeDB and assisted MGN in the preparation of Mutants of Maize, and Jack Gardiner to integrate CIMMYT data, as well as GRASSIUS and UniformMu resources. The quality of existing mutant images is being assessed by MGN and LB, and where image quality is determined to be inferior, new images are being generated. We are using Jack's expertise with the Maize Microarray Project (Arizona) to begin integrating sequence-based gene expression datatypes, including both microarray and RNA_seq, and others as they are made available. Literature curation is largely based upon recommendations from the Editorial Board input, which averages 5 articles/month, with additional inputs from community curator, Ed Coe and requests from the community. We have just begun a new collaboration with Truman State University to provide Gene Ontology (GO) annotation for interesting genes as revealed by the SAM (shoot apical meristem) Project (see also http://sam2.truman.edu/index.html for more information).

Outreach has been greatly enhanced by several a new tutorials on both the use of MaizeGDB, and the understanding of new datatypes for maize. The list of tutorials includes 4 focused on the B73 sequence: MaizeGDB Genome Browser; How was each B73 BAC sequenced; BAC based B73 Genome Assembly Cavets; and All about B73 RefGen_v1. These are prominently featured on the homepage, next to our RSS feed and Facebook links. The tutorial page also provides links to other tutorials of interest to maize researchers. We continue to provide on-site personal tutorials on request by locations with several maize research groups.

We have made a major upgrade to our infrastructure by implementing a virtual server environment. This aligns with Executive Order 13423: Strengthening Federal Environmental, Energy and Transportation Management, sec. 2(h); sec.3(a and f) by improving energy efficiencies and extending computer replacement lifecycles, by reducing the number of physical computers, while maximizing existing hardware capacity. The virtual servers interact with each other and users just like physical servers, without dedicating new of specified hardware to a specific purpose. New virtual servers allow the testing of new operating systems, software patches and application interactions with the ability to roll changes out of the testing procedure without starting over. The virtual environment is made up of three identical servers; each acts as host and can pick up services provided by a failing host, with priority given to production virtual servers

Noteworthy: Lawrence, Sen, Andorf and Campbell were members of the group awarded the USDA-ARS Midwest Area's Outreach, Diversity and Equal Opportunity Award in 2009 for Outreach to American Indians (led by C. Lawrence and C Gardner; funded by the NSF). Campbell was awarded the USDA-ARS Midwest Area's Research Support Award in 2009.

Publications 2009, 2010

Sen, TZ; Andorf, CM; Schaeffer, ML; Harper, LC; Sparks, ME; Duvick, J; Brendel, VP; Cannon, E; Campbell, DA; Lawrence, CJ (2009) MaizeGDB becomes 'sequence-centric'. Database (Oxford) 2009:bap020.

Sen, TZ; Harper, LC; Schaeffer, ML; Andorf, CM; Seigfried, TE; Campbell, DA; Lawrence, CJ (2010) Choosing a genome browser for a Model Organism Database: surveying the maize community. Database (Oxford) 2010:baq007

Andorf, CM; Lawrence, CJ; Harper, LC; Schaeffer, ML; Campbell, DA; Sen, TZ. (2010) The Locus Lookup tool at MaizeGDB: identification of genomic regions in maize by integrating sequence information with physical and genetic maps. Bioinformatics 26:434-6.

Acknowledgements

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Submitted by the MaizeGDB Team: Schaeffer, ML; Andorf, CM; Birkett, S; Braun, B; Campbell, DA; Cannon, E; Gardiner, J; Harper, LS; Sen, TZ; and Lawrence, CJ

VI. MAIZE GENETICS EXECUTIVE COMMITTEE MGEC SURVEY OVERVIEW

Overview of the Maize Genetics Executive Committee report, 2010 Maize Genetics Conference, Riva del Garda, Italy

Reformatted by the editors from the slide presentation presented at National Corn Growers Association workshop April 2010 http://www.ncga.com/presentations. See also the MaizeGDB website for the MGEC for the original presentation.

Current Elected Postions in the MGEC: Tom Brutnell 2011 (Chair); Marty Sachs 2010; Virginia Walbot 2011; William Tracy 2012; Sue Wessler 2012; Jeff Bennetzen 2013; Becky Boston 2013.
Apppointed positions (2 year terms): *Small college/university representative*, Brent Buckner (Truman State Univ) 2012*; Latin American representative*, Jean-Philippe Vielle-Calzada 2011; *European representative,* Roberto Tuberosa 2012; *Asian representative*, JinSheng Lai 2013.

Actions that Increase Visibility of Maize Genetics

- •Develop press-worthy summaries from community to highlight recent advances in maize genetics (e.g. completion of the maize genome, high provitamin A corn, outreach efforts)
- •Arrange formal visits to Congress/funding agencies to inform politicians and program directors of the exciting advances in maize genetics
- •Work more closely with NCGA to identify common goals and share materials (one-pagers) that could help increase funding to maize genetics research.

Future Directions

- •Conducted a survey of Maize Genetics Community to identify research and outreach needs
- •149 respondents = about 10% of the community.

What resources and tools are important for maize genetics

- •Generate sequence annotation of high quality
- •Improve reverse genetics resources
- •Improve assemblies of B73 genome
- •Improve maize transformation in multiple backgrounds
- •Increase bioinformatics and computational resources and tools
- •Support for training in maize genetics, genomics and bioinformatics should increase

What would be a valuable strategy for handling annotation?

- •Combination of different types of curatorial efforts
- •Interoperability among dispersed sites
- •Decentralized annotation where individual groups or teams contribute

What would drive a successful annotation effort?

- •Some form of centralized coordination (via MaizeGDB), but driven by community
- •Needs to include structural features of genes (e.g. introns, exons, splice variants) and link to a single reference genome (e.g. A.thaliana vs. rice)
- •Decentralized annotation where individual groups or teams contribute

What are the priorities for MaizeGDB and the Stock Center?

- •MaizeGDB should continue to develop an effective genome browser as a major focus
- •Scope of the data currently made available through MaizeGDB should be expanded
- •Seed curation and distribution is an essential need for the maize research and breeding communities

Specific suggestions for crop improvement and increased international cooperation.

- •What are the top three resources needed to specifically improve corn as a crop?
- •Please make suggestions for programs that would increase opportunities for international cooperation among maize researchers
- •Enhancing marker-assisted selection
- •Fine-mapping QTL/positional cloning
- •More efficient exploitation of genetic diversity

Provide specific examples of how maize genome sequencing has or will directly impact crop improvement.

•Resequencing (cultivars, expression data, SNPs)
•Phenotyping standards and repositories
•Reverse genetics resources
•Transformation for multiple inbreds
•A well-supported Maize GDB
•Students

What are the top three resources needed to specifically improve corn as a crop?

•International annotation effort
•Coordinate international efforts at funding-agency level (e.g. joint USDA-NSF program)
•Engage China (sequencing at BGI, maize meeting) for more joint ventures
•More international exchange of students/postdocs esp. with developing countries

Please make suggestions for programs that would increase opportunities for international cooperation among maize researchers.

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b-70 4
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This newsletter shares current research on genetics, cytogenetics, molecular biology, and genomics of maize. Information is shared by Cooperators with the understanding that it will not be used in publications without their specific consent.

Send your notes for the 2012 Maize Genetics Cooperation Newsletter now, anytime before Mar 31, 2012. Your MNL Notes will go on the Web verbatim, promptly, and will be prepared for printing in the annual issue. Be concise, not formal, but include specific data, tables, observations and methods. Notes that require extensive editing will be returned. Check MaizeGDB for the most current information on submission of notes. Send your notes as attachments or as the text of an email addressed to MaizeNewsletter@missouri.edu (we will acknowledge receipt, and will contact you further if necessary). If email is not feasible, please mail a double-spaced, letter-quality copy of your note, preferably with a disk containing the electronic version. Please follow the simple style used in this issue (city /institution title / --authors; tab paragraphs; give citations with authors' initials --e.g., Maizer, BA et al., J Hered 35:35, 1995, or supply a bibliography). Figures, charts and tables should be compact and camera-ready, and supplied in electronic form (jpg or gif) if possible. To separate columns in tables, please tab instead of using spaces, to ensure quality tabulations on the web. Mailing address:

Mary Schaeffer
203 Curtis Hall
University of Missouri
Columbia, MO 65211-7020

SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

MNL 51ff. on line	MaizeGDB - http://www.maizegdb.org
Author and Name Indexes (and see **MaizeGDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeGDB**)	
Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue, updates only after No 78, and MaizeGDB
Rules of Nomenclature (1995)	MNL69:182; MNL82:84: and **MaizeGDB** (2006 update)
Cytogenetic Working Maps	MNL 52:129-145; 59:159; 60:149 and **MaizeGDB**
Gene List	MNL69:191; 70:99 and **MaizeGDB**
Clone List	MNL 65:106; 65:145; 69:232 and **MaizeGDB**
Working Linkage Maps	MNL 69:191; 70:118; 72:118; 77:137; 78:126; 79:116; 80:75; 82:87; 83: 103 {Map tutorial) and **MaizeGDB**
Plastid Genetic Map	MNL 69:268 and **MaizeGDB**
Mitochondrial Genetic Maps	MNL 70:133; 78:151 and **MaizeGDB**

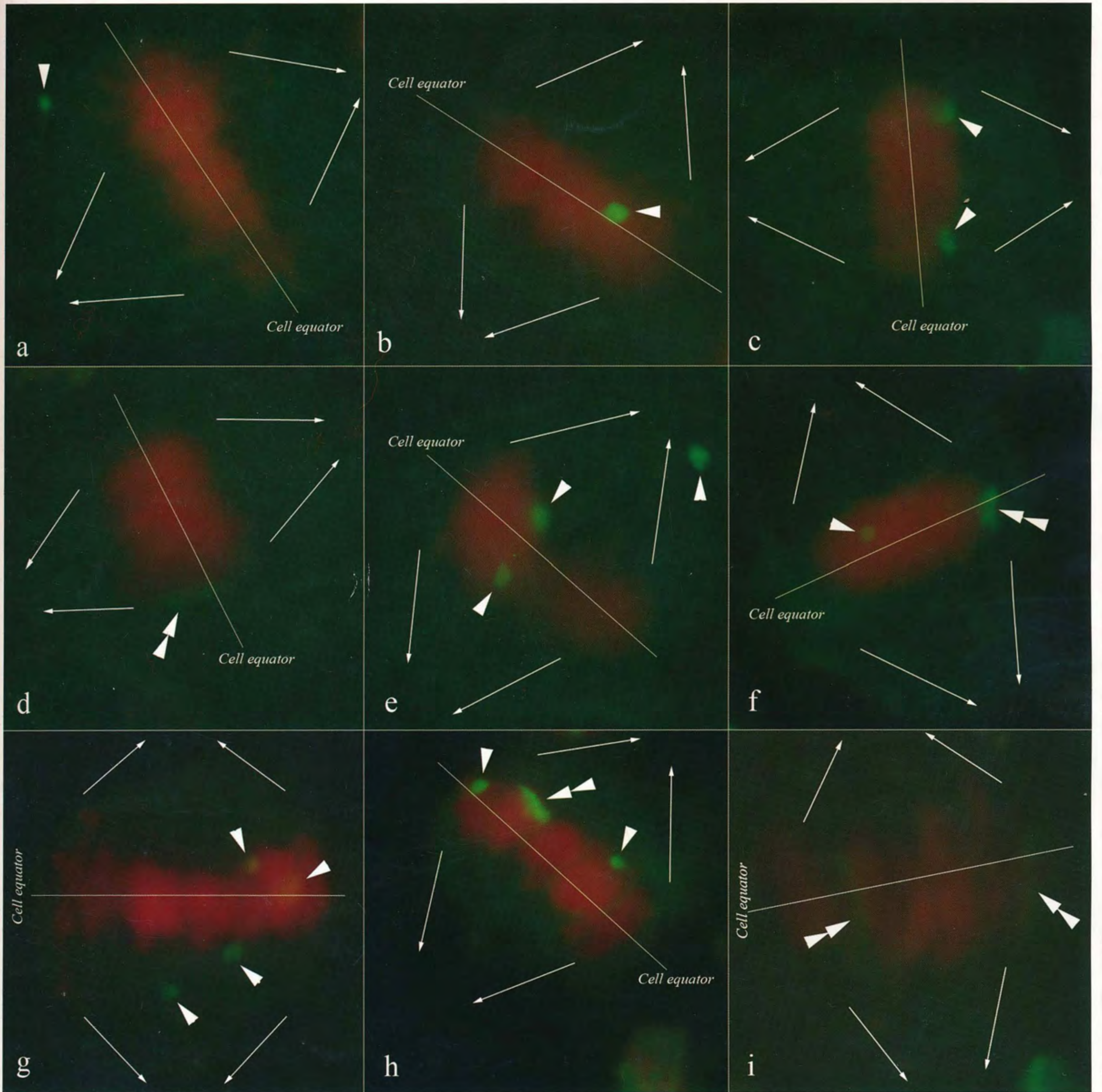
Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you report in this Newsletter or in publications, and stocks of new combinations, to the collection.
(2) Inform the Stock Center on your experience with materials received from the collection.
(3) Acknowledge the source, and advice or help you received, when you publish.

MaizeGDB needs Cooperators (this means you) to:

(1) Contact Carolyn Lawrence if you are preparing a grant that will generate large data-sets that you wish to be stored at MaizeGDB. Do this before submission to allow appropriate budgeting.
(2) New genes? Send email to MaizeGDB [http://www.maizegdb.org/newgenes.php] with details of **NEW GENES**. OR request access to the community curation tools and add your data to the database directly.
(3) Look up "your favorite gene or expression" in **MaizeGDB** and send refinements and updates via the public **annotation** link at the top of all MaizeGDB pages.
(4) Compile and provide mapping data in full, including, as appropriate, map scores; phenotypic scoring; recombination percentage and standard error; any probes and primer sequences; and other details significantly useful to colleagues. If not published, submit a note to this Newsletter, along with data for inclusion in **MaizeGDB**.
(5) Contribute to the community genome annotation effort. See **MaizeGDB** for updates.
(6) Contribute to the ***MNL maize gene review*** (www.maizegenereview.org). These data will be transferred to MaizeGDB.

May you find a Unique corn in MM!


a
Cell equator
b
Cell equator
c
Cell equator
d
Cell equator
e
Cell equator
f
Cell equator
g
Cell equator
h
Cell equator
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