MAIZE GENETICS COOPERATION

NEWSLETTER

81

June 4, 2007

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and
Division of Plant Services
University of Missouri
Columbia, Missouri

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NOTE: The 50th Maize Meeting will be held at Washington, DC February 27 – March 2, 2008.
Registration will be due Nov 27, 2007
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.

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 entered into MaizeGDB. We set an arbitrary cutoff of January 1, 2008 for the next print copy, volume 81. 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.

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. This year, we call special attention to a number of special reports: the Maize Genome Sequencing Project (see pages 71-72); and the Molecular and Functional Diversity Project (see pages 73-74).

As in the past, Shirley Kowalewski has been responsible for final redaction and layout of the copy. She has performed this task with speed, 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

BEIJING, CHINA
Chinese Academy of Sciences

RAPD analysis on the variation and mutant types induced by the special environment of space in maize (*Zea mays* L.)

--Zeng, M

In our previous papers, we have observed and obtained some variation and mutant types of qualitative and quantitative traits induced by the special environment of space. For example, mutants include kernel types, multi-ear, ear length, leaf-blade, plant height, and resistance to *B. maydis* and *E. turcicum*, among others (Chinese Space Sci. Technol. 23(6):64-68, 2003; MNL 74:2-3; NNL 79:3). This paper deals with the RAPD analysis of these mutants.

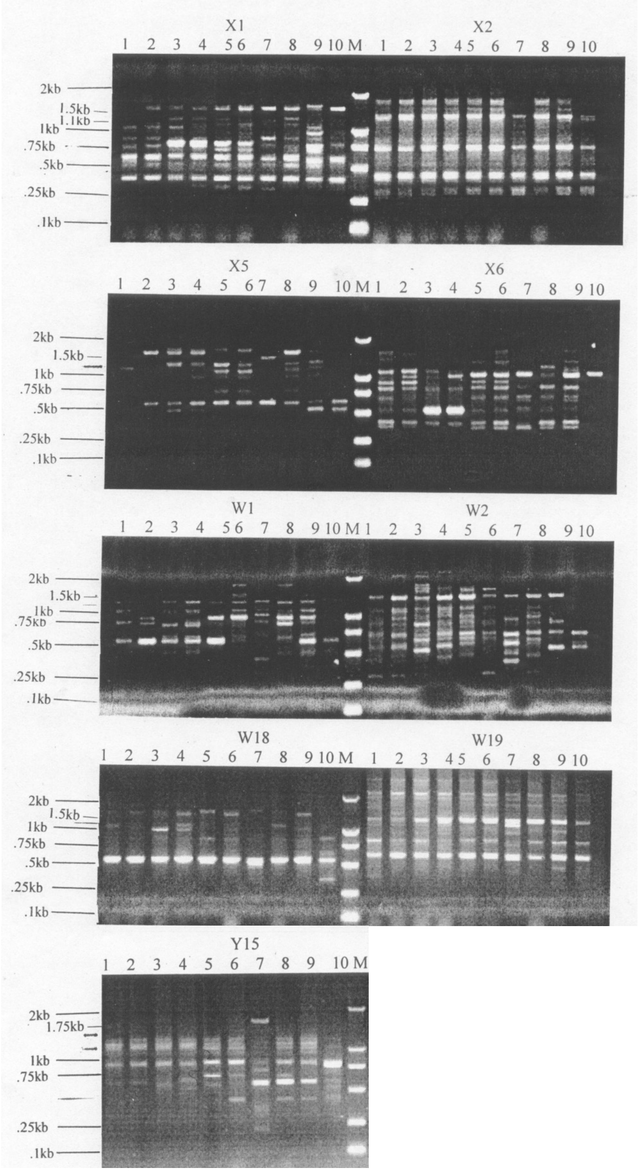

Figure 1. Amplification products from operon primers *Opx1*, *Opx2*, *Opx5*, *Opx6*, *Opw1*, *Opw2*, *Opw18*, *Opw19*, *Opy15*, respectively. 1. U8112CK, 2. *Mut7*, 3. Me141CK, 4. *Mut10*, 5. Yi01-4CK, 6. *Mut1*, 7. XHCK, 8. XH3, 9. Zi24CK, 10. *Mut4*.

Experimental materials included: (1) *Mut7* from U8112 and U8112CK; (2) *Mut10* from Me141 and Me141CK, (3) *Mut1* from Yi01-4 and Yi01-4CK, (4) XH3 from [(XH×TSPT-HZ4 Pop.)×*Mut2*]F2 population, (5) *Mut2* from Yi01-4, and (6) Mut4 from Zi24 and Zi24 CK. Nine operons were used (Figure 1, Table 1). Molecular weight standards backgrounds are lane DL2000: 2.0Kb, 1.0Kb, 0.75Kb, 0.5Kb, 0.25Kb, 0.1Kb from top to bottom, respectively.

The results show clearly that there are changes at the molecular DNA level. Mutants (*Mut7*, *Mut10*, *Mut1*, *Mut4*) possess different amplification products compared to their CK comparison. The inbred line XH3 also possesses different amplification products.

Table 1. Comparison of the amplification products clearly amplified from operon primers.

	1	2	3	4	5	6	7	8	9	10
	U8112CK	*Mut7*	Me141CK	*Mut10*	Yi01-4CK	*Mut1*	XHCK	XH3	Zi24CK	*Mut4*
	—	More 1.5Kb	—		—		—	More 1.5Kb	—	
Opx1				Less 1.0Kb		Less 1.1Kb				Less 1.1Kb
	—		—		—		—	More 1.5Kb	—	
Opx2										Less 1.1Kb
	—	More 0.5, 1.5Kb	—		—		—	More 1.0,1.5 Kb	—	
Opx5		Less 1.1Kb								Less 1.1Kb
	—		—		—		—	More 1.5Kb	—	
Opx6		Less 1.5Kb								Less 0.5,0.75,1.0Kb
	—		—		—	More 1.0,1.5 Kb	—	More 0.5,1.5 Kb	—	
Opw1		Less 1.0Kb								Less 1.0,1.1, 1.5Kb
	—	More 1.0, 0.75Kb	—	More 0.5Kb	—	More 0.25Kb	—		—	
Opw2								Less 0.35Kb		Less 1.1Kb
	—		—	More 1.1Kb	—		—	More 1.0Kb	—	More 0.25Kb
Opw18										Less 1.0,1.5 Kb
	—		—		—		—		—	
Opw19								Less 1.1Kb		Less 0.75Kb
	—		—		—	More 0.4Kb	—	More 0.5Kb	—	More 0.5Kb
Opy15								Less 1.75Kb		

BERGAMO, ITALY
CRA - Istituto Sperimentale per la Cerealicoltura

Evaluation of maize hybrid genotypes for resistance to *Aspergillus flavus*

--Balconi, C; Berardo, N; Ferrari, A; Pisacane, V; Della Porta, G; Verderio, A; Motto, M

The development of plants able to overcome damage caused by fungal pathogens has been a significant challenge for maize breeders. Although selection eliminates genotypes particularly susceptible to diseases, cultivated hybrids frequently show serious fungal infection (Munkvold, Annu. Rev. Phytopathol. 41:99-116, 2003).

Aspergillus flavus and *Aspergillus parasiticus* are responsible for both pre- and post-harvest accumulation of aflatoxins (AF) in maize; concern about aflatoxin contamination is due to its potential carcinogenicity (Counc. Agric. Sci. Technol. Rep., CAST, Ames, IA, 2003). Aflatoxin B1 is the principal member of the family; it has an extremely high carcinogenic potential to some species of animals and a widespread occurrence in some food (Moreno et al., Plant Breed. 118:1-16, 1999).

In Italy, attention was focused on aflatoxins in 2003, when particularly favourable climatic conditions caused heavy *A. flavus* attack of maize. Milk produced by farm livestock fed with maize grains contaminated by *A. flavus* showed an unusual presence of aflatoxin M_1 (AFM_1 *milk toxin*) (Piva and Pietri, Informatore Agrario 14:7-8, 2004).

Some limiting factors in breeding for aflatoxin resistance are the spatial and temporal variations in aflatoxin accumulation that require inoculation and a high number of plants, the lack of a reliable and inexpensive screening methodology, and the low metabolic activity of maize plants after physiological maturity (Payne, Crit. Rev. Plant Sci. 10:423-440, 1992). In maize, resistance to aflatoxin is under genetic control and large genotype variability for this trait has been found. Studies in this field allowed identification and development of sources of genetic resistance, such as inbred lines (Mp420, Mp313E, Mp715, Tex6, LB31, CI2) and populations (GT-MAS: gk) (Betran et al., Crop Sci. 42:1894-1901, 2002). However, the majority of these sources of resistance lack acceptable agronomic performance and adaptation which precludes their direct use in commercial hybrids. Current efforts are to map and characterize the genetic factors involved in resistance and to transfer them through marker-assisted selection to more suitable elite genotypes (Rocheford and White, Proc. Aflatoxin/Fumonisin Workshop 2000, Yosemite, CA, http:www.nal.usda.gov/fsrio/ppd/ars06.pdf, 2002).

Beneficial secondary traits such as husk covering and tightness, physical properties of the pericarp, and drought or heat stress tolerance are factors contributing to aflatoxin resistance. In general, the hybrids with good husk cover show a greater resistance to insect damage and accumulate lower levels of aflatoxins (Betran et al., Crop Sci. 42:1894-1901, 2002). The incidence and severity of *A. flavus* infection and aflatoxin contamination are highly dependent on genotype, cultural practices, and environmental conditions (Brown et al., In K. K.Sinha and D. Bhatnagar (eds.), Mycotoxins in Agriculture and Food Safety, Marcel Dekker, New York, 1998).

Reliable methods for screening and evaluation of maize genotypes for improving tolerance to *Aspergillus* attacks are a valuable tool in breeding programs to increase crop protection against fungal diseases. Accordingly, the aim of our research was to evaluate and compare 34 maize hybrids (FAO 300-400-500-600-700) for *A. flavus* resistance and for aflatoxin accumulation in field trials. The test included: i) self-pollinated *A. flavus* inoculated ears, ii) self-pollinated non-inoculated ears (SIB), iii) sterile water inoculated ears. The inoculation experiment was replicated at two different planting dates. Environmental conditions, such as temperature and rainfall, were recorded.

At pollination, silk channel (region within the husk between the tip of the cob and tip of the husk where the silks emerge) length was recorded for each hybrid; variability for this trait was observed among the genotypes, with values ranging from 3.1 cm to 10.6 cm (average: 7.0 ± 1.8). Ten hand-pollinated plants per plot were inoculated with a fresh spore suspension (mixture of 5 *A. flavus* isolates from Northern Italy, supplied by Dr. Battilani-University of Piacenza), 7 days after pollination (DAP) using the non-wounding Silk Channel Inoculation Assay (SCIA method, Zummo and Scott, Plant Disease 73:313-316, 1989). The silks of each primary ear were inoculated with 1.5 ml of 10^8 spore/ml fungal suspension; controls were non-inoculated and sterile water-inoculated plants.

At maturity, ears were manually harvested and husk cover was evaluated using a visual rating ranging from 1 (good: tight long husks extending beyond the tip of the ear) to 5 (poor: loose short husks with exposed ear tips). Also at this stage, variability among hybrids was recorded for this husk morphological trait; for this parameter 9 hybrids scored 1 (ear tip un-exposed), 21 scored between 1 and 2 (1-2 cm ear tip exposed), and 4 scored between 2 and 3 (2-4 cm ear tip exposed).

After hand de-husking, the severity of ear *A. flavus* attack was evaluated using rating scales (% of kernels with visible symptoms of infection, such as rot and mycelium growth; Disease Severity Rating, DSR, ranging from 1=0%-no infection, 2=1-3%, 3=4-10%, 4=11-25%, 5=26-50%, 6=51-75%, 7=76-100% visibly infected kernels/ear; see Reid et al., Technical Bull., 1996-5E, Research Branch, Agriculture and Agri-Food Canada, 1996). Individual ear rating using a visual scale, as described above, allowed a discernible screening of the 34 hybrids tested for *A. flavus* resistance; variability in the hybrid response was observed (DSR: 2.45 ± 0.96). For all entries, non-inoculated (SIB) and sterile water-inoculated ears, as control, had no or very low disease symptoms (DSR respectively, 1.02±0.06 for SIB and 1.01 ± 0.03 for water-inoculated). This result indicates that the non-wounding silk channel inoculation technique applied was effective in inducing *A. flavus* attack.

After visual inspection, ears of each plot were dried, shelled, and the kernels bulked. To evaluate internal kernel infection, 50 kernels, randomly chosen from each sample, were surface-disinfected and plated on DRBC agar (King et al., Appl. Environ. Microbiol. 37: 959-964, 1979). Seven days after plating, percentage of kernels showing visible *Aspergillus* mycelium was calculated. Variability among inoculated hybrids was also observed for this parameter, with the value of contaminated kernels ranging from 0 to 88% (average 16.4 ± 1.5). In contrast, controls showed a percentage of internal contaminated kernels lower than that observed in the corresponding inoculated hybrids (SIB: 0.94 ± 1.81, water-inoculated control: 0.6 ± 1.03).

The level of AFB_1 in ground grain samples of the hybrids under study was evaluated using enzyme-immunoassay-ELISA kit (Kit Ridascreen-Aflatoxin B_1 30/15-R-Biopharm-Art. Not: R1211). AFB_1 level for inoculated hybrids ranged from 0 to 80 µg/kg (average: 27 ± 4.8), while in the controls AFB_1 was present in trace amounts or absent (SIB: 2.0 ± 2.8; water-inoculated control 2.0 ± 5.0). In this case, variability also occurred among hybrids under investigation.

Studies of the correlations between visual ear rot ratings, internal kernel infection evaluation, aflatoxin content, silk channel length at pollination, husk cover ratings, are in progress.

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Transcriptome analysis of *opaque2* and *opaque7* mutants in maize endosperm

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

In maize, the zein synthesizing system is particularly adapted for the study of the regulating mechanisms of plant genes because i) its expression is restricted to specific tissues and stages of seed development and ii) because of the availability of mutants useful in dissecting the regulatory processes taking place in the developing seed (Pirona et al., Maydica 50:515-530, 2005). Studies on genetic mutations that affect the accumulation of different zeins have demonstrated the existence of several regulatory signals controlling the expression of specific members of the zein family which confer an opaque phenotype to the endosperm (Motto et al., pp: 479-522, In: B. A. Larkins and I. K. Vasil (eds.), Cellular and Molecular Biology of Plant Seed Development, Kluwer Acad. Publ., The Netherlands, 1997). For example, the recessive mutations *opaque2 (o2) and opaque7 (o7)* induce specific decreases in accumulation of 22 and 19-kD alpha-zeins, respectively, while the *opaque15 (o15)* mutation exerts its effect primarily on the 27-kD gamma zeins. The recessive mutation *opaque6 (o6)* and the dominant or semi-dominant mutations *Floury (Fl2*), *Defective endosperm *B30 (De*B30*), and *Mucronate (M*c) cause a more general reduction in accumulation of all zein classes.

The *o2* mutation has been widely studied at the molecular, genetic, and biochemical levels (see Pirona et al., 2005). The product of the *o2* gene is a basic leucine zipper (bZiP) transcriptional regulator that is specifically expressed in the endosperm and activates the expression of 22 kDa alpha-zein and 15 kDa gamma-zein, together with the *B-32* gene, encoding an endosperm specific ribosome nactivating protein. Other possible direct or indirect target genes of the *o2* factor have been shown to belong to various metabolic pathways, suggesting that *o2* may play an important role in the developing grain, as a coordinator of the expression of storage protein, and nitrogen and carbon metabolism genes.

In recent years, the development of extensive maize cDNA libraries, along with computer software to systematically characterize them, has made it possible to analyze gene expression in developing maize endosperm more thoroughly. Accordingly, we have used cDNA microarray technology to investigate the transcription profiles and differential gene expression of maize endosperm from two different opaque mutants (*o2* and *o7*) and in double mutant combination (*o2o7*).

Microarrays were assembled using clones obtained from the EC ZeaStar project (Edwards et al., unpublished results). Briefly, 20 part-normalized cDNA libraries were prepared from tissues covering 5 key stages in both endosperm and kernel development. Approximately 20,000 ESTs were sequenced, aligned, assembled into contigs using a similarity score of 80%, and annotated using BLASTA and TBLASTN software. Contigs and singleton cDNAs were used to construct a unigene set of 8,950 sequences. EST sequences were analyzed with the BLAST2GO software (http://www.blast2go.de). First, homology searches using public domain non-redundant databases were performed and identified significantly homologous sequences for 48.4% of the ESTs considered. These ESTs represented 3,090 single hit (71.3%) and 1,240 multiple hit sequences. Subsequently, an attempt was made to associate biological functions to each of the ESTs showing sequence homology using the gene ontology (http://www.geneontology.org) and KEGG databases (http://www.genome.jp/kegg). Approximately 85% of the ESTs analyzed could be associated with GO database entries. The results of this analysis permitted us to divide the aforementioned ESTs into 24 functional groups with a total of 7,250 clones identified as duplicates.

Microarray slides containing the entire Zeastar unigene set were hybridized with probes derived from endosperm tissue harvested 15 days after pollination (DAP) and derived from the A69Y*wt*, A69Y*o2*, A69Y*o7*, and A69Y*o2o7* isogenic lines. To reduce hybridization artifacts, all probes were labelled both with Cy3 and with Cy5 and used in dye-swapping experiments on a series of three independent slides. The expression data obtained were assayed for consistency by performing T-tests at 95% confidence levels.

All microarray experiments were performed in triplicate using dye swapping, hence giving rise to 12 independent measurements for each EST, considering the presence of duplicate spots on each slide. Raw measurements of spot fluorescence intensities were collected from hybridized slides using a Genepix 4100A scanner and Genepix Pro4 software (Axon Instruments, Union, CA). Subsequently, the spot values obtained were corrected for background fluorescence and analyzed using the Vector Xpression3 software (Informax, Frederick, MD). The data were log2 transformed and normalized by equalizing the mean intensity of each channel to 1. To verify reproducibility between spots and between channels, T tests were performed applying a 95% confidence threshold and allowing us to remove inconsistent hybridization results. Ratios between wild type and mutant expression levels were calculated and ESTs exhibiting ratios below 0.5 or over 2 were selected for further analysis.

Average signal values derived from the four probes used were graphed using a logarithmic scale. The graphical representations clearly showed the prevalence of genes with distinct expression patterns in the A69Y*wt* and A69Y*o2* genotypes. Conversely, the A69Y*wt* and A69Y*o7* genotypes show less evident differences in expression levels. The A69Y*o2o7* double mutant exhibits differences in expression patterns resembling those obtained for the A69Y*o2* genotype. A plot of A69Y*o2* vs. A69Y*o7* expression levels showed the cumulative effect of both genotypes, revealing a high number of genes with distinct expression patterns.

Consistently performing spots in T-tests were selected and used to calculate *wt*/ mutant expression ratios. Among the ESTs considered, 17.1% exhibited a down-regulated expression profile. The *o2* mutation may be associated with 649 down-regulated ESTs. 508 down-regulated ESTs were identified in the A69Y*o7* background, whereas 759 ESTs showed a reduced expression pattern in A69Y*o2o7.* Up-regulated expression profiles were found for 3.23% of the ESTs considered. One hundred and thirteen up-regulated ESTs were identified in the A69Y*o2*, 26 in the A69Y*o7*, and 86 in an the A69Y*o2o7* backgrounds, respectively. Among the ESTs identified, 36.7% exhibited relevant homology with sequences deposited in public databases and could be univocally associated with known biological processes related to amino acid and carbohydrate metabolism, signal transduction, protein turnover, transport and protein folding. In addition, three transcription factors other than *o2* appear to be down-regulated. Collectively, the results may provide a framework for investigating a common mechanism that underlies the *o2* and *o7* kernel phenotypes.

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Identifying low-copy loci by FISH on chromosomes in 3-D: Position of *p1,* the 22kDa alpha zein cluster, and the *5S rDNA* locus

--Harper, LC; Wang, R; Cande, WZ

As part of an effort to cytologically map single copy genes to maize pachytene chromosomes, we developed a fluorescent in situ hybridization (FISH) method for identifying low-copy loci on chromosomes in 3-D. The advantage of this 3-D method is that nuclear architecture is preserved, and important structural information, such as the relative position of chromosomes, is maintained. We have routinely used this FISH protocol to analyze the degree of homologous pairing in various maize meiotic mutants; for example, *pam1* (Golubovskaya et all., Genetics 162:1979-1993, 2002), *phs1* (Pawlowski et al., Science 303:89-92, 2004), *sgo1* (Hamant et al., Curr. Biol. 15:948-954, 2005), *afd1* (Golubovskaya et al., J. Cell Sci. 119:3306-3315, 2006), and others (Pawlowski et al., Plant Cell 15:1807-1816, 2003). In this article, we report a detailed protocol for this 3-D FISH method. This method, however, is not suitable for routine cytological mapping of single copy genes. To do that, we developed a 2-D FISH protocol which is suitable for cytological mapping, but does not preserve nuclear architecture (Wang et al., Plant Cell 18:529-544, 2006).

Here we report the cytological position of three loci on maize pachytene chromosomes; *p1* on chromosomes 1, the 22kDa alpha zein cluster (*z1C/SF4/az22z1* cluster http://www.maizegdb.org/cgi-bin/displaylocusrecord.cgi?id=9017693) on chromosome 4, and the *5S rDNA* loci on chromosome 2L. These loci are genetically mapped, allowing us to use them as anchor points for a cytogenetic map. Some of this data has been used to confirm the RN map generated by Laurie Anderson (Anderson et al., Genetics 166:1923-1933, 2004). As a further check, we used the Morgan2McClintock translator (Lawrence et al., Genetics 172:2007-2009, 2006) to compare our empirically determined positions to those calculated based on the RN maps. We report a detailed protocol of the method, and incorporate the modifications we currently use.

The *5S rDNA* locus is located at 2L.85. We routinely detect the *5S rDNA* locus with probes directly labeled with fluorescent nucleotides, so we used this probe as a test to see if TSA amplification would work. We compared DIG labeled *5S rDNA* probes detected with anti-DIG FITC, or with the TSA amplification method. TSA amplification increased the average signal pixel intensity up to 15-fold above background, which is roughly 2-3 times better detection than with anti-DIG-FITC (data not shown), or with a directly labeled probe (Figure).

To determine the cytological position of the *5S rDNA* locus, we traced and computationally straightened chromosome two from seven nuclei (Figure, chromosome 2). The figure is presented in color at (http://www.agron.missouri.edu/mnl/81/06harper.htm; in the near future also at www.maizegdb.org/mnl/81/06harper.htm), where everything can be seen better. In the top three chromosomes in this panel, DIG-labeled probe was detected with TSA, while in the bottom 4 we used probes directly labeled with fluorescent nucleotides. The *5S rDNA* locus is at an average position of 2L.85 (Table). The most difficult part of this procedure is the successful tracking of the complete length of a chromosome before entering it into the straightening program. Our position of the *5S rDNA* locus is in good agreement with that found previously using a radioactively labeled probe (Wimber et al., Chromosoma 47:353-360, 1974).

To check if our cytological position was in good agreement with the Recombination Nodule map (RN map, see Anderson et al., 2004), we used the Morgan2McClintock translator (Lawrence et al., 2006) (http://golem4.zool.iastate.edu/Morgan2McClintock/), and put in the Genetic 2005 2 map (http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=940881) for translation. The translator puts the *5S rDNA* locus at 2L.88, in good agreement with our position of 2L.85.

5S	2L arm length	Distance: cen-5S	position
	2576	2224	0.863354037
	2208	1920	0.869565217
	2464	2072	0.840909091
	2408	2000	0.830564784
	2584	2240	0.866873065
	2528	2128	0.841772152
		ave	**0.852173058**
		SD	**0.016404664**

Lengths are reported in pixels

Detection of the 22kDa alpha zein cluster with TSA, at 4S.95. In order to determine if this signal amplification method was sufficient to allow the detection of a much lower copy gene, we used a zein coding sequence from the 22kDa alpha zein cluster, located on 4S, as a probe. This locus contains about 22 copies of the small alpha zein gene, in tandem array (Llaca and Messing, Plant J. 15:211-220, 1998), and we reasoned that this should allow more probe to hybridize to the target sequence. With a DIG-labeled probe, we were not able to detect this locus with anti DIG-FITC, but we were able to detect this locus routinely using the TSA amplification (top 4 chromosomes in the chromosome 4 panel). We can also detect the 22kDa alpha zein cluster, albeit less robustly, by using a zein probe directly labeled with fluorescent nucleotides (bottom 4 chromosomes in the chromosome 4 panel). In both cases, one bright spot was found on a single chromosome very near the telomere. Following chromosome tracing and straightening, we determined its position as 4S.95 (Figure and Table). In order to determine whether the correct chromosome was hit, we used a centromere probe that is specific to centromere 4, and in all cases, the zein probe hybridized to the same chromosome as the centromere 4 probe (data not shown).

Our results consistently put the 22kDa alpha zein cluster at 4S.95 (in the A344 and KYS inbred)--very close to the telomere. The first mapping of the zein cluster was done in the BSSS53 inbred, and zein genes were found 0.4 and 2.2 cM from the *drz1* locus (Chaudhuri and Messing, Mol. Gen. Genet. 246:707-715, 1995). The genes in the 22kDa alpha zein cluster are not on the Genetic 2005 map, but the *drz1* locus is. We used the Morgan2McClintock translator to determine if our position is similar to that found on the RN map. Translating the Genetic 2005 4 map on Morgan2McClintock, puts *drz1* at 4S.93. This is consistent with our cytological position. However, in other mapping data using the Pioneer composite map, *az22z1*, a single gene in the 22kDa alpha zein cluster, was found completely linked with *csu235* (http://www.maizegdb.org/cgi-bin/displayrecombrecord.cgi?id=9017699). On the translated Genetic 2005 RN map, *csu235* is at 4S.83. Translating the Pioneer composite 1999 map places *csu235* at 4S.78; even further from our empirically determined cytological position. Possibly, using mapping data from one inbred (BSSS53), a cytological position from another (A344), and an RN map from yet a third inbred (KYS) may not yield a usable mark to anchor the genetic and cytological map. Alternatively, if the mapping in the BSSS53 inbred is the most accurate genetic mapping, there may be no real discrepancy.

zein	4S arm length	Distance: cen-zein	position
	2016	1928	0.956349206
	1784	1696	0.950672646
	1776	1664	0.936936937
	1936	1816	0.938016529
		ave	**0.945493829**
		SD	**0.009553181**

Lengths are reported in pixels

<u>The *p1* locus is 1L.53</u>. The *p-wr* allele of *p1* contains an array of 6 tandem copies of the *p1* gene (Zhang and Peterson, Plant Cell 17:903-914, 2005). Using this allele as a target, we hybridized *p1* probes to pachytene chromosomes and used TSA amplification for detection. We were able to routinely detect the *p1* gene. We selected 4 cells (two from W22 and two from W23), and completely straightened chromosome 1 from these cells (Figure, chromosome 1 panel). The cytological position of the *p1* locus is the same in both inbreds (Table): 1l.53. The *p1* gene has been previously mapped relative to many translocations, and *p1* was found to be distal to T1-5(6899) (1S.32) (Auger and Sheridan, MNL67:46, 1993) and to T1-2b (1S.43) (Anderson, Genetics 26:452--459, 1941), yet proximal to T1-4b (1S.55)(Auger and Sheridan, 1993). This places *p1* between 1S.43 and 1S.55, and in good agreement with out direct findings.

To determine the position of *p1* on the RN maps, we translated the Genetic 2005 1 map (http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=940880) and found *p1* at 1L.63. This discrepancy, 10% of the arm length, seems high to us but we have no explanation for this discrepancy.

p1	1S arm length	Distance: cent - p1	position
	2696	1392	0.516320475
	2544	1274	0.500786164
	2464	1360	0.551948052
	2736	1488	0.543859649
		ave	**0.528228585**
		SD	**0.023817591**

Lengths are reported in pixels.

We developed a 3-D FISH strategy to detect multi- and low-copy genes on maize prophase chromosomes in intact nuclei where chromosome organization is preserved. Acquiring data in 3-D allows us to correlate biological events, such as the position of genes and defined heterochromatic blocks (i.e., centromeres, telomeres and knobs) during homologous pairing, recombination and synapsis. We use 3-D FISH (without TSA amplification) and the 5S rDNA probe routinely to assess the degree of homologous pairing in meiotic mutants. We have tried 3-D FISH with and without TSA amplification to detect a number of single copy genes, included *kn1, su1, ahd1, bz1* and other. However, we have not been able to reliably detect single copy genes. Thus, this 3-D FISH method is not sensitive enough to use for routine cytogenetic mapping. For that purpose, we recommend using HRgeneFISH (Anderson et al., 2004).

<u>Maize lines and DNAs used</u>. Inbred line A344 was obtained from Inna Golubovskaya (UC Berkeley) and was used for *5S rDNA* and zein gene experiments, KYS was obtained from the National Plant Germplasm System (now GRIN) and was used for *5S rDNA* and zein gene experiments, *p-wr* lines in W23 and W22 were obtained from Tom Peterson (University of Iowa) and these were used for the *p1* experiments.

The 22 kDa alpha zein gene was generously provided by Victor Llaca and Jo Messing (Rutgers, NJ). The *p1* gene was generously provided by Tom Peterson (Iowa State Univ., Ames), and the *5S rDNA* gene was generously provided by Elizabeth Zimmer (Smithsonian Inst.).

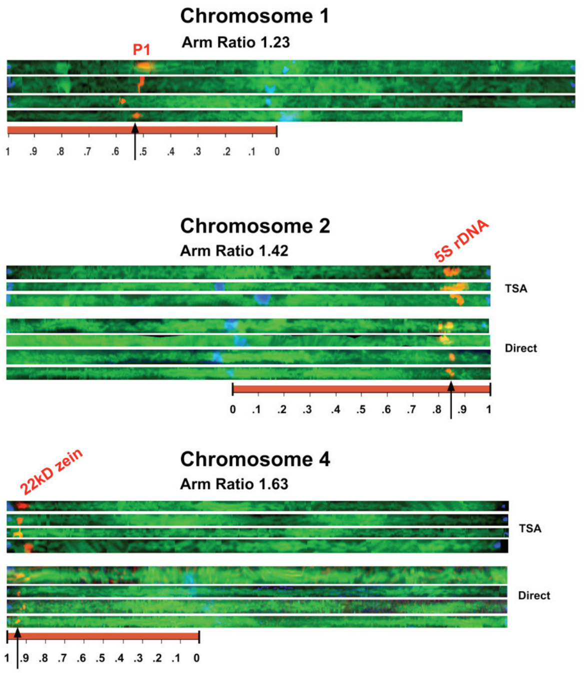
<u>Probe labeling</u>. The three probes used in this study where labeled with alkali- stable digoxigenin -11-dUTP (Roche) (DIG) by PCR. Approximately 1 to 10 nanograms of template DNA was added to a standard PCR reaction mix: 2 µl 10x buffer with 15 mM $MgCl_2$ from Perkin Elmer, 2 µl forward primer 10 pmol/µl, 2 µl reverse primer 10 pmol/µl, 2 µl 1mM dATP, dGTP, dCTP, a mixture of dTTP and dUTP-dig, 2 Units Amplitaq (Perkin-Elmer) and water to 20 µl. Labeling reactions were made in these proportions in various amounts from 20 to 100 µl. A PTC-100 PCR machine (MJ Research, Inc.) was used. For each labeling reaction, a 20 µl unlabeled control reaction was performed, and an aliquot of equal molar volume was run side by side with the labeling reaction in a gel of appropriate concentration for the fragment sizes expected. Incorporation of DIG could be seen visually from the gel shift, and the amount of DIG incorporation was occasionally calculated based on the degree of the gel shift. In addition, gels were blotted and developed as a western with anti-DIG-AP followed by NBT/BCIP detection (Roche protocol). This allowed us to estimate the degree of DIG incorporation in each probe.

Incorporation of DIG-11-dUTP by Taq polymerase was very sensitive to fragment length. We could label fragments of up to 200 bp with a ratio of 1:1 of dTTP and DIG-11-dUTP ("highly-labeled"). Fragments of 500-700 bp were labeled with a 2:1 ratio, and 2 kb fragments could be labeled with a ratio of 9:1 dTTP and DIG-11-dUTP ("low-labeled"). Intermediate sizes required intermediate ratios. We found that a mixture of highly-labeled and low-labeled probe often gave the best results for the zein probe. We also found PCR labeling can give better FISH results than random priming, terminal transferase and nick-translation labeling. We routinely use both PCR labeling and random priming to label probes for use in 3-D FISH.

<u>Fixation and embedding of meiocytes</u>. Maize anthers were removed from living immature tassels and fixed for 30 minutes with 4% formaldehyde (EM grade) in a special buffer designed to preserve chromatin structure, "buffer A" (15 mM Pipes-NaOH, pH 6.8, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 2 mM EDTA, 0.15 mM spermine tetra HCl, 0.05 mM spermidine, 1 mM DTT, 0.32 M sorbitol) (Belmont et al., J. Cell Biol. 105:77-92, 1987; see also Dawe et al., Cell 76:901-912, 1994; Bass et al., J. Cell Biol.

137:5-18, 1997). After fixation, anthers were rinsed in 1X buffer A three times for 30 minutes each. Anthers are stored after fixation and rinsing in the fridge in the dark. For the experiments reported here, anthers were used within 3 weeks after fixation. We have subsequently found that anthers can be used up to two years after fixation with no signs of degradation if they are stored in completely dark, airtight containers at 4 degrees. For FISH, meiocytes were extruded from anthers into 1X buffer A. 10µl of meiocytes in buffer A were transferred by a BSA-coated pipette tip onto a glass cover slip. 100µl of polyacylamide mix (50µl 30:.8% bis-acrylamide, sterilized, filtered stock, kept at 4C and 50µl 2X buffer A) was catalyzed with 5µl of ammonium persulfate (20%) and 5µl of Na_2SO_3 (20%) and then vortexed; 5µl of this was added to the 10µl of meiocytes on the cover slip and then mixed with the pipette tip very quickly. Another cover slip was immediately placed on top and sometimes a small weight was added to slightly flatten the meiocyte and the contents. The polyacrylamide was allowed to polymerize for 30 minutes. The cover slips were then separated and the resulting pad of meiocytes embedded in polyacryamide was placed in a well containing a prehybridization solution of 50% deionized formamide in 2X SSC (in a standard 6 well plate). This solution was changed three times over the course of 1 hour, and then the hybridization was started.

Fluorescence in situ hybridization (FISH). 50 µl of a probe solution containing labeled DNA (usually 1 µl, but amount determined empirically for each new batch of probe) in 50% formamide and 2X SSC was used for each pad. Probe solution was added to each pad, then the pad was covered with a cover slip and sealed with rubber cement. Probe was allowed to penetrate for 30 to 60 minutes at 36C. Strand separation was induced by placing the slide on a PCR block for 6 minutes at 95C. Pads were then incubated at 30C overnight to allow hybridization. After hybridization, the pads went through a series of 10 to 20 minute washes to remove weakly hybridized probe and excess fluorescent molecules: 2X SSC; 1X SSC, 1X PBS; 1X PBS + 0.1% tween-20; 1X PBS*. To detect single and low copy probes, we developed a tyramide signal amplification method as follows: Pads FISHed DIG-labeled probes were treated with a blocking solution (1% bovine serum albumin, 1X Roche block, in 1XPBS) for 1 hour. Then this block was removed and 150mUnits was added per pad of anti-DIG-POD Fab fragments from Roche (1µl of anti-DIG-POD-poly at 50mUnits per pad and 1µl of anti-DIG-POD as supplied) and left overnight in a humid chamber. Then, excess antibody was removed with at least 5 hours of washing with 1X PBS, changing to fresh solution at least every hour. 100 µl of tyramide-Cy3 solution containing 2 µl tyramide-Cy3 in 98 µl “amplification diluent”(from a tyramide signal amplification kit from NEN) was added to each pad, and allowed to catalyze for 10 minutes. We optimized this time with various probes, testing times from 3 minutes to 2 hours. We found that the longer times simply increased background, and that 10 minutes was optimal for the several probes we used. Following the TSA step, pads were washed with 1X TBS plus 0.05% Tween20, three times immediately, then 4 times, 15 minutes each. Then pads were washed in 1X TBS with no Tween20, two times 10 minutes each. TBS was used instead of PBS so that the DAPI would not precipitate in the next step. DNA was then stained with DAPI (5µg/µl in 1X TBS) for 30 minutes and washed out with 1X TBS three times, 10 minutes each. Pads were mounted in DABCO, by adding and removing the DABCO three times to allow penetration into the pad. Then a 22x22 cover slip was placed on top and sealed with nail polish. (To use directly labeled probes, after this step, we wash in 1X TBS, then complete the DAPI staining step. We do not use the TSA amplification step for analysis of homologous pairing.)



(For color see online.)

Meiocyte Imaging and Deconvolution. Cells were viewed with an Applied Precision Delta Vision Microscope system, consisting of an Olympus 1X70 inverted fluorescence/bright-field microscope and Olympus 100x 1.35 UPlanApo oil-immersion lens. Images were recorded by a Sensys Ch250 CCD camera, controlled by computer. 0.2-0.4µm sections in the z plane were collected; image size was 34µm x 34µm. A single maize meiocyte nucleus is usually 15 to 25 microns in thickness in our preparations (the small weight added during pad polymerization can flatten the nuclei to 15 microns). Three-dimensional data stacks representing individual nuclei were reiteratedly deconvolved using Deltavision 2.1 software (from Applied Precision). Deconvolved three-dimensional images were analyzed with Softworx 2.50 (from Applied Precision) software. The program '3D Model' was used to trace the chromosomes by hand in three dimensions through the x, y and z plane. The program 'Straighten' was used to straighten and then flatten the straightened chromosomes into two dimensions.

Mu killer locus available in multiple inbred backgrounds

--Lisch, D; Slotkin, RK

Mu killer is a dominant locus that can silence the *Mutator* system of transposons heritably and reliably. Lines carrying *Mu*-tagged alleles can be crossed to lines homozygous for *Mu killer* resulting in a rapid loss of activity. Subsequent backcrosses to low-copy *Mu* lines (such as commonly used inbreds) result in a rapid reduction in the number of *Mu* elements, making isolation of linked *Mu* insertions much easier. The loss of activity can also make PCR-based efforts to isolate insertions considerably easier, as somatic *Mu* activity is eliminated. By crossing a given *Mu*-induced mutation to both *Mu killer* and a related line without *Mu killer*, it is also possible to rapidly test for the possibility that a given mutation is suppressible. In response to requests by cooperators, we have now introgressed *Mu killer* into five commonly used inbred backgrounds: Mo177, A188, W22, W23 and B73. In each case, the *Mu killer* locus was introgressed five times into each inbred background and then selfed twice to produce *Mu killer* homozygotes. These lines will be available in the spring of 2007, when they will be deposited at the Maize Genetics COOP Stock Center (http://maizecoop.cropsci.uiuc.edu/). We also have available homozygous *Mu killer* lines that carry the *bz-mum9* or the *a1-mum2* reporter alleles, as well as low copy, or minimal *Mutator* lines that carry a single *MuDR* element and a single *Mu1* element. Further information is available at our web sites: http://plantbio.berkeley.edu/~mukiller/ and http://plantbio.berkeley.edu/~mukiller/mutator/Mutator%20Home.html. Seed requests can be sent directly to Damon Lisch (dlisch@berkeley.edu) or to the Maize Stock Center.

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Mapping of *ragged* (*rg) mutation using classical and molecular markers**

--Whalen, RH; Brozik, M; Auger, D

A recessive mutation resulting in abnormal leaves and shorter plants was found in the inbred W22. This apparently spontaneous mutation was informally designated "*ragged*" (*rg**) in reference to its cut or torn leaves (Fig. 1). It was found to be due to a single recessive gene, and using B-A translocations, was found to be on the long arm of chromosome 5. We mapped it more precisely in a three-point linkage test with *red aleurone* (*pr*) and the SSR molecular marker *umc1221*. The reference allele for *umc1221* has seven tandem copies of CT. Determination of polymorphisms was accomplished through electrophoretic separation in a 3.5 % agarose TAE gel of polymerase chain reaction (PCR) products that included the SSR. The mapping sample was produced by crossing an F1 heterozygous for all three loci by a homozygous testcross parent (Fig. 2). The data from the mapping sample are presented in Table 1.

Figure 1. Leaf of *rg*/rg** plant. (For color see online.)

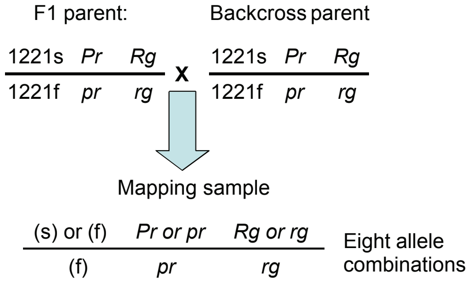

Figure 2. Mapping scheme. 1221s refers to the slower band of *umc1221*; 1221f refers to the faster band of *umc1221*. *Pr* refers to the wild-type allele of *pr1*; *pr* refers to the red allele. *Rg* refers to the wild-type allele of the *rg** locus; *rg* refers to the mutant allele.

Table 1. Recombination data.

		Family 1	Family 2	Total	
Parentals	*umc1221-f Pr Rg*	70	80	157	315
	umc1221-s pr rg	77	88	158	
SCOs	*umc1221-f pr rg*	7	7	14	25
Region 1	*umc1221-s Pr Rg*	3	8	11	
SCOs	*umc1221-f Pr rg*	5	2	7	21
Region 2	*umc1221-s pr Rg*	8	6	14	
DCOs	*umc1221-f pr Rg*	3	0	3	4
	umc1221-s Pr rg	0	1	1	
Totals		173	192	365	

The *rg** locus is located on the long arm of chromosome 5, 6.8 ± 1.4 cM from *pr1*, which is 7.9 ± 1.4 cM from *umc1221*. The map is shown in Fig. 3. This places the new locus distal to *pr1*.

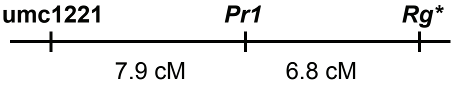

Figure 3. Map of new locus relative to *pr1* and *umc1221*.

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Inheritance of ear shank length in maize (*Zea mays* L.)

--Ji, HC; Lee, JK; Choi, GJ; Kim, KY; Seong, BR; Seo, S; Kim, SH; Lee, HB

The ear shank in maize corresponds to the lower portion of the lateral branch and comprises several nodes and shortened internodes. The inheritance of ear shank length was investigated in sweet corn inbreds Ia453*sh2* (with a long shank) and Hi38*c1* (a tropical Hawaiian super sweet, with the *brittle1* gene and a short shank). Ear shank lengths of the parent lines were 4.59 cm (Hi38*c1*) and 13.25 cm (Ia453*sh2*). The ear shank length of F1 hybrids was 14.66 cm, while the length of F2 lines was 12.69 cm (Fig. 1). The ear shank lengths in BC1 and BC2 were 8.88 cm and 15.32 cm, respectively. The average coefficients of variation (CV) were as follows: P1 40%, P2 27%, F1 35%, F2 52%, BC1 56%,

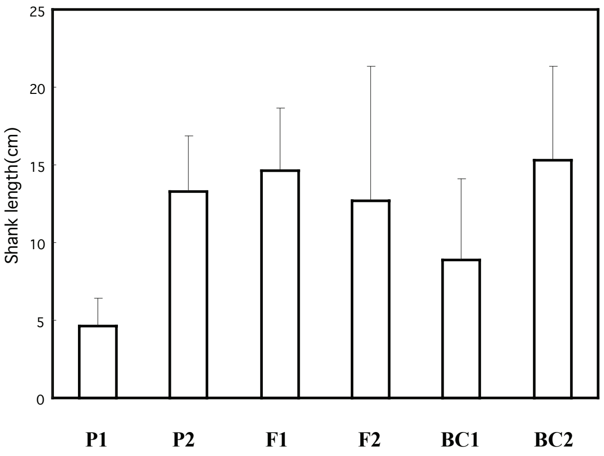

Figure 1. Distribution of ear shank length in parents, F1, F2 and Backcross (BC1, BC2) generations. F1, F2 and BC1 and BC2 populations were derived from the parental inbreds Hi38-71 (short shank) and Ia453*sh2* (long shank). P1: Hi38-71, P2: Ia453*sh2*.

BC2 39%. These results were analyzed by the Generation Mean Analysis (GMA) Method modified from Mather and Jinks (1977). Generation mean analysis of the six generations (Table 1) revealed a highly significant additive effect. The aa [additive x additive] and dd [dominance x dominance] effects were not significant but ad [additive x dominance] effects were significant.

Table 1. Estimates of additive (a), dominance (d), and interaction parameters for the cross Hi38*c1*(*bt*) x Ia453*sh2*.

Parameter+	Estimate (±SE)	t test	
m	12.69±0.27	18.36	**
a	-6.44±0.43	-6.39	**
d	3.38±1.42	0.97	NS
aa	-2.36±1.38	-0.69	NS
ad	-2.11±0.45	-2.03	*
dd	1.12±2.13	0.22	NS

+m=midpoint, a=additive effect, d=dominance effect, aa=additive x additive effect, ad=additive x dominance effect, dd=dominance x dominance effect
*, **, ^{NS}; levels of significance.

Broad-sense heritability was 60.51% and narrow-sense heritability was 58.5%. The estimated minimum number of gene loci, using the Castle and Wright formula, was 0.54. Therefore, ear shank length might depend on a single gene acting without any dominance effect, but more study is needed on the relationship between ear shank length and other characters.

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The influence of ear age on the frequency of maternal haploids produced by a haploid-inducing line

--Rotarenco, VA; Mihailov, ME

Large-scale production of haploids in maize became possible when the ability to induce maternal haploids was revealed in the Stock 6 line. Stock 6 and its derivatives have a wide distribution among maize breeders, who, besides using haploids for breeding work, try to improve the haploid-inducing ability and explain the nature of this phenomenon. Producing haploids every year by applying the same inducer, MHI (Chalyk, MNL 73, 1999), we have noticed that their frequency significantly varies: depending on the method of pollination, artificial or natural (Rotarenco, MNL 76, 2002), in different maternal genotypes and even within one ear (Chalyk, MNL 73, 1999). It is known from the literature that delayed pollination increases the frequency of haploids (Randolph, 1946; Seaney, 1954; Chase, 1969). However, the opposite results have been obtained in our experiments. The main purpose of our work was to estimate the influence of delayed pollination on the percentage of kernels with haploid embryos when a haploid-inducing line was used.

A number of hybrids and inbred lines were crossed with the MHI inducer line by hand pollination. Plants were divided into four groups within one maternal genotype--with two-day, four-day, seven-day and ten-day ears. The start day of the ear-age recording was the day of silk emergence, and such ears were considered one-day ears. When the ears reached the desired age, they were pollinated with the inducer.

After harvesting, those genotypes that had rather good seed set and the best expression of the *R1-nj* gene (a marker gene allowing kernels with haploid embryos to be identified) were selected. Thus, four inbred lines and four hybrids were used for the experiment (Table 1). The number of ears in each ear-age group varied from 10 to 15. Therefore, the total number of ears analyzed for each genotype exceeded 40. In the lines MK01 and Mo17, a total number of plants of less than 40 were divided into three and two groups, respectively. In the Mo17xB73 hybrid there were 37 plants and they were divided into two groups.

Table 1. Frequencies of haploid induction (%) in the four groups of ears and in general for each genotype, coefficients of correlation.

Maternal genotype	Day of pollination after silk emergence				General average	Coefficient of correlation, r
Inbred lines	2 days	4 days	7 days	10 days		
A464	7.3	5.6	3.9	4.6	5.4	-0.45**
A619	7.1	6.1	4.8	5.3	5.8	-0.34*
MK01	10.3	6.2	6.2	-	7.6	-0.56**
Mo17	6.82	5.4	-	-	6.11	-0.27
On average for lines					6.2***	
Hybrids						
Modavian450	6.2	4.4	4.0	3.8	4.5	-0.39*
Porumbeni295	4.3	2.9	4.5	3.3	3.7	-0.15
Porumbeni359	7.0	4.2	2.6	2.9	4.1	-0.61**
Mo17xB73	5.5	4.2	-	-	4.8	-0.43*
On average for hybrids					4.3	

*, **, *** significant at 5%, 1% and 0.1% level, respectively.

In all genotypes the delay of pollination caused a decrease of frequency of kernels with haploid embryos (Table 1). According to the coefficients of correlation, this decrease was statistically significant for most genotypes. Additionally, a significant difference was revealed between thee averaged percent of haploids in the inbred lines (6.2) and the hybrids (4.3). The average number of haploid kernels per ear did not change significantly, except for the MK01 line (Table 2).

Silk is known to appear gradually in maize. In our experiment, the frequencies of haploid kernels on the bottom and top half of ears were estimated (Table 3). The coefficients of correlation had negative values and in most cases were statistically significant. This kind of estimation at a greater degree showed the decrease of haploid-kernel frequency due to the delayed pollination. The highest percentage of kernels with haploid embryos was on the top half of the two-day ears, 11.3 on average for the lines, and 9.2 for the

Table 2. Average number of haploid kernels per ear in the four groups of ears and in general for each genotype, coefficients of correlation.

Maternal genotype	Day of pollination after silk emergence				General average	Coefficient of correlation, r
Inbred lines	2 days	4 days	7 days	10 days		
A464	7.8	7.2	7.8	5.1	7.0	-0.18
A619	10.4	13.8	10.1	8.2	10.6	-0.17
MK01	16.1	9.4	10.7	-	12.1	-0.37*
Mo17	11.2	7.5	-	-	9.3	-0.31
On average for lines					9.7	
Hybrids						
Modavian450	9.4	8.8	10.0	9.6	9.4	0.01
Porumbeni295	11.4	10.6	14.5	9.8	11.6	-0.04
Porumbeni359	11.4	11.2	6.3	6.4	8.8	-0.36
Mo17xB73	14.7	15.0	-	-	14.8	0.02
On average for hybrids					11.1	

* significant at 5% level.

hybrids. Thus, the highest frequency of haploid kernels was in ovules/silks that at the time of pollination were the youngest.

We hypothesize that the reason for the different influence of delayed pollination on the frequency of haploid kernels in our work and in the common opinion (delayed pollination increases haploid frequency) is connected with the unique way of haploid-kernel occurrence caused by inducers. Sarkar and Coe (1966), working with the Stock 6 inducer, found a higher frequency of haploid kernels at the top half of the ears. A spontaneous frequency of haploid induction in maize is 0.1% (Chase, 1951), whereas using inducers allows haploids with frequencies from 2.3% (Coe, 1959) up to 6% (Sarkar et. al, 1994; Shatskaya et. al, 1994; Chalyk, 1999) to be produced. In our experiment, several two-day ears at the top half had frequencies that exceeded 20%. Such essential distinction between the frequencies of spontaneous and induced occurrence of haploids and the contradiction of the influence of delayed pollination can be connected with the different causes of haploidy in these two cases.

Each year among haploids we find plants that have expression of marker genes which belong to the inducer; however, the plants do not differ from other haploids by their phenotype. Probably, these results of gene transformation have some causal reasons, but there is an opinion that it might be a product of the haploid induction. In other words, instead of one normal sperm there are some fragments of its DNA molecule in the embryo sac, and one of these fragments fertilizes the ovule which provokes its development. If we take this as a fact, then the plants produced by the inducers are not real haploids.

The assumption above needs to be proved experimentally. Now, we would like to discuss the possible reasons for the decrease of haploid-kernel frequency caused by delayed pollination in our experiment. The reason that might have an influence on the frequency of haploids is heterofertilization. It was found earlier that the frequency of heterofertilization in the MHI inducer is much higher than in a genotype without the haploid-inducing ability (Rotarenco and Eder, MNL 77, 2003). Additionally, this year, an experiment with the goal of revealing the influence of delayed pollination on the frequency of heterofertilization was carried out.

Two groups of plants of a heterogeneous population with two-day (21 ears) and ten-day (11 ears) ears were pollinated by a pollen mixture made (50/50) of the pollen collected from two lines, X28C (possessing the *R1-nj* gene) and 092 (no marker genes); neither line was a haploid inducer. The frequency of heterofertilization in the two-day-ear group was 0.48 %, and in the ten-day ears 1.97 %. The difference between the groups was significant at the 0.1% level. The average number of kernels per ear was 250. This establishes that the delay of pollination influenced the increase of heterofertilization frequency. Most likely, it is connected with an increase in the number of pollen tubes that penetrate into an embryo sac in older silk. Probably, this occurs because of an increase in the silk diameter during the plant vegetation. Therefore, in the case of single fertilization after pollination with a haploid-inducer in older silk, the opportunity of compensation of missing sperm from another pollen tube is high in comparison with young silk. This might be the reason for the decrease in haploid-kernel frequency.

Probably, the significant difference in the haploid frequency between the lines and the hybrids in our experiment is connected with heterofertilization (Table 1). Theoretically, the silk diameter in hybrids is bigger than in inbred lines, and consequently, the frequency of heterofertilization might be higher in hybrids, resulting in a negative influence on haploid induction.

Some additional experiments are needed to before reaching a final conclusion on this problem, but these results might be useful, especially for improving haploid inducers.

Table 3. Frequencies of haploid induction (%) at bottom and top half of ears in the four ear groups, coefficients of correlation.

Maternal genotype	Day of pollination after silk emergence								Coefficient of correlation, r	
	2 days		4 days		7 days		10 days			
	bottom	top	bottom	top	bottom	top	bottom	top	bottom	top
A464	4.2	8.1	4.8	4.9	3.3	5.2	1.1	4.2	-0.53*	-0.37*
A619	4.9	10.4	3.3	9.1	1.9	7.6	1.8	7.3	-0.54**	-0.37*
MK01	7.7	16.2	4.0	11.9	4.1	11.2	-	-	-0.54**	-0.45*
Mo17	4.0	10.4	2.7	8.9	-	-	-	-	-0.37*	-0.1
Modavian450	4.0	9.0	3.0	6.8	2.9	5.3	3.1	5.0	-0.19	-0.46*
Porumbeni295	2.3	7.4	1.7	4.4	2.5	7.1	1.7	5.5	-0.1	-0.17
Porumbeni359	4.3	11.2	1.6	7.3	0.9	4.6	2.3	3.9	-0.47*	-0.61**
Mo17xB73	3.5	9.1	2.2	6.8	-	-	-	-	-0.4*	-0.37*

*, ** significant at 5% and 1% level, respectively

Using oil content to identify kernels with haploid embryos

--Rotarenco, VA; Kirtoca, IH; Jacota, AG

To identify haploids in the dry-seed stage, the *R1-nj* marker gene (anthocyanin coloration of the top of the endosperm and embryo) is widely used. However, there are some inhibitor genes (*C1-I* and others) that are able to block the expression of the marker gene with the result that the selection of kernels with haploid embryos becomes impossible. These inhibitors are especially widespread in flint maize. Thus, there is a need to find an alternative way for the screening of haploid kernels.

Haploid plants differ significantly from diploids by their phenotype (Chalyk and Ostrovsky, 1993). Most likely, an embryo with the haploid number of chromosomes should differ from a diploid embryo by size, too. An embryo is known to contain up to 80% of the oil of a whole kernel, and the oil content has a positive correlation with the embryo size. Therefore, it was supposed that there might be a difference in oil content in kernels with diploid and haploid embryos. The purpose of our work was to compare the oil content in kernels with diploid and haploid embryos.

Eight genotypes (4 inbred lines and 4 hybrids) were selected for the analysis. First, they were crossed with the MHI haploid-inducing line (Chalyk, MNL 73, 1999). The selected maternal genotypes had rather good expression of the *R1-nj* gene that allowed the kernels with haploid embryos to be identified easily.

Diploid hybrids of the maternal genotypes and MHI (with colored embryos), and the haploid kernels (with colorless embryos) were used for the analysis of oil content. The sample size for each variant was 100 kernels. The analysis was carried out on the Sakslet's device modified by Rushkovskiy (1962).

The oil percentage of the haploids was lower than the diploids in all genotypes. The results of the analysis are shown in the Table. The averaged excess of the diploids over the haploids was 19.4%. The coefficient of correlation was 0.76 (significant at the 0.1% level).

Table. Oil content in kernels with haploid and diploid embryos and differences between them.

Genotype	Oil content, %		Difference, %
	n	2n (hybrids with MHI)	
Inbred lines			
A464	4.00	5.23	31.0
A619	4.60	5.44	18.3
MK01	4.16	4.75	14.2
Mo17	4.01	5.04	25.7
Hybrids			
Modavian450	4.04	4.92	21.8
Porumbeni295	4.73	5.33	12.7
Porumbeni359	3.78	4.47	18.3
Mo17xB73	3.86	4.37	13.2
On average for all genotypes	4.14	4.94	19.4

Our method of oil testing required the kernels to be ground up. However, there is a method of biochemical analysis (spectral analysis) that does not destroy kernels so that they can be used for further work. Thus, an oil test can be applied as a marker to identify kernels with haploid embryos. Besides solving the problem connected with the *R1-nj*-gene inhibitors, this kind of analysis might be used to mechanize haploid seed selection.

The first attempt to identify haploids by oil content was carried out at the Bavarian State Institute for Agronomy in 2002 (Germany), and the author of this note is very grateful to Dr. Eder for help.

Gamma-irradiation of seeds with haploid and diploid embryos

--Rotarenco, VA; Maslobrod, SN; Romanova IM; Mihailov, ME

Haploid plants have recently gained wider utilization in maize breeding programs. This was feasible after highly effective inducers of maternal haploids were discovered (Coe, 1959).

All genes, both dominant and recessive, are expressed at the level of haploid plants due to the absence of the second gene allele. Thus, the use of haploids for induced mutagenesis may allow more efficient identification of mutations. In addition, a higher mutation number may appear at the level of haploid plants in comparison with diploids--the reduction of gene repair efficiency in haploids might be one possible reason.

In order to induce mutations in haploids, their reaction to a mutagenic factor should be evaluated. The aim of this work was to establish the impact of different γ-irradiation doses on seeds with haploid embryos. Both haploid and diploid seeds of the heterogeneous SA population were irradiated. Dry seeds were irradiated at doses of 20, 40, 60, 80 and 100Gy. Soaked seeds (24 hours) were irradiated at doses of 2, 4, 6, 8 and 10Gy. 50 kernels were used in each treatment. Following irradiation, seeds were divided into two replications and grown in a growth chamber for 4 days (28°C). Root (main root) and coleoptile length were measured in seedlings, and the number of roots was counted. The results of the experiment are shown in the Table.

The irradiation of haploid seeds (soaked) resulted in a significant decrease of root length at doses of 4, 6, 8 and 10Gy as compared with control. A similar regularity was found for coleoptile length; however, significant differences were observed only at doses of 6 and 10Gy. This tendency was maintained for the parameter of root number, but the differences were not significant. The dose of 2Gy showed a tendency to stimulation for the three indices mentioned.

The irradiation of haploid dry seeds resulted in a decrease in root length, accompanied by intensification of the inhibiting effect beginning from a dose of 40Gy to 100Gy. As for coleoptile length, a significant difference at a dose of 100Gy was found. A significant reduction was discovered for root number beginning from a dose of 40Gy. A tendency toward stimulation was found at a dose of 20Gy.

The irradiation of diploid seeds (soaked) resulted in a decrease

Table. The parameters of 4-day seedlings after γ-irradiation of seeds.

Haploids					Diploids		
#	Dose, Gy	Coleoptile length, mm	Root length, mm	Number of roots, no.	Coleoptile length, mm	Root length, mm	Number of roots, no.
Soaked seeds							
1	Control	53.0±4.97	104.4±6.81	3.4±0.23	70.4±4.62	124.6±7.61	3.7±0.27
2	2	60.8±3.97	107.0±3.43	3.6±0.18	73.1±4.36	133.4±6.54	3.9±0.18
3	4	55.0±4.89	75.9**±5.79	3.0±0.22	71.9±3.66	132.1±4.70	4.3±0.18
4	6	42.5*±4.26	66.3***±5.49	3.0±0.22	73.1±5.11	114.6±7.42	3.3±0.20
5	8	53.0±3.26	76.0**±5.94	3.3±0.17	78.0±3.47	118.5±5.43	4.1±0.16
6	10	44.6*±2.92	50.7***±4.42	3.0±0.16	54.2*±5.25	75.3***±7.50	2.8*±0.26
Dry seeds							
1	Control	23.7±2.56	71.3±3.79	3.1±0.15	28.2±2.85	74.3±6.06	3.0±0.19
2	20	29.0±2.83	69.2±3.59	3.4±0.14	38.3±2.70	90.2*±4.58	3.3±0.15
3	40	18.9±2.05	49.1***±3.80	2.7±0.17	31.7±2.43	86.5±5.12	2.9±0.17
4	60	21.9±2.26	40.4***±3.30	2.3***±0.18	34.3±2.99	76.3±5.88	3.0±0.24
5	80	17.6±2.19	41.0***±3.06	2.3***±0.16	29.9*±2.74	79.9±5.23	2.9±0.19
6	100	11.0**±2.00	27.7***±2.62	1.8***±0.16	19.5*±2.23	53.1**±5.06	1.8**±0.16

*, **, *** significant at 5%, 1% and 0.1% level, respectively

in root length beginning at 6Gy; however, a significant difference was observed only at 10Gy. A similar trend towards stimulation at a dose of up to 8Gy, and a significant decrease at 10Gy was found for coleoptile length. A similar regularity was discovered for root number. The tendency towards the stimulating effect was observed up to 4Gy for root length, and up to 8Gy for coleoptile length and root number.

The irradiation of diploid dry seeds yielded an increase in root length, the reliable increase being found at 20Gy and 40Gy. A dose of 100Gy yielded a significant decrease. The tendency towards stimulation was observed at a dose of up to 80Gy for coleoptile length, but significant stimulation was established at a dose of 20Gy. A significant decrease was found at a dose of 100Gy. A significant decrease was observed at 100Gy for root number. Significant differences were not observed in germination rate between the treatments and regardless of the ploidy of the kernels; average germination rate was 95%.

Based on the results obtained, some conclusions can be made: a significant difference in sensitivity to irradiation was recorded in haploids in comparison with diploids; stimulation of seedling growth was found at minimal doses of irradiation in both diploid and haploid seeds, however, the stimulation was insignificant in most cases. This experiment allowed us to identify doses of γ-irradiation that can be employed to induce genetic variation using haploids.

COLUMBUS, OHIO
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GRASSIUS: A first step in establishing regulatory networks in maize and other grasses

--Palaniswamy, S; Gray, J; Davuluri, R; Grotewold, E

The increasing amount of genome sequence information in maize and other grasses makes it possible to start building genome-wide regulatory networks. Towards this goal, we have initiated the development of GRASSIUS, the Grass Regulatory Information Server (http://grassius.org/). The ultimate goal of GRASSIUS is the integration of information on transcription factors and *cis*-regulatory elements into regulatory motifs, the building blocks of regulatory networks, across the grasses. Thus, GRASSIUS contains growing databases on maize, rice, sorghum and sugarcane transcription factors (GRASSTFDB) and promoter elements (GRASSPROMDB). As a first step towards the generation of GRASSIUS, a uniform nomenclature system for proteins corresponding to transcription factor was adopted, in which an organismal identifier (e.g., Zm) is followed by a letter code corresponding to the transcription factor family member (e.g., MYB), followed by a number. In this way, for example, a synonym for the *C1*-encoded protein would be ZmMYB1. Transcription factors from these grasses are currently being grouped into 43 families (Fig. 1), representing the major classes of regulatory proteins identified in other plants (e.g., *Arabidopsis*, see http://arabidopsis.med.ohio-state.edu/). GRASSIUS is expected to significantly benefit from community input, for example through voluntary curation contributions. The Grass Annotation Tool (GAT) is under implementation, which would facilitate easy submission, curation and validation of new and existing experimental data. Experimental data will include plasmid clone descriptions/maps and validated sequences from full-length cDNAs for each transcription family member. Distribution information for clones generated will be made available, translating into time and cost savings for community users. Web services provide a standard way of accessing publishing applications and data sources over the internet, enabling mass acquisition and dissemination of knowledge. We would be making use of existing web services (*WSDbfetch, CitationExplorer)* to semi-automate the process of acquiring new data for GRASSIUS from literature and from other existing resources and we would also be making GRASSIUS data available as web services (Bio Moby) in a phased manner when a specific plant module in our database is completed. The Web interface for GRASSIUS was developed using the Java J2EE platform, and the databases were developed using MySql. The GRASSIUS database will be constantly updated, thus stay tuned.

The current release of Grass TFDB classified into 43 families

ABI3VP1
ARF Family
BBR/BPC (BBR) Family
CAMIA (CAM) Family
CCAAT-HAP3 (HAP) Family
E2F-DP (E2F) Family
GeBP (GBP) Family
Homeobox (HB) Family
JUMONJI (JMJ) Family
MYB-related (MYB-R) Family
SBP Family
TUB Family
ZF-HD Family

Alfin like (ALF) Family
ARID Family
bHLH Family
CCAAT-DR1 (CDR) Family
CCAAT-HAP5 (CAP) Family
EIL Family
GRAS Family
HRT Family
MADS Family
NAC Family
TCP Family
VOZ Family
Zinc Finger Families
C2C2-CO-like, C2C2-Dof,
C2C2-GATA, C2C2-YABBY,
C2H2, C3H

AP2-EREBP (EREBP) Family
ARR-B (ARR) Family
bZIP Family
CCAAT-HAP2 (CHP) Family
CPP Family
G2-Related (G2-R) Family
GRF Family
HSF Family
MYB Family
PHD Family
Trihelix (THX) Family
WRKY Family

Figure 1. Initial classification of regulatory proteins into 43 families. (For color see online.)

CORVALLIS, OREGON
Oregon State University

Microarray evidence for ABA-GA antagonism during embryo maturation

--Carroll, KA; Kulhanek, D; Fowler, J; Rivin, C

Complex developmental processes are often controlled by the interplay of positive and antagonistic, or modulating, signaling pathways. The switch between embryogenesis and the maturation phase of maize embryo development involves the interaction of antagonistic signaling pathways governed by abscisic acid (ABA) and gibberellic acids (GAs). Abscisic acid (ABA) is a highly conserved hormone signal required to induce maturation phase in developing plant embryos. During embryonic development in cereals, bioactive GAs accumulate, peaking prior to the ABA peak that initiates maturation phase. Although ABA's role in maturation is highly conserved in plants, a preceding GA peak is found only in cereals, and its significance is unclear. We have previously used both genetics and manipulation of hormone levels in culture to support our idea that the pre-maturation GA peak antagonizes ABA in controlling maturation-phase processes in maize (White and Rivin, Plant Physiol. 122:1089-1097, 2000; White et al., Plant Physiol. 122:1081-1088, 2000). In these studies, we found that ABA-deficient kernels are viviparous (germinating precociously on the ear) and desiccation-sensitive, but that mutants deficient in *both* ABA and GA exhibit the wild-type phenotypes of quiescence and desiccation tolerance. Thus, the early GA peak may either intercept the ABA signaling pathway to modulate ABA sensitivity, or participate in a negative regulatory mechanism to suppress maturation independently of ABA.

The wildtype behavior of ABA / GA double-deficiency embryos suggests that gene expression in this genotype is more like that in wildtypes than in ABA-deficient mutants. To test this proposition, we collected early maturation (stage 3) embryos from two types of ears: 1) ears segregating for *vp5* (ABA-deficient kernels) and 2) *d1* homozygotes (bioactive GA deficient) segregating for *vp5* kernels. mRNA was isolated from sibling wildtype and *vp5* homozygous embryos from each type of ear for comparison by microarray analysis. A loop-design hybridization scheme was used to compare the message profiles of the four genotypes, using the maize oligonucleotide array produced by the University of Arizona. Bioconductor and Limma software packages were used to identify genes with significantly different expression based on an adjusted P value $p < 0.05$.

In a comparison of wildtype and ABA-deficient sibling embryos at Stage 3 of embryogenesis, 75 moderate to highly expressed genes were found to be significantly different in expression between the normal and hormone-deficient condition. Of these genes, 70 were also found to be significantly different in a comparison of sibling embryo mRNAs from the double ABA/GA vs. single ABA-deficient ears, an 89% overlap in expression patterns indicating that gene expression in the double hormone mutants is very similar to wildtype on a broad scale. The differentially expressed genes included well-known maturation genes like the storage globulins and LEA proteins previously shown to be regulated by ABA and the *Vp1* transcription factor, but a wide variety of other genes, not known to be ABA regulated, also appeared in this gene set.

Practical advice on using the maize oligonucleotide microarray

--Carroll, KA; Rivin, C

Microarrays have become a popular method to monitor gene expression levels on a genomic scale. We have been using the array produced by the Maize Oligonucleotide Array Project at University of Arizona. We have generally followed the protocols provided on the project website (www.maizearray.org), and we have also tried modifications. Based on our experience with microarray experiments, we have the following recommendations for people who may be interested in starting a microarray experiment. Please feel free to contact us if you have any questions or would like more information.

1. Successful modifications to the project protocols. We used the protocols for cRNA targets provided on the website with the following alterations.

a. During RNA purification step we adjusted the total elution volume to 100 µl (65 1st, 45 2nd) instead of the recommended 60 µl. Our yields ranged from 20-40 µg aRNA.
b. We experienced up to 50% reduction in yield during the Cy Dye coupling step due to cRNA adherence to the column. To help alleviate this we used 50ºC DEPC water for the elution steps and also heated the entire column during the elution for ~ 5 minutes in a 50ºC hybridization oven. This increased the yield of labeled aRNA to about 80%.
c. In fear of washing the oligos off the microarray slides, we opted to skip the rehydration steps as recommended in the protocol under *DNA Probe Immobilization* and simply cross-linked and washed the slides as described.

2. Use of aliens as a control feature for cRNA targets. Aliens are control RNAs that can be added to the total RNA as a standard for data normalization and scanning. Stragene alien sequences 1-10 are printed on the maize array. To take advantage of this control, we used mRNA spikes from the Stratagene SpotReport® Alien® cDNA Array Validation System in our amplification and hybridizations. In our hands, the aliens created problems during scanning as they drastically reduced the signals from other features. We also found that the aliens could not be used to manually adjust the scanner for equal red and green intensities. Our core facility has a Perkin Elmer ScanArray 4000 and Genepix software for microarray scanning and analysis. Using this scanner and software the auto PMT setting was found to be optimal for adjusting the signal intensities for all scans (the saturation levels were adjusted from the default settings of .05% to .005 % when using the auto PMT setting).

*3. Use of Dyesaver for fluor preservation***.** Dyesaver, by Genesphere, is a toluene-based material coating which is applied to the slides after hybridization and washing. It is recommended to help preserve the fluorochromes from degradation, especially the Cy5 which is more easily degraded than the Cy3 dye. Our experience is that Dyesaver is expensive and may not be necessary for repeated scanning. As an experiment, we used the "practice" slides supplied to us by the Maize Array Project for two identical hybridizations, one with Dyesaver and the other without. The data from both slides produced similar results. The slide without the Dyesaver was scanned at least 4 times with only a minor loss in fluor intensity, using the auto PMT setting, with laser power settings between 70-90%. Slides that were coated in Dyesaver did maintain their integrity for several months, unlike untreated slides which expire rapidly. The major disadvantages of Dyesaver were the toxic toluene fumes which made it unpleasant to work with, high evaporation rate of the dye during storage drastically reducing the number of slides on which one can actually use the dye, and the overall green hue it gives to the slides.

4. Data analysis using Bioconductor freeware (bioconductor.org), which uses the R computing environment (www.r-project.org), requires writing customized Perl scripts. The main advantage to using Bioconductor is that it is one of the most powerful software packages to use for microarray statistical analysis. The main disadvantage, however, is that it uses a command driven user interface and therefore is not user friendly for most scientists. We needed to create customized scripts effectively filter and normalize our data. In Bioconductor we used the Limma package (also available at www.r-project.org), which uses the empirical Bayesian method to create a linear model to evaluate genes with significant differential expression. We would be happy to share our scripts for filtering, normalization and linear model analysis.

DAEGU, SOUTH KOREA
Kyungpook National University and the International Corn Foundation

Severe epidemics of downy mildew (*Perosclerospora sorghi*) on maize in Cambodia, East Timor and Vietnam

--Kim, SK; Yoon, NM; Kim, HJ; Kim, YB; Chhay, N; Kim, SM; Oeun, KS; Bora, P; Glaudino, N; Fontes, L; Tam, TT; Cho, MC

Downy mildew (*Perosclerospora sorghi*) is still considered the most damaging disease of maize (*Zea mays* L.) in South Asia. Since the early 1990s, downy mildew (DM) has been a minor problem in Thailand, Indonesia, the Philippines and Taiwan. Breeding for resistance materials and the uses of chemicals such as Ridomil have played a catalytic role in reducing the spread of the disease in the region. However, the same disease has produced epidemics in Cambodia, East Timor and Vietnam, recently. A study of DM was carried out in East Timor and Ben Tre Province in Vietnam for three years, and at the ICF/Cambodia Banteay Dek Agricultural Research Station, for two years (2005-2006). This paper reports the results of DM infections at the station and farmers' fields.

In East Timor, severe infections of DM were observed in farmers' fields and the Lapos station of the CIMMYT/Australia maize trials in February 2003. DMR lines evaluated include: a Thailand open-pollinated variety (OPV), Suwan 1, and lines from IITA, Nigeria and CIMMYT, Mexico. Among the IITA DMR materials, TZDMR-ESR-Y appears to be the best. Using Suwan 5, Kalinga (Indonesia), TZDMR-ESR, the project has developed several DMR variety crosses. Both OPV and variety crosses are being tested at research stations and in farmers' fields. DMR materials will be recommended to East Timor.

In Ben Tre Province of Vietnam (the first province of the Mekong Delta), DMR materials from IITA and Thailand have been tested for three years (October, 2002) with the assumption that DM would be the key biotic constraint of maize cultivation in the country. However, DM was observed in March 2006 to be widely present in the country. Waxy hybrids introduced from Thailand and locally bred field corn hybrids were found to be highly susceptible. Five DMR OPVs were selected. The best known DMR OPV, Suwan 1 showed an unknown black ear rot. Maize programs in Vietnam must focus on DMR breeding to block further spread of DM nation wide.

In Cambodia, DM is the number 1 production constraint for maize cultivation. Severe epidemics of DM infection were observed from several farmers' fields in the Phnom Penh area in August, 2005. The program has focused on DMR materials for Cambodia. Ten different plantings have been made to screen DMR and segregating materials using the ICF/Cambodia Banteay Dek Agricultural Research Station. The station was established by the Government of Hungary 15 years ago. Among the 50 materials tested, four DMR OPVs showed an acceptable level of tolerance. They are Suwan 5 (coded as KC35), Suwan 1 (coded as KC6) from Thailand and TZDMR-ESR-Y (coded as KC25) and TZDMR-LSR-Y (coded as KC4) from IITA. A CIMMYT DMR conversion, EV28-DMR, and several other DMR materials segregate for resistance. DMR genes are being incorporated into farmers' preferred local waxy materials.

GUELPH, CANADA
University of Guelph

http://www.MaizeLink.org: A searchable database linking maize experts from around the world

--Makhijani, R; Wight, C; Radford, D; Kajenthira, A; Papineau, E; Raizada, MN

We have developed a searchable database, online at www.MaizeLink.org, linking maize experts with their colleagues from around the world. Developed at the University of Guelph, the aim of MaizeLink.org is to provide an online environment where researchers can share their challenges, questions and resources with other researchers across sub-disciplines.

MaizeLink.org provides a very easy to use searchable interface to access researcher information from 80 sub-disciplines, including molecular genomics, abiotic stress, agronomy, breeding, ecology, food safety, nutrition, plant disease, agribusiness and social issues. Users have the ability to search by name, institution, country, research keyword or area of technical expertise. Alternatively, users may browse by research subject area. Query results consist of a researcher list linking to individual profile pages. A profile page contains the following customized information, some of which is entered into the database by researchers at the time of registration:

- researcher contact details
- a summary of a researcher's key challenges
- a summary of the researcher's interests
- links to the best introductory publications or URLs in the researcher's area of interest or technical expertise
- links to a researcher's favorite URLs
- links to useful technical protocols

In addition, a query auto-generates the following information:

- links to researcher publications in popular databases (e.g., PubMed, Agricola, MaizeGDB)
- links to researcher patents (e.g., U.S., European, Japanese and worldwide patent databases)
- researcher grant abstracts from around the world

MaizeLink.org integrates a bulletin board, a forum to post messages or questions and exchange information online. The bulletin board is completely searchable. Our objective is to make the MaizeLink bulletin board the central communication portal for the world's maize research community by pooling expertise from diverse sub-disciplines.

MaizeLink also includes LifeSciLink, a function that provides access to publications, patents and grant abstracts for all researchers in the life sciences, not only those registered in MaizeLink. A user simply enters a researcher's name, and the system automatically searches a collection of public databases from around the world, returning results on a single page. This functionality is also available separately at http://www.LifeSciLink.org.

In addition, MaizeLink incorporates EquipmentLink, a place for researchers to donate and seek equipment and materials to/from other researchers and educators around the world. The infrastructure required to perform research can be expensive, especially for researchers in developing nations. We hope that EquipmentLink will help to meet this challenge.

Over the coming months, MaizeLink.org will grow to include at least 20 major crops and research model systems, part of the CropLink Global Initiative. Our goal is to make CropLink into the world's most comprehensive online researcher-to-researcher portal for plant science and agriculture.

All of these databases are publicly accessible, and registration is not required to conduct searches. An effort has been made to include Open Source journal databases. Having a customized profile page does require registration, but is open to all graduate students, research fellows and associates, faculty, and private sector scientists with a shared interest in maize. Because many researchers, particularly in the developing world, do not have a webpage/lab page, the profile page is an attempt to provide a basic, free website for all of the world's agricultural researchers. All of our databases are secure and designed to prevent third-party users from sending batch Spam emails. MaizeLink.org is a nonprofit initiative intended solely for research purposes.

We must feed more people in the next 40 years than we have in the last 10,000 years combined. This great challenge will require more extensive collaboration between researchers across diverse crops, subdisciplines and nations. It is our hope that MaizeLink, CropLink, LifeSciLink and EquipmentLink will be useful tools in this endeavor.

HONOLULU, HAWAII
University of Hawaii

Near isogenic lines (NIL) of inbred Hi27

--Brewbaker, JL; Josue, AD

The Hi27 NIL series was initiated in 1967 to provide tropically-adapted mutants to scientists working in the tropics (MNL 42:37-38). Each mutant was to be backcrossed at least six times to Hi27, a hardy tropical flint inbred that we selfed out of inbred CM104, created in India as a sib-line from the Colombian flint Amarillo Theobromina (pedigree = A Theo 21-B-6#-15-7#). Hi27 generally tolerates most tropical diseases and environmental insults, and is homozygous for loci such as *A1 A2 b Bz C-I Mv p-ww Pl pr r Y*. In 1995, we published a list of mutant loci that had been entered through backcrossing (MNL 69:58-59). All of the mutants listed at that time have now been backcrossed six or more times to Hi27, with the following changes:

(1) Mutants that could not be maintained from the 1995 list: *bk, bt2, lc, mn, pg2, rf, rt, v2* and *w3*

(2) Mutants that have been added to this list: *a2, bk2, bt1-A, c2, j2, ms6, ms8, o5, Tlr, y8* and *y11*

(3) New mutants (temporary symbols) under study: blo (blotch), bst (brown-stripe), dcb (double-cob), lc2 (leaf-color), lfl (leaf-fleck), nl3 (narrow-leaf), os (opaque-small), sky (skinny) and zb232 (zebra)

The complete NIL set now includes 97 mapped genes and the 9 mutants under study. More than 200 genotypes are now available, including many digenic and multi-genic combinations such as *bm3 gt* and *C sh bz wx*. All are being provided to the Maize Genetics Coop, e.g., symbolized *sh2*^Hi27.

Grassy tiller and sweet corn

--Brewbaker, JL

Tillering is a characteristic of early American sweet corns (*sugary1*), but is rarely found in other races or types of maize. We report here that all of the early sweet corns we've tested carry the gene, *grassy tiller (gt)*. Grassy-tillered plants also produce leaves that extend the husks ("husk leaves" or "flag leaves"), a feature not noted in the genetic literature but of utility to processors of temperate sweet corn for removal of husks.

Our breeding of tropical sweet corns in Hawaii has been based entirely on hybrids of temperate (tillered) and tropical (non-tillered) types. All of the >20 open-pollinated populations we've released of this type segregated tillered plants as a recessive trait (Brewbaker, HortSci. 33:1262-4). Tillered plants were also marked by presence of husk leaves that segregated as a recessive monogene (MNL 79:14), now known also to be *gt1*.

In the present study, temperate sweet corn inbreds provided by Bill Tracy (U. Wis.) were crossed with two sources of *gt*, one based on population WGRComp2 from Jim Coors (U. Wis.) and one, *gt*^Hi27 from our near-isogenic line series (MNL 69:58-9). The temperate inbreds were:

sugary1:	101t, C5, C40, Hotevilla AZ, P39, P51
shrunken2:	Ia453*sh2*

Hybrids of these sweet inbreds with *gt* stocks were all highly tillered, with long flag leaves (Figure 1). All F2 populations grown from these hybrids were also 100% tillered. One recombinant inbred population (SET M) based on the cross of Ia453*sh2* (tillered) with Hi38*bt* (no tillers) segregated 19 tillered and 27 non-

Figure 1. Grassy tillered hybrid of NIL *gt*^Hi27 with sweet corn inbred P51. (For color see online.)

tillered RILs, while the F2 of this cross segregated 3:1 for normal to grassy tillered. One of the tillering NILs, M23, was crossed to a *gt* stock and produced only grassy-tillered hybrids.

The number and size of tillers and husk leaves is highly correlated with plant vigor. Experimental trials at Waimanalo, Hawaii, are planted year-round, and corn biomass yields in summer are roughly double those in winter. Yields are reduced largely by low light in our wet winters (Jan. avg. 275 cal/cm^{-2}day^{-1}) vs. the dry summers (July avg. 450 cal/cm^{-2}day^{-1}). Tiller numbers are reduced in winter; the tiller heights of inbred *gt*^Hi27 were reduced to <6" in winter vs. >18" in summer. Vigorous *+/gt* hybrids often produce small flag leaves in the summer also. High plant density and low nitrogen fertility reduce the expression of tillers and flag leaves. Husk-leaf extension increased greatly in Hawaii's summer trials for many hugely tillering Korean genotypes (MNL 59:14).

Other highly tillering genes include *Tlr* (tillering) and *tb* (teosinte-branched), and both of these mutants also have long flag leaves. The genes are on long arm of chromosome 1 and possibly allelic. Both genes have a major effect on ear morphology, unlike *gt*. The *Tlr/Tlr* homozygote is extremely grassy in Hawaii and has abortive ears. It resembles *Cg* (corngrass), a mutant that also leads to tillering and flag leaves.

Teosinte species tiller abundantly like most grasses, presumably based on genes like *Tlr*. Our hybrids of maize with Jutiapa teosinte and with *Zea diploperennis* were all highly tillered, showing tillering to be dominant (cf. Fig. 1, Srinivasan and Brewbaker, Maydica 44:353-370). In the referenced study Srinivasan produced 11 hybrids between tropical maize inbreds (with only the single main culm) and *Z. diploperennis* (avg. 18.3 tillers in winter, 29.3 in summer). In summer plantings the F1 plants averaged 5.4 tillers, F2's averaged 3.4, backcrosses to maize averaged 1.6 and backcrosses to Z.d. averaged 2.9. Generation mean analysis showed that narrow-sense heritability was high (81%) and based largely on dominance and epistatic (dd) interactions. At least two loci were inferred. Winter data for the Z.d. x maize populations showed that tillers were reduced an average of 13.8% for the four generations, with similar reduction in heritability.

We have bred a broad-based population, HIC9d, from backcrosses of these Z.d. hybrids to maize. It segregates about 10% tillered plants. The population is highly heterogeneous for tiller and husk leaf extension, and for vigor, prolificacy and many ear traits. It is being tested for allelism of tillering genes to *gt* and *Tlr*.

The Maize Genetics Coop gene *gt* is located near the centromere on chromosome 1 and is attributed to Don Shaver (MNL 39:18-22), who writes (pers. commun.) "Earl Patterson had told me that E. G. Anderson found it or discovered it (at Cal Tech)". The *gt* in our NIL set (reported in MNL 69:58-9) derives from the MGC stock *gt/gt id/id* (66Cal, 3327x28) that seems to have the same origin, out of mutants from Bikini in Anderson's collection in 1948, a nursery in which I was privileged to work with Earl, Ed Coe and Andy. However, Walt Galinat (pers. commun.) notes his early interest in tillering and the possibility that the N.E. sugary lines in his program provided the *gt* locus of Shaver, who made hybrids of Galinat's sweet corns with *id* (also found on chromosome 1L) and *pe* stocks in studies of perennialism in maize (Shaver, J. Hered. 58:270-273; MNL 79:39-41). In any event, the two sources appear to be identical alleles.

In view of the rarity of tillering in maize, the independent origin of *gt* in early American sweet corns or their progenitors appears highly probable. Mysteriously eluding early authors on this subject was the fact that *gt* also controls husk-leaf extension, a feature that became of value to the temperate sweet corn industry by facilitating husk removal during processing. In Thailand, the tropical supersweets with Hawaiian ancestry (many husks but no husk leaves) from 150,000 A. annually are husked following sprays with hot water (Taweesak Pulam, pers. commun.). It is unclear whether genotypes exist with flag leaves but no capacity or totipotency for tillering. We suspect that source of cytoplasm must be considered in unravelling the perennialism of *Z. diploperennis* that has been elusive in maize hybrids with genes like *gt, Tlr, id* and *pe*.

Heterosis among near-isogenic lines of Hi27

--Josue, AD; Brewbaker, JL

Ten mutants in our Hi27 NIL series, one on each of the 10 chromosomes, were chosen for a diallel analysis of heterosis. Each mutant had been backcrossed at least six times to Hi27, hardy tropical flint inbred (see above). Our NILs are sibbed following backcrossing, allowing preservation of some heterozygosity (1.5625% >BC6, 0.0977% >BC10). However, there is much evidence of linkage drag in such conversions, linkage that could also be associated with inter-NIL heterosis. Linkage drag with loci *na1*^Hi27 (3L-101) and *lg2*^Hi27 (3L-113) led us (Ming et al., MNL 69:60) to the *Mv/mv* locus on chromosome 3L-80 (all temperate corn carries allele *mv* for susceptibility to the tropical maize mosaic virus). Current studies in Hawaii seek to use linkage drag in spotting other QTLs of importance to corn breeders.

It can be conjectured that QTLs for yield heterosis are often linked to mutant genes we've backcrossed into Hi27. To test our hypothesis, ten NIL (one per chromosome) were crossed in a diallel manner, including parent Hi27 (Griffing method 2). Mutants selected were located at 1S-55 (*gt*^Hi27), 2S-11 (*lg*^Hi27), 3L-149.0 (*a*^Hi27), 4S-(55) (*bm3*^Hi27), 5S-41 (*bm*^Hi27), 6L-17 (*y*^Hi27), 7S-16 (*o2*^Hi27), 8-(0) (*rf4*^Hi27), 9S-31 (*bz*^Hi27) and 10L-64 (*R-nj*^Hi27). Mutant *rf4* had been advanced 12 backcrosses. The diallel entries were planted in single-row 5m plots in Field S1-4 at Waimanalo on May 23 and June 21, 2006. Data were taken from two samples per row of 5 plants, with months treated as replications.

Heterosis among the 53 hybrids (two were omitted due to poor stand) was universal for measured traits. Highly significant differences (P<0.001) were observed for yield (as gm. per plant), for ear length and ear diameters in cm. (Table 1), and also for plant heights (not shown). The Experimental Error interaction of NIL x "Reps" (months) was never significant when tested against sampling error, a reflection of the homogeneity of Waimanalo soils on which our breeding nurseries have been grown since the 1960's.

Table 1. ANOVA for yield, ear length and ear diameter.

Source	df	Yield	EL	ED
Entries	63	456.3**	1.60**	0.05**
Reps	1	420.9 ns	2.41*	0.09*
NIL & Parent	10	277.6*	2.04**	0.06**
F1s	52	375.3**	1.10**	0.04**
Heterosis (NIL vs F1s)	1	6,456.4**	22.93**	0.39**
EE (Ent x Rep)	63	115.7 ns	0.39 ns	0.01 ns
SE	128	584.4	3.47	0.13

**,* - Significant at the 5% and 1% level of probability

Means and standard deviations of these three sets of data are summarized in Table 2. The NILs were similar to their parent inbred Hi27, while all hybrids were significantly higher in yield and ear traits. Relative homogeneity characterized all traits, as evident in the standard deviations and CV values.

Table 2. Means and standard deviations for yield and ear traits.

	Yield	EL	ED
Hi27	92.5 ± 16.9	13.8 ± 1.4	3.95 ± 0.17
NILs	93.8 ± 20.4	12.7 ± 1.7	4.05 ± 0.32
Hybrids	121.1 ± 17.8	14.4 ± 1.3	4.25 ± 0.27

Individual variations were seen in GCA (general combining ability) and mid-parent heterosis values, and these will be studied in greater detail following duplicate plantings in 2007. Mutant *bz*^Hi27 had the highest GCA for yield, but it is in a known linkage group with *C*, and would be expected to have greater linkage drag in our conversions to Hi27 (which is *Bz C-I*). GCA for hybrid yield minimized for *rf4*^Hi27 (97.6 gm/plant). However, the *rf4* conversion represented BC12 (~ .0244% heterozygosity) and the male-sterile hybrids were grown as a block, both facts helping account for their reduced apparent heterosis for yield.

Heterosis among NIL hybrids clearly can reflect the remnant of heterozygosity among their very distinct temperate dent and tropical flint parents; indeed, we make much use of modified sister-single crosses in our supersweet tropical breeding to exploit this kind of heterosis. But it is similarly clear that linkage drag with QTLs affecting vigor and yield may play a role in this heterotic response. The dent x flint heterosis is very widely exploited in tropical corn breeding, and localization of significant QTLs may improve our genetic advance.

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Presumable redox control of phosphorylation of the mitochondrial chaperonin hsp60

--Subota, IY; Arziev, AS; Sengenko, LP; Tarasenko, VI; Konstantinov, YM

It was shown previously (MNL 80:14-15) that phosphorylation/dephosphorylation of serine/threonine or histidine residues of the target mitochondrial proteins is presumably involved in the metabolic response of mitochondria under the changes of redox conditions. To date redox-dependent phosphorylation of mitochondrial proteins has not been sufficiently elucidated. Although this modification has been observed in our experiments for at least 8 maize mitochondrial proteins (MNL 80:14-15), the nature of the polypeptides and the function of phosphorylation for these proteins remain poorly understood. In this work, we show that one of the mitochondrial phosphoproteins is the heat shock protein 60 (hsp60).

The mitochondria were isolated from 3-day-old etiolated maize seedlings of hybrid VIR42MV, by a standard method of differential centrifugation. Protein phosphorylation assays were carried out according to Struglics et al. (FEBS Lett. 475:213-217, 2000) with the use of [γ32P]ATP at 6000 Ci/mmol.

By immunoblotting with specific antibodies, we have identified one of 8 mitochondrial phosphoproteins as mitochondrial chaperonin hsp60 (Fig. 1). Mitochondrial chaperonin hsp60 is required for ATP-dependent folding of precursor polypeptides and complex assembly. It also prevents aggregation and mediates protein refolding after heat shock. There is also some evidence of hsp60 involvement in the structure and transmission of mitochondrial DNA nucleoids in *Saccharomyces cerevisiae* (Kaufman et al., J. Cell. Biol. 163:457-461, 2003). We suggest that this evolutionarily conserved hsp60 participates in redox regulation of mitochondrial genome expression and is possibly mediated by reversible redox-dependent phosphorylation.

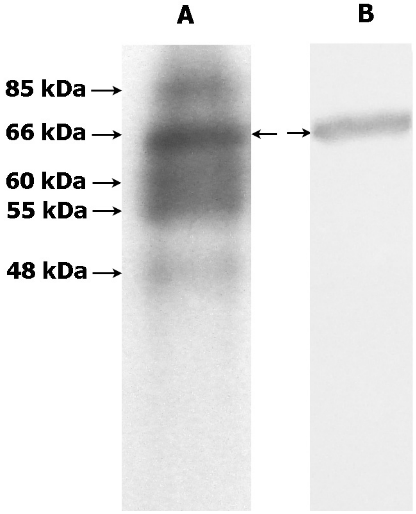

Figure 1. In vitro phosphorylation of redox-sensitive phosphoproteins including 66 kDa (A) were resolved by 12% SDS/PAGE and were immunoblotted (B) with antibody against hsp60.

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Adding B-chromosomes of *Zea mays* L. to the genome of *Avena sativa* L.

--Kynast RG; Galatowitsch, MW; Huettl, PA; Phillips, RL; Rines, HW

B-chromosomes (Bs) are supernumerary dispensable chromosomes described in hundreds of animal and plant species, including maize (*Zea mays* L.). However, Bs have not been reported to exist in hexaploid oat (*Avena sativa* L.).

In order to transfer maize Bs sexually from maize to oat genomes, we chose the maize cultivar Black Mexican Sweet (a well known sweet corn line hosting Bs in different numbers) as the B donor (male parent) and the oat cultivars Starter, Sun II and Paul as potential B recipients (female parent) for inter-species cross-hybridizations. Since all of these direct crossings of Black Mexican Sweet to each of the oat cultivars failed to produce vigorous F1 offspring, we used in a further experimental series as the male parent a backcross line of the maize inbred B73 harboring Bs from Black Mexican Sweet. The $B73^B$ derivative is the 5th backcross generation of the F1 (B73 × Black Mexican Sweet) hybrid to B73. BC5 seeds with hexasomic B addition (BC5-$B73^B$, $2n = 2x+6_B = 26$) were generously provided by J. A. Birchler, University of Missouri Columbia. This genotype based on B73 germplasm seemed more promising because, recently, Kynast et al., PNAS 101:9921-9926, 2004) had reported the successful crossing of B73 without Bs to different oat genotypes.

All parental plants were cultivated in growth chambers to deliver favorable environmental conditions for germination and plant growth, and to synchronize the peak of pistil receptiveness in oat plants with the peak of pollen grain release in maize plants. For inter-species crossing, the stigmas of emasculated oat florets were hand-pollinated with freshly shed maize pollen by using a fine camelhair brush. The panicles with pollinated florets were isolated in glassine bags, then 24-48 hours after pollination sprayed with a mixture of 50 ppm 2,4-D and 50 ppm GA_3 and again bagged during further cultivation in the growth chambers.

From 2341 ovaries, 115 immature F1 (oat × maize) embryos (Table 1) were in vitro rescued 14-15 days after pollination. The F1 embryos were cultivated on modified one-half strength MS-medium. A total of 31 F1 embryos germinated and developed into vigorous plantlets large enough for molecular and cytogenetic analyses. Plantlets were further grown in growth chambers with optimal growing conditions to produce F2 seeds for testing (1) fertility of (aneu)haploid oat plants with added maize Bs and (2) transmission of maize Bs to the offspring in an oat background.

Table 1. Plant material for crossing three different oat cultivars (2n = 6x = 42) by the maize $B73^B$ ($2n = 2x+6_B = 26$) and results of maize B-positive offspring production.

Oat cultivars	Starter	Sun II	Paul	Total
Oat panicles	40	53	3	96
Oat florets, emasculated and hand-pollinated	1177	1094	70	2341
F1 proembryos, rescued 14-15 dap*	62	52	1	115
F1 embryos, geminated**	14	16	1	31
Maize (A and/or B)-positive juvenile F1 plantlets (shoot- and root-tested)	7	6	1	14
Maize B-positive adult F1 plants*** (tiller-tested)	2	0	0	2
Maize B-positive F2 offspring / Total F2 offspring (shoot- and root-tested)	20 / 30	0 / 0	0 / 0	20 / 30

*Days after pollination; **Embryos that formed shoot and root with enough tissue for molecular and cytogenetic analyses; ***Plants represent tillers that are clone parts of two clones after extensive tiller cloning of both F1 plants allowing for more F2 seed production

Two F1 plantlets (5811-1 and 5845-1) were found to have retained maize chromosomes in shoot tissues based on results from a PCR assay for *Grande1*, a dispersed LTR-type retrotransposon which is abundant on all A chromosomes (As) and Bs of maize, but absent from all chromosomes of the oat genotypes used in our crossing program. PCR assays involving two B-specific markers (primer pair *p-2ndB1* + *p-2ndb4* and primer pair *p-brpt2* + *p-taralb1*; generously provided by J. A. Birchler, University of Missouri Columbia) and a selected set of A-specific markers for maize [chromosome arm-specific SSR markers selected from the 'Maize Genetics and Genomics Database' (http://www.maizegdb.org/)] showed that in both genotypes the *Grande1*-positive PCR products represented the presence of maize Bs and not maize As (Fig. 1).

Cytological analyses on very young, juvenile plantlets revealed that in the F1 plant 5811-1, all ten maize As had been eliminated and a complete set of 21 oat chromosomes plus three maize Bs ($2n = 3x+3_B = 24$) were retained in its primary root meristem. In the primary root meristem of the F1 plant 5845-1, all ten maize As had been eliminated with a complete set of 21 oat chromosomes and a single maize B retained ($2n = 3x+1_B = 22$).

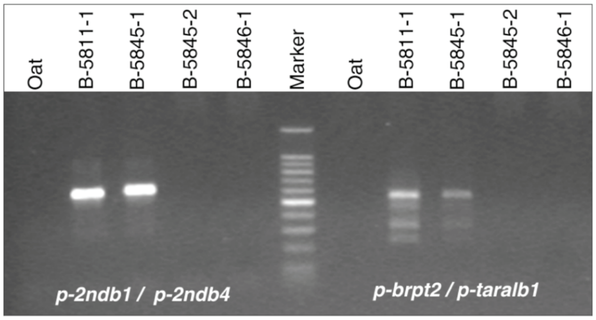

Figure 1. PCR products of B-chromosome-specific markers after electrophoresis in 1.5% agarose; both markers demonstrate the presence of B-chromatin in the two F1 plants 5811-1 and 5845-1 and the absence of B-chromatin in two examples of B-negative F1 plants (5845-2 and 5846-1).

Both F1 plants were kept under short-day conditions to allow plants to tiller extensively for continuing tiller cloning. Tiller cloning provides an extended source of leaves for the extraction of genomic DNA and RNA, and for further seed production. Both genotypes are descended from a (Starter × $B73^B$) cross. Hence they represent B additions in a Starter background. The phenotypes of both mature F1 plants did not differ from those of haploids of Starter without Bs at any point in time during their growth period.

After shifting individual tiller clones into long-day growing conditions, self-pollination produced F2 seed of both genotypes (Table 1). This fertility could be attributed to the frequent formation of numerically unreduced female and male gametes. High fertility had already been observed in oat haploids of Starter, Sun II and Paul without and with individual maize *As* of B73 (Rines et al., In*:* Jain, Sopory, Veilleux (eds.) Kluwer Acad. Publishers, Dordrecht, The Netherlands, In vitro haploid production in higher plants 4, 205-221, 1997; Kynast et al., 2004).

Cytological and molecular analyses of 20 F2 offspring plants showed that the F1 plant 5811-1 carrying 3 Bs produced three F2 plants with 1 B, six F2 plants with 2 Bs, one F2 plant with 3 Bs, one F2 plant with 4 Bs, and nine F2 plants with highly chimeric root meristems showing cells with 1-5 Bs in different frequencies. All chromosome counts were based on ten cells of root meristems of each F2 offspring. The presence of Bs in root meristem cells was visualized by GISH at high (85%) stringency using Alexa Fluor 488-labeled genomic DNA of maize as the probe without oat competitor DNA (Figure 2).

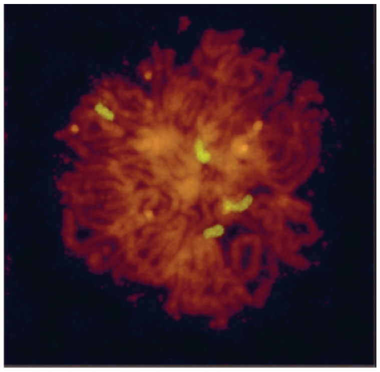

Figure 2. Root prometaphase cell from the F2 plant K1188; the tetrasomic addition of maize B-chromosomes to Starter oat is demonstrated by green fluorescence after GISH with Fluor 488-labeled genomic DNA of maize well contrasted against the red-brown counterstained oat chromosomes. (For color see online.)

For the F1 plant 5845-1 carrying one B, none of the 10 F2 offspring tested by cytological and molecular means had Bs, indicating a transmission failure. However, since more F2 seeds were produced, we will analyze a larger offspring population of F1 plant 5845-1 to evaluate the transmission data.

Taking all the data together, our results showed that Bs from maize Black Mexican Sweet can be sexually transferred to the genome of Starter oat, and that haploid oat plants hosting one or three maize Bs are fertile and can set seed after self-pollination. In addition, our results of 30 tested F2 offspring from two maize B-positive F1 plants showed that maize Bs can be transmitted to offspring even when in the presence of only oat chromosomes.

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Maize quality breeding in Argentina. I. Chemical analysis of waxy maize starch

--Corcuera, VR; Caro Solís, C; Garcia-Rivas, G; Tortoriello, C; Salmoral, EM

In 1990, a breeding program was initiated at the Instituto Fitotécnico Santa Catalina and CIGEN, located in Llavallol, province of Buenos Aires, Argentina (22 m.a.s., 34° 48´S; 58° 31´W), with the purpose of obtaining new starch and quality protein genotypes. During the first stage of the program, several maize inbreds were developed, tested and selected following the classic methodology. These inbreds carry single recessive (*wx1, o2, o5, O9, O11, sh4*) or double recessive (*wx1 sh4; wx1 o2*) genes. Using some of the inbreds developed, single crosses were obtained and tested in several complete randomized block design field trials with three replicates conducted in Llavallol. During the last year, the starch composition (% amylose and % amylopectin) of twenty-six inbreds and four single crosses was analyzed through molecular fractionation (Curá and Krisman, 1990) and spectrophotometry. The results of the starch molecular fractionation performed in inbreds and single crosses are shown in Tables 1 and 2. The length of the external chains of each α-D glucane may be estimated from the ratio (A) of peaks and shoulder absorbances for the polysaccharide in the presence of I_2•KI in a saturated $CaCl_2$ solution. Long external chains of glucopolysaccharides (amylose) usually yield a high "A" value (around 2-4). Smaller values of "A" (around 1-1.8) are typical of the short- and branched-chains of amylopectin. Our results (Table 3) show that the amylose contained in these genotypes has some degree of branching and therefore is not completely linear. The amylopectin of the different genotypes has a similar degree of branching and is moderately branched. The single crosses CIG10, CIG25 and CIG45 have a higher amylopectin content (81 to 88%). As CIG10 was obtained by crossing a "normal" inbred (female) x *wx1* (male), its endosperm genotype is *Wx Wx wx*. The amylopectin content in normal maize (*Wx Wx Wx*) is about 70 to 75%, thus the expression of only one recessive allele (*wx1*) raises the content of this α-D glucane by ca. 8 to 10 %.

Table 1. Starch content and composition of inbreds.

Genotype	Starch %	Amylose %	Amylopectin %	R
3002 a	68.8	24.2	75.5	3.2
3008	67.8	26.4	73.6	2.8
3014a	69.3	22.0	78.0	3.5
3016a	70.1	20.1	80.0	4.0
3016 b	69.2	29.3	69.9	2.4
3020/1	69.5	21.0	79.0	3.8
3020a	70.7	20.3	79.7	3.9
3022a	73.1	18.0	82.0	4.6
3022a/1	70.0	11.1	89.0	8.0
3022a/2	70.7	16.0	84.0	5.3
3022 b	69.7	15.8	84.2	5.3
3022c	69.5	18.0	82.0	4.6
3043 b	70.9	29.9	70.1	2.3
3074a	69.9	9.0	91.0	10.1
3074b	71.3	17.1	82.8	4.8
3074c	71.9	20.0	80.0	4.0
3078a	71.6	9.0	91.0	10.1
3088	70.1	25.0	75.0	3.0
3092	69.0	20.0	80.0	3.0
3096 b	70.2	17.0	82.0	4.8
3098	69.1	24.0	76.0	3.2
3109	69.4	22.0	78.0	3.6
3115	70.4	29.0	71.0	2.3
3119	69.0	21.6	78.5	3.6
3132 a	70.7	27.1	72.8	2.7
3139a	70.2	24.0	76.0	3.2

R is the ratio of amylopectin to amylose.

Table 2. Starch content and composition of single crosses.

Genotype	Starch %	Amylose %	Amylopectin %	R
CIG10	69.6	18.8	80.7	4.3
CIG24	73.4	20.0	79.9	4.0
CIG25	71.1	12.2	87.8	7.2
CIG45	71.0	11.1	88.9	8.0
CIG47	70.0	22.0	78.0	3.5
CIG50	72.4	20.3	78.7	3.9
CIG66	71.4	29.0	71.0	2.4

R is the ratio of amylopectin to amylose.

Table 3. Spectrophotometric analysis of starch molecular components.

	AMYLOSE			AMYLOPECTIN		
	λmax	λmax shoulder	A	λmax	λmax shoulder	A
Inbred						
3002a	590.0	415.0	1.43	497.5	408.0	1.22
3016b	614.0	415.0	1.48	521.0	411.0	1.27
3022b	560.0	415.0	1.35	494.5	410.0	1.21
3043b	596.0	415.0	1.44	506.0	410.0	1.23
3096b	617.0	414.0	1.50	490.0	408.0	1.20
3115	589.0	414.0	1.42	525.5	411.0	1.28
3132a	620.0	415.0	1.49	500.5	411.0	1.22
Single-cross						
CIG25	567.0	415.0	1.45	496.0	412.0	1.20
CIG47	590.0	410.0	1.48	522.0	411.0	1.27
CIG50	561.0	410.0	1.45	496.0	409.0	1.21
CIG66	599.0	410.0	1.46	521.0	408.0	1.28

A is the ratio of absorbances at the wavelengths λmax and λmax shoulder.

Maize quality breeding in Argentina. II. Determination of lysine and fatty acids by chromatography

--Corcuera, VR; Giraudo, M; Bernatené, EA; Sánchez Tuero, H; Malcowski, I

Within the last decade at the Instituto Fitotecnico Santa Catalina and CIGEN located in Llavallol, a province of Buenos Aires, Argentina (22 m.a.s., 34° 48´S; 58° 31´W), a maize quality breeding program has been underway. Normal genotypes previously developed in Argentina were reconverted to quality protein maize through the incorporation of the *o2, o5, o11 or o12* genes from Illinois and Bergamo inbreds used as donors. Lysine content in endosperm flour of three inbreds and a single cross has been determined via rp-HPLC. Simultaneously, the germ fatty acid composition of 4 inbreds and three single crosses were analyzed through gas chromatography.

Normally, maize has only 0.3% lysine in endosperm flour, but the expression of the *o2* gene can double or treble it. High lysine contents were found in the first inbreds studied (3088: 1.3%; 3098: 0.9% and 3139 II: 0.6%). These inbreds have a high oil content as previously detected by NIR using an Isotec 1227 device (3088: 5.8%; 3098: 6.0% and 3139 II: 7.3%). Also, the single cross 3152, obtained by crossing one of these inbreds as female x a *wx1 o2* double recessive male, was a complete success in relation to its high lysine content (0.7%). This hybrid has a good agronomic performance as demonstrated through its average yield during three years running in multilocation trials (9,100 kg/ha). The fatty acid composition of the different genotypes studied may be seen in Tables 1 to 3. Generally, the endosperm of maize without expression of single mutant genes has a composition of 60% linoleic acid and 20 to 27% oleic acid (3:1 ratio). In the case of the high lysine and double recessive *wx1 o2* genotypes analyzed, we found a 1.3:1 to 2.2:1 ratio between linoleic and oleic acid. The narrower ratio (1.3:1) was expressed by the *o11* mutant inbred 3115. All inbreds and single cross hybrids studied showed very low levels of eicosenoic and linolenic acid. The levels of myristic, palmitic, heptadecanoic and arachidic acids found in the single cross 3257 (see Table 3), obtained after crossing the inbreds 3096b (♀) x 3135a (♂), show maaternal inheritance. In contrast, the levels of oleic, linoleic and linolenic acids are paternally inherited. On the other hand, the stearic acid content of the hybrid does not differ significantly from the mid-parent value, showing additive inheritance.

Table 1. Fatty acid composition in single mutant gene inbreds.

		% content	
Fatty acid		**3115***	**3016b****
16:0	Palmitic	10.04	7.47
16:1	Palmitoleic	1.25	0.21
18:0	Stearic	2.44	1.05
18:1	Oleic	32.16	35.25
18:2	Linoleic	42.85	50.18
18:3	Linolenic	1.04	0.8
20:0	Arachidic	0.56	0.53
20:1	Arachiidonic	0.32	0.4
22:0	Behenic	0.39	0.41
22:1	Erucic	0.32	0.21
24:1	Lignoceric	1.37	0.95

**o11* inbred ***wx1* inbred

Table 2. Fatty acid composition in single-cross hybrids.

		% content	
Fatty acid		**3165***	**3166****
16:0	Palmitic	9.36	12.83
16:1	Palmitoleic	1.05	no data
18:0	Stearic	2.68	3.77
18:1	Oleic	33.2	27.49
18:2	Linoleic	52.2	38.36
18:3	Linolenic	0.33	no data
20:0	Arachidic	0.68	no data
20:1	Eicosenoic	no data	no data
22:0	Behenic	1.06	no data
22:1	Erucic	0.64	no data
24:1	Lignoceric	4.54	no data

**wx1 o2* ***waxy*

Table 3. Fatty acid composition of a single-cross and its parents.

		% content		
Fatty acid		**3096b***	**3135****	**3257**
14:0	Myristic	3.00%	0.04	0.03
16:0	Palmitic	8.66	12.25	9.46
16:1	Palmitoleic	0.09	0.09	0.08
17:0	Heptadecanoic	0.06	0.1	0.05
18:0	Stearic	2.03	1.8	1.56
18:1	Oleic	35.84	25.12	28.53
18:2	Linoleic	45.13	55.45	55.87
18:3	Linolenic	0.94	0.82	0.8
20:0	Arachidic	0.54	0.35	0.58

**wx1 o2* female parent ***wx1 o2* male parent

Heterosis percentage of yield traits in quality maize single cross hybrids developed in Argentina

--Corcuera, VR; Bernatené, EA; Poggio, L

During the growing season 2005/06, twenty single cross hybrids, generically termed CIG, and their parents were evaluated in a completely randomized blocks field trial design (three replicates) at the Instituto Fitotécnico de Santa Catalina and CIGEN placed in the location of Llavallol province of Buenos Aires, Argentina (22 m.a.s., 34° 48´S; 58° 31´W). Genotypes were evaluated for minimun potential yield expressed in kilograms/hectare (Y), ear weight (EW), kernel weight per ear (KWE) and cob percentage (% C). All genotypes evaluated were developed within a maize quality breeding program initiated during the 90´s. Yield was calculated as follows:

Y= average kernels weight per ear x 71,500 plant per hectare

High parent heterosis (%) was determined for each trait mentioned according to the formula:

$$HP\,\% = \frac{F1 - HP}{HP} \times 100$$

(F1: single cross value; HP: high-parent value)

The single crosses may be divided into three groups: 1) *wx1*, 2) *o2* and 3) double recessive (*wx1 o2* hybrids). Minimum yield varies from 5,326 kg/ha to 8,701 kg/ha for the hybrids CIG133, CIG141, CIG144, CIG158, CIG159 and CIG161. Significant HP heterosis values (%) were found for yield in these hybrids (102.0% to 169.9%; Table 1). Two hybrids (CIG109: 3,632 kg/ha and

Table 1. High Parent heterosis (%) of single cross hybrids.

Genotype	Hybrid	EW	KWE	% C	Yield
wx1	CIG46	2.0	3.2	-39.9	3.2
	CIG67	32.3	35.6	-23.3	35.5
	CIG25	100.0	20.0	-39.4	20.1
	CIG70	82.6	90.9	-18.2	90.9
	CIG77	58.1	63.8	-14.6	64.0
	CIG141	123.8	169.9	-37.1	169.9
o2	CIG186	35.4	39.8	-14.6	40.0
	CIG8	18.8	13.3	-34.3	13.3
	CIG10	45.8	40.4	-7.3	40.4
	CIG187	-20.6	-19.8	-12.2	-19.8
	CIG159	88.4	114.7	-32.6	114.7
	CIG3	29.0	38.1	-47.7	38.1
	CIG109	-41.9	-38.8	-20.0	-38.8
wx1 o2	CIG82	33.5	31.3	-17.1	44.5
	CIG127	-3.4	2.8	-22.2	2.8
	CIG133	74.2	109.0	-38.5	102.0
	CIG144	104.4	151.1	-44.5	151.3
	CIG158	112.8	167.8	-50.4	168.1
	CIG161	135.7	148.9	-36.0	148.9
	CIG164	4.1	8.6	-24.2	8.7

CIG187: 4,762 kg/ha), obtained by crossing very closely related inbreds, showed negative HP heterosis values for all the traits as were. All hybrids showed a negative HP heterosis value for % C. This is due to the fact that the hybrid ears are completely fertile whilst the inbreds normally lack some kernels. Correlation between yield and kernel weight per ear is highly significant (r: 0.99; p: 0.01) and the regression equation between these parameters is Y: 0.887 + 0.99(KWE). In contrast, the correlation coefficient between yield and ear weight (including cob weight), is significant; it is lower (r: 0.83; p: 0.01). As seen in Table 2, the highest heterosis values for Y and KWE were found in the double-recessive hybrids. Yields varied from 3,331 kg/ha for CIG109 to 9,404 kg/ha for the waxy hybrid CIG70. Considering that these single crosses carry one or two mutant genes usually associated with lower yield, the high HP heterosis values and minimum potential yield found in some makes them candidates for commercial release.

Table 2. Average HP heterosis for different groups of hybrids.

Genotype	EW	KWE	% C	Yield
wx1	66.41	63.89	-28.74	63.94
o2	22.12	26.8	-24.1	26.84
wx1 o2	65.89	88.5	-33.26	90.46

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Quantitative trait loci for ruminal starch degradability of *opaque2* maize (*Zea mays* L.)

--Lebaka, NG; Coors, JG; Gutierrez, A; Menz, MA; Betran, JF

The *opaque2* (*o2*) gene that alters protein composition in maize also influences starch digestibility in ruminants. The softer, less dense kernel texture of *o2* grain improves starch digestibility. Unfortunately, the softer kernels of *o2* maize also adversely affect agronomic performance (Vasal, Specialty Corns. 2nd Ed., 2001). Breeding efforts were initiated to improve the yield and kernel characteristics of *o2* genotypes as part of the Quality Protein Maize (QPM) project at the International Center for Maize and Wheat Improvement. Ruminal starch degradability of *o2* maize has been reported (Phillipeau et al., J. Sci. Food Agric. 80:404-408, 2000). Most of the published research results show that ruminal digestibility is inversely related to kernel hardness (Philippeau et al., J. Anim. Sci. 77:238-243, 1999; Philippeau et al., J. Sci. Food Agric. 80:404-408, 2000 and Correa et al, J. Dairy Sci. 85:3008-3012, 2002). To our knowledge there are no published research studies on quantitative trait loci (QTL) that model ruminal starch digestibility in *o2* maize.

One-hundred and thirty-six *opaque2* recombinant inbred lines (RILs) spanning a wide range of kernel hardness were evaluated for in situ ruminal degradabilities. The RILs were derived from the cross CML161 *o2/o2* (hard kernels) x B73 *o2/o2* (soft kernels). The inbreds were raised, genotyped and rated for kernel hardness (light box) by Dr. J. Betran's lab at Texas A & M University. A 2.0 g sample (90% dry matter) of kernels ground through a 6 mm Wiley mill screen was used for measurement of in situ ruminal dry matter degradation (RDMD) at 0 and 14-hr incubation (1.5 g per bag by 2 bag replicates per corn sample in 5 cm x 5 cm dacron bags of 50 micron pore size) in 3 mid- to late-lactation dairy cows fitted with ruminal cannulae and fed ad libitum a total mixed ration comprised of 60% forage (60% corn silage to 40% haylage mix) and 40% concentrate (DM basis).

Correlation analysis was done to determine the relationship between kernel hardness and in situ starch disappearance. Kernel hardness was determined using the score of 1 to 5 (where 1 – hard and 5 – soft). QTL analysis was done using composite interval mapping (Liu, Statistical Genomics, 1998) of QTL cartographer (version 2.5) using Kosambi mapping function and assuming no gene interaction with the threshold LOD score of 2.5.

Dry matter disappearance was positively correlated ($r=0.73$, $p<0.05$) with kernel hardness. Results for composite interval mapping analysis are shown in Table 1. The analysis revealed significant QTLs on chromosomes 1, 6 and 7 for 14-hr dry matter disappearance. The QTLs on chromosome 7 occupy about the same position with the *opaque5* locus located near the centromere of the long arm of chromosome 7 (Gibbon and Larkins, Trends Genet. 21(4):227-233, 2005). This suggests the effect of particle size on starch digestibility. The results indicate the positive contribution of B73 (soft endosperm) to improved ruminal starch degradability. For 0-hr DM disappearance, QTLs were detected on chromosomes 3 and 6. Three QTLs were detected for the difference between 14-hr and 0-hr DM disappearance.

Table 1. Detected QTLs for 0, 14 hours and (14 - 0 hr) starch disappearance in *opaque2* maize RILs.

Chromosome	Position (cM)	LOD	Additive (%)	R-square
		14-hr incubation		
1	131	2.94	-2.7	0.08
6	96	4.30	-3.9	0.18
7	75	3.40	3.0	0.11
7	83	3.00	2.7	0.08
		0-hr incubation		
3	114	3.23	2.0	0.08
6	98	5.07	-3.0	0.19
		(14-hr - 0-hr) incubation		
7	41	4.37	2.8	0.04
7	56	3.55	1.6	0.11
7	61	3.44	1.6	0.11

14-hr DM disappearance includes both 0-hr and 14-hr minus 0 h disappearance. 0-hr DM disappearance represents an instantaneously soluble part of the total DM that dissolves instantly in the ruminal fluid. The 6-mm ground softer endosperm generally has a higher component of finer particles (instantaneously soluble particles) than harder endosperm. The difference between 0-hr and 14-hr disappearance represents the DM that is degraded in the rumen and therefore the actual ruminal DM degradability. One to three QTLs for the difference were detected on the shorter arm near the centromere of chromosome 7. This position coincides with the position of one of the modifiers located between the locus for *opaque2* and the centromere on the shorter arm of chromosome 7 (Lopes et al., Mol. Gen. Genet. 247:603-613, 1995 and Gibbon and Larkins, Trends Genet. 21(4):227-233, 2005). QTLs for the difference did not overlap with QTLs for the other traits, suggesting that the true DM degradability can probably be selected for independent of the other traits. Alleles for improved digestibility came from B73 and those reducing digestibility were contributed by CML161. However, some RILs performed better or worse than the best or worse parent, respectively, indicating the presence of transgressive segregation and possible additive by additive gene interaction.

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Linkage data for *sml*

--Manzotti, PS; Gavazzi, G

The *sml* gene is a recessive mutation affecting shoot apical meristem maintenance and lateral organ formation. Its introgression in different genetic backgrounds has highlighted the epistatic interaction between *sml* and the unlinked *distorted growth* (*dgr)* locus. Seeds homozygous for both *sml* and *dgr* loci have a shootless phenotype whereas *Dgr/-sml/sml* seeds produce plants with altered phyllotaxis and abnormal leaf morphogenesis.

Previous data had shown that *sml* lies on the long arm of chromosome 10, and it was established that there is a distance of 21 cM between s*ml* and the molecular marker *umc1084* (Pilu et al., Plant Physiol. 128:502-511, 2002); in order to define if its position is centromeric proximal or distal, we made a three-point linkage test of *sml* using the chromosome 10 markers *r* and *o7*. Since homozygous *sml* seedlings are lethal, the test was set up as a modified testcross, as outlined below.

Heterozygous *sml R-st O7/Sml r o7* females were outcrossed to *Sml r O7*/*Sml r O7* male parents and kernels from the cross were separated into stippled (*R-st/r*) and colorless (*r/r*) classes, planted, and the resulting plants self-pollinated. The F2 ears were scored for the presence of *o7* and *sml* (upon germination of a sample of 50 seeds from each ear), thus allowing us to trace the chromosomal constitution of the outcrossed heterozygous females in terms of the three markers.

The results, obtained by the analysis of 145 ears, were tabulated and linkage values were calculated (see Table 1).

Table 1. Modified three point linkage data for *sml-r-o7*.

Region	Phenotype	No. of ears	Totals
0	*sml R-st O7*	50	
	Sml r o7	33	83
1	*sml r o7*	11	
	Sml R-st O7	13	24
2	*sml R-st o7*	14	
	Sml r O7	16	30
1+2	*sml r O7*	6	
	Sml R-st o7	2	8
total			145

The gene order and distances obtained are as follows:

sml – 22 ± 3.4 - *r* – 26 ± 3.6 - *o7*

A successive test was performed using the chromosome 10 markers *r* and *v18*. Heterozygous *Sml R-r V18/sml r v18* plants were selfed and the kernels obtained were divided into coloured and colourless, germinated and scored for the presence of *sml* and *v18.* The results obtained are shown in Table 2.

Table 2. Linkage data between *sml*, *r* and *v18* as determined in the progeny of *Sml R-r V18/sml r v18* selfed plants.

	Phenotype			
	Sml R	*Sml r*	*sml R*	*sml r*
n.	697	55	77	138
	R V18	*R v18*	*r V18*	*r v18*
n.	619	135	51	89
	Sml V18	*Sml v18*	*sml V18*	*sml v18*
n.	588	164	82	60

This allows us to establish this linkage result:

sml – 16 ± 1.3 – *r* – 24.5 ± 1.7 – *v18*

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***Bg* transposon transcription from both strands: two products similar to NFI and SET domain proteins may be involved in transcription and chromatin modulation**

--Koterniak, VV

<u>*Bg* transposon sequence and probable *Bg*-encoded proteins</u>. Analysis of the *Bg* transposon sequence suggests that this mobile element encodes several proteins (designated as PPBg1-PPBg3), described previously (MNL 79:32-35; MNL 80, submitted). The analysis also shows certain regions of *Bg* sequence may form Z-DNA and that *Bg*-encoded proteins have Z-DNA binding properties, indicating a possible autoregulation of this transposon at the transcriptional level (MNL 80, submitted). Structure of all above-mentioned proteins was deduced from the strand of *Bg* transposon containing the two longest ORFs. However, some mobile elements (e.g., the maize *MuDR* autonomous element) are transcribed from both strands (Hershberger et al., Genetics 140:1087-1098, 1995). Further analysis of the other sstrand for *Bg* indicates this mobile element may encode 2 further transcription and chromatin modulation proteins.

<u>*An 87 amino acid protein encoded by the second strand of Bg element is similar to Nuclear Factor I family of transcriptional regulators*</u>. The ORF of the second strand of *Bg*, from position 724 to 460 (positions for both strands according to the first strand, the sequence of GenBank accession X56877.1), encodes an 87 amino acid protein, designated hereafter as PPBg4 (Fig. 1a). It is unusually rich in tryptophan (7 residues) and has several PS dipeptide residues.

This protein shows significant similarity with the transcription regulators of the nuclear factor I (NFI) family (Fig. 1a), using CLUSTALW analysis at the European Bioinformatics Institute (http://www.ebi.ac.uk) using default parameters. In the human genome, the promoter sites of NFI and Z-DNA forming regions (ZDRs) are near transcriptional start sites (Champ et al., Nucl. Acids Res. 32:6501-6510, 2004). In the case of the *PPBg4* gene, possible ZDRs are located just downstream of the *PPBg4* gene at positions 120 and 402. In addition, a perfect canonical NFI binding site (5'-TGG(N)$_6$GCCAA-3'; Zorbas et al., J. Biol. Chem. 267:8478-8484) is present at position 1775 of the *Bg* sequence; i.e., at -1051 bp upstream on the opposite strand in relation to the translation start site of *PPBg4*. The SP-rich stretch S29-S35 of PPBg4 (SPSPSTS, Fig. 1b) is similar to the SPTSPSYSP motif contained in the NFI transcriptional activation domain (Wendler et al., Nucl.

```
a)
PPBg4  1  MRQQLQWS-CAAWRQQPHLP------WRR---------------TCWFWLSPSPSTS----  35
NFI   109 MEEDVDTSPGGDYYTSPNSPTSSSRNWTEDIEGGISSPVKKTEMDKSPFNSPSPQDSPRLS 169

PPBg4  36 ----------CCSRGLA-TPRGT-----PQTDLHVNEVAVSWS-----LP---EPSSTLT-  47
NFI   170 SFTQHHRPVIAVHSGIARSPHPTSALHFPATPILPQTASTYFPHTAIRYPPHLNPQDPLKD 230

PPBg4  48 LWEMELLSRRVADGD----G  87
NFI   231 LVSLACDPATQQPGPSWYLG 250

b)
NFI      -SPTSPSYS
PolII    YSPTSPS---
PPBg4 29 -SP-SPSTS- 35
```

Figure 1. (a) Alignment of PPBg4 with the transcription factor NF I of *Mus musculus* (GenBank accession AAK21332.1). The T23-S35 sequence similar to the P-4 peptide (Fujii et al., 2003) is underlined. (b) Similarity of the S29-S35 region of PPBg78 with the SPTSPSYSP motif of NFI transcription factor (NFI) and with repeat YSPTSPS of the RNA polymerase II (PolII). Identical residues are shown in a black background, similar ones are in a grey background.

```
PPBg5          1   MSCTGLPCNVWIQSNELSTCLLIVGPIICNNLNDILHPNLINNHLSNT     48
CAG25109.1  3427                 NNMNNMNNIMNNIMNNNMNNIMN-NIMNNNMNNI   3459

PPBg5         49   IINKFCILNTTSCIYRLVKKHPSIAIYHEINNAHHGRT     86
CAG25109.1  3460   INNNNIFNNDVSNNVDMQHKSDQICIFNS-NNIH       3492
```

Figure 2. Similarity of PPBg5 with a SET-domain protein of *Plasmodium falciparum* (GenBank accession CAG25109.1). Identical residues are shown in a black background, similar ones are in a grey background.

Acids Res. 22:2601-2603, 1994). Another indication that PPBg4 regulates transcription is an unanticipated similarity between the PPBg4 sequence TCWFWLSPSPSTS (residues T23-S35) and the P-4 peptide (TWFWPYPYPYHLP) which is known to inhibit transcriptional regulation (Fujii et al., Clin. Cancer Res. 9:5423-5428, 2003).

An 86 amino acid protein, PPBg5, encoded by the second strand of the Bg element is similar to SET-domain proteins. The ATG codon on the *Bg* second strand, starting from position 2350, may determine the translational start site of another second strand *Bg* encoded protein. BLAST analysis of this 86 amino acid protein (referred to as PPBG5) revealed its similarity with SET-domain proteins (Fig. 2). Based on known SET domain involvement in histone methylation, transcription activation of repression (reviewed in Schotta et al., Genes Dev. 18:1251-1262, 2004) and transcription elongation in *Saccharomyces cerevisiae* (Krogan et al., Mol. Cell. Biol. 23:4207-4218, 2003), the SET domain in PPBg5 may be involved in chromatin remodeling processes connected with transcription of *Bg*.

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Prediction of maize (*Zea mays* L.) combining ability using molecular markers and mixed linear models theory

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Predicting the performance of untested single crosses is important in hybrid breeding programs. The cost involved in field testing makes it impossible to evaluate all new inbreds and posible combinations. The traditional fixed linear model, coupled with the ordinary least squares estimation used for most plant breeders, is too restrictive because of the independence assumption. Error structure is often more complex than the one used in standard linear models (Balzarini, In Quantitative Genetics, Genomics and Plant Breeding, 2002). In contrast, the general linear mixed model (Henderson, Applications of Linear Models in Animal Breeding, Univ. Guelph, 1984) can easily accommodate covariances among observations. The inclusion of a numerator matrix generates unbiased heritability estimations when maximum likelihood methodologies are used (ML, REML and Bayes), mainly because it takes account of the correlation between observations due to covariance between relatives and the variation due to genetic drift, which is important in finite populations under selection (Sorensen and Kennedy, Theor. Appl. Genet., 1983). The objective of this study was to analyze the effectiveness of best linear unbiased prediction (BLUP) based on molecular (microsatellite) marker data. Field data was obtained from Nestares et al. (Pesq. Agropec. Bras. 34:1399-1406, 1999): topcrosses between a collection of 48 inbred lines and four tester populations (sB73 and sMo17 from the Reid x Lancaster pattern, and HP3 and P5L2 from the local orange flint pattern) were evaluated for grain yield during the 1991/92 season at four environments. All lines but two (B73 and Mo17) were orange flint germplasm developed by INTA from twenty different sources (synthetics, composites, landraces, planned crosses and a commercial hybrid). Molecular data were obtained for twenty-six (26/48) parent lines and the four tester populations using 21 microsatellite markers evenly distributed in the genome (Morales Yokobori et al., MNL 79:36, 2005). We had some problems in molecular characterization of the testers HP3 and P5L2, but used the data, considering the robustness of blup predictors (Bernardo, Crop Sci. 36:862-866, 1996)

Relatedness (r) between parents was estimated using MER

(Moment Estimate of Relatedness) software (Wang, Genetics 160:1203-1215, 2002). $r = 2\Theta$, where Θ, the coefficient of coancestry, is the probability that, for any autosomal locus, a random gene taken from individual *x* is identical by descent with a random gene taken from individual *y.* Three different variance-covariance structures were compared using molecular and/or pedigree data and under the following linear mixed model (Henderson, 1984):

$$\mathbf{y} = X\hat{\mathbf{a}} + Z_l\mathbf{a}_l + Z_t\mathbf{a}_t + Z_d\mathbf{d} + Z_{ge}(ge) + \mathbf{e}$$

Where: **y** is the response vector (yield data of hybrids derived from the crosses between lines and testers), and X, Z_l, Z_t, Z_d and Z_{ge} are known design matrices. β is the vector of fixed parameters and a_l, a_t, d are vectors of random effects associated with additive effects of lines, additive effects of testers and dominance effects, respectively. e is the vector of residuals. (ge) is a random effects vector associated with genotype-environment interaction. For the sake of simplicity, we assumed that C_{ge}, the covariance matrix for (ge), is an identity matrix (no correlation between interactions). Residuals were also considered independent.

Assumptions regarding relatedness between parents allows the definition of the covariance matrices A_l, A_t and D:

Model 1) Variance components, Parents unrelated. A_l, A_t and D are identity matrices.

Model 2) Lines and testers are derived from two different ancestral populations, so: $A_l=\{r_{ij}^{(l)}\}$, $At=\{r_{ij}^{(t)}\}$ and $D=\{d_{ij}=0.25\ r_{ij}^{(l)}\ r_{ij}^{(t)}\}$ (given hybrids i and j, $r_{ij}^{(l)}$ is the relatedness between parent lines and rij(t} is the relatedness between testers).

Model 3) Lines and testers are derived from the same ancestral population. al and at can be combined in one vector a of additive effects of parents, $A=\{a_{xy}\}$, a_{xy} =relatedness between parents (lines and/or testers). $D=\{d_{ij}=0.25(r_{ij}^{(ll)}r_{ij}^{(tt)}+r_{ij}^{(tl)}r_{ij}^{(lt)})\}$, $r_{ij}^{(ll)}\}$ $r_{ij}(xx)$ is the relatedness between parents of hybrids i and j; (xx): l stands for lines and t stands for testers.

All models were evaluated by restricted likelihood (resLL) and Akaike's information criterion (AIC) (Table 1). Cross-validation statistics were calculated to assess and compare the predictive ability of some of the proposed models. For each genetic model, the set performance of m missing crosses was predicted based on the formula (Balzarini, 2002):

Table 1. Variance analysis of proposed models.

	additive variance	dominance variance	GE variance	error	-2resLL	AIC
Model 1	$\sigma^2_l=3.23$ $\sigma^2_t=7.00$	$\sigma^2_d=15.33$	$\sigma^2_{ge}=3.87$	$\sigma^2_e=151.36$	6348.8	6358.8
Model 2 Pedigree	$\sigma^2_l=3.38$ $\sigma^2_t=14.00$	$\sigma^2_d=15.47$	$\sigma^2_{ge}=3.88$	$\sigma^2_e=151.36$	6348.7	6358.7
Model2 microsatellite	$\sigma^2_l=3.23$ $\sigma^2_t=11.11$	$\sigma^2_d=15.60$	$\sigma^2_{ge}=3.78$	$\sigma^2_e=151.36$	6348.6	6358.6
Model 3 Pedigree	$\sigma^2_a=13.88$	$\sigma^2_d=14.43$	$\sigma^2_{ge}=3.83$	$\sigma^2_e=151.36$	6349.7	6357.7
Model 3 microsatellite	$\sigma^2_a=12.34$	$\sigma^2_d=14.59$	$\sigma^2_{ge}=3.82$	$\sigma^2_e=151.37$	6349.3	6357.3

* Variance components were estimated via restricted maximum likelihood (REML) using SAS (Sas Institute) PROC MIXED.

**σ^2_l additive variance due to parent lines, σ^2_t additive variance due to parent testers, σ^2_a additive variance of testers and lines (both groups belong to the same population).

$$\mathbf{y_M} = CV^{-1}\mathbf{y_P}$$

Where yM = m x 1 vector of predicted yields of missing crosses, yP a p x 1 vector of average yields of predictor hybrids, C m x p matrix of genetic covariances between missing and predictor hybrids and V (p x p) phenotypic variance-covariance matrix among the predictor hybrids. We performed predictions for the (25 x 4) hybrids (m=4, p=100) and did not consider 4 hybrids based on a missing line. Effectiveness of predictions was measured by Spearman correlation (Table 2).

Table 2. Spearman Rank Correlation between observed (BLUP) and predicted hybrid yields (model 2).

Population	Pedigree data	Microsatellite data
26 lines	0.40**	0.36**
lines derived from synthetics	0.45*	0.44
lines derived from composites	0.52**	0.49**
Lines unrelated or highly divergent	0.08	0.02

* Indicates significance at P = 0.05.

** Indicates significance at P =0.01.

Conclusions. Inclusion of a numerator matrix (using pedigree or molecular data) generates more precise variance estimates and higher values of heritability when compared with traditional fixed effects models. Molecular data used in these types of crosses (genetically divergent parental populations) did not provide any additional information to that provided by pedigree data.

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Suggested guidelines for naming helitrons

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<u>PREAMBLE:</u> Helitrons are a novel class of transposable elements discovered recently by computational analysis of the complete genome sequences of *C. elegans*, Arabidopsis, and rice (Kapitonov and Jurka, Proc. Natl. Acad. Sci. USA 98:8714-8719, 2001). It has become apparent that, in maize, helitrons are both abundant and highly variable in sequence. There are currently no guidelines for naming these elements, yet they are highly diverse in size and sequence because they can pick up different gene fragments from the maize genome. Their diversity in sequence is presently matched by their diversity in names. For example, they have been named according to the gene where they insert (helitron *sh2-7527*; helitron *ba1-ref*) or the names of the locus and ferried gene fragments (helitrons 9002NOPQ and 9008 HI) or identified with a letter (*HelA*; *HelB*). In an attempt to introduce some order into this chaos, we would like to suggest a nomenclature system for maize helitrons at the onset of the maize genome sequencing project. These guidelines arose from informal discussions at the Maize Genetics Conference in Asilomar in March, 2006. We will adopt these guidelines in our future publications and hope that other researchers working with helitrons will adopt them, as well.

<u>NOMENCLATURE</u>: Although they differ greatly in internal sequences, maize helitrons share substantial sequence homology at their 5' and 3' ends, the latter being more highly conserved. By

comparing the 3' terminal 30 nucleotides of maize helitron sequences currently in the database, it is clear that the elements group into two major clades, *Hel1* and *Hel2*. The elements in the larger *Hel1* clade share at least 70% sequence identity; those in the smaller *Hel2* clade are less related, sharing around 50% identity. We anticipate that many new elements will be identified as helitrons because of conserved sequence features at their 5' and 3' ends (Kapitonov and Jurka, 2001). We propose that new elements be grouped into either *Hel1* or *Hel2* superfamilies based on the relationship of their 3' terminal 30 bp to the respective consensus sequences. Presently, the consensus sequences for *Hel1* and *Hel2* correspond to the sequences of *HelA* and *HelB*, respectively, in the *bz* locus of line McC (Lai et al., Proc. Natl. Acad. Sci. USA 102:9068-9073, 2005). We also propose a criterion of 50% identity as the cutoff to assign helitrons to a particular superfamily. If, by this criterion, additional helitron superfamilies are identified in the future, they should be named *Hel3*, *Hel4*, and so on.

Following the symbol designating the superfamily to which a helitron belongs would be a number provided by a clearing house for helitron nomenclature (see below) and an identifier, in parentheses, consisting of the locus or mutation where the element is found, if known, separated by a colon from the name of the maize line. For simplicity, the entire helitron symbol should be italicized. Thus, the helitron in *sh2-7527* would be *Hel1-1(sh2-7527)*, the first one discovered, and the one in the *bz* genomic region of McC would be *Hel1-3(bz:McC).* The NOPQ element in locus 9002 of B73 would be *Hel1-x(9002:B73)*, where x would stand for a number assigned by the helitron nomenclature clearing house.

If a helitron is discovered that is virtually identical to a previously described helitron, but at a different locus, than the letter "a" is placed after the number assigned to the first helitron and a letter "b" is placed after the number of the second helitron. Again, the parenthetical identifier would include the locus and line carrying the new helitron. For example, B73 has an almost identical copy of *Hel1-3(bz:McC)* in chromosome 5S, at the same map location as *umc1260.* The *Hel1* element in the *bz* locus would become *Hel1-3a(bz:McC)* and the one in 5S, *Hel1-3b(umc1260:B73)*. If a helitron is discovered that is virtually identical to a previously described helitron at the same locus, but in a different line, then it should be given the same designation as the first one, specifying in the parenthetical identifier the names of the locus and line where found. For example, McC and W22 have a copy of *Hel1-3* at the same site in the *bz* genomic region. The helitron in W22 would then be named *Hel1-3a(bz:W22)*. Note that this designation does not imply absolute sequence identity of the two helitrons (which is, actually, not the case here). As with genes, it will be up to the individual investigator to assess sequence relatedness from the sequence database records. Finally, if a helitron is identified in a BAC sequence not yet associated with any locus, the number of the BAC in the GenBank record can substitute temporarily for the locus name.

Dr. Shailesh Lal at Oakland University, MI, has agreed to serve as clearing house for assigning blocks of numbers to investigators, institutions, or multi-institutional projects, such as the maize genome initiative, that have identified new helitrons.

AUTONOMOUS HELITRONS. An autonomous helitron has not been discovered. Following maize genetics convention, an autonomous helitron can only be defined by a functional test. Therefore, helitrons should not be designated as "autonomous" solely on the basis of sequence homology. If, based on its sequence content, e.g., an intact replicase and helicase, a helitron is considered to be potentially autonomous, it could be called a putative autonomous helitron, yet given a symbol based on the general nomenclature guidelines. It is suggested that, once confirmed, an autonomous helitron be designated *aHel*, followed by an identifier as described above.

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A machine learning approach for heterotic performance prediction of maize (*Zea mays* L.) based on molecular marker data

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A number of statistical methods based on molecular data are currently available for assigning new inbreds to heterotic groups in maize (*Zea mays* L.) with variable results (Reif et al., Crop Sci. 45:1-7, 2005; dos Santos Diaz et al., Genet. Mol. Res. 3:356-368, 2004). We conjecture that the main flaw of traditional statistical models is that they do not capture the non-linear relation between parental data and progeny performance (Tollenar et al., Crop Sci. 44:2086-2094, 2004); alternatively, experimental results show that such non-linearity can be easily captured by supervised machine learning models, i.e., by multiclassifiers (Witten and Eibe, Data Mining: Practical machine learning tools with Java implementations, Morgan Kaufmann, San Francisco, 2000).

The field data analyzed in this study was taken from Nestares et al. (Pesq. Agropec. Bras. 34:1399-1406, 1999). Briefly, our investigation involved 26 inbred lines (all lines but one, B73, were orange flint germplasm developed by INTA from twelve different sources: synthetics, composites, landraces, etc) from a total of 48 evaluated for their combining ability with four testers: sB73 & sMo17 from the Reid x Lancaster pattern and HP3 & P5L2 from the local orange flint pattern. The 48 lines were grouped according to their combining ability with the tester populations into 4 heterotic groups (H1-H4) using the SAS-Fastclus procedure (Nestares et al., 1999). The 26 lines were characterized using 21 SSR (simple sequence repeats) evenly distributed in the genome (Morales Yokobori et al., MNL 79:36, 2005).

A dataset comprising 42 attributes corresponding to the 21 SSR (2 alleles of each locus per line) were generated. This dataset contains 26 instances (26 lines) and 4 classes defined by the four heterotic groups (H1 = 4 instances, H2 = 8 instances, H3 = 6 instances and H4 = 8 instances). Finally, we considered six standard multiclassifiers provided by the Java WEKA library (Witten and Eibe, 2000): Naïve Bayes, Support Vector Machines with Radial Basis function kernel-one against all (SVM-RBF), Decision Tree (J48 and random forest), AdaBoost Decision Stumps and Multilayer Perceptron. Classifiers' performances were evaluated by 3-, 5- and 10-fold Cross Validation (3-CV, 5-CV and 10-CV) (we run all classifiers with WEKA's default values). Results are presented in Table 1.

Table 1. 3-, 5- and 10-fold CV error on the heterosis dataset using multiclass classifiers.

Multiclassifier	3 CV error	5 CV error	10 CV error
Naive Bayes	0.654	0.692	0.769
SVM-RBF	0.654	0.769	0.769
Decision Tree (J48)	0.808	0.769	0.769
Decision Tree (random forest)	0.731	0.846	0.769
Adaboost-Decision Stump	0.731	0.610	0.770
Multilayer Perceptron	0.770	0.770	0.692
Error Expected by Chance	0.774	0.774	0.774

Considering that our classification results are preliminary, they suggest the usefulness of a molecular based, machine learning approach for solving general heterotic group assignation problems; we must consider the effect of population structure (parents highly divergent) which affects linkage disequilibrium between DNA markers and genes involved in the expression of target traits (Charcosset and Essioux, Theor. Appl. Genet. 89:336-343, 1994). Alternatively, and based on previous work, we hypothesize that further application of feature selection methods, i.e., the selection of highly discriminant molecular markers, might improve heterotic group assignation. This hypothesis is supported in the observed similarity between classification problems involving microsatellite marker and those involving microarray data. In both cases, missing and noisy features might be present in scarce data samples. This type of classification noise can be properly limited by feature selection methods so that resulting data sets can be safely managed by binary based, Coding Theory inspired multiclassifiers (Ornella et al., VIII Argentine Symposium on Artificial Intelligence, Mendoza, Argentina, 2006).

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Marker-assisted selection without QTL mapping: prospects for genome-wide selection for quantitative traits in maize

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The availability of cheap and abundant molecular markers in maize has allowed breeders to ask “How can molecular markers best be used to achieve breeding progress?” without conditioning this question on how breeding has traditionally been done. Exploiting molecular markers in breeding has involved finding a subset of markers associated with one or more traits, i.e., QTL mapping. In contrast, genome-wide selection refers to marker-based selection without first identifying a subset of markers with significant effects. Our objectives were to assess, in simulation studies, the response due to genome-wide selection compared with marker-assisted recurrent selection (MARS), and to determine the extent to which phenotyping can be minimized and genotyping maximized in genome-wide selection. We simulated genome-wide selection that comprised evaluating doubled haploids for testcross performance in cycle 0, followed by two cycles of selection based on markers. Individuals were genotyped for a set of 128, 256, 512, or 768 markers, and breeding values associated with each of the markers were predicted and were all used in genome-wide selection. We found that across different numbers of QTL (20, 40, and 100) and levels of heritability, the response to genome-wide selection was 18 to 43% larger than the response to MARS. Responses to selection were maintained when the number of doubled haploids phenotyped and genotyped in cycle 0 was reduced and the number of plants genotyped in cycles 1 and 2 was increased. Such schemes that minimize phenotyping and maximize genotyping would be feasible only if the cost per marker data point is reduced to about 2 cents. The convenient but incorrect assumption of equal marker variances led to only a minimal loss in the response to genome-wide selection. We conclude that genome-wide selection, as a brute-force and black-box procedure that exploits cheap and abundant molecular markers, is superior to MARS in maize.

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Studies on genetic variability, correlation and path analysis in maize (*Zea mays* L.)

--Sofi, PA; Rather, AG

A number of studies in maize have been conducted to elucidate the nature of the association between yield and its components which identified traits like ear length, ear diameter, kernels/row, ears/plant, 100-seed weight and rows/ear as potential selection criteria in breeding programmes aiming at higher yield (Debnath and Khan, Pakistan J. Sci. 2nd Res. 34:391-394, 1991; Agrama, Plant Breed. 115:343-346, 1996; Mohan et al., Natl. J. Plant Improv. 4:75-76, 2002; Tollenaar et al., Crop Sci. 44:2086-2094, 2004). The present study was undertaken to elucidate such character association in local and CIMMYT inbred line crosses of maize in the temperate valley of Kashmir. The present study was carried out in 2004-05. The materials were generated by crossing 15 diverse white maize inbred lines (4 local and 11 exotic) to three phenotypically diverse testers (W_3, W_5 and W_3 x W_5) in a line x tester design, at trhe winter maize nursery in Amberpet, Hyderabad (India). The parental lines and test crosses were evaluated at two diverse locations of the Kashmir valley, namely Larnoo and Wadura, representing distinct climatic zones. Each genotype was replicated thrice at each location in randomised block design. Each entry was grown in two rows of 2 m length with row to row and plant to plant spacing maintained at 60 and 25 cm, respectively. The recommended practices were followed to ensure a good crop. Data were recorded from 10 randomly selected competitive plants from each replication for 11 quantitative traits, and the data were statistically analysed for correlation coefficients and path analysis as per the methods of Al-Jibouri et al. (Agron. J. 50:633-637, 1958) and Dewey and Lu (Agron. J. 51:515-518, 1959). There was substantial variability for all traits. Grain yield, ear length, ear height, 100-seed weight and ear diameter had high GCV estimates, with high heritability. The genetic advance was higher for plant height, ear length, grain/row and grain yield. The genotypic correlation coefficient revealed that ear diameter, 100-seed weight, ear length, kernel rows/ear and kernels/row had the highest significant correlation with grain yield. The path analysis revealed that the highest direct effect on grain yield was exhibited by 100-seed weight, followed by kernels/row, kernel rows/ear, ear length and ear diameter. Most of the traits exerted their positive indirect effects through 100-seed weight, kernel rows/ear and kernels/row.

Genotypic relationships among traits affecting grain yield elucidate true association as they exclude environmental influences. In the present study, the highest significant positive correlation with grain yield was shown by ear diameter, followed by 100-seed weight. Days to 50% silking and days to pollen shed had significant negative correlation with grain yield. Similar results have been reported in maize by Mohan et al. (2002), Vasic et al. (Acta Agron. Flung. 49:337-342, 2001), Mohammadia et al. (Crop Sci. 43:1690-1697, 2004), Neto and Miranda (Sci. Agric. 58:99016-9018, 2001).

Using the path coefficient analysis revealed positive direct effects on grain yield, with the highest direct effect exhibited by 100-seed weight followed by kernels/row, kernel rows/ear, ear length and ear diameter. Days to pollen shed, days to 50 percent silking and ear height showed negative direct effects on grain yield even though ear height had a positive correlation with grain yield. These traits also shared positive indirect effects on grain yield through other yield traits such as ear length and ear diameter. Ear diameter had the highest indirect effect on grain yield through kernels/row (0.362), followed by ear height (0.316) through rows/ear. In fact, the bulk of the indirect effects on grain yield was exerted by the traits studied through these two traits. Similar results in maize have been reported by Wang et al. (Field Crops Res. 61:211-222, 1998), Vasic et al. (2001), Broccoli and Burak (MNL 74:43-44, 2000), Abdmishani et al. (Maize Genet. Conf. Abst. 46:1-2, 2004) and Mohammadia et al. (2003). Thus, in light of the results obtained in the present study, it can be suggested that the traits such as kernels/row, 100-seed weight, kernel rows/ear, ear length and ear diameter should be used as target traits for improvement of grain yield in maize. Thus, it can be emphasized that the ideal plant type should have higher values of the traits described above, whereas the traits showing negative effects on grain yield should be selected for lower values. In fact, Vasic et al. (2001) used various indices of selection for improvement of grain yield, and were able to show that even with a simple selection for improvement of grain yield.

The conventional path analysis, or the one carried out in the present study, suffers from the limitation of non-independence of predictor variables, often leading to high multicollinearity. In fact, Samonte et al. (Crop Sci. 38:1130-1136, 1998) proposed a sequential path analysis which is based on minimising multicolinearity due to complex interaction of yield component traits, and which delineates the importance of predictor variables into various orders based on their direct effects. Thus multiple regression based path analysis can be improved by stepwise regression analysis by sequentially removing the non-significant predictor variables from analysis. Besides, more and more traits can be included in the path analysis in order to reduce the residual effects.

Triple test cross analysis for detection of epistasis for ear characteristics in maize (Zea mays L.)

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Additive genetic variance is typically considered the most important process in the inheritance of quantitative traits, followed by dominance, whereas epistasis is of minor significance (Hallauer and Miranda, Quantitative Genetics in Maize Breeding, Iowa State Press, Ames, IA, 1988). However, there is growing evidence that epistasis is an important component of genetic variance. In fact Eta-Ndu and Openshaw (Crop Sci. 39:346-352, 1999) opined that failure to include epistasis in the estimation of genetic components causes bias in such estimates of expected genetic gain under selection. The present investigation was undertaken to characterise the genetic architecture of ear characters in maize by using triple test cross analysis.

The triple test cross procedure is an efficient genetic model and is applicable to segregating and non-segregating populations arising from F2, backcross or homozygous lines. Besides, it is independent of gene frequency, linkage relationship and degree of inbreeding. In addition to the detection of epistasis, it provides unambiguous estimates of additive and dominance components in the absence of epistasis. The material for the present study was generated by crossing 15 white inbred lines (3 local and 11 exotic) of maize viz., WI9, W6, W7, GLET7, GLET27, CML77, CML79, CML111, CML138, CML173, CML213, CML214, CML240, CML244 and CML463 with three testers W3, W5 and W3 x W5. The test crosses were generated in 2004 at the winter maize nursery at Amberpet (Hyderabad). The parental lines, testers and crosses were evaluated at two diverse locations in the Kashmir valley, Larnoo and Wadura, in RBD with three replications at each location. Data was recorded for six ear characters (ear height, ear length, ear diameter, ears/plot, kernel rows/ear, seed weight/ear) from 10 competitive plants from each replication and analysed as per the procedure of Ketata et al. (Crop Sci. 16:1-4, 1973) which is based on the original model proposed by Kearsey and Jinks (Theor. Hered. 23:403-409, 1968).

The epistatic components were tested against their environmental interactions which in turn were tested for significance against their block x environment interactions. The degree and the direction of dominance were determined.

The analysis of variance due to genotypes, lines, testers, crosses, and parents v/s crosses indicates that substantial variability exists in the parental lines for ear traits, and that there were significant differences between parents and crosses. The environmental component was significant for all traits except ears/plot whereas, G x E interaction was significant for all traits except ear diameter, ears/plot and seed weight/ear indicating that environment plays an important role in the expression of these traits as is expected for quantitative traits. Similar results have been reported in maize for ear traits by Satyanarayan (Madras J. Agric. 30:204-208, 1999) and Dodiya and Joshi (Crop Res. 26:114-118, 2003)

Analysis results (Table 1) revealed significant epistasis for all traits except ears/plot, further establishing the fact that epistasis cannot be excluded in the estimation of genetic parameters.

The portioning of epistasis and its fixable [I] and non-fixable [j + l} components revealed the significance of both components for all traits except ear length, ears/plot and seed weight/ear for which additive interaction was non-significant and ears/plot for which (j + l) type was non-significant. Epistasis, as well as its components, interacted significantly with environment for most of the traits. The comparative analysis revealed that the non-fixable [j + l] component of epistasis was greater than its corresponding fixable com-

Table 1. Analysis of variance for detection of epistasis for and ear characteristics in maize.

Source of variation	d.f.	Ear height (cm)	Ear length (cm)	Ear diameter (cm)	Ears/plot	Kernel rows/ear	Seed weight/ear (g)
Epistasis (L1i + L2i-2L3i)	15	451.36**	55.68**	2.07	146.48	4.53**	95.59**
[i] type epistasis	1	493.455**	24.52	2.58*	10.21	5.53**	0.66
[j +l] type epistasis	14	448.367**	57.90**	2.03*	152.13	4.45**	102.38**
Epistasis x blocks	30	56.28	7.04	0.16	44.42	1.15	37.59
[I] type x blocks	2	38.93	3.97	0.91	13.73	0.03	52.85
[j + l} type x blocks	28	57.52	7.25	0.17	42.13	1.23	36.50
Epistasis x environments	15	127.42**	18.09**	0.93**	86.12**	1.13**	29.14**
[i] type x environments	1	103.18*	18.64**	1.12**	13.31	2.10	4.35
[j + l] type x environments	14	129.13**	18.05**	0.91**	91.32**	1.42**	30.91**
[i] type x blocks x environments	2	13.96	3.19	0.06	6.91	3.71	1.34
[j + l) type x blocks x environments	28	46.80	7.25	0.17	15.14	0.12	10.48
Epistasis x blocks x environments	30	44.61	6.98	0.16	14.37	0.24	9.87

ponent for all traits except ear height, ear diameter and kernel rows/ear, where the reverse was the case. The preponderance of non-additive epistasis indicates that hybrid breeding can be employed to exploit this component. However, both components, i.e., [I] and [j + l], can be exploited in intra- as well as inter-population improvement. Similar results in maize have been reported by Wolf and Hallauer (Crop Sci. 37:763-770, 1997) and Leon et al. (Crop Sci. 45:1370-1378, 2005)

The presence of epistasis for almost all ear characters indicates that the estimates of components of variation would be biased to an unknown extent if they are estimated by genetic models assuming absence of epistasis. Regardless of the type of epistasis, the bias tends to be greater in the additive component than the dominance component (Viana, Genet. Mol. Biol. 28:67-74, 2005), which causes over-estimation of narrow sense heritability. Consequently the predicted genetic gain would have an additional bias proportional to that of heritability. It would thus be logical to search for epistasis rather than attributing it to leftover variance after additive genetic variance and dominance are accounted for.

Combining ability analysis for maize (*Zea mays* L.) lines under the high altitude temperate conditions of Kashmir

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Maize, though widely considered a warm weather crop, is currently grown between 55^0 north and south latitudes (Shaw, In Corn and Corn Improvement, ASA, Madison, WI, 1988). However, due to a limited frost-free season, earliness assumes a considerable significance in tailoring maize cultivars suitable in high altitude areas.

Ten inbred lines, namely PMI-1, PMI-26, PMI-47, PMI-53, PMI-83, PMI-135, PMI-198, PMI-199, PMI-224 and PMI-401 (designated as P_1 to P_{10}), were evaluated in a half diallel mating design to generate 45 F1 crosses. The parents and their crosses were evaluated at two locations: the High Altitude Maize Research Sub Station, Pahalgam (2222m asl;341/2^0N,741/2^0E) and the Regional Research Station of Sher-e-Kashmir University of Agricultural Sciences and Technology in Khudwani (1542m asl;341/2^0N,741/2^0E), Kashmir, India during Kharif 2005. The experimental material was arranged in a randomized complete block design with three replications per location. Lines and crosses were randomized separately in each experiment. Each entry was represented by two rows of 4m length with a crop geometry of 60 x 20cm and a plant density of 83333 plants per hectare. The data were recorded and analyzed for six quantitative traits (Table 1). Grain yield was calculated using fresh ear weight at harvest, assuming 80% shelling and adjusted to 15% moisture content. Ear height was recorded for the primary ear. Combining ability analysis was performed using Griffing's (Aust. J. Biol. Sci. 9:463-493, 1956) method 2 model II. Pooled analysis over environments was carried out following Singh (Indian J. Genet. 33:469-481, 1973; Indian J. Genet. 39:383-386, 1979)

Pooled analysis of variance for combining ability (Table 1) revealed the presence of highly significance of mean squares due to GCA and SCA for all the characters studied indicating thereby the differences among parental lines for GCA and among crosses for SCA effects. The diversity of test locations was revealed by their highly significant mean squares. Both GCA and SCA effects showed significant interaction with location for all traits. This suggested the differential response of lines and crosses for GCA and SCA effects, respectively, implying thereby that different parental lines are needed to synthesize hybrids for different ecological situations. The SCA effects for grain yield , 50% silking, plant height and ear placement were relatively stable over locations as indicated by lower estimates of SCA x location interaction, whereas the reverse was the case for days to pollen shed and moisture content. The ratio of estimated GCA to SCA variances indicated the preponderance of the latter component in controlling the expression of all traits.

Table 1. Pooled analysis of variances for different traits in a diallel cross of maize.

Source of variation	Df	Grain yield per plot (Kg)	Days to 50% pollen shedding	Days to 50% silking	Moisture content (%)	Plant height (cm)	Ear placement (cm)
	Mean squares						
Locations	1	0.76**	0.13**	0.13**	0.22**	0.49**	0.44**
GCA	9	0.87**	0.75**	0.44**	0.32**	0.70**	0.89**
SCA	45	0.86**	0.18**	0.19**	0.24*	0.45**	0.58**
GCA x Locations	9	0.66**	0.28**	0.87**	0.30**	0.46**	0.38**
SCA x Locationst	45	0.13	0.30**	0.30**	0.45**	0.14	0.11
Pooled error	108	0.18	0.05	0.04	0.08	0.13	0.14
σ^2s/ σ^2g		0.021	0.353	0.127	0.065	0.080	0.075

*,**significant at 5% & !% level respectively
GCA = general combining ability
SCA = specific combining ability

The perusal of GCA effects (data not shown) reveals that P_{10} was an ideal general combiner for all characters followed by P_1, whereas P_9 was a good general combiner for all traits except pollen shed. P_4, though a good combiner for grain yield, showed positive significant GCA effects for moisture content and plant height. P_8 x P_9 is the most desirable cross combination for SCA effects, closely followed by P_6 x P_{10} and P_8 x P_{10}. An important inference that can be drawn from these results is that cross combinations involving P_{10} as one of the parents recorded desirable SCA effects for all or most of the traits studied. P_{10} thus could serve as a potential donor for all these desirable attributes, and therefore has a special value in the maize improvement programme of the high altitude temperate ecology of Kashmir.

Genetic divergence among local maize (*Zea mays* L.) cultivars of the Kashmir valley

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Jammu and Kashmir state is a source of biological heritage and 90% of the maize area in the state is under the cultivation of local cultivars with distinct morphological differences for plant type, grain shape and grain colour. These cultivars have adoptability on account of early maturity and resistance to biotic and abiotic stresses. The present investigation was undertaken to study the extent of variability and identify divergent lines for their future use in a breeding programme.

The experimental material was comprised of 50 local maize cultivars collected from maize growing areas of the Kashmir valley, and 4 high yielding maize composites (C_8, C_{15}, C_6) and super-I were used as controls. 50 cob samples were collected from the villages of Khag, Yusmarg, Neilnag, Dalwash, Kralpathri (District of Budgam); Shopian, Balpora, Malanpora, Tral, Turkwangwam (District of Pulwama); Uri, Gurez, Bandipora, Pattan, Wagoora, Tangmarg, Farozpura (District of Baramula); Qazigund, Dooru, Verinag, Kulgam, Pombai, Pahalgam, Mattan, Kokernag (District of Anantnag); and Kangan Wangat, Satrain, Babanagri, Sonmarg, Dara (District of Srinagar). 25 sampled villages show preference for different grain colours such as orange yellow, yellow, creamish, white and purple. Progeny rows from each sampled cob were planted at K D Research Station. Each plot was 2 rows of 5m length. All recommended agronomic practices were followed to raise an ideal crop. Data on 5 competitive plants from each plot was recorded for maturity, morphological and yield related traits, e.g., days to 50% silking, days to 50% anthesis, days to husk browning, ear height (cm), ear length (cm) and grain yield (kg/ha). Data was subjected to analysis of variance, and coefficient of variability, heritability and genetic advance was estimated as per the methods of Johnson et al. (Agron. J. 47:314-318, 1955). Divergence analysis was computed following Mahalanobis (Proc. Natl. Inst. Sci. India 2:49-55, 1936). The individual contribution of characters towards the D^2 for each cultivar was determined. Genotypes were grouped into clusters following Tocher's method as described by Rao (Sankhya 19:201-408, 1958)

Genotype variance was highly significant for all characters (Table 1). Days to 50% silking ranged from 71 days (C_{15}) to 87 days (Pul-Tral 26) with a population mean of 78 days, whereas days to 50% anthesis ranged from 69 days (Bar-Bandipora 33) to 85 (Sgr-Sonamarg 16). Pul-Malangpora 30 cultivar recorded the minimum days for husk browning (131 days), whereas the maximum number of days was recorded by Bar-Bandipora 33 (148 days). Population mean for this trait was recorded as 140 days. Most of the local cultivars were observed to be early compared to high yielding composites. Controls recorded a range of 136-143 days for husk browning. Irrespective of the origin, plant height ranged from 118.50 cm (Sgr-Wangat 12) to 218.50 cm (Bud-Khag 3), with a population mean of 185.91 cm. A similar trend of cob samples collected from the districts of Srinagar and Budgam was recorded for minimum and maximum ear height (99.50 cm). The mean ear height was 81.04 cm. Ear length, which is an important yield attributing trait, was minimum in the case of local cultivars (7.93 cm) and maximum in the controls (20.02 cm). A wide range of variability was recorded for yield (kg/ha). The highest yielder (C_6) recorded a yield of 5828.50 kg/ha, whereas a local cultivar for district Pulwama (Pul-Turkwangan 29) recorded the lowest yield (1330.50). Average population yield was recorded as 2052.11 kg/ha. It should be possible to isolate superior genotypes during the selection process (Tables 1-3).

Table 1. Components of variance for maturity, morphological and yield related traits in local maize cultivars.

Components of variance	Days to 50% silking	Days to 50% anthesis	Days to husk browning	Plant height (cm)	Ear height (cm)	Ear length (cm)	Yield (kg/ha)
σ^2p	26.30	26.18	27.94	189.74	127.71	3.12	271878.74
σ^2g	21.74	13.26	21.93	187.08	125.91	3.12	271855.22
PCV	6.52	6.62	3.78	7.41	13.94		12.93
GCV	5.92	4.72	3.35	7.35	13.84	10.09	12.93
h^2	0.82	0.51	0.78	0.98	0.98	0.99	0.99
Genetic Advance	8.73	5.34	8.54	27.97	22.95	3.63	1074.03
Genetic Advance as % of mean	11.09	6.91	6.11	15.04	28.31	20.77	26.63
Mean	79.00	77.00	185.91	140.00	81.04	12.98	2032.11
Range	71.00 to 87.00	71 to 85	118.90 to 218.50	131 to 148	48.50 to 99.50	7.93 to 20.02	1330.50 to 5428.50

Table 2. Classification of local maize cultivars into different clusters.

Cluster	**Cultivars included**
Cluster 1	35, 39, 3, 23, 22, 4, 5, 45, 51 C_8, 18, 43, 34, 42, 24, 8, 15, 36, 44
Cluster 2	9, 41, 1, 10, 47, 14, 50, 16, 52, 27, 17, 26
Cluster 3	19, 32, 29, 31, 30, 48, 11, 12, 20, 7, 13, 38, 40, 21, 33
Cluster 4	2, 49, 46, 6, 37
Cluster 5	28
Cluster 6	53
Cluster 7	25
Cluster 8	54 Super-1

Bd-Khag 1, Bd-Khag 2, Bd-Khag 3, Bd-Yusmarg 4 , Bd-Neilnag 5, Bd-Yusmarg 6, Bd-Dalwash 7, Bd-Dalwash 8, Bd-Kral Pathsia 9, Bd-Kral Pathsia 10, Sgr-Khag 11, Sgr-Wangat-12, Sgr-Satrain 13, Sgr-Babanagri 14, Sgr-Dara 15, Sgr-Sonamarg 16, Sgr-Sonamarg 17, Sgr-Gund 18, Sgr-Gund 19, Sgr-Gutlibagh 20, Pul-Shopian-21, Pul-Shopian-22, Pul-Balpora 23, Pul-Malangpora 24, Pul-Tral 25, Pul-Tral 26, Pul-Tral 27, Pul-Turkwangam 28, Pul-Turkwangam 29, Pul-Malangpora 30, Bar-Uri 31, Bar-Gurez 32, Bar-Bandipora 33, Bar-Bandipora 34, Bar-Pattan 35, Bar-Wagoora 36, Bar-Wagoora 37, Bar-Tangmarg 38, Bar-Fareozpur 39, Bar-Uri 40, Ant-Qazigund 41, Ant-Dooru 42, Ant-Verinag 43, Ant-Verinag 44, Ant-Kulgam 45, Ant-Pumbai 46, Ant-Pahalgam 47, Ant-Pahalgam 48, Ant-Mattan-49, Ant-Kokernag 50

Table 3. Cluster means for different traits in local maize cultivars adapted to different temperate conditions of Kashmir.

Cluster no.	Number of cultivars in cluster	Yield (kg/ha)	Days to husk browning	Days to 50% silking	Days to 50% anthesis	Plant height (cm)	Ear height (cm)	Ear length (cm)
1	18	3983.72	181.44	86.81	83.61	152.64	83.14	18.10
2	12	4665.25	173.29	86.88	85.25	157.83	87.63	19.55
3	15	4581.60	173.50	86.33	83.57	151.73	85.00	15.63
4	5	3585.60	167.30	89.30	87.00	155.30	88.50	15.58
5	1	4923.50	167.00	91.50	82.00	157.50	124.00	17.84
6	1	5763.00	189.00	95.50	93.00	156.50	123.00	16.65
7	1	3104.50	194.50	82.50	81.50	162.00	99.00	19.53
8	1	5851.00	163.50	81.50	81.50	158.60	94.50	18.27

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University of Craiova

Studies concerning the heredity of some characteristics of the corncob

--Bonea, D; Urechean, V

The great productivity of corn relies heavily on heterosis. In this paper we explore heterosis with the potential to contribute to the creation of valuable new corn hybrids. We performed direct diallel hybridization of five corn inbred lines and analyzed certain characteristics of the corncob in the simple hybrids. We evaluated heterosis using capacity relation (CR) calculated using the formula of M. Jost (1976):

$$CR=\frac{F1-Max.P}{\frac{1}{2}(P2-P1)}$$

where Max P is maximum parental value.

We found (Table) strong heterosis for all the characteristics of the corncob most notably the weight of the corncob for Lc406x Lc407 (222g) and Lc402xLc406 (215.7g) hybrids; the length of the corncob for Lc402xLc 406 (8.35 cm) and Lc402xLc407 (6.5 cm) hybrids; and the number of grains/corncob for Lc402xLc406 (524) and Lc406xW153R (444.5). Our data shows that there is no heterosis effect in the number of grains per corncob for Lc403xLc407 (-77.5) and Lc407xW153R (-7.0) hybrids.

Table 1. The difference between F1 and parent's average and capacity relation of some simple corn hybrids.

No.	Hybrid	Corncob weight (g)		Corncob length (cm)		Number of grains per corncob	
		$F1-\bar{x}$	CR	$F1-\bar{x}$	CR	$F1-\bar{x}$	CR
1	Lc403xLc402	26.5	+0.74	5.05	-0.83	205	-0.68
2	Lc406xLc402	193	+26.57	5.75	-22.0	428	+1.56
3	Lc407xLc402	195	-194	5.7	-3.38	166.5	+29.2
4	W153RxLc402	138	-5.43	3.8	-1.11	147.5	+1.47
5	Lc402xLc403	86	-0.15	3.25	+0.18	167	+0.36
6	Lc406xLc403	137.5	+0.28	2.3	-0.08	252	-0.13
7	Lc407xLc403	68.8	-0.31	3.55	+1.46	48.5	-0.62
8	W153RxLc403	34.2	-0.46	1.06	+0.10	137.5	-0.24
9	Lc402xLc406	**215.7**	+27.8	**8.35**	+32.4	**524**	-2.43
10	Lc403xLc406	92.5	+0.14	3.6	-0.44	202	+0.30
11	Lc407xLc406	155.3	-18.4	2.05	-0.95	286.5	-0.77
12	W153RxLc406	135.4	-2.07	2.55	-0.64	391.5	-2.64
13	Lc402xLc407	207	+206	**6.5**	+4.0	283.5	-50.5
14	Lc403xLc407	74.5	+0.25	0.55	+0.62	-77.5	+1.60
15	Lc406xLc407	**222**	+26.75	4.95	+3.71	384.5	+1.38
16	W153RxLc407	158	-3.38	3.5	-6.0	231	+3.27
17	Lc402xW153R	187.8	+4.07	5.8	+2.22	324.5	-4.45
18	Lc403xW153R	64.5	-0.02	4.25	-3.47	98.5	+0.45
19	Lc406xW153R	147	+2.34	5.95	+2.83	**444.5**	+3.13
20	Lc407xW153R	129.5	+2.59	2.50	+4.0	-7.0	-1.12

In summary, we find that for corncob weight, eleven hybrids are positive for heterosis and nine are negative; for corncob length, ten hybrids are positive and ten are negative; for the number of grains per corncob, ten hybrids are positive and ten are negative. Positive hybrids are considered to be valuable and will be promoted through improvement programs.

TURDA, ROMANIA
Agricultural Research Station

Breeding implication of intra- and interheterotic group crosses as a source of new inbred lines in maize

--Has, V; Has, I

Advanced cycle pedigree breeding is the most common method for developing maize inbreds. Many of the current elite maize inbreds are derived from only a few progenitor inbreds; this breeding process systematically leads to a narrow maize germplasm within heterotic groups. Maize breeders have sometimes used commercial hybrids as a source of new inbreds. The effects of disrupting heterotic patterns in maize, by selfing from commercial hybrids, are not well understood.

The objective of this study was to compare intra- and interheterotic group crosses as sources of new inbred lines. We evaluated 425 inbred lines, created at the Agricultural Research Station in Turda, Romania. The inbred lines have been derived from different sources of germplasm using conventional breeding techniques of pedigree selection and early-generation yield testing. We used the following sources of germplasm for inbred line development: local varieties 3%, composites 14%, improved elite inbred lines 47% and commercial hybrids 36%.

Twelve of these inbred lines were selected by the year when they were finalized (Table 1). The inbred lines have been crossed with two testers--inbred lines belonging to a flint heterotic pattern. The testcrosses were evaluated in randomised complete block design in two locations for 2 years. Analysis of variance was performed for grain yield, stalk and root lodging, kernel dry matter and selection index (Table 2).

Table 1. Turda inbred lines listed by four decades of important use.

Inbred line	Decade of important use	Year finalized	Source of germplasm*	Origin of initial material
Maternal inbreds (m)				
T248	1961 – 1970	1964	LCS	Commercial hybrid KS3
T291		1966	RYD	Local variety Ungheni 247
T243		1965	RYD	Commercial variety VIR42
T169a	1971 – 1980	1972	RYD x ?	(W153R x W37A) x Mihalţ256
T158		1971	RYD x ?	(W153R x W37A) x Mihalt 1745
T160		1971	RYD	Commercial hybrid KC3VI
TC243	1981 – 1990	1989	WF9 Group x RYD	Commercial hybrid
TB366		1987	LSC	W182B x T248-I
TC316		1988	? x LCS	S54 x MO17
TC344	1991 – 2000	1995	RYD	Commercial hybrid
TC335		1994	(LSC x RYD) x ID	(T248 x T291) x TB329
TE203		1996	RYD	TD261^2 x T291
Paternal inbreds (n)				
LO3	-		ELF	Pop de Lostrano
PI187	-		ELF	PTF x Pop Italia

RYD - Reid Yellow Dent; LSC - Lancaster Sure Crop ID - Iodent; ELF - European Late Flint

Table 2. Additive genetic effects ($\hat{g}m$) for m=12 inbred lines, n=3; a factorial crossing system m x n (12 x 3) x 2 locations x 2 years.

Trait Inbred lines (m)*	Grain yield	Dry matter of grain at harvest	Percent of plants not stalk lodging at harvest	Selection index
T248^3	- 0.61	- 0.07	6.77	6.09
T291^1	2.17	- 0.34	4.62	6.45
T243^3	- 1.84	- 0.84	- 6.29	- 8.97
-created in '1960	**- 0.28**	**- 1.25**	**5.10**	**3.57**
T169a^2	- 11.55	2.00	4.88	- 4.67
T158^3	1.33	0.17	- 9.14	- 7.64
T160^3	- 12.98	0.59	2.13	- 10.26
-created in '1970	**- 23.20**	**2.76**	**- 2.13**	**- 22.57**
TC243^3	8.26	- 1.04	- 2.06	5.16
TB366^2	- 3.07	1.09	- 4.78	- 6.76
TC316^2	- 0.87	- 2.10	0.56	- 2.41
-created in '1980	**4.32**	**- 2.05**	**- 6.28**	**- 4.01**
TC344^3	7.52	- 0.58	- 0.92	6.02
TC335^2	4.97	0.79	2.80	8.56
TE203^2	6.67	0.36	1.45	8.48
-created in '1990	**19.16**	**0.57**	**3.33**	**23.06**
DL 5%	3.14	0.40	3.47	-

*Inbred lines were derived from: [1]open-pollinated varieties; [2]improved elite inbred lines; [3]commercial hybrids.

The new elite inbred lines were crossed with more testers (7-8 inbred lines per year) from different heterotic patterns. They were evaluated (Table 3) by their GCA for the main characters.

The testcross means showed good results for grain yield: TC385A, TA428, TE203, TD268, TC365, TC344, TD345; stalk and root lodging resistance: TD273, TD268, TC335, TC365, TC344; kernel dry matter: TD273, TC335, TE210, TC344, TD345, TD348;

Table 3. General combining ability (GCA) specific to 11 new Turda inbred lines in maize.

Inbred line	Year of testing	No. crosses	Grain yield (DMG = 15.5%)		Percent of plants not stalk lodged at harvest		Dry matter of grain at harvest		Selection index %*
			q/ha	%*	%	%*	%	%*	
Source: BK of elite inbred lines									
TC385 A	1999	41	101.3	98	92.0	99	72.0	103	100
	2001	27	114.6	98	91.2	99	81.3	99	95
	2002	35	113.1	109	91.6	95	80.2	99	102
GCA/ TC385A			**108.8**	**99**	**91.9**	**98**	**76.2**	**101**	**99**
TA428	1999	66	103.5	100	92.0	99	71.9	103	102
	2001	3	127.3	108	82.0	89	81.1	98	95
	2002	67	110.9	106	95.0	99	79.7	98	104
GCA/ TA428			**107.7**	**103**	**93.3**	**99**	**75.9**	**100**	**103**
TE203	1999	35	98.7	95	92.5	100	76.6	109	104
	2001	85	115.1	98	88.5	96	82.5	100	94
	2002	100	100.9	97	94.3	97	81.9	101	95
GCA/ TE203			**106.0**	**97**	**91.8**	**97**	**81.3**	**102**	**96**
TD273	1999	13	110.2	97	91.1	98	70.6	101	96
	2001	23	110.5	94	96.3	100	82.6	100	99
	2003	77	81.2	97	98.6	100	84.6	100	98
GCA/ TD273			**90.5**	**96**	**97.3**	**100**	**82.6**	**100**	**98**
TD268	2001	91	115.2	98	97.1	106	80.8	98	102
	2003	61	86.5	103	96.5	98	82.9	98	100
GCA/ TD268			**103.4**	**100**	**96.9**	**103**	**81.6**	**98**	**101**
TC335	1999	21	94.9	92	94.0	102	73.3	105	97
	2001	34	108.4	92	93.6	102	82.4	100	94
	2003	29	85.1	102	96.4	98	84.1	100	99
GCA/ TC335			**97.0**	**95**	**94.7**	**101**	**80.7**	**101**	**96**
TC365	1999	38	109.3	106	93.5	101	70.7	101	108
	2001	22	125.2	107	97.7	106	81.5	99	112
	2002	40	109.5	105	95.7	99	80.2	99	103
	2003	38	88.9	106	95.8	98	84.5	100	106
GCA/ TC365			**106.3**	**106**	**95.4**	**100**	**79.0**	**100**	**107**
Comparison mean: Saturn, Helga (commercial hybrids)	1999	50	103.5	100	92.5	100	70.1	100	100
	2001	50	117.4		92.0		82.4		
	2002	50	104.2		96.5		80.9		
	2003	50	83.8		98.2		84.4		
Source : commercial hybrids									
TE210	1999	29	102.6	99	92.5	100	72.9	104	103
	2001	44	111.5	95	89.5	97	83.5	101	94
	2003	73	86.1	103	92.2	94	85.9	102	98
GCA/ TE210			**97.0**	**100**	**91.4**	**96**	**82.6**	**102**	**98**
TC344	1999	70	104.0	100	94.0	102	72.8	104	106
	2001	125	119.9	103	92.4	101	82.1	100	102
	2002	112	111.6	107	96.6	100	80.4	99	107
	2003	55	90.8	108	94.4	96	84.5	100	104
GCA/ TC344			**109.8**	**104**	**94.3**	**100**	**80.1**	**100**	**105**
TD345	1999	34	102.7	99	91.5	98	74.3	106	103
	2001	28	112.7	96	92.0	100	83.1	101	97
	2002	56	107.7	103	95.0	98	82.0	101	103
	2003	37	85.3	102	91.8	93	85.0	101	96
GCA/ TD345			**102.2**	**101**	**92.9**	**97**	**81.2**	**102**	**100**
TD348	1999	10	102.1	99	95	103	70.2	100	101
	2002	55	101.3	97	96.7	100	81.9	101	99
	2003	60	84.6	101	93.1	95	84.1	100	95
GCA/ TD348			**93.3**	**99**	**94.8**	**98**	**82.0**	**100**	**97**
Comparison mean: Saturn, Helga (commercial hybrids)	1999	50	103.5	100	92.5	100	70.1	100	100
	2001	50	117.4		92.0		82.4		
	2002	50	104.2		96.5		80.9		
	2003	50	83.8		98.2		84.4		

*GCA of the Turda inbred lines has been compared with the mean of the two hybrids.

selection index: TA428, TD268, TC365, TC344, TD345.

In conclusion:

1) The last years were characterized by a genetic gain in inbred lines development.

2) GCA effects for the main characters were more favourable for inbred lines derived from improved elite inbreds and commercial hybrids.

3) The local populations would be used as sources of inbred lines only after they were improved in a special program by recurrent or reciprocal-recurrent selection.

4) The relative usefulness of intra- versus intergroup populations as sources of new inbreds depends on the particular inbreds used and/or on finding a suitable tester.

Effects of different cytoplasms on quantitative characters in maize

--Has, V; Has, I

The study of cytoplasmic effects on the expression of quantitative characters is important to understanding cytoplasmic-nuclear interactions and their influence on breeding and genetic programs. The genotypes of six early-maturing maize inbreds T248, TC243, TC209, TB367, A654, TC102, T291 were backcrossed eight times into ten cytoplasms other than their own. Thus, each cytoplasmic source of seed was assumed to have the same nuclear genotype. Five characters were studied over two years and two locations. Significant differences among the cytoplasms occurred for plant vigor, date of tasseling and silking, stalk lodging resistance and kernel dry matter.

Table 1. Mean percent of stalk lodging resistance at harvest for each of the ten cytoplasmic sources with six genotypes.

Pair no.	Inbred genotype	TC209	T291	T248	TC243	TB367	TC102
	Cytoplasmic source	%	%	%	%	%	%
1.	Own cytoplasm	61.1	32.6	68.6	68.0	66.1	42.6
2.	T 291	61.0	x	71.4	60.8	70.2	53.8**
3.	T 248	61.3	49.9**	x	68.8	69.6	59.1***
4.	TC 243	55.0	30.7	66.4	x	72.1*	58.9***
5.	A 654	61.3	56.7***	70.9	60.2	71.5	59.0***
6.	TC 221	61.5	35.5	66.8	63.5	63.3	55.8**
7.	TC 102	55.4	41.4	67.1	69.9	70.5	x
8.	TC 209	x	56.6***	69.0	65.5	71.4	44.2
9.	K 1080	58.3	40.2	68.3	62.8	62.5	48.3
10.	TC 316	60.2	30.7	58.8^{00}	64.7	74.9**	40.4
	LSD 5%	9.0	9.9	7.0	10.7	5.8	7.9
	1%	12.0	13.4	9.4	14.6	7.8	10.7
	0.1%	15.8	17.7	12.4	19.5	10.3	14.1

Differences in grain yield among cytoplasms were not significant, or their expression was of low magnitude, and probably due to interaction with environments. Differences that did exist were in the direction expected on the basis of inbred performance. The results indicate that differences in inbred performance were not necessarily an indication of cytoplasmic effects in hybrid combination. Results from these experiments provide further support for cytoplasmic effects on some agronomic characters in maize. They also emphasize that the environment can influence the performance of cytoplasm from one year to another.

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Maize Genetics Cooperation • Stock Center

Allelism testing of miscellaneous stocks in Maize COOP phenotype only collection

--Jackson, JD

This report summarizes allele testing of miscellaneous stocks characterized by phenotype only in the Maize Genetics COOP Stock Center collection. These particular mutants were sent in by M. G. Neuffer. If possible, crosses were made between known heterozygotes and homozygous plants. Plants were scored at the seedling stage and again at maturity. Ears were scored at maturity. Proposed new designations have been assigned to these alleles. These stocks have been increased and placed on our stocklist. It is expected that with further sorting and allelism testing of mutations characterized by phenotype only, additional alleles of characterized mutants will be discovered and placed in the main collection.

POSITIVE TESTS:

previous designation	allelism test with *g1*	new designation	MGCSC: stock #
yg-N1856A*	positive: (*g1*) x *yg**)	*g1-N1856A*	X05L

previous designation	allelism test with *gl1*	new designation	MGCSC: stock #
gl-N1843C*	positive: (*gl**) x *gl1*	*gl1-N1843C*	704J
gl-N1845*	positive: (+/*gl**) x *gl1*	*gl1-N1845*	704K

previous designation	allelism test with *sh1*	new designation	MGCSC: stock #
sh-N399A*	positive: (+/*sh**) x *sh1*)	*sh1-N399A*	912AK

previous designation	allelism test with *dek5-N874A*	new designation	MGCSC: stock #
sh-N961*	positive: (+/*sh**) x (+/*dek5*)	*dek5-N961*	333AB

previous designation	allelism test with *cp2-N1324A*	new designation	MGCSC: stock #
sh-N1105B*	positive: (+/*sh**) x (+/*cp2*)	*cp2-N1105B*	426E

Additional new alleles 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). These new alleles were 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 stocklist. It is expected that with further sorting and allelism testing of other stocks characterized by phenotype only, additional *ps1* alleles will be discovered.

previous designation	allelism test with *ps1*	new designation	MGCSC: stock #
y-vp-8105*	5 positive	*ps1-8105*	526H
vp-8107*	5 positive	*ps1-8107*	526I
vp-8115*	1 positive	*ps1-8115*	526J
vp-8208*	3 positive	*ps1-8208*	526K

A survey of viviparous stocks in Maize COOP phenotype-only collection for new alleles of *viviparous9*

--Jackson, JD

This report summarizes allele testing of viviparous stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here, pale kernels linked to the viviparous trait characterized all stocks. Crosses were made as follows: +/*vp** X +/*vp9-R* or +/*vp** X +/*vp9-Bot100*. Ears were scored at early maturity for the segregation of pale kernels. In crosses, most kernels were dormant for the viviparous phenotype. This could be due to the fact that ears were scored at an early stage of maturity. In almost all crosses, pale kernels gave the characteristic greenish almost fluorescent cast found in the *vp9-Bot100* allele (Jackson, MNL 73:86, 1999). This color slowly faded with exposure to sunlight.

New designations have been assigned to these alleles and these have been placed in the main collection. It is expected that with further sorting and allelism testing of viviparous stocks characterized by phenotype only, additional alleles of *vp9* will be discovered. Stocks with this same phenotype that were found to complement *vp9* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	Allelism test with *vp9*	New designation	MGCSC: stock #	Dormant allele
pale y-vp-85-3140-15*	3 positive	*vp9-85-3140-15*	721A	no
pale y-vp-87-2286-1*	3 positive	*vp9-87-2286-1*	721B	yes in F2 w/ B73
pale y-vp-87-2286-2*	5 positive	*vp9-87-2286-2*	721C	yes in F2 w/ B73
pale y-vp-87-2286-3*	4 positive	*vp9-87-2286-3*	721D	yes in F2 w/ B73
pale y-vp-87-2286-18*	5 positive	*vp9-87-2286-18*	721E	yes in F2 w/ B73
pale y-vp-87-2286-25*	4 positive	*vp9-87-2286-25*	721F	yes in F2 w/ B73
pale y-vp-88-3177-14*	5 positive	*vp9-88-3177-14*	721G	no
y-vp-88-89-3613-25*	10 positive	*vp9-88-89-3613-25*	721H	variable
y-vp-99-2226-1*	4 positive	*vp9-99-2226-1*	721I	no
y-vp-6961*	3 positive	*yp9-6961*	721J	variable
y-vp-8701*	3 positive	*vp9-8701*	721K	no
vp-8113*	2 positive	*vp9-8113*	722A	no
y-vp-81-15*	5 positive	*vp9-81-15*	722B	no
y-vp-1982-2*	7 positive	*vp9-1982-2*	722C	no
vp-8204*	5 positive	*vp9-8204*	722D	no
y-vp-8206*	5 positive	*vp9-8206*	722E	no
y-vp-8207*	5 positive	*vp9-8207*	722F	no
y-vp-83-1A*	3 positive	*vp9-83-1A*	722G	no
pale y-85-3005-22*	5 positive	*vp9-85-3005-22*	722H	yes in F2 w/ B73
pale y-85-3069-6*	4 positive	*vp9-85-3069-6*	722I	yes in F2 w/ B73
pale y-89-90-1525-23*	2 positive	*vp9-89-90-1525-23*	722J	yes in F2 w/ B73/Mo17

Additional *r1* haplotype-specific aleurone color enhancer mapping results

--Stinard, PS

In last year's MNL (80:31), we reported that three mutable and two stable *r1* haplotype-specific aleurone color enhancers map to the same location on chromosome 2 and are likely allelic. We tested the mutable factors *Fcu* with *arv-m594*, *arv-m594* with *arv-m694*, and *Fcu* with *arv-m694*. We also tested the stable full color enhancer *Arv-V628#16038* with a full color *Fcu* revertant, *Fcu-R2003-2653-6*. However, direct linkage tests had not been performed between the mutable factors and the stable factors. We report the results of such tests in this article. We tested linkage of the mutable factor *arv-m594* with the stable factor *Arv-V628#16038*, and the linkage of the mutable factor *Fcu* with the stable *Fcu* revertants *Fcu-R2003-2653-2* and *Fcu-R2003-2653-6*.

All tests were conducted as follows: Lines homozygous for the two factors, and homozygous for either *r1-g*, or for a responsive *r1* haplotype, were crossed together. The resulting F1's were outcrossed as males to the responsive *r1* haplotype *R1-r(Venezuela559-PI302355)* without any factors present. The parental classes would be expected to have either mutable or full colored aleurones, the double factor recombinant class would be expected to have full colored aleurones, and the recombinant class lacking both factors would be expected to have stable pale aleurones. Kernels from these crosses were classified as mutable, colored, or pale/colorless. The exceptional pale or colorless kernels were planted last summer and the resulting plants self-pollinated and outcrossed to *R1-r(Venezuela559-PI302355)* in order to test the genotypes of these kernels. Since the only recombinant class that can be detected by these experiments is the class lacking both factors, we doubled the number of recombinants in this class in order to account for the double mutant class for the purpose of calculating linkage values. The results are presented below:

***arv-m594* with *Arv-V628#16038*.** From the linkage cross, we isolated 29 pale and 8 colorless kernels from a population of 7,475 kernels. Upon further testing of the pale and colorless exceptions, 6 proved to carry *Arv-V628#16038* and 21 carried *arv-m594* and were thus members of parental classes and not recombinants. Ten kernels did not germinate and were not tested. Based on our failure to recover recombinants from among the high proportion of exceptions tested, we calculate that the linkage between these two factors is less than 0.03 ± 0.02 cM. Thus, we conclude that these two factors are very tightly linked and most likely allelic.

***Fcu* with *Fcu-R2003-2653-2*.** We isolated one colorless kernel from a population of 3,936 kernels in this test. Upon further testing, this kernel proved to carry *Fcu-R2003-2653-2* and was not a recombinant. We calculate that the map distance between these two factors is less than 0.05 ± 0.04 cM.

***Fcu* with *Fcu-R2003-2653-6*.** We isolated two pale and three colorless kernels from a population of 2,040 kernels in this test. Upon further testing, two kernels proved to carry *Fcu-R2003-2653-6* and two carried *Fcu*; one kernel failed to germinate and was not tested. Based on our failure to recover recombinants from among the exceptions tested, we calculate that the linkage between these two factors is less than 0.10 ± 0.07 cM.

While we had strong reason to believe, based on indirect evidence, that the mutable enhancers map to the same location as these stable enhancers, these tests provide direct evidence for this. The two *Fcu* revertants we examined mapped to the same location as *Fcu*, as expected. We report elsewhere in this MNL the identification of two enhancers that map to linked sites. We plan to test these and other enhancers isolated from the Brink *r1* haplotype collection for linkage in order to place them into linkage or “complementation” groups and identify the number of enhancer loci segregating in these lines.

An *r1* haplotype-specific aleurone color enhancer expressed only in female outcrosses

--Stinard, PS

Members of the *Fcu*/*Arv* system of *r1* haplotype-specific aleurone color enhancers increase aleurone pigmentation in crosses to specific weakly pigmented *r1* haplotypes (Kermicle, MNL 77:52; Stinard, MNL 77:77-79). The enhancers characterized to date have mapped to the same location on chromosome 2, and express equally well in male and female outcrosses to susceptible testers (Stinard, MNL 78:63-64; MNL 79:45; MNL 80:31; MNL 80:33). However, during the course of propagating an accession (*R1-r(Venezuela459#16039)*) from the Brink *r1* haplotype collection donated to the Stock Center by Jerry Kermicle, aberrant ratios appeared in crosses to tester stocks, indicating the possible presence of multiple enhancers. The ratios were not simple Mendelian ratios, and differed depending on the direction of the cross. 1:1 ratios of colored to pale kernels, or ears with completely pale kernels, were invariably obtained when a segregating *Arv* parent was used as the male parent, but when used as a female, ratios were either 1:1, or showed an excess of colored kernels. Subsequent generations of outcrossing allowed the separation of two enhancers, one (*Arv-V459A*) that behaves as previously characterized enhancers, producing colored kernels in both male and female outcrosses, and one (*Arv-V459B*) that produces colored kernels in female outcrosses, but only pale kernels in male outcrosses. The ratios of colored and pale kernels on ears of female outcrosses of plants heterozygous for both enhancers deviate from 3:1 ratios and indicate linkage, with an approximate separation of 25 centiMorgans.

Data from testcrosses are presented in Tables 1, 2, and 3; and linkage data are summarized in Table 4. All stocks are in a W22 inbred background, and tests for *Arv* status were conducted using testers and backgrounds carrying responsive *r1* haplotypes (either *R1-r(Venezuela459#16039)* or *R1-r(Venezuela559-PI302355)*).

Data presented in Table 1 summarize the results of reciprocal crosses of plants carrying *Arv-V459A* and/or *Arv-V459B* with *arv* testers. The *Arv* parents were grown from the male outcross to an *arv* tester of a plant heterozygous for *Arv-V459A* and homozygous for *Arv-V459B*. Under the model presented above, colored kernels from such a cross would be expected to carry both *Arv-V459A* and *Arv-V459B*, and pale kernels would be expected to carry *Arv-V459B* only. The results of reciprocal crosses of such progeny bear this out. All plants grown from colored kernels produced an excess (from a 1:1 ratio) of colored kernels in female outcrosses, and a 1:1 ratio of colored to pale kernels in male outcrosses. This result can be explained if both *Arv-V459A* and *Arv-V459B* are expressed in female outcrosses, but only *Arv-V459A* is expressed in male outcrosses. All plants grown from pale kernels produced a 1:1 ratio of colored to pale kernels in female outcrosses, and only pale kernels in male outcrosses. Again, this result can be explained if *Arv-V459B* is expressed in female, but not male, outcrosses. The kernel counts of female outcrosses of plants heterozygous for both *Arv-V459A* and *Arv-V459B* showed an excess of colored kernels from a 1:1 ratio, but significantly less than a 3:1 ratio ($p < 0.05$, calculations not shown), suggesting linkage in coupling of these two factors.

Data presented in Table 2 summarize the results of reciprocal crosses of plants carrying *Arv-V459B* with *arv* testers. The *Arv-V459B* parents were grown from kernels from the self-

Table 1. Counts of colored (Cl) and pale kernels from reciprocal crosses of plants carrying *Arv-V459A* and *Arv-V459B* with *arv* testers. Families 2005-603 and 2005-604 were planted from the cross: *arv* X *Arv-V459A*/+; *Arv-V459B*. Plants crossed in 2005-603 were grown from colored kernels, and plants crossed in 2005-604 were grown from pale kernels.

	Kernel No.; Female OC			Kernel No.; Male OC			
Arv parent	Cl	pale	1:1 χ^2	Cl	pale	1:1 χ^2	Arv status
source: 2004-830-8/827-6; Cl planted							
2005-603-1	247	115	48.133[1]	146	153	0.164	A + B
2005-603-2	213	142	14.200[1]	145	132	0.610	A + B
2005-603-4	162	124	5.049[1]	147	151	0.054	A + B
2005-603-6	170	112	11.929[1]	99	74	3.613	A + B
2005-603-7	186	100	25.860[1]	124	122	0.016	A + B
2005-603-8	217	121	27.266[1]	112	125	0.713	A + B
2005-603-9	220	105	40.692[1]	85	114	4.226	A + B
source: 2004-830-8/827-6; pale planted							
2005-604-1	89	87	0.023	0	309		B
2005-604-6	171	166	0.074	0	248		B
2005-604-7	147	139	0.224	0	287		B
2005-604-9	221	186	3.010	0	317		B
2005-604-10	183	155	2.320	0	285		B
2005-604-11	147	168	1.400	0	191		B
2005-604-12	120	155	4.455	0	340		B

Two-sided χ^2 value significant at $p = 0.05$ level.

Table 2. Counts of colored (Cl) and pale kernels from reciprocal crosses of plants carrying *Arv-V459B* with *arv* testers. Families 2005-605 and 2006-606 were planted from the cross: [*Arv-V459B*/+] selfed. Plants crossed in 2005-605 were grown from pale kernels, and plants crossed in 2006-606 were grown from colored kernels.

	Kernel No.; Female OC			Kernel No.; Male OC		
Arv parent	Cl	pale	1:1 χ^2	Cl	pale	Arv status
source: 2004-827-7@; paleplanted						
2005-605-4	47	36	1.458	0	263	B
2005-605-5	103	89	1.021	0	216	B
2005-605-6	0	66		0	312	-
2005-605-8	119	128	0.328	0	333	B
2005-605-11	66	51	1.923	0	254	B
2005-605-12	49	62	1.523	0	196	B
source: 2004-827-7@; Cl planted						
2005-606-1	165	0		0	351	B
2005-606-3	45	41	0.186	0	250	B
2005-606-4	79	78	0.006	0	262	B
2005-606-5	27	31	0.276	0	335	B
2005-606-6	60	56	0.138	0	284	B
2005-606-9	139	0		0	378	B
2005-606-10	74	91	1.752	0	280	B
2005-606-11	54	0		0	333	B
2005-606-12	48	44	0.174	0	297	B

pollination of a plant heterozygous for *Arv-V459B*. Colored kernels from such a cross would be expected to be either heterozygous or homozygous for *Arv-V459B*, with one copy of *Arv-V459B* transmitted through the female; pale kernels would be expected to carry one copy of *Arv-V459B* transmitted through the male, or be homozygous *arv*. The results of reciprocal crosses of such progeny bear this out. Plants grown from pale kernels produced either 1:1 ratios of colored to pale kernels (parent heterozygous for *Arv-V459B*), or only pale kernels (parent homozygous for *arv*) in female outcrosses; and only pale kernels in male outcrosses. Kernels grown from colored kernels produced either all colored kernels (parent homozygous for *Arv-V459B*) or 1:1 ratios of colored to pale kernels (parent heterozygous for *Arv-V459B*) in female outcrosses, and only pale kernels in male outcrosses.

Tests were carried out another generation in order to confirm these inheritance patterns. The data presented in Table 3 summarize the results of these tests. The plants tested in family 2005P-94 were grown from colored kernels from the male outcross to *arv* of a plant heterozygous for both *Arv-V459A* and *Arv-V459B*. All such plants would be expected to carry *Arv-V459A*, and half should also carry *Arv-V459B*. The female outcrosses of 3 out of 7 plants tested had ratios of colored to pale kernels that did not deviate significantly from 1:1, indicating parental heterozygosity for *Arv-V459A* only, and the remaining 4 plants had an excess of colored kernels (but not a 3:1 ratio) in female outcrosses, indicating heterozygosity for both *Arv-V459A* and *Arv-V459B*. The male outcrosses of 3 plants segregated 1:1 for colored to pale kernels as expected. The remaining 4 plants were not outcrossed as males.

The plants tested in family 2005P-95 were grown from colored kernels from the female outcross to *arv* of a plant heterozygous for *Arv-V459B*. All such plants would be expected to be heterozygous for *Arv-V459B*. The female outcrosses of all plants tested segregated 1:1 for colored to pale kernels, and the male outcrosses produced pale kernels only, as expected.

Table 3. Counts of colored (Cl) and pale kernels from reciprocal crosses of plants carrying *Arv-V459A* and *Arv-V459B* with *arv* testers. Family 2005P-94 was planted from the cross: *arv* X *Arv-V459A*/+; *Arv-V459B*/+. Family 2005P-95 was planted from the cross: *Arv-V459B*/+ X *arv*. Both families were grown from colored kernels.

	Kernel No.; Female OC			Kernel No.; Male OC			
Arv parent	Cl	pale	1:1 χ^2	Cl	pale	1:1 χ^2	Arv status
source: 2005-622-2/603-6; Cl planted							
2005P-94-2	107	107	0	45	60	2.143	A
2005P-94-3	146	71	25.922[1]	-	-		A + B
2005P-94-4	128	119	0.328	33	40	0.671	A
2005P-94-5	93	110	1.424	40	46	0.419	A
2005P-94-7	157	117	5.839[1]	-	-		A + B
2005P-94-8	135	88	9.906[1]	-	-		A + B
2005P-94-10	120	85	5.976[1]	-	-		A + B
Source: 2005-604-6/619-2; Cl planted							
2005P-95-2	147	132	0.806	0	161		B
2005P-95-3	82	94	0.818	0	52		B
2005P-95-4	135	112	2.142	0	67		B
2005P-95-6	108	130	2.034	0	47		B
2005P-95-7	101	101	0	0	106		B

Two-sided χ^2 value significant at p = 0.05 level.

All female outcrosses of plants heterozygous for both *Arv-V459A* and *Arv-V459B* in coupling produced ears with ratios of colored to pale kernels deviating from the 3:1 ratio expected of independent segregation ($p < 0.05$, calculations not shown). Kernel counts from all such ears examined are summarized in Table 4. A chi-square test of homogeneity revealed that the kernel color ratios are reasonably homogeneous in this data set, being homogeneous at the p = 0.01 level, but not at p = 0.05. Since the phenotypes of the parental class carrying both *Arv-V459A* and *Arv-V459B* cannot be distinguished from the two crossover classes carrying the individual factors, the number of individuals in this parental class was approximated as being equal to the parental class lacking both factors, namely the pale kernel class. Based on this assumption, the map distance between these two factors is calculated to be 25.2 +/- 0.8 centiMorgans. Direct multi-point linkage tests will be conducted with factors on chromosome 2 in order to verify this result and to better place both factors.

Table 4. Summary of counts of colored (Cl) and pale kernels from the cross: *Arv-V459A*/+; *Arv-V459B*/+ X *arv*.

	Kernel counts		
Arv parent	Cl	pale	freq. of Cl
2005-603-1	247	115	0.68
2005-603-2	213	142	0.60
2005-603-4	162	124	0.57
2005-603-6	170	112	0.60
2005-603-7	186	100	0.65
2005-603-8	217	121	0.64
2005-603-9	220	105	0.68
2005P-94-3	146	71	0.67
2005P-94-7	157	117	0.57
2005P-94-8	135	88	0.61
2005P-94-10	120	85	0.59
Totals	1973	1180	0.63

Homogeneity $\chi^2 = 22.767$, df = 10.

Data are homogeneous at the p = 0.01 level, but not at the p = 0.05 level.

Linkage between *Arv-V459A* and *Arv-V459B* = 25.2 +/- 0.8 cM.

Tests have not yet been performed to determine whether the differential expression of *Arv-V459B* is due to a dosage effect or to imprinting—this will be the subject of further study. Mapping will be conducted with chromosome 2 markers to determine which (if either) of these two factors maps to the same position as *Fcu*, and to determine a more precise location for the second factor.

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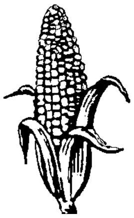
IV. MAIZE GENETICS COOPERATION STOCK CENTER



Maize Genetics Cooperation • Stock Center

USDA/ARS/MWA - Soybean/Maize Germplasm, Pathology & Genetics Research Unit

&

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3865 seed samples have been supplied in response to 292 requests, for 2006. A total of 82 requests were received from 24 foreign countries. More than 90% of our requests were received by electronic mail or through our on-line order form. Popular stock requests include the IBM RIL mapping populations, Hi-II lines, *ig1* lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, transposable element lines, Maize Gene Discovery Project lines, and Chromatin stocks.

Approximately 11.2 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Favorable weather in the early spring allowed the timely planting of our first crossing nursery. However, cool, rainy weather hindered germination and emergence in our early plantings, resulting in reduced stands and necessitating the replanting of a few lines. Rainfall was adequate, but like last year, redwing blackbirds started feeding on our second crossing nursery. As soon as we noticed this, we irrigated our second field, which seemed to reduce feeding. Subsequent rainfall seemed to solve the problem, and few rows were lost. Growing conditions were generally good, and supplemental irrigation was not necessary. Moderate temperatures and low plant stress resulted in a good pollination season.

Special plantings were made of several categories of stocks:

1. In the ‘Phenotype Only’ collection, we have made available an additional 48 stocks in 2006. This low number is due to the reduced stand of our second crossing nursery in 2005. We are still working on 119 phenotype-only stocks from the large collection sent to us by Gerry Neuffer in 1996 and 1997.

2. Plantings were also made from donated stocks from the collections of Alice Barkan (photosynthetic mutants), Ed Coe (*pg15*, *o16*, and *v29* alleles), Jerry Kermicle (various *r1* alleles), Robert Lambert (defective kernel mutants), Rob Martienssen (MTM material), Gerry Neuffer (recent EMS-induced mutants), the North Central Regional Plant Introduction Station (brown midrib and anther ear traits that were found in various PI accessions), Ron Phillips (mutants in various inbred backgrounds), Pat Schnable (*rth1*), Margaret Smith (male sterile cytoplasm lines), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

3. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We identified additional alleles of *albescent1*, *Factor Cuna* (*Fcu*), *glossy1*, *defective kernel5, collapsed2, pink scutellum1, viviparous5*, *viviparous9*, and *pale yellow9*. We plan to test additional members of the viviparous, spotted leaf, and pale endosperm classes of mutants. In this manner, we hope to move more stocks from our vast collection of unplaced uncharacterized mutants into the main collection.

4. 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.

5. We further characterized the *Fcu* system of *r1* aleurone color enhancers. We are working on linkage stocks to refine the map position of *Fcu* alleles using visible kernel and seedling markers on Chromosome 2, and we continued a series of crosses to transposon tag *Fcu* using one of Tom Brutnell’s transposed *Ac* lines. We are collecting and characterizing additional alleles of *Fcu* and other *r1* aleurone color enhancers and inhibitors.

6. Two acres were devoted to the propagation of the large collection of cytological variants, including A-A translocation stocks and inversions. In this collection is a series of *waxy1*-marked translocations that are used for mapping unplaced mutants. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have completed testing on these stocks and can now supply good sources proven by linkage tests to include the correct translocated chromosomes. Additional translocations we have received from W. R. Findley and Don Robertson marked with *wx1* are being checked by linkage tests as we did for the main series of *waxy1*-marked translocations. Some of these may replace ones from the main collection that were found to be bad.

7. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0110189) were grown this summer. Families that were observed in 2005 to segregate mutations were

selected to be increased in the nursery. These increases help to confirm the presence of the mutation and maintain adequate seed stock to fill future requests. These mutants are being added to our phenotype-only collection.

We continue to grow a winter nursery of 0.5 acres at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico. We had an excellent winter crop last year, and all indications are that the crop will perform well this year. We plan to continue growing our winter nurseries at this location.

We have received 579 additional EMS lines from various inbred backgrounds produced by Dr. Gerry Neuffer (Regulation of Inflorescence Architecture in Maize project). There are sufficient seed for all of these for distribution. We have also received an additional four lines from the Functional Genomics of Maize Chromatin project (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0421619) from Karen McGinnis.

The 579 lines from Gerry Neuffer's EMS material that were screened for ear and kernel mutations in the lab, were planted in observation fields on the University of Illinois Crop Science Research facility for observation of seedling and adult plant mutations during our annual mutant hunt. In addition to these lines, 2300 lines of MTM material from Rob Martienssen (http://mtm.cshl.edu) were also planted this year to be observed for new adult mutant phenotypes. Unfortunately, the MTM material was apparently extremely susceptible to the cool damp conditions that we had during the few weeks after planting, and therefore had extremely poor stands and was subsequently plowed under. We plan to have another mutant hunt next summer.

Our IT specialist, Josh Tolbert, 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. They are also used for our internal database (e.g., inventory, pedigrees, requests). Development of new tools is underway to improve the efficiency of the input processes for pedigree information, and creation of forms required for planting and harvest information. Our web site has also been updated (http://www.uiuc.edu/ph/www/maize).

Samples of 1239 stocks were sent to the National Center for Genetic Resources Preservation in Fort Collins, Colorado for back up. These represent new stocks that had not been previously backed up. Our new inventory system has made selecting ears to be sent and producing a packing list to accompany them a much more efficient procedure.

The new greenhouse space in Urbana has been completed and is being used this winter. Our new seed storage space (which will double our capacity) is presently being built.

Marty Sachs	Philip Stinard	Janet Day Jackson	Shane Zimmerman	Josh Tolbert
Director	Curator	Biol Res Tech (Plants)	Agric Sci Res Tech (Plants)	Information Tech Specialist

ADDITIONS TO OUR CATALOG OF STOCKS SINCE MNL80

(For a complete list of our stocks, see: http://maizegdb.org/cgi-bin/stockcatalog.cgi)

CHROMOSOME 1 MARKERS
116B bz2-m::Ds; Ac2
116BA bz2-m::Ds; Ac2-strong
128DB pg15-N495B

CHROMOSOME 2 MARKERS
213K w3-N1907

CHROMOSOME 4 MARKERS
402G tga1

CHROMOSOME 5 MARKERS
504H v36-N1835
520L nec3-85-3457-40

CHROMOSOME 7 MARKERS
704K gl1-N1845

CHROMOSOME 8 MARKERS
804F elm1-ref

CHROMOSOME 10 MARKERS
X02M Oy1-N1460
X06G Og*-Catlin-yel
X17G R1-r(standard)
X27KA v29-N1224C

CHROMDB STOCKS
3201-22.1 T-MCG4291.007
3201-28.2 T-MCG5297.020
3201-42 T-MCG3832.001
3201-43 T-MCG4585.005
3201-43.1 T-MCG4585.009
3201-44 T-MCG6071.022
3201-45 T-MCG6432.027
3201-45.1 T-MCG6432.034
3201-45.2 T-MCG6432.046

UNPLACED GENES
U139J d4

TETRAPLOID
N108B Autotetraploid; Oh43

CYTOPLASMIC-STERILE / RESTORER
CX36E Wf9 (C) Sterile; cms-C rf1 rf2 rf3 rfC

TOOLKIT
T3301-56 Ac-im; r1-sc:m3::Ds

INVERSION
I543E Inv5e (5S.21; 5L.75)

PHENOTYPE ONLY

adherent leaf
3608L ad*-N247A
3610O ad*-N2507

anther ear
5802CA an*-PI595561

bleached leaf
3612G blh*-N2325

collapsed endosperm
3602G cp*-N1225B

defective kernel
3706E de*-N1234A
3706EA de*-N1222A

discolored kernel
3605Q dsc*-N1362

etched endosperm
3804M et*-N1344
3804Q et*-N1941

germless
3807LA gm*-N2485B

luteus yellow seedling
3810J l*-N1229B
3811A l*-N1879

miniature kernel
338-02 mn*-MTM5910
438-05 mn*-MTM11139

necrotic leaf
6106K nec*-91g-6045-25

opaque endosperm
3904F o*-N1071
3906D o*-N1246A
3906M o*-N1350A
3907A o*-N1354
3907E o*-N1360

pale green seedling
4301H pg*-N760A
4304L pg*-N2404

shrunken kernel
4006P sh*-N1307C
4007K sh*-NA695

small kernel
4004D smk*-N1432

spotted leaf
4107M spt*-N474A
4107O spt*-N537A

stiff leaf
4010L stf*-N227A

virescent seedling
4506P v*-N688A
4510H v*-N1395B
4511M v*-N2286B

white stripe leaf
3512F whv*-N2288

yellow streak leaf
3812P Ysk*-N2324

yellow stripe leaf
6005L yel-str*-W23

zebra leaf
6006D zb*-78-695

zebra necrotic leaf
6006E zn*-PI228181

V. MAIZE GENETICS AND GENOMICS DATABASE

(www.maizegdb.org)

New Personnel in 2007

Lisa Harper USDA-ARS Plant Gene Expression Center, Albany, CA
Feb 2007, Part-time Curator and Outreach Coordinator
In her first year on staff, Lisa plans to visit 3 cooperator sites: University of Florida, University of Georgia, and University of Arizona. One of her first curation tasks is to better integrate data from the RescueMu and Maize Inflorescence Architecture Projects with the rest of MaizeGDB so that these datasets can be searched via the site's integrated mechanisms.

Taner Sen USDA-ARS at Iowa Sate University, Ames, IA
To begin June 2007, Computational Biologist
Early on, Taner will be working to incorporate a genome browser into MaizeGDB to display the B73 sequence and to serve as a basis for representing gene models. Be on the lookout for inquiries from Taner on your preferences for genome browsing capabilities!

Data Improvements

MaizeGDB has added and facilitated the addition of a wide variety of new data, along with incrementally improving the existing data through regular manual and automated updating. Some of our most noteworthy newer initiatives in this area are described below.

Sequence Pipeline

Public sequence data for all of the *Zea* species are updated from Volker Brendel's PlantGDB on a monthly basis and linked with relevant manually-curated data within MaizeGDB. Individual sequences are also linked to contigs generated by external projects that include PlantGDB and the Dana Farber Cancer Institute. The Maize Genome Sequencing Consortium's B73 sequences are associated to BACs on a monthly basis from the data releases posted at maizesequence.org.

Editorial Board

We have initiated and currently maintain an Editorial Board whose members contribute a paper each month to be highlighted at MaizeGDB. Perhaps most exciting are reports that the Editorial Board has directly led to the founding of journal clubs on various campuses! Students and faculty alike download the recommended papers and meet to discuss them. The 2006 Editorial Board was made up of: Tom Brutnell (chair), Surinder Chopra, Karen McGinnis, Wojtek Pawlowski, and Jianming Yu. The 2007 Board consists of: Marja Timmermans (chair), Guri Johal, Damon Lisch, Gael Pressoir, and Moira Sheehan.

Data Additions – Larger Sets

TILLING: We have worked extensively with Cliff Weil's team at Purdue to include the output of the Maize TILLING project in MaizeGDB. This includes integrated primer, probe, locus, variation, and gene product data, along with an integrated interface for ordering stocks from the TILLING project. The current schedule (see http://www.maizegdb.org/data_schedule.php) is to update TILLING data twice yearly.

New maps: The Maize Mapping Project and a number of community members have volunteered a number of new maps for inclusion in MaizeGDB. These include new QTL maps, continued refinements of the IBM and IBM Neighbors maps, and maps that describe the structure of the AGI physical maps. The current schedule is to update maps once each spring.

Contributing your data to MaizeGDB

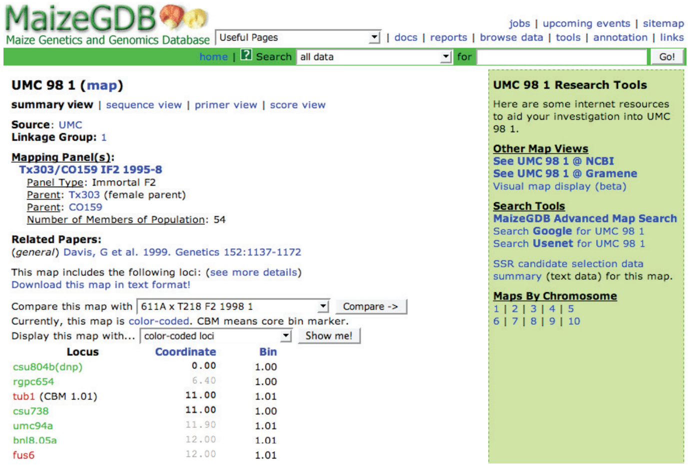
You may contribute data in a number of ways to MaizeGDB. The easiest is very like a 'wiki', where you simply add a comment using the annotation tool. You will first need to register, using the menu item 'annotation' on the top menu bar of the homepage. Once registered, every time you access MaizeGDB, you will be able to annotate any page. Annotations will appear in the monthly updates of the database. A second way is to use the community curation tools. Inquire at mgdb@iastate.edu for access.

If you are developing a project that will generate large datasets and that you would like to submit to MaizeGDB, you need to contact Carolyn Lawrence before you submit the proposal.

New Tools

We have continued our commitment to providing a consistent and clean interface, continued maintenance and improvement of that interface, and integration of new interface options where appropriate. Some noteworthy changes include new map displays and a stand-alone tool to compare cytological and genetic maps.

Map Display Update: One major interface addition is the inclusion of new map displays designed with the aid of commentary from a number of maize community members. We have added three new options that enable interesting new ways of viewing maps without cluttering the interface: a sequence view, a primer view, and a scores view.



UMC 98 1 (map)

summary view | sequence view | primer view | score view

Source: UMC
Linkage Group: 1

Mapping Panel(s):
Tx303/CO159 IF2 1995-8
Panel Type: Immortal F2
Parent: Tx303 (female parent)
Parent: CO159
Number of Members of Population: 54

Related Papers:
(*general*) Davis, G et al. 1999. Genetics 152:1137-1172

This map includes the following loci: (see more details)
Download this map in text format!

Compare this map with 611A x T218 F2 1998 1 Compare ->
Currently, this map is color-coded. CBM means core bin marker.
Display this map with... color-coded loci Show me!

Locus	Coordinate	Bin
csu804b(dnp)	0.00	1.00
rgpc654	6.40	1.00
tub1 (CBM 1.01)	11.00	1.01
csu738	11.00	1.00
umc94a	11.90	1.01
bnl8.05a	12.00	1.01
fus6	12.00	1.01

UMC 98 1 Research Tools

Here are some internet resources to aid your investigation into UMC 98 1.

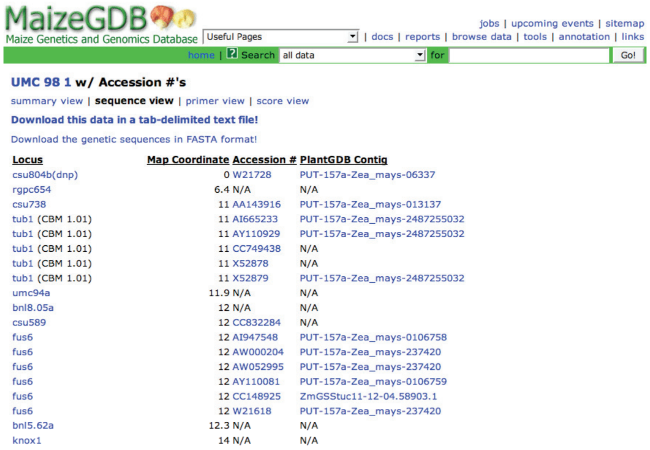
Other Map Views
See UMC 98 1 @ NCBI
See UMC 98 1 @ Gramene
Visual map display (beta)

Search Tools
MaizeGDB Advanced Map Search
Search **Google** for UMC 98 1
Search **Usenet** for UMC 98 1

SSR candidate selection data summary (text data) for this map.

Maps By Chromosome
1 | 2 | 3 | 4 | 5
6 | 7 | 8 | 9 | 10

Figure 1. This is a map view of UMC 98, arrived at by clicking on UMC 98 on the *tub1* locus record. There are two things to note here. First, right below the name of the map there is a line with "summary view" in bold and links to "sequence view", "primer view", and "score view."



UMC 98 1 w/ Accession #'s

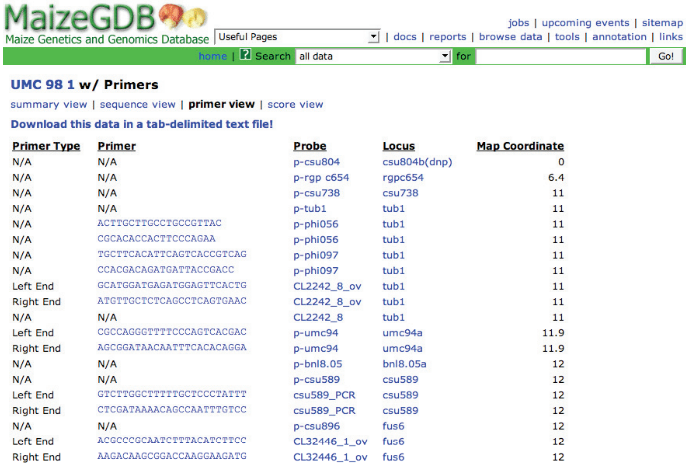
summary view | **sequence view** | primer view | score view

Download this data in a tab-delimited text file!

Download the genetic sequences in FASTA format!

Locus	Map Coordinate	Accession #	PlantGDB Contig
csu804b(dnp)	0	W21728	PUT-157a-Zea_mays-06337
rgpc654	6.4	N/A	N/A
csu738	11	AA143916	PUT-157a-Zea_mays-013137
tub1 (CBM 1.01)	11	AI665233	PUT-157a-Zea_mays-2487255032
tub1 (CBM 1.01)	11	AY110929	PUT-157a-Zea_mays-2487255032
tub1 (CBM 1.01)	11	CC749438	N/A
tub1 (CBM 1.01)	11	X52878	N/A
tub1 (CBM 1.01)	11	X52879	PUT-157a-Zea_mays-2487255032
umc94a	11.9	N/A	N/A
bnl8.05a	12	N/A	N/A
csu589	12	CC832284	N/A
fus6	12	AI947548	PUT-157a-Zea_mays-0106758
fus6	12	AW000204	PUT-157a-Zea_mays-237420
fus6	12	AW052995	PUT-157a-Zea_mays-237420
fus6	12	AY110081	PUT-157a-Zea_mays-0106759
fus6	12	CC148925	ZmGSStuc11-12-04.58903.1
fus6	12	W21618	PUT-157a-Zea_mays-237420
bnl5.62a	12.3	N/A	N/A
knox1	14	N/A	N/A

Figure 2. If you click on the "sequence view," you are shown columns for: the locus name, the map coordinate, an accession number, and a PlantGDB contig.



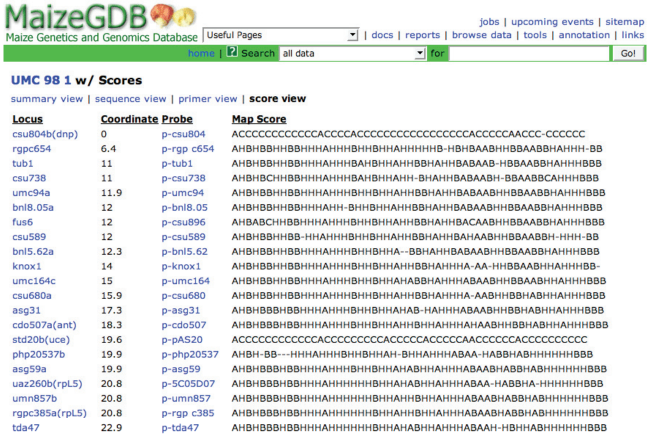
UMC 98 1 w/ Primers

summary view | sequence view | **primer view** | score view

Download this data in a tab-delimited text file!

Primer Type	Primer	Probe	Locus	Map Coordinate
N/A	N/A	p-csu804	csu804b(dnp)	0
N/A	N/A	p-rgp c654	rgpc654	6.4
N/A	N/A	p-csu738	csu738	11
N/A	N/A	p-tub1	tub1	11
N/A	ACTTGCTTGCCTGCCGTTAC	p-phi056	tub1	11
N/A	CGCACACCACTTCCCAGAA	p-phi056	tub1	11
N/A	TGCTTCACATTCAGTCACCGTCAG	p-phi097	tub1	11
N/A	CCACGACAGATGATTACCGACC	p-phi097	tub1	11
Left End	GCATGGATGAGATGGAGTTCACTG	CL2242_8_ov	tub1	11
Right End	ATGTTGCTCTCAGCCTCAGTGAAC	CL2242_8_ov	tub1	11
N/A	N/A	CL2242_8	tub1	11
Left End	CGCCAGGGTTTTCCCAGTCACGAC	p-umc94	umc94a	11.9
Right End	AGCGGATAACAATTTCACACAGGA	p-umc94	umc94a	11.9
N/A	N/A	p-bnl8.05	bnl8.05a	12
N/A	N/A	p-csu589	csu589	12
Left End	GTCTTGGCTTTTTGCTCCCTATTT	csu589_PCR	csu589	12
Right End	CTCGATAAAACAGCCAATTTGTCC	csu589_PCR	csu589	12
N/A	N/A	p-csu896	fus6	12
Left End	ACGCCCGCAATCTTTACATCTTCC	CL32446_1_ov	fus6	12
Right End	AAGACAAGCGGACCAAGGAAGATG	CL32446_1_ov	fus6	12

Figure 3. Clicking on the "primer view" link takes you to a map view that has four columns: primer, probe, locus, and coordinate. This table identifies probes that detect each locus on the map and also notes those that have primers available.



UMC 98 1 w/ Scores

summary view | sequence view | primer view | **score view**

Locus	Coordinate	Probe	Map Score
csu804b(dnp)	0	p-csu804	ACCCCCCCCCCCCACCCCACCCCCCCCCCCCCCCCCACCCCCAACCC-CCCCCC
rgpc654	6.4	p-rgp c654	AHBHBBHHBBHHHAHHHBHHBHHAHHHHHB-HBHBAABHHBBAABBHAHHH-BB
tub1	11	p-tub1	AHBHBBHHBBHHHAHHHBAHBHHAHHBBHAHHBABAAB-HBBAABBHAHHHBBB
csu738	11	p-csu738	AHBHBCHHBBHHHAHHHBAHBHHAHH-BHAHHBABAABH-BBAABBCAHHHBBB
umc94a	11.9	p-umc94	AHBHBBHHBBHHHAHHHBHHBHHAHHBBHAHHBABAABHHBBAABBHAHHHBBB
bnl8.05a	12	p-bnl8.05	AHBHBBHHBBHHHAHH-BHHBHHAHHBBHAHHBABAABHHBBAABBHAHHHBBB
fus6	12	p-csu896	AHBABCHHBBHHHAHHHBHHBHHAHHBBHAHHBACAABHHBBAABBHAHHHBBB
csu589	12	p-csu589	AHBHBBHHBB-HHAHHHBHHBHHAHHBBHAHHBAHAABHHBBAABBH-HHH-BB
bnl5.62a	12.3	p-bnl5.62	AHBHBBHHBBHHHAHHHBHHBHHA--BBHAHHBABAABHHBBAABBHAHHHBBB
knox1	14	p-knox1	AHBHBBHHBBHHHAHHHBHHBHHAHHBBHAHHHA-AA-HHBBAABHHAHHHBB-
umc164c	15	p-umc164	AHBHBBHHBBHHHAHHHBHHBHHAHABBHAHHHABAABHHBBAABHHAHHHBBB
csu680a	15.9	p-csu680	AHBHBBHHBBHHHAHHHBHHBHHAHHBBHAHHHA-AABHHBBHABHHAHHHBBB
asg31	17.3	p-asg31	AHBHBBBHBBHHHAHHHBHHBHHAHAB-HAHHHABAABHHBBHABHHAHHHBBB
cdo507a(ant)	18.3	p-cdo507	AHBHBBBHBBHHHAHHHBHHBHHAHHBHHAHHHAHAABHHBBHABHHAHHHBBB
std20b(uce)	19.6	p-pAS20	ACCCCCCCCCCCCACCCCCCCCCACCCCCACCCCCAACCCCCCACCCCCCCCCC
php20537b	19.9	p-php20537	AHBH-BB---HHHAHHHBHHBHHAH-BHHAHHHABAA-HABBHABHHHHHHBBB
asg59a	19.9	p-asg59	AHBHBBBHBBHHHAHHHBHHBHHAHABHHAHHHABAABHABBHABHHHHHHBBB
uaz260b(rpL5)	20.8	p-5C05D07	AHBHBBBHBBHHHAHHHHHHBHHAHABHHAHHHABAA-HABBHA-HHHHHHBBB
umn857b	20.8	p-umn857	AHBHBBBHBBHHHAHHHHHHBHHAHHBHHAHHHABAABHABBHABHHHHHHBBB
rgpc385a(rpL5)	20.8	p-rgp c385	AHBHBBBHBBHHHAHHHHHHBHHAHHBHHAHHHABAABHABBHABHHHHHHBBB
tda47	22.9	p-tda47	AHBHBBBHBBHHHAHHHHHHBHHAHABHHAHHHABAAH-HBHHABHHHHHHBBB

Figure 4. Clicking on "score view" allows you to see the markers used to generate a particular map along with associated map scores, enabling you to review the raw mapping data for the experiment. Note that not all maps in MaizeGDB have associated scores; if you see the "score view" option, you're in luck!

Morgan2McClintock: The Morgan2McClintock Translator was developed through our continued collaboration with Hank Bass and a new collaboration with Lorrie Anderson. The tool utilizes the maize Recombination Nodule map (Anderson et al., 2003 and 2004) to calculate approximate cytological positions for loci given a genetic map, and to calculate approximate genetic positions for loci given a cytological map (Lawrence et al., 2006). Morgan2McClintock is a stand-alone tool and can be run on any machine enabled to serve PHP. You can use it online at MaizeGDB: from the home page, choose "maps", then choose Recombination Nodule Map to arrive at http://www.maizegdb.org/RNmaps.php). Alternatively, go to: http://www.lawrencelab.org/Morgan2McClintock.

Maize Community Support

The MaizeGDB team offers support to the maize community in a variety of fashions. This support aids the annual Maize Genetics Conference, provides community addresses for mailings, an abstract submission interface, assembly and printing of the program, and integrates the abstracts into MaizeGDB. It supplies address lists for this Newsletter, and hosts the Newsletter, with links to the database. We facilitate community interaction with the Maize Genetics Executive Committee, including community surveys, elections and community-wide messaging on important issues. We also maintain a community job board (which has had dozens of job postings and has significantly aided at least ten job placements since its initiation), as well as a community calendar of upcoming events that may be of interest to the larger community.

Copies and Schema of MaizeGDB

Full copies of the database as well as individual tables and custom-formatted dumps are provided to individuals who make requests to the MaizeGDB team at mgdb@iastate.edu. Copies support Oracle, MySQL, and Microsoft Access. The current MaizeGDB schema can be accessed at http://www.maizegdb.org/MaizeGDBSchema.pdf.

Five-Year Plan

We are in the process of drafting our five-year plan for the USDA-ARS. Objectives were developed with input from the MaizeGDB Working Group and are available online at http://www.maizegdb.org/objectives.php.

Acknowledgements

MaizeGDB is guided by members of the community of maize geneticists through feedback sent to us through the website, and by guidance from the MaizeGDB Working Group. Current membership includes Volker Brendel, Ed Buckler, Karen Cone, Mike Freeling, Owen Hoekenga, Lukas Mueller, Marty Sachs, Pat Schnable, Tom Slezak (chair), Anne Sylvester, and Doreen Ware.

Citing MaizeGDB

MaizeGDB may be cited using any or all of these references:

Lawrence CJ, Schaeffer ML, Seigfried TE, Campbell DA, Harper LC, 2007. MaizeGDB's new data types, resources and activities. Nucleic Acids Res. 35:D895-900.
Lawrence CJ, Seigfried TE, Brendel V, 2005. The maize genetics and genomics database. The community resource for access to diverse maize data. Plant Physiol. 138:55-58.
Lawrence CJ, Dong Q, Polacco ML, Seigfried TE, Brendel V, 2004. MaizeGDB, the community database for maize genetics and genomics. Nucleic Acids Res. 32: D393-397.

Submitted by the MaizeGDB team May 8, 2007
Mary Schaeffer
Lisa Harper
Trent Seigfried
Darwin Campbell
Carolyn Lawrence

VI. MAIZE GENOME SEQUENCING PROJECT

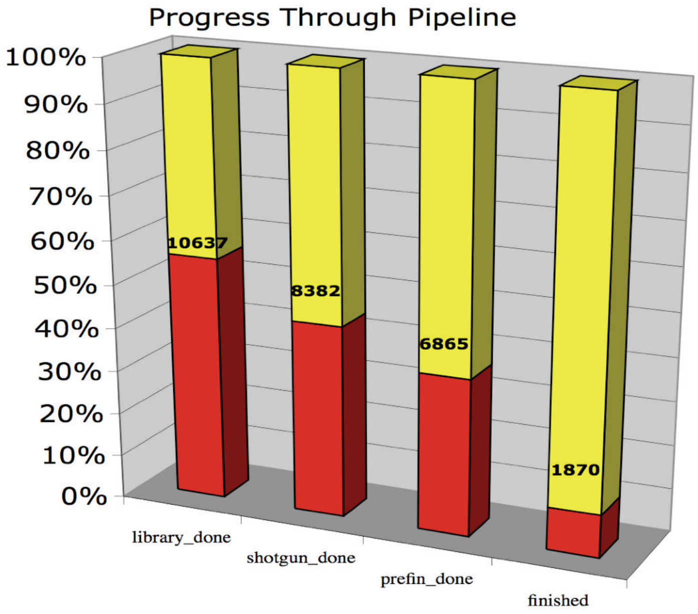
As we enter the second half of the 3-year Maize Genome Sequencing Project, we have begun to significantly accelerate production of draft sequence and are poised to do an effective ramp on improved sequence and submissions. The maize BAC DNA enters our pipeline from shipments of purified and fractioned BACs chosen from the tilepath at Arizona Genomics Institute (AGI). As of April 27, 2007, Washington University Genome Sequencing Center (WUGSC) has received slightly over 12,000 of the predicted 17,000 BAC clones to cover the genome. From each BAC, a plasmid library is created and plated and colonies are picked. We have over 10,630 BACs that have completed this step. Each BAC is given light sequence shotgun of 4-6X coverage, and the sequence is assembled along with fosmids end sequences produced at WUGSC and the original BAC end sequence (BES) performed at AGI. An automated system has been developed to confirm coverage, assembly, and incorporation of BES before completing the production phase. At this stage, the consensus sequence of the assembly is deposited in Genbank as phase I (draft), and 8,382 BACs at this stage now are available. The draft assembly then is screened with a program that will identify repetitive sequence, in order to exclude this sequence from improvement efforts. An automated improvement, or prefinishing, is performed on each BAC, using directed primer walks on subclones that span the gaps for two rounds, if necessary. After completion of the automated prefinishing, a program to utilize genome survey sequence in the form of methyl filtered and high coefficient of time (high C_0t) subtractive libraries, along with sequences from mRNA and cDNA libraries, is run to incorporate existing sequence information. A limited manual improvement effort is then made, using directed primer walking on plasmids along with PCR by finishers located at WUGSC, AGI, and Cold Spring Harbor Laboratory (CSHL). The development and refinement of software for tagging repeats, incorporating existing data, and navigating gene regions of the sequence has taken some extra time, but it is now working smoothly, and we expect to see a rapid increase of clones passing through this stage of the pipeline. We currently have completed 1,870 improved BAC clones. After improvement is finished, an automated pipeline submits the sequence to Genbank as phase I, HTGS_IMPROVED. There are currently 1,251 entries in Genbank with this designation.

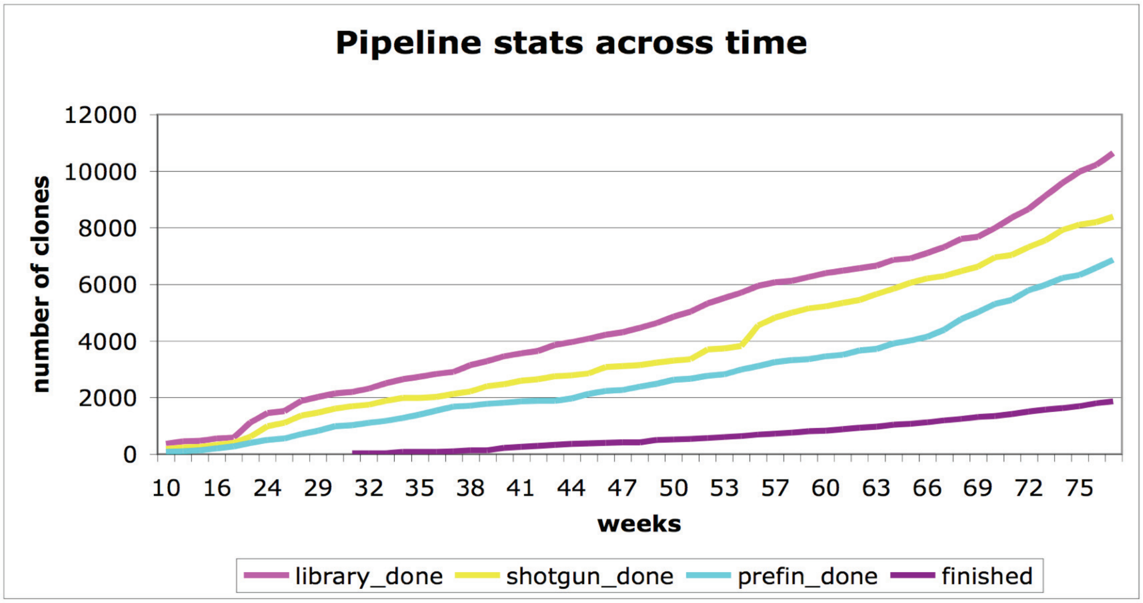
The sequence read data is immediately available in the NCBI Trace Archive and can be downloaded from there (http://www.ncbi.nlm.nih.gov.library.vu.edu.au/Traces/trace.cgi#). In addition to the Phase-1 improved category mentioned above, (HTGS_IMPROVED), others available for download from GenBank immediately upon completion are: 1) HTGS_FULLTOP-2 x 384 paired-end attempts (4-5X coverage); completed shotgun phase; initial assembly; 2) HTGS_PREFIN-completed automated improvement phase (AutoFinish); 3) HTGS_ACTIVEFIN-active work being done by a finisher.

In March 2007, the maize annotation pipeline became a fully automated system, analyzing incoming maize clones on a weekly basis. All maize BACs tagged as Phase I HTGS_IMPROVED (1,251 as of April 25, 2007) have been analyzed to date. The maize browser, available at http://www.maizesequence.org provides public access to maize BACs and their underlying annotations. The website is tightly integrated with Gramene (http://www.gramene.org) and provides cross-linkage for comparative analysis with other cereal genomes. Maize BACs are anchored on the agarose FPC map. Each BAC provides an independent sequence map displaying known order-and-orientation and underlying annotations. Presently, BAC annotations include: ab initio gene prediction using Fgenesh, transposon classification of gene models, MIPS repeat annotation, annotation of mathematically defined repeats, and alignment to a variety of cereal data sets, including maize physical markers. All annotations can be viewed graphically and downloaded through the browser. The browser now also provides BLAST functionality over all maize sequences. These include the sequenced maize BACs, as well as peptide translations of predicted genes. The browser also provides access via DAS to remote annotations produced independently by maize collaborators. One such data set includes TWINSCAN predictions curated through a collaboration between Brad Barbazuk and Michael Brent (NSF #0501758).

As the maize sequencing project enters its second half, annotation and visualization efforts are primed for significant milestones. All maize clones, regardless of status, will be automatically annotated on a regular basis to provide users with preliminary annotations. Mature (improved) BACs will be analyzed using an effective evidence-based gene build strategy in collaboration with Gramene that will provide higher-quality gene models. Improved sequences will also undergo peptide-based analysis, such as InterPro/GO, to provide greater context for gene models. As longer tiles of maize sequences become available, the maize BAC sequence maps will be integrated with the FPC map. This will provide a unified view of the physical and sequence map. Other data sets, such as the maize optical map and full-length cDNAs, will also be integrated into the browser as they become available. In the beginning of 2008, it is also expected that whole genome alignment to related organisms, such as rice and sorghum, will be made available. Finally, preparations are being made for whole genome assembly of the maize genome near the end of the project. The assembly will validate the order-and-orientation of the maize *genespace* and will isolate problem regions.

For further general information, please visit the GSC web site, http://genome.wustl.edu/genome.cgi?GENOME=Zea%20mays%20mays%20cv.%20B73. Weekly updates, usually posted on Friday afternoon, in the form of bar and line graphs are available there, (http://genome.wustl.edu/genome.cgi?GENOME=Zea%20mays%20mays%20cv.%20B73&SECTION=research). For access to the Cold Spring Harbor Browser, please visit www.MaizeSequence.org.





Top-line, library_done; next to top line, shotgun_done; next to bottom line, prefin_done; bottom line, finished.

Submitted by Sandy Clifton
Washington University, St Louis, MO
Apr 27, 2007

VII. MOLECULAR AND FUNCTIONAL DIVERSITY OF MAIZE
(www.panzea.org)

Panzea's Nested Association Mapping Population: A Powerful Phenomics Resource for the Maize Community

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"If you build it they will come." Well, the Panzea project has built it:

The ultimate germplasm resource to date for localizing QTLs in maize!

The Panzea project (a.k.a. 'Molecular and Functional Diversity of the Maize Genome'; NSF DBI 0321467) is a five-year NSF project headed by John Doebley and involves eight additional investigators at seven institutions (University of Wisconsin, Cornell University, North Carolina State University, University of Missouri, University of California-Irvine, Cold Spring Harbor Laboratory and USDA-ARS). Our overall objectives are to address the two major questions *'How has selection shaped molecular diversity?'* and *'How does this molecular diversity relate to functional trait variation?'* To address these questions, we have performed large-scale SNP discovery in maize and its wild progenitor, teosinte, in more than 3000 randomly chosen genes and in more than 1000 candidate genes for traits of agronomic, developmental, evolutionary and ecological importance. Based on this sequence data, we found that about 2-4% of genes in the maize genome were detectably influenced by artificial selection during the domestication and subsequent improvement of maize (Wright et al., Science 308:1310-14, 2005; Yamasaki et al., Plant Cell 17:2859-72, 2005). You can read more about the results to date from this project (and from its five-year forerunner) via our project publications web page: http://www.panzea.org/lit/publication.html.

Now that we have completed our objectives relating to our first major question, we are devoting our focus to the characterization of functional diversity in both teosinte and maize. To this end, we are engaged in QTL and association mapping experiments both in modern maize and in crosses between teosinte and maize. We are currently working with two teosinte association mapping populations, three teosinte-maize backcross QTL mapping populations, a maize association mapping population and a maize 'Nested Association Mapping' (NAM) population.

The maize NAM population is the centerpiece of our project. Of the numerous resources that we are generating, we expect the NAM population to be the most significant to the maize research community. Nested Association Mapping is a powerful new method for localizing QTL which uses a multifamily RIL mapping population derived from crosses to a common parent (i.e., B73) in order to perform a joint QTL and association analysis. By employing a genomic scan of common parent-specific SNPs in the progeny RILs combined with high density genotyping (or sequencing) of the parental lines, the NAM strategy captures the best of both worlds: the statistical power of QTL analysis is combined with the high chromosomal resolution of association analysis.

We are pioneering the NAM approach in maize. Our NAM population consists of >5000 RILs from 25 families, with 200 RILs per family, all being genotyped at 1500 SNP loci. It forms a permanent QTL mapping resource for the benefit of the maize community. The families were generated by crossing 25 diverse maize inbred lines with B73 as a common parent. Additionally, the well-known IBM mapping population is included as the 26th family. Finally, a collection of 280 diverse maize inbreds from around the world that serves as an association mapping platform for maize has also been included in these experiments.

This summer we will be planting out all of these RILs in three locations (Raleigh, NC; Ithaca, NY; Champaign-Urbana, IL). The parental lines are being sequenced over the next year and a half--these data will make it possible to analyze all populations as one unified experiment, potentially with gene-level QTL resolution. A list of the traits that we are scoring in the NAM population will soon be available from www.panzea.org. We are scoring a number of the most obvious agronomic and developmental traits in these populations, but are unable to score some of the more complex traits. We have created this NAM resource with the hope that maize researchers working on complex, specialty traits will use it to uncover the genetic basis of these traits in a broad sample of maize inbreds.

We invite interested maize researchers to score their own phenotypes of interest in one or more of our NAM 'Fields of Dreams'.

Our only stipulation is that your data set must be deposited in our project database, Aztec, where it will be held privately for two years (members of the Panzea group will not be allowed to analyze it either, without prior permission from you), and then released to the public via www.panzea.org.

We also have extensive experience in creating barcoding tools for phenotyping, and can provide help with this. For further details or to arrange your phenotyping visit(s), please contact:

Ed Buckler, USDA-ARS/Cornell University (esb33@cornell.edu),
Jim Holland, USDA-ARS/North Carolina State University (James_Holland@ncsu.edu), or
Torbert Rocheford, Univ. of Illinois (trochefo@uiuc.edu).

If you are unable to make it to one of our fields this summer, do not fret! Plans are afoot to have public grow-outs of the NAM population for as many as four subsequent years, as part of the maize phenomics initiative touted by the MGEC. However, at this point funding support is guaranteed only for this summer's grow-out. For educators at institutions focusing primarily on undergraduate teaching, the NAM resource provides an ideal opportunity for research involving undergraduate or Masters students, potentially funded via the NSF's 'Research Opportunity Awards'. Resources from these awards could potentially be pooled with those from other sources to fund one or more future 'public' grow-outs of the NAM population. In addition, for researchers interested in growing out all or part of the NAM population themselves, seed from the entire, fully-genotyped NAM population will be available from the Maize Stock Center in 2008. So if you miss out on this summer, it should not be too late to realize your maize phenomic dream!

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IX. SYMBOL INDEX

X. AUTHOR INDEX

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Stock Catalogs	Each issue, updates only after No 78, and MaizeGDB
Rules of Nomenclature (1995)	MNL69:182 and **MaizeGDB** (1996 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
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 mgdb@iastate.edu with details of **NEW GENES**. Also request access to the community curation tools so that you could 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) Provide BAC-probe/gene relationships for BACs on public physical map (http://www.genome.arizona.edu/maize), especially if probes/genes have been genetically mapped. This will improve the genome sequence and its annotation.

May you find a Unique corn in MM!