MAIZE GENETICS COOPERATION

NEWSLETTER

82

February 1, 2008

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and
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Columbia, Missouri

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NOTE: The 51st Maize Meeting will be held at St. Charles, IL. March 12-15, 2009.

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 have set an arbitrary cutoff of December 1, 2008 for the next print copy, volume 83. 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 color reproduction of images submitted with notes (pp. 32-34), and a number of special reports: Sequencing the Maize B73 Genome Progress Report (pp. 103-109); Sequencing the Codifying Genome of the *Palomero Toluqueño* Mexican Landrace (p. 110); the Allerton Report 2007 (pp. 111-117); and Genetic Maps 2007 (pp 87-102). In celebration of the 50th Maize Genetics Conference we provide a historical perspective that includes information about the first meeting in January 1959 (pp. 39-44), and various snapshots since that time supplied by various members of the community (pp. 35-38).

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. We also thank Arturo Garcia for maintaining the staging site, www.agron.missouri.edu, and this year, the front cover with color images of mutants of maize phenotypes.

Mary Schaeffer (Polacco)
James A. Birchler
Co-editors

Edward H. Coe, Jr.
Distinguished Editor

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AMES, IOWA
USDA-ARS and Iowa State University

Maize genetics outreach to American Indians

--Lawrence, CJ; Gardner, CAC; Widrlechner, M; Brendel, V

Maize is an excellent vehicle for plant genomics outreach to those American Indian tribes that use and appreciate it nutritionally, culturally, and spiritually. Thanks to NSF funds and USDA-ARS in-kind resources, we mentored four to six Native American Indian students for eight weeks during each of the summer 2006 and 2007 field seasons, and plan to continue offering the program for at least three more years. All students worked at the USDA-ARS North Central Regional Plant Introduction Station in Ames, Iowa learning about plant genetic resource conservation. Half of the students also worked on developing molecular markers for Abnormal Chromosome 10 in our lab and three worked with the MaizeGDB team to create project data storage and presentation solutions. The project website can be viewed at http://www.lawrencelab.org/Outreach/. Note that maize was not a central element in all tribes' cultures, so this sort of project may not be useful for outreach to tribes who do not revere maize! We encourage others to pursue plant genomics outreach to American Indians and invite inquiries on how to get started.

Caption: Ray Lee (Navajo medicine man) and Lula Jackson (Ray's wife) led a corn pollen blessing during the summer of 2006 at the NCRPIS. Pictured (left to right) are: (front) Carolyn Lawrence, (middle) Lula Jackson (Navajo), Regina Sanchez (Navajo), Ray Lee (Navajo), Sharon Garfield (Navajo), (back) Alexandra Volker (Cherokee), Nathan Etsitty (Navajo), Titus Harrison (Navajo), Mark Widrlechner, Lisa Burk, and Von Mark Cruz. (For full color, see p. 32.)

AMES, IOWA
Iowa State University

Linkage disequilibrium in a maize F2 population of B73 x Mo17

--Cook, KA; Hallauer, AR

The average level of dominance ($\bar{d}$) of genes conditioning quantitative traits is an important indicator of the genetic expression of heterosis in maize. Previous studies suggested that linkage disequilibrium causes overestimation of $\bar{d}$, sometimes designated as pseudooverdominance. Estimates of $\bar{d}$ within F2 populations intermated to reduce linkage disequilibrium have suggested that partial to complete dominance of genes is of greater importance to expression of heterosis in maize. F2 populations of elite, widely used hybrids, however, have not been extensively studied. The contribution of overdominant loci and linkage disequilibrium to the expression of heterosis in the hybrid B73 x Mo17 were studied in the (B73 x Mo17)F2 and (B73 x Mo17)F2 Syn. 10 (250 plants of the F2 population intermated for 10 generations) populations with the use of the North Carolina Design III mating design. It seems the sample of 250 individuals intermated for each of the 10 generations was adequate because the trait means of the backcrosses of F2 plants to each parental line were very similar (no significant differences) for the F2 and the F2 Syn. 10 populations for each trait (Table 1).

Differences of the estimates of additive genetic (σ^2_A) and dominance (σ^2_D) variances for the F2 and F2 Syn. 10 populations were not significant (confidence intervals are not included) for the nine traits studied (Table 2). Estimates of dominance variances were generally lower in the F2 Syn. 10 population compared with the F2 population. For grain yield, estimate of σ^2_D decreased 60% in the F2 Syn. 10 population compared with the F2 population. The direction of change for the estimates of σ^2_A for the two populations was not consistent among traits; e.g., estimates of σ^2_A decreased 16% from F2 to F2 Syn. 10 and increased 67% for plant

Table 1. Means of 100 males of (B73 x Mo17)F2 and (B73 x Mo17)F2 Syn. 10 populations backcrossed to B73 and Mo17 for nine traits averaged across the three Iowa environments.

Male plants	Inbred	Grain		Days planting to	
of population	parent	Yield	Moisture	Anthesis	Silk emergence
		t/ha	%	---------------no.---------------	
F2	B73	4.56	23.2	87.4	89.0
F2	Mo17	3.31	22.3	86.4	89.5
F2 Syn. 10	B73	4.30	22.8	87.3	88.8
F2 Syn. 10	Mo17	3.22	22.3	86.6	89.1

		Height		Lodging		
		Plant	Ear	Root	Stalk	Dropped ears
		-------cm-------		---------%---------		%
F2	B73	228	113	0.4	10.2	5.5
F2	Mo17	215	100	0.2	13.1	6.7
F2 Syn. 10	B73	227	112	0.4	10.7	5.8
F2 Syn. 10	Mo17	218	104	0.2	16.2	7.3

Table 2. Estimates of additive genetic (σ^2_A) and dominance (σ^2_D) variances, average level of dominance ($\bar{d}$), and heritability (h^2) for nine traits of the (B73 x Mo17)F2 and (B73 x Mo17)F2 Syn. 10 populations obtained from analysis combined across three environments.

		Estimates			
Trait	Population	σ^2_A	σ^2_D	$\bar{d}$	h^2
Grain yield (t/ha)	F2	60.4	41.7	1.17	0.48 ± 0.11
	F2 Syn. 10	50.5	16.2	0.80	0.57 ± 0.14
Grain moisture (%)	F2	1.0	-0.1	‡	0.33 ± 0.15
	F2 Syn. 10	3.0	0.2	0.41	0.82 ± 0.14
Days to anthesis (no.)†	F2	2.2	0.4	0.61	0.78 ± 0.19
	F2 Syn. 10	2.4	0.2	0.42	0.85 ± 0.20
Days to silking (no.)†	F2	3.0	0.8	0.71	0.73 ± 0.18
	F2 Syn. 10	2.8	0.2	0.35	0.84 ± 0.21
Plant height (cm)	F2	151.3	20.7	0.52	0.80 ± 0.13
	F2 Syn. 10	226.6	14.9	0.36	0.89 ± 0.14
Ear height (cm)	F2	136.8	9.5	0.37	0.87 ± 0.14
	F2 Syn. 10	169.8	6.1	0.27	0.92 ± 0.14
Root lodging (%)	F2	-0.1	0.1	‡	‡
	F2 Syn. 10	0.3	0.1	0.87	0.41 ± 0.20
Stalk lodging (%)	F2	68.8	4.6	0.37	0.78 ± 0.15
	F2 Syn. 10	45.5	5.9	0.51	0.64 ± 0.15
Dropped ears (%)	F2	13.4	0.8	0.36	0.64 ± 0.17
	F2 Syn. 10	11.9	-0.1	‡	0.56 ± 0.18

†Data collected at only one environment.
‡Average level of dominance was not estimable because of negative estimates of variance.

height from F2 to F2 Syn. 10 population. The estimate of $\bar{d}$ for grain yield decreased from 1.17 in the F2 to 0.80 in the F2 Syn. 10 population, but neither estimate deviated significantly from 1.0. Estimates of $\bar{d}$ were not greater than 1.0 for the other traits in either population before and after intermating. But estimates of $\bar{d}$ decreased for all traits where $\bar{d}$ was estimable, suggesting that linkage disequilibrium caused overestimation of σ^2_D in the F2 population. Evidence for the presence of significant overdominant effects for grain yield were no greater in B73 x Mo17 populations than estimates reported previously in other populations derived from crosses of inbred lines (Hallauer and Miranda, Quantitative Genetics in Maize Breeding, p. 122, 1988).

AMES, IOWA
Iowa State University and CIMMYT

Direct and correlated responses to selection for earliness in a tropical maize population

--Narro, LA; Hallauer, AR; Pandey, S

Earlier flowering is desired for many tropical maize cultivars for production for brief rainy seasons or for specific cropping sequences. Half-sib family selection was used in Compuesto Seleccion Precoz, a tropical cultivar, to decrease the number of days from planting to pollen shed but to retain the relatively high yield. Half-sib family recurrent selection was conducted at two locations in Mexico with primary emphasis given to selection for earlier flowering (days to pollen shed). Two cycles of selection were completed each year. Data for evaluation of response to selection were obtained at 12 locations (11 tropical and one temperate). Cycles C0, C3, C6, C9, C12, and C15 were evaluated to determine direct response to selection for earlier flowering and the correlated response (indirect effects of selection) for seven agronomic traits. The C15 selection cycle was earlier than checks at all tropical environments. Average response to selection for earlier flowering was about 0.5 days per cycle of selection (Table 1). The additive genetic effects, measured as the linear component of the model when partitioning cycles per se, accounted for 89% of the total genetic variation. Parameters from the Smith model (Crop Sci. 23:35-40, 1983) showed that the homozygotes and heterozygotes were highly significant for the C0 and selected cycles, but the homozygous contribution was more important. For the one temperate environment (Ames, Iowa), the average number of days from planting to male flowering was 79.6 days, decreasing from 85.3 days for C0 to 75.7 days for C15, nearly 10 days earlier with selection in tropical environments.

Table 1. Means of selection cycles in Compuesto Seleccion Precoz tropical cultivar for days to flower (direct response) and correlated responses for seven agronomic traits with selection for earlier flowering.

	Traits[1]							
Cycle of	Days to	Grain		Lodging		Height		Leaf
selection	flower	Yield	Moisture	Root	Stalk	Plant	Ear	area
	no.	t ha^{-1}	%	-------%-------		-------cm-------		cm^2 x 10
C0	58.2	5.53	19.6	3.4	1.4	199	95	813.1
C3	54.2	4.70	19.6	2.4	3.1	190	63	681.3
C6	53.8	4.74	20.1	2.7	3.7	184	78	613.1
C9	53.1	4.75	18.9	1.2	3.2	180	71	633.2
C12	51.3	4.01	18.7	2.3	3.6	169	53	628.4
C15	50.4	3.98	18.7	1.2	1.2	161	62	508.7
$\bar{X}$	53.5	4.62	19.3	2.2	2.7	180	70	646.3
2(ALI + DLI)	-0.42	-0.09	-0.10	0.07	0.12	-2.29	-2.21	-15.2
R^2	0.89	0.99	0.99	0.91	0.94	0.99	0.99	0.99
Linear (b)	-0.46	-0.10	-0.09	-0.12	-0.05	-2.22	-2.00	-16.2
R^2	0.89	0.81	0.63	0.30	0.46	0.27	0.51	0.71
(C15 - C0)/15	-0.55	-0.10	-0.06	-0.07	-0.10	-2.50	-2.26	-203.0

[1]Direct responses is for earlier male flowering measured in 12 environments. Changes for other traits are the correlated changes with selection for earlier male flowering with data from eight environments.

Information from eight environments were used to determine the indirect effects of selection for earlier flowering (Table 1). Selection for earlier flowering resulted in correlated changes for all traits except root and stalk lodging. The estimates of the additive genetic effects varied among traits: 81% for grain yield, 63% for grain moisture, 27% for plant height, 50% for ear height, and 79% for leaf area. The correlated changes in means were negative (decreased) for all traits except root and stalk lodging (Table 1). For the one temperate location, grain yield increased from 1.86 t ha^{-1} (C0) to 3.67 t ha^{-1} (C15) because of the effects of earlier flowering of the C15 (10 days earlier than the C0) selection cycle. Correlated responses to selection for earlier flowering on a per cycle basis showed that yield decreased about 95 kg/ha, plant and ear heights decreased about 2 cm, grain moisture decreased about 0.08%, and leaf area decreased about 172 cm^2.

Direct response for earlier flowering in the tropical cultivar Compuesto Seleccion Precoz was about 0.5 days per cycle vs. about 3.0 days per cycle for mass selection within tropical cultivars in temperate areas. The C15 was about 5 days earlier than the C0 when evaluated in tropical environments, but C15 was 10 days earlier than the C0 in the one temperate environment with a corresponding 97.3% increase in grain yield. The objective of selection for developing an earlier maturity strain of Compuesto Seleccion Precoz, but retaining yield, was not as effective as desired: the C15 was 5 days earlier but yield decreased 28% (Table 1).

Molecular studies for determination of quantitative trait loci for acid soil tolerance in maize

--Navas, AA; Hallauer, AR; Pandey, S

Acid soils include approximately four billion hectares of the earth's surface. Soils with pH < 5.6, deficiency of calcium, magnesium, phosphorous, molybdenum, iron, and aluminum saturation > 35% with phosphorous level < 16 parts per billion are considered acidic for maize growth and production. Because of acid soils, fewer and smaller roots are produced, which reduces the maize plant's capacity to uptake water and nutrients from the soil. Objectives of the study were to develop a marker linkage map for a segregating F2 population derived from a cross of acid tolerant and acid susceptible lines to dissect the quantitative trait loci (QTLs) for several traits, and to determine if the QTLs could be used in a marker assisted selection program for acid tolerance in maize. Based on previous evaluation of 783 S4 yellow lines and 755 S_6 white lines, representing two heterotic groups, in normal-fertile and acid soils, six acid tolerant and six acid susceptible lines from the heterotic groups were selected. The 12 lines were evaluated as lines themselves in one normal-fertile and two acid soil environments. Based on the performance of the 12 lines, one acid tolerant line and one acid susceptible line were selected as the parental lines for this study. An F2 population of 221 individuals was genotyped for 118 simple sequence repeats (SSRs) and 214 S1

progenies were evaluated in an alpha lattice design (22 x 10) at five environments (three acidic and two normal fertile) in Colombia, SA. Data were collected for dates of male and female flowering, anthesis-silking interval, grain yield, ears per plant, and plant and ear heights. The genomic DNA isolation protocol was based on the method of Saghai-Maroof et al. (PNAS 81:8014-8018, 1984), and the details of SSR protocol were given by Hoisington et al. (2nd ed., CIMMYT, 1994). The linkage map was constructed using the computer program MapMaker/EXP 3.0. QTL detection was performed with complete interval mapping, a software program developed by Jiang (CIMMYT, 1998).

Average grain yield of 214 S1 progenies was 0.7 t ha^{-1} for the three acid soil locations, which was an 84.3% lower yield than the best normal-fertile soil location (4.5 t ha^{-1}). Acid soil environments tended to reduce the genetic variability among S1 progenies for all traits (Table 1). The average heritability estimate of grain yield, for example, was 2.2 times greater for the normal-fertile soil environments compared with the acid-soil environments; the differences in heritability estimates were similar for all traits. Phenotypic correlations between the seven traits were similar in magnitude and sign for both the acid soil and normal-fertile environments (Table 2). Correlations between days to pollen shed and silk emergence, between grain yield and ears per plant, and between plant and ear height had the largest positive correlations.

Table 1. Average broad-sense heritabilities (h^2) for 214 S1 progenies evaluated at three acid-soil and two normal-fertile soil environments evaluated in Colombia, SA.

Trait	Acid soils	Normal-fertile soils
	h^{2*}	h^{2*}
Date of male flowering, no.	0.22	0.68
Date of female flowering, no.	0.23	0.73
Anthesis-silking interval, no.	0.17	0.37
Grain yield, t ha^{-1}	0.32	0.71
Ears per plant, no.	0.31	0.52
Plant height, cm	0.18	0.68
Ear height, cm	0.10	0.48

*Broad-sense heritabilities calculated as $\sigma^2_g/(\sigma^2/re + \sigma^2_{ge}/e + \sigma^2_g)$, where σ^2_g is genetic variation among S1 progenies, σ^2_{ge} is interaction of S1 progenies with environments, σ^2 is experimental error, r is number of replications, and e is number of environments.

Table 2. Average phenotypic correlations between seven maize traits for 214 S1 progenies evaluated in three acid soils (below diagonal) and two normal-fertile (above diagonal) environments in Colombia, SA.

	Traits						
Traits[1]	Male	Female	ASI	Yield	Ears	Plant	EAR
Male	-----	0.78**	-0.38**	-0.31**	-0.17*	0.14	0.03
Female	0.80**	-----	0.28**	-0.28**	-0.20**	0.14	0.02
ASI	-0.18*	0.43**	-----	0.10	0.07	-0.03	-0.03
Yield	-0.33**	-0.47**	-0.29**	-----	0.49**	0.19**	0.12
Ears	-0.36**	-0.42**	-0.31**	0.67**	-----	0.24**	0.26**
Plant	-0.18*	-0.27**	-0.19**	0.36**	0.30**	-----	0.57**
Ear	-0.08	-0.17*	-0.17*	0.29**	0.24**	0.72**	-----

[1]Traits included days to pollen shed (Male) and silk emergence (Female), pollen-silk-interval (ASI), grain yield (Yield), ears per plant (Ears), and plant (Plant) and ear (Ear) heights.

There were 66 QTLs identified across each environment, based on the composite interval mapping analyses (CIM-model 4) with LOD = 2.5. Thirteen QTLs were detected for acid soils, 33 QTLs for normal-fertile soils and 40 QTLs for the combined across environments. No QTLs with major effects were identified. QTLs had low single and total R^2 values for individual environments and combined across the five environments. QTLs were estimated across the five environments (three acid and two normal) and the total phenotypic variance (R^2) explained across five environments was 10% and 7%, respectively, for days to male and female flowering, 1% for ASI, 3% for grain yield, 4% for ears per plant, and 4% and 15%, respectively, for plant and ear height. There were few QTLs common for single environments and combined across environments. Ten QTLs were detected in all single environments for grain yield (Table 3). Single R^2 values ranged from 0.3% to 11% and the largest total R^2 was 19%. The main goal of the study was acid soils and grain yield, but 10% was the highest total R^2 value at acid environment 3 with a QTL on chromosomes 1 and 5. The complex acid soil environment showed that Al toxicity is important but Al toxicity is not the only factor affecting grain yields. Acid soils had significantly lower grain yields, greater genotype by environment interactions, and decreased genetic variability which affected QTL detection. No QTLs with major effects were identified.

Table 3. QTLs associated with grain yield expression at five environments.

Environment	Chromosome	QTL (cm)	SSR locus	LR score[1]	Additive[2] t ha^{-1}	Dominance[3] t ha^{-1}	R^2 %
Acid soils[4]							
1	1	103	*bmc1273*	13.3	0.05	-0.18	8
	2	96	*bnlg1887*	12.2	-0.15	0.00	0
2	1	181	*mmc0011*	16.4	-0.45	-0.30	2
3	1	137	*dup12*	13.0	0.03	0.10	5
	5	100	*bnlg2323*	12.4	-0.06	0.10	5
Combined	1	116	*bmc1273*	19.4	0.04	0.02	7
Normal soils							
1	2	2	*bmc1017*	11.9	-0.39	0.10	5
	5	79	*dup10*	13.3	-0.57	0.01	6
	6	19	*bngl1371*	15.8	-0.57	0.01	11
2	4	118	*umc1031*	12.6	0.27	0.10	5
	8	25	*bmc1067*	15.0	0.33	0.20	5
Combined	4	118	*umc1031*	18.9	0.23	0.17	2
	8	28	*bmc1067*	18.8	0.29	0.27	3

[1]LR scores $\geq$ LR critical values at LOD = 2.5 by CIM-model 4. LR critical values were 11.5, 18.7, and 15.3 for single environments and combined across acid and normal-fertile environments.
[2]Substitution effect of "A" allele from tolerant parent for "B" allele of susceptible parent to either reduce (-) or increase (+) grain yield at this locus.
[3]Effect that mean of heterozygote is either less (-) or more (+) than the mean of homozygous parents at this locus.
[4]All saturation was 55% at environment 1 and 65% at environments 2 and 3.

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Allelism testing of Maize Coop Stock Center lines containing unknown brown midrib alleles

--Haney, LJ; Hake, S; Scott, MP

This report summarizes the allele testing of brown midrib stocks characterized by phenotype only in the Maize Coop Stock Center collection. Maize seeds obtained from the Maize Coop Stock Center were planted in the summer nursery of 2006 and 2007 at the Agronomy Farms of Iowa State University (Boone County, IA). Each of the lines containing a homozygous recessive unknown brown midrib allele was crossed to a series of lines, each containing a different homozygous recessive known brown midrib allele. The F1 plants were screened for reddish-brown pigmentation in the leaf midrib at the V8 stage of growth. Presence of this pigmentation was indicative of the brown midrib mutant phenotype. Two of the lines carrying “phenotype only” brown midrib alleles failed to complement a line carrying the *bm1* allele, one line failed to complement a line carrying the *bm3* allele, and three lines complemented lines carrying each of the four known brown midrib alleles. Proposed new designations have been assigned to the alleles tested. The three lines that complemented all four of the

known brown midrib alleles were not crossed to each other so they may or may not be different brown midrib alleles. Further testing of these genetic stocks will provide insight into these brown midrib mutant alleles.

Positive tests:

Previous Designation	New Designation	MGCSC Source Number
5803D *bm*-PI251009*	*bm1*	MGSC94-4844-9 ((M14 x W23) x *bm**))self
5803L *bm*-N2331B*	*bm1*	MGSC2001-2659-4 ((B73 x Mo17) x *bm**))self
5803K *bm*-2001PR-1*	*bm3*	MGSC2002-335-3 ((M14 x W23) x *bm**))self

Negative tests:

Previous Designation	New Designation[a]	MGCSC Source Number
5803F *bm*-PI251930*	*bm5*	MGSC94-4829-2 (*bm**)self
5803H *bm*-PI262485*	*bm**	MGSC94-4827-1 (*bm**)self
5803J *bm*-86-87-8875-6*	*bm**	MGSC2002P-22-2 ((W23 x M14) x *bm**))self

[a]These lines complemented lines carrying alleles *bm1*, *bm2*, *bm3*, and *bm4*.

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Unità di Ricerca per la Maiscoltura

Evaluation of inoculation techniques for *Fusarium verticillioides* ear rot and fumonisin contamination of maize hybrid genotypes

--Balconi, C; Ferrari, A; Berardo, N; Verderio, A; Mascheroni, S; Laganà, P; Motto, M

In breeding programmes, reliable methods for the screening and evaluation of maize plants for improving tolerance to *Fusarium* attacks are an invaluable tool in increasing crop protection against fungal infection. Some *Fusarium* strains produce mycotoxins which can be formed in infected plants before harvesting, or in grain during post-harvest storage (Bottalico, J. Plant Pathol. 80:85-103, 1998). The occurrence of mycotoxins in cereal grains is a great concern worldwide, because their presence in feed and foods is often associated with chronic or acute mycotoxicoses in livestock and also in humans (Placinta et al., Animal Feed Sci. Technol. 78:21-37, 1999).

Our research is focused on the screening of maize genotypes for tolerance to *Fusarium,* a widely distributed pathogen of maize, causing diseases of roots, seedlings, stalks and grain. In addition to their effects on yield, *Fusarium* species, particularly *F. verticillioides*, can decrease grain quality and produce a number of toxic compounds, including fumonisins. Before starting a breeding program for resistance, efficient techniques for inoculating ears with *Fusarium* ssp. and for detecting mycotoxins are needed to differentiate hybrids as resistant or susceptible under natural infection. Compared with natural infection, inoculation increases disease severity and decreases variability within and among treatments. High levels of resistance identified from inoculated trials will enable breeders to develop more resistant hybrids which are useful to producers during normal growing seasons and growing seasons when disease development is favoured.

Additionally, effective inoculation and subsequent evaluation of corn germplasm may lead to a reduction of fumonisins in grain of commercial corn hybrids and minimize economic disruptions when fumonisin production is favoured (Clements et al., Plant Dis. 87:147-153, 2003). The concentration of mycotoxins and severity of *Fusarium* ear rot are moderately correlated. In fact, fumonisins have been reported at levels of concern in asymptomatically infected grain (Bacon et al., Can. J. Bot. 74:1195-1202, 1996; Robertson et al., Crop Sci. 46:353-361,2006). Therefore, inoculation techniques that are effective for ear rot may not be effective for the assessment of fumonisin content.

The objective of this study was to identify an inoculation technique suitable for the efficient evaluation of a large number of maize hybrids for resistance to *F. verticillioides* ear rot and fumonisins in grain. Furthermore, each entry tested in the artificial inoculation experiments was evaluated in field tests at different locations in North Italy, in order to compare the response of hybrids in different environmental conditions.

For this purpose, 33 commercial maize hybrids (FAO 300-400-500-600-700) were grown in experimental plot designs at 4 different locations. Three inoculation techniques designed to promote the development of *Fusarium* ear rot were applied to self-pollinated and open-pollinated plants. For each inoculation method, 10 replicates for each plot were inoculated with a fresh spore suspension (10^6 spore/ml mixture of 2 *F. verticillioides* isolates from Northern Italy, supplied by Dr. Battilani-University of Piacenza); controls for all the inoculation techniques were non-inoculated and sterile water-inoculated primary ears.

The infection treatments were: i) wounding silk channel inoculation assay (SCIA): syringe injection of 2 ml fungal suspension in the silk channel (region within the husk between the tip of the cob and tip of the husk where the silks emerge) of the primary ear 7 days after pollination (DAP); ii) non-wounding-SCIA: silks of the primary ear were sprayed with 1.5 ml of fungal suspension 7 DAP (Clements et al., 2003); iii) kernel inoculation method: the inoculum was applied 15 DAP by dipping a stainless steel fork in the fungal suspension and stabbing the central-basal of the ear through the husks and into 3 kernels, thus producing a point source of infection from which the fungus may spread (Reid et al., Technical Bull. 1996-5E, Research Branch, Agriculture and Agri-Food Canada, 1996).

Beneficial secondary traits such as husk covering and tightness are factors contributing to fungal pathogen resistance; in general, it was reported that the hybrids with good husk cover show a greater resistance to insect damage and in turn accumulate lower levels of mycotoxins (Betran et al. Crop Sci. 42:1894-1901, 2002). Therefore, in this study, silk channel length at pollination and husk covering at maturity were recorded for each hybrid.

At maturity, ears were manually harvested. For husk cover, visual ratings ranging from 1 (good: tight long husks extending beyond the tip of the ear) to 5 (poor: loose short husks with exposed ear tips) were recorded (Betran et al., 2002). After hand dehusking, the severity of *F. verticilliodes* ear attack was evaluated using rating scales based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth. As reported by Reid et al. (1996), for the SCIA (wounding and non-wounding) method the visual rating scale consists of 7 classes based on percentage of visibly infected kernels (Disease Severity Rating-DSR-: 1=0%-no infection; 2=1-3%; 3=4-10%; 4=11–25%; 5=26-50%, 6=51-75%; 7=76-100%). For the kernel inoculation method, the number of visually infected kernels at the inoculation

point were recorded. Individual ear ratings, using a visual scale as described above, allowed a discernible screening of the genotypes tested for *F. verticillioides* resistance.

After visual inspection, ears were dried and shelled and the kernels were bulked within replicates. Ground grain was analyzed for the concentration of fumonisin with an enzyme-linked immunosorbent assay (ELISA). Correlation analyses between visual ear rot ratings, fumonisin content, and ear morpho-physiological parameters (silk channel length at pollination, husk cover ratings) in genotypes derived from the three different inoculation technique experiments are in progress.

*This research was developed in the Program MICOCER.

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Screening male-sterile mutants in Berkeley for anther development mutants

--Harper, L; Timofejeva, L; Wang, R; Golubovskaya, I; Walbot, V; Cande, Z

We are interested in identifying mutants that have specific defects in early anther development that occur in the window of developmental time between initiation of the anther and initiation of meiosis. Studying these mutants will allow us to identify processes involved in anther cell fate acquisition, including acquisition of meiotic cell fate. In order to identify appropriate mutants, we are screening all known male sterile mutants that we can find, including those at the Maize Genetic Stock Center, the majority of which contain *RescueMu*, and other resources. **Please send us your male sterile mutants!!** This screen is part of a new NSF sponsored plant genome research project, Cell Fate Acquisition in Maize which we call "The Anther Project" (PI: Virginia Walbot, CoPI: Zac Cande). Here we describe our screen of existing male sterile mutants for mutants with defects in early anther development.

The Screen. In the summer of 2007, we planted 117 families segregating for male sterile mutants from the coop and from the mutants Inna Golubovskaya identified as male sterile from the MTM project (identified summers of 1999 and 2000), representing 6 known male sterile mutants and 57 uncharacterized male sterile mutants. In order to determine the nature of the male sterile phenotype, we collected immature anthers at the stage of early meiosis for every plant, and fixed in 3:1 ethanol:acetic acid. This took about 17 person hours per 100 plants in the field. About two weeks later, when the plants started to shed pollen, plants were scored for male sterility and appropriate crosses were made. During the scoring, it was possible to identify many families segregating for very late shedders. In the initial screens performed by others, such lines were probably identified as male steriles if sterility was checked only at the normal shedding time, or alternatively, the mild Berkeley climate allowed these male steriles to escape. After the field season, the immature anthers from identified male sterile plants where examined microscopically. For a screen of this size, it is too time consuming to embed and section anthers from each male sterile plant. Instead, acetocarmine squashes were performed on several stages of anther development (based on anther length) and the organization and viability of cells in the anther wall, tapetum and meiocytes could be determined. This turned out to be a very productive way to screen the male sterile anthers and identify mutants with early anther development defects.

Results. From 57 uncharacterized mutants screened so far, 7 segregate for classic male sterile pollen development defects, 3 have defects in meiosis, and 5 have defects in early anther development (the class we want). Thus, of the 57 uncharacterized mutants examined, 5 warrant further analysis in our study; almost 9%! The rest appeared normal in the acetocarmine squashes, probably indicating post meiotic defects. After we complete complementation tests, we will know how many new genes these 5 mutants represent.

Conclusion. Screening male sterile mutants will be an extremely productive way to identify early anther defects. Examining immature anthers by acetocarmine squashes is a productive way to identify even mutants with abnormal anther wall morphology. During the winter, we will continue to examine the 5 early anther defects by careful microscopic examination of embedded and sectioned immature anthers.

Where to find information. All information from this screen, including images and the criteria used to determine which class mutants fall into, will be deposited at MaizeGDB.

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The β-glucosidase null phenotype in maize is due to a jacalin-related chimeric lectin and its lectin domain is responsible for β-glucosidase aggregation

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

β-glucosidases catalyze hydrolysis of alkyl and aryl-β-D-glucosides as well as β-linked oligosaccharides. In maize, two isozymes of β-glucosidase (Glu1 and Glu2) have been identified and are thought to be involved in the defense against pathogens and herbivores by releasing toxic aglycones, such as hydroxamic acids, from their glucosides. The predominant hydroxamic acid glucoside in maize is 2-glucopyranosyl-4-hydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOAGlc), whose aglycone DIMBOA is the primary defense chemical against aphids and the European corn borer (*Ostrinia nubilalis*).

Certain maize inbred lines were reported to be devoid of β-glucosidases (Biochem. Genet. 15:383-394, 1977). These inbreds were originally thought to be homozygous for a null allele at the *glu1* locus. However, we found that they have β-glucosidase activity but the enzyme in them occurs as large insoluble complexes (Biochem. Genet. 28:319-336, 1990). Furthermore, we identified a 32 kD protein, referred to as β-glucosidase aggregating factor (BGAF), which specifically interacts with maize β-glucosidases to form insoluble complexes (Plant Physiol. 122:563-572, 2000). BGAF is a chimeric protein consisting of an N-terminal dirigent domain and a C-terminal Jacalin-related lectin (JRL) domain (J.

Biol. Chem. 276:11895-11901, 2001; J. Biol. Chem. 282:7299-7311, 2007).

To gain an insight into lectin and β-glucosidase aggregating activities of BGAF, native BGAF free of β-glucosidase was isolated from maize null-line H95 and its recombinant version was produced in *E. coli*. Both native and recombinant BGAF agglutinated rabbit erythrocytes, and their hemagglutinating activity, was inhibited preferentially by galactose, lactose and glycoproteins containing N-acetyl-D-galactose- amine and N-acetyllactosamine residues (Table 1). BGAF binds to maize Glu1 even in the presence of saturating concentrations of galactose, indicating that the sugar and the β-glucosidase binding sites are distinct (Fig. 1). When kinetic constants (*K*m and *k*cat) for Glu1 were determined (using *para*-nitrophenyl-β-D-glucopyranoside as substrate) in the absence and presence of BGAF, no differences in the *K*m and *k*cat values were observed, suggesting that BGAF does not have any effect on β-glucosidase activity. Of the two domains in BGAF (expressed separately in *E. coli*), only the JRL domain was able to retard the mobility of Glu1 on the native gel (Fig. 2), clearly indicating that the binding site(s) for β-glucosidase are in the JRL domain. Replacing the JRL domain of a BGAF homolog from sorghum (non-binder) with the JRL domain of maize BGAF (binder) resulted in a chimera with high affinity for maize Glu1 (Fig. 3),

Table 1. Inhibition of hemagglutination activity[a] of native BGAF, recombinant BGAF and BGAF-Glu1 complex by saccharides and glycoproteins.

	Native BGAF			
Saccharides	MIC mM	rP[b]	rP rBGAF	rP BGAF-Glu1 complex[c]
Galactose	7.8	1	0.50	0.25
Methyl-α-D-galactopyranoside	3.9	2	1	1
Methyl-β-D-galactopyranoside	31.2	0.25	0.25	0.12
Galactosamine	15.6	0.50	0.50	0.12
N-acetyl-D-galactosamine	31.2	0.25	0.25	0.62
N-acetylneuraminic acid	31.2	0.25	0.25	NI
Mannose	15.6	0.50	0.50	NI
Methyl-α-D-mannopyranoside	7.8	1	1	NI
Lactose	1.9	4.1	4.1	1
N-acetyllactosamine	3.9	2	2	1
Raffinose	1.9	4.1	4.1	2
Stachyose	7.8	1	1	ND[e]
Glycoproteins				
Ovalbumin	0.0013	6000	6000	6000
Horseradish peroxidase	NI	-	NI	NI
Asialofetuin	<0.001	>7800	>7800	>7800
PSM	0.001	7800	7800	7800

[a]Inhibition of hemagglutination was assayed by serially diluting saccharide and glycoprotein solutions in the microtiter wells, followed by the addition of 4 units of the lectin, and then the addition of a 2% suspension of trypsinized rabbit erythrocytes after 30 min. The lowest concentration of saccharides or glycoproteins that visibly decreased the extent of agglutination was defined as the minimum inhibitory concentration (MIC). The MIC values were obtained from two independent measurements.
[b]Relative potency, relative to D-galactose with native BGAF.
[c]Isolated from the maize H95 "null" line.
[d]NI, No detectable inhibition.
[e]ND, not determined.

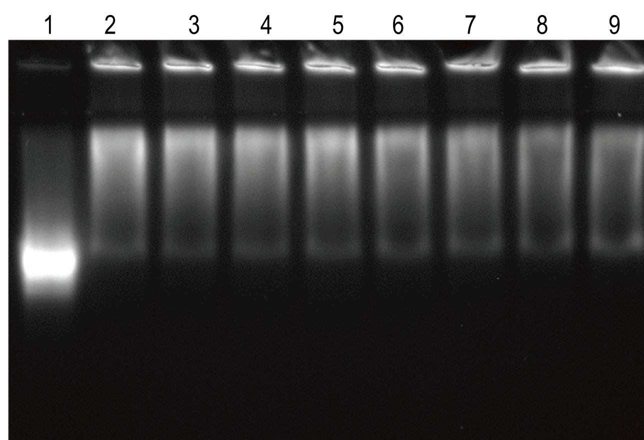

Figure 1. Gel-shift assay to detect binding of BGAF to maize Glu1 in the presence of increasing concentrations of galactose. rBGAF (14 nM) was incubated with increasing concentrations of galactose (0-125 mM) at room temperature for 1 h in PBS, pH 7.4. Following addition of Glu1 (58 nM) and incubation for 2 h, aliquots were withdrawn and electrophoresed on an 8% native gel. β-glucosidase activity was detected by staining with 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG). Lane 1, Glu1; lane 2, BGAF + Glu1 with no sugar, lanes 3-9, rBGAF plus Glu1 in the presence of 1.9-125 mM galactose. In the presence of BGAF, β-glucosidase activity zones (smearing) detected with 4-MUGlc are retarded in a region extending from the top of the resolving gel to the sample well in the stacking gel (lane 2). Note the same pattern is observed even in the presence of saturating concentrations of galactose (lanes 3-9), indicating that carbohydrate and β-glucosidase binding sites are distinct.

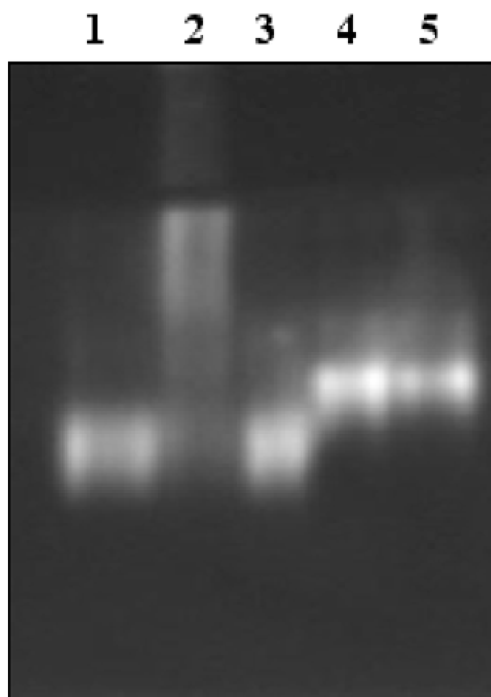

Figure 2. Gel-shift assay to detect binding of dirigent and the JRL domain to maize Glu1. Glu1 (58 nM; lane 1) was incubated with rBGAF (14 nM; lane 2), dirigent (100 nM; lane 3), JRL (100 nM; lane 4) and dirigent plus JRL (100 nM each; lane 5) at room temperature for 2 h in PBS, pH 7.4. Electrophoresis and staining was done as described in the legend for Figure 1. Note that only the JRL domain retards the mobility of Glu1 (lanes 4 and 5).

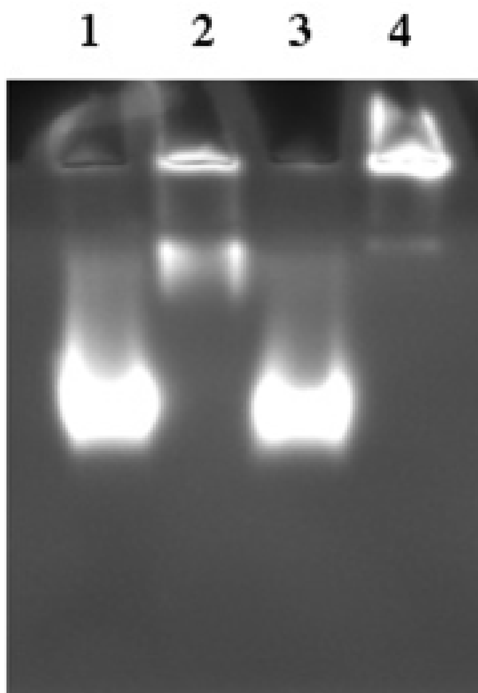

Figure 3. Gel-shift assay to detect binding of chimeric BGAF (consisting of the dirigent domain of the BGAF homolog from sorghum and the JRL domain of maize BGAF) to maize Glu1. Glu1 (58 nM, lane 1) was incubated with 200 nM maize rBGAF (lane 2), sorghum rBGAF (lane 3) and chimeric BGAF (lane 4) at room temperature in PBS, pH 7.4. Electrophoresis and staining was done as described in the legend for Figure 1. Sorghum rBGAF itself does not bind Glu1 (lane 3), whereas chimeric BGAF formed complexes with Glu1, which are retained in the sample well (lane 4).

indicating that the JRL domain is responsible for β-glucosidase aggregation and hence the null-phenotype in maize. The facts that BGAF had no adverse effect on β-glucosidase activity and that the sugar-binding site is free in the complex to interact with sugars suggest that the BGAF-β-glucosidase complex might protect maize against pests by binding to glycoproteins and producing a local burst of DIMBOA in the oral cavity or by damaging the peritrophic membrane lining the midgut of insect larvae.

BOLOGNA, ITALY
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A Gaspé flint × B73 introgression library suitable for the genetic dissection of flowering time and other agronomic traits

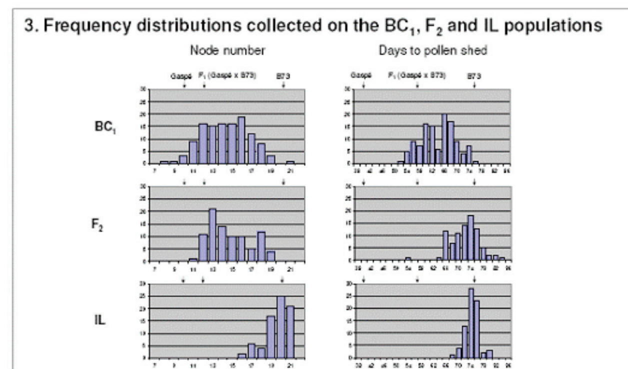
--Salvi, S; Corneti, S; Giuliani, S; Sanguinati, MC; Talamè, V; Tuberosa, R

An introgression library (IL) is a collection of lines each carrying a well-defined chromosome segment introgressed from a donor accession into the genetic background of a common line (Zamir, Nat. Rev. Genet. 2:983-989, 2001). Introgression libraries are ideal for mapping and cloning QTLs (Zamir, 2001; Salvi and Tuberosa, TIPS 10:297-304, 2005), two increasingly important goals in maize breeding in view of the importance of quantitative traits for the improvement and sustainability of yield. We are interested in identifying major QTLs influencing flowering time and root architecture, two important features for the adaptation of maize to different environments and to counteract the negative consequences of global climate change (e.g. increase in temperature, drought, flooding, etc.). B73 and Gaspé flint differ greatly in flowering time (25-30 days in our environment) and in a number of other architectural features, including root architecture, an important trait in improving maize performance under lower-input agriculture (e.g., less irrigation and fertilizer application). Additionally, B73 was chosen because of its importance in maize breeding and the vast amount of genetic and genomics information that is available for this line.

Materials. The [(B73 x Gaspè flint) x B73] BC_1 to BC_5 generations were grown from 2002 to 2005. In each generation, plants heterozygous for a given chromosome interval were identified based on SSR analysis and backcrossed to B73. At BC_5, one plant homozygous for the Gaspé flint introgression for each one of the 70 BC families was chosen to assemble the IL. On average, outside the introgressed regions ca. 98% of the B73 genome should have been recovered for each line. The following materials were evaluated: the 88 BC_1 plants and 126 B73 x Gaspé flint F_2 plants, along with the 70 IL lines.

Molecular markers. 165 SSRs (maizeGDB at www.maizeGDB.org) were used, 86 (52%) of which were polymorphic between B73 and Gaspé flint. Among the polymorphic SSRs, 72 were utilized to produce a linkage map based on the BC_1 population.

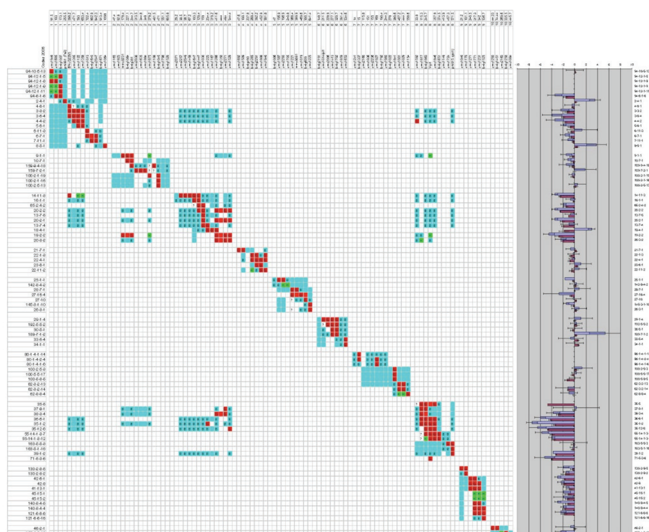
Traits analyzed. Days to pollen shed (DPS), node number (ND) and other traits were measured in the BC_1, F_2 and IL populations. The BC_1 population was grown in 2002, and the F_2 and IL lines in 2006. The 70 IL lines were grown in four reps with 10 plants per plot. The figure below presents the frequency distribution of flowering time of the BC_1, F_2 and IL populations.



Root traits were evaluated at the seedling stage and in pots. Preliminary observations indicate the presence of extensive variability for root features (Salvi et al., unpublished). The data are being analyzed.

Structure and phenotyping of the Gaspé flint x B73 introgression library. In the figure below, rows represent the IL lines while columns indicate the SSR markers. Red, blue and green squares with/without letters indicate homozygosity for Gaspé, homozygosity for B73 and heterozygosity, respectively, as per SSR analysis. White squares indicate homozygosity for B73 ($P > 0.98$). The blue dashed lines indicate the approximate position for newly identified QTLs for flowering time, herein named *Vgt (Vegetative to generative transition) 3 to Vgt6*, after the previously described *Vgt1* and *Vgt2* (Vladutu et al., Genetics 153:993--1007, 1999, Salvi et al.,

Plant Mol. Biol. 48:601-603, 2002). Recently, *Vgt1* has been positionally cloned (Salvi et al., PNAS 104:11376-11381, 2007). (NOTE: the figure below has viewable detail if zoomed, online.)



Conclusions and perspectives. We produced a Gaspé flint x B73 introgression library through five marker-assisted backcrosses. The 70 introgression lines cover ca. 70% of the Gaspé flint genome in a B73 genetic background. The evaluation of the IL lines confirmed the important role of *Vgt1* and *Vgt2,* and revealed the presence of four additional QTLs for flowering time. The fine mapping of *Vgt3* is underway. Extensive phenotyping, both as lines per se and testcrosses, is in progress to identify additional QTLs for the genetic control of yield and other agronomically important traits. The IL lines are available for collaborative studies.

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CASTELAR, ARGENTINA
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Effect of ethyl methane sulfonate (EMS) on ear weight in maize

--Kandus, MV; Menéndez, Y; Salerno, JC; Delucis, M; Boggio Ronceros, RE; Díaz, DG; Prina, A; Almorza, D

The use of induced mutants is an important tool in plant breeding. The effect of EMS on individual ear weight was analysed after seven generations of open pollination.

Two hundred seeds of an inbred flint line were treated with EMS at two concentrations (0.5% and 0.75%) for 13 hours. After seven generations of open pollination, 300 ears were measured for each treatment and the same line without treatment as a check. The mean weight of ears was higher for the descendants of the EMS-treated material: 99.80 grams for 0.5%, 95.76 grams for 0.75% and 75.53 grams for the check (Figure 1). Also, the ear weight range was higher for the treatment: 40 to 200 grams for 0.5%; 30 to 180 grams for 0.75% and 30 to 130 grams for the check (Figure 2). The populations derived from these EMS treatments should be a useful source for inbred line selection with respect to ear weight.

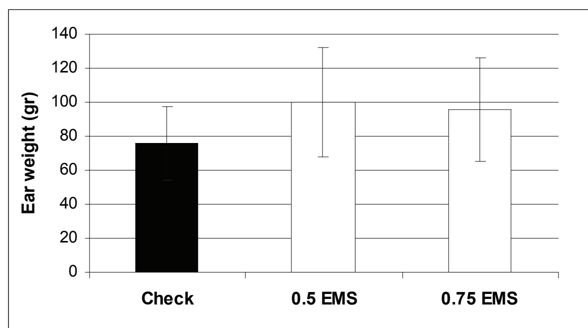

Figure 1. Mean ear weight (grams/ear).

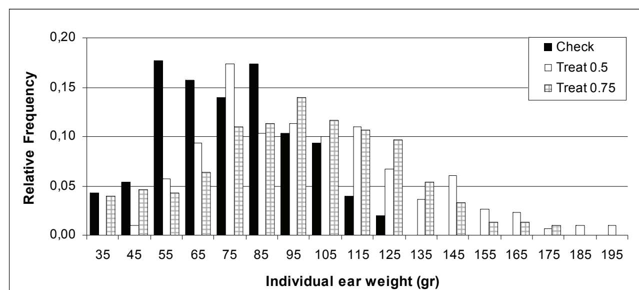

Figure 2. Distribution of ear weight (grams/ear).

Increase of chlorophyll deficient mutants in a maize line induced by ethyl methane sulfonate (EMS)

--Kandus, MV; Menéndez, Y; Salerno, JC; Delucis, M; Boggio Ronceros, RE; Díaz, DG; Prina, A; Almorza, D

The action of EMS in a maize line was studied to see the effect on the number of chlorophyll deficient mutant seedlings after seven generations of open pollination.

Two hundred seeds of an inbred flint line were treated with EMS at two concentrations (0.5% and 0.75%) for 13 hours. The results for 300 ears harvested after seven generations, for each treatment and the same line without treatment as a check, revealed that the mean of chlorophyll deficient mutant seedlings was higher in both treatment groups than in the check (65.7% for 0.5%, 64.00% for 0.75% and 62.33% for the check). The check showed 100% white seedlings, while the seeds treated with EMS in different concentrations showed white, yellow and virescent seedlings (Figure 1).

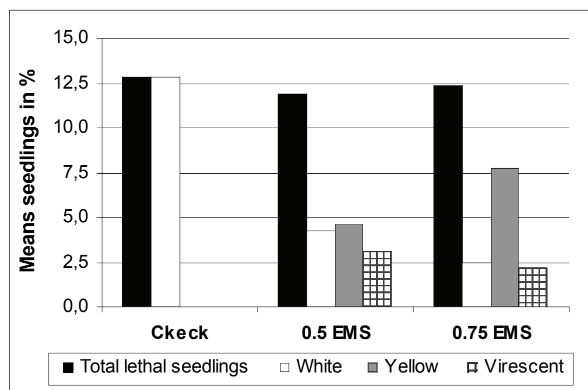

Figure 1. Mean lethal seedlings in % (white, yellow, virescent).

Performance of inbred lines considering different agronomic traits

--Kandus, MV; Salerno, JC; Boggio Ronceros, RE; Almorza, D

In order to evaluate the relationship among different traits of the ear of maize inbred lines, and to group genotypes according to performance, ten inbred lines developed at IGEAF (INTA Castelar) and five public inbred lines used as checks were evaluated in a field trial. At harvest, individual weight (P.E.), diameter (D.E.), row number (N.H.) and length (L.E.) of ears were assessed, using a principal component analysis, PCA (Infostat 2005). Principal components 1 and 2 (CP1 and CP2) explained 90% of the data variability. CP1 was correlated with P.E., L.E. and D.E., while CP2 was correlated with N.H. It was found that individual weight (P.E.) was more correlated with diameter of the ear (D.E.) than with length (L.E). Five groups of inbred lines were distinguished: high P.E. and mean N.H., high P.E. but less N.H., mean P.E. and N.H., high N.H. but less P.E. and low P.E. and low N.H. The use of PCA showed which variables had the greatest correlation with ear weight and what the correlation was among them. Moreover, the different groups discovered with this analysis allow the evaluation of inbred lines by several traits simultaneously.

Mal de Río Cuarto virus in maize: QTL mapping analysis

--Kreff, ED; Pacheco, MG; Díaz, DG; Robredo, CG; Puécher, D; Céliz, AE; Salerno, JC

Mal de Río Cuarto (MRCV) is the major viral disease of maize (*Zea mays* L.) in Argentina. The causal agent, Mal de Río Cuarto virus, is vectored by *Delphacodes kuscheli* Fennah (Homoptera: Delphacidae). Identification of Quantitative Trait Loci (QTL) conferring resistance to MRCV could aid in selecting for this trait.

The characterization of the disease response of maize genotypes under natural infestations in order to localize QTLs for resistance to MRCV was made in local lines of maize, genotyping with simple-sequence. The symptoms analyzed were tassel symptoms (TS), presence and size of enations (PE), internode shortening (IS), leaf shortening (LS) and leaf narrowing (LN), plant height (PH), ears healthy (ES).

In each line the symptoms were expressed by their incidence, severity, and intensity, calculated as follows: (1) Symptom incidence: Σ (number of plants with symptoms)/number of plants. (2) Symptom severity: Σ (rating of each plant with symptoms)/number of plants with symptoms. (3) Symptom intensity: Σ (rating of each plant with symptoms)/number of plants. Severity and intensity of symptoms were calculated for tassel and ear symptoms, and presence of enations. Plant height average of each line was calculated.

The most important QTLs mapped for marker-assisted selection or for QTL cloning and expression profile studies were on chromosomes 1 and 4.

Chromosome segments involved with hybrid vigor in maize (*Zea mays* L.)

--Salerno, JC; Kandus, MV; Boggio Ronceros, RE; Almorza, D

It is known that hybrid vigor (heterosis) expression in maize depends on the heterozygous condition of a portion of the genome. A method for studying the relative contribution of different chromosome segments to hybrid vigor is through balanced lethal systems that permit a heterozygous quasi-permanent condition of some chromosome segments, while the rest of the genome becomes homozygous by continuous selfing. In this way, balanced lethal systems were isolated in two lines of maize, and were evaluated in a yield trial using the public lines B14, B73, MO17 and N28 (Table 1). A randomized block design was used, with three replications in a plot of two rows of 3.50 meters for 1.40 meters. Seventy thousand plants were planted per hectare. Through the years, these balanced inbred lines showed a high yield with the public lines, suggesting the usefulness of genetic load in the maize population in a natural way. After 5 generations of backcrosses of these balanced lethal systems to the public lines mentioned before, grain yield trials were conducted to study the evolution of grain yield in the inbred lines with respect to the same lines per se. The grain yield of the backcrosses was higher than the respective original lines (Table 2). The manipulation of these genetic factors where genes important for yield are in heterozygous segments can give rise to high grain yield in the inbred lines that produce hybrids.

Table 1. Grain yield in kg/ha of the two balanced lethal system lines of maize, with the public lines B14, B73, MO17 and N28 as a check.

	Generations					
Lines	1	2	3	4	5	6
BLS 14	3967 A	3313 A	3685 A	4017 A	3852 A	1135 A
BLS1	3747 A	3243 A	3579 A	3537 A	3245 A	2469 A
N28	2240 B	1709 C	2252 B	2675 B	1652 BC	473 B
B73	2060 B	853 D	1976 B	2573 B	1580 BC	722 B
MO17	2007 B	1503 C	1776 B	2566 B	1864 BC	242 D
B14	1860 B	811 D	1718 B	2506 B	2156 B	294 D

Means with the same letter are not significantly different at 5%.

Table 2. Grain yield in kg/ha of the public lines after 5 generations of backcross, with the introgression of the two balanced lethal system lines of maize.

Backcross 5	Per se	BLS1A	BLS14
B73	722	1536*	3570*
B14	294	1237*	2252*
N28	473	1025*	1831*
MO17	242	1971*	2505*

Markov chain in the analysis of the chromosome segment in maize

--Salerno, JC; Kandus, MV; Boggio Ronceros, RE; Almorza, D

A biometric model was designed using the mathematical formalism relating to the discrete absorbent Markov chain in the canonical form, in order to analyse the evolution of the chromosome segment, taking recessive lethal genes linked with the grain yield factors through the generations, as a different approach to the classical mathematical ones.accordance with the theory of absorbent Markov chains, genotypes A, C, F, G, H and I act as absorbent states. Selfing genotype A produces the same cross in the next generation, therefore AA is also an absorbent non-lethal state. The transient states are BB, DD, EE and KK (Figures 1 and 2).

Afterwards, beginning with selfing crosses for independent segregation (loci with very weak linkage, practically independent for the segregation), the selfing crosses AA, CC, FF, GG, HH and II can be recognised as absorbent states, while BB, DD, and EE are equivalent to KK.

Under these conditions, and following Mendel's laws to calculate the resulting probabilities for selfing in the next generation, the

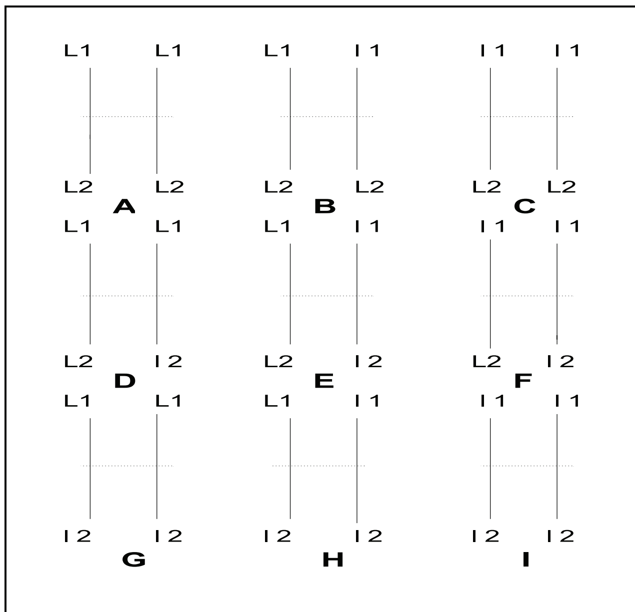

Figure 1. Mean separated chromosomes (really in the same chromosome with negligible linkage).

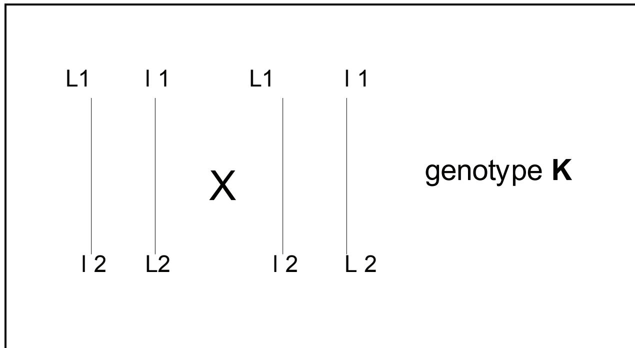

Figure 2. Balanced system (lethal in opposite chromosomes) fully linked loci.

results are given by the transition matrix P written in canonical form. For an absorbing chain, only transient and absorbent states must be considered. The transition matrix P can be written as four sub-matrices: sub-matrix O, a zero matrix of $s\cdot(r–s)$ order; sub-matrix Q, providing the probabilities for the transition between transient states; sub-matrix R, denoting the probabilities between transient and absorbent states; and sub-matrix S, $(r\text{-}s)\cdot(r\text{-}s)$ the identity matrix (Figure 3).

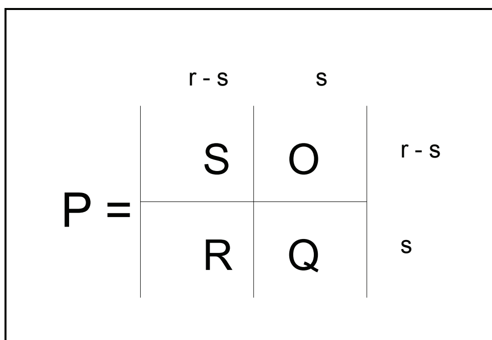

Figure 3. Matrix P, in canonical form; r = 9, s=3.

A chromosome segment containing only recessive alleles remains in the population of two genotypes which have been denoted by A and K. These genotypes can be distinguished easily, with genotype K being an excellent marker for the study of chromosome segments and their association with yield in grain production.

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Using haploid plants for the creation of high yield populations in maize

--Rotarenco, VA; Mihailov, ME; Dicu, G

Haploid breeding is of interest to maize breeders, which by chromosome doubling of haploids will reduce the expense and time for the production of homozygous lines.

However, there are some papers in which a wider use of haploid/doubled-haploid plants in breeding work is being discussed (Bouchez and Gallais, 2000; Rober, Gordillo and Geiger, 2005; Bordes et.al., 2006). The main point of the papers is that at the haploid level, due to the absence of allelic gene interactions, selection for favorable genes with non-allelic effects might be more effective in comparison with the diploid level.

Chalyk and Rotarenco used this feature of haploids in a scheme of recurrent selection to improve seed productivity of two synthetic populations - SA and SP (Chalyk and Rotarenco, 1999; Chalyk and Rotarenco, 2001). The selection was carried out by ear size in haploid plants. After three cycles of haploid recurrent selection, the values of the seed productivity and other quantitative traits in both diploid populations were significantly increased (Rotarenco, Chalyk and Eder, 2004).

During the last years, the fourth cycle of such recurrent selection has been carried out in the SP population. Thus, the seed productivity of this population (SPC4) reached a rather high level and, in our opinion, it might be comparable to F1 hybrids.

The main purpose of our work was to compare the SPC4 population (FAO – 330) with three simple hybrids that are widely used in local maize production: Moldavian 291, Porumbeni 295 and Porumbeni 359. The F2 progenies of these hybrids, and an inter-population hybrid (SPC4xSAC3), were also included in the estimation. The trial was conducted in three replications in Fundulea, Romania. Each genotype was grown in a two-row plot with a length of 7.8 meters. Plant density was 50, 000 plants per hectare. After flowering, two plant traits, plant height and ear height, were measured. Ear length, number of seed rows and seed productivity were estimated after the drying of the ears. Because of drought, the seed productivity of the estimated genotypes was much lower (about 40%) in comparison with favorable years. Nevertheless, the results obtained can be used according to the goal of the experiment. The results are presented in the Table 1.

Table 1. Seed productivity and parameters of ear and plant traits in estimated genotypes.

Traits	Genotypes							
	Population SPC4	Interpopulation hybrid SPC4xSAC3	Moldavian 291	Porumbeni 295	Porumbeni 359	Moldavian 291 F2	Porumbeni 295 F2	Porumbeni 359 F2
Seed productivity, gr/plant	101.6±3.9	102.2±3.3	121.3±4.1^^^	122.8±5.0^^^	104.0±4.4	95.5±4.0	94.2±4.7	96.2±3.6
Ear length,cm.	18.9±0.3	18.4±0.2	20.1±0.1^^^	18.7±0.2	18.1±0.2**	17.1±0.2***	17.2±0.3***	17.0±0.2***
Number of seed rows, no.	16.9±0.2	16.4±0.2	14.6±0.2***	15.5±0.2***	14.8±0.2***	14.8±0.2***	15.4±0.2***	14.5±0.2***
Plant height, cm.	222.1±2.6	239.9±2.2^^^	225.4±2.8	191.7±2.0***	185.1±2.1***	202.4±2.3***	181.4±2.4***	181.2±1.5***
Ear height, cm.	90.2±1.8	96.6±1.5^^	79.2±1.1***	68.5±1.0***	71.7±1.1***	75.1±1.3***	65.6±1.4***	73.8±1.0***

^ - the excess over the SPC4 population; ^^, ^^^ significant difference at 0.01 and 0.001 probability level, respectively

* - the excess of the SPC4 population; **, *** significant difference at 0.01 and 0.001 probability level, respectively

Two hybrids, Moldavian 291 and Porumbeni 295, exceeded the SPC4 population for seed productivity. There were no significant differences between the population and the F2 progenies of the hybrids for this trait. Ear length was significantly greater in the Moldavian 291 hybrid, whereas the SPC4 population exceeded all the simple hybrids and their F2 progenies in the number of seed rows.

Most likely, the excess of the seed productivity of the two F1 hybrids over the population was caused by their superiority in size of seeds (weight of 1,000 seeds); however, an estimation of this parameter was not carried out.

The values of seed productivity and ear traits in the interpopulation hybrid did not differ significantly from the SPC4 population. However, this hybrid significantly exceeded the population for the plant traits. In other words, there was no heterosis for the ear traits, whereas a rather high heterosis for the plant traits.

As was previously revealed, the SPC4 population significantly exceeded the SAC3 population for seed productivity and other quantitative traits. Therefore, the SPC4 population can be considered the best parent in the interpopulation hybrid SPC4xSAC3.

The influence of allelic gene interactions, dominance and overdominance, could be the reason for the high heterosis for plant traits in the interpopulation hybrid. At the same time, these gene interactions did not have a significant influence on the ear traits and consequently on the seed productivity of this hybrid.

The improvement of a heterogeneous population per se occurs due to an increase of the frequency of favorable genes with non-allelic effects (Hallauer and Miranda, 1986). Based on the results obtained, it can be concluded that the selection among haploids is a very efficient and relatively quick way to increase the frequency of favorable genes with non-allelic effects in a population.

We plan the further improvement of the SPC4 population. However, because of the decrease of genetic variability in the population, there is a large chance that further improvement will not be as efficient as it was during the four cycles of haploid recurrent selection. Therefore, a new germplasm with favorable genes will be introduced into the population. For the estimation and selection of this germplasm haploid plants will also be used.

In our opinion, haploidy might be a very useful tool for the improvement of synthetic populations. High yield populations could be widely used both as an initial material in breeding work and in production.

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Recording field narratives

--Kazic, T

This note describes a simple system for recording narrative data in the field.

To permit the eventual use of voice-recognition software to translate the recording into text, a system compatible with the software (MacSpeech 1.7) was needed. It consists of a fifth-generation video iPod (Apple; 80 GB model, MA450LL), connected to a Belkin TuneTalk stereo microphone (F8Z082-BLK) via the iPod's dock connector. A Plantronics VXi TalkPro headset/microphone is connected in turn to the TuneTalk using a standard microphone jack. The headset microphone's audio quality is higher than that of the TuneTalk itself, probably because extraneous noise from moving plants is sharply reduced. The microphone is placed approximately 2 inches away from, and slightly below, the narrator's mouth. The ``audio quality'' setting on the iPod's voice recorder menu is set to ``high''; the ``autogain'' on the TuneTalk is set to ``on''. Both the iPod's and TuneTalk's batteries are recharged nightly `via their USB connectors.

Commercial shields for the iPod tend to push the TuneTalk out of the dock connector, losing the audio signal. They also do not protect the iPod very well in field conditions. For these reasons, a simple enclosure was built from a 4X6 inch Plano fly box and Oasis rippled closed-cell adhesive foam used to hold trout flies (# 3583 and # 38-421-922-30, respectively; both available from Bass Pro Shops). The top layer of foam was trimmed to hold the iPod snugly when the box is closed while permitting inspection of the screen. Holes just large enough to accommodate the microphone and headset leads when the lid is closed were drilled through the base and lid and buffed with a Dremel. The enclosure is illustrated in Figure 1.

In use, the iPod is switched on and recording started, then placed in the enclosure and the latter closed and snapped shut. The enclosure is then placed in a pollinating apron. Surprisingly, even during extremely hot weather the ventilation is adequate; checking that the iPod is still recording every hour or so seems sufficient to ventilate it. The iPod also serves as a back-up disk for other field data.

Figure 1. The fly box enclosure with iPod, TuneTalk, and VXi headset/microphone.

I am grateful to Karen Cone for suggesting that voice recognition software might be useful in transcribing field notes.

A collection of *les* mutants

--Kazic, T

The production of lesions by lesion mimic mutants offers a rich system for understanding complex phenotypes and developing algorithms to infer their underlying network of biochemical reactions and physiological and developmental events. Several other notes in this issue of the Newsletter describe our progress in photographic methods, recording, and scripts to generate tags for plant management and inventory. But the most fundamental step we have taken so far is to collect *les* mutants and begin their introgression into Mo20W, W23, and M14.

Table 1 shows the current mutants as of the 2007 field season and the list of those I am certain I do not have. The phenotype of the *Les15* mutants in the collection is uncertain; I would appreciate additional instances of this gene. I would like to collect these and any other mutants that exhibit differential health of patches of leaf tissue at different times in development, or whose expression is affected by genetic background, weather, or latitude. As time permits, I will begin their introgression as well.

Table 1. Current and needed genes.

Current Genes	Missing Genes
Les1	*Les5*
Les2	*Les14*
Les3	*Les16*
Les4	*Les20*
Les6	*Les21*
Les7	
Les8	
Les9	
Les10	
Les11	
Les12	
Les13	
Les15	
Les17	
Les18	
Les19	
les23	
lls1	

I am grateful to Gerry Neuffer for giving us lines and sage advice, and to Marty Sachs and Guri Johal for lines and thoughtful discussion. My debt to the entire maize community at Missouri, especially Ed Coe, Karen Cone, Georgia Davis, and Mary Schaeffer, is profound.

Collection of quantitative images of leaves in the field and greenhouse

--Foard, A; Hopfenblatt, J; Kalaga, S; Lutes, M; Powell, D; Raithel, D; Roberts, P; Young, C; Clements, A; Cornelison, C; Fourman, K; Orozco, D; Sutterfield, J; Rose, A; Thawani, N; Thieman, M; Harnsomburana, J; Green, J; Jones, C; Paulsell, D; Frey, B; Chipley, M; Kataria, A; Venkataraman, A; Kazic, T

This note describes the photographic apparatus and procedures we have used to collect quantitative images of leaves from les mutant plants in Missouri.

Apparatus. The apparatus consists of an imaging platform, a digital single-lens reflex (SLR) camera, and a means to evenly illuminate the platform.

Platform. The platform joins a photographic plane and a support for the camera. The plane is a sheet of 3/8 in plywood, 14 3/16 x 10 3/16 in, screwed into a V-shaped aluminum bracket. The bracket's base extends beyond the platform and admits a 1/2 x 48 in steel rod; a set screw holds the rod in place. 3/16 in thick foam board (Borden Elmer's), cut to match the plywood, is covered with blue cloth; the cloth is taped to the back of the foam board with duct tape. The covered foam board is mounted to the plywood using four small pieces of industrial-strength velcro. (We change the entire foam board as the cloth gets dirty.) On the side of the cloth facing the camera, a GretagMacBeth mini-color checker (now a Munsell color checker available from X-Rite, # M50111) is mounted on a small piece of velcro so that it is approximately 1.25 in from the edge of the platform next to the bottom of the V, and centered left to right. The color checker is mounted so that its greyscale is toward the rod. The cloth is blue, matching the blue square of the color checker as closely as possible (RGB = (56,61,150)).

The camera is suspended from a Manfrotto ball-jointed, quick release tripod mount (# 486RC2). The joint comes with the corresponding quick release plate that threads into the base of the camera. The threaded end of the mount is screwed onto a 1/2 x 6 in aluminum rod. This short rod is clamped to the steel rod using a perpendicular rod clamp. 1/4-20 x 5/16 in cup-point alloy steel hex socket set screws are used in the clamp and at the base of the V to tighten joints. The rods and ball joint are arranged so that the platform just fills the camera's field of view and so that the plane of the camera lens is parallel to that of the platform. Once set, the suspension need be checked only at the beginning of a photographic session. The platform and key components are shown in Figure 1.

Camera. We have used Nikon D70S (2006) and D80 (2007) cameras, both with a fixed lens. We strongly prefer the D80: the autofocusing algorithm is much faster and more robust; data collection and writing to the memory card are faster; the noise correction algorithm is substantially better; and the camera's resolution is much higher (10 MP). A 60mm f/2.8D AF micro-Nikkor lens (# 51214) is used with a UV filter (lately, a ProMaster digital). This

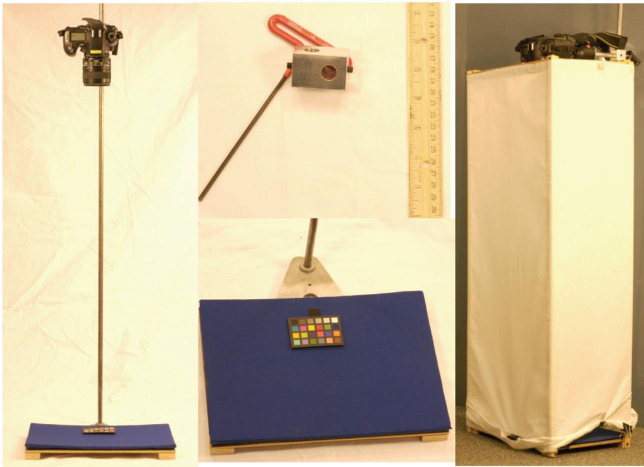

Figure 1. The imaging platform. The left panel shows the unshaded platform used in the greenhouse and for time-course photographs in the field. The middle panel shows the perpendicular clamp and its wrench (upper) and the platform bottom (lower). The right panel shows the shaded platform for collection of end-point photographs of detached leaves in the field (the "box kite"). (For full color, see p. 32.)

lens combines good field of view with depth of field, helping to project the waviness of the margins of the mature leaf into the photographic plane. Since we minimize the distance of the lens to the leaf, mature leaves must be shot in three sections, to be joined together digitally in the laboratory.

If the leaf is centered in the camera's field of view with the color checker close to it, we have found we can rely on the D80's autofocusing, but not on that of the D70S. We routinely check the focus prior to shooting each leaf, however. Data upload to the computer is via the camera's USB port.

All of our images are collected in RAW format at the highest possible resolution, and we under-expose the images by 2 - 3 "bars" on the camera's internal light meter. In the 2007 field season, the remaining parameters were ISO 200-400 to minimize noise; f8-11 for good depth of focus; and shutter speeds between 1/20-1/160. We adjusted the white balance periodically throughout the day using an ExpoDisk digital white balance filter, shooting the platform shade as a standard.

Illumination. All photographs to date have been taken using reflected light, except for a few experimental transmitted light ones. It is imperative that the leaf and platform be evenly illuminated, with no dark or light areas. In the field, we use ambient light exclusively: the waxiness of the leaves produces bright spots unless a flash is directed at the underside of a reflective umbrella, and in that case the quality of the illumination was not improved. In the 2006 field season, photography was confined to the morning (ca. 0800 - 1200) and late afternoon (1600 - 1900) to avoid excessively warm or cool light. In the 2007 season, we were able to shoot throughout the day provided we adjusted the white balance while the light color was rapidly changing.

In the field, even illumination is provided by carefully shading the platform. For time-course photographs, this has been done by using one to two photographic umbrella(s) to shade both the camera and the platform (2006). On bright, clear days this method works well, but it fails badly on overcast days: the light is reflected by the clouds so much so that it falls on the platform from all directions. In that case, our only recourse has been to surround the platform with people to block the light. We are now considering alternative shading devices for the 2008 field season (essentially, a lightweight beach umbrella with sides). For end-point images, we circumvented this problem by surrounding the platform with partially opaque drapery liner cloth (Roc-Lon rain no-stain # 184-9801), stapled to a wooden rectangular frame attached to the platform and open on its short sides to permit one to slide the leaves onto the platform (the "box kite"). The cloth hangs approximately 6 in below the platform, and the top of the kite, camera, and photographer's head are covered with a large piece of the same material. The wooden frame does not cast any shadows on the platform, even in very strong sunlight.

Since the greenhouse is designed to reflect light from every possible surface and the color of the light from the grow-lights is too orange (even with a yellow filter), we built a 68 x 60 x 115 in tent of drapery liner (more opaque than that used in the field, Roc-Lon special shade white # 796-2517). Sections of fabric were glued together with heat-sensitive fabric glue to avoid introduction of light through needle holes. The drape was tacked to the frame on three sides, with the fourth side suspended from a rod by shower curtain rings so that it opens easily to permit people and plants to move in and out of the tent. The lightweight tent is suspended from the greenhouse's structure (we use an electrical conduit) by a series of pulleys and is lowered away from the lights when not in use. Light is supplied by 4 to 6 150-200 W fluorescent bulbs that fit an incandescent socket (e.g., n:vision) in aluminum or white plastic clamp reflectors mounted inside the tent, either on light stands or a frame attached to a small sawhorse. The lights are aimed so that they cross-illuminate the leaf to prevent shadows.

Leaf Preparation. Several minutes before photography, leaves are cleaned by spraying them with water from a hand-held misting sprayer and wiping them dry with a soft cloth. The leaves are wiped from the culm to the tip, one side at a time. A heavy dew usually suffices to clean the leaves. Only the top surface is sprayed; the bottom surface is wiped with the damp cloth. Leaves are then left to air-dry before imaging.

To provide an external reference for joining leaf sections, in the 2007 field season we taped one to two fiducial marks to the underside of the leaf. These project 0.5-1 in perpendicular to the length of the leaf, on the side opposite the color checker, and are placed so that they evenly divide the leaf. The marks should be red: we used 2 in lengths of 22g insulated wire attached with 3M gloss finish multitask tape (# 34-8507-5365-7). With care, the same tape can be reused several times before replacing it.

While the box kite shades the platform extremely well, it prevents all members of the photographic team from seeing the leaf, making photography very slow and clumsy. For end-point photographs in 2007, we therefore cut the clean leaf from the culm using a small pair of bandage scissors less than a minute before it was to be photographed.

Photography. Photographs are collected by three to four person teams. In the field, the platform is either carried to the plant to image the leaves in situ (both time-course and end-point photographs, 2006), or cut and brought to a photography station set up between the rows (end-point, 2007). For in situ photography, one person holds the platform, one the leaf, one the umbrella, and the fourth keeps other plants out of the way and records the data.

Everyone sees the leaf on the platform and the field of view of the camera, and checks leaf alignment, lighting, and obstructions. The shutter is triggered by an infrared remote control. For ex situ photography, two folding lawn chairs are placed a platform's length apart; one person holds the leaf on the platform, resting the platform on his knees, and records the image number for each successful shot while the other photographs it. The photographer must direct the leaf-holder in aligning the leaf, since only he can see it. The camera's shutter button is used. The third person cleans and collects leaves and records data on the plant and leaf.

In the greenhouse, plants are placed on plant caddies and rolled to the tent for in situ photography. Short plants are brought completely into the tent, while tall plants are tipped in through the curtain. The platform is placed so that its angle is parallel to that of the plant. For short plants, it is simply held as in the field; for tall plants, it rests on a sawhorse at a constant position, marked by duct tape on the 1/2 in steel rod and on the sawhorse. The photographer stands on a box, if needed, and at a marked spot behind the sawhorse, so that the angle of the platform relative to the lights is constant.

For all images, the leaf is placed so that its long axis is parallel to the long axis of the platform; adjacent to the color checker; and with its tip at the leaf-holder's right hand. The leaf is gently flexed and unrolled so that it is as flat as possible over as much of its length as possible; the presence of fingers in the image is minimized. A leaf is photographed in as many sections as needed to cover its entire length (1-4), but always starting at the tip end and finishing at the culm. We make sure the fiducial marks are seen in the image. In in situ photography of the stem section, we place the leaf on the platform so that the latter is as close as possible to the stem without breaking the leaf. Focus and white balance are checked and changed as needed. A skilled team can photograph a leaf in approximately a minute, exclusive of travel of people or plants.

Data Collection and Quality Control. All plants are identified by a 15-character, 128-bit barcode that includes year, crop, family, row, and plant number. This barcode is scanned for each set of data recorded. Symbol Technologies' SPT 1800 barcode scanner running Portable Technologies Solution's TracerPlus/TracerPlusPro is used for the bulk of data collection. Occasionally paper forms pre-printed with barcodes are used for recording image numbers if the scanners are busy elsewhere. The data are later entered by scanning the forms or direct entry into a spreadsheet.

Several types of data relevant to the photographs are recorded: absolute and relative leaf numbers; plant identifiers; type of section; image number; photographer; and date and time. The absolute leaf number is determined as soon as possible in the season by tagging fifth, tenth, and fifteenth leaves (the fifth while the coleoptile is still visible). In the 2007 season we used 9 in paper-covered, white, red, and blue twist ties, respectively, to mark the different leaves, loosely encircling the stem above that leaf and twisting them closed. (Usually marking the fifteenth leaf is not necessary.) We have found marking the leaves with twist ties, rather than ink spots, very significantly speeds up data recording and improves quality control. Relative leaf number is counted with respect to the first ear leaf (e0) and the tassel. We record the relation between absolute and relative leaf numbers for each plant in mutant lines when we collect other anatomical data on the plant.

During photography, the plant barcode, absolute leaf number, section, and image number are recorded. The first two are scanned in prior to leaf collection and retained in the scanner so that they can be reused for other images of that leaf. The image numbers are entered once they have been shot and checked. For ex situ photography, the fastest method was to use two scanners per photographic team, so that a different scanner was associated with the current and next leaves. We check the images by viewing them on the camera's display after each leaf, and only record the numbers of images we intend to use. This lets us re-shoot any poor sections before we move away from the plant or discard the leaf. The other fields of the image menu (photographer, camera, light, etc.) are set to the session's defaults or taken directly from the scanner's internal clock (date, time; scanners are synchronized to within a minute). The photographic details are collected in the EXIF data the camera bundles with each image.

To help ensure quality, each member of the photographic team has specific responsibilities. The photographer is responsible for directing leaf positioning, focus, lighting, and image quality. The leaf-holder is responsible for placing fiducial marks, checking leaf cleanliness, and aligning the leaf on the platform. In ex situ photography, this person also records the section and image data. The leaf collector is also responsible for gathering the plant and leaf data; during in situ photography, this job falls to the plant- or umbrella-holder. The other team members monitor the data collection and photography processes and point out errors and confusions. During a photographic session, the images are periodically uploaded to a laptop computer and visually checked for quality by the team. We do this early in the session to confirm settings, and at 1-2 other times to monitor quality and team fatigue.

Scripts and methods for tag production

--Kazic, T

This note describes the methods we use to generate and manufacture a variety of barcoded tags and labels for use in the field and seed room.

Generation. A set of Perl scripts and modules that generate barcoded plant tags, seed packet labels, row tags, inventory labels, harvest tags, cross tags, and leaf emergence tags has been written. All scripts take a comma-delimited file dumped from a spreadsheet or database and are easily adapted for different tag layouts, barcode encodings, and other media. Several libraries have been written, including subroutines that lay out boxes on several different types of Avery labels to guide the placement of elements and others that typeset genotypes. The current versions of the scripts and sample input and output files are available at http://www.maizelesions.org/scripts.html.

The scripts rely on several freely available open source packages in addition to Perl: LaTeX, GNU barcode, and any PostScript or PDF viewer. Like Perl, these packages are available for any platform (Unix, MacIntosh, Windows, VMS, etc.), often as binaries, and their installation is straightforward (GNU barcode requires a simple compilation in C). Linux users running the KDE windowing environment and not wishing to edit Perl scripts may wish to try KBarcode (http://www.kbarcode.net/).

All scripts generate a 128-bit barcode to make it more robust to degradation. Each barcode is accompanied by an alphanumeric translation for people. For labelling plants, a 15-character barcode is used. Rows, seed packets, bags, sleeves, and boxes are labelled with a 6-character barcode; the first character is a prefix denoting the type of object (r, p, a, v, and x, respectively). Depending on the use of the tag or label, successive tags are printed vertically or horizontally; the vertical arrangement lets a stack of tags fit in an apron pocket. Each stack is labelled with a number to help keep the tags in order.

Plant Tags. We use an 8-up 8.5 x 14 in perforated sheet of tags to label plants, available from the University of Missouri print shop. These have three sections: a long section that is wrapped around the stem, pulled through a hole in the tag, and twisted to lock the tag in place; and two smaller tear-off tags. Barcodes, rowplant identifiers, crop, and genotypes are repeatedly printed on the three sections.

We use the tear-off tags during pollinations to label the bags with female and male parents; in the seed room these are removed from the pollination bag and stapled together to form the harvest tag. (A simple staple remover can be built by trimming a flexible, soft-handled putty knife with tin snips to form a tongue just narrower than the staple; a file is used to bevel the front of the tongue and dress the other edges.) Though these labels are quite robust to the weather, some barcodes do degrade to illegibility to the scanner or eye and must be replaced before constructing the composite harvest tag in the seed room. A separate script prints the equivalent of the tear-off, cross tags, together with the maternal numerical genotype, on 8.5 x 11 in card stock in 30-up layout.

While the current design is very useful, our experience this past summer has suggested several improvements. The most important is to increase the robustness of the design to errors in feeding the tag stock through the printer. If the sheets are not laid snugly in the tray, tear-off tag barcodes can be clipped by the perforations; thus, quality output depends on vigilant monitoring of the printing. I am currently modifying the layout to improve robustness, to enable all the redundant barcodes on the long section to function as tear-off tags, and to improve the abbreviation of genotypes on the tag. The modified script should be available this winter and a notice will be posted on the web page.

Seed Packet Labels. The script prints the packet barcode, numerical genotype, row number, sleeve number of the source ear, number of kernels, and row length on Avery 30-up 1 x 2 5/8 in labels (# 5160).

Row Tags. These have the row number, crop, investigator name and address, field, and family. In the 2006 season we printed these on the 2 x 4 in labels and mounted the labels on wire-threaded shipping tags. For the 2007 season we printed these directly on card stock. I may modify this to include the row's barcode for harvest management; if so, the revised script and a notice will be posted to the web page.

Harvest Tags. These have the maternal and paternal barcodes, the numerical and abbreviated symbolic genotypes of the parents, ear number, and the date of pollination. For the 2006 crop we printed the tags on Avery 10-up 2 x 3.5 in (# 5871) business card stock; we now print these on card stock. A subroutine to print lines to guide the cutting is available for this label arrangement; if useful it will be extended to other tags printed on card stock.

Inventory Labels. Barcodes to identify bags and sleeves for seed and ear storage are printed on the small 30-up labels. The bag labels are affixed to a shipping tag or a leaf emergence tag, and the sleeve labels are pasted directly on the inside back of the sleeve. During inventory, the harvest tag and the bag or sleeve label are scanned. The box labels are printed in pairs on Avery 20-up 1 x 4 in (# 5161) labels. The left label gives the investigator's surname, crop, and box number. The right label has room for a description and the box's barcode. During inventory, each bag or sleeve is scanned with its box. Thus, each ear or packet is located to its sleeve and box.

Leaf Emergence Tags. To help monitor the emergence of leaves, we printed the plant's barcode, row-plant identifier, and a table of leaf numbers on the small 30-up labels. The table has space to write the date the leaf emerged; the observer only scans the tag when a new leaf appears in the whorl. The labels were affixed to our manufactured leaf emergence tags. These tags can also serve as pollination tags in a pinch.

Manufacture. In organizing our manufacture of tags, we balance ease of printing, the number of steps in construction, and cost. For example, we could print the labels directly on the seed packets, rather than on labels that must then be transferred to the packets. However, feeding packets through the printer is slower and more tedious than sheets of labels. Whenever feasible, we print the data directly on 54 lb card stock, then drill and cut the stock. This stock is the heaviest that Hewlett-Packard laser printers are designed to feed. We use a straight-through paper path to minimize wear; periodically refilling the paper tray helps pace the printing so that the printer can cool. This path also lets us avoid punching the chad from the plant tag sheets before printing. Harvest and replacement tear-off tags are simply cut; row and leaf emergence tags must first be drilled to accommodate a twist tie and cut apart.

To drill holes, a cardboard or thin plywood template is prepared and the stack of printed stock clamped tightly between it and another piece of wood. A long 1/4 in augur bit in an electric drill is used. For cutting, a backsaw, knife, or paper cutter can be used; sawing requires the stock be tightly clamped on both sides of the saw, but is fastest if the number of sheets is large. Drilling and cutting can also be performed at many office supply stores on relatively short notice.

We use twist ties to attach tags to plants or mesh bags. The leaf emergence tags have two holes approximately an inch apart for reinforcement; the tag is folded between them and the twist tie threaded through and tightly twisted. The label is affixed; tags for each row are threaded onto another twist tie; and the bundles are hung from a coat hanger in row sequence for transport to the field. In our summer nursery we only attach row tags to mesh bags at harvest; tags for rows without pollinations are set aside for monitoring the completeness of the harvest.

I am grateful to Georgia Davis and Arturo Garcia for introducing me to the idea of barcoding plants; Doug Davis and Theresa Musket for helping us with the commercial package for the plant tags our first year; Susan Melia-Hancock for suggesting row tags; and Ed Coe for suggesting barcoding the rows.

ITHACA, NEW YORK
Cornell University

Centennial Celebration: Cornell's Department of Plant Breeding, 1907-2006
--Kass, LB; Murphy, RP

The Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY, celebrated its centennial on July 26-28, 2007. Many students who studied the genetics and breeding of maize, small grains, perennial forages and vegetable crops, reunited at Cornell with their former student colleagues and professors to present or attend talks at the day and a half symposium in honor of the Department's 100th anniversary (Mark Sorrells, Chair, Department of Plant Breeding and Genetics, presiding).

The program featured a historical perspective on the "Evolution of Plant Breeding at Cornell" by R. P. Murphy, former Chair of the Department (1953-1964), in addition to talks by graduate student alumnae and alumni. The symposium was videotaped and soon will be edited and available for viewing.

Norman Borlaug, winner of the Nobel Peace Prize (1970), unfortunately could not present the keynote address, scheduled for the first day of the symposium. Still, we were all proud that earlier this month, on July 17, 2007, Borlaug, in his 93rd year, was awarded the Congressional Gold Medal. Murphy, the second speaker on the program and Borlaug's University of Minnesota graduate school colleague of the same age, filled the gap with his first PowerPoint presentation, after which the audience of more than 250 strong gave him a standing ovation.

Murphy and reunion participants fondly recalled the speakers for the Department's 75th anniversary symposium on the "Golden Age of Corn Genetics" (Figure 1). All had been trained at Cornell in maize genetics or cytogenetics (see Rhoades, Annu. Rev. Genet. 18:1-29, 1984).

Figure 1. 1982, Synapsis, 75th Plant Breeding Department reunion, speakers for the "Golden Age of Corn Genetics Symposium". Left to Right: Marcus M. Rhoades, Charles R. Burnham, Barbara McClintock, Harriet B. Creighton, George F. Sprague, Harold S. Perry. (Courtesy Department of Plant Breeding, Cornell University)

The afternoon of July 27 featured lab and field tours of the Department's facilities. One of the highlights was a field trip to the Emerson Garden, where Margaret Smith, Denise Costich and Owen Hoekenga had planted a Living Map of Mutants of Maize (Figure 2). This was an updated reproduction of the living chromosome map, which had been planted by Marcus Rhoades for the 1932, 6th International Congress of Genetics held in Ithaca, the year of the Department's 25th anniversary celebration.

Figure 2. Plant Breeding Centennial reunion attendees visit the Living Map of Mutants of Maize, July 27, 2007, Cornell University, Emerson Garden, Cornell Plantations. (Photo by Denise Costich.) (For full color, see p. 32.)

Many participants attended the Department picnic at Stewart Park, the evening of July 27, where they made their own ice cream sundaes, a treat invented in Ithaca, and gave recollections of times past at the "Open Mic." Tours of the Geneva Experiment Station and other local attractions were scheduled for the following day, Saturday, July 28.

All attendees received the recently published Evolution of Plant Breeding at Cornell University, A Centennial History, 1907-2006, by Royse P. Murphy in cooperation with Lee B. Kass (Figure 3). The story begins at the turn of the 20th century with the exciting applications of the new science of genetics to plant breeding and continues with R. A. Emerson and students' contributions to the field. Chairs of the Department also contributed memoirs to the centennial history volume. The book includes a photo section of over 65 photographs and snap shots of the Department and the Synapsis Club, beginning in 1907 through 2006. A copy of the centennial history can be ordered from the Department of Plant Breeding and Genetics office (Cynda Farnham, clf4@cornell.edu, phone: 607-255-2180) at $15 per copy.

EVOLUTION of PLANT BREEDING
at CORNELL UNIVERSITY

Spring of 1909

A CENTENNIAL HISTORY
1907-2006

Figure 3. Evolution of Plant Breeding at Cornell University: A Centennial History, 1907-2006 (Murphy & Kass 2007).

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Jala maize - giant?
--Karl, JR

Jala is a race of maize that has the longest cobs (Collins, J. Hered. 9:147-154, 1918). It is not the tallest (Karl, unpubl.). Jala is topically from the town of Jala in the Jala Valley of the Ceboruco volcano in the southeast of the state of Nayarit in Mexico (Kempton, J. Hered. 15:337-344, 1924). Phylogenic evaluation suggests that the race has only been around for recent centuries (Duncan, Crop Sci., 1968). Early publication regarding the race specified the plants to be "twenty feet or more" in height and ears "three feet in length" (Mangelsdorf, Texas Ag. Exp. Sta. 574:1-315, 1939). There has never been a cob documented that has exceeded the length, 24 inches, of those in the field of the town at that time. Multiple races have been grown there (Weatherwax, MNL 22:22-23, 1948). The race with the giant morphology was named by the scientific community after the town, but was known locally as Maíz de Humedo (Listman, Diversity 9:34-36, 1993). The cobs only reached this length indigenously, 15" otherwise (Kempton, J. Agric. Res. 29:311-312). After another race, Tampiqueño, began to be planted in the town before 1947, the cob length diminished to 15". A decade best has been 18". The tallest segregation group (exotically) has been 27' (Figure 1), the tallest individual plant has been at least 28.5'.

Figure 1. 29' stalk displayed at Canadian Royal Winter Fair, November 1999, right of center. 27.5' Jala stalk, left. (For full color, see p. 32.)

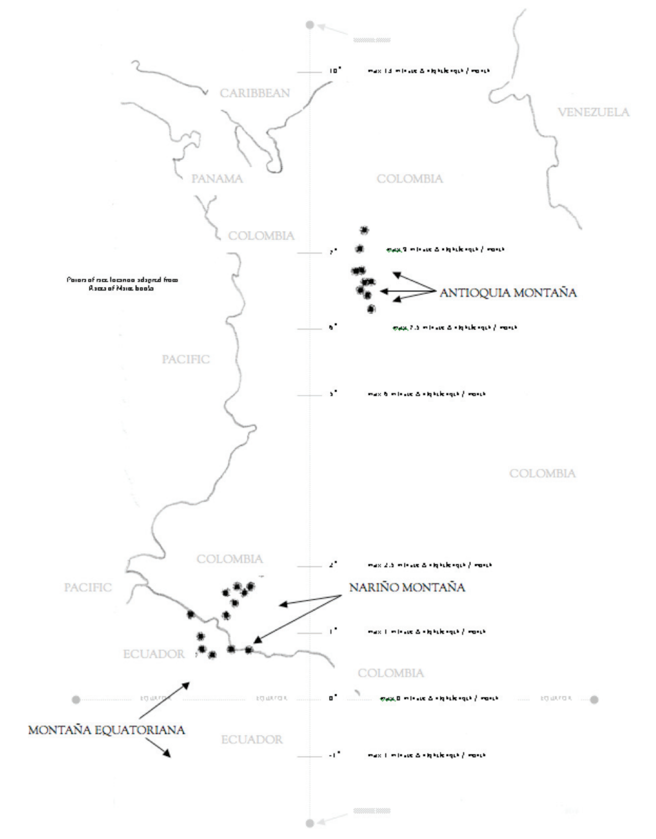
Getting seed from the maize race Montaña
--Karl, JR

Montaña may not make seed when grown under decreasing night length.

The tallest maize types make fine seed during the winter in Florida with the exception of Montaña, an equatorial maize race. When grown in a December planting when the night length was decreasing, similar to a June planting in the southern hemisphere, Montaña made no seed. However, when planted in late September, the race makes some seed, presumably because the night length is increasing from the 12-hour autumnal equinox toward the 13.5 hours of the winter solstice.

A similar phenomenon was described in the tallest teosinte, Huehuetenango (Modena, MNL 57:38, 1983). Huehuetenango and Montaña are peerless in this sensitivity. Huehuetenango has neither male nor female flowers, while Montaña tassels but does not make ear shoots or protrude silk. It appears that Huehuetenango has an obligate, increasing night length and long-night requirement for flowering, and that Montaña has this nature just for the ears, while the tassel has the basic, photoperiodic-tropical-maize, facultative long-night nature.

Most of the Montaña race derived in a location (see accompanying map, zoomable online) that is within one or two degrees (Maps.com, http://i.infoplease.com/images/mcolombia.gif, 2007) (namely as much as 1.75˚, 122 miles) of the equator, where the maize crops develop (2 months*) under night length that does not change by more than (at 2˚) 5 minutes (Baker and Baker, http://www.orchidculture.com/COD/daylength.html#5N) (relatively



2.5 min per degree latitude, 15 min "annually" at 7.5 min each degree). The reproductive problem may be focused in this geographic majority of the race because of the negligible night length change there, which entailed negligible selection pressure against night length sensitivity. The discrete Antioquian (6-7˚ latitude) group of Montaña, in the northern half of Colombia, experiences a night length change of less than 18 minutes.

Photoperiodicity seems to be primitive, and shed by maize as it was carried by the human pole-ward. Selection pressure against photoperiodicity decreased in the southbound efflux of maize from (its creation at) the Balsas river to the equator, permitting this stretch of geography to be the semblance of a tallest maize corridor. Balsas latitude American maize had selection pressure to silk despite night length change; this pressure never existed against equatorial Montaña. That makes it a first generational selection against Montaña when it is grown under long nights that are decreasing in length.

Artificial long nights of unchanging length make good seed on equatorial Montaña in New York. The required duration of long-night treatment, of course, is likely between 11-20 days (Emerson, J. Hered. 15:41-48, 1924), beginning at the stage of 5 visible leaves (Tollenaar and Hunter, Crop Sci. 23:457-460, 1983)--when the sensitivity to night length has begun in maize--and lasting until the plants are less than 1.5', 12 leaves. 2 layers of 4-mil black plastic works, drawn over a 2 - 3', 2x2"/1x4" wood frame.

constant night length, long night → seed

12-hr night September to 13.5-hr December → seed

13.5-hr December to 12-hr March → no seed

*First 5 leaves in 12 days (unpublished data) (heterotic maize); + 11-20 days for initiation, @ 1 leaf per 3 days; = 12 leaves and 32 days; + days from tassel initiation to ear initiation (Struik, Breeding Strategies for Maize Production and Improvement in the Tropics, 1982); 2 months is safe parameter.

Thanks to M. Goodman's warm generosity in sharing that his Montaña made seed in a September 7th planting in Homestead. This inspired the idea for a resolution of the problem.

KEW, UNITED KINGDOM
Royal Botanic Gardens, Kew
SAINT PAUL, MINNESOTA
University of Minnesota and USDA-ARS
MÜNSTER, GERMANY
Westphalian Wilhelms University Münster

Maternal and paternal transmission to offspring of B chromosomes of *Zea mays* L. in the alien genetic background of *Avena sativa* L.

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

B chromosomes (Bs) are supernumerary dispensable chromosomes with highly host-specific organization, behavior and mode of inheritance described in hundreds of animal, fungal and plant species. We transferred native Bs of maize (*Zea mays* L. ssp. *mays* cv. Black Mexican Sweet) to oats (*Avena sativa* L. ssp. *sativa* cv. Starter) (Kynast et al., MNL 81:16, 2007) since native Bs of oats have not been reported to exist in wild and cultivated populations of hexaploid oat species. However, native Bs of maize belong to the first-discovered (Kuwada, Bot. Mag. Tokyo 39:227-234, 1925), and presumably molecularly and cytogenetically best-described (Jones and Diez, The B chromosome database, http://www.bchromosomes.org/bdb/, 2004), Bs in the plant realm. Among their extraordinary features of structure and function, native Bs of maize are capable of prevailing in populations by balancing selfish drive and counteracting factors which are genetically controlled by different genes/factors that have been assigned to the Bs themselves, as well as to the host genome. We address the question in our research objectives: How will a native B of maize behave after being converted into an alien B by transferring it into hexaploid oats – a very remotely related species that has not been exposed to a native B during its entire evolution?

Hybridization experiments of the three common oat cultivars Starter, Sun II and Paul (2n = 6x = 42, *Avena sativa* L. ssp. *sativa*) by the maize line $B73^B$ – a dent corn inbred B73 derivative that carries six Bs of the sweet corn cultivar Black Mexican Sweet ($2n = 2x + 6_B = 26$) generously provided by J. A. Birchler, University of Missouri-Columbia – generated 14 F1-plants with complete sets of 21 oat chromosomes and different numbers of individual maize chromosomes, resulting from incomplete uniparental genome loss (UGL) during early stages of the F1-plants' embryogeneses. The retained maize chromosomes were found in shoot tissues based on PCR results for *Grande-1*, a dispersed LTR-type retrotransposon, which is abundant on all A-chromosomes (As) and Bs of maize but absent from all chromosomes of the three oat genotypes used in our crossing program. Two of these 14 F1-plants (5811_1 and 5845_1) proved to carry maize Bs in shoot and root tissues. 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) 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 plants the *Grande-1*-positive PCR products resulted from the presence of maize Bs and the absence of maize As (Figure 1). Cytological analyses by the use of fluorophore-labeled genomic DNA of maize in GISH assays on primary root meristems of very young, juvenile plantlets revealed that in the F1-plant 5811_1 all ten maize As were eliminated, and three maize Bs were retained along with the complete set of 21 oat chromosomes ($2n = 3x+3_B = 24$). In the primary root meristem of the F1-plant 5845_1 all ten maize As were eliminated, and a single maize B was retained along with the complete set of 21 oat chromosomes ($2n = 3x+1_B = 22$).

Self-pollination of the F1-plants 5811_1 and 5845_1 has produced up to this point, a total of 132 F2-seeds in both genotypes due to frequent formation of unreduced female and male gametes (Table 1). Partial fertility had already been observed in haploids of Starter, Sun II and Paul oats without (Rines et al., In: Jain, Sopory, Veilleux (eds) Kluwer Acad Publishers, Dordrecht, The Netherlands, In vitro haploid production in higher plants 4, pp. 205-221, 1997) and with (Kynast et al., PNAS 101:9921-9926, 2004) the addition of individual As of B73 maize. Cytological and molecular analyses of 30 F2-offspring plants showed that the F1-plant 5811_1 – carrying three Bs – produced six F2-plants each without Bs (2n = 6x = 42), three F2-plants each with one B ($2n = 6x + 1_B = 43$), nine F2-plants each with two Bs ($2n = 6x + 2_B = 44$), one F2-

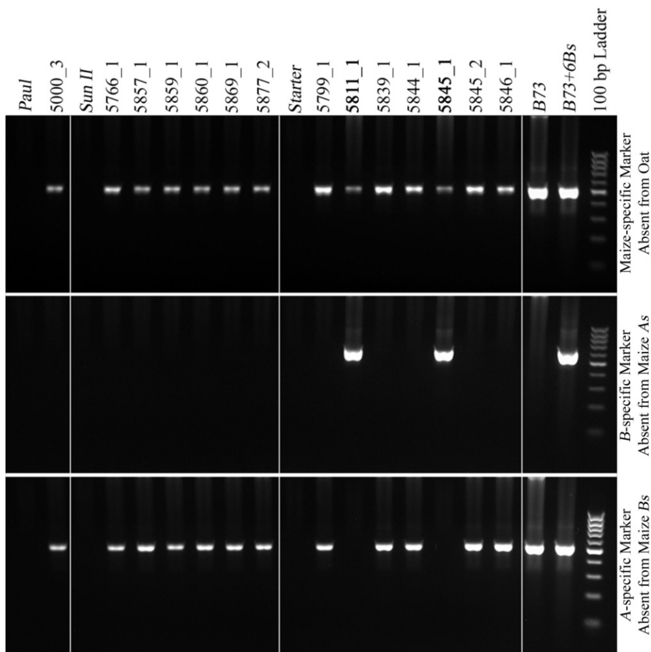


Figure 1. PCR products from genomic DNA of three oat plants, 14 F1 (oat × maize) plants, one maize plant without B, and one maize plant with six Bs by using a maize-specific, a B-specific and an *A*-specific marker; electrophoresis in 1.5% agarose

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
Oat panicles	40	53	3
Oat florets, emasculated and hand-pollinated	1177	1094	70
F1-proembryos, *in vitro* rescued 14-15 days after pollination	62	52	1
F_1-embryos, geminated*	14	16	1
Maize (A and/or B)-positive juvenile F1-plantlets (shoot- and root-tested)	7	6	1
Maize B-positive adult F1-plants (shoot- and root-tested)	2	0	0
F1 5811_1** (tiller-tested)	1	n/a	n/a
F1 5845_1** (tiller-tested)	1	n/a	n/a
Total F2-offspring of F1 5811_1, harvested to date	59	n/a	n/a
Total F2-offspring of F1 5845_1, harvested to date	73	n/a	n/a
Maize B-positive / Tested F2-offspring of F1 5811_1 (shoot- and root-tested)	24 / 30	n/a	n/a
Maize B-positive / Tested F2-offspring of F1 5845_1 (shoot- and root-tested)	0 / 30	n/a	n/a

*Embryos that formed shoot and root with enough tissue for molecular and cytogenetic analyses; **Plants represent clonal tillers from two clones after extensive tiller cloning allowing for more F2-seed production

plant with three Bs (2n = 6x + 3$_B$ = 45), two F2-plants each with four Bs (2n = 6x + 4$_B$ = 46), and nine F2-plants with highly chimeric root meristems showing cells with one to five Bs (2n = 6x + 1$_B$ … 5$_B$ = 43 … 47) in different frequencies (Figure 2). In contrast, none of the 30 F2-offspring of the F1-plant 5845_1 – carrying one B – had Bs based on the results of cytological and molecular tests (Table 1). Taking all data of the F1 and F2 analyses together, our results show that (1) maize Bs can be added to the complete haploid genome of oats via inter-species (oat × maize) hybridization and successive incomplete UGL, (2) haploid oat plants hosting one or three maize Bs are partially fertile, mainly because of frequent formation of unreduced gametes of both sexes, and (3) maize Bs can be transmitted to F2-offspring, which has been observed as being doubled haploid (=hexaploid) oat plants without and with the addition of one to four Bs, and occasionally up to five Bs in chimeric root meristems.

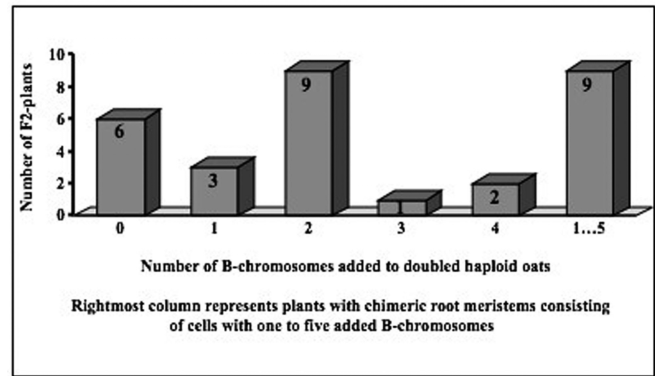


Figure 2. Numbers of F2-plants without and with an added maize B chromosome.

However, the transmission of added maize Bs from haploid F1-oats to doubled haploid (=hexaploid) F2-oats is very special due to two particularities: Firstly, the frequency of transmission from F1 to F2 does not correlate with the frequency of retention during the incomplete UGL process in the primary inter-species hybrid. Secondly, the transmission from F1 to F2 does not necessarily reflect alien B transmission in general due to the meiotic restitution process leading to unreduced gametes and doubled haploid F2-plants. Therefore, transmission rates from F1 to F2 apparently do not allow transmission rates being predicted for successive offspring generations. Thus, transmission of maize Bs added to oats was analyzed also in F3- and BC1-offspring. Investigations of F3-offspring of three selected F2-plants with one, two, and three added maize Bs to their oat genomes showed that the alien Bs neither became immediately eliminated from the oat genome nor became excessively accumulated in the oat genome (Table 2). The frequencies of B transmission from F2 to F3 did not correspond with Mendelian expectation values for chromosome segregation common for As in monosomic, disomic, and trisomic condition with regular meiotic behavior. For instance, the offspring of the trisomic B addition F2 1188_20 generated only monosomic and disomic B additions indicating a tendency of B loss. However, the F3-offspring 1390_2 of the disomic B addition F2 1188_19 "gained" one B by becoming a trisomic B addition. This accumulation certainly indicates irregular transmission conditions. In order to characterize B transmission in more detail, we backcrossed F3-plants with monosomic and disomic B addition (male parent) to Starter oat (female parent). Both offspring populations showed successful B transmission. Eight tested BC1-offspring descending from the monosomic B addition accounted for two euploid oat

Table 2. Chromosome numbers of F3-offspring plants descended from three F2-plants with one, two, and three added B chromosomes.

F2-Genotype	2n =	F3-Offspring	2n =
1188_21	6x + 1$_B$ = 43	1392_1	6x + 0$_B$ = 42
		1392_2	6x + 0$_B$ = 42
		1392_3	6x + 0$_B$ = 42
		1392_4	6x + 1$_B$ = 43
1188_19	6x + 2$_B$ = 44	1390_1	6x + 2$_B$ = 44
		1390_2	6x + 3$_B$ = 45
		1390_3	6x + 1$_B$ = 43
		1390_4	6x + 1$_B$ = 43
1188_20	6x + 3$_B$ = 45	1391_1	6x + 1$_B$ = 43
		1391_2	6x + 0$_B$ = 42
		1391_3	6x + 2$_B$ = 44
		1391_4	6x + 2$_B$ = 44

plants, three monosomic and three disomic B addition plants. Besides the herewith proven paternal transmission of maize Bs in an oat background, the 3/8 frequency of BC1-genotypes with an increased number of Bs demonstrates the competitive strength of male oat gametes hosting maize Bs and a tendency to prevail similar to the situation in maize – the native host species. Among eight tested BC1-offspring descending from the disomic B addition, three plants were euploids, four plants were monosomic B additions, and one plant was a tetrasomic B addition. B accumulation took place, although at a low 1/8 frequency. In order to test for maternal maize B transmission in the oat background, we backcrossed F2-plants with a disomic B addition (female parent) by Starter oat (male parent). Among six tested BC1-offspring, three plants were euploid oats and three plants had monosomic B additions. These data prove that maize Bs can be maternally transmitted in oats. However, the limited number of offspring tested to date does not show whether female sporogenesis and/or megagametogenesis may also accumulate maize Bs in an oat background. Thus, further offspring genotypes are being characterized. Analyses of meiosis and gametogenesis are in progress.

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Tomato and *Arabidopsis* plants overexpressing the *Ramosa1* maize gene show cell expansion

--Cassani, E; Landoni, M; Pedretti, G; Pilu, R

With the aim of studying the possible application of the *Ramosa1* gene as a modifier of inflorescence and branching architecture in plants, the coding region of the maize gene *Ramosa1* was cloned under the control of the (CaMV) 35S promoter and the resulting construct containing the expression cassette *35S::Ra1* was used to transform *Arabidopsis* and tomato plants.

Transformed plants showing the most severe phenotype were characterized by a dramatic reduction of inflorescence elongation and a bushy appearance, i.e. the leaves were larger than wild type and often swollen and curled (Figure 1 A and B). In *Arabidopsis*, floral organs such as sepals, petals, stamens, pistils, seeds and pollens were bigger compared to wild type. These data indicate that the effects of *Ra1* overexpression act in every tissue of plants, even in the gametophyte. The effects of the *Ra1* ectopic expression were easily detectable even in seedlings because cotyledons and leaves are bigger than those of the control.

To investigate at the cellular level whether the increased size of the leaves and flower organs is a consequence of enhanced cell expansion or of enhanced cell division, we analyzed leaves and petals from normal and from *Ra1* overexpressing plants using differential interference contrast imaging microscopy. Apparently only the size, but not the shape, is abnormal in transgenic plants, as observed in the morphology of transgenic leaves and flowers studied at the whole organ level.

Epidermal cells of tomato (Figure 1D) and *Arabidopsis* (Figure 1F) transgenic leaves are bigger than those of the wild type (Figure1 C tomato and Figure 1E *Arabidopsis*) while still maintaining the correct organization inside the tissues. A good correlation between increased organ size and increased cell size recorded in the transgenic plants for primary root diameter and petal length confirms that the increase in organ size is caused primarily by the cells being larger (data not shown).

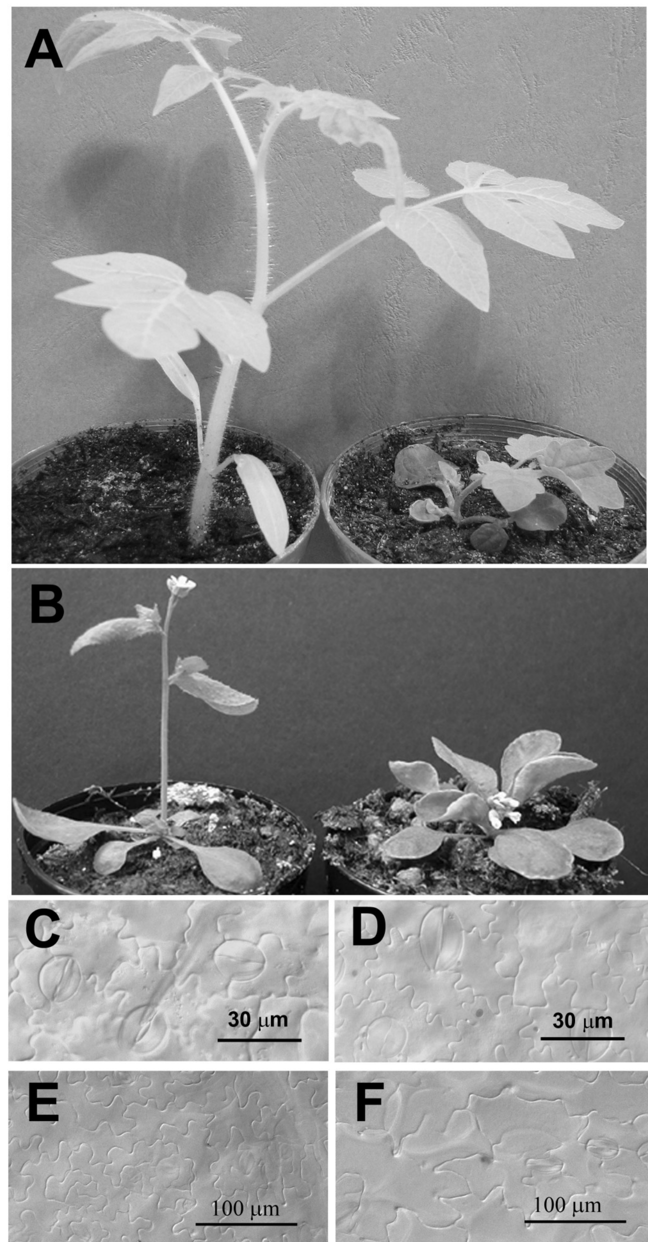

Figure 1. Phenotype of transgenic tomato (A) and *Arabidopsis* (B) plants overexpressing the *Ra1* gene (right) and control (left). Interference contrast photographs of wild type epidermis cells of tomato (C), *Arabidopsis* (E) and transgenic plants overexpressing the *Ra1* gene (tomato, D; *Arabidopsis*, F).

These results suggest that the RA1 protein is able to upregulate cell expansion in all organs of *Arabidopsis* and tomato plants. Further studies will be necessary to understand the interaction between the *Ra1* exogenous gene and the final target genes in *Arabidopsis* and tomato plants, and the behaviour of the *Ra1* gene when overexpressed in other plant species.

First evidence of non-Mendelian inheritance of the *lpa1* trait in maize

--Pilu, R; Panzeri, D; Cassani, E; Landoni, M

In maize, three loci have been isolated so far which are involved in phytic acid biosynthesis: *lpa1*, *lpa2* and *lpa3.* These low phytic acid (*lpa*) mutants produce seeds in which the chemistry of seed P, but not the total amount of P, is greatly altered. In 2007, a gene tagging experiment performed by Shi et al. found that the *ZmMRP4* (multidrug resistance-associated proteins 4) gene mutation causes the *lpa1* phenotype.

In our lab, we have isolated and described a single recessive *lpa* mutation (MNL 76:46) which was allelic to the *lpa1-1* mutant, and was consequently renamed *lpa1-241*. In order to quickly follow the *lpa1* trait segregation, we scored for the free inorganic phosphate seed content using Chen's assay performed in microtitre (Chen et al., 1956; Raboy et al., 2000; Pilu et al., 2003). We defined four phenotypic classes corresponding to the level of free inorganic phosphate in the seed, expressed as mg of atomic P per g of flour. These classes, wild type (0–0.3), weak (0.3–0.5), intermediate (0.5–1.4), and strong (>1.4), are easily scored by visual inspection if the assays are performed in microtiter. Originally, the mutant phenotype has been observed in a selfed *ACR* family, where the "strong" class segregated 1:3 as expected for a recessive mutation. In this progeny, heterozygotes showed a weak phenotype although crosses to wild type *ACR* plants resulted in 100% wild type progeny. With the aim of better understanding this behavior, we crossed heterozygous *ACR Lpa1/lpa1-241* families with wild type plants from the B73 inbred line and as expected, only the wild type phenotype was detected in F1. Selfing heterozygous *Lpa1-B73/lpa1-241ACR* plants we obtained a segregating F2 generation in which the phenotype classes were similar to those obtained in segregant F2 *ACR* families. However, in the following generation we observed a general decrease in the number of wild type and weak classes in F3 families, associated with a general increase of the intermediate and strong classes. The segregation data regarding F2, F3 and F4 families showed a consistent increase of the strong/intermediate phenotypes that was correlated to the number of selfings (Figure 1A). This non-Mendelian segregation could be explained with a progressive *Lpa1* allele silencing occurring in *lpa1-241* families over subsequent generations.

To support these data we crossed several heterozygous plants of subsequent generation *Lpa1'B73/lpa1-241ACR*, *Lpa1''B73/lpa1-241ACR* and *Lpa1'''B73/lpa1-241ACR* (*Lpa1* alleles have an apostrophe when exposed in trans to the *lpa1-241* allele for one generation, after two generations of exposure to the paramutagenic allele we give two apostrophes and so on) to homozygous stable *lpa1-1* lines. In these crosses we expected a segregation ratio of 1:1 for the *lpa1* phenotype as expected for a backcross of a monogenic recessive mutation. But even in this case, phenotype segregation of the progeny showed a decrease in the wild type and weak classes, and a correlated increase of intermediate and strong classes associated with progressive exposure of the *Lpa1* allele to the paramutagenic *lpa1-241* allele (Figure 1B).

Genetic and phenotypic data collected are compatible with a gene silencing phenomenon which seems somewhat similar to the *r1* locus paramutation (Brink, 1956). Further genetic and molecular characterization will be needed to better understanding this non-Mendelian inheritance of the *lpa1* trait.

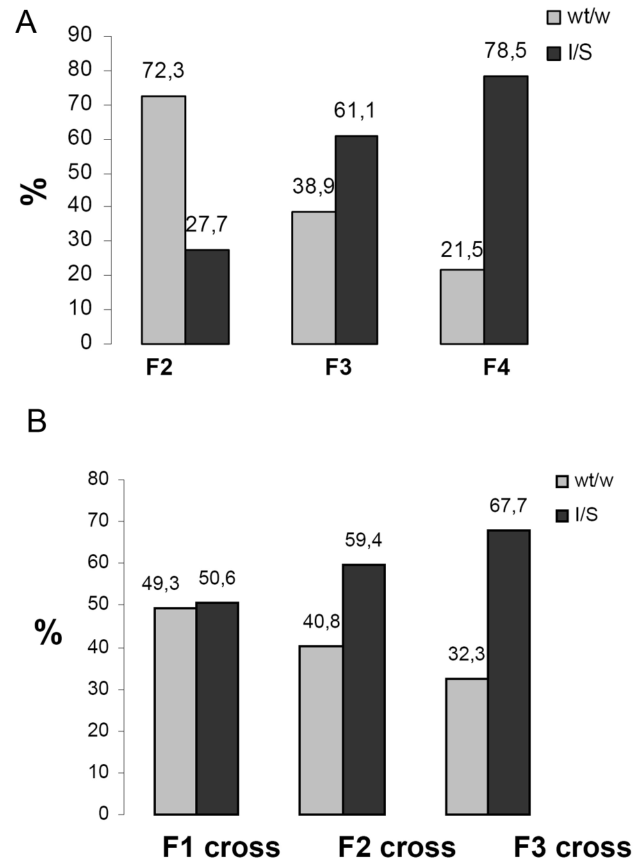

Figure 1. Changes in phenotypic class segregation ratios over generations in the presence of the *lpa1-241* allele. (A) Seed free phosphate phenotypic classes are presented in the histogram as a percentage of occurrence in selfed heterozygous progenies, where F2 refers to *Lpa1'/lpa1-241* selfed progeny, F3 refers to *Lpa1''/lpa1-241* selfed progeny and F4 refers to *Lpa1'''/lpa1-241* selfed progeny. (B) Histogram representation of phenotypic classes segregation ratios in the progenies of the same families crossed with homozygous *lpa1-1*: F1 cross refers to *Lpa1'/lpa1-241* x *lpa1-1/lpa1-1* progeny, F2 cross refers to *Lpa1''/lpa1-241* x *lpa1-1/lpa1-1* progeny, and F3 cross refers to *Lpa1'''/lpa1-241* x *lpa1-1/lpa1-1* progeny. The sums of wild type and weak (wt/w) phenotype occurrence (gray bars) versus intermediate plus strong (I/S) phenotype occurrence (black bars) are shown.

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Progress in transformation and regeneration of tropical inbred maize lines in Kenya

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Tropical inbred maize lines have a reputation of being difficult to transform, mainly as a result of their inherent limitations associated with resistance to *Agrobacterium* infection and their recalcitrance to in vitro regeneration. To enhance the capacity for public sector maize transformation, the Plant Transformation Facility at Kenyatta University, Kenya, embarked on a program to improve

transformation of diverse tropical inbred maize lines using *Agrobacterium tumefaciens*. We evaluated both N6 (Frame et al., Plant Physiol. 129:13-22, 2002) versus LS (Negrotto et al., Plant Cell Rep.19:798-803, 2000) media with different hormone regimes and optimized transformation and regeneration protocol for tropical inbred maize lines. Using immature embryos as explants, four Kenyan tropical inbred lines TL21, TL22, TL23 and TL18; two Sudanese inbred lines IL1, IL2 and CIMMYT inbred lines CML 216 and CML 244 have been investigated for their tenability to transformation and regeneration. Transformation frequencies (callus resistant events over total explants) and efficiencies (plantlet regenerating events over total explants) for the recovered events were used to evaluate successful transformation (Figures 1 and 2).

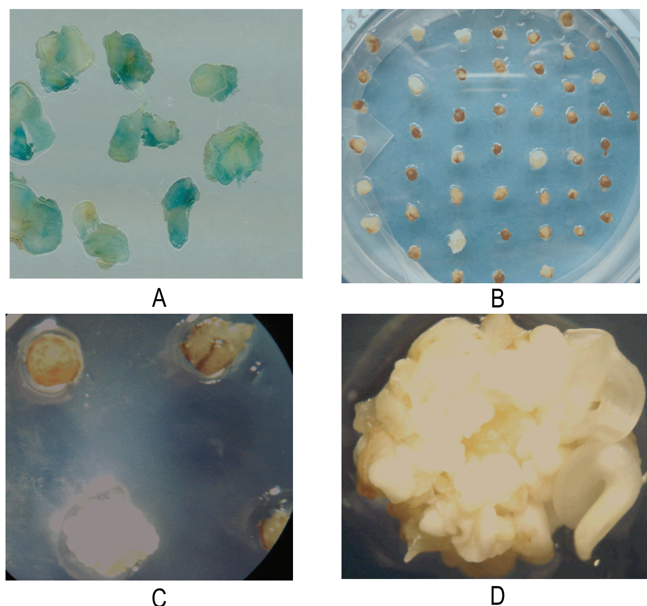

Figure 1. Selection and regeneration of transformed tropical maize inbred lines. A: Positive *Gus* staining calli after four weeks on selection II. The immature embryos were transformed with EHA101 *Agrobacterium* strain harboring pTF102 vector containing the p35S-GUS gene, described in Frame et al. (2002). B, C and D: Dying and surviving calli after four weeks on selection II medium (3mg/L Bialaphos). (For full color, see p. 33.)

Transformation frequency as high as 70% was recorded, transformation efficiency was lower and ranged between 20-30% for all the genotypes. There were no regenerants from non-transformed control plates.

The success in transformation and regeneration has led to the application of approaches to reduce dehydration stress in tropical inbred lines. A novel artificial miRNA (amiRNA) approach, as an alternative to RNAi, has been used to negatively regulate the ZmPARP1 gene in tropical maize genotypes, thereby enhancing energy use efficiency in the transgenic lines. As an adaptive mechanism to regulate growth in response to drought stress by integrating stimuli to alter transcriptional activity, one of the Elongator components has been overexpressed in tropical maize and is being tested for its ability to increase stress tolerance.

The same maize inbred lines are being considered for genetic upgrading to combat maize streak virus (MSV) disease. Several peptide aptamers have been isolated that specifically interact with viral replication proteins using yeast two hybrid assays. The best interacting peptide aptamer candidates are being evaluated in

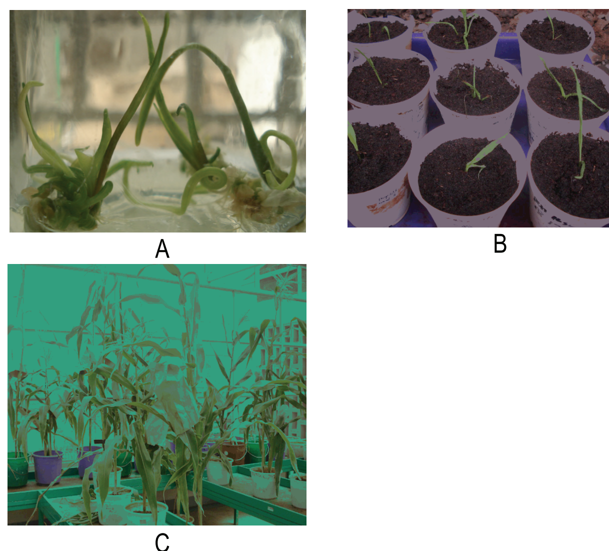

Figure 2. Putative transformed T1 tropical maize plantlets. A: Plantlets growing after one week in regeneration medium. B: Acclimatization of putatively transformed plantlets in the growth chamber. C: Selfing of T1 plantlets growing in the greenhouse. (For full color, see p. 33.)

maize suspension cells to confirm interference with the function of the viral replication proteins. The aptamer genes will be introduced into the maize germplasm via *Agrobacterium tumefaciens*, and the transgenic lines evaluated for resistance to the disease.

In conclusion, the transformation of tropical inbred lines has progressed to a level at which agronomically useful genes can now be introduced into the tropical maize genome employing *Agrobacterium* as a vehicle of DNA delivery, suggesting a remarkable improvement in extending *Agrobacterium*-mediated transformation systems to elite cultivars of economically important tropical Kenyan maize. The application of this technology has the potential to significantly impact maize production systems that experience drought stress in Sub-Saharan Africa.

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Effect of mutagens on imprinting expression in apomicts

--Sokolov, VA; Blakey, CA; Tarakanova, TK; Abdyrahmanova, EA

It has been long believed that apomixis is determined by two components – apomeiosis and parthenogenesis. The current work shows the considerable role of imprinting in viable seed formation of pseudogamic asexual species. Of significance to note, in apomicts the embryo sacs develop out of diploid cells, and the central cell is tetraploid until fertilization. In the initial endosperm cell development, the ratio of female to male genomes will be 4F:1M in its nucleus. Such a combination of male and female genomes in the cell nuclei of grain storage tissue is abnormally different from that of the regular 2F:1M necessary for viable seed formation. Thus, only 20% of asexual *Tripsacum* (gamagrass) florets actually give rise to viable seeds. Despite a considerable

deviation of maize genomes in endosperm cells from that of regular 2F:1M, we obtain up to 50% viable seeds in the apomictic maize x *Tripsacum* hybrids produced.

In this case, grain development was connected with a suppression of imprinting dosage effect manifestations in the presence of *Tripsacum* chromosomes. In addition, grain size and weight dependence on the pollinator's ploidy and its quality was also revealed. Grains produced with one pollinator have a large range of weight and viability. This observation can be explained by a complex interaction of genes imprinted on male and female types in endosperm cell nuclei and environmental effects on their expression. As the average weight of grains is reduced in the presence of a large number of *Tripsacum* parent chromosomes, an attempt was made at using mutagens to effectively modify their expression.

For this purpose, aerated dry seeds of 39-chromosome apomictic maize x *Tripsacum* hybrids (30 Zm + 9 Td) were soaked in a chemical mutagenic solution, e.g., ethyl methanesulfonate, 5-azacytidine, natriumbutyrate, or trichostatine A; and they were also gamma-radiated. Comparative studies of grain weight in M2 allowed us to determine that only in the 5-azacytidine-treated (0.33 μM solution) families resulted in variants having a considerable average seed weight increase. The 5-azacytidine treatment was carried out twice--in the greenhouse (2002-2003) and the hothouse (2003) of the ICG SB RAS. In 2004, the control and M2 were grown in the experimental field of Krasnodar, Russia; M3 and M4 were grown at the Kuban VIR experimental station in 2005 and 2006, respectively. Di- and tetraploid maize--Mangelsdorf (2n=2x=20) and Tester purple (2n=4x=40)--were used as pollinators in M1 (greenhouse 2002-2004 and hothouse 2003); only tetraploid pollinator C-435 was used in M2-M4.

The results of these experiments are presented in Tables 1, 2, and 3, (also Figure 1: bronze = seed from 39-chromosome hybrid, white = 4n maize parent). As is seen from the data presented in Table 1, about 30% of the germs die from the 5-azacytidine treatment. We observed significant weight differences of the seeds produced (Table 2) in the pollination of 39-chromosome lines with di- and tetraploid maize. Only the seeds of the second experiment were used for further research of the effects of 5-azacytidine treatment in M1 and subsequent generations; observations are

Table 1. Effect of 5-azacytidine on treated grain viability.

	Experiment 1 (Greenhouse, 2002-2003)		Experiment 2 (Hothouse, 2003.)	
	K	AZ	K	AZ
Grains set	24	37	20	33
Grains germinated	24	36	20	33
Died in Petri dishes	0	6	0	3
Died after planting	0	6	1	7
Died total	0	12 (33.3%)	1	10 (30.3%)
Adult plants	24	24	19-3B_{III}	23-1B_{III}
Unpollinated (late)	8	7	1	7
Total of pollinated plants	16	17	15	15

Table 2. Grain weight in M1 of 5-azacytidine-treated (AZ) apomictic plant line 4x-6 (30Zm+9Td) as compared to Control (K), Experiment 1 (AZ), Greenhouse, 2002-2003.

Line	Pollinator (2n)	Grain weight (x ± m)	min	max	n		Pollinator (4n)	Grain weight (x ± m)	min	max	n
39 (K)	Mangelsdorf (K)	0.020±0.0031	0.005	0.095	30		TP (K)	0.074±0.0052	0.040	0.135	18
	Mangelsdorf (AZ)	0.029±0.0037	0.002	0.085	44		TP (AZ)	0.083±0.0039	0.005	0.130	64
							TSh(AZ)	*0.081±0.0032*	*0.010*	*0.125*	*74*
39 (AZ)	Mangelsdorf (K)	0.036±0.0035	0.010	0.105	54		TP(K)	0.069±0.0064	0.030	0.120	15
	Mangelsdorf (AZ)	0.033±0.0027	0.005	0.105	75		TP(AZ)	0.079±0.0055	0.015	0.135	32
							TSh(AZ)	*0.064±0.0025*	*0.005*	*0.130*	123

Table 3. Average grain weight of apomictic plant line 4x-6 (30Zm+9Td) treated with 5-azacytidine at 0.33 μM (AZ) in pollination with tetraploid maize (2n=4x=40).

Family No.	M1 (AZ)			M2 (AZ)			M3 (AZ)			M4 (AZ)		
	Grain weight (g)	min-max (mg)	n	Grain weight (g)	min-max (mg)	n	Grain weight (g)	min-max (mg)	n	Grain weight (g)	min-max (mg)	n
87	0.094±0.007	7-155	36									
88	0.084±0.006	3-140	37	0.092±0.004***	4-170	167	0.067±0.005	2-130	42	0.086±0.005***	2-165	116
90	0.113±0.006***	15-165	34	0.093±0.003***	2-195	279	0.065±0.003	5-115	92	0.069±0.005	2-140	74
94	0.061±0.006	4-115	26	0.069±0.002	2-175	345	0.052±0.003	2-110	109			
95	0.091±0.007	10-145	33	0.081±0.002***	2-175	472	0.061±0.013	10-100	47			
97	0.097±0.005	4-140	43	0.075±0.004	3-175	119	0.063±0.010	10-110	11			
98	0.081±0.005	3-130	59	0.032±0.002***	1-125	199						
99	0.084±0.005	3-135	39									
100	0.116±0.005***	3-155	56	0.048±0.005***	5-155	77						
103	0.080±0.006	7-140	60	0.048±0.002***	2-185	448						
106	0.088±0.006	3-145	45	0.075±0.002	2-175	403	0.057±0.007	3-100	20			
108	0.088±0.005	6-125	61	0.058±0.003**	3-130	125						
114	0.067±0.006	3-125	45	0.061±0.004**	2-140	112						
115	0.090±0.004	3-145	73	0.091±0.003***	4-165	164	0.070±0.003	4-140	126	0.092±0.004***	2-175	144
116	0.100±0.006*	3-150	58	0.088±0.003***	2-170	204	0.040±0.004***	10-85	22			
Control	0.088±0.002	1-170	578	0.069±0.002	2-170	414	0.060±0.004	6-130	55	0.066±0.003	2-135	167

Figure 1. Bronze = seed from 39-chromosome hybrid, white = 4n maize parent. (For full color, see p. 34.)

presented in Table 3. Three families were studied in M4, and two families showed a significant gain in grain weight.

The mechanism of the 5-azacytidine effect has been connected with methyl-cytosine demethylation, one way of imprint marking. Therefore, grain weight increase can be explained due to signal amplification changes. The results obtained in these experiments allow us to dwell on the 5-azacytidine effect on imprinting expression, the latter being revealed both in M1 and the following generations. However, due to complicated interactions, its expression is most likely to be unstable.

It is necessary to emphasize that, in earlier data, when pollinating with hybrid pollen, the weight of produced seeds was almost equal to that of seeds in the pollination with the tetraploid. Thus, it is possible to conclude that imprinting expression and its strict dosal dependence is different from that observed in diploid maize of apomictic maize x *Tripsacum* hybrids in the presence of some of the wild parent (*Tripsacum*) chromosomes. The presence of *Tripsacum* chromosomes is likely, in some way, to affect the imprinting signal-setting and modify its expression. In this connection, we tried to influence its expression experimentally with 5-azacytidine treatment of germinating seeds. Therefore, the DNA involved in replication in the presence of the chemical agent results in a decrease in the degree of methylation of cytosine. Thus, the elimination of the marker signal and modification by imprinting expression proceeds. Such changes can be traced in M1 and, possibly, in the following generations by comparing average grain weight to that of the control and experimental variants. In this present contribution, we are now left to dwell on the results of our investigation and consider those of the future (Figure 2, plant height modification) in using 5-azacytidine for modification of imprinting expression in apomictic maize x *Tripsacum* hybrids.

Figure 2. Plant height modification. (For full color, see p. 34.)

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Mapping, positional cloning and expression profiling of mutants affecting endosperm development

--Pasini, P; Carletti, G; Marocco, A

The maize caryopsis is specialized to convert assimilate solutes rapidly to provide a carbohydrate and protein reserve for the germinating seed. The endosperm tissue has in the course of this specialization process acquired a distinctive pattern of gene expression. Our efforts have concentrated on the role of two mutant types, a set of viable reduced endosperm mutants (*de*), and miniature-like mutants (*cp*, *mn* and *rgf*). They all exhibit a reduced growth rate of the endosperm and smaller seed size compared to the wild type. In mutants, protein content and zein fraction appear to be strongly correlated to endosperm weight. Mutations *de1*, *de34*, and *de127* induce a lower than normal accumulation of zeins. Some of the *de* mutants alter auxin level, thus changing endosperm development. These mutations are important for detection of genes involved in seed development, and transport and accumulation of reserve products.

This report summarizes the results obtained in a collaborative project between UCSC, Dipartimento Produzione Vegetale, Università di Milano, Italy (F. Salamini) and ISTA, Lodi, Italy (M. R. Stile and E. Puja). The objectives of this study were: 1) to identify

AFLP markers linked to individual mutant alleles and to integrate them into the reference genetic map; 2) to isolate the *rgf1* gene using a map-based cloning technique; and 3) to reconsider the effects of mutations on endosperm cell size and indole-3-acetic acid (IAA) content in the seed.

A collection of viable mutants, including defective endosperm (*de*), miniature (*mn*), collapsed (*cp*), and reduced grain filling (*rgf*) was obtained by selfing plants of open-pollinated maize varieties or from mutagenized and random tagging materials (Manzocchi et al., Maydica 25:105-116, 1980). The following mutants, *de1, de3, de6, de10, de18, de21, de22, de34, de76, de90, de127, de301, de302, mn2, mn3, cp1* and *rgf1* were introgressed into the B37+ and A69Y+ background through 5-6 backcrosses. Allelism tests showed the mutations were not linked. Mutant phenotypes of genetic loci controlling endosperm size and structure are reported in Figure 1.

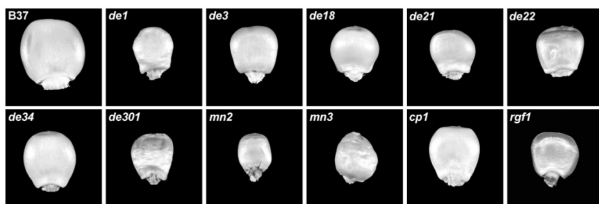

Figure 1. Phenotypes of wild type and *de*, *mn*, *cp* and *rfg* kernels.

Bulked segregant analysis was used to identify AFLP markers linked to mutants (Michelmore et al., Proc. Natl. Acad. Sci. USA 88:9828-9832, 1991; Vos et al., Nucl. Acids Res. 23:4407-4414, 1995). On average, 182 *Eco*RI/*Mse*I primer combinations were used to screen parentals (B37/A69Y, mutant, B73 and Mo17) and F3 family pools (wild type ++ and mutant --). Markers associated with the mutations were used to build specific linkage maps with the program MapMaker 3.0. The AFLP markers linked to a mutation and polymorphic between B73 and Mo17 were integrated in the IBM2 reference map using the program MapMaker 3.0. The assignment of AFLP markers and mutations to a specific chromosome was confirmed by an SSR-based step (Castiglioni et al., Genetics 149:2039-2056, 1998). The mutants were linked to the closest molecular makers by distance ranging from 0 to 22 cM. We detected six chromosomal regions in which the mutants affecting seed weight are located.

In order to clone *Rgf1* (Maitz et al., Plant J. 23:29-42, 2000), a map-based approach was initiated.Using 135 BC1S1 plants and SSR markers, *rgf1* was mapped to bin 2.04 of chromosome 2 within a 5 cM interval (Zhang et al., Maydica 47:277-286, 2002). A group of 1406 F3 plants consisting of 302 *rgf1*/*rgf1*, 322 *Rgf1*/*Rgf1* and 782 *Rgf1*/*rgf1* was used to screen for recombinants around the genetic locus *rgf1*. Based on the molecular alleles at the two flanking SSR markers *bnlg1613* and *bnlg1140*, 114 recombinants were found. AFLP fragments were generated to search for markers tightly linked to *rgf*1. Preliminary results indicate that 16 candidate AFLP markers allow further reduction of the interval mapping to 0.5 cM.

Some of the *de* mutants alter auxin level, thus changing endosperm development. The mutants, reducing more or less drastically the growth rate of the kernel, exhibit a highly variable level of IAA in the endosperm. It is known that auxin is involved in enhancing post-mitotic nuclear DNA synthesis, and endoreduplication is positively correlated with cell enlargement and cell volume. Endosperm tissue of the *de18* mutant had substantially lower free and bound IAA than wild type counterparts (Torti et al., Theor. Appl. Genet. 72:602-605, 1986). The level of IAA is at least 15 times lower in the mutant *de18* than in the wild type. We have identified differentially expressed genes related to *de18* phenotypes using the microarray technology. Only relative changes greater than –2.0 (mean log2 <–1.0) were considered. We compared the number of down-regulated and up-regulated genes in the seed of the *de18* mutant to that of the wild type at four developmental stages. *de18* showed delayed gene expression until 21 days after pollination (DAP). The expression level of auxin regulated genes was reduced in *de18* from 7 to 28 DAP.

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Screening for male-sterile mutants that act before meiosis to disrupt normal cell fate specification in maize anthers

--Walbot, V; Skibbe, D; Young, C; Culbertson, T; Nan, G; Morrow, D; Wang, D; Fernandes, J; Ritter, M; Wong, J; Bolduan, R; Gay, J; Wang, R; Timofejeva, L; Harper, L; Cande, Z

To learn more about cell fate acquisition in the maize anther, we are focusing on mutants disrupted after organ specification in the tassel, laying accessory vegetative structures and the stamens. Mutants of interest have a normal filament, but one or more anther locule cell types mis-differentiate prior to the start of meiosis. During this approximately 7-10 day period of anther development of interest here, mitotic divisions establish populations of L1 (epidermal) and L2 (all other cell types) cells within anther locule primordia. In a transverse section a normal anther locule will contain just 5 cell types organized in rings. Starting from the outside, these layers are epidermis, endothecium, middle layer, tapetum, and microsporocytes. This developmental window is currently bracketed by *msca1* (formerly *ms22*) in which no anther locule cell types are normal and *am1* in which the centrally located presumptive meiotic cells conduct mitosis instead. Anthers in which one or more cell types fail to achieve normal function, placement, or numbers are of interest. In the mutants studied thus far, lack of normal somatic cell types seems to result in meiotic arrest, suggesting that the anther is a “system” and if any component is missing or defective, development arrests. We will be evaluating the transcriptional profiles in defined cell layers collected with laser microdissection to fine tune our understanding of early anther development.

Targeted *Mu* tagging is being used as a prelude to cloning and analysis of key genes. Highly active *Mu* lines heterozygous for a mutable *bz2* reporter allele are crossed to multiple *ms-reference//ms-reference* individuals with colorless or *bz2* seed for construction of tagging populations and to *bz2* tester (validation test performed on the same pollen collection). Seed resulting from validated pollen donors yielding ~50% spotted progeny kernels in the *bz2* test cross are pooled to organize families for the screening field. This screen is conducted at Cal Poly-SLO and is organized to provide an undergraduate summer training program. The tagging population should consist mainly of male fertile plants (*ms-reference//+*) with rare sterile individuals (*ms-reference//ms-Mu*). Fields of up to 100,000 plants are screened daily, and all tassels shedding pollen are clipped off. Newly identified mutants are photographed, shoot bagged, and ultimately crossed by *a1 Bz2 Mu killer* stocks to promote epigenetic silencing of the Mutator transposon family. In this cross, progeny seed are purple (*A1//a1 Bz2//bz2* or *bz2-mu*), while contaminating pollen from unclipped fertile tassels should result in colorless, bronze or spotted kernels. Validation of individual "puton" male-sterile plants is done with two molecular tests: PCR assay for the *bz2-mu* allele (expected in 50% of authentic mutants) and/or RT-PCR assay for a high level of the *mudrA* transposase transcript. In a number of cases, multiple sterile plants are identified within large families, consistent with pre-meiotic *Mu* insertion. Segregation of the *bz2-mu* reporter allele is tested on the group. Lessons learned from an initial tagging effort with *mac1* in 2005 have nearly eliminated false positive "contaminating" *ms-reference* carriers in the tagging populations: detassel all sources of *ms-ref* allele fertile plants and construct populations in the field, not the greenhouse. Table 1 summarizes the current screening results.

Table 1. Tagging populations and yield of putons and confirmed *Mu*-induced mutants.

		Tagging population		Male-sterile individuals		
Locus	Year(s)	No. of families	No. of kernels	Putons	*Mu* parent confirmed	Frequency
mac1	2005	210	31,237[c]	10	3	1.4×10^{-4}
	2007	34	23,083[c]	13	12	6.9×10^{-4}
ms25	2006	140	35,665	6	6	1.7×10^{-4}
msca1	2006	120	40,265	4	4	1.0×10^{-4}
ms8	2007	22	23,083	6	5	2.2×10^{-4}
ms23	2005	79	19,981	0	0	0
	2007	27	26,565	8	8	3.0×10^{-4}

The second strategy involves assessing already reported *ms* mutants of maize, primarily from phenotypic screens in the Maize Gene Discovery Project (*RescueMu* tagging), UniformMu, MTM, etc. Under the milder growing conditions of California, compared to the initial screening sites in the Midwest, about ½ of the reported ms mutants fail to exert anthers. Among these, a smaller percentage show arrested anther development (2-3 mm) consistent with problems in cell fate acquisition or maintenance. Mutants with abnormal anther length, shape, or integrity are recovered for more detailed cytological analysis to identify mutants with the characteristics important to this screen.

A third strategy involves using transcriptome profiling data for maize anthers generated on 22K and 44K Agilent arrays and the database of sequenced *RescueMu* insertion sites. The tagging grids utilized in the *RescueMu* strategy yielded plasmid DNA sequencing templates from both the Rows and the Columns of the grid, however, only the Row *RescueMu* plasmids were sequenced to reasonable depth; approximately 2-4 columns per grid were sequenced as a check that some *RescueMu* insertion sites were found in both a Row and a Column, thus specifying a particular plant within the original grid. Several hundred Row + Col sequence matches were identified for anther expressed genes and thousands are available for Row only matches; the corresponding column(s) for each insertion site are being identified by PCR of library plates containing the rescued plasmids for each row and each column of a grid. The grid plants were self-pollinated, hence the expectation is that if a *RescueMu* insertion disrupts a gene required for normal anther development prior to meiosis, ¼ of the progeny will be male-sterile and these individuals should be homozygous for the *RescueMu* insertion at the target anther expressed gene.

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Two new PCR based polymorphic markers in bin 5.09

--Slewinski, TL; Braun, DM

In our effort to positionally clone a gene located near the telomere of the long arm of chromosome 5, we have developed two PCR based co-dominant polymorphic markers in bin 5.09. Figure 1 shows CDPK DEL, a marker derived from a 21 bp deletion found in a putative calcium dependent protein kinase (Gen Bank Accession DV541158) located at Sbi.0.23295 in sorghum and LOC_Os02g58520 in rice. Figure 2 shows BZIP DEL, a marker derived from a 24 bp deletion found in a putative bZip transcription factor (Gen Bank Accession CK370734) located at Sbi.0.23306 in sorghum and LOC_Os02g58520 in rice. PCR products for both

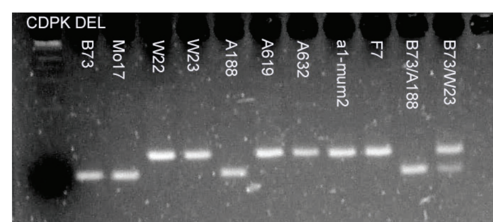

Figure 1. CDPK DEL.

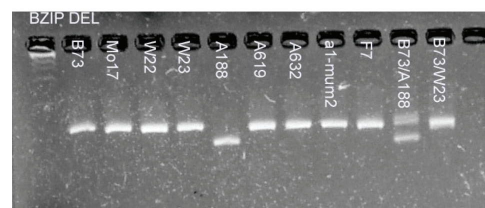

Figure 2. BZIP DEL.

markers are ~150 bp for the larger and ~130 bp for the smaller fragment and can be clearly resolved on a 4% APEX gel. In both figures several common inbreds, the *a1-mum2 Mutator* minimal line and two equal mixtures of DNA demonstrate the markers are polymorphic and co-dominant.

<u>Primers</u>:

CDPK DEL forward

TGATCCCAGGCCCAGCGATGC

CDPK DEL reverse
CGACAGGGCGATGCTGTTGCTGCTG

BZIP DEL forward
CAGCTGAGCCTGAGCGGCTGCAGC

BZIP DEL reverse
CGCCGAGCGTGAGCGACAGGAGAGG

PCR Conditions: Both use standard PCR reaction mixture with the addition of glycerol to a final concentration of 6% and DMSO to a final concentration of 3%.

Thermal Cycler Program:

1) 94ºC	2 min	1 cycle
2) 94ºC	30 sec	
3) 66ºC	30 sec	
4) 72ºC	30 sec	repeat 2-4 for 35 cycles
5) 72ºC	5 min	1 cycle

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Identification and characterizations of *P1-wr* epialleles in maize that show a gain in pericarp function

--Robbins, ML; Peterson, TA; Chopra, S

Homologous recombination mechanisms at *p1* have been proposed to be involved in the allelic diversity at *p1.* For example, the *P1-wr* allele has six tandemly-repeated gene copies, and is thought to be derived by gene duplication events from an ancestral allele. However, the *P1-wr* may be refractory to recombination since ears with pericarp pigmentation sectors are not present in *P1-rr/P1-wr* X *p1-ww* screens (Brink, Annu. Rev. Genet. 7:129-152, 1973; Chopra et al., Mol. Gen. Genet. 260:372-380, 1998). Toward this objective, a similar screen was performed to identify recombination events at *P1-wr* [W23]. In place of the wild type *P1-rr* allele, a loss of function mutant allele called *p1-ww-10:443-3* (Athma and Peterson, Genetics 128:163-173, 1991) was used. This approach allows for the easy identification of hypothetical *P1-wr/ p1-ww-10:443-3* recombinants exhibiting a gain of pericarp pigmentation.

However, we show by PCR and Southern analysis that recombination did not occur between these two alleles. Nevertheless, three heritable gains of pericarp pigmentation epialleles were identified and are characterized herein.

Genetic screen for gain of pericarp pigmentation. An intragenic transposition of *Ac* from *P1-ovov-1114* produced the *P1-vv*-83934* allele. The *P1-vv*-83934* has an 8-bp target duplication within the *p1* reading frame (Athma and Peterson, 1991). The excision of *Ac* from the *P1-vv*-83934* engendered a frame shift mutant allele called *p1-ww-10:443-3* (Grotewold et al., Proc. Natl. Acad. Sci. USA 88:4587-4591, 1991). The standard *p1* alleles used in this study are *p1-ww* [4co63], *P1-wr* [W23], and *P1-rr-4B2*. The *P1-rr-4B2* allele was introgressed into the W23 background by over six generations of backcrossing.

The *p1-ww-10:443-3* was crossed with *P1-wr* [W23] and the F1 progeny plants were then crossed with *p1-ww* [4co63] (Figure 1).

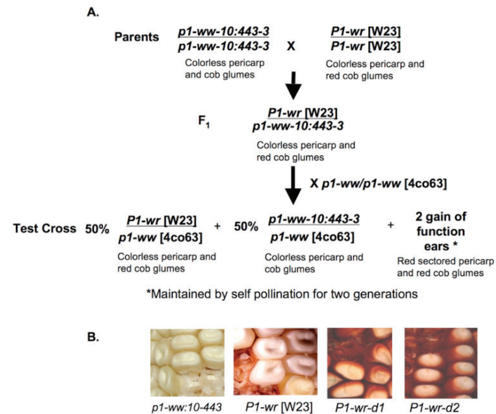


Figure 1. Gain of pericarp pigmentation crossing scheme used to generate new *P1-wr* epialleles. **A.** Diagram showing crosses performed for a gain of pericarp pigmentation screen. This screen generated two epialleles of *P1-wr* [W23] called *P1-wr-d1* and *P1-wr-d2* (see Figure 1D). **B.** Pericarp and cob glume pigmentation of *P1-wr-d1* and *P1-wr-d2* are shown. The phenotypes of the *P1-wr* [W23] and *p1-ww:10-443* parents that were used in this screen is also shown. (For full color, see p. 34.)

Approximately 80,000 test cross ears were open-pollinated and examined for a gain of pericarp pigmentation. Two parental ears produced progeny exhibiting kernel sectors of red pericarp pigmentation. F1 kernels from the red and colorless sectors were planted and the progeny was self-pollinated. The F2 progeny plants were self-pollinated and out-crossed to *p1-ww* [4co63]. Following this, there were two additional generations of self-pollination. During each generation, the pigmentation phenotype was examined on both the pericarp and cob for each allele.

DNA gel blot analysis. Seedling genomic DNA was prepared using a modified CTAB method (Saghai-Maroof et al., Proc.Natl. Acad. Sci. USA 81:8014-8018, 1984). PCR genotyping of the genomic DNA was done with standard conditions using primers listed in Table 1. Restriction digestion was achieved by using enzymes, reagents and protocols from Promega (Madison, WI). Restricted genomic DNA was fractionated on 0.8 % agarose gels and subsequently transferred to nylon membranes. Membranes were pre-hybridized for four hours and then hybridized for 15 h at 65°C in buffer containing NaCl (1 M), SDS (1%), Tris-HCl (10 mM) and 0.25 mg/ml salmon sperm DNA (Athma and Peterson, 1991). The *p1* probe fragments used include F8B, F8C, F13, and F15, and have previously been described (Chopra et al., 1998; Lechelt et al., Mol. Gen. Genet. 219:225-234, 1989; Sekhon et al., 2007) Blots were stripped of previous signal by boiling in 0.1% SDS before they were reused.

Table 1.

Primer Name	Sequence	Purpose	*P1* Alleles Amplified	Product Size
MRF	5'TGGAGCTCTTGCGTATCTAACGCT 3'	Genotyping	*P1-vv, P1-rr-4B2, p1-ww:10-443*	[a]481 bp
MRR	5' AGTGTGCACAGGGACACTTGAGTA 3'			
WRJ	5' CTGTCGGCTACTATCCCCTTGGTGA 3'	Genotyping	*P1-wr-d1, P1-wr-d2, P1-wr* [W23],	618 bp
WRK	5' GATCGCGAGCTGGAGGCGTTCGAGAC 3'			

[a]Another ~900 bp product was also amplified in all genotypes (*P1-vv, P1-rr-4B2, p1-ww:10-443, p1-ww* [4co63], *P1-wr* [W23], *P1-wr-d1,* and *P1-wr-d2*), but its sequence had only a limited homology with *p1* at its 5' end.

Pigmentation patterns of the gain-of-pericarp-function alleles. The two new *p1* alleles, called *P1-wr-d1* and *P1-wr-d2*, were discovered in a phenotypic screen as red kernel sectors on *P1-wr* [W23]/ *p1-ww-10:443-3* X *p1-ww* [4c063] pericarp (Figure 1). The red phlobaphene pigmentation on both alleles was limited to the silk attachment point and kernel gown. In subsequent generations, the pericarp pigmentation was no longer sectored; it instead encompassed the entire ear (Figures 1 and 2). However, there were some instances of ears with mosaic sectors on the pericarp gown (see second ear from left in Figure 2A). Variable pericarp pigmentation intensities and patterns were observed for both alleles. For example, some ears only had silk scar pigmentation, while other ears only had gown pigmentation (Figure 2). Furthermore, some ears had very low pigmentation levels that resembled *P1-wr.* Since the background of the stocks containing these alleles is heterogeneous, it might be that the variability in pigmentation levels depends on the genetic constituency of modifier alleles.

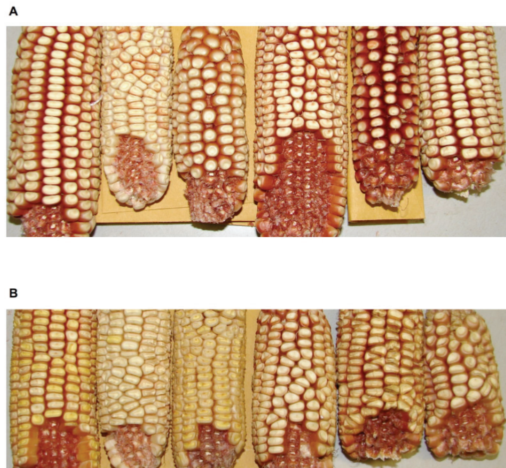


Figure 2. Pigmentation patterns and intensities in *P1-wr* [W23] epialleles. Ears show pericarp and cob glume pigmentation of **A.** *P1-wr-d1* and **B.** *P1-wr-d2.* Note that ectopic pericarp pigmentation can occur at the silk attachment point and/or kernel gown. (For full color, see p. 34.)

Gain-of-function *pericarp color1* alleles share an identical gene structure with *P1-wr* [W23]. Genomic DNA of the two gain of function alleles was compared by PCR genotyping with *p1-ww-10:443-3* and *P1-wr* [W23] (Figure 3). The RRF and RRR primer pair was used to amplify a 481 bp region in *p1-ww-10:443-3* (between 6349 and 6830 of accession Z11879) that is located in exon 3 and includes the 3'UTR and some downstream sequence (Figure 3A). The RRF and RRR primer pair does not amplify the 481 bp region in *p1-ww* [4co63] or *P1-wr* [W23], but does yield another ~900 bp product that is also present in *p1-ww-10:443-3.* The *P1-wr* genomic sequence of a *P1-wr* [W23] (accession EF165349) does not contain the 481 bp region, whereas the origin of the ~900 bp product has only a limited homology with *p1.* Conversely, the WRJ and WRK primer pair amplifies a 618 bp band in *P1-wr* [W23] that is located in the 3'UTR and downstream sequence (Figure 3A). The WRJ and WRK primer pair does not amplify *p1-ww-10:443-3* or *p1-ww* [4co63]. The *P1-wr-d1* and *P1-wr-d2* alleles had an amplification pattern identical to that of *P1-wr* [W23] (Figure

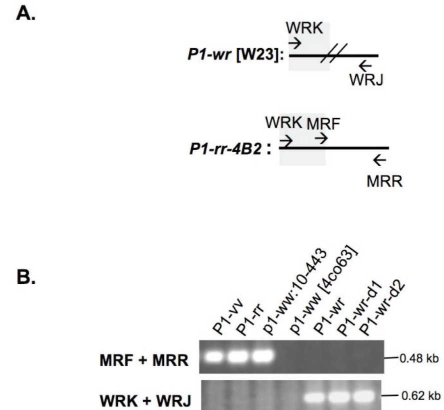


Figure 3. Molecular comparison of *P1-wr-d1* and *P1-wr-d2* with standard *p1* alleles **A.** Diagram showing the 3' ends of *P1-wr* [W23] and *P1-rr-4B2.* The location of PCR primers used for genotyping is shown. The grey shaded boxes represent the end of the gene sequences (i.e. exon 3). In the case of *P1-wr* [W23], the sequence 3' of the shaded box is the distal promoter of the downstream copy. The hash marks in *P1-wr* [W23] signify the junction between two repeated copies. Note that the MRF and MRR primers are not present in *P1-wr* [W23] and the WRJ primer is not contained in *P1-rr-4B2.* **B.** PCR analysis was done to compare *P1-wr-d1* and *P1-wr-d2* alleles with the parental *p1* alleles used in the gain of pericarp pigmentation screen (genetic screen is shown in Figure 1). Based on the amplification patterns, the *P1-wr-d1* and *P1-wr-d2* alleles could be classified as similar to *P1-wr* [W23].

3B). Conversely, no evidence of the presence of sequence originating from *p1-ww-10:443-3* was found.

To identify gene structural differences that may have led to the gain of function in pericarp tissue we assayed *P1-wr-d1* and *P1-wr-d2* by DNA gel blot analysis (Figure 4). Seedling genomic DNA was digested with ten restriction enzymes and hybridized with different *p1* homologous probes. This analysis revealed that *P1-wr-d1* and *P1-wr-d2* had an identical tandem-repeat gene structure with *P1-wr* [W23] (Figure 4A and B). Moreover, when the distal enhancer region of *p1* was examined, there were no *Sal*I fragments that resemble *P1-rr* (Figure 4C and D). Rather the bands (12.5kb and 1 kb) resemble the pattern expected for *P1-wr.* Additional results showed that there were no diagnostic *p1*-F15 *Sal*I fragments that resembled *P1-rr* (Figure 4C and D). Rather the bands (12.5kb and 1 kb) showed the pattern expected for *P1-wr.* Collectively, these results suggest that *p1-ww-10:443-3* has segregated from *P1-wr* and has not recombined with *P1-wr.* Hence, this data suggested that the gain of gown pigmentation in *P1-wr-d1* and *P1-wr-d2* may have arisen by spontaneous small nucleotide polymorphisms or epimutations in *P1-wr* [W23]

***P1-wr-d1* and *P1-wr-d2* are hypomethylated epialleles of *P1-wr* [W23].** Since *P1-wr-d1* and *P1-wr-d2* had no genetic differences from *P1-wr* [W23], we considered the possibility that epigenetic differences may have led to the gain of function in pericarp tissue. Hence, DNA gel blot analysis was used to construct a DNA methylation map comparing *P1-wr-d1, P1-wr-d2,* and *P1-wr* [W23] (Figures 5). We detected partial hypomethylation at the distal floral organ enhancer as evidenced by the diagnostic 500 bp F15-homolgous *Hpa*II band (Chopra et al., Genetics 163:1135-1146,

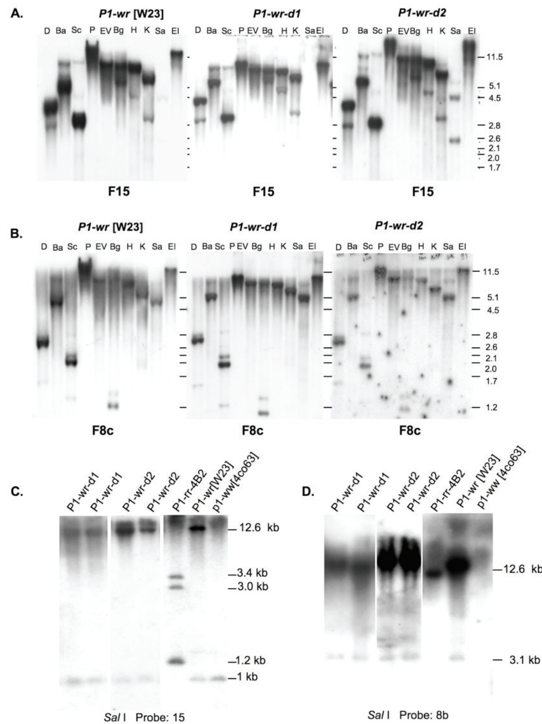
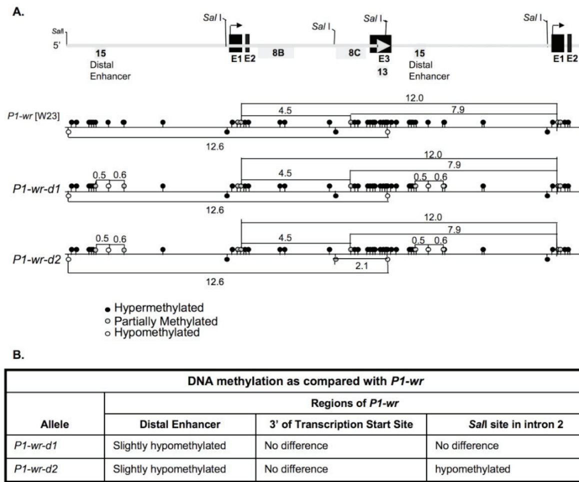


Figure 4. Structural comparison of *P1-wr-d1* and *P1-wr-d2* with *P1-wr* [W23] and *P1-rr-4B2*. Gene structure blots were made by digesting seedling leaf DNA with ten diagnostic restriction enzymes. Enzyme names are abbreviated as follows: D, *Dra*I; Ba, *Bam*HI; Sc, *Sca*I; P, *Pst*I; EV, *Eco*RV, Bg, *Bgl*II, H, HindIII; K, *Kpn*I; Sa, *Sac*I; El, *Eco*RI. Blots were hybridized with *p1* probes corresponding with **A.** the distal floral organ enhancer (F15) and **B.** intron 2 (F8C). The blots in **C** and **D** show *Sal*I digested DNA that has been hybridized with distal enhancer probe F15 and intron 2 probe F8B, respectively. For a diagram showing the location of these probes in *P1-wr* [W23] refer to Figure 5. The sizes of molecular weight marker bands are indicated in kilobase pairs to the right of the blots.



DNA methylation as compared with *P1-wr*			
	Regions of *P1-wr*		
Allele	Distal Enhancer	3' of Transcription Start Site	*Sal*I site in intron 2
P1-wr-d1	Slightly hypomethylated	No difference	No difference
P1-wr-d2	Slightly hypomethylated	No difference	hypomethylated

Figure 5. *P1-wr-d1* and *P1-wr-d2* are hypomethylated epialleles of *P1-wr* [W23]. **A.** *P1-wr* [W23] was used as a template to construct the DNA methylation map for *P1-wr-d1* and *P1-wr-d2.* The intron/exon structure of *P1-wr* is provided on a line diagram above the methylation maps. The large grey arrow on the line diagram represents the end of a copy in the tandem array. The bent arrows indicate the location of the transcription start sites. Exons are abbreviated as E1, E2, and E3. The placement of *p1* probes (grey shaded boxes) is shown immediately below the line diagram. DNA methylation maps are shown below the *P1-wr* [W23] gene structure. On the DNA methylation maps, black circles indicate hypermethylated sites; grey circles indicate partially-methylated sites; non shaded circles represent hypomethylated sites. **B.** Summary of the DNA methylation changes in *P1-wr-d1* and *P1-wr-d2* relative to that of *P1-wr* [W23].

2003) (Figure 6A). However, the presence of high molecular weight bands such as a 7.9 kb *Hpa*II band indicates that most copies remained hypermethylated at the distal enhancer region (Figure 5A).

At the intron 2 region of *p1,* we did not observe any differences using *Hpa*II digestion; however, differences were detected using *Sal*I (Figure 6B). Digestion with *Sal*I in *P1-wr* [W23] produces a 12.6 kb band that extends the entire length of the gene. This is because two of the three *Sal*I sites in *P1-wr* [W23] are hypermethylated. Interestingly, in *P1-wr-d2* we detected an additional 2.1 kb band which suggested that a *Sal*I in intron 2 (site 10,310 of *P1-wr* [W23] accession EF165349) was partially hypomethylated (Figure 5). This difference (2.1 kb band) was not observed in *P1-wr-d1* and therefore can be used to differentiate between the two epialleles (Figure 6B).

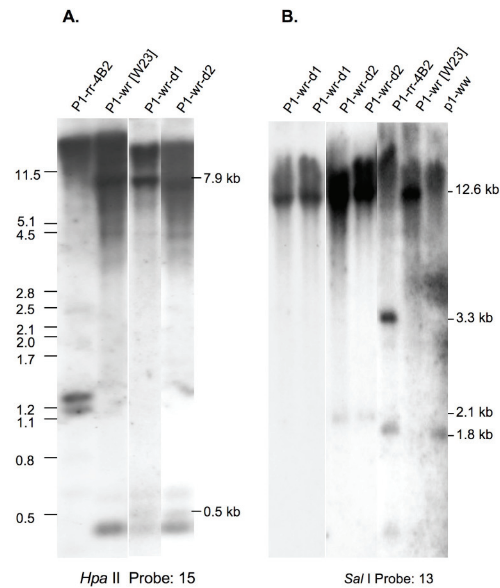


Figure 6. DNA gel blot showing methylation differences between *P1-wr* [W23] and its derived epialleles. **A.** Gel blots showing *Hpa*II digested genomic DNA hybridized with the *p1* distal enhancer probe 15. **B.** Gel blots showing *Sal*I digested genomic DNA hybridized with *p1* exon 3 probe 13. These and other blots were used to construct the DNA methylation maps for the *P1-wr-d1* and *P1-wr-d2* epialleles shown in Figure 5.

Notably, several *P1-wr* alleles from R. A. Brink's collection which have pericarp pigmentation confined to the kernel gown are also partially hypomethylated at this distal floral enhancer (Brink and Styles, MNL 40:149-160, 1966; Cocciolone et al., Plant J. 27:467-478, 2001). It was thus suggested that DNA hypomethylation was important for the gain of pericarp function (Cocciolone et al., 2001). However, Brink's *P1-wr* alleles were collected from numerous genetic sources and therefore would likely have subtle genetic differences such as SNPs. Herein, this gain of function screen employed *P1-wr* [W23], and hence, the resulting alleles are likely genetically identical to *P1-wr* [W23]. Therefore, these results help strengthen the theory that an epimutation in *P1-wr* can lead to the presence of silk scar and gown pigmentation.

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Maize Genetics Cooperation Stock Center

Allelism testing of phenotype only stocks in Maize Coop collection uncovers new *viviparous5*, *waxy1* and *shrunken2* alleles

--Jackson, JD

This report summarizes allele testing of various stocks characterized by phenotype only in the Maize Genetics Coop Stock Center collection. Some of these mutants have been found in other Coop stocks and some were sent to us by our cooperators. Proposed designations have been assigned to these new alleles and they have been placed on our stocklist. It is expected that with further sorting and allelism testing of stocks characterized by phenotype only, additional alleles of characterized mutants will be discovered and placed in the main collection.

Previous designation	Allelism test with vp5	New designation	MGCSC: stock number
y-vp-60-153*	1 positive	*vp5-60-153*	123C
y-vp-0730*	2 positive	*vp5-0730*	123D
y-vp-8103 Funk*	3 positive	*vp5-8103 Funk*	123E

Previous designation	allelism test with wx1	New designation	MGCSC: stock number
wx-98-1406-6*	4 positive	*wx1-98-1406-6*	924I
wx-0208*	4 positive	*wx1-0208*	924J

Previous designation	allelism test with sh2	New designation	MGCSC: stock number
sh-vp-8806*	6 positive	*sh2-8806*	333DA

New alleles of *pale yellow9* found in viviparous stocks in Maize Coop phenotype-only collection

--Jackson, JD

This report summarizes allele testing of viviparous stocks characterized only by phenotype in the Maize Genetics Coop Stock Center collection. Here pale kernels linked to the viviparous trait characterized all stocks. They had previously given negative results in tests with *vp9*. Since *y9* is also characterized by pale endosperm and is slightly viviparous, allelism tests were next conducted with this stock. The *y9* stock also gives green to pale green seedlings and plants. This and zebra striping had been noticed previously in the phenotype-only stocks. Crosses were made as follows: [+/vp*]@ X *y9* and +//+/vp* X *y9*. Ears were scored for the segregation of pale kernels. In all crosses, pale kernels were selected and planted in the field for observation. Seedlings were pale green and had white-tipped leaves. These pale green zebra plants grew to maturity.

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 *y9* will be discovered. Stocks with this same phenotype that were found to complement y*9* will be tested for allelism with other stocks associated with a pale endosperm phenotype.

Previous designation	allelism test with y9	New designation	MGCSC: stock number
lw-8513*	5 positive	*y9-8513*	X34C
pale y-vp-83-3124-33*	3 positive	*y9-83-3124-33*	X34D
pale-y-vp-85-3240-5*	3 positive	*y9-85-3240-5*	X34E
pale y-vp-85-3267-6*	3 positive	*y9-85-3267-6*	X34F
pale-y-vp-86-1316-27*	3 positive	*y9-86-1316-27*	X34G
vp-86-1573-27*	7 positive	*y9-86-1573-27*	X34H
y-vp-87-2340-36*	7 positive	*y9-87-2340-36*	X34I
lw-82-1*	3 positive	*y9-82-1*	X34J
y-pg-pale y*-84-5275-14*	3 positive	*y9-84-5275-14*	X34K
y-pg-pale y*-85-3042-7*	3 positive	*y9-85-3042-7*	X34L
y-pg-pale y*-85-3078-41*	3 positive	*y9-85-3078-41*	X34M
y-pg-pale y*-85-3562-31*	1 positive	*y9-85-3562-31*	X34N
y-pg-pale y*-85-86-3533-9*	4 positive	*y9-85-86-3533-9*	X07CB
y-pg-pale y*-86-1151-7*	2 positive	*y9-86-1151-7*	X07CC
y-pg-pale y*-87-2160-16*	7 positive	*y9-87-2160-16*	X07CD

III. COLOR FIGURES SUPPLIED WITH NOTES

Caption: Ray Lee (Navajo medicine man) and Lula Jackson (Ray's wife) led a corn pollen blessing during the summer of 2006 at the NCRPIS. Pictured (left to right) are: (front) Carolyn Lawrence, (middle) Lula Jackson (Navajo), Regina Sanchez (Navajo), Ray Lee (Navajo), Sharon Garfield (Navajo), (back) Alexandra Volker (Cherokee), Nathan Etsitty (Navajo), Titus Harrison (Navajo), Mark Widrlechner, Lisa Burk, and Von Mark Cruz.

FROM LAWRENCE ET AL., p. 2.

Figure 2. Plant Breeding Centennial reunion attendees visit the Living Map of Mutants of Maize, July 27, 2007, Cornell University, Emerson Garden, Cornell Plantations. (Photo by Denise Costich)

FROM KASS AND MURPHY, p. 17.

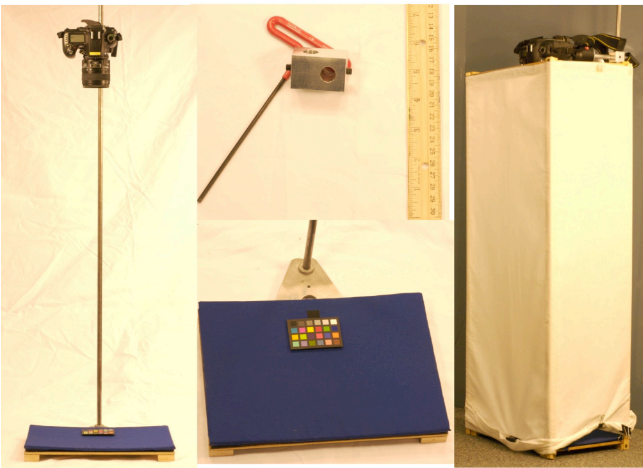


Figure 1. The imaging platform. The left panel shows the unshaded platform used in the greenhouse and for time-course photographs in the field. The middle panel shows the perpendicular clamp and its wrench (upper) and the platform bottom (lower). The right panel shows the shaded platform for collection of end-point photographs of detached leaves in the field (the "box kite").

FROM FOARD ET AL., p. 14.

Figure 1. 29' stalk displayed at Canadian Royal Winter Fair, November 1999, right of center. 27.5' Jala stalk, left.

FROM KARL, p. 18.

Caption: Maize fields, March 6, 2006, near Trece Aquas, Guatemala. This is 20 miles west of the southern end of Lake Izabel and in a region known for 20”-24” long cobs (Kempton, J. Hered. 15:337-344, 1924).

PHOTOS COURTESY OF JASON KARL.

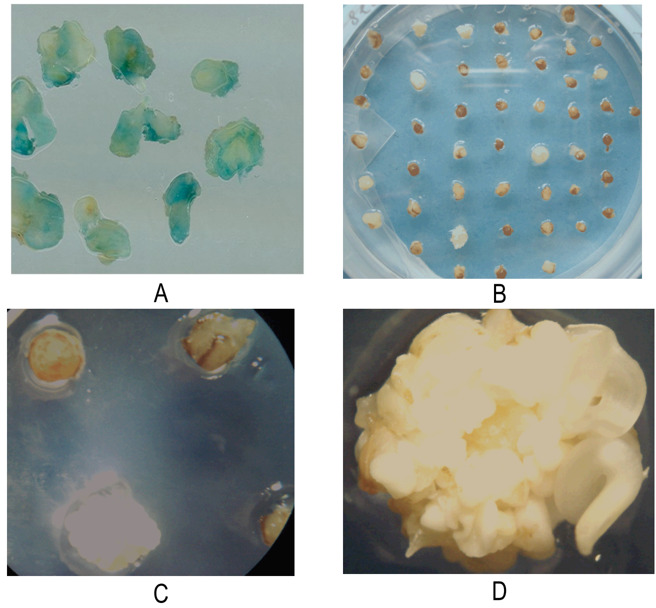


Figure 1. Selection and regeneration of transformed tropical maize inbred lines. A: Positive *Gus* staining calli after four weeks on selection II. The immature embryos were transformed with EHA101 *Agrobacterium* strain harboring pTF102 vector containing the p35S-GUS gene, described in Frame et al. (2002). B, C and D: Dying and surviving calli after four weeks on selection II medium (3mg/L Bialaphos).

FROM ANAMI ET AL., p. 23.

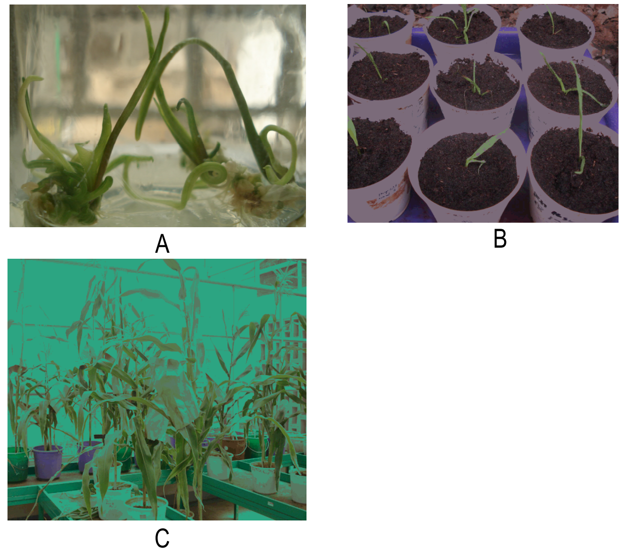


Figure 2. Putative transformed T1 tropical maize plantlets. A: Plantlets growing after one week in regeneration medium. B: Acclimatization of putatively transformed plantlets in the growth chamber. C: Selfing of T1 plantlets growing in the greenhouse.

FROM ANAMI ET AL., p. 23.

Figure 1. Bronze = seed from 39-chromosome hybrid, white = 4n maize parent.

FROM SOKOLOV ET AL., p. 25.

Figure 2. Plant height modification.

FROM SOKOLOV ET AL., p. 25.

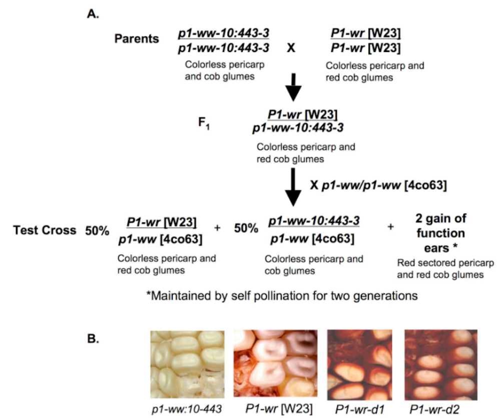


Figure 1. Gain of pericarp pigmentation crossing scheme used to generate new *P1-wr* epialleles. **A.** Diagram showing crosses performed for a gain of pericarp pigmentation screen. This screen generated two epialleles of *P1-wr* [W23] called *P1-wr-d1* and *P1-wr-d2* (see Figure 1D). **B.** Pericarp and cob glume pigmentation of *P1-wr-d1* and *P1-wr-d2* are shown. The phenotypes of the *P1-wr* [W23] and *p1-ww:10-443* parents that were used in this screen is also shown.

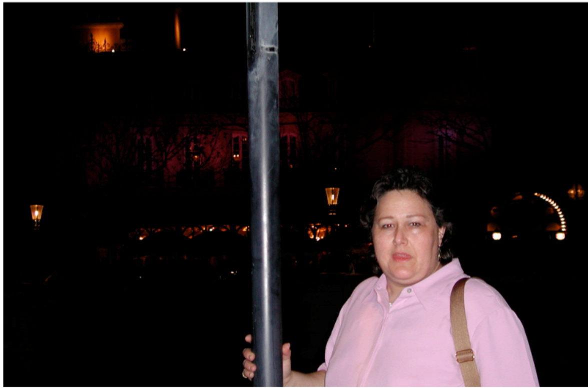
FROM ROBBINS ET AL., p. 28.

Figure 2. Pigmentation patterns and intensities in *P1-wr* [W23] epialleles. Ears show pericarp and cob glume pigmentation of **A.** *P1-wr-d1* and **B.** *P1-wr-d2.* Note that ectopic pericarp pigmentation can occur at the silk attachment point and/or kernel gown.

FROM ROBBINS ET AL., p. 29.

IV. PHOTOGRAPHS FROM PAST MAIZE MEETINGS >>>

Italian. From antipasto to tiramisu, choose from an elaborate menu featuring the best of innovative Italian cuisine, with a wine list to match.



TELEPHONE
TELEPHONE

UNIVERSITY OF ILLINOIS
DEPARTMENT OF BOTANY
URBANA, ILLINOIS

November 26, 1958

Dr. Edward H. Coe, Jr.
302 Curtis Hall
University of Missouri
Columbia, Missouri

Dear Colleague:

I believe it is the general concensus that, for various reasons, the annual meetings of the Genetics Society of America do not afford adequate opportunity for maize geneticists, and particularly for graduate students, to talk with colleagues about maize genetics.

An informal discussion of this problem at the Montreal meeting of the Genetical Congress led to the suggestion that we consider an annual, informal get-together of maize geneticists on a regional basis. It was also suggested that we rotate the sponsoring institution from year to year.

The purpose of this letter is to indicate that we would be happy to sponsor such a meeting at Illinois and would suggest that January 8 and 9, 1959, might be satisfactory dates.

We think there should be maximum opportunity for participation by graduate students and, in view of the number of people who might attend such a meeting, have restricted invitations to maize geneticists at Indiana, Iowa State, Minnesota, Missouri, Purdue and Wisconsin.

We would appreciate having your comments, and those of your colleagues in maize genetics, on the following:

a. Would such meetings, if properly organized and conducted, serve a useful purpose?

b. How many people at your institution would attend such a gathering on the proposed dates, January 8 and 9, 1959?

c. Would you and/or others at your institution be willing to give an informal account of maize genetics research work now in progress? We invite some indication of speakers and their general topics at this time.

d. If you give an affirmative answer to part "a" above, we should like to have your suggestions about organization of program, alternative dates for scheduling, and participating institutions; in short, we should like any comments which would aid in making such meetings a success.

May we have your comments at your earliest opportunity?

Very sincerely yours,

John R. Laughnan

John R. Laughnan

University of Illinois
Urbana, Illinois

COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION—EXTENSION SERVICE IN AGRICULTURE AND HOME ECONOMICS
Department of Agronomy

December 12, 1958

Dr. Edward H. Coe, Jr.
302 Curtis Hall
University of Missouri
Columbia, Missouri

John Laughnan, Earl Patterson and I examined the letters in response to John's letter of November 26 inquiring about interest in conducting a meeting of Maize Geneticists here at Illinois in January, 1959. The consensus of opinion was that such a meeting should be productive, although some preferred a time other than January 8-9.

Accordingly, we decided that the most appropriate time for holding this meeting would be the originally suggested date, namely, January 8 and 9, 1959. We have reserved the Allerton House at Monticello, Illinois for this period. We believe that the environment provided by these facilities should enhance this meeting considerably. The Allerton House is a country villa given to the University by a wealthy Chicago manufacturer. The facilities are such that both sleeping and dining facilities are in connection with a number of comfortably appointed meeting rooms.

It is necessary, however, that we make reservations for you at the Allerton House no later than Monday, January 5. By that date we must provide them with a list of those who intend to remain at Allerton House for either or both nights as well as a list of individuals desiring meals there. Individuals arriving on Wednesday evening, January 7, will be housed and fed if they wish to be.

The cost for both room and board at Allerton House amounts to $9.00 a day.

A number indicated that they would be interested in discussing certain phases of their research at this meeting. However, it would be useful to this committee if you would indicate the individuals and their general topics so that we can arrange some kind of an informal program or agenda. Accordingly, will you please inform us soon about this?

It appears that a Thursday morning meeting (January 8) is desirable. Therefore, it would be desirable that all participants be at Allerton House by 9:30 a.m. Thursday, January 8. Individuals arriving from some distance might find it desirable to arrive Wednesday evening. We have included a map which will enable you to drive to Allerton House. If you plan to fly in or arrive by train, we shall be glad to pick you up on arrival.

Also attached, you will find a form which can help us materially in planning for a successful meeting. Therefore, will you please fill this in and return it at your earliest convenience. Note, however, that we must have your reservation by Monday, January 6.

Alex

D. E. Alexander, Assoc. Prof.,
Plant Genetics

DEA:clk

Enc.

TENTATIVE PROGRAM

Maize Genetics Conference
Allerton Park
January 8-9, 1959

Thursday, A.M., January 8 (Conversion and Paramutation)

9:30 - 10:00	Coe
10:00 - 10:45	Brink
10:45 - 11:00	Break
11:00 - 11:45	Blackman and Ashman

Thursday, P.M., January 8 (Mutation)

1:30 - 3:00	Kramer, Hsu
3:00 - 2:30	Break
3:30 - 5:00	Nuffer, Nelson

Thursday Evening, January 8

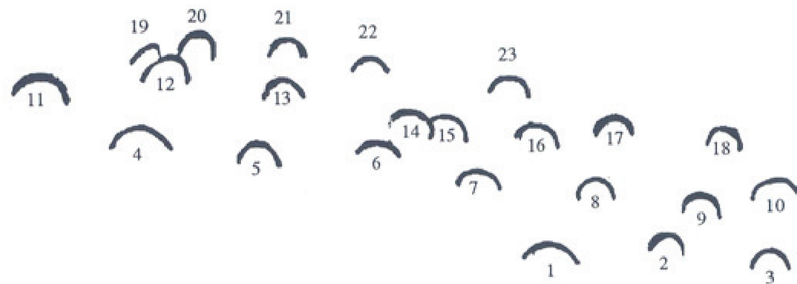
1. Organizational meeting
2. Maize Genetics Coop. Business

Friday, A.M., January 9 (Complex Loci)

9:00 - 10:15	Laughnan, Peterson, Sarma
10:15 - 10:30	Break
10:30 - 11:00	Richardson
11:00 - 11:30	Emmerling

Friday, P.M., January 9 (Cytogenetics)

1:15 - 2:00	Burnham
2:00 - 3:00	Rhoades, Dempsey
3:00 - 3:15	Break
3:15 - 3:45	Alexander
3:45 - 4:15	Doyle



1. S.K. Sinha; 2. Ed Coe; 3. Ikuko Misukami; 4. H.H. Kramer; 5. Maggie Emerling; 6. Marcus Rhoades; 7. Jack Beckett; 8. Ralph Singleton; 9. Margaret Blackwood; 10. Dwayne Richardson; 11. Shu; 12. George Hanson; 13. D.E. Alexander; 14. Charles Burnham; 15. Helen Peterson; 16. Don Robertson; 17. Alexander Brink; 18. ???????????????????; 19. Ellen Dempsey ? ; 20. John Laughnan; 21. Robert Jugenheimer; 22. Gerry Neuffer; 23. ????????

Jack Axtell

Oscar Miller

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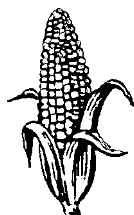
VII. MAIZE GENETICS COOPERATION STOCK CENTER



Maize Genetics Cooperation • Stock Center

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

&

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3,795 seed samples have been supplied in response to 287 requests, as of December 18, for 2007. A total of 86 requests were received from 22 foreign countries. 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 Inflorescence Project EMS lines, and Chromatin stocks.

Approximately 6.2 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. 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, probably due to it making the kernels more difficult to pull out of the damp compacted soil. Subsequent rainfall seemed to solve the problem and only a few rows were lost. Warm spring weather resulted in rapid germination and plant growth, and we had an earlier than normal pollination season. However, mild drought conditions necessitated supplemental irrigation prior to pollination. Moderate temperatures and low plant stress during and following pollination resulted in excellent yields.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of Joe Colasanti (*id1-m1*), Jerry Kermicle (various *r1* alleles), Major Goodman (rare isozyme lines), Frank Hochholdinger (*rtcs1*), Robert Lambert (*orange endosperm1*), Rob Martienssen (MTM material), Damon Lisch (*Mu-killer*), Gerry Neuffer (recent EMS-induced mutants), the North Central Regional Plant Introduction Station (*Lfy1*), 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.

2. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We identified additional alleles of *albescent1*, *Factor Cuna* (*Fcu*), *waxy1*, *shrunken2, viviparous5*, and *pale yellow9*. In 2008, we plan to test additional members of the viviparous, spotted leaf, and pale endosperm classes of mutants. In this manner, we hope to incorporate more stocks from our vast collection of unplaced uncharacterized mutants into the main collection.

3. Occasionally, requestors bring to our attention stocks that do not carry the traits they are purported to carry. We devote field space each year to analyzing these stocks, fixing or enhancing those we can, and soliciting replacements from researchers for those we can't. In those rare instances in which a particular variation or combination of variations cannot be recovered, we modify our catalog to reflect this.

4. We 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.

5. In the 'Phenotype Only' collection, we have made available an additional 48 new stocks since November 2006. Most of these are from the Primary Neuffer Collection sent to us by Gerry Neuffer in 1996 and 1997. This brings us down to 119 stocks we are still trying to recover from the original sources. Of the 234 stocks in this collection we had been unable to recover from Dr. Neuffer's sources, we received new sources for 20 of them in 2007 from the collection of Ed Coe.

6. Two acres were devoted to the propagation of the large collection of cytological variants, including A-A translocation stocks and inversions. Additional translocations received from W. R. Findley and Don Robertson marked with *wx1*, *y1*, *su1* and *o2* were added to our collection. For those marked with *wx1* we conducted linkage tests to confirm the chromosome arms involved. We were also able to recover several old translocations thought to be lost. These were recovered from E. G. Anderson sources and listed in MaizeGDB in 2007.

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. Approximately 2300 kernels of M1 families that were produced in 2006 were grown to produce M2 lines and for the observation of dominant mutations. From these plants, 800 M2 generation ears were produced and 5 adult plant dominant mutations were observed and pollinated. These 800 families will be planted for observation of recessive plant phenotypes in 2008.

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 a reduced winter crop last year due to spray damage, but received an adequate increase of most lines. This situation was brought

to the field manager's attention, and all indications are that we will have a good crop this year. We plan to continue growing our winter nurseries at this location.

We have received 265 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 seven lines from the Functional Genomics of Maize Chromatin project (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0421619) from Karen McGinnis. The 510 'Phenotype Only' Stocks from the UniforMu project received from Don McCarty/Mark Settles in 2004 and 2005 have been assigned stock numbers and permanent drawer locations. In addition, there are 27 new Sequence Indexed stocks from Mark Settles available, and 2,072 Maize TILLING stocks produced by Cliff Weil and Rita Monde.

The 265 lines from Gerry Neuffer's EMS material were screened for ear and kernel mutations in the lab, but were not planted in observation fields on the University of Illinois Crop Science Research facility. These lines, in addition to the 800 produced in Illinois in 2007, will be planted for phenotypic observation in 2008.

Our IT specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our collection. They are also used for our internal database (e.g., inventory, pedigrees, requests). A new tool for pedigree data entry is in the final stage of development and should greatly cut down the amount of time required to enter pedigree data. Work towards easier and faster generation of field notes and harvest tags is ongoing. Our web site has also been updated (http://www.uiuc.edu/ph/www/maize).

Additional samples of stocks will be sent this winter to the National Center for Genetic Resources Preservation in Fort Collins, Colorado for backup. These represent 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 for our second winter crop. The space has proven to be excellent for growing material that doesn't do well under our field conditions. Our new seed storage space (which doubles our capacity) was completed in March 2007. We presently have 476 seed storage drawers of the 1,584 the room will eventually hold (pending funding).

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 MNL81
(For a complete list of our stocks, see: http://maizegdb.org/cgi-bin/stockcatalog.cgi)

CHROMOSOME 1 MARKERS
115A rth1-1
131G id1-CSH::Ds2
131H rtcs1-1

CHROMOSOME 3 MARKERS
301B d1-4
301G Lfy1
305B K3L; High Loss; B chromosomes absent
305C tac2-116; bz2-m

CHROMOSOME 5 MARKERS
505G vp15-DR1126
505H vp15-MJ7546
505I vp15-umu1

CHROMOSOME 7 MARKERS
703CA o2-m(r)

CHROMOSOME 9 MARKERS
901F DfK9S(33166) C1

CHROMOSOME 10 MARKERS
X08J vp10-mu2
X08K vp10-4
X12F DfK10(C) R1
X12G DfK10(F) R1
X12H DfK10(H) R1
X12J DfK10(K) R1
X12K DfK10-II(M) r1
X12L DfK10-II(Q) r1
X12M High Loss with B Chromosomes; R1 K10-I
X15A r1 Hopi1-Brink; Pl1

MULTIPLE GENES
M541Q phyB1-563 PhyB2
M541R PhyB1 phyB2-F2
M541S phyB1-563 phyB2-F2
M541T PhyB1 PhyB2

TOOLKIT
T0318AK cms-T; ig1 R1-nj
T3301-56 bti03545::Ac
T3301-57 bti03702::Ac
T3301-58 bti04066::Ac
T3301-59 mon00030::Ac
T3301-60 mon00108::Ac
T3301-61 mon00218::Ac

STOCKS CHARACTERIZED ONLY BY PHENOTYPE

anthocyanin
3611E atc*-N2350

colorless floury
3604A clf*-N758D

dwarf plant
4407C d*-N2373B

golden plant
6008C g*-PI262475

miniature kernel
438-14 mn*-MTM12286
638-56 mn*-MTM22805

narrow leaf
3911K nl*-N812A

necrotic leaf
4104P nec*-N2267

opaque endosperm
3906F o*-N1288

pigmy plant
4408C py*-N2270

small kernel
4002G smk*-N1066

torn leaf
4107U trn*-N2298

white striped seedling
6004B str*-56-3012-10

yellow green leaf
4309K yg*-N2373C

yellow streak leaf
3812R ysk*-N2417

yellow stripe leaf
3812N ys*-N2379

Additionally, we received 2,072 stocks from the Maize TILLING project and additional stocks from other maize genome projects.

VIII. MAIZE GENETICS AND GENOMICS DATABASE

(www.maizegdb.org)

The five-year plan for MaizeGDB is under review in early 2008 by the ARS peer review process. The plan was developed with inputs from our Working Group and the Allerton 2007 Report (MNL 82:111-117). The primary objective is to integrate new maize genetic and genomic data in the database, specifically: (1) expand mutant and phenotype data and tools; (2) expand structural and genetic map sets, notably integration of the IBM genetic maps with the B73 genome sequence; (3) creation of views that convey the substantial variation in maize genome structure; (4) integration of next-generation genetic maps being generated by the Maize Diversity Project into a genomic view to enable its effective use by plant breeders; (5) provide access to gene models calculated by leading gene structure prediction groups; and (6) compile and make accessible at MaizeGDB this Newsletter. A second objective is to provide community support activities, such as lending help to the community of maize researchers to ensure their data can be made available through MaizeGDB; coordinating annual meetings; and conducting elections and surveys.

Data Highlights for 2007

Genetic maps: The NAM (nested association maps) maps, based on SNP (single nucleotide polymorphism) genotyping from the Maize Diversity Project are in process; all coordinates have been entered with links to allele alignment displays at www.panzea.org. Full documentation will include probe details, map scores for 26 mapping populations, and allele information, with links to GRIN, GenBank and Panzea. Maps anticipated in the near future include the ISU Map7 (Indel) maps from Pat Schnable and an updated Genetic Map (MNL 82:87-102) prepared by Ed Coe. Neighbors software has been updated, in collaboration with Hector Sanchez-Villeda and will facilitate updating this Consensus Map Product in the future.

New links: MaizeGDB now maintains deep links to www.panzea.org for allele alignments and to www.maizesquence.org for contigs and sequenced BACs. NCBI now links RefSeq and Gene records to MaizeGDB locus records, and has imported official names, full names, synonyms and probes.

Mutants and phenotypes. We are processing data from the EMS populations generated by the Maize Inflorescence Architecture Project (~4700 stocks), and The RescuMu population generated by the Maize Gene Discovery Project (~42,000 stocks). These data utilize the Plant Ontology terms, and regular updates of our associations to genes and stocks are supplied to that project, www.plantontology.org.

Data entry templates. We have customized spreadsheet templates for import of data from the Maize Tilling Project and the Maize Chromatin Project. Similar datasets should be able to use these templates.

Outreach

Nov 2007 was our first trip to an off-site location, the University of Florida, to provide a tutorial and explore data access issues. It was well received by persons at that site and we plan up to 3 such visits each year. Persons interested in scheduling a trip to their location should contact Lisa Harper or Carolyn Lawrence. Presentations in 2007 included the Plant and Animal Genome XV; Allerton & Maize Genetics Conference; GMOD meeting; Genome; 2nd Int. Biocurator Meeting; and Maize Crop Germplasm Committee. MaizeGDB hosted an exhibit booth at PAG XVI 2008; the Plant Genome DB Outreach Consortium at that involved 10 database groups in total.

Editorial Board

We have initiated and currently maintain an Editorial Board whose members contribute a paper each month to be highlighted at MaizeGDB. The 2008 Board consists of: Marja Timmermans (Chief), Kelly Dawe, Jim Holland, Mike Kolomiets and Damon Lisch. For full details go to http://www.maizegdb.org/editorial_board.php.

Stakeholder Input

In addition to the outreach activity referred to above, we respond quickly, typically within 24 h, to direct inputs by email, phone or comments submitted from each page in the database. The Maize Genetics Executive Committee (MGEC) and our Working Group provide oversight and work with us on developing surveys of the community. Most recently the MGEC surveyed the community preferences for a Genome Browser. The results support use of GBrowse, an open source software in use by many model organism databases, including TAIR (Arabidopsis).

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. At this time we have subcontracts from two NSF-funded projects: (1) Construction of Comprehensive Sequence Transposon Resources for Maize with Don McCarty as PI; and (2) The Grass Regulome Initiative: Integrating control of gene expression and agronomic traits across the grasses", with Erich Grotewold as PI.

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.

Acknowledgements

We are deeply grateful to our Working Group: Tom Slezak (Chief), Ed Buckler, Karen Cone, Mike Freeling, Owen Hoekenga, Lukas Mueller, Pat Schnable, Anne Sylvester, Doreen Ware and, Ex Officio, Volker Brendel and Marty Sachs. We will miss Tom Slezak, who retires from this group, and welcome his replacement, Mihai Pop, for the coming year. Most recently, the group met with MaizeGDB staff and observers during the Plant and Animal Genome XVI meetings in San Diego, CA. For more details see http://www.maizegdb.org/working_group.php.

Submitted by the MaizeGDB staff January 29, 2008
Mary Schaeffer
Darwin Campbell
Trent Seigfried
Lisa Harper
Taner Sen
Carolyn Lawrence (PI)

IX. A STANDARD FOR MAIZE GENETICS NOMENCLATURE

(From MNL 69:182-184 (1995), as updated Sep 1996; Apr 2000; Apr 2002; Oct 2006.)

PREAMBLE: We wish to have a system that is consistent, compatible with the historical background of maize genetics (insofar as these two goals can be reconciled), is easily understood by plant geneticists working with other species, and forms the basis for the importation of maize data into a general plant genetics database so that the basic knowledge concerning maize genes is available to researchers with other species and vice versa. We believe that this goal is best implemented by the researchers in each species having their own working vocabulary, while the identification of genes that catalyze the same functions in all species should rely on entry into a relational database of the genes' function as an E.C. number (2.4.1.13), trivial name (sucrose synthase), and systematic name (UDPglucose:D-fructose 2-glucosyltransferase). The situation can be less completely categorized for genes whose products are transcription factors, structural proteins, storage proteins, etc.

If one accepts the premise outlined above that the common ground between species need not reside in the working vocabulary of geneticists using any species as a model system but in the manner in which their data are expressed in the database, then the previously adopted names for maize genes can be retained. It will not be necessary to rename the genes previously named on the basis of the mutant phenotype produced as soon as the function of the nonmutant alleles becomes known, but we should proceed to define more precisely words or terms whose meanings need clarification and to decide how we wish to deal with the new information becoming available.

1. DEFINITIONS: The words "locus" and "gene" should not be treated as synonymous. A locus can be defined as "a chromosomal site of variable size at or within which is located a gene, a restriction site, a knob, a breakpoint, an insertion, or other distinguishable feature". This necessitates specifying whether we mean a gene locus or an RFLP locus, etc. We can then define a plant gene as "a DNA sequence of which a segment is regularly or conditionally transcribed at some time in either or both generations of the plant. The DNA is understood to include not only the exons and introns of the structural gene but the cis 5' and 3' regions in which a sequence change can affect gene expression". This treats the gene as a functionally defined entity that is not circumscribed by the transcribed region or other fixed limits.

2. ANONYMOUS TRANSCRIPTS[1]: For most of the history of genetics, the existence of a gene was recognized when a mutation occurred, and the gene was then named by a word/term that was descriptive of the mutant phenotype. That will continue to be the practice except with isozyme markers, for which the designation will be the enzyme in question, or the instances in which the biochemical lesion responsible for the mutant phenotype is identified before the locus is reported. The loci of these genes have then been placed on chromosome maps in relation to other mapped loci. However, we now have the possibility of recognizing genes in which no mutation has been detected through the construction of cDNA libraries. These anonymous cDNAs are often used as probes in RFLP mapping. When such a probe hybridizes to a single band, it is clear that the RFLP loci circumscribe the transcriptional unit that encodes the message represented by the cDNA, and these RFLP loci with other RFLP loci can be used as the basis for mapping the gene. Mapping a locus in this fashion is encouraged as a means of obtaining maximum coverage of the genome. As long as the locus retains an anonymous status (unknown function or no mutant phenotype), the symbol for the locus should be assigned according to the convention used for RFLP loci (as umc148, see Section 8). Further information about the probe and its derivation is best provided in tabular or database form rather than in the symbol itself.

A gene name identifying function for a locus detected with a cloned sequence should be given only when there is unambiguous evidence that this is the site by which that function is encoded. Particular caution should be taken in identifying genes (and their function) from several RFLPs hybridizing to a gene-specific probe from another organism. Until a sequence has been shown to encode the function in question, the gene designation should be that of an RFLP locus (see Section 8).

3. STANDARD NOMENCLATURE AND SYMBOLS: The names and symbols that have been used for maize genes should be retained. The name and symbol of a gene locus should be represented with lower-case, italic characters (*defective kernel12, dek12*). Note that no hyphen separates the gene name from a numerical suffix, which is a change from previous usage. We use a hyphen in the case of mutant alleles to separate the allele designation from a suffix specifying the particular allele (see Section 5). We advocate strongly that all genes identified in the future be given a three letter symbol. Newly detected maize genes that have been previously identified in other plant species should be named where appropriate (see the last paragraph in Section 2) with reference to the list of generic names compiled by the Commission on Plant Gene Nomenclature.

When designating homozygous genotypes with two or more unlinked genes, the genes are separated by semicolons, e.g., *a1;a2;c1;c2;r*. If linked, the genes are separated by spaces, e.g., *C1 sh1 bz1 Wx1*. Heterozygous genotypes should be written with a slash separating the sets of linked genes, e.g., *C1 Bz1/c1 bz1*. If the genes are unlinked, the proper designation is *Sh2/sh2; Bt2/bt2*.

4. LOCI WITH THE SAME GENE NAME: Where we have more than one nonallelic mutant with the same gene name, the earlier recommendation was that the first one to receive that name should not have a numerical suffix but the second has *2* as a suffix. Thus we have *shrunken* (*sh*), *shrunken2* (*sh2*), and *shrunken4* (*sh4*) mutants. Geneticists outside the maize community are apt to misinterpret this convention. We recommend that we be consistent and write *shrunken1* or *sh1* and advocate that even if a new locus is identified and given a unique name, it be designated as *1*. This has the definite advantage in maintaining databases and indices that no retrospective correction would be necessary if a second gene locus receives the same designation.

5. ALLELIC DESIGNATIONS[2]: Where a mutant allele is recessive, it should be designated by an italicized symbol (lower case) as *dek12*, which is the same as the symbol of the locus. Since it is unlikely that any two mutant or nonmutant alleles in a highly polymorphic

species such as maize have identical sequences, maize geneticists are encouraged to specify the particular allele with which they are working (see in this Section, Alleles of Independent Mutational Origin and Designation of Nonmutant Alleles). The symbol for dominant, nonmutant (i.e., conditioning a normal phenotype) alleles will be the same italicized three letter symbol as the mutant alleles but with the first letter capitalized (*Dek12*). The symbol of the gene product should not be italicized and should be written with all letters capitalized (e.g., ADH1). The name of the gene product (alcohol dehydrogenase) should neither be capitalized nor italicized.

When the mutant alleles of a gene are dominant, the first letter of the mutant symbol is capitalized. The nonmutant symbol has all the letters lower case. For example, the *corn grass1* (*cg1*) gene locus has several dominant mutant *(Cg1*) alleles as well as nonmutant (*cg1*) alleles. The reference mutant allele is designated as *Cg1-R* or *-1*.

Codominant alleles such as isozymes where the variants are functional and distinguished from each other by electrophoretic mobility, should be designated by symbols with the first letter capitalized and identified by allelic specifications as *Pgm2-5* or *Pgm2-7*.

ALLELES OF INDEPENDENT MUTATIONAL ORIGIN: The unambiguous designation of mutant alleles that have arisen as independent mutational events is increasingly important. It is generally understood that a gene symbol followed by a hyphen plus a letter or number(s) specifies a particular recessive allele at that gene locus. We have referred to the mutation by which the gene was identified as the reference allele; e.g., *bz1-Ref* or *bz1-R*. It is equally appropriate to refer to that allele as *bz1-1*. The mutations in any gene that were identified subsequently have been categorized in various idiosyncratic ways. Alleles that have arisen by independent mutational events have been designated by letters, numbers, a letter plus numbers, the name of the inbred in which the mutation occurred, and sometimes all of these applied to a group of alleles at a gene locus. While all of these designations served the purpose of indicating that these alleles had independent mutational origins, there is a clear advantage to greater standardization. As in the 1973 Nomenclature Standard, it is recommended that new alleles be identified by a laboratory number that might indicate the year of isolation as *sh2-6801*. This has the definite advantage that two laboratories are unlikely to designate two new mutations of the same gene by the same number. However, if two laboratories are targeting the same locus in mutagenesis experiments, they should consult before naming their new alleles to avoid giving the same designation to different alleles. Also recommended is the convention of referring to a new mutation of a given phenotype by a provisional designation as bt-lab number until it is ascertained whether the mutant is a new allele of a known gene or identifies a previously unidentified gene. In the first instance, the proper gene symbol *(bt1 or sh2*) replaces *bt*, but the lab number is retained (e.g., *bt1-8711*). In the second instance (a previously unidentified locus), a new gene name and symbol would be selected, and this mutant would become the reference allele *(-R or -1*).

When mutant alleles are referred to in the generic sense without specification of their origin, a hyphen without further designation (e.g., *bz1-, dek12-)* is desirable to make it clear that one is referring to an allele or alleles, not the gene locus.

DESIGNATION OF NONMUTANT ALLELES: Since it is now apparent that in a species as polymorphic as maize, nonmutant alleles from different sources are apt to have a number of sequence differences one from the other, and these differences can be reflected in gene action (nonmutant isoalleles), it is desirable to specify the nonmutant allele being investigated or used as a control. Incorporating the name of the inbred as part of the allelic designation, *Bz1-W22*, is an appropriate method of doing this. However, mutant alleles should not be designated by the inbred in which they arose (e.g., *bz1-W22*) to avoid confusion with the progenitor allele. Also, there may eventually be numerous mutant alleles of a particular gene isolated in that inbred if a researcher uses that inbred in a mutagenesis experiment. A particular nonmutant allele may be found in an exotic race or other accession that is not an inbred. A unique designator (e.g., a PI number or Bolivia #) should be part of the allelic designation.

RFLPs AND RAPDs AS ALLELES: The presence or absence of a restriction site or a primer-amplifiable sequence at a particular locus represent Mendelian alternatives. They fall under the broadest definition of an allele, and it is appropriate to refer to these alternatives as alleles as has already been done in some reports.

6. NAMING DELETIONS: When it is clear that a mutation results from a deletion that has removed all or part of two gene loci, it would be appropriate to indicate this in the following manner. For *an1-6923*, this would be *def(an1..bz2)-6923*, and for *sh-bz-X2*, *def(bz1..sh1)-X2*. When molecular evidence indicates that a deletion has removed all of the structural portion of a gene as is true of *wx1-C34*, it should be indicated in the same manner; i.e., *def(wx1)-C34*.

7. MUTATIONS RESULTING FROM TRANSPOSABLE ELEMENT INSERTIONS: There is one further point concerning allelic specification. Maize in particular has many mutable alleles resulting from the insertion of a transposable element. These have been designated by the mutant symbol, a hyphen, a lower case "m", and an isolation number; e.g., *wx-m1*. When the transposable element insertion *[Ac, Ds, Spm(En), dSpm(I), Mu1..MuX, etc.*] is known, it is suggested that this be indicated by a double colon following the allele as *wx-m1::Ds1*. Since a maize stock may have more than one transposable element family active at the same time, firm genetic and/or molecular evidence is necessary to ascribe mutability to a particular transposable element family. Further, mutable alleles generate both stable nonmutant and stable mutant alleles when the transposable element excises from the gene locus. Since the mutant derivatives are certain to differ in sequence from the nonmutant progenitor allele around the site of the transposable element insertion and the nonmutant derivatives are very likely to differ at that site, researchers should be certain to indicate the origin of such alleles in their reports. One means of doing this is to indicate such an origin by an apostrophe following the locus symbol as *Bz1'-7801* or *bz1'-8905.* The specifics of its origin including the transposable element involved could then be included in the text and entered in the Maize Genome Database. Since transpositions of a transposable element from a site within a gene often insert in locations where they have no phenotypic effect but can be useful markers, it is desirable to have a standard to refer to such insertions. Designate them as RFLP's would be designated (see Section 8), but follow the institutional symbol and number with a double colon and the symbol of the transposable element (e.g., *dnap2094::Ac*).

8. NAMING RFLPs AND RAPDS: In naming RFLPs and RAPDs, use a lower case three or four letter code designating the originating university or company followed by a laboratory number (no space between the code and the number). When the probe used is a cDNA or a subclone of a gene, the gene symbol should be added in parentheses after the RFLP locus designation, as *umc000(a1).* Since a probe not infrequently recognizes RFLPs on two or more chromosomes, these should be designated by the same institutional code, number, and probe followed immediately by A, or B, or C. In so far as possible, the locus with the strongest hybridization should be designated A and the more weakly hybridizing loci be designated B, C etc. in descending order of signal strength.

9. CHROMOSOME REARRANGEMENTS: The conventions for dealing with chromosomal rearrangements are well established and adequate for the purpose. To designate particular reciprocal translocations as T1-2a or T1-9(4995) etc. with the breakpoints noted parenthetically or in a table of supporting information is explicit and sufficient. Additional information (the fact that the translocation stock is homozygous for *wx1*) can be incorporated by prefacing the translocation number with the gene symbol as the Coop does in its stock lists (e.g., *wx1* T1-9c). Translocations with B chromosomes have designations that indicate the arm of the A chromosome involved (L or S) as well as a lower case letter distinguishing that translocation from any others involving that particular chromosome arm, as TB-5Sc. The cytological breakpoint in the A chromosome as well as the loci uncovered when the TB translocation is used as a male parent can be noted in the text or in a table of supplementary information. The designations for inversions (e.g., Inv9b again with the breakpoints, 9S.05-L.87, listed in a supporting table) are succinct and convey the necessary information.

10. ORGANELLAR GENES: For chloroplast and mitochondrial genes, we accept for the present the proposals already in place. For chloroplast genes, this is Hallick and Bottomley, Plant Mol. Biol. Rep. 1(4):38-43, 1983, as updated at SwissProt <http://www.expasy.ch/cgi-bin/lists?plastid.txt> or by the Chloroplast working group for the Commission on Plant Gene Nomenclature. For mitochondrial genes, this is Lonsdale and Leaver, Ibid. 6(2):14-21, 1988, updated by the Mitochondrion working group for the Commission on Plant Gene Nomenclature. For brevity's sake, these are not summarized here.

11. TRANSCRIPTION FACTORS: (Oct 2006 addition) We define here TFs as proteins that contain a DNA-binding domain and that fall within one of the families described in http://arabidopsis.med.ohio-state.edu/AtTFDB/.

There is currently no coherent effort in maize for a rational and organized naming of transcription factors (TFs). The use of GenBank accession numbers, EST names or locus identifiers provides an impractical mechanism, which often leads to ambiguities, for example because of multiple entries in GenBank or of several ESTs for the same protein. Thus, we propose here to create a uniform nomenclature for maize TFs, following the lead from *Arabidopsis*. A similar proposal is being adopted by the TIGR rice annotation group and by the SUCEST-FUN sugarcane annotation group.

Recommendation: GENE PRODUCTS: Each transcription factor will have an organism identifier (Zm) to be used only in the context of other organisms, followed by letters that represent the TF family (e.g., MYB, bHLH, HD, bZIP) and by a number that will start with '1'. A similar strategy is currently being applied to other maize gene families (e.g., the kinesins, see Lawrence et al., J. Cell Biol. 167:19-22). Since we realize that many TFs are known by their genetic names, this nomenclature will permit the use of synonyms. For example, KNOTTED could be named HD1(KN) (or ZmHD1(KN) when being compared to HDs of other species) and C1 would be MYB1(C1) (or ZmMYB1(C1)). In addition, whenever possible, we will try to have the numbers provide a historic perspective of which TFs have been first identified. In that regard, since KN and C1 correspond to the founding members of their respective families in maize, they are assigned the number '1'. Prior genetic nomenclature will be incorporated in the database.

GENES: Existing names for genes encoding TFs will not be altered. If necessary, and only as a way to provide coherence with the naming of the gene products, the synonym strategy described above would be used. In that regard, *c1* would continue to be *c1* but could also be cross-referenced as *c1(myb1)*. New genes will be named according to their products. If mutant phenotypes are identified at a later date, gene names derived from mutant phenotypes will be added as synonyms, but the original name will not be changed. As indicated for the gene products, the use of the prefix *Zm* in front of the gene's name will only be used when comparing maize genes with related genes from other species (e.g., *Zm myb1*).

For generating a position for transcription factors, Erich Grotewold served on the Nomenclature Committee in an ad hoc capacity.

12. CLEARING HOUSE FOR NOMENCLATURE: We also believe that it is desirable to initiate a clearing house for maize nomenclature so that a researcher wishing to name a recently identified gene can ascertain almost immediately that no one has used the proposed designation and symbol. This clearing house can, in principle, function through the MaizeGDB <http://www.maizegdb.org/>, which will be refereed by a cooperator. The same facility could be used to insure that allelic designations are not duplicated or to answer questions concerning nomenclature.

Current Members Include: Hugo Dooner, chair; Tom Brutnell; Vicki Chandler; Curt Hannah; Toby Kellogg; Marty Sachs; Mike Scanlon; Mary Schaeffer (Polacco); Philip Stinard.

Footnotes:

[1]ANONYMOUS TRANSCRIPTS: 1996 decision made not to utilize the parenthetic 'gfu' designation for "gene, function unknown". RATIONALE: in common usage, the 'gfu' suffix has proven confusing, implying 'known function', especially to researchers from other species. The confusion arises from the practice in RFLP naming to include parenthetic acronyms where sites are detected by probes with an assigned or putative identity with a particular gene product.

[2]ALLELIC DESIGNATIONS: 1996 decision made to use '-', rather than '+', in designations of non-mutant alleles. RATIONALE: use of '+' has met with resistance by journal editors; definition of non-mutant alleles can be a grey area.

X. GENETIC MAPS 2007

The following maps are based on the high-resolution framework of the IBM2 map, aided by physical mapping. The loci shown are genes that have been defined by function or phenotype. All of the accompanying markers on IBM2, and the core bin markers, will be included in maps that are to be posted in MaizeGDB, as will supporting data notes. The IBM coordinates have been adjusted to represent conventional cM by dividing by 4, following the close approximation for short intervals per Winkler et al. (Genetics 164:741, 2003), which the present analysis has found to bear markedly close relationship to conventional classical mapping, e.g., with testcross data.

The maps were developed as follows:

1. All loci mapped on the IBM population itself, which included some genes, served as the main framework. See IBM2 maps in MaizeGDB.
2. Genes for which base sequences existed, which could be uniquely placed by BLAST matching to a Cornsensus overgo in the NCBI nr database, were added to the framework based on the physical map location in IBM2 FPC0507 maps, supported by other mapping data whenever possible. Matching BACs in the NCBI htgs database were sought in AGOL WebFPC contigs and were used to define location relative to neighboring loci within the same contig in IBM2 FPC0507.
3. Genes matching Genoplante gpm, ISU IDP, and UMC Indel/SNP sequences that could be identified by BLAST were added based on IBM2 FPC0507.
4. For genes lacking sequences, or not otherwise placed, recombination data and map locations from other populations were employed in relation to common markers. Orders and distances were estimated and loci were added for which uncertainty was no greater than 4 cM. Less-certain loci are not included in these maps.
5. Core bin markers lacking in IBM2, and the centromeres, were placed as in (2) and (4) above.

Again, the complete maps are to be posted in MaizeGDB soon. I need and request your FEEDBACK, now or after, on the gene placements and content. IS YOUR GENE MISSING? I hope to make further updates as new data are reported and to keep the map dynamic as new research, sequencing, and annotation advance. It is worth nothing that, up to the present stage of sequencing, fully 96% of 1,448 sequenced genes could be placed reliably onto a sequenced BAC.

Ed Coe, December 2007

KEY TO SYMBOLS

Symbol	Full Name
a	anthocyanin
aaa	adenosylmethionine aminotransferase
aap	acylaminoacyl-peptidase
abc	ABC(yeast) homolog
abp	auxin binding protein
acc	acetyl-CoA carboxylase
aco	aconitase
acp	acid phosphatase
acpt	acyl carrier protein
act	actin
ad	adherent
adh	alcohol dehydrogenase
adk	adenylate kinase
ae	amylose extender
ago	argonaute
agp	ADP glucose pyrophosphorylase large subunit embryo
agpsl	ADP glucose pyrophosphorylase small subunit leaf
ahh	adenosyl homocysteine hydrolase
aic	auxin import carrier
akh	aspartate kinase homoserine dehydrogenase
akr	aldo/keto reductase AKR
al	albescent plant
ald	aldolase
aldh	aldehyde dehydrogenase
alp	aluminum-induced protein homolog

Symbol	Full Name
als	acetolactate synthase
amo	amine oxidase
amp	aminopeptidase
amy	alpha amylase
amy	beta amylase
an	anther ear
an	kaurene synthase
ane	androgenic embryo
anl	anthocyaninless lethal
ant	adenine nucleotide translocator
aoc	allene oxide cyclase
aos	allene oxide synthase
aox	alternative oxidase
ap	clathrin coat assembly protein AP1
aprl	adenosine 5'-phosphosulfate reductase
apx	ascorbate peroxidase
ar	argentia
arf	ADP-ribosylation factor homolog
as	asynaptic
ask	aspartate kinase
asn	Zea asparagine synthetase homolog
atp	ATP synthase
atpc	ATP synthase gamma subunit
b	colored plant
ba	barren stalk
baf	barren stalk fastigiate

Symbol	Full Name
bap	basal layer antifungal protein
bas	beta alanine synthase
bd	branched silkless
bet	glycinebetaine
betl	basal endosperm transfer layer
bf	blue fluorescent
bgaf	beta glucosidase aggregating factor
bif	barren inflorescence
bip	Binding protein homolog
bk	brittle stalk
bm	brown midrib
bn	brown aleurone
br	brachytic
brk	brick
brn	brown kernel
bsd	bundle sheath defective
bss	bundle sheath strands specific
bt	brittle endosperm
btf	putative transcription factor
bv	brevis plant
bx	benzoxazin
bz	bronze
bzip	bZIP transcription factor
c	colored aleurone
caat	CAAT box binding protein
cad	cinnamyl alcohol dehydrogenase
caf	crs2 associated factor
cal	calmodulin
cas	cycloartenol synthase
cat	catalase
cax	calcium exchanger
cbp	calmodulin binding protein
ccp	cysteine protease
ccr	cytochrome c reductase
cdj	chaperone DNA J
cdpk	calcium dependent protein kinase
cef	CEFD homolog
cenH	centromeric histone H
cenpc	centromere protein C
cent	centromere chromosome
cesa	cellulose synthase
cg	corngrass
cgs	cystathionine gamma-synthase
ch	chocolate pericarp
chi	chalcone flavanone isomerase
chn	chitinase
chr	chromatin complex subunit A 10
chs	chitin synthase homolog
cka	CK2 protein kinase alpha
cko	cytokinin oxidase
cks	CMP-KDO synthetase
cl	chlorophyll
cld	cold regulated protein homolog
clx	calnexin homolog
cncr	cinnamoyl CoA reductase
cop	coatomer protein

Symbol	Full Name
cp	collapsed kernel
cpn	chaperonin
cpx	coproporphyrinogen III oxidase
cr	crinkly
crp	chloroplast RNA processing
crr	cytokinin response regulator
crs	chloroplast RNA splicing
crt	calreticulin
csy	chloroplast SecY-
ct	compact plant
cta	chitinase A
cx	catechol oxidase
cyc	cyclin
cyp	cytochrome P450
cys	cysteine synthase
czog	cis-zeatin O-glucosyltransferase
d	dwarf plant
dap	dappled aleurone
dar	monodehydroascorbate reductase
dba	DNA binding activity
dcl	dicer-like
dek	defective kernel
der	derlin
dfr	dihydroflavanoid reductase-like
dhn	dehydrin
dia	diaphorase
dmt	DNA methyl transferase
dnp	diphosphonucleotide phosphatase
dof	DNA binding with one finger
drh	DEAD box RNA helicase
dsc	discolored kernel
Dt	Dotted
dts	aspartyl-tRNA synthetase
du	dull endosperm
dxs	deoxy xylulose synthase
dzs	delta zein structural1
e	esterase
ebe	embryo-sac basal-endosperm-layer embryo-surrounding-region
ech	enoyl-CoA hydratase
ef	endosperm factor
eg	expanded glumes
eif	eucaryotic initiation factor
ein	ethylene insensitive
elfa	elongation factor alpha
elfg	elongation factor gamma
emp	empty pericarp
eno	enolase
enp	endopeptidase
eoh	E. coli origin of replication homolog
eps	enolpyruvylshikimate phosphate synthase
ers	ethylene receptor1-2
esp	embryo specific protein
esr	embryo surrounding region
et	etched
exg	exoglucanase

Symbol	Full Name
expa	alpha expansin
expb	beta expansin
f	fine stripe
fab	fatty acid biosynthesis
fad	fatty acid desaturase
fat	fatty acyl thioesterase
fcr	ferric-chelate reductase (NADH)
fdad	false DAD
fdh	formate dehydrogenase
fdx	ferredoxin
fea	fasciated ear
fer	ferritin
fgp	folylpolyglutamate synthetase
fht	flavanone 3-hydroxylase
fie	fertilization independent endosperm
fl	floury
fnr	ferredoxin NADP reductase
ftr	ferredoxin-thioredoxin
g	golden plant
ga	gametophyte factor
gar	gibberellin responsive
gbp	GTP binding protein
gcsh	glycine cleavage system protein H
gdcp	glycine decarboxylase
gdh	glutamic dehydrogenase
gl	glossy
glb	globulin
glct	glucose translocator
gln	glutamine synthetase
glu	beta glucosidase
gly	glycine
gn	gnarley
gol	goliath
got	glutamate-oxaloacetate transaminase
gpa	glyceraldehyde-3-phosphate dehydrogenase A
gpb	glyceraldehyde phosphate dehydrogenase B
gpc	glyceraldehyde-3-phosphate dehydrogenase C
gpdh	glucose-6-phosphate dehydrogenase
grf	general regulatory factor
grp	glycine-rich protein
grx	glutaredoxin homolog
gs	green stripe
gsh	gamma-glutamylcysteine synthetase
gsr	glutathione reductase
gst	glutathione-S-transferase
gtr	glutamyl-tRNA reductase
hag	histone acetyl transferase GNAT/MYST 10
hat	histone acetyltransferase
hb	hemoglobin
hcf	high chlorophyll fluorescence
hex	hexokinase
hfi	corn-activated Hageman factor inhibitor
hir	hypersensitive induced response
his	histone
hm	Helminthosporium carbonum susceptibility
hmg	high mobility group protein

Symbol	Full Name
hmga	high mobility group family A
hon	histone
hox	homeobox
hp	histidine-containing phosphotransfer protein
hrg	hydroxyproline rich glycoprotein
hsbp	heat shock factor binding protein
hsbp	herbicide safener binding protein
hscf	heat shock complementing factor
hsf	hairy sheath frayed
hsk	high-sulfur keratin homolog
hsp	heat shock protein
hstf	heat shock transcription factor
ht	Helminthosporium turcicum resistance
htn	Helminthosporium turcicum resistanceN
hyp	hybrid proline-rich protein
iaglu	indol-3-ylacetyl glucosyl transferase
ibp	initiator binding protein
icl	isocitrate lyase
id	indeterminate growth
idc	iron deficiency candidate
idh	isocitrate dehydrogenase
ids	indeterminate spikelet
ig	indeterminate gametophyte
igl	indole-3-glycerol phosphate lyase
ij	iojap striping
im30p	IM30 protein
imd	isopropylmalate dehydrogenase
imp	importin
in	intensifier
incw	invertase cell wall
irl	isoflavone reductase-like
isp	iron-sulfur protein
isr	inhibitor of striate
ivr	invertase
j	japonica striping
K6L	Knob on 6L
kik	kinase interacting kinase
kin	kinesin-like protein
kin	knotted1 induced
kn	knotted
knox	knotted related homeobox
kpp	kinase associated protein phosphatase
kri	ketol-acid reductoisomerase
krp	kinesin-like protein
l	luteus
la	lazy plant
lc	red leaf color
ldp	luminidependens protein
leg	legumin
lem	lethal embryo mutant
les	lesion
lg	liguleless
lhca	light harvesting complex A
lhcb	light harvesting chlorophyll a/b binding protein
li	lineate leaves
lip	low temperature-induced protein1

Symbol	Full Name
lls	lethal leaf spot
ln	linoleic acid
lo	lethal ovule
lon	LON peptidase
lop	lo1 pI allergen homolog
lox	lipoxygenase
lpa	low phytic acid
lpe	leaf permease
lrk	Ser/Thr receptor-like kinase
lrs	liguleless related sequence
ltk	leucine-rich transmembrane protein kinase
lw	lemon white
lxm	lax midrib
mac	multiple archesporial cells
mads	MADS
mas	malate synthase
mde	mouse DNA EBV homolog
mdh	malate dehydrogenase
mdm	maize dwarf mosaic virus resistance
mdr	maternal derepression of R
me	NADP malic enzyme
meg	maternally expressed gene
mek	MEK homolog
mez	enhancer of zeste
mgs	male gametophyte specific
mha	membrane H(+)-ATPase
mha	plasma-membrane H+ATPase
mip	major intrinsic membrane protein
mir	maize insect resistance
mlg	lea protein group
mlo	barley mlo defense gene homolog
mmm	modifier of mitochondrial malate dehydrogenases
mn	miniature seed
mop	mediator of paramutation
mpl	miniplant
mrp	Myb related protein
ms	male sterile
msf	mRNA splicing factor
mst	modifier of R1-st
msv	maize streak virus tolerance
mtl	metallothionein
mus	mismatch binding protein Mus
mus	MutS homolog
myb	myb transcription factor
myc	myc transcription factor
na	nana plant
nac	NaCl stress protein
nad	NADH dehydrogenase
nad	NADH ubiquinone oxidoreductase
nbp	nucleic acid binding protein
ndk	nucleotide diphosphate kinase
nec	necrotic
nii	nitrite reductase
nl	narrow leaf
nnr	nitrate reductase
nrt	nitrate transport

Symbol	Full Name
nrx	nucleoredoxin
ns	narrow sheath
ntf	nuclear transport factor
o	opaque endosperm
obf	octopine synthase binding factor
ocl	outer cell layer
odo	alpha keto dehydrogenase candidate
oec	oxygen evolving complex2
og	old gold stripe
ohp	opaque2 heterodimerizing protein
ole	oleosin
omt	Caffeoyl CoA O-methyltransferase
opr	12-oxo-phytodienoic acid reductase
orc	origin recognition complex
orp	orange pericarp
ost	oligosaccharide transferase
oy	oil yellow
p	pericarp color
pac	pale aleurone color
pal	phenylalanine ammonia lyase
pao	polyamine oxidase
parp	poly(ADP-ribose) polymerase
pbf	prolamin-box binding factor
pck	phosphoenolpyruvate carboxykinase homolog
pcna	proliferating cell nuclear antigen
pcr	protochlorophyllide reductase
pdc	pyruvate decarboxylase
pdh	pyruvate dehydrogenase
pdi	protein disulfide isomerase
pdlk	pyruvate dehydrogenase (lipoamide) kinase
pep	phosphoenolpyruvate carboxylase
pex	pollen extensin-like
pfk	phosphofructose kinase
pg	pale green
pgd	6-phosphogluconate dehydrogenase
pgm	phosphoglucomutase
phb	prohibitin
phi	phosphohexose isomerase
pho	phosphate regulatory homolog
phot	blue-light receptor phototropin
php	chloroplast phosphoprotein
phs	poor homologous synapsis
phyA	phytochromeA
phys	phytase
piip	physical impedance induced protein
pin	PIN-formed protein
pk	S-domain class receptor-like kinase
pki	protein kinase inhibitor
pl	purple plant
ploc	plastocyanin homolog
pls	phospholipid synthesis
plt	phospholipid transfer protein homolog
pm	pale midrib
pme	pectin methylesterase
pmg	phosphoglycerate mutase
po	polymitotic

Symbol	Full Name
pop	organelle permease
por	porin
ppi	peptidyl-prolyl isomerase
ppo	polyphenol oxidase
ppp	pyrophosphate-energized proton pump
ppr	pentricopeptide
ppt	plastid phosphate/phosphoenolpyruvate translocator
pr	red aleurone
prc	proteasome component
prf	profilin homolog
prg	pitted rough germless
prh	ser/thr protein phosphatase
prl	protease PrlC candidate
pro	proline responding
prp	pathogenesis-related protein
prr	putidaredoxin reductase homolog
ps	pink scutellum
psa	photosystem I reaction center
psad	photosystem I subunit d
psan	photosystem I subunit N
psbs	photosystem II subunit PsbS
psk	phytosulfokine peptide precursor
psy	phytoene synthase
pt	polytypic ear
ptd	pitted endosperm
ptk	receptor-like protein kinase
pur	pollen ubiquitin regulator
px	peroxidase
pyd	pale yellow deficiency
r	colored
ra	ramosa
rab	responsive to abscisic acid1
ras	ras related protein
rca	RUBISCO activase
rcm	rectifier
rcp	root cap protein
rcph	root-cap periphery
rd	reduced plant
ren	reduced endosperm
rf	restorer of fertility
rg	ragged leaves
rgd	ragged seedling
rgh	rough kernel
rhm	resistance to Helminthosporium maydis
rip	ribosome-inactivating protein
ris	iron-sulfur protein
rlc	rindless culm
rld	rolled leaf
roa	replication origin activator
rop	Rho-related protein
rp	resistance to Puccinia sorghi
rpl	60S ribosomal protein L1
rpo	RNA polymerase
rpot	RNA polymerase T phage-like
rpp	acidic ribosomal protein P
rpp	acidic ribosomal protein P4

Symbol	Full Name
rpp	resistance to Puccinia polysora and Puccinia sorghi
rps	40S ribosomal protein S2
rs	rough sheath
rt	rootless
rth	roothair defective
rtp	root preferential
rws	RNA recognition water-stress protein
rxo	reaction to X. oryzae
sad	shikimate dehydrogenase
sam	S-adenosyl methionine decarboxylase
saur	small auxin up RNA
sbe	starch branching enzyme
sbp	SBP-domain protein
sca	short chain alcohol dehydrogenase
sci	subtilisin-chymotrypsin inhibitor homolog
scl	scarecrow-like
sdg	SET domain group
sdh	sorbitol dehydrogenase homolog
sdw	semi-dwarf plant
se	sugary-enhancer
ser	seryl-tRNA synthetase
serk	somatic embryogenesis receptor-like kinase
sfb	SF1 binding protein candidate
sgo	shugosin centromeric cohesion
sh	shrunken
si	silky
sig	sigma-like factor
sk	silkless ears
sks	suppressor of KYS sterility
sl	slashed leaves
sm	salmon silks
smh	single myb histone
smt	sterol methyl transferase
sn	scutellar node color
snr	small nucleolar RNA
sod	superoxide dismutase
sos	Suppressor of sessile spikelets
spc	speckled
spk	salt-inducible protein kinase
spp	sucrose-phosphatase
spr	signal recognition particle receptor homolog
sps	sucrose phosphate synthase
spt	spotted
sqs	squalene synthase
sr	striate leaves
srk	S-receptor kinase
ssu	ribulose bisphosphate carboxylase small subunit
stc	sesquiterpene cyclase
stk	serine threonine kinase
stp	sugar transport
su	sugary
sum	siroheme uroporphyrinogen methyltransferase
sus	sucrose synthase
sut	sucrose transporter
sxd	sucrose export defective
tacs	terminal acidic SANT

Symbol	Full Name
tak	Triticum aestivum kinase
tan	tangled
tb	teosinte branched
tbp	TATA-binding protein
tcb	teosinte crossing barrier
td	thick tassel dwarf
te	terminal ear
tga	teosinte glume architecture
tgd	dTDP-glucose dehydratase homolog csu21
tha	thylakoid assembly
thi	thiamine biosynthesis
thl	thiolase
thr	threonine synthase
tif	translation initiation factor
tip	tonoplast intrinsic protein
tlk	tousled protein kinase
tls	tasselless
tm	transmembrane protein
toc	translocon at outer membrane of chloroplast
tp	teopod
tpi	triose phosphate isomerase
tps	terpene synthase
trap	transposon associated protein
trg	trigonelline
trm	thioredoxin M
trn	torn
ts	tassel seed
tu	tunicate
tua	alpha tubulin
tub	beta tubulin
tufm	elongation factor TU mitochondrial
ubi	ubiquitin
uce	ubiquitin conjugating enzyme
uck	UMP/CMP kinase
ufo	unstable factor for orange
ugp	UDP-glucose pyrophosphorylase
umi	ustilago maydis induced
v	virescent
va	variable sterile
vacs	vacuolar sorting receptor homolog
vg	vestigial glume
vgt	vegetative to generative transition
vp	viviparous
vpe	vacuolar processing enzyme
vpp	vacuolar proton pump
w	white seedling
wc	white cap
wd	white deficiency
whp	white pollen
wip	wound inducible protein
wlu	white luteus
wlv	white leaf-virescent
wrk	wrinkled kernel
ws	white sheath
wsm	wheat streak mosaic virus resistance
wt	white tip

Symbol	Full Name
wx	waxy
xet	xyloglucan endotransglycosylase homolog
xth	xyloglucan endo-transglycosylase/hydrolase
xyl	xylanase
y	yellow endosperm
yab	yabby homolog
yg	yellow-green
ypt	ypt homolog
ys	yellow stripe
yy	yin-yang
zag	Zea AGAMOUS homolog
zap	Zea apetala homolog
zb	zebra crossbands
zfl	zea floricaula/leafy
zfp	putative zinc finger protein3
zl	zygotic lethal
zmm	Zea mays MADS
zn	zebra necrotic
znod	Zea nodulation homolog
zp	zein alpha protein
zpb	zein familyB3
zpu	pullulanase-type starch debranching enzyme

CHROMOSOME 1	
Locus Name	cM
tub1	0.63
btf3	2.75
fus6	6.08
dcl101	11.00
mads1	12.26
knox1	12.26
gpb1	17.00
mlo1	17.08
cat2	20.76
gst11	20.76
gst10	21.26
gst12	21.30
smt2	21.51
gst31	21.51
prc3	22.26
lls1	25.75
ct2	27.00
rab30	28.05
gsr1	28.52
ms26	31.18
esr2	31.25
esr3	31.26
esr1	31.26
T1-2(4464)(1)	36.00
T1-2c(1)	36.00
T1-3(5597)(1)	36.00
les2	39.25
pg15	39.25
sr1	39.25
ltk1	40.25
pds1	40.25
vp5	40.25
grp2	42.02
lpa1	42.51
zmm14	42.52
gln6	42.52
hsp26	52.75
sig2B	57.53
ibp2	57.54
tgd1	57.55
zb4	62.00
pck1	64.30
imd1	64.81
fad8	65.18
asn1	65.31
pdc3	65.31
ms17	68.00
ms9	69.00
T1-4b(1)	69.00
hcf3	69.54
ts2	69.57
dek1	69.58
rth3	69.58
p1	69.58
p2	69.60

CHROMOSOME 1	
Locus Name	cM
zl1	70.60
mez3	71.76
nad1	71.76
T1-2b(1)	72.00
kri1	72.52
cal3	72.52
sbip2a	72.52
l16	72.60
T1-9c(1)	73.00
pki1	73.00
T1-5(6899)(1)	74.00
nec2	79.00
les22	82.27
sus2	82.27
sod4	84.38
T1-3k(1)	85.00
T1-6c(1)	87.00
pdlk1	87.81
T1-3a(1)	88.00
nrx1	90.25
msv1	91.00
agp1	94.76
an2	98.00
aoc1	98.26
gst42	99.51
gst32	99.51
mlo2	101.27
mtl2	101.27
pop1	107.77
myc7	107.80
lpa2	108.00
ocl4	108.53
aic1	109.78
TB-1Sb(1)	110.00
obf1	110.07
eno2	113.51
cent1	113.52
bsd2	113.52
as1	114.00
rs2	114.25
cp3	118.96
TB-1La(1)	120.71
bif2	121.52
cys2	121.54
grp1	127.01
uce1	127.01
bx9	127.01
dof1	127.03
myb6	128.68
ptk3	136.03
zmm6	139.76
ntf1	142.70
pdh2	146.75
br2	151.26
hm1	151.78

CHROMOSOME 1	
Locus Name	cM
T1-6a(1)	155.00
wlu5	157.00
amp2	158.00
kin4	163.25
acpt1	163.26
T1-9(4995)(1)	164.00
cncr1	164.77
hmga102	164.79
crs1	164.82
agpsl1	164.82
T1-6b(1)	165.00
T1-9b(1)	166.00
br1	170.00
T1-7c(1)	170.00
T1-7a(1)	171.00
vg1	173.00
f1	174.00
nip3a	175.25
yab10	178.01
dcl102	180.35
amp1	181.00
mdh4	186.15
T1-2a(1)	189.00
cpn2	190.00
mmm1	191.00
ad1	192.00
id1	194.80
an1	196.33
bz2	196.33
cyp8	198.28
sdg123	198.35
vp14	202.51
rpo1	202.51
cdj2	203.08
T1-9(8389)(1)	204.08
ptd1	207.00
glb1	209.83
gbp2	210.50
rth1	210.51
gst40	210.74
iaglu1	212.26
lpe1	212.26
ts3	217.00
tb1	220.68
w18	220.68
ole4	220.75
ers25	220.76
mpl1	220.76
d8	220.76
zb7	221.30
gln2	221.77
pgm1	221.77
phyA1	222.53
chr101	222.53
kn1	222.79

CHROMOSOME 1	
Locus Name	cM
knox3	222.79
tua1	222.88
tua2	222.88
gs1	224.00
lw1	224.00
tbp1	224.26
adh1	225.53
exg1	228.32
mta1	231.75
cka1	232.50
cka2	232.50
tlk1	233.63
lem1	237.76
atp3	241.01
phi1	241.01
fnr1	243.26
gst5	243.26
cdj1	243.26
gdh1	246.77
vp8	249.00
rd1	250.49
igl1	253.73
ohp1	253.75
hon110	254.75
ij2	255.05
sqs1	257.76
ccr1	257.76
cesa5	258.51
bm2	259.00
dia2	259.24
ts6	260.04
ids1	262.77
chi1	262.77
tls1	264.00
T1-4g(1)	270.00
cesa6	274.01
fdx3	274.60
phb4	275.76
tufm1	280.48
acp4	281.66
spc2	283.00
sdh1	284.00
akin1	284.00
gbp1	284.01

CHROMOSOME 2	
Locus Name	cM
mlo9	0.00
pcr1	0.00
crr1	0.01
ws3	2.00
al1	6.00
fht1	7.76
lg1	11.75
T2-3a(2)	12.00
kin6	23.25
aaa1	23.52
cad1	23.52
gl2	30.52
cpx1	30.52
eks1	30.60
d5	35.00
nec4	35.00
T2-3(5304)(2)	36.00
T2-5g(2)	37.00
aox1	37.01
zfl2	37.01
myb5	37.30
T2-9c(2)	40.00
hon101	41.25
ago106b	46.14
czog1	49.26
b1	49.30
gs2	50.00
T2-3c(2)	52.00
T2-9a(2)	53.00
ole1	54.13
rws1	54.27
ivr1	55.28
px14	55.29
sk1	57.00
les1	60.00
wt1	64.00
sam2	66.70
ba2	67.00
ts1	67.00
cta1	67.52
mas1	68.51
gpdh1	68.51
rab2a	68.78
mop1	68.78
stk2	68.79
T1-2(5255)(2)	71.00
prp2	71.18
gl11	72.00
grf1	73.13
mlo3	74.03
opr5	74.03
fl1	75.70
les10	77.00
T2-3(6270)(2)	78.00
T2-9b(2)	79.00

CHROMOSOME 2	
Locus Name	cM
aba1	79.18
T2-5a(2)	80.00
mn1	80.79
hrg1	80.83
T2-10a(2)	81.00
sdg112	82.23
clx1	82.27
sks1	83.00
hcf106	83.01
mde1	84.76
acc2	85.26
les15	86.00
cent2	86.01
ssu2	86.05
zpu1	86.25
gl14	87.00
v4	87.00
T2-3(7285)(2)	87.00
ns1	87.80
T1-2(4464)(2)	88.00
hsbp1	88.05
ask2	89.00
l18	90.00
cdpk2	90.00
pbf1	92.03
parp2	93.53
hfi1	93.55
cesa4a	93.55
dia1	93.70
hp2	93.83
dar1	93.83
phb1	94.36
agp1	94.73
akh2	94.76
ppt1	94.76
hir2	95.51
emp2	95.75
tha8	95.77
his2b3	95.78
amy3	100.51
dof2	103.52
tpi2	107.00
ugp1	110.00
v24	111.00
kin2	113.51
px1	114.00
spt1	119.00
T2-9d(2)	121.00
w3	122.00
ht1	126.00
tua5	127.26
eif5a	127.27
fdx5	137.30
tacs1	140.81
pur1	142.81

CHROMOSOME 2	
Locus Name	cM
apx2	143.51
ap17	144.51
rDNA5S	145.00
whp1	148.00
ser1	148.01
betl1c	150.27
mha1	150.53
pex1	150.55
rf3	152.54
wlv1	156.00
srk1	162.50
ch1	162.60
se1	177.00
gn1	181.26

CHROMOSOME 3

Locus Name	cM
g2	0.50
ein2	7.01
e8	11.58
zag4	16.78
brn1	18.00
me3	19.78
cr1	20.00
me1	21.00
eif3	23.85
cg1	24.40
cko1	24.40
hex1	24.98
hsp18f	27.25
mus2	27.26
d1	30.00
ra2	32.76
ccp1	39.76
tpi4	42.02
e4	44.00
ocl1	47.75
ltk3	47.76
cl1	52.00
rt1	52.00
cyc2	53.82
rf1	54.00
ago105	54.43
wrk1	55.00
cef1	57.02
lg3	57.04
caf2	57.26
bet1	58.00
rps25	58.00
tha1	58.00
TB-3Sb(3)	58.00
rp3	68.75
rg1	69.00
cent3	70.02
TB-3La(3)	71.00
ys3	72.00
incw4	72.79
T1-3(8995)(3)	74.00
TB-3Lf(3)	74.00
TB-3Lg(3)	74.00
wsm2	76.14
phys2	76.75
betl3	76.79
eif6	76.79
abp1	76.80
rad51b	76.80
pm1	77.00
mv1	78.00
ts4	78.00
gst18	78.26
zag2	78.26
cop1	78.76

CHROMOSOME 3

Locus Name	cM
atp1	79.50
cyp7	79.52
rcph1	79.52
prc4	79.53
gst4	80.00
rps27	81.51
gl6	82.00
T3-9c(3)	82.00
crr5	82.76
gst28	82.76
gst7	82.76
cko2	84.00
pgd2	86.00
TB-3Lh(3)	86.00
TB-3Ld(3)	86.00
ldp1	86.01
myb2	86.70
agp3	86.75
sps2	89.58
sdw2	91.00
vp1	92.75
drh1	92.76
te1	92.77
rd4	97.00
im30p1	97.85
ig1	98.00
spc1	99.00
lxm1	99.35
zmm16	99.55
psbs1	102.90
T1-3k(3)	103.00
lg2	103.25
T1-3(5597)(3)	105.00
TB-3Lc(3)	106.00
TB-3Li(3)	108.00
TB-3Lj(3)	108.00
plt1	108.50
ba1	109.00
his2b4	113.50
expa1	115.27
na1	120.00
obf3.2	122.03
tub6b	122.03
cyc4b	122.78
gos1	123.54
K3L	124.00
wlu1	128.00
hox3	136.01
cesa11	136.26
dnp2	136.27
dhn6	136.27
mek1	140.51
T2-3(6270)(3)	142.00
smh4	142.51
y10	145.28

CHROMOSOME 3

Locus Name	cM
gst2	149.50
thr1	149.51
gst20	149.51
gpm3	149.52
chn1	154.76
bzip1	154.76
mdh3	158.10
a3	166.00
w19	174.75
a1	174.75
x1	174.75
sh2	174.75
sum1	174.75
cyp10	175.51
cenpc1	175.51
thi2	175.51
lhcb1	188.69
aldh3	188.91
et1	190.01
ga7	196.00
phot1	201.77
rpl10	201.77
plt2	206.85
cyp1	207.25

CHROMOSOME 4

Locus Name	cM
mtl1	0.73
rca1	2.48
msf1	3.95
gst6	5.76
cyp3	6.26
bx4	9.38
cyp4	9.51
bx8	9.51
cyp5	11.90
bx1	12.01
pex2	12.01
zpl1a	15.00
zpl1d	18.00
rp4	19.00
zpl1b	19.00
zpl1c	19.00
dzr1	19.80
pls1	20.25
zp22.1	20.25
zpl1e	21.00
sos1	24.00
zpl1f	25.00
ga1	27.00
sbip1a	29.02
bss1	29.50
adh2	36.78
pdi1	38.26
sig2A	39.76
ts5	48.00
gl5	49.00
pgd3	50.08
wip2	51.25
fl2	51.50
la1	52.00
ahh1	53.25
Dt6	55.00
psb3	57.02
ocl5a	58.01
cld1	58.01
zp1	58.05
gl7	60.00
tcb1	61.00
v17	61.00
aco1	61.50
cp2	61.50
bm3	62.75
bap2	62.76
fie1	62.77
orp1	62.77
dsc1	63.00
gpc1	63.50
expa2	63.55
su1	63.55
rpl44	63.77
tga1	69.26

CHROMOSOME 4

Locus Name	cM
nnr1	69.98
T4-9g(4)	70.00
v23	70.00
bt2	70.02
zpl3a	71.00
his2b2	71.51
akh1	71.77
hda108	71.77
zpl2a	72.00
TB-4Sa(4)	72.00
TB-4Sg(4)	72.00
hir1	73.52
eng1	73.54
pep7	73.54
su3	74.00
als1	74.50
cent4	74.51
spk1	75.01
serk3	75.06
ypt3	75.07
spr1	75.08
gl8b	75.50
gpc3	75.51
fea2	75.51
mtl3	75.51
zb6	77.00
umc156a	78.50
nfa104	78.73
krp1	83.04
hmg3	86.00
lw4	90.35
tip2a	90.50
zag3	90.75
his2b5	93.26
aldh2	93.2825
gln5	93.29
ant2	94.83
gl4	95.85
pip1e	98.05
prh1	98.06
abc1	98.06
trg1	98.10
pcna2	102.82
lkrsdh1	103.58
nii2	107.10
dek31	108.00
umc127c	110.85
rpl29	110.86
o1	115.00
pdh1	115.76
fer1	115.76
gol1	115.83
tu1	118.00
v8	118.00
rtp1	119.02

CHROMOSOME 4

Locus Name	cM
cax1	119.02
j2	123.00
ns2	126.00
gl3	128.00
rcph2	129.00
ssu1	130.53
T4-7(4698)(4)	131.00
ms44	133.00
c2	133.01
kin8	134.52
mlo4	139.77
mdr1	142.00
T1-4b(4)	142.00
ane1	143.76
T4-9b(4)	150.00
ris2	150.26
rpd3	150.28
mgs2	150.50
ms41	154.00
vpp2	154.27
smh3	154.50
zfp30	154.53
lox1	158.76
knox7	158.76
cbp2	158.76
sbp2	161.08
vpp3	164.25
ubi2	167.51
dba1	167.51
eif5	167.51
cas1	167.51
rop2	167.51
rpl32	167.51
wee1	182.26
cpn10	182.26
bip2	184.45
nrt2	185.26
cat3	186.03

CHROMOSOME 5

Locus Name	cM
sig6	0.00
arf1	13.77
sca1	17.13
ms42	18.00
psad1	18.02
ohp2	18.02
zap1	31.26
hcf108	33.00
tua4	36.88
umc90	37.75
tua3	37.75
pgm2	39.26
knox10	47.45
d9	47.50
ers14	47.50
ole3	47.50
rab15	47.51
tub4	49.25
tbp2	49.25
rps15	49.26
csy1	50.00
na2	57.00
mdh5	58.25
eif7	59.01
amy2	59.01
cpn1	59.01
px13	60.75
hag101	61.51
ugu1	61.51
anl1	62.00
nl2	62.00
T5-6d(5)	65.00
gly1	65.01
xet1	67.88
eif4	69.76
arpp3	69.76
td1	70.28
piip2	70.28
gl17	71.00
chn3	71.50
sbe1	71.50
cat1	71.50
hmg1	71.50
nec6	72.00
T5-6(5685)(5)	72.00
T5-6(5906)(5)	72.00
T5-9(022-11)(5)	72.00
a2	72.01
ivr2	72.01
bip1	72.02
rop9	72.03
TB-5Sc(5)	73.00
dts1	73.78
T5-6b(5)	74.00
vp2	75.00

CHROMOSOME 5

Locus Name	cM
T4-5i(5)	75.00
T5-9c	75.00
ps1	76.00
T5-6(4933)(5)	76.00
sbp1	76.90
amp3	77.00
T2-5a(5)	78.00
T5-6(8590)(5)	78.00
knox6	78.78
nec3	79.00
bm1	79.25
dmt3	79.25
T1-5(8041)(5)	79.50
bt1	80.01
pep2	80.01
cent5	80.05
sxd1	80.52
pho1	80.76
ris1	80.78
mip1	80.81
cbp4	80.82
rps24	80.83
v3	81.00
T1-5(6197)(5)	81.00
T1-5b(5)	81.00
T5-9(4817)(5)	81.00
T1-5e(5)	81.00
T1-5f(5)	81.00
TB-5La(5)	82.00
TB-5Lb(5)	82.00
TB-5Ld(5)	82.00
T2-5d	82.00
bv1	84.00
ga2	85.00
als2	86.75
ppi1	86.77
ae1	87.80
ms5	89.00
myb3	89.73
dek33	90.67
yy1	92.79
incw1	94.10
csu93b	96.75
pr1	98.00
sh4	98.00
cbp1	98.18
gst17	99.25
lw2	100.00
prg1	100.00
gl8	100.51
nbp35	103.45
gpc4	103.51
pal1	105.00
phb3	107.02
serk2	107.08

CHROMOSOME 5

Locus Name	cM
pip2d	108.00
v12	110.00
lw3	113.00
snr14	116.76
yab15	116.76
ys1	116.77
vpe1	116.80
ypt2	117.51
rpl19	117.75
cal1	119.26
hmg2	120.02
pac1	122.01
prr1	125.27
zag5	126.00
ren1	128.00
lec1	129.04
hsf1	130.00
ant1	130.03
gln4	132.18
rop1	132.26
pin2	132.26
atpc1	132.27
lhcb4	147.51
ppp1	147.51
dap1	148.00
T5-9a(5)	148.00
nnr2	149.88
eg1	150.00
gst24	150.01
yg1	152.00
K5L	155.00
v2	156.00
zb1	156.00
got2	161.00
T5-6(8696)(5)	164.00
pcna1	164.26
fdh1	167.26
brk1	167.27
prl1	167.27
rop4	169.18

CHROMOSOME 6	
Locus Name	cM
fdx1	0.01
fdx2	0.01
adk1	4.50
rps13	7.50
rhm1	11.00
gpc2	14.18
rxo1	16.51
po1	17.00
NOR	17.27
mdm1	17.27
TB-6Sa(6)	17.27
wsm1	17.27
rgd1	18.00
idc1	18.01
lip15	18.25
gsh1	18.25
cent6	23.00
mn3	24.00
uck1	24.00
myb1	24.01
pgd1	24.01
rcp1	24.60
zp15	25.02
leg1	25.04
dof3	25.26
TB-6Ld(6)	26.50
TB-6Lc(6)	27.00
w15	28.00
T4-6(8428)(6)	28.00
T6-9(6019)(6)	28.00
T6-9e(6)	28.00
T6-9(6270)(6)	28.00
l12	29.00
omt1	29.01
mez1	29.02
y1	30.13
cyc3	30.28
chn2	30.28
cdpk1	30.29
si1	30.29
enp1	31.00
l10	31.00
saur1	31.26
eif4a	31.26
oec33	31.27
mir1	31.78
mir2	31.78
ms1	33.00
T4-6(6623)(6)	35.00
T6-9(043-1)(6)	35.00
T1-6(4456)(6)	35.00
T6-9b(6)	35.00
sbp3	38.43
l15	40.00
pg11	44.00

CHROMOSOME 6	
Locus Name	cM
ln1	45.48
gst19	45.50
elfg1	45.50
kin5	47.51
ploc1	51.27
pl1	52.88
ebe2	53.01
l11	54.00
nip2b	57.26
su2	57.26
tm20	59.04
dzs18	59.05
vpp1	59.06
hex2	59.90
pfk1	61.32
kin9	61.33
sm1	63.00
imp1	63.83
cesa2	63.84
T1-6a(6)	64.00
pt1	67.00
zag1	67.45
K6L2	67.51
TB-6Lb(6)	68.00
rop3	69.25
ptk1	69.26
psan1	71.02
sod3	71.02
rps21	73.00
dhn1	74.03
me2	74.29
esp1	75.51
esp5	75.51
tan1	77.75
K6L3	77.75
gst41	78.77
mlo8	78.77
dxs1	79.65
w1	80.00
pdk1	80.88
pmg1	81.48
w14	85.00
nfy2	93.51
roa2	93.52
fcr2	98.52
cenH3	98.52
rop6	106.50
prf1	108.76
hsp101	108.76
hox2	108.76
mlg3	113.18
lhcb48	128.51
gbf1	133.00
idh2	133.00
mdh2	133.00

CHROMOSOME 6	
Locus Name	cM
agp2	134.10
asg7a	134.10
ago104	135.75
hir3	136.13

CHROMOSOME 7

Locus Name	cM
hsp3	1.38
cka4	3.45
ppr2	3.51
psk1	11.25
rs1	17.25
umi11	17.25
psa6	17.28
w17	20.00
hda110	21.50
mdh6	28.00
dmt101	30.52
o2	30.60
his1a	31.30
meg3	31.53
y8	34.00
v5	37.00
sgo1	37.88
rcm1	38.00
rpl39	38.01
zds1	38.02
smt1	38.02
vp9	38.02
sgo1	38.02
cesa9	38.02
zpl2b	38.02
ost1	38.02
embp1	38.02
kpp1	38.95
in1	39.00
crt2	42.70
bgaf1	42.76
cesa8	42.77
w16	43.00
TB-7Sc(7)	43.00
cent7	44.00
mn2	44.00
cesa7	44.25
cyp6	44.98
cncr2	45.27
nbp1	45.27
caf1	45.30
pip2c	45.30
TB-7Lb(7)	45.50
ppt2	46.52
pep4	47.76
hat1	48.27
piip1	57.30
mus3	62.26
rs4	63.00
zpb36	63.00
ra1	63.01
o5	64.00
his2b1	64.50
les9	65.00
ccp2	65.51

CHROMOSOME 7

Locus Name	cM
gl1	66.00
gst23	74.50
tp1	76.00
sbp6	80.78
ij1	82.78
dfr1	82.78
sl1	84.00
va1	84.00
ocl3	87.77
T7-9a(7)	88.00
T6-7(013-8)(7)	90.00
lon1	91.28
lhcb2	95.29
gst16	95.30
bas1	95.55
rpl15	95.56
ndk1	95.65
grx1	95.75
zmm7	97.01
icl1	98.02
tif1	99.83
amy5	101.53
oec17	101.53
crp1	102.00
kik1	102.00
pk3	102.00
psb29	102.76
K7L	103.01
tpi1	107.77
tua6	107.77
rad51a	107.77
bn1	110.00
e1	112.00
ren2	113.00
mus1	118.25
rip2	118.25
rpot1	123.77
gst37	129.53
sod2	137.04
bd1	137.05
akr1	152.06
ra3	154.29
oec6	154.56
kin1	154.57

CHROMOSOME 8	
Locus Name	cM
hsp1	1.51
rpl30	1.51
rf4	4.00
cal2	6.77
rnp2	13.00
rps5	13.79
ms23	21.00
ptk5	26.30
lrk1	26.30
lrk pseudo	26.30
crs2	28.82
glct1	32.32
tpi3	33.08
pdc2	34.33
hsp18c	44.01
zmm2	45.02
ech1	51.01
parp1	51.03
lhcb3	51.51
cent8	51.77
cks1	51.77
rph1	51.77
atp2	51.78
gpa1	52.00
mdh1	54.00
bif1	54.00
irl1	60.18
rpl17	61.53
stp1	62.76
rpl12	62.76
scl1	62.76
rps28	67.50
oec23	68.26
zmm18	71.25
zmm29	71.25
fl3	72.00
T8-9d(8)	72.00
tub2	72.50
TB-8Lc(8)	73.00
pdk2	73.75
der1	73.76
rip1	73.83
act1	74.00
pro1	74.00
spp1	76.04
gl18	77.00
caat1	78.75
pdc1	79.00
cyc1	79.01
nec1	81.00
TB-8La(8)	82.00
rop7	84.25
hox1	84.26
trap1	84.26
kin3	84.26

CHROMOSOME 8	
Locus Name	cM
prf2	84.26
hox1	84.30
mrp1	88.52
dmt106	88.52
lg4	89.53
lg4b	89.53
sci1	91.80
sdw1	92.00
ht2	93.00
thi1	94.57
por1	95.75
smh1	95.75
a4	97.02
idh1	97.02
dba2	97.03
rap2	97.05
vgt1	97.05
nad2	97.51
gstIIB	98.00
v16	99.00
aba2	102.75
bss1	102.75
ebe1	102.77
htn1	106.00
sbe3	108.00
sps1	108.01
ald1	108.01
ms8	113.00
lrs1	116.76
tpi5	120.00
j1	122.00
psy2	122.43
tub6	128.76
emp3	131.00
gst1	142.88
rgh1	154.00
hstf2	155.51
kin16	155.52

CHROMOSOME 9

Locus Name	cM
K9S	-4.50
Dt1	-4.00
pyd1	-3.50
wd1	-3.00
yg2	-3.00
rop5	1.26
pin1	1.26
aap1	6.03
c1	16.18
ftr1	16.25
sh1	20.08
stc1	21.01
stk1	22.51
bz1	22.53
orc1	23.95
l6	24.00
g6	30.00
hyp1	31.51
prc1	31.51
ss1	32.77
omt2	32.78
zb8	35.00
znod1	35.78
thl1	37.03
mgs3	37.04
l7	40.00
les8	40.00
eno1	40.79
v31	41.00
lo2	42.00
baf1	46.00
TB-9Sb(9)	47.00
wx1	47.93
dhn2	48.00
d3	50.07
rpo2	50.07
fdad1	50.10
hsk1	51.00
TB-9Sd(9)	52.00
T9-10b(9)	53.00
w11	54.00
cent9	56.16
fdh2	56.51
dzs10	56.51
phs1	56.52
TB-9Lc(9)	56.60
ar1	57.00
pg12	57.00
ago101	57.52
hsbp2	57.52
fat1	57.52
rf2	57.76
ms2	58.00
pep1	58.00
v1	58.00

CHROMOSOME 9

Locus Name	cM
dxs2	58.28
elfa4	59.00
obf2	59.00
gl15	59.56
acp1	60.75
knox2	63.00
T8-9a(9)	64.00
trn1	64.00
hm2	64.60
tub7	65.26
sbp4	66.50
hscf1	66.50
expb1	74.52
cgs1	54.52
lop1	74.52
sus1	77.88
TB-9La(9)	78.00
bk2	78.50
sod9	80.78
v30	83.00
hsp18a	85.50
vacs1	88.54
mez2	93.26
fad7	96.27
ibp1	96.50
ms45	105.50
pck2	105.51
rps22	107.51
toc34	107.52
lhca1	107.52
dba4	108.00
psk3	116.02
aos1	116.25
gln3	123.03
zmm8	123.03
hb1	123.08
sbip1b	131.50
xyl1	133.51
eps1	134.25
gst30	134.75
ltk2	138.51
rpp40	138.51
prc2	140.76
sut1	144.65
gst9	144.77
gst13	144.77
wc1	146.98
T4-9(5788)(9)	147.00
bf1	151.03
rld1	151.03
gst35	159.29
bm4	162.00

CHROMOSOME 10	
Locus Name	cM
fer2	0.0024
rp5	11.5000
rp1	13.2638
rpp9	15.0000
rp6	15.4000
alp1	16.0177
gdcp1	24.4750
cr4	26.0000
oy1	33.7586
vpp4	33.7601
mac1	41.0000
y9	41.0000
gs4	41.0000
rlc1	41.0000
sr3	41.0000
T9-10b(10)	41.0000
og1	42.0000
TB-10Sc(10)	42.0000
dnp1	42.0060
trm1	42.0089
cent10	42.0150
gcsh1	42.0500
TB-10L18(10)	43.0000
TB-10L19(10)	43.0000
chs1	43.5222
ms11	44.0000
zn1	44.0000
TB-10L26(10)	45.0000
glu1	45.0085
php1	45.0100
aprl6	45.2540
sad1	45.2627
abp5	45.7508
abp4	45.7513
zmm1	45.7775
eoh1	46.0000
ufo1	46.0000
du1	46.7925
ppo1	46.7930
cx1	47.0000
TB-10Lb(10)	48.0000
pao1	48.8500
T8-10b(10)	49.0000
xth1	50.2936
nl1	52.0000
rps3	52.1250
TB-10L22(10)	53.0000
T6-10a(10)	53.0000
tps5	53.0163
der2	53.2536
ane3	53.7575
bap1	53.7600
ef1	54.0000
fie2	54.5100
orp2	54.5100

CHROMOSOME 10	
Locus Name	cM
TB-10L36(10)	55.0000
mpk6	57.0049
nac1	57.0105
fgp1	58.5750
ef2	59.0000
li1	61.0000
T3-10a(10)	61.0000
TB-10L20(10)	62.0000
acc1	62.0104
amo1	63.0000
ef3	63.0000
TB-10L10(10)	63.0000
gar1	63.5073
bf2	63.5300
fab1	63.5432
TB-10L1(10)	64.0000
TB-10L25(10)	64.0000
TB-10L28(10)	64.0000
TB-10L31(10)	64.0000
TB-10L4(10)	64.0000
TB-10L5(10)	64.0000
TB-10L8(10)	64.0000
odo1	64.3023
hcf106c	64.3082
TB-10L16(10)	65.0000
TB-10L17(10)	65.0000
mgs1	65.1250
isp1	65.4750
ms10	66.0000
TB-10L7(10)	66.0000
TB-10L11(10)	66.0000
TB-10L29(10)	66.0000
TB-10L3(10)	66.0000
TB-10L30	66.0000
TB-10L34(10)	66.0000
TB-10L35(10)	66.0000
TB-10L9(10)	66.0000
sfb1	67.7508
TB-10L14(10)	68.0000
TB-10La(10)	68.0000
T5-10(4801)(10)	68.0000
incw3	68.0500
TB-10L23(10)	69.0000
TB-10L12(10)	70.0000
TB-10L13(10)	70.0000
TB-10L16(10)	70.0000
TB-10L24(10)	70.0000
TB-10L33(10)	70.0000
TB-10L37(10)	70.0000
TB-10L38(10)	70.0000
TB-10Ld(10)	70.0000
tip1	71.0372
TB-10L6(10)	72.0000
serk1	72.0025
gpa2	72.0055

CHROMOSOME 10	
Locus Name	cM
pme1	72.0055
grf2	72.0093
phb2	72.0096
ras1	72.0155
TB-10L21(10)	74.0000
TB-10L27(10)	74.0000
tp2	76.0000
TB-10L15(10)	76.0000
sam1	77.0000
TB-10L2(10)	77.0000
dcl103	77.5000
g1	78.0000
TB-10L15(10)	78.0000
wsm3	84.0000
kin15	86.2525
TB-10L32(10)	89.0000
ago106a	93.0000
isr1	95.0000
l1	95.0000
por2	95.2506
r1	95.2537
sn1	95.2537
ocl2	95.2625
lc1	97.0000
tip5	98.1250
zfl1	100.0045
mst1	101.0000
cpx2	102.7539
rps11	102.7540
gtr1	112.7520
cop2	112.7550
w2	113.0000
o7	121.0000
mha2	122.5041
gln1	122.5053
kin11	122.5063
l13	126.0000
crr2	126.5118
dba3	126.5150
sr2	130.0000

XI. MAIZE GENOME SEQUENCING PROJECTS

Sequencing the Maize B73 Genome Progress Report

--PI: Richard K. Wilson (WUGSC), Co-PIs/Key Personnel: Doreen Ware (CSHL), Rodney Wing (AGI), W. Richard McCombie (CSHL), Patrick Schnable (ISU), Sandra W. Clifton (WUGSC), Srinivas Aluru (ISU), Lincoln Stein (CSHL), Robert Martienssen (CSHL), Robert Fulton (WUGSC)

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CSHL Cold Spring Harbor Laboratories, Cold Spring Harbor, NY; URL: maizesequence.org
ISU Iowa State University, Ames, IA; URL: www.plantgenomics.iastate.edu

From the Oct 2007 Annual Progress Report, posted in more detail at MaizeGDB: http://www.maizegdb.org/sequencing_project.php.

Overview of Original Project Goals:

<u>Project Objectives:</u>

1. Provide the complete sequence and structures of all maize genes and their locations (in linear order) on both the genetic and physical maps of maize.
2. The gene space of B73 maize (gene sequences and adjacent regulatory regions) should be finished to high quality according to currently acceptable standards
3. If applicable, the sizes of gaps between the genes should be estimated and draft sequences of repetitive DNA between genes presented where possible.
4. The sequence will be fully integrated with the genetic and physical maps.
5. Annotation will include gene models, predicted exon/intron structure, incorporation of EST and full-length cDNA data, gene ontology, and relationship with homologs in other organisms, including but not limited to, the other sequenced plant genomes.
6. Annotation will be coordinated with existing maize community and comparative databases with the eventual goal of generating complete curation of the genomic sequences to a standard set by established model organism databases.

Research Activities and Results:

1. *Choosing minimal tiling path (MTP) clones from maize physical map preparing DNA for sequencing. Responsible PI: R. Wing, AGI.*

<u>Goals:</u> The objective for AGI for year 2 was to complete MTP clone selection in consultation with the WUGSC. AGI prepared all DNA from the selected clones, sheared the DNA, and confirmed identity by generating end sequences for each clone. Once validated, the sheared DNA was shipped to the WUGSC for shotgun library construction and sequencing.

<u>Progress:</u> In Year 1, we built a framework for MTP selection, which combined all the data from the physical map, blastn searches of seed BACs against a BES (BAC end sequence) database, and trace display. We used this pipeline for cloning walking from 3,400 seed BACs chosen in Year 1. We also chose clones according to their fingerprints along contigs due to project time constraints and limited sequence availability.

In year 2 we selected ~8,000 MTP clones to complete the first round of picking. In total, we selected 16,224 BACs (169 96-well plates). Of the selected clones, DNA from 15,400 clones was prepared, sheared and validated for shotgun library construction. The difference between the two numbers is due to failed clones in selection and DNA preparation.

Our initial estimate of 19,000 MTP clones still stands, but we anticipate that number will be reduced to approximately 16,000 MTP clones due to the fact that we selected very large seed BACs. The additional ~600 clones will be used to fill physical gaps between sequences. This selection is ongoing.

<u>Plans:</u> We will manually check all the neighboring clones for any potential gaps. A new BAC or fosmid clone will be selected to fill each gap. We expect 500-1,000 clones will be selected for gap filling. DNA of these clones will be prepared, sheared, verified, and delivered to WUGSC for shotgun library construction and sequencing. In the meanwhile, we will merge map contigs according to the available BAC sequence information.

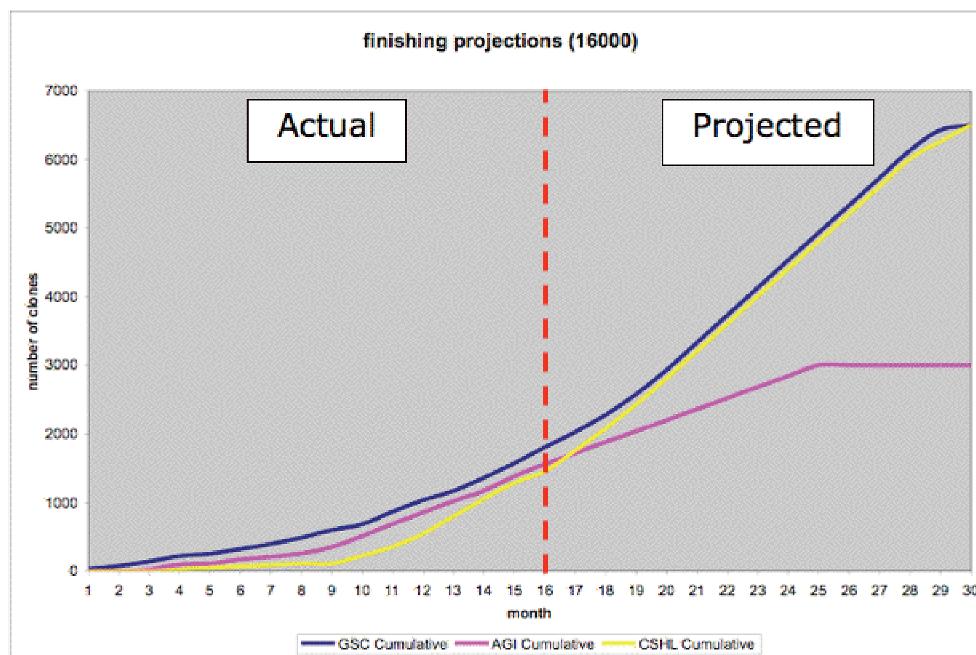
2. BAC sub-clone libraries, production sequencing and assembly, "genespace" boundary determination, automated sequence improvement, and shared finishing of "genespace" in BAC clones. Responsible PI: R. Wilson, WUGSC

<u>Goals</u>: Provide the complete sequence and structures of all maize genes. The gene space of B73 maize (gene sequences and adjacent regulatory regions) should be of finished quality according to currently acceptable standards. If applicable, the sizes of gaps between the genes should be estimated and draft sequences of repetitive DNA between genes presented where possible.

Progress: In year 1, the first 4-6 months of the grant year were spent optimizing procedures and improving computer access and communication among the three Centers (WUGSC, AGI and CSHL) sharing production and finishing tasks. Bimonthly or monthly conference calls among the three centers involved in the production/finishing procedures have been and are continuing to be used as an effective means of anticipating and dealing with any problems in a timely manner. A smoothly operating protocol is now in place and functioning well. In year 2, we followed and improved upon the operating protocol. This includes DNA intake, library construction, the production process, automated sequence improvement, and manual sequence improvement of the gene space. When projects reach the "shotgun_done" status, they proceed to the pre-finishing process where automated directed improvement techniques are employed to improve the overall sequence quality of the projects. Finally, BAC projects are queued for manual improvement (finishing), which involves the complete resolution of all sequences deemed "do finish" to a standard of less than one error per 10,000 bp. The sequence improvement is divided forty percent, forty percent, twenty percent respectively among WUGSC, CSHL, and AGI, but these divisions are very flexible and adjustable, enabling appropriate and effective distribution of the workload. The improvement effort has been steadily increasing over the last half of year two with very comfortable ramp rates in place to complete the remainder of the improvement territory in the timelines established by the project. Details of procedures described above can be accessed in the Year 1 progress report, posted at MaizeGDB with this report.

As of Jan 28, 2008, 15,233 of an estimated 16,000 BAC clones have progressed through the library_done stage, 15,002 projects are shotgun_done, 13,115 are prefin_done, and 5782 are improved. {*EDITOR note*: *At GenBank these categories are called HTGS_FULLTOP, HTGS_PREFIN and HTGS_IMPROVED, respectively. A 4th designation, HTGS_ACTIVEFIN indicates clones being worked on by a finisher.*}

Plans: The Oct. 2007 line graph below shows a projected ramping for the finishing queue.



3. Bioinformatics CSHL. Responsible PI: Doreen Ware.

The CSHL team collaborates closely with teams at WUGSC, AGI, and ISU to define requirements and deliverables.

The project has presented various challenges that have been successfully overcome. One such challenge is the unique nature of the sequencing project. Sequence data and mapped annotations from the project are made immediately available for public use, which is almost unprecedented for a genome project. In 2007 the project focused on refining and automating the sequence annotation pipeline, releasing and improving the project website, as well as outreach activities. The initial target this year was to provide annotation on the *improved* BACs. This objective was broadened to include all BAC sequence, now (Jan 2008) amounting to over 15,000 analyzed clones available for viewing. In addition, the current browser maintains previous versions for public viewing of the BACs at different phases.

Data Analysis:

The analysis builds on the Ensembl analysis pipeline software system (Potter et. al., Genome Res. 14:934-941, 2004). The primary unit of data being analyzed by the annotation pipeline is an intra-BAC contig. Because much of the sequenced BAC clones consists of

unordered stretches of sequence, each is annotated in isolation so as to minimize the mis-annotation of features that span neighboring contigs.

The system for analyzing and visualizing the maize genome is nearly fully automated. The BAC sequence records are retrieved from GenBank on a nightly basis. The analysis pipeline is run for the newly acquired clones on a semi-weekly basis, accessing 160 of the 2,000 processors in a high-performance IBM BlueHelix cluster. Finally, the staging server is synchronized each week with the production server, with backups of the older databases in case of failure.

Ab initio Gene Predictions

The repetitive nature of the genome required the team to explore alternate methods of analysis. Because many analyses are uninformative when they are run on unmasked sequence, there exists the concern that a masked portion of the genome may contain an important gene-related feature. Ab initio gene prediction is performed on non-masked individual contigs with FGENESH using the Monocot parameter (Salamov and Solovye, Genome Res. 10:516-522, 2000; http://softberry.com). Predicted genes are named according to the BAC accession/version, a method code (FG) and a 3-digit number: An example FGENESH predicted gene is "AC177916.2_FG023". Names containing 'FGT' and 'FGP' codes refer to the associated transcript and protein sequences, respectively. Two additional modules, namely Runnable::FgeneshGene and RunnableDB::FgeneshGene, are implemented for the Ensembl analysis pipeline software system to automate the FGENESH gene prediction of genomic sequences as Ensembl gene objects.

Peptide Classification and Protein Annotation

The gene models predicted by FGENESH are classified into three categories by comparing their peptide translations to known proteins. The three categories are TE (Transposable Element: transposon-like gene models), WH (With Homology: similar to known genes), and NH (No Homology: no similarity to known genes). BLASTP is used to generate alignments for all the predicted models against the NCBI Non-Redundant Amino Acid database (NR). Alignments are processed using a confidence level cutoff (E-value of 1e-5) to identify a known protein in the database. A curated list of transposon-like proteins in NR is used to identify the prediction as TE. If a high-confidence alignment corresponds with an NR protein not on the TE list, the prediction is classified as WH. Otherwise, it is classified as NH.

Since the incorporation of non-improved BACs into the analysis pipeline in April 2007, faulty translations have been detected in a subset of gene predictions. These aberrant predictions make up 4.8% of the total predictions on the generated sequence thus far. The error occurs in an isolated condition where the FGENESH gene prediction tool attempts to predict gene model structure without the presence of a starting exon. The aberrant translations are therefore generated from low-fidelity gene predictions. While the issue is being addressed, the browser provides such predictions, but demarcates them as “Corrupt Translations”.

All translations of the FGENESH gene predictions are run through a Protein Annotation Pipeline. The pipeline provides the same annotation modules that are provided through InterProScan, such as PFAM, Prosite, and SignalP. However, present annotations are provided with module-specific IDs; InterPro IDs are not being assigned as of yet. A system is being put into place to extract specific InterPro IDs for each annotated translation. This will allow for the assignment of Gene Ontology (GO) terms to describe protein function. It is anticipated this functionality will become available in December 2007.

Mathematical Repeats

As discussed in the 2006 report, the group has continued to use mathematical repeat as a form of annotation. We have taken a lead from an approached used on rice (Yu et al., “The Genomes of *Oryza sativa*: A History of Duplications”, PLoS Biology, February, 2005) that analyzes the repeat content based on genome-wide oligomer copies. Using an unbiased whole genome shotgun library of maize sequence generated by the Joint Genome Institute that accounts for a genome coverage of 0.25, we built an oligomer index that stores the number of occurrences of each encountered oligomer, at varying sizes. The index is then used to query a given maize sequence for the repeat number at any given basepair. We use a sliding window to average a neighborhood of oligomers. We found that an oligomer size of 20 and a window size of 50 yield as good a specificity as the ISU repeat library while drastically reducing the number of false positives (unique regions that are deemed to be repetitive). Repeats are currently available as discreet tracks in the genome browser.

Repeat Masking

RepeatMasker is run on all sequences using the MIPS repeat library (http://mips.gsf.de). The annotated repeats are classified using the MIPS Repeat Element Catalog (REcat), which provides a hierarchical tree structure of repeat families. The repeat families break down into high-level classes: Class I Retroelements, Class II DNA Transposons, Class III RNA Transposons, and Other. These classes can then be used for more downstream analysis of the genome, such as validation of TE-classified gene predictions. The RepeatMasker annotations are available from the genome browser and provide links back to the MIPS website for details of the repeats.

Cereal Alignments

Sequenced clones are aligned to 54 different cereal data sets. The data sets include important maize data sets such as the maize EST and BAC-end libraries. The current strategy uses the BLAT alignment tool to analyze the incoming maize sequences. Different data sets require different alignment parameters. Several libraries, particularly those that demonstrate high sensitivity to repetitive DNA, are aligned to repeat-masked maize sequences. Also different selection criteria are applied to procured alignments, depending on the nature of the data set. A set that contains uniquely-mapped markers, such as maize BAC ends, are processed using a best-hit approach — only one hit

would appear across the genome. Alignments from data sets that contain more prevalent markers are selected using a cutoff criterion. The analysis makes use of Gramene's BioPipe pipeline. BioPipe (Hoon et al., Genome Res. 13:1904–1915, 2003) at this time is no longer being supported. Based on the lack of support of BioPipe the Maize Sequencing project and Gramene have made a strategic decision to develop resources that will leverage the Ensembl infrastructure. At this point in time cereal alignments are partially automated and evaluation of Exonerate (Slater and Birney, BMC Bioinformatics 6:31, 2005) and the Ensembl runnables to support these features are being evaluated.

Electronic SSR Analysis

The current release of the database supports annotations for mined Simple Sequence Repeat markers (SSRs) from BAC end sequences. The nomenclature for the SSRs was done in conjunction with the Maize community database, MaizeGDB and as part of NSF Project #0333074. The analysis assigns universally unique IDs to novel SSR markers to allow for specific localization of SSR markers. The mined SSR markers are ultimately provided to MaizeGDB in order to provide meaningful entry points to the data. The eSSRs are available as part of CytoView where a drop down menu from the features provides links to both MaizeGDB and Gramene for details. The analysis has been integrated into the annotation process for all sequenced BACs, but the quality control is not complete. Release of this data through the BAC ContigView is anticipated for December 2007.

Analysis for Primary Sequencing Effort:

Minimal tiling path clone selection

Working with project participants a system has been developed for employing BAC-end sequences to aid in ordering contigs near clone ends that provide insight about minimal tiling path (MTP) clone selection. BAC end sequences generated by the FPC map assembly, as well as those produced by post-sequencing clone-ends as a quality control measure, are aligned to contigs from improved sequence. This serves to inform the relative position and orientation of the FPC BAC end sequences.

Repeat Tagging

The knowledge gained through the mathematical *k*-mer analysis has been implemented within a repeat tagger that has been exported to WUGSC. Repeat tags are placed on BAC assemblies at each finishing iteration in order to terminate contig extension at repetitive endpoints.

The Maize Genome Browser:

The maize browser is based on the Ensembl browser (http://ensembl.org). The browser, available at http://maizesequence.org, provides several views of the maize genome, namely, a high-level MapView providing a topographic overview of each chromosome along with associated features, a chromosome-based FPC view, CytoView, presenting updated clones that have been sequenced by the project, and a lower-level BAC view, ContigView, that provides all underlying annotations that have been procured for the clone sequence maps. In the past 9 months the site has upgraded from Version 40 to version 45, providing enhancement to existing views as well as increased website performance. The website has evolved beyond a genome browser and provides various types of data about the sequenced maize genome. Beyond visual genome browsing, users can access the data via sequence search (BLAST), marker name search, FTP access, or data syndication via RSS. The website's index page provides a set of entry points into the browser, and includes documentation about the project, its standards, nomenclature, and overall progress.

A common starting point is MapView, which provides, for each chromosome, a high level view of the chromosome's state. Vertically-drawn density plots provide a rudimentary visualization of all FPC clones, the relative number of sequenced clones, the SSR markers discovered on the FPC map, and the core maize bins, aligned with the corresponding chromosome karyotype view of the FPC bands.

To facilitate entry, "bins" were identified using available markers on the IBM 2 Neighbors map, which includes the anchored overgos. These bins are displayed as part of MapView, CytoView and the main navigation bar on the left hand side of the Website.

The FPC view provides a bird's-eye view of the underlying agarose FPC map that composes the maize genome. It features the FPC assembly, including all the clones. A prominent track displays clones sequenced or in-progress by WUGSC as part of the project. Historical clones from previous sequencing efforts are also displayed for posterity using a specific color code. The FPC view provides a set of marker tracks, distinguished by function and origin. A set of high-confidence color-coded overgo markers are also included. The maize core-bin markers present a cytological context to the physical map. Finally, a track displays syntenic rice regions, linked to Gramene, that were determined based on contact points to overgo probes on the FPC map. The FPC view can be accessed through specific FPC contigs shown on a universal navigation bar, or by clicking on a region on MapView.

The BAC view, reachable either through the entry page or by selecting individual clones from the FPC view, is now available for all clones that have been submitted to GenBank, at every level of sequencing. The BACs provided by the browser reflect the same sequence that is available in GenBank, despite the fact that underlying intra-BAC contigs are unordered. GenBank records for HTGS_IMPROVED clones include higher-level scaffolding information. These contig scaffolds attempt to distinguish contigs that are known to be ordered with respect to each other from those that aren't. Several ordered scaffolds may exist within the same clone, even though the relative position of the scaffolds may not be known. Such scaffolds are prominently distinguished on the browser using an appropriate color scheme.

The BAC view graphically displays all data that has been generated through the annotation pipeline. These include classified gene ab initio predictions, cereal alignments, mathematically defined repeats, as well as curated MIPS repeats. Externally curated sequence-based markers, such as TWINSCAN transcripts, are presented via Distributed Annotation System (DAS) tracks. From the gene models, users can obtain additional annotation on the gene models from the GeneView. In the next year users will be able to obtain orthologue and paralogue information as the BACs move from primary to secondary annotation.

Finally, the browser includes SyntenyView, a graphical visualization of synteny derived between maize and rice. The views are universally accessible through the navigation bar. The views provide linkages to corresponding FPC views on the maize browser as well as to the rice browser on Gramene (http://gramene.org).

Data Export: All genome browser views provide export functionality specific to the region being displayed, in contrast to the genome-wide FTP dumps available on the site. Users can choose to export data as either raw sequences or meta-information for a particular region, such as the genomic coordinates of included markers.

FTP Site

The website now includes an FTP server that provides access to sequenced clones, underlying sequence contigs, as well as gene predictions and their associated translations, broken down by class. Finally, an FTP sequence report that indicates which clones on the FPC map are being sequenced through the genome sequencing project is also included.

The FTP dumps are generated every week to coincide with the latest update of sequences and underlying clone information. As resources allow, the dumps from previous weeks are preserved.

BLAST

The BLAST server initially (March, 2007) provided alignments to all sequenced clones. The server has evolved to provide a variety of other options. Users are now able to search a variety of data sets, including BAC clones, GSS sequences such as BAC ends, as well as the predicted gene models either through nucleotide BLAST (BLASTN) or translated BLAST (TBLASTN).

RSS Notification

With the changing nature of the maize sequence and with the addition of new annotation tools to analyze the underlying sequences, it becomes cumbersome for users to track updates. As part of the plan to address this, the website now provides data syndication feeds. Built on RSS technologies (Really Simple Syndication, http://www.webreference.com/authoring/languages/xml/rss/intro/), the system provides notifications of updates for a given region in a syndicated fashion, meaning that it is accessible without having to visit the website. Users can "subscribe" to feeds through a number of freely available applications. A standalone news aggregator, for example, can poll the website for updated items. Several established browsers such as Firefox and Internet Explorer (version 7) also include RSS support. The website allows users to subscribe to any region specified through a notification form.

For the initial release, web feeds are available for FPC markers specified through chromosome coordinates. The system is being rapidly enhanced to include all types of sequence data on both the FPC map as well as individual clone maps.

DAS

The Ensembl browser provides inherent support for DAS (Distributed Annotation System; http://biodas.org) functionality. Sequence annotations that are stored on an external server can be readily visualized on the browser without having to import and maintain the data into the maize database. The browser can be configured with any number of external data sources that can be displayed as separate tracks.

Users can readily use DAS to visualize their own private annotations on the sequenced BACs. In such cases, the DAS tracks remain unpublished. However, DAS tracks that are intended for public use are configured as part of the browser and are easily accessible.

As a pilot project, TWINSCAN transcript annotations (NSF #0501758; http://maize.danforthcenter.org/abinitio.htm) are provided on the BAC views. Visual placement of such transcripts has been enabled by the preservation of the GenBank record both on the browser end and within the originating transcript data source (at the Danforth Center).

Other projects and collaborations are being considered for providing annotations through a DAS server.

Plans: For the upcoming year, the annotation pipeline will incorporate higher-level annotations into the analysis of individual clones. The existing protein annotation pipeline will be extended to provide Gene Ontology (GO) terms as well as value-added correspondences to established protein databases such as SwissProt using the Xref system. Sequence-based whole genome alignment will be conducted between maize and other mature cereal genome assemblies, such as rice, sorghum, and Arabidopsis. Other sequence-mining tools will be incorporated to discover potential SSR sites, tRNA genes, and other interesting sequence features. Externally curated data sets, such as maize full-length cDNAs and the maize optical map, will be integrated into the genome. Finally, leveraged by the integration of external data, evidence-based gene builds will be conducted to provide mature mRNA transcripts.

4. Bioinformatics ISU. Responsible PI: Pat Schnable

Goals: The Iowa State University group is focusing on refining strategies for BAC assembly. Our long-term goals are to develop computational strategies to use the project's high quality sequence data to generate even better BAC assemblies. In addition, we desire to

develop a community-accessible visualization of assembled BACs that will allow scientists to evaluate the data used to make specific assembly decisions. In this manner community members will be able to decide for themselves the degree of confidence to which to assign particularly contigs and scaffolds. During the current project our primary goal is to determine whether the approaches described have the potential to generate improved assemblies relative to traditional approaches.

Progress: Although traditional assembly approaches typically work well, the maize genome presents some unique assembly challenges including the presence of "NIPs" (Nearly Identical Paralogs) and large numbers of long, high-copy, highly conserved elements. NIPs are groups of low-copy genes that exhibit >98% sequence identity (Emrich et al., Genetics, 2007). Conservatively, 1% of maize genes have a NIP. Members of a given family of NIPs can be arranged in tandem arrays or distributed across the genome. Tandemly arrayed NIPs could potentially collapse during BAC assembly. Collapse means that two (or more) adjacent copies of a sequence are inadvertently assembled into a single copy. We have previously shown that assembled maize sequences (MAGIs) can contain collapsed NIPs.

To test whether this occurs during BAC assembly, we used seven NIP families whose members were shown by genetic analyses to be tightly linked in recombination experiments involving 91 recombinant inbred lines. The sequences of the seven NIP families were used to blast against assembled BACs that had been deposited in Genbank. Hits were obtained for all seven NIP families. Shotgun sequences from each BAC were downloaded from the trace archive of Genbank and aligned to these BACs. The resulting multiple sequence alignments were examined for the presence of paramorphisms (Emrich et al., Bioinformatics, 2004) which provide evidence of collapsed NIPs. In two of the seven cases, evidence of NIP collapse could be detected. Although we have developed a general strategy to use paramorphisms to prevent NIP collapse during BAC assembly, this strategy has not yet been implemented.

Just as NIPs can collapse during assembly, other kinds of repeats can complicate traditional BAC assembly pipelines. In traditional assembly approaches, sequence alignments between reads are identified. Contigs are assembled by starting at a good alignment and then extending the ends of contigs one sequence at a time. Clone pair information is used to scaffold contigs *after* contig construction.

In an attempt to develop improved BAC assembly strategies, we are exploring the idea of integrating clone pair data into the contig assembly process. To do so we are modeling sequence alignments and clone pair relationships as a graph. First, we construct an alignment graph in which sequence reads are nodes. A (black) edge is drawn between a pair of nodes if there is a valid sequence alignment. Next we introduce two additional types of edges into the graph. Clone pair edges are drawn (in red) between paired nodes. "Path edges" (green) are drawn between two nodes if the nodes are close together in the graph AND their clone pairs are also close to each other. Path edges identify sequence alignments that are more likely to be relevant to correct assemblies than are unfiltered sequence alignments.

We can then use various graph transformations to ensure that black edges (sequence alignments) represent *correct* genomic overlaps, and resolve entries into and exits out of repeats. For example:

Use clone pairs to validate alignments in repeat regions if the corresponding clone pairs are anchored to unique regions and exhibit alignment.

Use paramorphisms to break spurious alignments due to NIPs.

Use clone pairs to match entries into and exits out of repeats.

Use clone pairs and validated alignments to guide contigs

Use graph min-cuts to find correct assignment of reads to the complementary strands.

This will allow for annotation of paths (contigs) via walking through the graph. In doing so we make use of three levels of pointers:

Black edges: show what steps are available

Green edges: indicate the best path

Red edges: indicate the final destination

This graph approach was applied to three random (Stage 3) maize BACs that had been deposited in Genbank. Multiple lines evidence established the existence of repeat-induced "knots", collapsed repeats and NIPs, and missed scaffolding opportunities in the three BACs.

Collaborations:

MaizeGDB (http://maizegdb.org) Ames, IA

Deep reciprocal links are present between both websites, allowing maize researchers to easily navigate between maps and features. The WUGSC and CSHL have jointly written a letter of collaboration regarding providing updated information for the 2008 MaizeGDB Project Plan, an internal USDA-ARS document for continued funding.

Gramene (http://gramene.org) Cold Spring Harbor, NY

Gramene has been a valuable resource in many aspects.

Development efforts have heavily leveraged the Gramene project. Source code is heavily shared between both projects. Initially the Ensembl codebase was inherited from Gramene. Subsequent modifications and enhancements have propagated back into the Gramene codebase. Likewise, the maize annotation pipeline has been progressively adapted for use by Gramene and is presently repeatedly run on other cereal genomes. Guidance and development effort by Gramene personnel has also proven invaluable over the course of the project. Exchanges of code and ideas are ongoing and drive further improvement of the website. Deep reciprocal links are maintained between both sites.

EBI Ensembl (http://ensembl.org) Hinxton, UK

The Ensembl team provides much needed software support. Reciprocally, new functionality introduced into the maize browser as well as improved software enhancements, have been incorporating into the main development branch of the Ensembl codebase.

Several workshops and meetings have taken place between both groups to further enhance website and pipeline code and to discuss future plans of both projects.

Affymetrix Maize Pilot Expression Array Project

A recent collaboration has been established with Dr. Roger Wise and Affymetrix to design a maize genome GeneChip for transcript detection. The project team will be furnishing mature mRNA transcripts from evidence-based gene predictions that will facilitate the design of accurate microarray probes. The microarray data that will be generated by the project will be readily available on the website.

Optical map Madison, WI

Proposals are being made to leverage the maize optical map (NSF #0501818) in validating the maize sequence and identifying potential sequence assembly errors. Concrete plans are also being made to visualize the optical map alongside the FPC map and individual sequence maps on the maize browser, in order to provide context-based public access to the optical map.

TWINSCAN (http://maize.danforthcenter.org/abinitio.htm) St. Louis, MO

TWINSCAN transcripts (NSF #0501758; http://maize.danforthcenter.org/abinitio.htm) are available for public access through a Distributed Annotation System (DAS) track. The transcripts are displayed alongside the predicted gene models and can be used for reciprocal validation of both data sets.

Vmatch (http://www.vmatch.de) Hamburg, Germany

A unique collaboration has been established with Dr. Stefan Kurtz, the principal developer of Vmatch (http://www.vmatch.de). Vmatch has been used extensively for mathematical analysis of the maize genome and is being adapted for cross-species analysis with other cereal genomes. The collaboration has allowed for specific software modifications in Vmatch in order to meet certain requirements of maize-related hypotheses.

Full-Length cDNA Project (http://www.maizecdna.org) Tucson, AZ, Stanford, CA

Full-length cDNAs will be provided on the maize browser as sequence-based alignments and used in evidence-based gene predictions.

Public Presentations: Project personnel have presented the project at various biological and maize-related conferences with involvement varying among informative project poster, project overview talks, and website tutorials. These conferences include the Maize Genetics Conference, Plant Genome Conference, Plant and Animal Genome Conference, Rice Functional Genomics Meeting, Gordon Conference on Agricultural Biotechnology, Banbury Meeting on Conifer Genomics, American Society of Plant Biologists Conference, Genome Biology Conference, Genome Informatics Conference, Intelligent Systems in Molecular Biology Conference, and the National Corn Growers Association Conference. Involvement in such conferences varied between informative project posters, project overview talks, and website tutorials. The WUGSC group also presented a project overview to the annual meeting of the National Corn Growers Research and Business Development Action Team in December 2007.

Sequencing the Codifying Genome of the *Palomero Toluqueño* Mexican Landrace

--**The Mexican Maize Genome Team (CENTLI)**. National Laboratory of Genomics for Biodiversity (Langebio); Cinvestav Campus Guanajuato, Km 9.6 Libramiento Norte Carretera Irapuato-Leon, Irapuato Guanjauato MEXICO.

In contrast to developed countries where it is fundamentally used for agro-industrial or animal production, maize in Mexico is mainly cultivated for human nutrition and under a wide range of climatic conditions. Its consumption represents the main source of protein and energy in rural regions, particularly in the poorest communities. Maize was domesticated from its wild progenitor teosinte (derived from "*teocintli*" in nahuatl language: "*teotl*"=sacred and "*cintli*"= dried ear of corn), a common name given to a group of annual and perennial species of the genus *Zea* native to Mexico and Central America (reviewed in Doebley, Ann. Rev. Genet. 38:37-59, 2004; Matsuoka, Breed. Sci. 55:383-390, 2005). As the center of origin and domestication, Mexico has the largest diversity of maize genetic resources. Maize biologists do not always agree on the total number of maize landraces that exist in Mexico. The classical monograph published by Wellhausen et al. (Folleto Técnico Número 55, 1951) has been an essential reference for all subsequent reports. Based on general architecture, kernel cytological traits, and physiological characteristics (time of flowering, yield, and disease resistance), they classified 25 landraces into 5 major groups. The first one included 4 Ancient Indigenous Races believed to have arisen from primitive pod corn (*Palomero Toluqueño*, *Arrocillo Amarillo*, *Chapalote*, and *Nal Tel*).

Large-scale sequencing efforts concentrated in B73 will not be sufficient to fully understand maize genome organization and identify all functional units available in the domesticated gene pool. To complement the large-scale B73 sequencing initiative and explore landrace genomic diversity, we undertook the structural and functional characterization of the *Palomero Toluqueño* genome after estimating its small genome size (20 to 25% smaller than B73; Mexican Maize Genome Team, unpublished results). *Palomero Toluqueño* is an ancient popcorn landrace originally classified by Wellhausen et al. (1951), and a member of Central and Northern Highlands Group composed of 15 landraces that most often produce short individuals (140-190 cm) and grow at elevations higher than 2000 meters. *Palomero toluqueño* accessions usually show conically shaped ears and high kernel row number, a low frequency of tassel branches, a weakly developed root system, and strongly pubescent leaf sheaths often pigmented by anthocyanins. A total of 1.2 million Sanger reads (10% HCot; 90% enzyme-based methyl-filtration) and 213 pyrosequencing runs (50% methyl-filtered, 50% whole genome sequencing) were sequenced at the National Laboratory of Genomics for Biodiversity (Langebio), in Guanajuato. The total sequence generated represents coverage of more than 3X the full genome; it has been complemented by in-depth pyrosequence-based global transcriptional analysis of the same genotype. As expected, a significant percentage of codifying transcripts are not reported in publicly available databases, suggesting that a large portion of the molecular and functional diversity contained in Mexican landraces remains unexplored; the structural annotation of resulting contigs will be completed by the end of 2007.

Figure 1. *Palomero Toluqueño* landrace. State of México (Valley of Toluca Valley). Identification: Wellhausen et al., 1951. Description: Wellhausen et al., 1951.

XII. ALLERTON REPORT 2007

The Future of Maize Genetics
Planning for the Sequenced Genome Era

A Maize Genetics Community Retreat
Allerton Park and Conference Center
March 20 - 22, 2007

Executive Summary:

Leaders in the maize community met for a two-day retreat to discuss the strengths, challenges, and initiatives that define the future of maize research. To guide strategic planning, the community first identified key questions in biology that can be best answered using maize as a model system. These biological questions were considered relative to the overarching goal of understanding the genetic basis of traits in maize. The research directions defined below and the plans to achieve the goals serve as the foundation for basic research and provide the tools for improving food, fuel, and crop yields in a changing environment.

Key biological issues define our research goals and directions:

- Maize is the pre-eminent model for studying genome evolution and trait variation due to its unsurpassed natural diversity, genome duplication history and range of adaptations.
- Because adaptation is critical to agriculture, maize research will continue to be a model for understanding the basis of genetic interactions with the environment.
- Study of maize heterosis will provide key information about how genes and alleles interact.
- Maize will continue to lead in the area of epigenetics. Imprinting, paramutation and transposons were discovered in maize and are readily studied with color markers.
- Maize is positioned as a leading model for developing cellulosic biofuels for the future.
- Maize is a model for the study of development and physiology of unique traits such as C4 photosynthesis, a persistent endosperm, inflorescence structure, etc.
- Maize cytogenetics is highly advanced and continues to provide tools for understanding mechanisms of meiosis and for developing the potential of chromosome manipulation.

Community resources will help achieve our research goals:

To advance these research areas, community resources must be created or strengthened. Short and long term planning will help leverage the sequenced maize genome and will position maize as a model for tool development and for hypothesis-driven and translational research.

Short Term Planning (Expect substantial progress in the next one to three years):

1. Current sequencing/annotation will be completed and additional map-based sequencing efforts initiated.
2. Centralized databases with increased funding are needed now.
3. Indexed reverse genetic resources need to be finalized and will accelerate many areas of research. Current mutagenesis libraries should be indexed with new technologies.
4. Expression platforms and informatic tools should be selected and developed.
5. Transformation capacity should be enhanced in the public sector. Continuous support mechanisms for public transformation need to be resolved.

Long Term Planning (Start now, with sustained efforts over the next decade)

1. Databases and stock center capacity will be enhanced, coordinated and supported.
2. Map-based sequences of other inbreds, races, and teosintes will be available.
3. A phenomics initiative will be underway, with large scale and multi-dimensional phenotyping capabilities for multiple environments available for the entire community.
4. The maize scientific community will be large, diverse, well-trained, and interactive.

Detailed Report:

Introduction

The maize genetics community held a two-day retreat to discuss the future of maize research in the post-genomics era. The meeting included principal investigators representing approximately 60 labs from universities, colleges, industry, and USDA-ARS. Participants were primarily from the U.S., but representatives from the United Kingdom, France, and Germany were also present. Staff from ARS, NRI, NSF, DOE, and NCGA participated in discussion on the second day of the retreat.

The goal of the meeting was to develop a strategic plan for the future of maize genetics research. To guide strategic planning, the community first identified important questions in biology that can be best answered using maize as a model system. These biological questions were considered within the context of an overarching goal to understand the genetic basis of traits in maize -- traits that are the foundation for improving food, fuel, and fiber crop yields in a changing environment. Second, the community considered the current research capacity for answering these biological questions and also explored how to translate the answers to practical outcomes. It was noted that recent research accomplishments have opened many new avenues of investigation. For example, advances in genetic mapping technology have enhanced functional genomics so that gene functions can be discovered using a single population of plants and inexpensive sequencing technology. This groundwork will allow rapid establishment of productive genomics programs in related agronomically important species and will advance research in biofuels. Following the plan presented here, maize researchers will be able to accelerate the rate of discovery considerably, delivering an expanding knowledge base for the needs of breeders, for biotechnology industry and for continued basic research.

Challenging unanswered questions in biology best addressed by research on maize

Discoveries in the current genomic era of biology have generated new questions and enabled new approaches to long-standing questions. The maize community discussed these issues in broad terms and then focused on the subset of questions that could clearly be addressed best in maize due to its unique development and biology, its genetic and evolutionary history and its genome architecture.

- How is genomic diversity maintained, and how does it change during evolution?
- What is the underlying molecular genetic basis for specific traits in a species?
- Can we use maize to predict what genes will regulate plant growth in related species?
- Can natural variation provide information to develop novel breeding traits?
- What drives genome evolution, and how are these processes impacted by interaction with the environment?
- What is the genetic, molecular and physiological basis of hybrid vigor (heterosis)?
- What are the impacts of sequence-independent inheritance (epigenetics) on the growth development and evolution of maize?
- How does cytogenetic variation impact genome architecture, agronomic traits, and plant breeding efforts?

Maize is particularly useful to answer questions about genome evolution, genetic diversity and selection because allelic variation in maize is greater than in any other eukaryote. Also, due to its duplication history, maize is a model monocot for understanding evolutionary mechanisms that surround genome duplication events. Gene content and order varies considerably between maize lines, reflecting local transposon-mediated rearrangements and tandem duplications. This great genetic diversity translates into differences in phenotype and variation in how maize interacts with the environment. The genetic diversity also provides a rich toolset for the study of heterosis. With the ability to compare maize alleles with those of wild *Zea* accessions (teosintes), maize also provides an excellent species to study selection. An understanding of selection will allow researchers to harness existing diversity for advancing biological understanding and crop improvement.

Maize is well suited to study epigenetics because of the accessible phenomena associated with transposition, imprinting, and paramutation, three processes that were first identified in maize. The study of epigenetics is facilitated by the separate male and female flowers, which simplifies the process of conducting controlled pollinations. Maize has a rich collection of active transposons in the genome and color markers that are simple to score. The large size of the plant allows researchers to sample specific tissues at distinct time points from a single individual. Furthermore, epigenetic events confer heritable phenotypes, and can therefore provide direct information for crop improvement, placing maize at the forefront of translating basic research for the agronomic community.

Maize is a genetic model for other grasses with its rich collection of mutants, genetic diversity and ease of moving between phenotype and genotype. Information from maize can be easily translated to other important, less tractable members of the grass family. For example, maize is a member of the Andropogoneae, and thus is closely related to other energy crops such as *Miscanthus*, switchgrass, sorghum, and sugarcane. Knowledge of cell wall synthesis and degradation can be obtained in maize and then transferred to these potential crops for which few genetic resources are available.

Arabidopsis thaliana has been a model for understanding principles behind growth and development, but some key developmental, cellular, and physiological processes do not occur in *Arabidopsis*. Biologically and economically important features such as C4 photosynthesis, a persistent endosperm, phase dependent epidermal differentiation, complex inflorescence structure, and sex determination are best studied in maize.

Finally, maize has been central to research in cytogenetics and continues to provide cutting edge information about genome architecture. Tools developed cytogenetically will be useful for future chromosome manipulations, which can benefit both basic and applied research. The combination of easily analyzed chromosomes, meiotic mutants, well-studied segregation phenomena, and increasingly sophisticated cytogenetic tools continues to position maize as a model system for this area of research.

Current Tools, Resources and Future Needs

A major goal of the Allerton Retreat was to assess the current state of research capabilities as the maize B73 genome becomes available. It is clear that fully sequenced genomes have revolutionized the corresponding research communities. With long term planning, we can learn from these past experiences and develop the tools and capacity to optimize and fully leverage the value of a sequenced maize genome. Topics that were considered most imperative to achieve this goal are summarized here and represent the starting point for further discussion.

Annotation of a fully sequenced B73 genome and additional genome sequencing is the foundation for future research

The maize B73 genome is currently being sequenced using a minimal tiling BAC approach with full display at http://www.maizesequence.org (a project site that will exist during the sequencing project's funding period). The sequenced genome promises to revolutionize maize research. With annotation, it will be the foundation upon which complementary resources and activities such as reverse genetics and phenomics will be built. To achieve this promise, a fully annotated, accessible, and centralized sequence database will be essential because all additional resources depend upon robust integration of sequence information. The sequence project site must be transitioned into a community-based permanent platform that will have robust long-term support. The community expressed the desire that **MaizeGDB should become the centralized sequence resource soon after the genome is complete (2009-2010).**

The maize community recognizes that nearly all relevant questions posed here will need sequence information beyond the annotated B73 genome. **Genomic sequence from additional maize lines is essential to advance crop improvements and to exploit maize for its unparalleled strength as a model system and as a fuel, food and fiber resource worldwide.** Developing map-based sequence information of additional genomes was identified as a priority due to the unique duplication history of the maize genome and due to the exceptional haplotype variability among inbred lines. A physical map from a second inbred line, and ultimately multiple lines, was considered important. Post-Allerton follow-up discussion will allow for continued assessment about the most efficient and effective way to accomplish synthesis of sequence information from multiple genomes.

Databases need to be centralized

Continued assessment and coordination of data deposition is essential to all advances in maize research. Currently, various types of plant database resources exist and are utilized by maize researchers, including Model Organism Databases (MODs), Clade Oriented Databases (CODs), Automatic Annotation Shops (AA), Static Repositories, and Laboratory Information Management Systems (LIMS; a category that includes coordinated project databases). A (non-exhaustive) list of databases used by maize researchers includes:

Resource Type	Funding Agency	Resource	Website
MOD	USDA-ARS	MaizeGDB*	http://www.maizegdb.org
MOD	NSF	TAIR	http://www.arabidopsis.org/
COD	NSF, USDA-ARS	Gramene	http://www.gramene.org
COD	USDA-ARS	GrainGenes	http://wheat.pw.usda.gov
COD/AA/LIMS	NSF	PlantGDB	http://www.plantgdb.org
COD/AA/LIMS	NSF, USDA-ARS	PLEXdb	http://plexdb.org
AA	NSF	TIGR	http://www.tigr.org/
AA/LIMS	NSF/USDA/DOE	MGSC's Maize	http://www.maizesequence.org
	Genome Browser*		
AA/LIMS	NSF	MAGI*	http://www.plantgenomics.iastate.edu/maize/
AA/LIMS	NSF	FPC-maize*	http://www.genome.arizona.edu/fpc/maize/
Static	NIH	NCBI	http://www.ncbi.nlm.nih.gov/
Static	NIH	UniProt	http://www.pir.uniprot.org/
Static/LIMS	USDA-ARS	GRIN	http://www.ars-grin.gov/
LIMS	NSF	Panzea*	http://www.panzea.org/
LIMS	NSF	ChromDB	http://www.chromdb.org/

* indicates a maize-specific resource

MaizeGDB is of particular interest because it is the MOD for maize. The MaizeGDB website serves biological information about the crop plant *Zea mays* ssp. *mays*. Genetic, genomic, sequence, gene product, functional characterization, literature reference, and person/organization contact information are among the datatypes accessible through MaizeGDB. Based upon community evaluation and input, MaizeGDB will continue to focus on the following areas of concentration over the next five years: 1) integration of new maize genetic and genomic data into the database, including expansion of phenotype data and tools, 2) expansion of structural and genetic map sets, 3) access to gene models calculated by leading gene structure prediction groups through the MaizeGDB interface, and 4) support of community services such as coordinating the Maize Meeting, MGEC Elections, Polls, etc.

Long-term support for MaizeGDB from USDA-ARS was recognized; however, **new and creative funding mechanisms are required now to provide sufficient resources to exploit a fully sequenced genome**. The fact that maize will become a model genome for other complex grass genomes necessitates even more careful planning. Coordination with Gramene is critical to success. With additional resources, MaizeGDB should be able to integrate project data from diverse studies, keep gene function data current, oversee community curation, and carry out gene and plant ontology as well as metabolic pathway curation. To improve access to maize sequence data, re-

sources that integrate various gene models and annotation sets must be made available to MaizeGDB. Complex datasets from federally funded projects should be deposited into MaizeGDB. However, collaborations should be established between MaizeGDB and the researchers who develop these complex datasets to insure efficient and cost-effective data flow directly into MaizeGDB. For the other database resources listed in the above table, recommendations from this group are in agreement with those cited by the Plant Database Working Group (see http://www.maizegdb.org/PDBNeeds.pdf).

Transformation technology needs to be advanced and costs reduced

Improved maize transformation resources remain one of the highest priorities for the community. A sequenced maize genome will continue to drive research hypotheses that require direct testing in transgenic plants. Furthermore, transformation capabilities will bridge the gap between basic and applied research. To achieve these goals, **several critical needs were identified: an increased capacity for public sector maize transformation, improved transformation of diverse lines and reduced transformation costs**. Improved regulatory transfer would also facilitate progress and communication among researchers. The community recommends cohesive action to evaluate ways to improve regulatory compliance within current and changing Federal guidelines.

Public researchers currently produce transgenic maize primarily by outsourcing to the Plant Transformation Facility (PTF) at Iowa State University or through facilities at their own institutions. These centers constitute a critical and reliable resource. The success and demand on the PTF clearly validates the ever-increasing need for maize transformation in the public sector. Costs remain higher than industry, however, reflecting both industry technologies that are unavailable to the public and differences in production scale. Thus, increased transformation capacity, properly implemented, will correlate with reduced costs per transgenic event.

There are several major limitations to capacity building in the public sector. First, more trained transformation experts are essential to insure quality outcomes. Second, more facilities, particularly greenhouses, are also necessary to grow transgenics to seed. Increased funding would be required to devote more resources to existing facilities, either concentrated in one main location or in multiple, collaborating centers that would allow for standardization of genotypes and transformation vectors. Third, reliable transformation of multiple genotypes is needed to reduce the time frame for post-transformation analysis by one or two years for every project.

In addition to improving the production pipeline, continued research is essential to advance methods of transformation. Ideally, this should be facilitated through dialogue with industry to address any bridgeable gaps that might exist between public and private sector methodologies. **Major investments should be made in training and facility improvement. A transformation task force that includes academic and industry representatives should be formed to facilitate this goal.**

Efforts to streamline the APHIS notification process would be beneficial. Such efforts could be accomplished within Federal guidelines. For example, the community could develop a standardized operating procedure (SOP) for transgenic lines so that users can quickly and consistently provide the required information. In particular, common notification requests for frequently used transgenic resources could be standardized by the community, then communicated to APHIS. This cooperation between the maize community and APHIS regulators would help researchers for whom regulatory compliance can be prohibitive, such as researchers at smaller institutions or researchers who experiment infrequently with transgenic maize.

Similar to the challenge of regulatory compliance, many researchers do not have the infrastructure to grow transgenic events to seed. Furthermore, in the future integrated genomics world, it is essential that all researchers should be able to navigate between *Arabidopsis* and maize to conduct transgenic experiments. Most *Arabidopsis* researchers lack both experience and facilities to carry out the intensive aspects of maize transformation. Multiple centralized field sites across the US dedicated to growing transgenic plants would both facilitate compliance and enable more researchers from diverse institutions to use this critical technology.

The community noted the importance of communication with the public sector to publicize the value of transgenic maize research. One mechanism to do this would be for qualified representatives to communicate directly with reporters or media outlets that are in place at most institutions, to widely publicize our message.

Reverse genetics resources need to be expanded

A sequence-indexed collection of mutations is essential for researchers to exploit the genome sequence fully. It was noted that multiple mutagens are necessary to insure broad coverage of the genome and generate a range of allelic lesions. These would include transposon insertions, small deletions, and point mutations. **It is imperative that these lines be accessible through a community web browser to facilitate dissemination of the resource.** Training in the use of the resource should be an essential and embedded component of dissemination. This collection should be searchable by BLAST, browsable, and linked to readily available seed stocks. **The sequence-indexing of transposon collections needs to be on validated germinal alleles so that seed are available for the community to advance the study of identified mutations.**

To date, several populations have been developed for forward and reverse genetics in maize inbred lines. The use of inbred materials greatly facilitates phenotypic analysis in near-isogenic lines and should be given strong consideration in population development. This is particularly relevant for maize, where a long generation time limits most researchers to propagating at most two generations/year. Large Uniform*Mu*, *Ac/Ds* and TILLING populations have been developed in the W22 inbred. The existence of these W22 populations provides a case for sequencing the W22 genome, which potentially could be one of the choices for a second physical map. The maize community considers that further discussion is needed to come to consensus about sequencing plans after the first-stage completion of B73.

TILLING populations have also been developed in B73. *Mutator* and *Ac/Ds* populations are nearly completed in B73 as an effort to exploit the genome sequence and provide greater accessibility for researchers across a broader geographic distribution. To achieve the goal of near-saturation mutagenesis (95% chance of a disruption in any given gene) additional line development is essential.

A number of approaches were discussed for chemical, radiation and insertional mutagenesis. There was much excitement over the potential for 454 and Solexa sequencing technologies to deliver quickly a near-saturation collection of *Mutator* insertions. Several *Mutator* populations exist with high copy number germinal *Mu* insertions. It was estimated that over 300,000 *Mu* insertions could be rapidly sequenced from Uniform*Mu* from the McCarty lab, and additional populations from the Schnable and Martienssen labs could provide similar levels of coverage. It was noted that a minimal input of resources could help accomplish the task of generating these essential resources.

The possibility of increasing *Ac/Ds* populations and developing fast neutron populations was also discussed to complement the non-transgenic *Mutator* populations. For instance, over 30% of maize genes are represented in tandem duplications, suggesting that a large number of potentially redundant paralogs are present in the maize genome; because many single gene mutations cause a phenotype, detailed analysis of locally duplicated genes in maize will address a key general question in biology, namely the mechanisms that permit subfunctionalization of duplicated genes. The task of recombining single gene insertions in tightly linked paralogs to create double mutant stocks is daunting and unlikely to succeed without a strong genetic selection. *Ac/Ds* can be used to sequentially mutagenize tandem gene clusters providing a resource to define functions for a sizeable fraction of the maize genes. Similarly, fast neutron mutagenesis programs will result in a range of deletion sizes, some of which will encompass multiple adjacent genes. Detailed genetic analysis of a locus is greatly facilitated by using an allelic series of mutants wherever possible. **Increasing effort in generating, expanding and integrating the data from these populations now will pay huge dividends in the coming years**.

Better access to gene expression profiling tools and datasets is needed

A number of platforms presently exist in the maize community for expression analysis. The 44K Agilent and 46K NSF-Arizona long oligo arrays, shoot apical meristem (SAM) cDNA arrays, and a first-generation 18K Affymetrix GeneChip are publicly available. However, the fully sequenced maize genome offers the opportunity to begin conducting gene expression profiling experiments using "all genes" platforms. Moreover, recent advances in cost effective deep sequencing (e.g. Solexa, NextGen) might yet provide another alternative for expression profiling in different tissues and variants including their microRNAs and alternative splice products. The maize community will continue discussion about which platforms will be best and what toolsets need to be developed to insure long-term utility of datasets generated by expression profiling. Tools must be developed that allow datasets to be browsed, queried, visualized, meta-analyzed and linked to the physical and genetic maps of maize. Development of cost-effective platforms is also paramount. **These ambitious goals will require substantial database efforts and funding, but are absolutely critical to optimize use of these cost-intensive datasets.**

Consistent with the emergence of the genome sequence, two "all genes" platforms are currently being designed by collaborative efforts of industry, maize biologists and informaticians, including an Affymetrix 100K GeneChip and an Agilent 105K *in situ* synthesized glass slide array. **Community input into the design of expression profiling platforms continues to be a high priority to the maize community.** The Agilent arrays will allow community input via customization, which is facilitated by its flexible format. A database of sequences, customizable formats and designs will be maintained by Agilent to allow results to be compared across experiments conducted on the various versions of Agilent arrays. The Affymetrix GeneChip will be developed in consultation with the community. It is predicted to include ~70K B73 gene models, allowing the remaining ~30K sequences to be used for evaluating allele-specific expression and the abundance of sRNA, transposon and retrotransposon transcripts according to community input. Each platform provides complementary approaches that together reduce bias associated with sequence variability among alleles.

These "all genes" arrays will be valuable for annotation of the genome, particularly for those sequences that were not represented as ESTs. It will be essential that all platforms adopt a common nomenclature and should be able to retrofit with updated annotations. These features will be a key to the longevity and widespread utility of the "all genes" arrays. The group considered that both of these platforms are cost-effective choices in the current climate and that competition will accelerate the improvement of database support and will lower costs. **Efforts should focus on incorporating community input, developing data integration tools and maintaining accessibility so diverse groups of researchers can benefit from public investment in profiling experiments and database tools.** The maize community also looks toward developing profiling platforms in the future that will accommodate advances in systems biology.

A major phenomics effort will contribute to basic and translational research

Understanding the function of genes and networks is a central research goal both currently and also in the post-genomics era. Phenotyping is one of the major strengths of the maize community. The maize community envisions carrying this capacity to the next level by **developing large scale and multi-dimensional phenotyping capabilities**. The group recognized that understanding adaptation and applying the information to improve agriculture can be best achieved through in-depth phenotyping in diverse environments for numerous traits.

The major goals of this recommended effort are to: 1) harness genetic diversity to assign biological functions to sequences, i.e., associating traits with genes, and 2) enable predictive biology via an iterative process of discovery and validation. This effort will involve broad community involvement to collect and analyze phenotypes in great depth and breadth on a common set of diverse genotypes. It likely will also require common center(s) for production and quality control of seed, shared planting locations and protocols for collecting phenotypes, and centralized quality control for experimental design and data analysis. A series of phenotypes, including agronomic, morphological, cellular and molecular traits will be measured. Genotypes will include combinations of the natural variants, transformants and mutagenized

populations developed by the research community. This effort will require development of new high throughput analytical tools, *e.g.*, remote sensing and image analysis. There will be very wide dissemination of collected data and efforts to coordinate sharing of results.

A unified phenotyping effort proposed here will require new scales of coordination within the community, will require continued advances in cyberinfrastructure, and further development of centralized databases for analysis, synthesis and dissemination of phenomics data. At the outset, researchers will guide the effort by establishing consistency of phenotyping language and by maintaining quality of experimental design and well-designed reporting mechanisms. First and foremost, database resources must be planned to provide for the integration and dissemination of data. Planning for large-scale phenotyping efforts should include industry, if mechanisms for shared and public access of data generated can be resolved unambiguously.

The maize community needs to broaden and strengthen its membership

The maize genome can be best leveraged by increasing the diversity of participants and strengthening the depth of training. The maize research community is committed to training creative, independent and collaborative scientists who conduct hypothesis-driven research, tool development and research translatable to agriculture. This can be achieved by **strengthening and diversifying graduate education and post-doctoral training.** It is clear from the experience of other research communities that establishing effective partnerships, nationally and internationally, is also essential to exploit a genome fully. The maize community considered types of partnerships that need to be developed and strengthened in the coming years including industry, international contacts, a wider spectrum of US-based researchers involved in maize research as well as other plant and non-plant biologists in general**.**

Enhance public - private interactions

Academic-industry partnerships have historically been strong for the maize community. A number of ideas were considered to strengthen our relationships further with industry. One idea was to provide mechanisms for industry to donate resources to the community, such as the EST database that was made accessible through an MTA, and funding of undergraduate summer internship programs. Another idea was to help young scientists make a transition to industry from academia by setting up a partnership with the private sector to facilitate tours and visits and by providing talks for web viewing on 'how to get trained for an industry position'. Issues related to public accessibility of data from new industry-academic partnerships need to be resolved. Adding a private sector member to the MGEC could be considered, as a mechanism to help facilitate interactions.

Enhance international dimension

International efforts should be coordinated to avoid duplication of effort and to foster dialogue. Ideas to enhance international collaboration included inviting one international speaker who has not been to the Annual Maize Genetics Conference to present each year. The community can also encourage and recognize greater efforts by PIs to become involved in Developing Country Collaboration supplements to NSF grants. Fellowship opportunities for graduate students abroad are currently lacking. Furthermore, to encourage the growth of maize research internationally, more meetings could be conducted outside of the US, such as in Mexico, South America, Europe, Asia, and Africa. International researchers might become more engaged in maize research if meetings are more accessible periodically. Independent or satellite workshop/short courses, such as the 2004 CIMMYT workshop conducted before the Annual Maize Genetics Conference, would further attract international participants.

Enhance local and national participation: Forming partnerships as outreach

The maize community is committed to improving science training in the US by reaching out to: small colleges and universities, traditionally under-funded research institutions, undergraduate institutions, minority-serving institutions, community colleges, tribal colleges, the K-12 system, and the general public. It was noted that maize genetics is an excellent "hook" for attracting new participants to science, because corn is such a familiar food item in the US and so many genetics tools are available. The goals of outreach activities should be 1) to integrate research and education and 2) to provide for a mutually beneficial partnership between members of the maize research community and new participants. These goals can be achieved by emphasizing relevance for all involved. Educational partnerships should be logical to the researcher's expertise/interest as well as to the recipients' needs and environment. **Communication among researchers with active and successful outreach programs should be strengthened to avoid duplication of effort.** To avoid continuous reinvention of methods, it might be useful to organize outreach advisory boards that can help guide new programs. Such advisory boards could be established and centralized through the current plant genome research outreach portal (plantgdb.pgrop). Best practices could also be highlighted at the Annual Maize Genetics Conference at the new designated poster session on Outreach and Training. The annual meeting should also be a central venue to bring new students and researchers from outside the maize field together with the current maize research community, thus enticing them to maize research. The Maize Genetics Meeting Steering Committee should continue to develop innovative ways to fund fellowships to new participants.

Summary and Next Steps

The sequenced maize B73 genome holds great promise for contributing to basic and translational research. To take full advantage of that promise we need to 1) make MaizeGDB the centralized sequence resource, 2) make plans and implement next level map-based sequencing efforts, 3) provide increased capacity and lower costs for maize transformation technology, 4) increase sequence-based reverse

genetics, 5) coordinate expression platforms so all data are easily shared, 6) conduct a major phenomics effort that is effectively integrated, and 7) increase participation in maize research. A timeline for completing these goals was discussed as follows:

Activity	**Timeline for completion**
Convert MaizeGDB to a sequence-oriented database	2011 (three years)
Implement additional sequencing efforts. Generate physical maps from other genomes and anchor their sequences to their maps	2013 (3-5 years)
Establish high capacity transformation facilities	2013 (5 years)
Establish a near-saturation reverse genetics resource	2013 (3-5 years)
Standardize expression platforms	2013 (5 years)
Phenomics project underway	2016 (8-10 years)
Increase and diversify the maize research community	2018 (10 years)

Allerton Retreat participants agreed on several first steps to begin to implement the long-term plan described here. **First**, this planning document will be disseminated to the broader maize research community by the MGEC via MaizeGDB. **Second**, this document, combined with an executive summary, will be presented to guests at the Allerton Retreat including representatives of the funding agencies (NSF, DOE, USDA) and the NCGA. **Third**, an article will be submitted to *The Plant Cell* to inform the broader plant biology community of the future directions of maize research in the genomic era. **Fourth**, taskforces will be formed to focus on solutions to particular research bottlenecks, including transformation, and to help shape future new research efforts, such as phenomics. Through MGEC guidance, we anticipate that taskforce action plans will help maintain dynamic assessment of progress and will guide maize research into the future.

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5803F bm*-PI251930 5
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5803L bm*-N2331B 5
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This newsletter shares current research on genetics, cytogenetics, molecular biology, and genomics of maize. Information is shared by Cooperators with the understanding that it will not be used in publications without their specific consent.

Send your notes for the 2009 Maize Genetics Cooperation Newsletter now, anytime before December 1. Your MNL Notes will go on the Web verbatim, and will be prepared for printing in the annual issue. Be concise, not formal, but include specific data, tables, observations and methods. Articles that require extensive editing will be returned. Check MaizeGDB for the most current information on submission of notes. Send your notes as attachments or as the text of an email addressed to MaizeNewsletter@missouri.edu (we will acknowledge receipt, and will contact you further if necessary). If email is not feasible, please mail a double-spaced, letter-quality copy of your note, preferably with a disk containing the electronic version. Please follow the simple style used in this issue (city /institution title / --authors; tab paragraphs; give citations with authors' initials --e.g., Maizer, BA et al., J Hered 35:35, 1995, or supply a bibliography). Figures, charts and tables should be compact and camera-ready, and supplied in electronic form (jpg or gif) if possible. To separate columns in tables, please tab instead of using spaces, to ensure quality tabulations on the web. Your MNL Notes will go on the Web verbatim promptly, and will be prepared for printing in the annual issue. Mailing address:

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SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

MNL 51ff. on line	MaizeGDB - http://www.maizegdb.org
Author and Name Indexes (and see **MaizeGDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeGDB**)	
Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue, updates only after No 78, and MaizeGDB
Rules of Nomenclature (1995)	MNL69:182 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; 82:87
MaizeGDB	
Plastid Genetic Map	MNL 69:268 and **MaizeGDB**
Mitochondrial Genetic Maps	MNL 70:133; 78:151 and **MaizeGDB**

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

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

MaizeGDB needs Cooperators (this means you) to:

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