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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.)

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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).

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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*.

BERGAMO, ITALY
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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.

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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.

BLACKSBURG, VIRGINIA
Virginia Polytechnic Institute and State University

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.

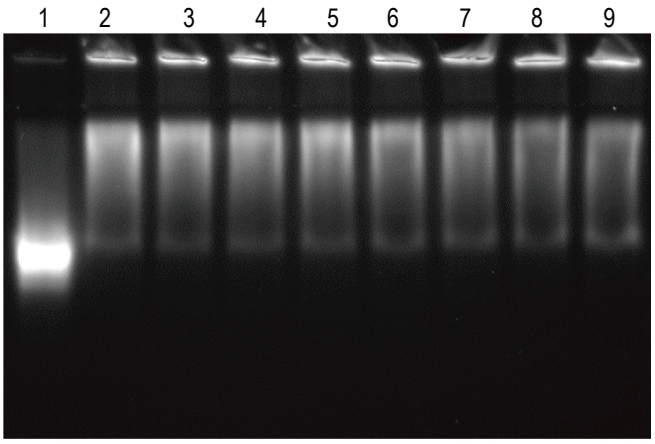


Figure 1. Gel-shift assay to detect binding of BGAF to maize Glu1 in the presence of increasing concentrations of galactose. rBGAF (14 nM) was incubated with increasing concentrations of galactose (0-125 mM) at room temperature for 1 h in PBS, pH 7.4. Following addition of Glu1 (58 nM) and incubation 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.

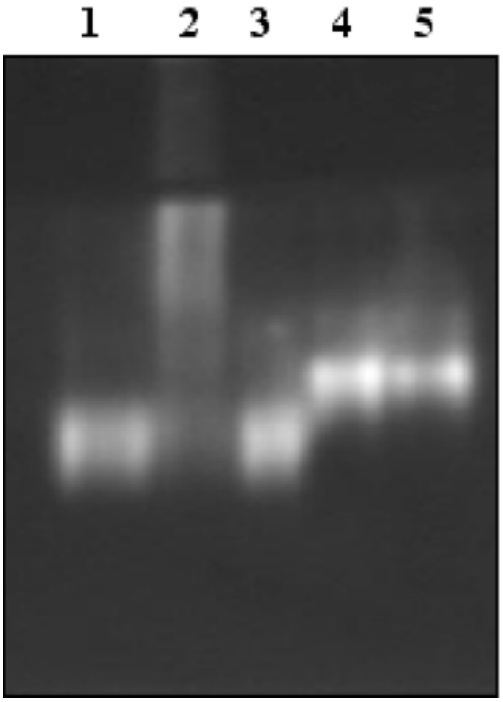


Figure 2. Gel-shift assay to detect binding of dirigent and the JRL domain to maize Glu1. Glu1 (58 nM; lane 1) was incubated with rBGAF (14 nM; lane 2), dirigent (100 nM; lane 3), JRL (100 nM; lane 4) and dirigent plus JRL (100 nM each; lane 5) at room temperature for 2 h in PBS, pH 7.4. Electrophoresis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

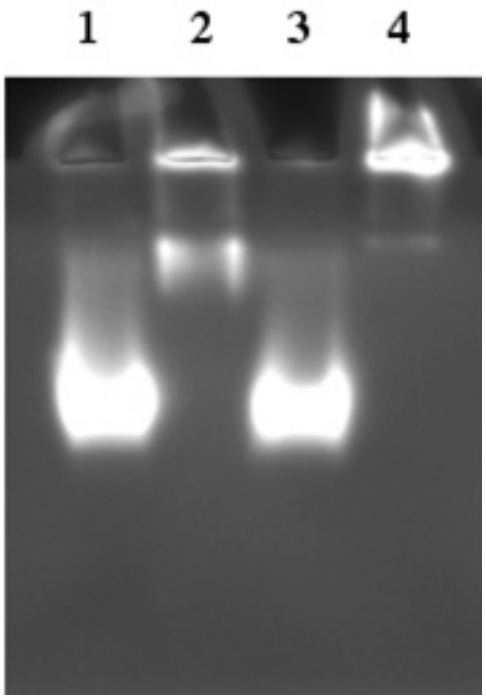


Figure 3. Gel-shift assay to detect binding of chimeric BGAF (consisting of the dirigent domain of the BGAF homolog from sorghum and the JRL domain of maize BGAF) to maize Glu1. Glu1 (58 nM, lane 1) was incubated with 200 nM maize rBGAF (lane 2), sorghum rBGAF (lane 3) and chimeric BGAF (lane 4) at room 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
University of Bologna

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

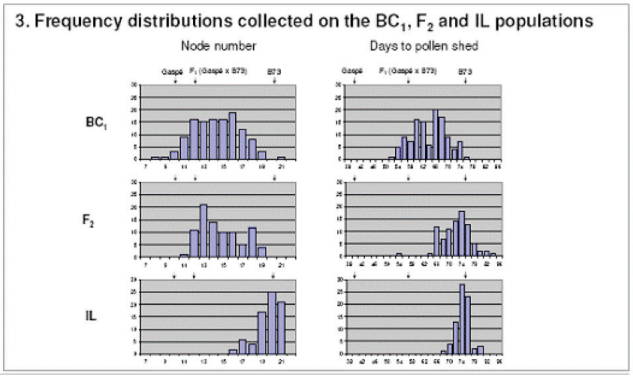
--Salvi, S; Corneti, S; Giuliani, S; Sanguinati, MC; Talamè, V; Tuberosa, R

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.

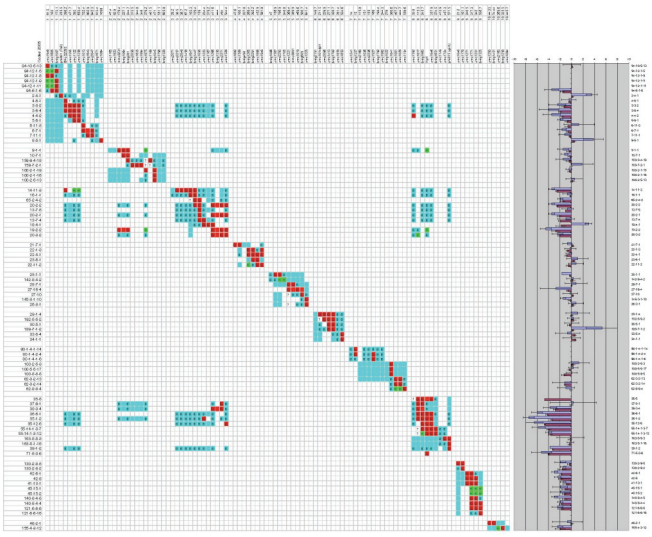
Traits analyzed. Days to pollen shed (DPS), node number (ND) and other traits were measured in the BC_1, F_2 and IL populations. The BC_1 population was grown in 2002, and the F_2 and IL lines in 2006. The 70 IL lines were grown in four reps with 10 plants per plot. The figure below presents the frequency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

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.

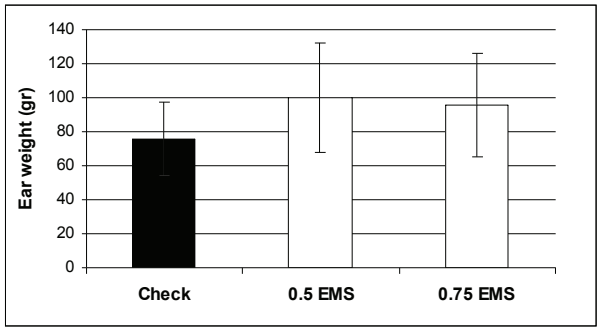

Figure 1. Mean ear weight (grams/ear).

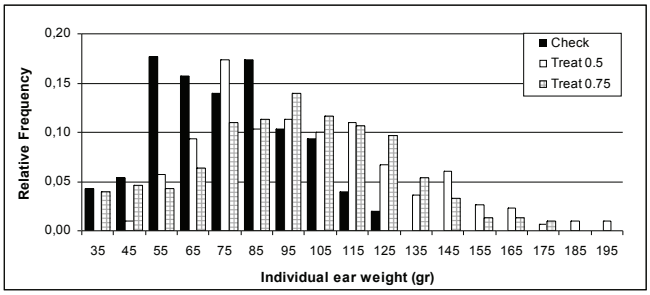

Figure 2. Distribution of ear weight (grams/ear).

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).

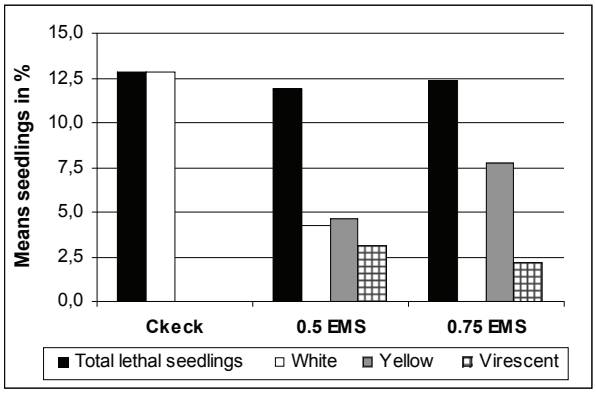

Figure 1. Mean lethal seedlings in % (white, yellow, virescent).

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

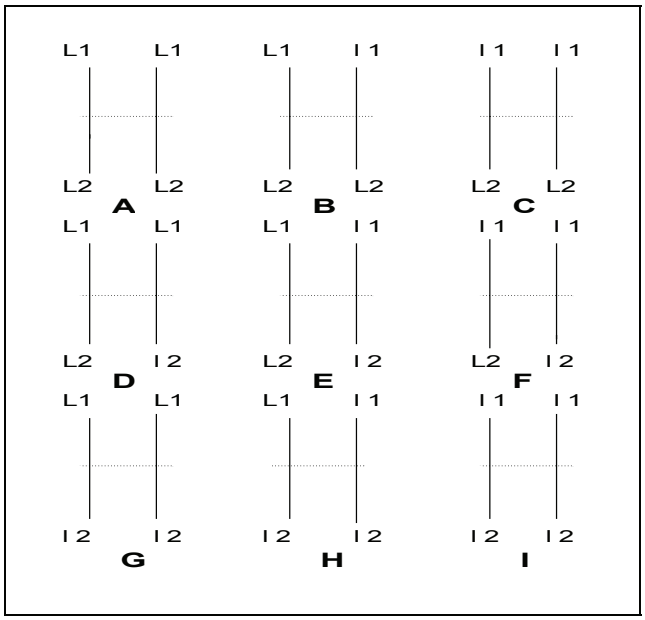

Figure 1. Mean separated chromosomes (really in the same chromosome with negligible linkage).

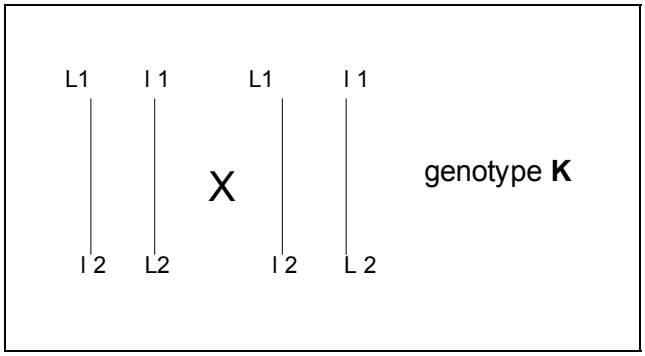

Figure 2. Balanced system (lethal in opposite chromosomes) fully linked loci.

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, $(r-s) \cdot (r-s)$ the identity matrix (Figure 3).

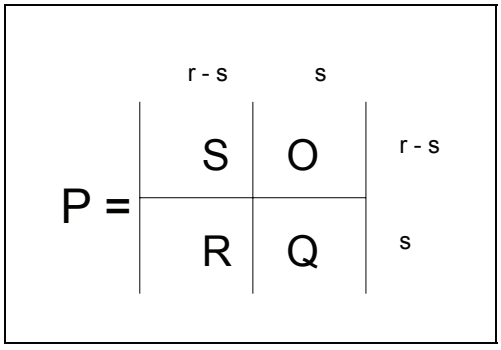

Figure 3. Matrix P, in canonical form; $r = 9$, $s=3$.

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.

CHISINAU, MOLDOVA
Institute of Genetics and Physiology of Plants
FUNDULEA, ROMANIA
Procera Agrochemicals

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.

COLUMBIA, MISSOURI
University of Missouri

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.

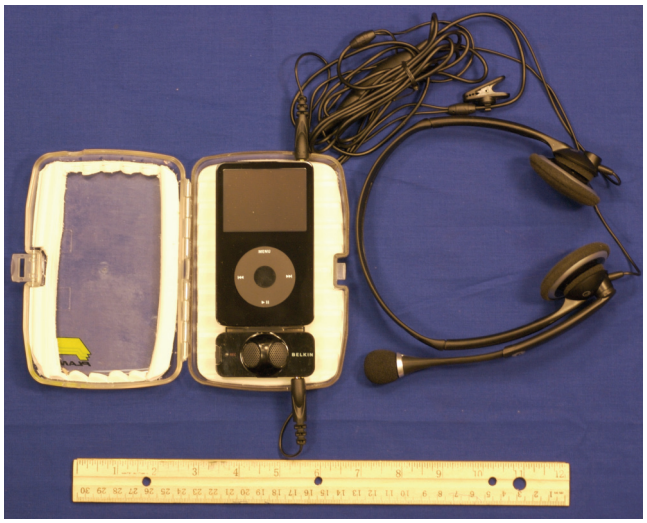

Figure 1. The fly box enclosure with iPod, TuneTalk, and VXi headset/microphone.

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

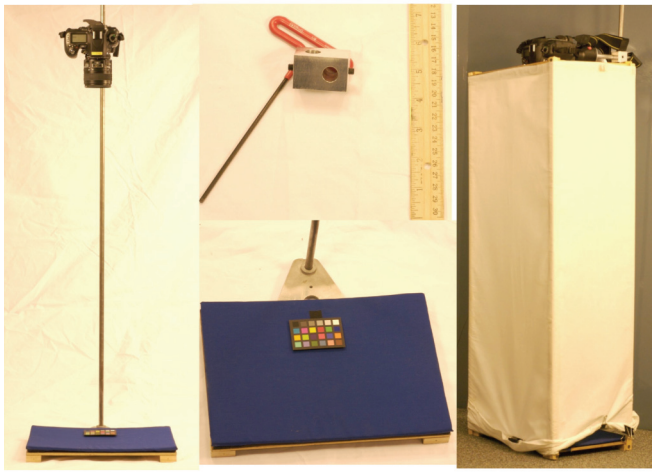

Figure 1. The imaging platform. The left panel shows the unshaded platform used in the greenhouse and for time-course photographs in the field. The middle panel shows the perpendicular clamp and its wrench (upper) and the platform bottom (lower). The right panel shows the shaded platform for collection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

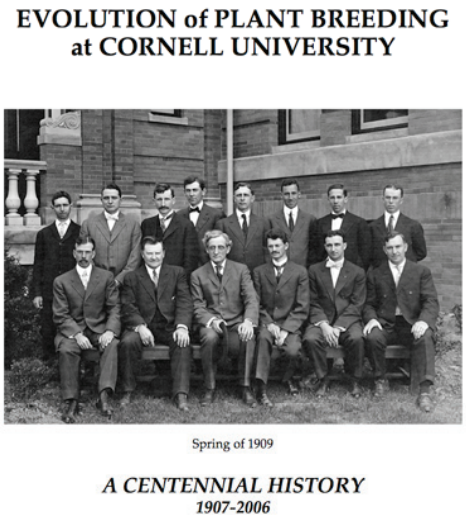

Figure 3. Evolution of Plant Breeding at Cornell University: A Centennial History, 1907-2006 (Murphy & Kass 2007).

ITHACA, NEW YORK
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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

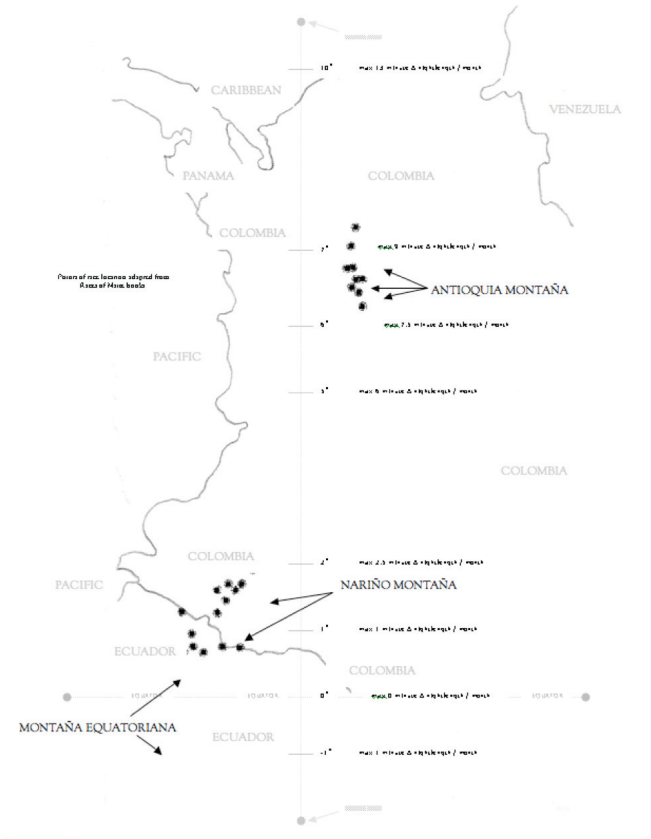
--Karl, JR

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.

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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-

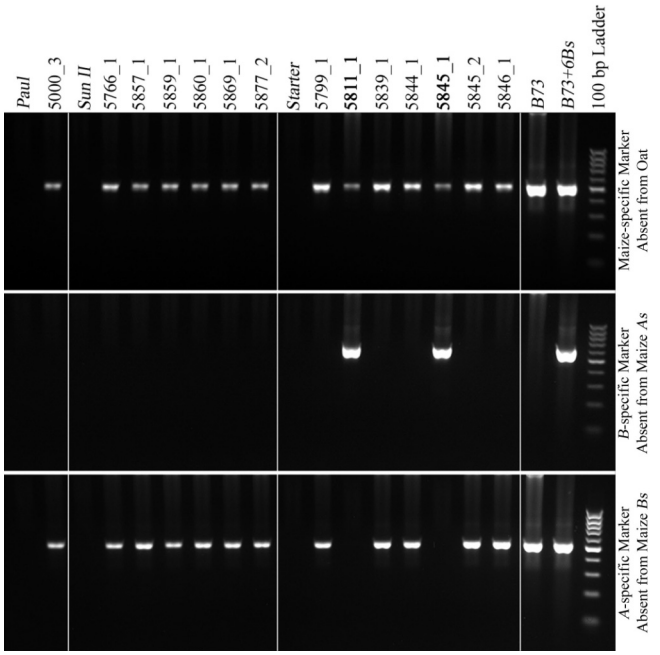

Figure 1. PCR products from genomic DNA of three oat plants, 14 F1 (oat × maize) plants, one maize plant without B, and one maize plant with six Bs by using a maize-specific, a B-specific and an *A*-specific marker; electrophoresis in 1.5% agarose

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.

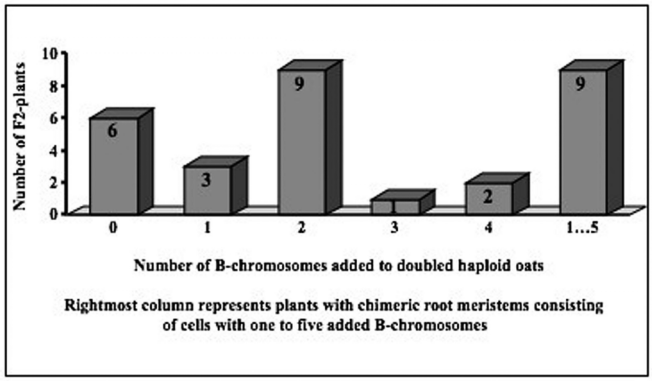

Figure 2. Numbers of F2-plants without and with an added maize B chromosome.

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.

MILAN, ITALY
University of Milan

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).

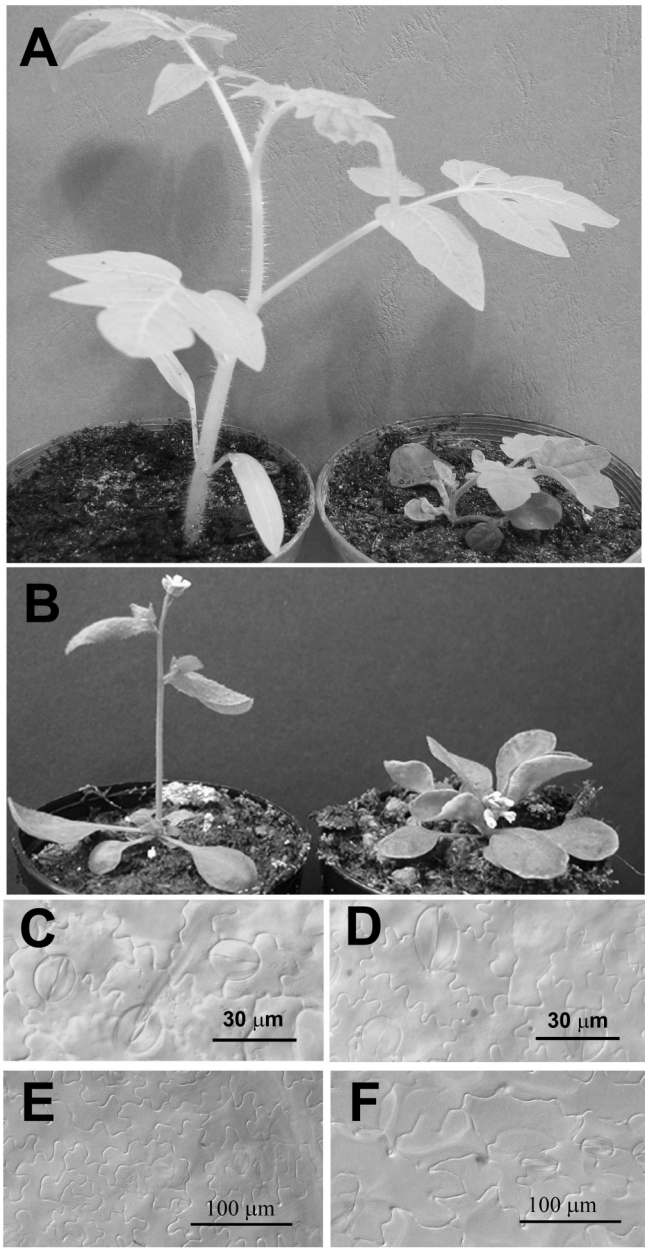

Figure 1. Phenotype of transgenic tomato (A) and *Arabidopsis* (B) plants overexpressing the *Ra1* gene (right) and control (left). Interference contrast photographs of wild type epidermis cells of tomato (C), *Arabidopsis* (E) and transgenic plants overexpressing the *Ra1* gene (tomato, D; *Arabidopsis*,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

These results suggest that the RA1 protein is able to up-regulate 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.

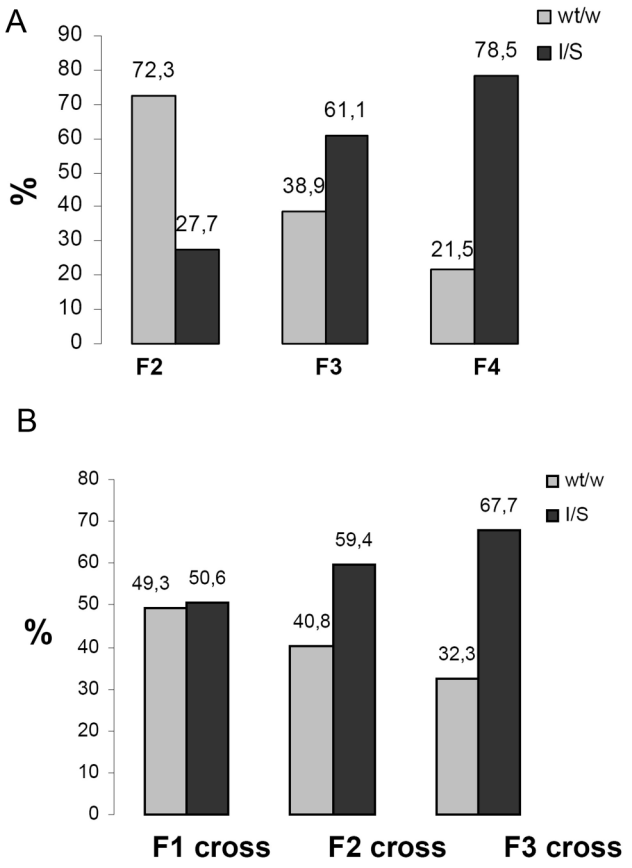

Figure 1. Changes in phenotypic class segregation ratios over generations in the presence of the *lpa1-241* allele. (A) Seed free phosphate phenotypic classes are presented in the histogram as a percentage of occurrence in selfed heterozygous progenies, where F2 refers to *Lpa1''/lpa1-241* selfed progeny, 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.

NAIROBI, KENYA
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GHENT, BELGIUM
Ghent University

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

--Anami, ES; Mgutu, JA; Hanley-Bowdoin L*; Rasha, AO; Nelissen, H; Inzé, D; Van Lijsebettens, M; Machuka, J
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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).

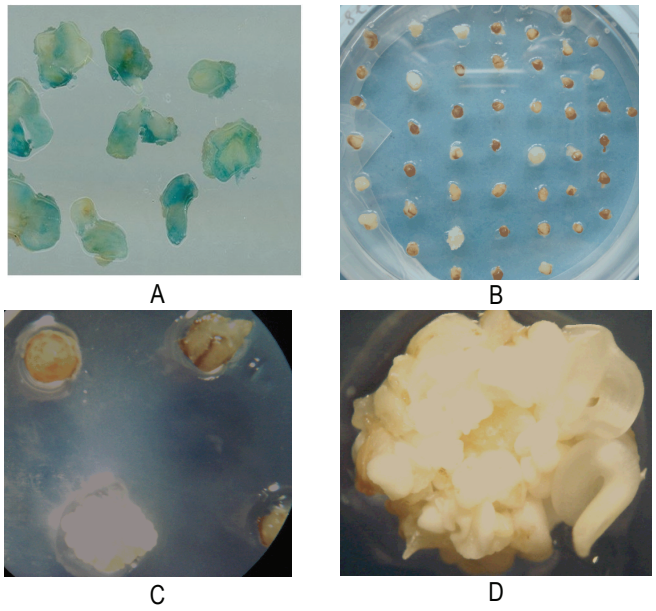

Figure 1. Selection and regeneration of transformed tropical maize inbred lines. A: Positive *Gus* staining calli after four weeks on selection II. The immature embryos were transformed with EHA101 *Agrobacterium* strain harboring pTF102 vector containing the p35S-GUS gene, described in Frame et al. (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

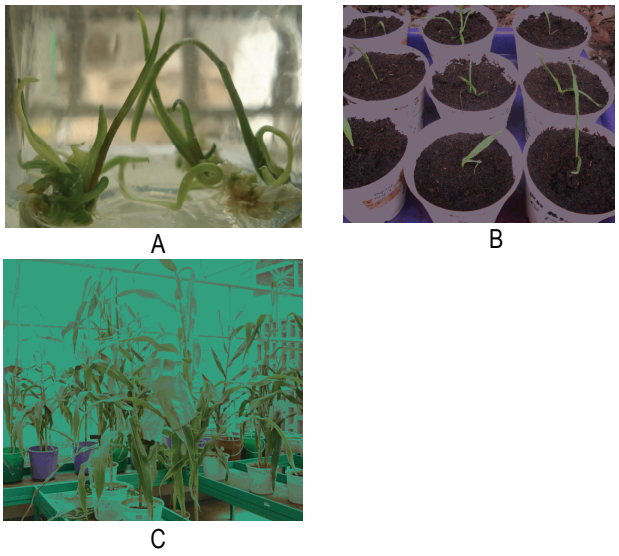

Figure 2. Putative transformed T1 tropical maize plantlets. A: Plantlets growing after one week in regeneration medium. B: Acclimatization of putatively transformed plantlets in the growth chamber. C: Selfing of T1 plantlets growing in the greenhouse. (For full color, see p. 33.)

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

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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.

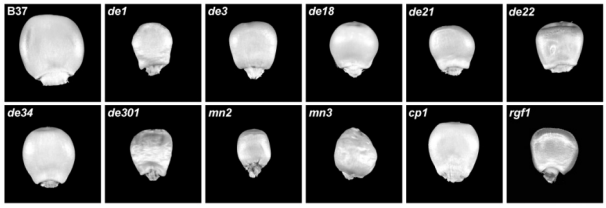

Figure 1. Phenotypes of wild type and *de*, *mn*, *cp* and *rfg* kernels.

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

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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

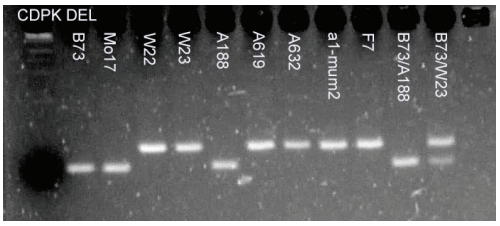

Figure 1. CDPK DEL.

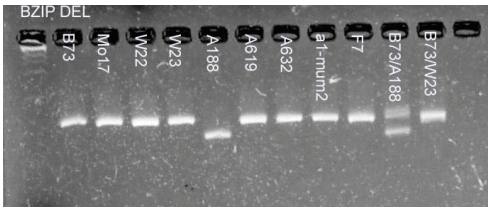

Figure 2. BZIP DEL.

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.

Primers:

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).

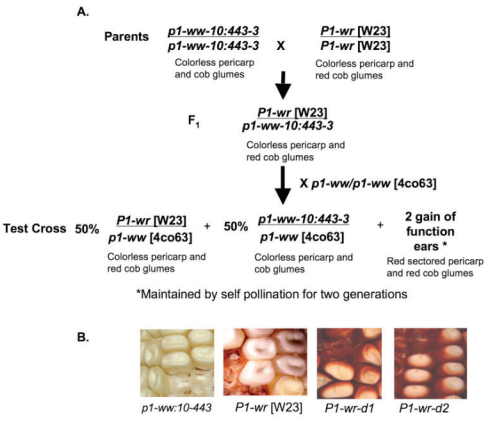


Figure 1. Gain of pericarp pigmentation crossing scheme used to generate new *P1-wr* epialleles. **A.** Diagram showing crosses performed for a gain of pericarp pigmentation screen. This screen generated two epialleles of *P1-wr* [W23] called *P1-wr-d1* and *P1-wr-d2* (see Figure 1D). **B.** Pericarp 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.

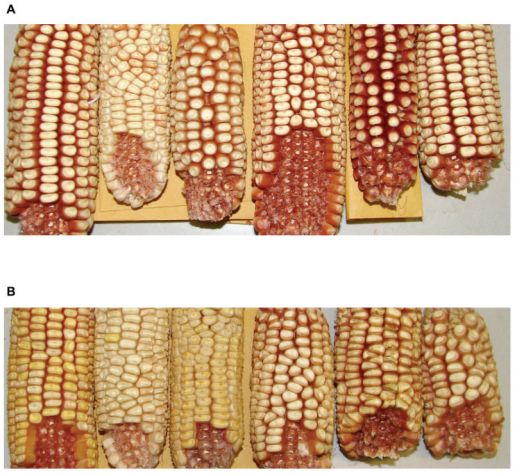


Figure 2. Pigmentation patterns and intensities in *P1-wr* [W23] epialleles. Ears show pericarp and cob glume pigmentation of **A.** *P1-wr-d1* and **B.** *P1-wr-d2*. Note that ectopic pericarp pigmentation can occur at the silk attachment point and/or kernel gown. (For full color, see p. 34.)

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

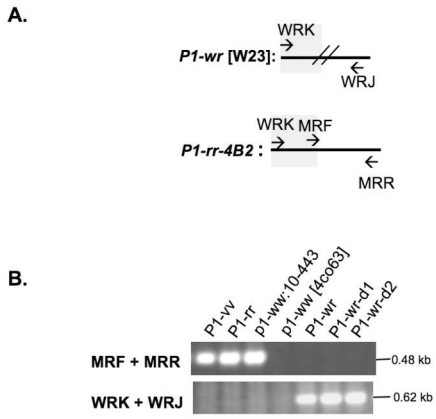


Figure 3. Molecular comparison of *P1-wr-d1* and *P1-wr-d2* with standard *p1* alleles **A.** Diagram showing the 3' ends of *P1-wr* [W23] and *P1-rr-4B2*. The location of PCR primers used for genotyping is shown. The grey shaded boxes represent the end of the gene sequences (i.e. exon 3). In the case 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].

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,

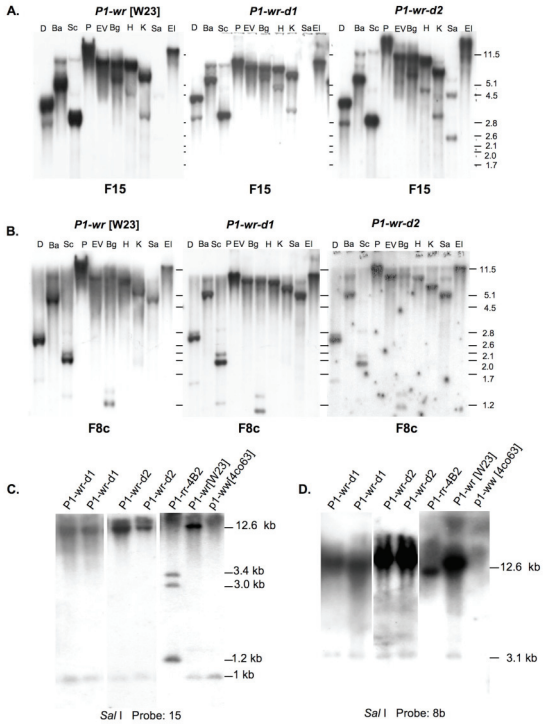
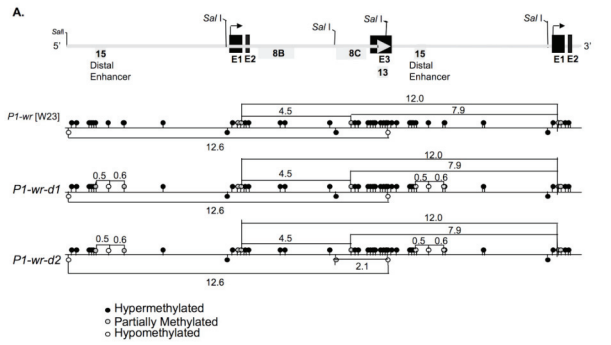


Figure 4. Structural comparison of *P1-wr-d1* and *P1-wr-d2* with *P1-wr* [W23] and *P1-rr-4B2*. Gene structure blots were made by digesting seedling leaf DNA with ten diagnostic restriction enzymes. Enzyme names are abbreviated as follows: D, *Dra*I; Ba, *Bam*HI; Sc, *Sca*I; P, *Pst*I; EV, *Eco*RV, Bg, *Bgl*II, H, HindIII; K, *Kpn*I; Sa, *Sac*I; EI, *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.



B.

	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).

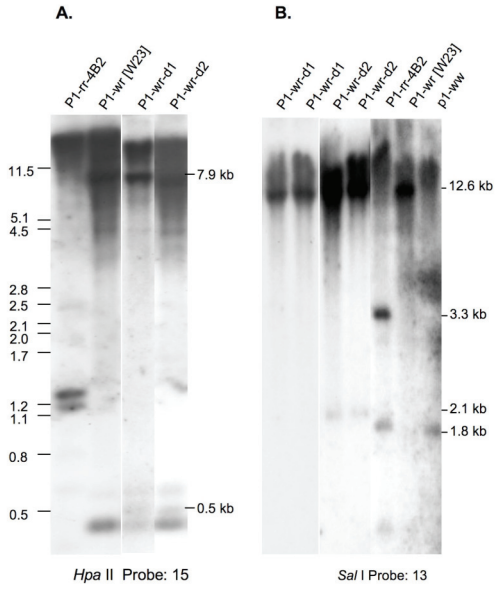


Figure 6. DNA gel blot showing methylation differences between *P1-wr* [W23] and its derived epialleles. **A.** Gel blots showing *Hpa*II digested genomic DNA hybridized with the *p1* distal enhancer probe 15. **B.** Gel blots showing *Sal*I digested genomic DNA hybridized with *p1* exon 3 probe 13. 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.

URBANA, ILLINOIS
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