MAIZE GENETICS COOPERATION NEWSLETTER 85

April 17, 2012

Division of Biological Sciences and Division of Plant Sciences, University of Missouri, Columbia, MO

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NOTE: The 55th Maize Meeting will be held at St Charles, IL USA March 14-17, 2013.

Check MaizeGDB for more details.

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COVER IMAGES: Front *ra2* tassel,from J Brewbaker pp Fig. 141.p 43 these notes ; Back cover kindly provided by G Consonni, *emp4* maize gene review, this volume.

I. FOREWORD

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

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

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes can be submitted at any time and are posted without editing at the staging site, which has access from MaizeGDB, mnl.mgdb.org. When the print copy is finalized, the staging site copies are updated and moved to MaizeGDB. **We set an arbitrary cutoff of May 1, 2012 for print copy of volume 86, per call sent by email earlier this year.** Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions must require minimal editing to be accepted.

Owing to use of electronic address scanning by the postal service, and its requirement for evolving address standards, a number of persons may not have received copies have not in recent years. Please email the editors: MaizeNewsletter@missouri.edu, if this is a concern, after first checking the staging site about printing status of the issue you were expecting. For USA subscribers, please provide your zip +4.

Special contributions to this volume include two letters written by B McClintock very early in her career and accompanied by a short note from Ed Coe; phenotype observations from Jim Brewbaker, Honolulu HI, for mutants in near isogenic lines of inbred Hi27; and several maize gene reviews kindly prepared by Alice Barkan, Gabriella Consonni, and Monika Frey. The maize gene reviews are now featured on the gene and person pages at MaizeGDB. The Maize Genetics Executive Committee report this year is accompanied by summaries of meetings with the NSF in 2010 and 2011 about many issues, including: the maize genome assembly and annotation, informatics, single investigator grants, graduate and postdoctoral training, and the Stock Center. These have also been posted at the MaizeGDB.

This year Sarah Strasburg, a Master's candidate in Journalism at the University of Missouri-Columbia, was responsible for final redaction and layout of the Newsletter Cooperators Notes. She has performed this task with precision, considerable good humor and much communication with authors. The maize community owes her much gratitude for her service.

Mary Schaeffer (Polacco)
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AMES, IOWA
Iowa State University[1]
USDA-ARS, Corn Insects and Crop Genetics Research Unit[2]

Comparison of tungsten carbide and stainless steel ball bearings for grinding single maize kernels in a reciprocating grinder

--Hoch, J[1]; Moran Lauter, A[2]; Scott, MP[2]

When grinding many corn kernels at once, there are several different options to choose from, but single kernel grinding is limited to few options with relatively low throughput. An attractive alternative is a reciprocating grinder with 3/8" grinding balls and a 4ml polycarbonate vial set. This format allows grinding of 24 samples simultaneously, which is a significant improvement to grinding throughput. A reciprocating grinder like the Talboys HT Homogenizer (Troemner, Thorofare, NJ), operates by violently shaking the 4ml polycarbonate vials containing a bearing and a single kernel. In our experience, stainless steel ball bearings do not lead to a satisfactory grind in this system. We reasoned that higher-density bearings should perform better. Tungsten carbide ball bearings are twice as dense as stainless steel and are available in the correct size. The objective of this experiment was to compare the grinding quality between stainless steel and tungsten carbide bearings.

Four corn genotypes with a wide range of hardness were selected. The genotypes chosen were, in order starting with the softest, *floury2*, a mutant with exceptionally floury kernels; *B73*, a typical corn- belt dent variety; commercial popcorn; and *Uruguay 16A*, a flinty variety. Kernels of each corn genotype were randomly selected from a single ear packet as follows: two kernels were blindly selected from each packet followed by a random coin flip to choose which kernel would be used between the two. For each corn genotype, this randomizing process was carried out six times resulting in selection of six kernels of each genotype. Every kernel from each genotype was weighed prior to the experiment so percent recovery could be calculated.

Each kernel was then placed into an individual 4ml polycarbonate grinding vial, with either one 3/8" tungsten carbide bearing (Dennis Kirk, Rush City, MN, manufacturer number: SSCBB) or one 3/8" stainless steel bearing (Troemner, Thorofare, NJ, serial number: 930156) placed on top of the kernel before twisting the lid shut. Next, the twenty-four samples were placed into the HT Homogenizer with a random vial arrangement. The samples were then ground in the Talboys HT Homogenizer (Troemner, Thorofare, NJ) for two minutes on maximum speed then the vials were inverted and ground for an additional two minutes (dial setting 10). Following grinding, particle size distributions were determined using a Sonic Sifter Separator by Advantech MFG, which had four different sieve sizes, ranging from 150 micron to 1000 micron as well as a collecting container at the bottom of the sieve screens for < 150 micron particles. The sieve screens were weighed in advance; therefore, the weight of the each corn sample on each sieve screen could be determined after sonication by weighing the screen and its contents after separation. The contents of each 4ml vial were separately placed into the Sonic Sifter Separator for a one minute and thirty second cycle on amplitude eight with sift-pulse.

Table 1. Average percent recoveries from the three kernels from each genotype ground for each bearing type.

Corn Genotype	Tungsten carbide	Stainless steel
B73	91.1	96.7
Uruguay 16A	91.6	92.8
Popcorn	92.2	87.3
fl2	90.6	93.9

Recoveries were high with both types of bearings (Table 1), with only one treatment falling below 90%. Tungsten carbide bearings gave more uniform recoveries, varying between 92.2% and 90.6%, while recoveries from grinds with stainless steel bearings ranged from 87.3% and 96.7%.

Table 2. Particle size distribution showing percentage of starting mass retained by each screen following grinding and sieving.

Sieve size (microns)	Tungsten carbide				Stainless steel			
	B73	*floury2*	Popcorn	*Uruguay 16A*	*B73*	*floury2*	Popcorn	*Uruguay 16A*
Fine <150	9.804	3.223	23.936	6.935	6.376	1.921	7.0453	5.133
150	16.630	8.269	33.536	21.893	12.518	14.719	3.695	9.346
250	20.953	14.380	21.786	18.966	9.371	16.583	6.931	9.563
500	19.994	24.726	8.193	18.033	10.706	24.200	7.479	7.622
Coarse 1000	23.393	40.090	8.0423	25.770	57.760	36.473	61.889	61.973

With stainless steel bearings more than half of the recovered material was in the coarsest fraction in three out of four genotypes (Table 2). By contrast, with tungsten carbide bearings the coarsest fraction contained less than half of the material in all genotypes. In addition, tungsten carbide bearings produced more material in the finest fraction in all genotypes than stainless steel bearings.

The *floury2* genotype was the softest grain in the study and there was very little difference in the distribution of mass between tungsten carbide and stainless steel bearings. This suggests that for very soft kernels, stainless steel bearings might be adequate. For all other genotypes, tungsten carbide bearings gave clearly better results.

The authors wish to thank Susan A. Duvick, (USDA-ARS North Central Region Plant Introduction Station, Ames, IA) for the use of her sonic sifter.

MaizeResearch.org: Engaging an audience beyond the journals

--Bodnar, AL[1]; Andorf, C[2]; Lawrence, CJ[2]

At the 52nd annual Maize Genetics Conference in Italy, the Maize Genetics executive committee called for improved public communication of research relevance and results (e.g., press releases, etc.) from the community at large. They argued there was a need to communicate basic and applied research in a way that is accessible and comprehensible to non-scientists. In pursuit of this goal, MaizeResearch.org was developed by Anastasia Bodnar with technical assistance from Carson Andorf. This website is designed to be an information hub for all things maize research. To our knowledge, this is the only broad initiative to discuss maize research on the web. The blog format allows scientists to interact with each other and with non-scientists in unique ways. The website promotes the work of maize researchers, provides a venue for communication between scientists, and makes the research more accessible to non-scientists. Scientists can use MaizeResearch.org to engage an audience beyond the scientific journals. We encourage others to participate in this outreach project. There are many opportunities, including providing summaries of published research, writing articles about research or current events, searching the web for interesting maize related stories, and much more. Please email Anastasia Bodnar at maizeresearch1@gmail.com for details.

BEIJING, CHINA
Institute of Genetics CA, Hua-Tai Use for Specific Corn R & D Center

Application of Maize Mutation Types Induced by Space Environment to Plant Genetic and Breeding

--Zeng, M; Zeng, Z

The authors' previous papers described that space environment has significant effects on maize hereditary variation and different mutation types (MNL 79:3, 2005; MNL 80:2, 2006; MNL 81:2, 2007). This paper deals with the application of three mutation types to the plant genetic and breeding, which are based on the precision scheme of heterotic breeding of the space inducement. The seven mutants belong in three mutation types: plant height, ear length and grain type. These types are used

Zeng & Zeng Fig.1. Breeding sketch map for 11 of inbred lines by using multiple cross and backcross methods.

1. Me12 {[（Mo17×Zi330）⊗×Y78599⊗]⊗S6 variation plant×Mut5}⊗S5 ×(PHG analogous plant⊗×NRRSP Ⅰb analogous plant⊗) F2 pop.⊗, Selected, Testcross…→S8 (Me12)	2. XH3 [HZGC1⊗S5×(HZGC2 analogous plant⊗×HZPC2 analogous plant⊗)] ⊗S4×(Mut4×NRRSP Ⅰa analogous plant⊗)⊗ F2 pop.⊗, Selected, Testcross…→S7(XH3)
3. Met88 [(NRRSP Ⅰb⊗×Me8)⊗S3×SW5P analogous plant ⊗S3] ⊗S3×Mut8 F2 pop.⊗, Selected, Testcross…→S7(Met88)	4. Mv02 [(Q1621×Y78599⊗)⊗S3×NRRSP Ⅰa ⊗S3] ⊗S3×Mut7 F2 pop.⊗, Selected ,Testcross…→S7(Mvo2)
5. Me196 [(Ye3189 variation plant×Ye478)⊗S3×NRRSPⅠb analogous plant⊗] ⊗S3×Mut8 F2 pop.⊗, Selected Testcross…→S8(Me196)	6. Met883 [(NRRSPⅡb⊗×Me8)⊗S3×(SW3⊗× SW3P an alogous plant⊗)] ⊗S3×Mut8 F2 pop.⊗, Selected, Testcross…→S8(Mett883)
7. Me06 {[(Q1621×Y78599⊗)⊗S3×NRRSPⅡa ⊗S3] ⊗S3×Mut6 F2 pop.⊗, Selected, Testcross…→S8 (Me06)	8. Met81 [(NRRSP Ⅰb⊗×Me81)⊗ S3×SW5P analogous plant⊗ S3] ⊗S3×Mut9 F2 pop.⊗, Selected, Testcross…→S7(Met81)
9. Me05 [(Q1621×Y78599⊗)⊗S3×NRRSP Ⅰa⊗ S3] ⊗S3×(Ki45×Mo17 variation plant⊗ S3) ⊗S3×Mut5 F2 pop.⊗, Selected, Testcross…→S8(Me05)	10. Me07 [(Q1621×Y78599⊗)⊗S3×NRRSP Ⅰ a⊗ S3] ⊗S3×(Ki21×M017 variation plant⊗ S3) ⊗S3×Mut7 F2 pop.⊗, Selected, Testcross…→S8(Me07)
11. Me887wx Li502wxF2 pop.⊗ S1 variation plant⊗ S3 ⊗S3×ThaiWwx pop.⊗ S3 ⊗S3×Mut12wx F2 pop.⊗, Selected, Testcross…→S7(Me887wx)	

(Z

Table 1.The characters and properties for space Environment induced maize mutant. (Zeng & Zeng p 3)

	Mut2	Mut5	Mut6	Mut7	Mut8	Mut9	Mut12
Original	Yi01-4-1 Sp2-3	U8112 Sp3-4	U8112 Sp3-2	U8112 Sp3-3	Me141 SP3-1	Me141 Sp3-2	Zi24 Sp4-5
Plant height (cm)	182.0	157.0	214.0	189.0	220.1	227.3	190.5
Ear height (cm)	75.1	50.1	93.0	82.5	97.2	100.1	88.7
Length of the leaf at the ear site (cm)	75.6	71.3	74.5	70.1	85.1	87.1	74.0
Width of the leaf at the ear site (cm)	9.1	10.0	9.4	9.5	10.0	10.1	10.0
Tassel length (cm)	50.0	43.1	46.2	45.1	47.2	52.6	41.0
Tassel branch number	15.0	9.0	15.1	15.2	15.3	14.0	8.5
Leaf number	20	21	21	21	22	22	21
Ear length (cm)	13.9	14.1	20.2	19.1	17.1	18.5	17.0
Ear diameter (cm)	4.8	4.7	5.0	4.7	5.0	5.2	4.4
Number of kernel row	12	14~16	14	14	16	16	14
Kernel number of each row	33.0	31.0	32.0	33.0	34.2	32.3	35
Weight of 100 kernel (g)	27.1	22.1	24.8	22.7	24.7	28.5	19.8
Each ear weight	120.1	105.7	143.0	130.0	172.4	174.0	135.2
Kernel weight per ear	99.9	87.2	120.3	107.5	141.8	142.9	116.3
Ear form, kernel type	Spindle SD	Spindle SD.D	Cycle SF.	Cycle F.	Cycle SF.	Cycle SD.SF	Cycle wx
Cob, kernel color	W.Y.	W.Y.	W.Y.	W.Y.	W.Y.	W.Y.	R.W.
Day from seeding to kernel maturing	55	57	57	57	64	64	58
Length for leaf-blade of husk top(cm)	No	No	No	No	No	No	No
Length of ear handle (cm)	6.0	6.0	6.0	6.3	6.9	6.4	5.0
Ear number per plant	1～2	1	1	1	1～2	1～2	1～2
Level of leaf-blade erect	Erect	Erect	Erect	Erect	General	General	More erect
Resistance to E.turcicum & B.maydis	HR	HR	HR	HR	HR	HR	R
to P.inflatum,P.aphanidermatum, F.moniliforme & F.graminearum to S.holci-sorghi to P.sorghi & P.polysora	MR HR HR	HR MR HR	HR MR HR	HR MR HR	HR HR HR	HR HR HR	MR HR HR

as main experimental materials. Nineteen new lines were developed, and six pairs of the superior genetic materials were developed and screened to use in the research of the machinery and regularity of space inducement. Among them, 11 elite lines (Tables 1 & 2, Fig. 1), where 25% to 50% of the genome had been subjected to space inducement, have been used to develop seven superior hybrids for regional and yield potential trials. (national or province tests; city or autonomous region tests) and/or obtained a certificate of new variety from the committee that examines and approves crop varieties. The accumulative total for spread areas per year reached more than 800,000 Mu (0.053 million ha). From the below data, we ought to discuss the problems of rising combing ability, biochemical component quality for space induced lines, and hybrids and construction of the breeding engineering system for space inducement. Prospective progress for flight aerospace engineering breeding should not be neglected.

Table 2. The characters and properties for the maize mutants came from inbred lines. (Zeng & Zeng p 3)

	Yio1-4-1	Zi:24	U8112	Me141
Plant height (cm)	143.1	191.5	190.0	181.5
Ear height (cm)	41.2	90.2	82.8	81.2
Length of the leaf at the ear site (cm)	67.2	75.2	41.8	88.6
Width of the leaf at the ear site (cm)	7.0	10.1	9.4	9.4
Tassel length (cm)	41.5	42.1	65.4	51.3
Tassel branch number	10.8	10.5	8.9	11.1
Leaf number	20	21	21	22
Ear Length (cm)	11.2	16.7	16.7	12.8
Ear diameter (cm)	3.5	4.2	4.6	5.1
Number of kernel row	12	14	12	18
Kernel number of each row	22	36	28	22
Weitght of 100 kernel (g)	19.1	24.0	21.5	25.0
Each ear weight	61.0	145.7	84.0	123.4
Kernel weight per ear	50.1	124.3	71.0	101.0
Ear form, kernel type	Cycle.SF	Spin-dle. SDSF	Cycle.D	Cycle.SD
Cob, kernel color	W.Y.	R.W.	W.Y.	W.YW
Day from seeding to kernel maturing	57	58	60	69
Length for leaf-blade of husk top(cm)	No	No	No	No
Length of ear handle(cm)	5.0	5.1	6.0	6.3
Ear number per plant	1～2	1～2	1	1～2
Level of leaf-blade erect	Erect	More erect	Erect	General
Resistance to E.turcicum & B.maydis	S	MR	MR	HR
Resistance to P.inflatum,P. aphanidermatum, F.moniliforme & F.graminearum	MR	MR	HR	HR
Resistance to S.holci-sorghi	HR	HR	MR	HR
Resistance to P.sorghi & P.polysora	HR	HR	HR	HR

BERGAMO, ITALY
Unità di Ricerca per la Maiscoltura

Analysis of insecticide seed coatings for protection of corn kernels, seedlings, and plants

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

As previously reported (Balconi C et al., MNL 84, 2010), in Italy and other European countries one of the risk factors for honeybee health and colony collapse disorder is supposed to be agrochemical treatments. The risk is described to be in the loss of active seed coatings ingredients from the fan drain of pneumatic seed drills during corn sowing operations (Greatti et al., Bulletin of Insectology 56:69-72, 2003; Greatti et al., Bulletin of Insectology 59:99-103, 2006). Because of this a precautionary suspension of using the four insecticide active ingredients registered for seed dressing was established in Italy from 2008 to 2010. The yield and vigor of a crop can be increased or improved in locations where the level of insect infestation indicates the need to use an insecticide for insect control purposes by treating a seed of the plant with a neonicotinoid compound (Bai et al., Pestic Sci 33:197-204, 1998; Nauen et al., Pestic Sci 51:52-56, 1998).

The aim of our research in the frame of the APENET project, a national research project financed by the Italian Ministry of Agriculture (Bortolotti et al., APOidea 6:2-21, 2009) is devoted: i) to compare the yield and agronomic traits of a commercial hybrid when grown without insecticide treatment (as untreated control) with the yield and agronomic traits when grown with the four insecticide seed coatings under study: thiamethoxam, imidacloprid, clothianidin, and fipronil. The study took place in several locations of north-central Italy; ii) to detect the presence of the four insecticide active ingredients registered for seed dressing, in leaves and other corn plant tissues periodically collected from the emerging seedling to the flowering stages.

Agronomic trials were undertaken in 17 locations during 2009 and in 19 locations during 2010. In each location 30 m^2-plots were sown with seeds prepared from a homogeneous lot of commercial maize hybrid seeds coated either with the four active ingredients plus the fungicide or with the fungicide alone (control). The five treatments were replicated four times in each location.

The following observations and the standard agronomic measurements were performed: grain humidity (%), yield (t/ha), grain density, plant height, ear height, percentage of plants with split stalk, percentage of lodged plants.

Statistical analysis performed with ANOVA showed that there are no significant differences among the five treatments for the measured parameters.

Experimental plots (50 m length) were sown with seeds prepared from a homogeneous lot of commercial

maize hybrid seeds coated either with the four active ingredients plus the fungicide, or with the fungicide alone (control). Tests were undertaken to analyze residues of the four active ingredients used for seed coating at various stages of maize plant development, sampling leaf tissues at different phonological stages (Leaves 2-3, Leaves 7-8, Leaves 13-14). Extraction, separation, and detection of thiamethoxam, imidacloprid, clothianidin, and fipronil were performed according to the quality assurance criteria of Good Laboratory Practice (G.P.L. Prot. CH-012-2010-Test Laboratory Prot. CH - 013/2010), by using HPLC coupled to tandem mass spectrometry (APCI-MS/MS) following with modifications (Bonmatin et al, Anal Chem 75:2027-2033, 2003). The results showed that amount of fipronil sharply decreased at early seedling stage development (Leaves 2-3), while the decrease in thiamethoxam, imidacloprid, and clothianidin was lower (Leaves 7-8); All four active ingredients were non-detectable at stages 13-14 of leaf development.

Research made within the project APENET: Monitoring and Research in Apiculture was funded by the Italian Ministry of Agricultural Food and Forestry Policies.

IDIAM Project: Identification of genetic variability and genes for the selection of genotypes tolerant to rootworm damage in maize

--Lanzanova, C; Valoti, P; Hartings, H; Motto, M; Balconi, C

Recently, *Diabrotica virgifera virgifera*, commonly referred to as the corn rootworm, spread in the Italian areas devoted to corn cultivation. Estimates indicate that the area affected by rootworm in 2009 was about 165,000 hectares, which includes 135,000 hectares in Lombardia that have a major impact in the corn-livestock industry and showed losses amounting to 100 million euros per year. Among the prevention and containment measures that appear effective are the use of some agricultural practices (hybrid selection, crop rotation, sowing early, good availability irrigation, earthing up, and insecticide treatments). However, these strategies of control and prevention appear poorly effective in containing pest damage in addition to their high costs and negative effects on the environment and the parasite's ability to evolve individuals tolerant of different active ingredients and their host plants.

One of the most promising strategies to deal with infestations of pests is given by the cultivation of resistant varieties. The establishment of plants that can produce their own insecticide is proposed as an effective strategy that is safe from an ecological point of view to counter the spread of insects. Plants resistant to insects lead to a reduction in production losses, a decrease of the costs of insecticide treatments and improved food safety for animal feed and human.

Studies aimed at evaluating the germplasm of maize and its ancestors allowed i) to identify plants with tolerance against rootworm larvae, useful in breeding programs (Hibbard et al., Maydica 44:133-139, 1999; Eubanks, Proc 2002); ii) to set up methods to test the susceptibility of plants (Moellenbeck et al., J Econ Entomol 88:1801-1803, 1995; Knutson et al., J Econ Entomol 92:714-722, 1999); iii) to elucidate the mechanisms of plant resistance to insect damage (Assabgui et al., J Econ Entomol 88:1482-1493, 1995; Prischmann et al,. J Appl Entomol 131:406-415, 2007). This research has also revealed a wide range of varieties and genetic variability in inbred lines, which shows a complex quantitative genetic basis and makes it difficult and expensive to select tolerant genotypes. Moreover, investigations of the genetic variability in corn hybrids have shown that indirect selection for large root systems can support the development of secondary roots and reduces larval damage (Simic et al., Maydica 52:425-430, 2007; Tollefson, Maydica 52:311-318, 2007; Marton et al., Hungary Tagung der Vereinigung der Pflanzenzuchter und Saatgutkaufleute Osterreichs 77-80, 2008; Ivezic et al., Proc XXIst IWGO, 2009). A promising strategy for biological control has recently been proposed by Degenhardt et al. (Proc Natl Acad Sci 106:13213-13218, 2009). These authors found that the recent maize crops have lost the ability to emit a volatile (E)-β-caryophyllene, which attracts specific nematodes (*Heterorhabditis megidis*), enemies of the larvae of corn rootworm. To restore the signal insect-radical damage, the corn was engineered by introducing in his genome, a gene that promotes oregano emissions (E)-β-caryophyllene. The results show that maize plants that form (E)-β-caryophyllene have a decrease (60%) of the number of rootworm larvae than non-transformed plants. Another promising strategy for biological control has been proposed by Meissl et al. (Appl Environ Microbiol 75:3937-3943, 2009). These authors found that the fungus *Metarhizium anisopliae* on maize plants can reduce the growth of larvae of corn rootworm.

It is therefore clear that the identification of genes and molecules underlying the defensive response of the plant against the corn rootworm products are of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae. To speed up the selection of plants with damage tolerance of larvae of corn rootworm, research has been recently addressed to clarify the genetic basis of character, with investigations aimed at identifying specific genetic or biochemical compounds and QTL underlying tolerance (Gray et al., Annu Rev Entomol 54:303-321, 2009). The main objective of this research aims to: i) detect and identify varieties and genes useful for the development of maize genotypes to reduce the damage caused by rootworm larvae on corn production through the development of plants with tolerance to insect infestations; ii) identify

genetic factors to speed up the selection of genotypes able to counteract the damage to the larvae of corn rootworm infestation; iii) develop analytical technologies capable of allowing identification of genotypes tolerant to the development of lines and hybrids to be used in areas north-central Italy with the risk of infestation.

Current studies are analyzing genetic variation to estimate the damage caused by rootworm tolerance of about 25 commercial maize hybrids from different maturity classes (FAO 500-700). The hybrids tested were tested in 20 locations representative of the maize Italian areas, using a randomized block experimental design with three replications. The basic plot consists of 4 rows, distant 75 cm and long 10 m. The following observations and the standard agronomic measurements were performed: Grain humidity (%), yield (t/ha), grain density, plant height, ear height, percentage of plants with split stalk, and percentage of lodged plants.

The findings relate to production and agronomic traits (flowering time, moisture at harvest, flattened crop production). At each location of agronomic trials was also determined the frequency of catches of adult insects by traps (Pherocon, AM) to correlate the production level and the insect spread in the locations. Regular sampling methods were used to assess plant response to *D. v. virgifera* larvae and determined the extent of the damage with the index node injury scale (Oleson et al., J Econ Entomol 90:1-8, 2005), frequency of active roots, root dimensions (weight biomass), and larval infestation of two of the hybrids tested at two locations. Larvae were extracted by collecting then floating roots and soil in water and drying the roots in Berlese funnels. These data will be integrated with other data (radical damage and larval infestation) obtained from other agronomic trials.

This research is part of a greater project, "IDIAM – Interventions to counterattack the spread and damage from rootworm in maize Italian crop", which is funded by the Italian Ministry of Agricultural Food and Forestry Policies.

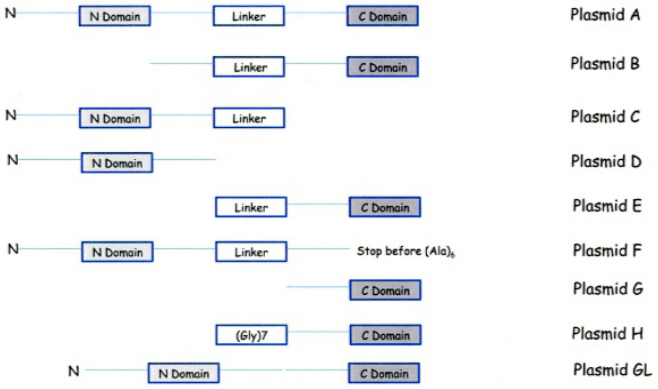
Use of bioactive proteins in plant protection against pathogens

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

One of the main topics of maize breeding is the improvement of plant protection against pathogens. Plants respond to attack by pathogenic fungi with a complex network of active responses such as the production and accumulation of proteins that are toxic or inhibitory to pathogens such as RIP (Ribosome Inactivating Protein). The role of RIP in the pathogen defense has been documented (Balconi C et al., *Plant Cell Monographs* 18:149-166, 2010).

In maize endosperm, a cytosolic albumin termed *b-32* is synthesized in temporal and quantitative coordination with the deposition of storage proteins. In past years *b-32* was shown i) to enzymatically inactive ribosomes modifying rRNA inhibiting protein synthesis in vitro (Maddaloni et al., Journal of Genetics and Breeding, 45:377-380, 1991); ii) to inhibit the growth of *Rhizoctonia solani* mycelia in an in vitro bioassay and plant assays (Maddaloni et al., Transgenic Research, 6:393-402, 1997). In this context, we have recently shown that maize *b-32* was effective in wheat transgenic lines as an antifungal protein by reducing *Fusarium culmorum* head blight (FHB) (Balconi C et al., European Journal of Plant Pathology, 117:129-140, 2007), and in maize transgenic lines reducing *Fusarium verticillioides* attack symptoms in leaf tissues assays (Lanzanova, C et al., European Journal of Plant Pathology, 124:71-482, 2009).

Figure 1. Genetic deletions of the *b-32* gene.



Similarly to other RIPs, maize RIP is accumulated in the seed as an inactive precursor that is converted into an active form by proteolytic processing, which removes peptide segments from the N (residues 1-16 of pro-RIP) and C (residues 295-301) termini and the center of the polypeptide (residues 162-186) (Hey et al., Plant Physiol. 107:1323-1332, 1995).

Aims of the BIORES project are devoted to deepen our knowledge of relationships between structure and substrate specificity of *b-32* protein in order to clarify the role of the processed segments of *b-32* gene on the ability of maize RIP to inhibit fungal growth and/or mycotoxin accumulation.

Thereby, a series of genetic constructions was made by selectively deleting the N-terminal, or C-terminal or internal linker domain (Fig. 1). Genetic deletions of the *b-32* gene, which is apparently responsible for suppressing enzymatic activity in the precursor, will be primarily expressed in *Escherichia coli* to produce sufficient quantities of modified proteins.

To assess the role of bioactive *b-32* modified protein in the protection against fungal pathogens (*F. verticillioides, Aspergillus flavus*), a series of in vitro bioassays are in progress, to analyze their effect on

the fungal growth and on mycotoxin accumulation in comparison with i) the unmodified *b-32* (control); ii) a commercial RIP (Saporin).

Research developed in the BIORES for use of bioactive proteins in plant protection against pathogens.

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A challenge: who/what/when/where/why are our telomeres?

--Coe, EH

Telomeres are significant structures. The maize telomeres are doubly significant in light of the evolutionary history of the maize genome and the many discoveries in maize about cytogenetic behavior, which include broken ends, rings, fragments, and the processes of synapsis (*n.b.* the role of base motifs in other species: Phillips C et al., Identification of chromosome sequence motifs that mediate meiotic pairing and synapsis in C. elegans, Nature Cell Biol 11:934-942, 2009). We do not know enough about maize telomeres yet. Mapping them genetically and physically would advance the field.

The updated Genetic 2008 maps in the Maize Genetics and Genomics Database provide best estimates for coordinates of the telomeres, which I reviewed and revised in September 2010 (Table 1). The v.2 genome-build erected by Fusheng Wei and presented in WebFPC, was the foundation for placements. The URL for his expert synthesis of the maize genome is: http://www.genome.arizona.edu/fpc/WebAGCoL/maize/WebFPC/. Version 1 was used in previous estimates (MNL 83:17, 2009) as of October 2008.

Current placement was inferred in silico from BLAST matches or hybridizations of telomere-specific sequences (e.g., pMTY9ER, pBF266) at one end of a contig, consonant with positions of genetically mapped markers. When these criteria were insufficient, best positions were inferred relative to markers on IBM2. Improved v.2 placements were defined for the 2S, 3L, 6L, 7L, 7S, and 9S telomeres. The evidence is strong, though indirect, for map locations of 2L, 3L, 5S, 5L, 6S, 6L, and 9S. Mapped markers that fall beyond the telomeres are mostly, if not entirely, artifacts of mapping analysis in particular studies.

The challenge posed here is much more significant than mapping coordinates or knowing their physical location, structure, and fluidity—it is having the tools necessary to unveil telomeres' functional involvement in central themes of cytogenetics. Among these themes are evolution of distinct synaptic partners, mechanics of synaptic "finding," roles of motifs and sequences, and repair of broken ends.

Table 1. Estimated Genetic 2008 map locations of telomeres as of September 2010.

Name	Ctg	cM	Evidence
telomere1S	1	0	Reviewed Sep 2010 (EHC): Mapped by inference from BLAST of pMTY9ER and pMTY7SC1, associated at the left end of ctg1, and BLAST of subtelomeric clones S46925 S46926 U39641 and U39642 to BAC c0014I07 at left end of ctg1 distal to IBM2 marker *umc1354*.
telomere1L	67	286+/-2	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated from BACs that hybridize with pMTY9ER, located at the end of ctg67 beyond the distal-most markers, anchoring and orienting this contig.
telomere2S	485	0	Reviewed Sep 2010 (EHC): Mapped by inference from BLAST of subtelomeric clones S46925 S46926 U39641 and U39642 to BAC b0500C18 in ctg485 at the end of 2S in v.2, and by RFLP placement of *bnl(tas1a)*.
telomere2L	110	183+/-2	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated from the right end of ctg110, distal to IBM2 marker *AY111236*.
telomere3S	111	0	Reviewed Sep 2010 (EHC): Mapped by inference from BAC filter hybridizations of subtelomeric clone U39641(pMTY7SC1) to BACs at the left end of ctg111, distal to *umc2118*.
telomere3L	153	209+/-1	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate revised, estimated from ctg153, placed by IBM2 marker *umc1594*; ctg152, the next-nearest, has BAC filter hybridizations of pMTY7SC1 and pMTY9ER that are ambiguously distributed.
telomere4S	154	0	Reviewed Sep 2010 (EHC): Mapped by inference from BAC filter hybridizations of subtelomeric clones S46925 S46926 U39641 and U39642 to BACs at left end of ctg154, distal to IBM2 marker *umc2278*.
telomere4L	203	189+/-2	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated in silico by inference from BLAST of subtelomeric clones S46925 S46926 U39641 and U39642 to BAC b0528L21 in ctg203 at its right end, distal to IBM2 markers *bnlg1890* and *umc1707*, orienting this contig.
telomere5S	204	0	Reviewed Sep 2010 (EHC): Mapped by inference from left end of ctg204, distal to IBM2 marker *AI676903*.
telomere5L	254	173+/-3	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated by inference from the right end of ctg254, distal to IBM2 marker *umc1153*.
telomere6S	256	0	Reviewed Sep 2010 (EHC): Mapped by inference from left end of ctg256, distal to IBM2 markers *fdx1*, *fdx2*, and *umc2310*.
telomere6L	291	138+/-2	Reviewed Sep 2010 (EHC): Revised Genetic 2008 coordinate estimated by inference from the right end of ctg291, distal to IBM2 markers *hir3* and *umc2324*.
telomere7S	714	0	Reviewed Sep 2010 (EHC): Mapped in silico by inference from BLAST of subtelomeric clones S46926 and U39642 to BAC c0203N21 in ctg714 at the end of 7S in v.2; markers bnl(tas1j), pMTY9ER, pMTY7SC1 are at the "right" end of ctg714, and IBM2 marker *umc2177* is in the adjacent contig, ctg293.
telomere7L	325	158+/-3	Reviewed Sep 2010 (EHC): Revised Genetic 2008 coordinate estimated by inference from the right end of ctg325, distal to IBM2 marker *AY109703*.
telomere8S	326	0	Reviewed Sep 2010 (EHC): Mapped by inference from left end of ctg326, distal to IBM2 marker *csu319*.
telomere8L	366	160+/-1	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate inferred from BLAST of subtelomeric clones S46925 S46926 U39641 and U39642 to BAC c0447I13 at right end of ctg366, distal to IBM2 marker *AY109853*.
telomere9S	441	0	Reviewed Sep 2010 (EHC): Mapped by inference beyond left end of ctg368, IBM2 markers *umc109* and *umc1957*; ctg441 is placed in v.2 to the left of ctg368 and contains BACs hybridizing with knob probes (i.e., *K9S*) but has no markers on IBM2.
telomere9L	391	164+/-4	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated by inference from the right end of ctg391, distal to IBM2 markers *AW216329*, *umc1505*, and *AI901738*.
telomere10S	392	0	Reviewed Sep 2010 (EHC): Mapped by inference from BAC filter hybridizations of pMTY9ER and pMTY7SC1, associated in parts of ctg392 with the distal-most IBM2 markers, *umc48a* and others, on 10S.
telomere10L	420	136+/-2	Reviewed Sep 2010 (EHC): Genetic 2008 coordinate estimated in silico from BLAST of subtelomeric clones S46925 S46926 U39641 to BAC b0310F06 at the "right" end of ctg420, with BACs that hybridize with *pMTY9ER* and *pMTY7SC1* probes.

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Path coefficient analysis of *Mal de Río Cuarto* disease components

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The *Mal de Río Cuarto* (MRC) is considered the most important viral disease of corn in Argentina because it causes increased damage and high yield losses to susceptible cultivars (Lenardón et al., Plant Dis 82:448, 1998; Di Renzo MA et al., J Agr Sci 139:47-53, 2002). The disease is caused by MRC virus, which belongs to the genus *Fijivirus* within the family *Reoviridae*, and is transmitted by the planthopper *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae) (Nome SF et al., Phytopathol Z 101:7-15, 1981; Ornaghi JA et al., J Gen Breed 47:227-282, 1993). Its symptomatology depends on the plant's phenological state when it is infected, the genotype, and the environmental conditions where the culture grown. This includes stunting, short internodes, galls or enations on the abaxial side of the leaves, size reduction and malformation of spikes, ears and leaves (Lenardón SL et al., Enfermedades causadas por virus y fitoplasmas en cultivos extensivos intensivos, 1999; Di Renzo MA et al., J Agric Sci 142:289-295, 2004). Because both economic and environmental reasons prevent an increase in agrochemicals usage, future cereal improvement will rely on germplasm that optimizes present genetic tolerance to plant pathogens (Abeledo LG et al., Euphytica 130:325-334, 2003). This is an effective way of both increasing and stabilizing production in affected areas.

Selection for one trait usually affects several traits. The correlated response to selection is a change in one or more traits due to selection of another as a result of genetic relations between them. Genetic correlations are useful to decide on selection strategies since they express the relative importance of pleiotropy and linkage between loci (Kang et al., Crop Sci 23:643-647, 1983; Kang, Applied quantitative genetics. MS Kang Publisher, Baton Rouge, LA, 1994). The genetic correlations can be estimated from phenotypic values, which are influenced by type of gene action, environmental effects, and genotype x environment interactions (Falconer and Mackay, Introduction to quantitative genetics. Longman Technical, Essex, UK, 1996).

Nevertheless, while the correlation coefficient only measures the association magnitude between variables, the path coefficient analysis (Wright S, J Agric Res 20:557-587, 1921; Wright S, Ann Math Stat 5:161-215, 1934) allows dissecting the correlation between them into direct and indirect effects. This method has been commonly used in crop breeding studies to establish the relationships between grain yield and its contributing components (Mohammadi SA et al., Crop Sci 43:1690-1697, 2003). In addition, there are many references of its application in plant pathology studies (Birhman and Singh, Ann Appl Biol 127:353-362, 1995; Desprez-Loustau and Wagner, Eur J Plant Pathol 103:653-665, 1997; Garcia D et al., Eur J Forest Pathol 29:323-338, 1999; Nayak P et al., J Phytopathol 119:312-318, 1987; Neher DA et al., Plant Dis 77:1106-1111, 1993; Van Bruggen and Arneson, Phytopathology 76:874-878, 1986). Therefore, it would be useful to get information regarding direct and indirect relationships among MRC disease tolerance and different related traits. The objectives of this work were i) to determine phenotypic and genetic correlation coefficients among MRC disease tolerance and different related traits and ii) to present a path coefficient analysis to show how these traits affect MRC disease tolerance.

Plant material: One hundred and forty four $F_{2:6}$ recombinant inbred lines (RIL) were developed from a cross between Mo17, a susceptible dent line, and BLS14, a partially resistance red flint line. The RILs were assessed to MRC disease and parental genotypes were used as controls in each plot.

Field trial: The RILs were tested during the 2004 summer cycle in field experiments at two locations belonging to the endemic area: Río Cuarto (R4) and Sampacho (S4). The field trials were conducted in a randomized complete block design with two replicates of single-row plots. Each plot consisted of 3 m rows with 0.7 m spacing. Plants were thinned to a distance of 0.15 m in the row. The sowing date determination was made through insect vector monitoring during spring in order for the *Delphacodes kuscheli* population reach the peak during early stages of maize development.

Symptoms observed and scored

Disease symptoms were scored 60 to 70 days after male flowering. Individual plants from each plot were phenotypically screened for traits related to MRC disease: plant height (PH), internodes (IN), enations (EN), flag leaf edge (LE), width (LW) and length (LL), panicle (PA), and ear (EA). A disease severity grade was estimated for each plant according to a 0 to 3 scale proposed by Ornaghi et al. (Maydica 44:219-223, 1999). The response variable is the disease severity index (DSI) based on the disease severity grades of individual plants. DSI was calculated for each plot and used to rate RIL for their reaction to MRC disease according to Grau et al. (Plant Dis 66:506–508, 1982). The details of the rating for MRC severity were described in Di Renzo MA et al. (J Agr Sci 139:47-53, 2002).

Statistical analysis

Data from each trial location were subjected to

variance and covariance analyses using the PROC GLM procedure of SAS (SAS Institute Inc. SAS/STAT User´s Guide, Version 9.1.3. SAS Institute Inc., Cary, NC, 2005). Genetic and phenotypic correlation coefficients among DSI and traits related to MRC disease were determined from variance and covariance components. The path coefficient analysis was performed to calculate direct and indirect effects among the response variable, DSI, and MRC related predictor variables. Direct and indirect path coefficients were calculated as firstly proposed Wright (J Agric Res 20:557-587, 1921; Ann Math Stat 5:161-215, 1934), and then Dewey and Lu (Agron J 51:515-518, 1959) and Li (Path analysis: A primer. Boxwood Press, Pacific Grove, CA, 1975). For each trait, direct path coefficient was solved by means of PROC IML (SAS Institute version 9.1.3). The product of appropriate correlation coefficient (*r*) and path coefficient values provides the indirect path coefficient. Correlations and path coefficient analyses based on genetic values more precisely define what factors affect DSI genetically. Previous to analysis, the original data was logarithmically transformed to satisfy the assumption of additivity among the components. The presence of multicollinearity among variables was measured using the variance inflation factor (VIF) and the condition number (CN). Residual effects and determination coefficients were estimated according to Kang (1994). The criterion followed to evaluate the extent of effects of MRC related traits on DSI magnitude was according to Board et al. (Crop Sci 37:879-884, 1997)

Results & Discussion: Phenotypic and genetic correlations coefficients among all pairs of traits are shown in Table 1. In general, phenotypic and genetic correlation coefficients agreed in sign and the magnitude of the phenotypic correlation coefficient was practically the same that the genetic correlation coefficient indicating that the influence of environment on these relationships was little or negligible. However, in most cases, the genetic correlation estimates between DSI and MRC disease-related traits were slightly high and showed that they are genetically associated or that they are physiologically related (Sidwell RJ et al., Crop Sci 16:650-654, 1976). At R4 location, EN had the highest positive phenotypic correlation on DSI (0.94) followed by IN (0.91) and EA (0.64) but, IN had the highest positive genetic correlation on DSI (0.96) followed by EN (0.95) and EA (0.69). However at S4 environment, both phenotypic and genetic correlation coefficients of EN on DSI showed the highest positive values (0.96 and 0.98, respectively) followed by IN (0.90 and 0.94, respectively) and PA (0.69 and 0.77, respectively).

Because of the similarity between phenotypic and genetic correlations, the phenotypic and genetic path coefficients were also quite similar. The path diagram for both phenotypic and genetic correlation coefficients as shown in Figure 1. The almost equal magnitude of EN and IN direct effects on DSI at both phenotypic and genetic levels indicated that these effects were under

Table 1. Phenotypic and genetic correlations among all pairs of traits at R4 and S4 environments.

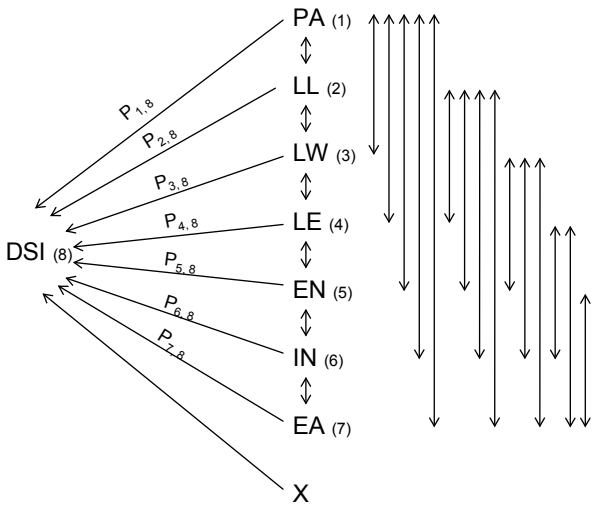
	PA	LL	LW	LE	EN	IN	EA	DSI
PA		0.490 **0.535**	0.408 **0.399**	0.237 **0.225**	0.576 ++ **0.596**	0.552 **0.581**	0.255 **0.298**	0.591 ++ **0.611**
LL	0.577 **0.703**		0.411 **0.412**	0.175 **0.128**	0.353 **0.358**	0.309 ++ **0.363**	0.019 **0.002**	0.352 ++ **0.375**
LW	0.444 **0.320**	0.362 **0.340**		0.119 **0.042**	0.155 **0.092**	0.148 **0.137**	-0.044 **-0.143** ++	0.189 **0.166**
LE	0.324 **0.350**	0.480 ++ **0.530**	0.128 **-0.038**		0.534 **0.558**	0.518 ++ **0.582**	0.596 ++ **0.679**	0.541 **0.556**
EN	0.666 **0.762**	0.622 ++ **0.747**	0.226 **0.243**	0.566 **0.714**		0.867 ++ ++ **0.913**	0.600 ++ **0.648**	0.937 ++ **0.955**
IN	0.616 ++ **0.697**	0.530 **0.646**	0.212 **0.221**	0.483 **0.603**	0.849 ++ ++ **0.891**		0.559 ++ **0.651**	0.914 ++ **0.961**
EA	0.427 **0.469**	0.233 **0.272**	0.049 **-0.016**	0.282 ++ **0.331**	0.528 **0.600**	0.486 **0.570**		0.642 ++ **0.691**
DSI	0.685 ++ **0.774**	0.597 ++ **0.707**	0.232 **0.240**	0.532 **0.660**	0.961 ++ **0.979**	0.898 ++ **0.941**	0.549 ++ **0.628**	

† R4 = Río Cuarto 2004; S4 = Sampacho. DSI = disease severity index; PA = panicle; LL = leaf lenght; LW = leaf width; LE = leaf edge; EN = e-nations; IN = internodes; EA = ear.

++, + Significant at the 0.01 and 0.05 levels, respectively, by bootstrap method.

§ Values above/below the diagonal: correlations among all pairs of traits at R4 and S4 environments, respectively. Genetic values are shown in bold.

Figure 1. Path diagram showing causal relationships between disease severity index (DSI) and Mal de Río Cuarto (MRC) disease related traits. One-headed arrows represent direct paths and double-headed arrows represent correlations (r).



genetic control with unimportant environmental effects. However, at R4 and S4 environments, the genetic direct effects of EN and IN increased hardly over their respective phenotypic direct effects, which suggests a negative relationship between DSI and environmental effects. In general, EN and IN were the primary and secondary direct determinants of DSI over both environments at the phenotypic and genetic levels (see supplemental data online). Nevertheless, at R4 location, IN showed the highest positive genetic direct effect on DSI (0.490) followed by EN (0.445). Similarly, the large positive phenotypic and genetic direct effects of EN and IN on DSI was counterbalanced by a small phenotypic and genetic indirect effects via the remaining traits included in the analysis. In most cases the remaining traits had negligible phenotypic and genetic direct effects, but the phenotypic and genetic indirect effects were intermediate via EN and IN on both environments (see additional data online).

The phenotypic and genetic path coefficients for DSI accounted for a large proportion of phenotypic and genetic variation on both environments as indicated by a large coefficient of determination and by the corresponding small residual effect. In the phenotypic and genetic path analysis, the residual effect represents the failure of the estimated genetic correlations among the variables to account for the total genetic variation in a trait (Sidwell RJ et al., Crop Sci 16:650-654, 1976).

The most important components of DSI, if we just consider phenotypic and genetic correlation coefficients, were EN, IN, EA, PA and LE. Nevertheless, phenotypic and genetic path coefficient analysis showed that among these traits only EN and IN were the most important to increase DSI. The direct effects of EA, PA and LE were little or negligible. The results of this study suggest that EN and IN are the best traits in determining DSI on both environments and might be useful as an indirect selection criteria in breeding and selection programs related to MRC disease.

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Dynamics of callusogenesis in Lancaster inbred lines

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Corn inbred lines in biotechnological and breeding investigations must be sensitive to cultivation *in vitro* to be able to proliferate morphogenic calli, maintain morphogenic potential during long periods of time, regenerate, and quickly accumulate fresh weight.

The dynamics of callusogenesis of ten inbred lines of *Zea mays L.*, which belonged to the commercial germplasm Lancaster, was investigated. Among them DK267, DK212, DK6080, and DK420-1 originated from subgermplasm Oh43; DK633/266 and DK298 originated from subgermplasms Mo17 and Oh43; DK633 originated from subgermplasm Mo17; DK3070 originated from subgermplasms Mo17 and O92; DK236 originated from subgermplasms Mo17 and F2; and DK633/325 originated from subgermplasms Mo17 and Mindszenpuszta. Inbred Chi31 (exotic germplasm) was taken as a standard.

Immature zygotic embryos, 1 mm to 1.5 mm in length, were harvested 10 to 12 days after self-pollination from field-grown plants and cultured on a callus induction medium. This medium included N_6 inorganic components, 100 mg/l inositol, 100 mg/l casein hydrolysate, 10 mg/l $AgNO_3$, 690 mg/l L-proline, 1 mg/l 2,4-dichlorophenoxyacetic asid, 0,1 mg/l abscisic acid, and 30 g/l sucrose. Embryos and calli were incubated in the dark and in temperatures between 27° C and 25° C.

The frequencies of morphogenic callus initiation, initiation of callus type I and type II, frequency of spontaneous regeneration, frequency of brown explants, and fresh weight callus at 30 and 60 days in culture (DIC) are presented in Tables 1 & 2. These characteristics were calculated in ratio to the total amount of responsive embryos (embryos with calli or swollen scutellia).

At 30 DIC inbred DK267, DK 212, DK6080, DK420-1, DK633/266, DK298, and Chi31 were suitable for callusogenesis, but the others appeared to have only swollen scutellums.

At 30 DIC the morphogenic callus frequency was the highest in DK633/266 and DK298, and the lowest frequency was observed in DK420-1. The frequency of callus type I initiation was the highest in DK267, and the frequency of callus type II initiation was the highest in DK633/266.

Table 1. Dynamics of callusogenesis of inbred lines in germplasm Lancaster.

Genotype	DIC	Number of cultivated embryos, among which		Frequency of morphogenic callus initiation %	Frequency of callus type I %	Frequency of callus type II, %
		Callusogenetic	Swollen scutellum			
DK267	30	396	26	76,54 ± 4,13	72,75 ± 4,34	3,79 ± 1,86
	60	396	26	53,08 ± 4,86	48,34 ± 4,87	4,74 ± 2,07
DK 212	30	224	0	44,67 ± 6,38	15,16 ± 4,60	29,51 ± 5,85
	60	224	0	13,39 ± 4,56	0	13,39 ± 4,56
DK6080	30	184	0	38,04 ± 7,18	0	38,04 ± 7,18
	60	184	0	3,26 ± 2,63	0,54 ± 1,09	2,72 ± 2,40
DK420-1	30	184	0	32,61 ± 6,93	2,72 ± 2,40	29,89 ± 6,77
	60	184	0	2,17 ± 2,16	1,09 ± 1,53	1,09 ± 1,53
DK633/266	30	379	0	85,49 ± 3,62	5,54 ± 2,35	79,95 ± 4,12
	60	379	0	35,88 ± 4,93	3,17 ± 1,80	32,72 ± 4,83
DK298	30	360	0	81,11 ± 4,13	35,83 ± 5,06	45,28 ± 5,25
	60	360	0	71,94 ± 4,74	31,94 ± 4,92	40,00 ± 5,17
DK633	30	0	294	0	0	0
	60	167	127	22,11 ± 4,85	13,61 ± 4,01	8,50 ± 3,26
DK3070	30	0	155	0	0	0
	60	96	59	15,48 ± 5,83	15,48 ± 5,83	0
DK236	30	0	429	0	0	0
	60	56	373	1,40 ± 1,14	0	1,40 ± 1,14
DK633/325	30	0	502	0	0	0
	60	336	166	9,96 ± 2,68	7,97 ± 2,42	1,99 ± 1,25
Chi31	30	432	0	64,81 ± 4,60	31,53 ± 3,96	43,29 ± 4,77
	60	432	0	16,90 ± 3,61	6,94 ± 2,45	9,95 ± 2,88

At 60 DIC the morphogenic callus frequency was the highest in DK298, and the lowest frequencies were observed in DK6080 and DK420-1. The frequency of callus type I initiation was highest in DK267, and the frequency of callus type II initiation was highest in DK298.

Inbred lines that showed callusogenesis before 30 DIC saw a substantial decrease in the frequency of morphogenic callus initiation between 30 DIC and 60 DIC. The decrease of frequency of callus type I can be explained by transformation into callus type II, or grown brown calli. Callus type II transformated into nomorphogenic callus. It should be noted that inbred DK6080 had the rare occurrence of forming callus type I at 60 DIC. Inbred DK633/266 and DK298 showed increased frequencies of spontaneous regeneration.

Inbred lines that showed swollen scutellum before 30 DIC had formed morphogenic callus between 30 DIC and 60 DIC. The higher ability to form morphogenic calluses was observed in DK633 and the lower one in DK236. It should be noted that some inbred lines had formed calli of both types (DK633, DK633/325), some inbreds had formed only calli of type I (DK3070) or type II (DK236). Inbred lines DK633, DK236, and DK633/325 at 60 DIC had grown brown explants. This process was connected with callus age though their callusogenesis ability appeared only after 30 DIC.

The comparison of fresh callus weight at 60 DIC showed that DK633/266 had the highest weight, and DK212 and DK236 had the lowest ones.

Thus, the responsive ability of immature maize embryos in a culture is strongly genetically dependent.

Inbreds DK633/266 and DK298 are recommended to use in future in biotechnological and breeding programs because of their ability to form morphogenic calli, maintain morphogenic potential during 60 DIC, and quickly accumulate fresh weight.

Table 2. Fresh callus weight, frequency of spontaneous regeneration and frequency of brown calli in culture of Lancaster inbreds.

Genotype	DIC	Fresh weight, g	Frequency of spontaneous regeneration, %	Frequency of brown explants, %
DK267	30	-	0	0
	60	0,0475 ± 0,0093	0	0
DK212	30	-	0	0
	60	0,0364 ± 0,0049	0	0
DK6080	30	-	0	0
	60	0,1383 ± 0,0187	0	0
DK420-1	30	-	0	0
	60	0,0473 ± 0,0068	0	0
DK633/266	30	-	15,57 ± 3,73	0
	60	0,3442 ± 0,0495	20,32 ± 4,14	0
DK298	30	-	0	0
	60	0,1500 ± 0,0156	5,83 ± 2,47	0
DK633	30	-	0	0
	60	0,0563 ± 0,0071	0	7,82 ± 3,14
DK3070	30	-	0	0
	60	0,0704 ± 0,0090	0	0
DK236	30	-	0	0
	60	0,0325 ± 0,0029	0	11,42 ± 3,07
DK633/325	30	-	0	0
	60	0,0509 ± 0,0054	0	3,78 ± 1,71
Chi31	30	-	0	0
	60	0,1021 ± 0,0136	0	0

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Descriptions of near-isogenic lines of inbred Hi27
--Brewbaker, JL

The following near-isogenic lines (NILs) of Hi27 have been developed in Hawaii through 6 or more backcrosses (BCs). Hi27 is a hard yellow flint of tropical origin from inbred CM104 (India) that was Colombian Amarillo Theobromina in origin, which we converted to *Mv* gene (3L-78) for resistance to maize mosaic virus (Brewbaker, Crop Sci 37:637, 1997). It is of medium height (2m) and mid-maturity (60 days to silk) (Fig. 1). Hi27 has bronze anthers on reddish glumes, pink silks, red blush on lower stems, and colorless seedlings and coleoptiles. Our genetic testcrosses generally confirm that it is of the following genotype:

A1 A2 b1 Bz1 C1-I C2 Mv1 P1-ww Pl1 Pr1 R-r Y1

Hi27 carries "wild-type" alleles of all other loci being introduced into NILs. It boasts a set of tolerances to diseases and pests virtually unknown in temperate germplasm, e.g., fusarium seedling and kernel rots, common and southern rusts, MDMVA and B viruses, MMV Virus, MCMV virus, maydis blight, ear and armyworms, and stalk borers (Brewbaker, Crop Sci 17:631-2, 1997). References for gene loci are from the classic “Mutants of Maize” (Neuffer MG, Coe EH, and Wessler SR, 1997), and gene locations are from Ed Coe’s excellent summary (MNL 82:87-102, 2007).

I’m eternally indebted to the Maize Genetics Cooperation Stock Center at University of Illinois and especially to Earl Patterson with whom I worked at Cal Tech in summer 1948, along with people such as Earl and also Ed and Mary Olga Coe, Charlie Burnham, Don Robertson, Edgar Anderson, and E.G. Anderson. Earl provided many of the stocks used to initiate this program in 1967, while Marty Sachs and Phil Stinard have continued to offer great support. NILs that have been provided to the Maize Coop are annotated here with their MGC stock numbers. Ultimately all NILs will also be preserved at the Maize Coop.

Photographs of these NILs, where available, are provided at the end of this manuscript and labeled as figures. Seasonal variations in phenotype relate to “winter” (71° F average temperature) and “summer” (78° F aver-

age temperature) at the Waimanalo Research Station at sea level on Oahu, Hawaii.

***a1*^Hi27 – anthocyaninless.** Location: 3L-174.75 (identical to *sh2*). MGC 322F. Origin: MGC 63-2665 sib (*a1 Dt1* segregating *sh2*). Hawaii stock represents 7 BCs to Hi27 over 35 cycles of breeding. Plants are distinguished from Hi27 and other NILs by the pure green plant, silks, tassels and anthers. Inbred Hi27 has some basal stalk color ("sun-red"), reddish silks, bronze tassels, and bronze anthers. All of our *C1* stocks also have green silks (Hi37 = *Cl-I*). Our *sh2*, *sh2 sl1*, and *sh2 y1* NILs are also homozygous for *a1* (Fig. 2).

a2 (cf. NIL **(*bt1 a2*)^Hi27**)

***ad1*^Hi27 (1:0, 1:1) – adherent tassel.** Location: 1L-192.0. MGC 110EA. Origin: MGC *ad1* stock (1969). The two Hawaii stocks (*ad1* homozygote and 1:1) represent 8 BCs to Hi27 over 29 cycles of breeding. In the Hi27 background adherent affects the tassels, but unlike the early report of Kempton, the leaves are not adherent. The homozygous stock often includes highly compressed tassels having little or no pollen. Plant height is about 10% below normal and ears are a bit high (Figs. 3 & 4).

***ae1*^Hi27 – amylose extender.** Location: *ae1* = 5L-87.8. Origin: This monogenic NIL was derived from the *ae1 wx1* double mutant (below) crossed with the (*bm1 ae1*) double mutant in cycle 41 of breeding for the *ae1 wx1* stock. Hawaii stock represents another backcross to Hi27 and selfing to homozygosity. Plants are tall, a few days late, and tassels are normal (not *flta1*, as in most of our waxies). Kernels are heavy and dark yellow in color, which resembles those of the (*bm1 ae1*) stock. Unlike *ae1 wx1*, they do not attract fusarium rots.

***ae1 wx1 flta1*^Hi27 – amylose extender, waxy endosperm, floppy tassel.** Two stocks; *ae1 wx1*^Hi27 and *ae1 wx1*^Hi27 3:1 (derived from cross of *ae1 wx1*^Hi27 x *wx1*^Hi27). Locations: *ae1* = 5L-87.8, *wx1* = 9S-47.9, *flta1* = 9?. MGC M541P. Origin: Penn State breeding stock 64-702-3 self (*ae1 su1 wx1*). Hawaii stock represents 6 BCs to Hi27 over 40 cycles of breeding. The double mutant is reduced ~20% in height compared with Hi27 or with *wx1*^Hi27 but has similar floppy tassels (linked to *wx1*). It produces typically compressed or collapsed kernels that are highly susceptible to kernel rot by fusarium (Fig. 5), which leads to entire ear rots. The homozygote has thus been difficult to maintain. In the 1960s we did a little (unsuccessful) breeding of *ae1 wx1* and *ae1 su1 wx1* as potential sweet corns. See also *ae1* (above) and (*bm1 ae1*), a mutant that is dwarfed and annoying to breed (Figs. 6 & 7).

***B1*^Hi27 – booster colored plant.** Locations: 2S-49.3. MGC M341BA. Origin: Carribean Composite (Thailand, 1967). Hawaii stock represents 8 BCs to Hi27 over 31 cycles of breeding. There is no reason to suspect that our *B1* is different from that reported in 1921 by the father of many corn genes, Cornell's R.A. Emerson. Our *B1* stocks all have dark purple stalks, sheaths and husks (Hi27 is *A1 Pl1*) with red anthers (versus bronze for Hi27) and green silks (versus red for Hi27). The cobs are strongly pigmented through indurate tissues, unlike the *P* locus cob colorations that occur only in soft floral tissues. Our *B1* NILs tend to be taller (long internodes) and higher eared, somewhat fragile in appearance, and with smaller ears. They succumb to premature senescence, especially when infected with southern rust, unlike Hi27 itself (Figs. 8-11).

***B1 C1*^Hi27 – booster colored plant, colored kernels.** Locations: *B1* = 2S-49.3, *C1* = 9S-16.18, MGC M341BB. Origin: Identical to *B1*^Hi27, separated after 28 cycles of breeding. Kernels have purple aleurone (Hi27 is *Cl-I R1 Pr1 Pl1*) on the dark red cob, and outcrossed kernels (from *Cl-I* pollen) are yellow. Plants are otherwise similar to *B1*^Hi27 with purple stalks and husks, red anthers and green silks, and the plants tend to be attenuated and with smaller ears. They are also more susceptible to rust.

***B1* (*C1 bz1*)^Hi27 – booster colored plant, colored kernels, bronze aleurone and stalk.** Locations: *B1* = 2S-49.3, *C1* = 9S-16.18, *bz1* = 9S-22.53. Origin: Hybrid of NILs *B1*^Hi27 and (*bz1 C1 flta1*)^Hi27. These plants have bronze-reddish stalks and husks that are easily distinguished from *B1*^Hi27 (above). Tassels are largely of floppy-tassel (*flta1*) type (cf. (*bz1 C1*)^Hi27). Ears have bronze aleurone and cob, and both anthers and silks are bronze-red. They are very pretty (Fig. 12).

***ba1*^Hi27 3:1 – barrenstalk.** Location: 3L-109.0. MGC 318B. Origin: MGC 62F-1116 self. Hawaii stock represents 6 BCs to Hi27 over 27 cycles of breeding. Expression is typical with missing ears and a single-spiked tassel with such poor pollen that maintenance of a 1:1 stock is difficult (Fig. 13).

***bd1*^Hi27 3:1 – branched silkless.** Location: 7L-137.05. MGC 705DA. Origin: MGC *ij1 bd1* stock 1962; exact record lost. Hawaii stock represens 6 BCs to Hi27 over 30 cycles of breeding. Expression is typical with multi-branched ears that often have a tassel-tip. The tassel itself appears very thick due to a great abundance of florets that are especially on central spike. However, tassel branch number is normal for Hi27, i.e., ~12 (Fig. 14).

***Bf1*^Hi27 – blue fluorescent.** Location: 9L-151.03. MGC 918F. Origin: MGC 63-3331 self. Hawaii stock represents 6 BCs to Hi27 over 21 cycles of breeding. Expression is typical of that described by Howard Teas and E.G. Anderson (1951). Anthers of heterozygotes show light blue fluorescence under a black light, but seedlings only fluoresce if homozygous.

***Bif1*^Hi27 1:1 – barren inflorescence.** Location: 8L-54.0. MGC 827C. Origin: MGC 90-69-4 x 27-3. Hawaii stock represents 8 BCs to Hi27 over 12 cycles of breeding. Expression is similar to those described by Gerry Neuffer and Bill Sheridan (1977) with tassels

showing partial sterility and reduced branching (Fig. 15).

(*bk2 wx1 flta1*)^Hi27 – brittle stalk, waxy endosperm, floppy tassel. Locations: *bk2* = 9L-78.5, *wx1* = 9S-47.9, *flta1* = 9?. MGC916CA (Fig. 16). Origin: MGC 59-1897 self (*bk2 wx1* stock). Hawaii's NIL represents 6 BCs to Hi27 over 26 cycles of breeding and continues to be a double mutant with waxy (segregating). Breakage is typical for leaves, stalks, husks, tassels, cobs, etc. Maintaining a homozygous *bk2* line has not been too difficult in Hawaii's gentle trade winds, and it makes excellent ears. Like our other *wx1* NILs, this has floppy tassels (Figs. 17 & 18).

***blo1*^Hi27 – blotched leaf.** Location: Unknown. Origin: Hawaii sweet corn AA8-163 (79-1143 sib) derived from 'Hawaiian Sugar' (Golden Bantam x Caribbean sugary). Hawaii stock represents 7 BCs to Hi27 over 21 cycles of breeding. This NIL shows chlorotic blotches as long stripes between veins and can often be identified within two weeks of planting. Expression is somewhat weaker in hot summer days. It is fully recessive and does not directly mimic any of the several lesion-mimic mutants (e.g., Gerry Neuffer) (Fig. 19).

***blo1 p1-wr*^Hi27 – blotched leaf, red cob.** Locations: *blo1* = unknown; *p1-wr* = 1L. Origin: This segregated as a single plant (mutant?) in our *p1-wr* stock, and it segregated originally in inbred Hi27 (see *p1-wr*^Hi27). Testcrosses to the *blo1* stock confirmed allelism, and homozygous *blo1 p1-wr* stock was selfed out.

(*bm1 ae1*)^Hi27 – brown midrib, amylose extender. Locations: *bm1* = 5S-79.25, *ae1* = 5L-87.8. MGC 515D. Origin: MGC 69-543 self. Hawaii stock represents 7 BCs to Hi27 over 26 cycles of breeding. Kernels are typically denty in appearance (Fig. 20) and testcrosses to *ae1 wx1*^Hi27 confirmed homozygosity for *ae1*. Unlike our *ae1* RIL, this mutant is quite susceptible to fusarium, which can lead to poor germination and to dwarfing, often with a delay of from 5 to 7 days to anthesis. I suspect a gene linked to *bm1* for fusarium susceptibility. Midrib colors are evident within 3 weeks of planting and become more intense before fading in older plants. They segregate both bronze and red anthers. See also *ae1* and *ae1 wx1* (Figs. 21 & 22).

***bm2*^Hi27 – brown midrib 2.** Location: 1L-259. MGC 119F. Origin: MGC 61-1956 sib (a stock with *br1, f1, y1 and p1-ww*). Hawaii stock represents 7 BCs to Hi27 over 27 cycles of breeding. This NIL is normal in height, maturity, and apparent yield, as reported by its discoverers R.A. Brink and Charlie Burnham. Midrib colors are evident within 3 weeks of planting, become intense, then fade in older plants. (Fig. 23).

***bm2*^Hi27 ("*bm3*") – brown midrib 2.** Location: 1L-259. MGC 408EA. Origin: Pete Baumann's Purdue hybrid H55 *bm3* x H49 *bm3* (1972), that later proved to be *bm2*, not *bm3*, in many types of testcrosses. Hawaii stock represents 7 BCs to Hi27 over 24 cycles of breeding. It is classical in appearance with early expression of color in midribs and sheaths, and of normal height. Similar misclassifications of *bm2* and *bm3* have been noted by J. Coors and others.

***bm2 gt1*^Hi27 – brown midrib 2, grassy tiller.** Location: *bm2* = 1L-259, *gt1* = 1S-?. MGC 408EB. Origin: Selfed out of hybrids in 2004 of our gt1^Hi27 and our *bm2*^Hi27 ("*bm3*") stock originally believed to be *bm3*. NIL shows early expression of brown color in midribs and sheaths, with typical grassy tillers and long flag leaves that express the color also (Fig. 24).

***bm4*^Hi27 – brown midrib 4.** Location: 9L-162. MGC 919A. Origin: MGC 86-1575 selfed. Hawaii stock represents 6 BCs to Hi27 over 20 cycles of breeding. NIL is classical in appearance with early expression of color in midribs and sheaths, as described in 1947 by Charlie Burnham, with whom I had the pleasure of working at Cal Tech in the summer of 1948. However my stock is quite dwarfed (30%) and late (4-6 days to silk) compared to Hi27. It also seems highly susceptible to fusarium as expressed through seedling mortality; in short it is an ugly stock. The hybrids with *bm4*'s from University of Wisconsin were also unimpressive (Fig. 25).

***br1*^Hi27 – brachytic plant.** Location: 1L-170.0. MGC 112H. Origin: MGC 65-193-1 x 186-7. Hawaii stock represents 7 BCs to Hi27 over 30 cycles of breeding. Extremely dwarfed (50%) with unusually erect and sharply-pointed leaves. Dwarfing mimics that of *d1* dwarfs. The mutant and the double mutant (*br1 f1*) have very poor root development and essentially lacks brace roots. Note that rootless (*rt1*) is on 3S (Fig. 26).

(*br1 f1*)^Hi27 – brachytic plant, fine-stripe. Location: *br1* =1L-170.0, *f1* = 174.0. MGC 113C. Origin: MGC 65-193-1 x 186-7 (cf. *br1*^Hi27 above). Hawaii stock represents 7 BCs to Hi27 over 27 cycles of breeding. This NIL is dwarfed somewhat less than *br1*^Hi27 (above) and has better brace roots. It has the same type of erect and pointed leaves, and fine-stripe is expressed exactly as in the single mutant NIL *f1*^Hi27 (Figs. 27 & 28).

***br2*^Hi27 – brachytic 2 plant.** Location: 1L-151.26. MGC 117A. Origin: Indian inbred CM104 *br2* (1967). Hawaii stock represents 6 BCs to Hi27 (an inbred that was derived from CM104) over 32 cycles of breeding. Dwarfing is moderate (25%), versus more than 40% in other inbreds I've studied. The *br2* gene shortens internodes below the ear. I took an immense amount of data on *br2* conversions of tropical inbreds when with Rockefeller in Thailand (1967-1968). Since many tropical inbreds have high ear location, *br2* converts them to more Corn Belt-looking inbreds. Nonetheless, the gene has no commercial significance. Comparable dwarfs include *ct1* (compact) and *br1* (Fig. 29).

***br3*^Hi27 – brachytic 3 plant.** Location: 5-?. MGC 520C. Origin: MGC 89-1674 sib. Hawaii stock represents 7 BCs to Hi27 over 22 cycles of breeding. In Hawaii this is moderately dwarfed (50% of normal), less than as noted by discoverer Ralph Singleton (1959). Heterozy-

gotes appear also somewhat dwarfed. Our stocks seem to segregate for defective endosperm alleles (Fig. 30).

***Brta1*^Hi27 – branched tassel.** Location: chromosome 2, linked to *fl1* and *v4*. Origin: (*fl1 Brta1 v4*)^Hi27 breeding stocks, first observed in 2007 (BC6). See also (*Brta1 v4*)^Hi27 and *fl1 Brta1 v4*^Hi27. Tassels have extra primary and secondary branches that approximate twice the normal number (13) of Hi27. Branches tend to be more erect but easily distinguished from mutants like *ra2* (more than 40 branches). Ears are not affected. A similar phenotype occurs in *Og1 B1*^Hi27 and *su1-jlb3*^Hi27. Gene inherits as a co-dominant, with heterozygotes intermediate to parental homozygotes. Branch number of all NILs is reduced by about half by Hawaii's short-day, low-light winters (Figs. 31 & 32).

(*Brta1 v4*)^Hi27 – branched tassel, virescent 4. Locations: *Brta1* = 2-?, linked to *fl1* and *v4* = 2L-87.0. Origin: (*v4 fl1*)^Hi27 breeding stocks, segregating since 2007 (BC6). See also *Brta1*^Hi27 and (*fl1 Brta1 v4*)^Hi27. Branched tassel is clearly linked to the *v4* and *fl1* loci in this region of chromosome 2, and essentially all combinations of the three have been created as NILs. This stock appears to segregate for a linked semi-dwarf locus and for double-cob (*dbcb1*) (Fig. 33).

***bst1*^Hi27 – brown stripe.** Location: Not known. Origin: Illinois sweet corn inbred 442 (in 1972). Hawaii stock represents 7 BCs to Hi27 over 24 cycles of breeding. It is distinguished from mutants like *f1, gs1, ij1, j1, j2,* and *li1* by linear stripes on leaves that turn rusty brown. It mimics *j2* in the irregularity of stripes. Expression is quite variable and often difficult to identify. Severe symptoms involve some stunting and irregular seed set. (Fig. 34).

(*bt1 a2*)^Hi27 – brittle anthocyaninless-2 endosperm. Locations: *bt1* = 5S-80.01, *a2* = 5S-72.01. MGC 511H. Origin: MGC 63-3023-8 x 3033-10 (segregating *a2*, *bt1*, *bm1* and *pr1* in an ACR background). Hawaii stock represents 7 BCs to Hi27 over 33 cycles of breeding. This is also the source of the *brittle-1* gene used in all Hawaii breeding of "Hawaiian Supersweets" (Brewbaker, 1977), where segregants lacking *a2* were used also in breeding but later discarded. The present double-mutant stock is dwarfed about 20% versus Hi27 and versus *bt1 A1*^Hi27 (Fig. 35), and dwarfing is dominant in hybrids (see *bt1 A1*^Hi27 below, that is normal height). All *bt1 a2* stocks lack color in stalk, silks, anthers, and tassels. They thus mimic the classic *sh2* stocks that are all double-mutants with the *a1* allele. Care was taken during breeding to select for high germination rates, thus favoring fusarium-tolerant lines with only occasional evidence of fusarium kernel rots (Fig. 36). Such rots are otherwise common in *sh2*, *o2*, and other NILs (Figs. 37-39).

(*bt1 a2*)(*sh2 a1*)^Hi27 – brittle anthocyaninless-2, shrunken-2 anthocyaninless-1 endosperm. Locations: *bt1* = 5S-80.01, *a2* = 5S-72.01, *sh2* and *a1* = 3L-174.75. This double mutant of the two "high-sucrose" loci and its closely linked partners were created by crossing the respective NILs (*bt1 a2*)^Hi27 x (*sh2 a1*)^Hi27. The subline chosen is normal height and has the expected green anthers and silks. The mature kernels have an unusual bronze color and are presumed to be in aleurone, though the stock must carry inhibitor *Cl-I*. At sweet corn stage, however, the kernels are yellow (Fig. 40).

(*bt1 a2*)(*sh2 a1*)*vir1*^Hi27 – brittle anthocyaninless-2, shrunken-2 anthocyaninless-1 endosperm, virescent. Locations: *bt1* = 5S-80.01, *a2* = 5S-72.01, *sh2* and *a1* = 3L-174.75, "vir" = unknown. This virescent mutant segregated in the (*bt1 a2*)(*sh2 a1*) stock above and is suspected to be *v4*. Plants resemble the originating stock, as do the kernels that are an unusual bronze color when mature.

(*bt1 a2*) *y1*^Hi27 – brittle anthocyaninless-2, white endosperm. Locations: *bt1* = 5S-80.01, *a2* = 5S-72.01, *y1* = 6L-30.13. This double mutant was created by crossing the respective NILs (*bt1 a2*)^Hi27 x *y1*^Hi27. The NIL mimics Hi27 in appearance and has the expected green anthers and silks. Kernels are not white; they are an unusual bronze color identical in appearance to that of the double mutants with (*bt1 a2*)(*sh2 a1*) above.

***bt1-A*^Hi27 – brittle-A endosperm.** Location: *bt1* = 5S-80.01. Origin: Stock TS93-130 selfed from Tom Sullivan of University of Wisconsin and originally from Curt Hannah. Seeds introduced in 1995 and backcrossed 6 times to Hi27. They carry allele *A2* and shows color in silks and tassels. Allelism to *bt1* in stock (*bt1 a2*)^Hi27 was verified. The two NILs differ about a foot in height, as do their hybrids, which are presumably related to a semi-dominant, linked dwarfing gene in the (*bt1 a2*) double mutant. Hybrids of the two NILs with supersweet breeding stocks were distinguished only by height and were similar in yield (excellent), tenderness (average) and sweetness (not bad). Some potentially commercial hybrids were placed into Hawaii yield trials.

(*bv1 pr1*) *C1*^Hi27 – brevis plant, red aleurone. Locations: *bv1* = 5L-84, *pr1* = 5L-98, *C1* = 9S-16.18. Origin: unknown MGC (*a2 bv1 pr1*) stock. Hawaii stock is in early stages of development and retains the closely linked *bv1* and *pr1* alleles. Plants are dwarfed about 50%, as described by Cornell's HW Li in 1931 (Fig. 41).

(*bz1 C1 flta1*)^Hi27 – bronze aleurone colored aleurone, floppy tassel (*bz1 C1 flta1*). Locations: *bz1* = 9S-22.53, *C1* = 9S-16.18, *flta1* = 9S-?. MGC 905C. Origin: MGC 68-1238-5 x 1238-4 (*C1 bz1 Wx1*). Hawaii stock represents 7 BCs to Hi27 over 30 cycles of breeding. Stock is marked by green silks (instead of red in Hi27) and bronze aleurones. It is homozygous, for or segregates the "floppy tassel" gene we've discovered to be linked closely to *wx1* locus (Brewbaker and Yu, MNL, 2009).

***C1*^Hi27 – colored (blue) aleurone**. Location: *C1* = 9S-16.18. MGC X17E. Origin: MGC 63-3065-5 self (a stock with *A1 A2 C1 R1 Pr1*). Hi27 lacks aleurone anthocyanins due to presence of the inhibitory allele *Cl-I* at the

C1 locus. Many of our NILs have this inhibitor removed. The *C1*^Hi27 stock represents 5 BCs to Hi27 over 28 cycles of breeding, and aleurone color is blue due to presence in Hi27 of alleles *R1* and *Pr1*. The *C1*^Hi27 stock is a classic and attractive flinty "blue corn" (aleurone), with no mottling evident. It is further distinguished by green silks versus red in Hi27. It does not carry *flta1* (cf. *bz1 C1 flta1* above). Other *C1* containing NILs include *B1 C1 p1-wr*, (*bz1 C1*), (*C1 sh1 bz1 wx1*), in *B1 C1 R1*, and *R1-nj C1*. Also we've two stocks, *R1 C1* and *R1 B1 C1*, that evidently have a unique *R1* allele from that in Hi27. All NILs with *C1* (not *Cl-I*) have green silks, somewhat unusual for tropical flints (Figs. 42 & 43).

(*C1 sh1 bz1 wx1*)^Hi27 – colored shrunken bronze waxy aleurone (*C1 sh1 bz1 wx1*). Locations: *C1* = 9S-16.18, *sh1* = 9S-20.08, *bz1* = 9S-22.53, *wx1* = 9S-47.93. MGC 903B. Origin: Barbara McClintock's stock 358-2 self, received in 1970. Hawaii stock represents 9 BCs to Hi27 over 30 cycles of breeding. Like all *C1* stocks, this is distinguished by green silks, versus red in Hi27. It is normally dwarfed ~20% versus Hi27, mimicking our *br2*^Hi27. It does not carry *flta1*, the "floppy tassel" gene common among our waxy stocks and also seen in (*bz1 C1 flta1*)^Hi27 (Fig. 44).

c2 (cf. stock (*o1 c2*)^Hi27).

***Cg1*^Hi27 1:1 – corngrass.** Stock is maintained by backcrossing to Hi27 and segregates 50% corngrass. Location: *Cg1* = 3S-24.4. MGC 301D. Origin: MGC 65F-383 x 421Cg. Hawaii stock represents more than 10 BCs to Hi27 over 33 cycles of breeding. Plants are grassy and have tillers and flag leaves. They produce many 2- to 4-rowed ears and variable tassels. Selfs of corn-like segregants produced a 3:1 ratio differing widely in expression, with homozygotes assumed to be the most severe and sterile, confirming Ralph Singleton's view (1951) that gene is partially (or co-) dominant (Figs. 45-47).

***Ch1*^Hi27 – chocolate pericarp**. Location: *Ch1* = 2L-162.6. MGC 219C. Origin: MGC 60-47 sib (a stock with *lg1 gl2 fl1* and *v4*). Hawaii stock represents 19 BCs to Hi27 over 28 cycles of breeding. Stock has attractive brown pericarp and cob, as first reported by E.G. Anderson and R.A. Emerson (1931). I had the pleasure of working a summer with "Andy" at Cal Tech in 1948, and his daughter enjoyed stealing shootbags from my hip pockets! In the Hi27 background this highly backcrossed NIL is notably vigorous from time of germination and larger and more erect than Hi27, which suggests linked QTLs (Figs. 48, 49).

***cr1*^Hi27 – crinkled leaf**. Location: *cr1* = 3S-20.0. MGC 301A. Origin: MGC 63-2495-9 self. Hawaii stock represents 6 BCs to Hi27 over 29 cycles of breeding. Stock has leaf-rolling or "crinkling" typical of that described by R.A. Emerson (1921) that is identifiable within two weeks of planting. Leaves are shortened and plants dwarfed ~20% in the Hi27 background. I regret greatly that Emerson passed away shortly before I joined the team at Cornell, for he and students like McClintock, Rhoades, and Burnham (all of whom I am pleased to have known) laid a solid foundation for maize geneticists (Figs. 51-53).

***ct1*^Hi27 – compact plant**. Location: *ct1* = 8L-?. Origin: MGC 91-1102-1. Hawaii stock represents 6 BCs to Hi27 over 27 cycles of breeding. It has colorless anthers, possibly due to linkage with *a4* locus (8L-97). In the Hi27 background, the plants are dwarfed ~15% but without obvious shortening of leaves, tassels, or ears. The gene was described by Oliver Nelson and Ohlrogge in 1957 as "dwarfing all parts of the plant proportionately". They found it to be near "proline-responsive" *pro1* (8L-74). Ollie Nelson contributed immensely to our knowledge of maize genetics, notably of endosperm genes like *fl2, o2* and *wx1* (Fig. 54).

***d1*^Hi27 – dwarf.** Location: 3S-30. MGC302E. Origin: MGC 65-245-7 x 244-5. Hawaii stock represents 7 BCs to Hi27 over 32 cycles of breeding. This NIL resembles the original of R.A. Emerson (1912) in height (dwarfed to about 35% of normal). Plants tend to be highly tillered, unlike other Hi27 NILs (exceptions include *gt1, tb1* and *tlr1*). Leaves are shortened about 25% and are unusually broad (25% more than Hi27) with 11-12 veins on each side of the midrib (versus 8 in Hi27) at one month in age. Tassels are compact and ears are short and often tassel-tipped. It's described in the literature as "andromonoecious" and with anthers buried in the glumes. In the Hi27 NIL the anthers emerge well and it sets seed well. The stock is notably the most susceptible of all of our NILs to southern rust (*Puccinia polysora*), and has been grown many years as an indicator of severity of the disease (Figs. 2 and 3). All other NILs have very high tolerance (2.5 on 1 to 9 scale, versus 7.6 to 8.5 for *d1*^Hi27). Our evidence is that a linked gene for susceptibility that is dominant or co-dominant came in from the MGC stock. The susceptibility might be related in part to the broad leaves and tillering of this mutant (Figs. 55-58).

***dbcb1*^Hi27 – double cob.** Location: 1S-?. Origin: Double-cob segregated from our *p1-vv*^Hi27 NIL derived from MGC 1969 *p1-vv* stock after 10 BCs to Hi27. The trait segregated as a simple recessive and has additional BCs to Hi27. The ears tend to split near the top of the cob, rarely producing more than two paired cobs with normal seeds. The plants are slightly more erect than Hi27 but otherwise normal in appearance and vigor. The eartips open up, which leads to much damage from insects and diseases, described in MNL (2009) by J.L. Brewbaker and Yu. This is unlike fascinated ear genes (*fae1*, *fae2*) that produce a multiply-branched ear (Hake and Viet, MNL, 1988). Gene *fae1* occurred in a *Mu*-containing family, and *Mu* (or *Ac*) might also account for this mutant (Figs. 59 & 60).

(*dbcb1 p1-rw*)^Hi27 – double cob, red pericarp.

Locations: *dbcb1* =1S-?, *p1* = 1S-69.58. Origin: cf. *dbcb1*^Hi27. This is a clearly mutable line with variations in leaf width, tassel color, sterility and anthesis-silk interval. Like most *p1-rw* stocks it has red pericarp, colorless cob and green anthers (versus bronze in Hi27). Otherwise the plants are normal in appearance and vigor, with ears similar to the *dbcb1*^Hi27 stock.

(*dbcb1 p1-vv*)^Hi27 – Double cob, variegated pericarp. Locations: *dbcb1* =1S-?, p = 1S-69.58. Origin: cf. *dbcb1*^Hi27. This is a clearly mutable line with variations in leaf width, striping similar to japonica, tasselseed, and sterility. It mimics the *dbcb1 p1-rw* stock but has variable anther color (Fig. 61).

***Dt1 a1*^Hi27 – dotted aleurone, anthocyaninless.** Locations: *a1* = 3L-174.75, *Dt1* = 9S-0. MGC 323A. Origin: MGC 63-2665 sib (*a1 Dt1* segregating *sh2*). Hawaii stock represents 6 BCs to Hi27 over 37 cycles of breeding. Plants are similar to *a1*^Hi27 with green plant, silks, tassels, and anthers. Aleurones are irregularly dotted and kernel abortion is common. Plants showed high susceptibility to an infection of bacterial leaf blight.

***du1*^Hi27 – dull endosperm**. Location: *du1* = 10L-46.7925. MGC X10A. Origin: MGC 63-2127 x 2129. Hawaii stock represents 6 BCs to Hi27 over 24 cycles of breeding. Plants closely resemble Hi27. Kernels resemble description of discoverer Paul Mangelsdorf (1947) as "tarnished" but do not show any denting, as we observe for *ae1*. See also (*li1 du1*)^Hi27.

***el1*^Hi27 3:1 – elongate (collapsed seed)**. Location: *el1* = 8L-?. MGC 805E. Origin: MGC 63-787 x 786. Hawaii stock represents 6 BCs to Hi27 over 25 cycles of breeding with selection for red cob, assumed to be *p1-wr*. Plants closely resemble Hi27 but segregate red cob, red anthers, and green silks. Ears segregate collapsed *el1* kernels that resemble *sh2* seeds and generally fail to germinate. The collapsed kernels evidently result from unreduced gametes leading to 5N endosperms, as identified by brilliant cytogeneticist Marcus Rhoades (Fig. 62, 63).

***et1*^Hi27 3:1 – etched endosperm**. Location: 3L-190.01. MGC 320E. Origin: MGC 63-2656 x 2655, open-pollinated in Thai nursery 67-141. Hawaii stock represents 6 BCs to Hi27 over 35 cycles of breeding, not an easy target. Kernels resemble those described by discoverer L.J. Stadler, expert on mutation and mutability. The kernels are covered with networks of collapsed pericarp above collapsed endosperm cells. Etched kernels in this inbred usually abort and the few that germinate produce lethal white seedlings. Virescent seedlings were seen in early generations of backcrossing when stock was much more vigorous and kernels large. Heterozygous plants closely resemble Hi27 but have green silks (Fig. 64).

***et1 C1*^Hi27 3:1 – etched and colored endosperm**. Locations: *et1* = 3L-190.01, *C1* = 9S-16.18. Origin: cf. *et1*^Hi27. During the 6 BCs to Hi27 we retained purple kernels on which the etched trait was more easily identified. Surprisingly the plants with purple kernels (*C1* locus, replacing *Cl-I* of Hi27) have always been dwarfed ~35% from normal. Dwarfing is recessive, and a linked gene to *C1* is thus inferred. Like other *C1* stocks, they have green silks. The etched kernels are colorful but resemble those of the *et1*^Hi27 NIL (Fig. 65).

***f1*^Hi27 – Fine stripe**. Location: 1L-174. MGC 127E. Origin: MGC 61-1956 self (*br1 f1 bm2 p1-vv* and *y1*). Hawaii stock represents 6 BCs to Hi27 over 21 cycles of breeding, with strong selection to separate *f1* from *br1* (cf. NIL (*br1 f1*)^Hi27, from a different origin). Plants resemble closely those described by discoverer E.W. Lindstrom (1918), and emerge as virescents that develop linear chlorotic stripes along leaf margins. Expression is seasonally variable and is most striking in winter and spring, as with most chlorotic mutants (Figs. 66 & 67).

(*fl1 Brta1 v4*)^Hi27 – floury endosperm, branched tassel, virsecent 4 plant. Locations: *fl1* = 2S-57, *Brta1* 2L-?, *v4* = 2L-87). MGC 215G. Origin: 63-2370 x 2369 (chromosome 2 stock with linked *lg1 gl2 b1 fl1* and *v4*). Stock involved 6 BCs to Hi27 over 25 cycles of breeding. Floury kernels resemble those described by Herbert Kendall Hayes (my father's professor) and his professor, E.M. East, in 1915, with classic dosage effect in the triploid endosperm. The *v4* mutants resemble those described by discoverer Milislav Demerec (1924) as noted in description of the NIL (*v4 fl1*)^Hi27, with best expression in Hawaii's cool winters. The branched tassel (*brta1*) was first observed segregating in Hawaii NILs carrying fl (Brewbaker and Yu, MNL, 2009); see discussion under *Brta1*^Hi27, a gene that co-segregates with genes *fl1* (2S-75.7) and *v4* (2L-87). See also pedigrees of (*gl2 lg1*) and (*v4 fl1*) mutants (Figs. 68 & 69).

(*fl1 v4*) *y8*^Hi27 – floury kernel, virescent seedling, pale yellow kernel. Location: *fl1* = 2S-75.7, *v4* = 2L-87.0, *y8* = 7S-34. Origin: 63-2370 x 2369 (chromosome 2 stock with linked *lg1 gl2 b1 fl1* and *v4*), similar to (*fl1 brta1 v4*)^Hi27 pedigree (= MGC 215G). This originates from BC6 by the selection of whitish kernels. Otherwise similar to (*fl1 v4*)^Hi27 but with very pale yellow kernels (allelism verified to *y8* locus). Tassels are normal, and lack the branched tassel gene *Brta1* in related stocks.

***fl2*^Hi27 – floury 2 endosperm**. Location: *fl2* = 4S-57.5. MGC 406C. Origin: Unknown stock from MGC crossed to CM104 in 1967. Hawaii stock represents 7 BCs to Hi27 over 29 cycles of breeding. Kernel type and inheritance are similar to those described by Ollie Nelson and colleagues (1965), with plants otherwise similar to Hi27. See also our lazy NILs that carry closely-linked *fl2* (Fig. 70).

flta1 – Floppy tassel. (see *wx1 flta1*^Hi27) (Fig. 71).

***g1*^Hi27 – golden plant**. Location: *g1* = 10L-78.0.

MGC X11F. Origin: MGC 60-161 x 162. Hawaii stock represents 7 BCs to Hi27 over 28 cycles of breeding. NIL is typical in appearance with yellowish sheaths, often identifiable within one month. The yellowing progresses up to about sweetcorn stage. Strikingly golden spots occur when infected by rusts or blights. They are moderately dwarfed (20% below Hi27), unlike the double mutant *g1 li1* (compared with *gli1*) (Figs. 72 & 73).

(*g1 li1*)^Hi27 – golden lineate. Locations: *g1* = 10L-78.0, *li1* = 10L-62.0. MGC X09G. Origin: From our hybrid of the *g1* and *li1* stocks prior to BC3, with 7 BCs over 24 cycles of breeding. Both genes are typical in expression, but unlike the moderately dwarfed *g1* stock (20% below Hi27) and the tall, attenuated *li1* stock (20% above Hi27), the double mutant *g1 li1* is more or less normal in height. Kernels are normal, not *du1*, as in (*li1 du1*)^Hi27 (Fig. 74).

***gl1* – glossy seedling**. See (*o51 gl1*).

(*gl2 lg1*)^Hi27 – glossy seedling, liguleless plant. Locations: *gl2* = 2S-30.52, *lg1* = 2S-11.75. MGC 205C. Origin: MGC 63-2370 x 2369 (chromosome 2 linkage stock *lg1 gl2 b1 fl1 v4*). Hawaii stock represents 6 BCs to Hi27 over 24 cycles of breeding. Plants are similar to *lg1*^Hi27. Seedling leaves are typically glossy in appearance, as first described by my father (Hayes and Brewbaker, 1928, Amer Nat 62:228-235).

***gs1*^Hi27 – green striped leaves.** Location: *gs1* = 1L-224. MGC 119C. Origin: MGC stock 81-030-71-6. Hawaii stock represents 6 BCs to Hi27 over 21 cycles of breeding. Chlorotic stripes on leaves are similar to those described by discoverer R.A. Emerson (1912), but do not show rolling or wilting (as described by Emerson). In cooler weather the phenotype is evident after a month of growth, but in warm summer days it's only observed later in development. On one occasion we observed significant wilting under summer heat. The plants are significantly dwarfed (~20%) with pointed erect leaves. Unexpectedly, both anthers and silks are green. Crosses with (*bt1 a2*)^Hi27 also had green anthers and silks (Fig. 75).

***gt1*^Hi27 – grassy tiller.** Location: *gt1* = 1L-?. MGC 127I. Origin: MGC 66Cal-2327 x 2328 (Don Shaver's *gt1 id1* stock). Hawaii stock represents 7 BCs to Hi27 over 30 cycles of breeding. Shaver's *id1* gene was never recovered. Plants are tillered and husks are tipped with flag leaves, each trait expressed in relation to growth vigor. In Hawaii's winters *gt1*^Hi27 will have 4 to 8 tillers growing 3 to 5 feet tall and husk leaves will be from 4 inches to 8 inches in length. Mid-winter plants might not even show tillers, and identification must rely on appearance of husk leaves (Hi27 having none). The gene obviously affects totipotency of significant embryonic tissues in stalk and husk. We also discovered that all tested, temperate, American sweet and supersweet corn have the *gt1* gene (Brewbaker and Josue, MNL, 2008). See also *bm2 gt1*^Hi27 (Figs. 76-79).

***h1*^Hi27 – horny soft starch endosperm.** Location: *h1* = 3S-?. MGC 330A. Origin: MGC 61-2372 x W23/L317. Hawaii stock represents 6 BCs to Hi27 over 34 cycles of breeding. Kernels are floury with soft starch, and resemble *o2* and *fl2* in the Hi27 background. We've noticed increased mortality of seedlings in fusarium-rich soils but no increased incidence of kernel rots. Plants are extremely late to flower (by more than 7 days). Since the temperate parents would have in fact been much earlier (7 to 14 days) than Hi27, the late maturity might be due to the high susceptibility to fusarium. Plants are not unusually tall or high in leaf number. In our image, *h1* is compared with *ij1* NIL (Fig. 80).

***ij1*^Hi27 – iojap.** Location: *ij1* = 7L-82.78. MGC 711B. Origin: MGC 94-1615-1 * 1616-1 (*ij1* 711B). Stock was derived from 6 BCs to Hi27 during 17 cycles of breeding. Stock is dwarfed 25%, has pale green foliage, and is often a bit later to silk than Hi27. Expression is excellent (better in colder weather). It mimics expression in Mo17 background or *japonica-1* in Hi27, but allelism tests confirmed their distinctness. *Iojap* does not transfer maternally in this background (Figs. 81 & 82).

***in1 C1*^Hi27 – intensifier (of kernel color).** Location: *in1* = 7S-39. MGC 707GA. Origin: MGC 70-1151 self (a red-leaf stock with dark blue kernels). Hawaii stock represents 7 BCs to Hi27 over 42 cycles of breeding. Hi27 is *A1 A2 Cl-I R1 Pr1*, thus segregations involved *C1* and other loci. The leaf-color trait (see our *lc2* stocks) was not retained in conversions. Kernels are shiny, deep blue-purple on a white cob. Kernels on ears with red cobs (*p1-wr*) appeared even more intensely colored but have not been bred out. Plants are vigorous and tall with normal bronze anther color but red silk color, unlike our other *C1* stocks (with colorless silks) (Fig. 83).

***j1*^Hi27 – japonica 1**. Location: *j1* = 8L-122.0. MGC 810B. Origin: see *ms8*. Stock was derived from 7 BCs to Hi27 during 18 cycles of breeding. Leaf striping is very bold and is evident 3 weeks after planting, and it is always chosen for demonstrations or class use. Plants otherwise resemble Hi27 in vigor (Figs. 84-87).

***j2*^Hi27 – japonica 2**. Location: *j2* = 4L-123.0. MGC 415A. Origin: MGC 90-1948-1. Stock was derived from 6 BCs during 19 cycles of breeding. Expression is not as strong as *ij1*^Hi27 or *j1*^Hi27 and is suppressed during winter to marginal leaf stripes similar to *f1*^Hi27. Plants otherwise resemble Hi27 in vigor (Fig. 88).

***Kn1 p1-wr*^Hi27 – knotted leaves, red cob.** Location: *Kn1* = 1L-222.79, *p1-wr* = 1L-69.58. MGC 117E. Origin: MGC *Kn1* stock, 1967. Hawaii stock represents 7 BCs to Hi27 over 24 cycles of breeding. This stock was bred by simple backcrossing for the dominant gene. It has has reddish tassels, husks, and cob, but green silks. This suggests that it is a double mutant with the linked but distant *P1* locus, where allele *p1-wr* is associated with red cob, red anthers and green silks. In contrast, Hi27 has a white cob, bronze anthers, and reddish silks.

The leaves are seasonally variable in expression of the characteristic knots or roughness that mimics waves on a choppy sea. Plants are usually somewhat dwarfed, leaves are shortened, and tassel branches very erect (Figs. 89 & 90).

(*la1 fl2*)^Hi27 – lazy plant, floury 2 endosperm. Locations: *la1* = 4S-52, *fl2* = 4S-51.5. Origin: MGC 59-1320 x 1326 (*a1 la1 su1 Tu1 gl3* stock evidently carrying *fl2*). Hawaii stock represents 6 BCs to Hi27 over 38 cycles of breeding, which selected to remove *su1* from this subline (selfs of heterozygous *la1 fl2 su1 la1 fl2* + segregated 15 sugary in 1243 seeds). Lazy is variably expressed in Hi27, with plants sub-erect in summer seasons and almost prostrate in others. Excellent seed production in this subline (in contrast to stock (*la1 fl2 su1*)^Hi27) (Figs. 91 & 92).

(*la1 fl2 su1*)^Hi27 – lazy plant, floury 2 and sugary endosperm. Locations: *la1* = 4S-52, *fl2* = 4S-51.5, *su1* = 4S-63.55. MGC 405DA. Origin: MGC 59-1320 x 1326 (*a1 la1 su1 Tu1 gl3* stock evidently carrying *fl2*). Hawaii stock represents 6 BCs to Hi27 over 36 cycles of breeding. Plants are otherwise identical to (*la1 fl2*)^Hi27 stock (see images above). Sugary seeds from this subline are often rotted by fusarium. The sugary gene was isolated and is represented by our *su1* NIL.

***lc2*^Hi27 – leaf color.** Location: Unknown. Origin: HIC9 (Hawaii Composite 9), previously known as "Kalakoa" (Brewbaker JPR, 2009), a stock carrying many color genes including *p1-vv*. Stock involved 8 BCs to Hi27 over 32 cycles of breeding. Mutants appear similar to those described by discoverer Bob Bray in Brink's lab (1964). They are of a dominant leaf-color gene called *Lc1* that also came out of a tropical line (*R-r* Ecuador). *Lc1* (now listed as 10L-97.0) is closely linked to *R* (10L-95.2537). The *lc2* lines have always acted as fully recessive and segregations are Mendelian, and the trait is easily recognized within 3 weeks of planting. Possible involvement of *R1* alleles (*R1-ch1*) is implied. Plant heights are slightly reduced ~10%, but maturity and fertility are normal (Figs. 93-97).

***lc2 p1-rr*^Hi27 – leaf color, pericarp and cob color.** Location: *lc2* = Unknown, *p1-rr* = 10L-69.58. Origin: See *lc2*^Hi27 (a stock originally carrying *p1-vv*). The pericarp/cob color variant was identified belatedly and is presumed to be *p1-rr*. Pericarp, cob (glumes) and anthers are red in this stock, and the pith of the cob is strikingly colored. Cross-sectioned stems (including tassels) also show red veins, as noted by E.D. Styles for *p1-rr* (1988). Plants are otherwise similar to *lc2*^Hi27, and a sub-line *p1-wr* is being bred.

***lfl1*^Hi27 – leaf fleck.** Location: unknown. Origin: Tuxpeño derived *o2* germplasm in Thailand, 1968. Hawaii stock represents 6 BCs to Hi27 over 30 cycles of breeding. Mature leaves (> 6 weeks) show small chlorotic (not necrotic) spots originally thought to be infection by *Curvularia maydis*. Flecks are similar to lesions described for the dominant *Les8* mutant (9S-40) of Bird and Neuffer (1985), but *lfl1* is completely recessive. See also (*sky1 lfl1*)^Hi27. Flecking becomes very prominent by sweetcorn stage under summer sun (Figs. 98 & 99).

***lg1*^Hi27 – liguleless.** Location: *lg1* = 2S-11.75. MGC 205B. Origin: MGC 63-2370 x 2369 (chromosome 2 linkage stock *lg1 gl2 b1 fl1 v4*). Hawaii stock represents 7 BCs to Hi27 over 26 cycles of breeding. Liguleless expression is as observed by author R.A. Emerson (1912) and gives plants a very erect and leggy appearance. Plant height exceeds Hi27 by 10%, and tassels are poorly branched and occasionally single spiked. This gene is present also in our *gl2* stock (*gl2 lg1*)^Hi27, see above (Figs. 100 & 101).

***lg2*^Hi27 – liguleless 2.** Location: *lg2* = 3L-103.25. MGC 320A. Origin: MGC 63-2564 sib (chromosome 3 linkage stock *ra2 pm1 lg2*). Hawaii stock represents 6 BCs to Hi27 over 20 cycles of breeding. As noted by founder R.A. Brink, *lg2* is not as completely liguless as is *lg1* and often is a bit difficult to identify until flowering apaproaches. Plants are somewhat taller than Hi27, tassels are normal (unlike *lg1*), and ears are occasionally "tassel-tipped." Early evidence that this stock carried recessive allele *mv1* of the locus *Mv1* (resistance to MMV, maize mosaic virus, homozygous in Hi27) led to identification by Ray Ming et al (1997) of the location of *Mv1* on chromosome 3 (~ 3L-78.0). This stock is now homozygous *Mv1/Mv1* (Figs. 102 & 103).

***Lg3*^Hi27 – liguleless 3.** Location: *Lg3* = 3S-57.64. MGC 312D. Origin: MGC *Lg3* stock (1967). Hawaii stock represents 7 BCs to Hi27 over 22 cycles of breeding. Liguleless is fully dominant and plants appear very erect and compact. Plant height is seasonally variable, very dwarfed in cooler seasons, and anthers are red (Hi27 is bronze). This mutant is homozygous *Mv1/Mv1* (cf. *lg2*^Hi27, *pm1*^Hi27, *ts4*^Hi27) but highly susceptible to seedling mortality by fusarium (Fig. 104).

(*li1 du1*)^Hi27 – lineate, dull. Locations: *li1* = 10L-62.0, *du1* = 10L-46.79. MGC X08F. Origin: MGC 86H-686-1. Hawaii stock represents 7 BCs to Hi27 over 29 cycles of breeding. As noted by authors Collins and Kempton (1920) this gene creates fine chlorotic stripes above the veins. They are quite similar to striping by the Maize Mosaic Virus that is so common in Hawaii. Expression can be seasonally variable, severe enough in winter to reduce plant and ear size and lead to partial infertility. In summer trials the *li1* stock is very tall (20% above Hi27, to 10 ft height), attenuated, and has narrow leaves. Kernels are dull-colored flints (not quite floury or starchy) and assumed to be caused by linked locus *du1*. Ears tend to lack clear rows. See also double mutant *g1* (Figs. 105-107).

***lw1*^Hi27 3:1 – lemon white**. Location: *lw1* = 1L-224.0. MGC 118C. Origin: Maize Coop received in 1967 but undocumented, though it must trace to Don Robertson. Hawaii stock represents 6 BCs to Hi27 over 18

cycles of breeding. Mutant is typical in appearance with lemon-yellow kernels with no vivipary that produce pure-white (lethal) seedlings. Hi27 kernels are flinty, dark yellow and noted for high beta-carotenoids. The *lw1* mutant is excellent for displays of carotenoid protection of chlorophyll. Our NIL is somewhat variable in maturity and in tassel color (bronze and colorless) (Fig. 108).

***ms6*^Hi27 (cf. *po1*^Hi27)**

(*ms8* 1:1 *j1*)^Hi27 – male-sterile 8, japonica 1 stripe. Locations: *ms8* = 8L-113, *j1* = 8L-122. MGC 805G. Origin: MGC 64-306/10 x 305/5, a chromosome 8 stock noted to be *v16 j1 ms8* (1:1) *gl1* (1:1). Hawaii stock was derived from 6 BCs to Hi27. This was followed by sibbing to retain a stock homozygous for *japonica-1* but segregating *ms8* over 32 cycles of breeding. Gene *v16* (8L-?) segregated but was lost during conversions. Gene *ms8* confers complete suppression of anthesis in this stock, though vigor is somewhat reduced due to *j1* (Figs. 109 & 110).

***ms10*^Hi27 1:1 – male-sterile 10**. Location: *ms10* = 10L-66.0. MGC X09F. Origin: MGC 71-1044 x 1059 in background of inbred N28. Stock was derived from 6 BCs to Hi27 over the course of 21 cycles of breeding. It shows considerable heterosis for ear length, and tassels have red anthers. Mutants are fully male sterile and have slightly reduced plant height. (Fig. 111).

ms1-si (see *si1*).

***na1*^Hi27 3:1 – nana dwarf.** Location: 3L-120.0. MGC 318G. Origin: MGC 65-253 x 252. Hawaii stock represents 7 BCs to Hi27 over 25 cycles of breeding. Mutant plants are dwarfed < 2 feet and are erect with broad, short leaves. The mutant is delayed over an additional week to silk but can produce tassels and tiny ears with abortive kernels (Figs. 112-114).

***na2*^Hi27 3:1 – nana dwarf 2.** Location: 5S-57.0. MGC 518C. Origin: MGC 61-1988 x 1985. Hawaii stock represents 7 BCs to Hi27 over 25 cycles of breeding. Mutant plants are dwarfed < 2 feet and are erect with broad, short leaves. The mutant is delayed 1+ week to silk but can produce tassels and tiny ears with abortive kernels (Figs. 115 & 116).

***nl3*^Hi27 – narrow leaf.** Location: 1S-?. Origin: (*nl3 p1-rw*)^Hi27 pedigree, BC8 selfed, 2003. Gene clearly linked to *P1* locus; cf. stock (*nl3 p1-rw*)^Hi27. Plants are normal in height and vigor. Leaves are ~12% narrower than Hi27, and measure 48 mm at one month (versus 55 for Hi27) and 77 mm at tasseling (versus 88 for Hi27). Vein numbers are identical, 8 on each side of leaf at one month. Young plants and tassels appear more erect. Height, maturity, and vigor are comparable to Hi27. Cob is white the anthers are red, as in *p1-wr* stocks (Fig. 117).

(*nl3 p1-rw*)^Hi27 – narrow leaf, pericarp color. Location: *p1* = 1S-69.58, *nl3* 1S-?. Origin: MGC 63-2656 sib (*A1 C1 R1 Dt1 p1-rr*). Hawaii stock represents 8 BCs to Hi27, and uses only red-pericarp female parents over 28 cycles of breeding. Plants are normal in vigor, height, maturity, and color, but the narrow leaf trait (cf. stock *nl3*^Hi27) became clear as homozygous lines were established for the (dominant) *p1-rw* allele. Cobs are white, pericarps are red, and anthers are also red.

(*o1 c2*)^Hi27 – opaque endosperm, colorless. Locations: *o1* = 4L-115, *c2* = 4L-133.01. MGC 418FA. Origin: MGC 63-2154 sib. Hawaii stock represents 7 BCs to Hi27 over 26 cycles of breeding. From BC4 it was evident that the opaque selection was carrying linked locus *c2*, making mutant plants colorless (green plant, silk, anther). Plants tend to be heterotic and prolific in vigor but normal in height and maturity. Kernels are not unusually susceptible to fusarium kernel rot (cf. *o2*).

***o2*^Hi27 – opaque 2 endosperm.** Location: *o2* = 7S-30.6. MGC 701D. Origin: MGC 64-258 x 267 (a stock with *o2 v5 ra1* and *gl1*). Hawaii stock represents 7 BCs to Hi27 over 34 cycles of breeding. The *v5* was not eliminated until BC5 (cf. stock (*v5 o2*)^Hi27). Plants tend to be very vigorous but normal in height and maturity. Ears (cobs) are unusually large. Kernels and young seedlings are much more susceptible to fusarium rot, as they have been in all backgrounds we've studied (Fig. 118).

***o2*^Hi27 (Tuxp) – opaque 2 endosperm.** Location: *o2* = 7S-30.6. MGC 704B. Origin: An *opaque2* conversion of Mexican race Tuxpeño that I crossed in 1968 with a white opaque stock 650 of Rockefeller Foundation in Thailand. Hawaii stock represents 7 BCs to Hi27 over 31 cycles of breeding. Allelism with *opaque2* was verified. This is a very pale-colored, denty type of kernel that was suspected to carry genes such as *y1, y8* or *y11*, but testcrosses disproved each. Like other opaques, it is quite susceptible to fusarium, and germination and emergence in Hawaii's fusarium-rich soils is often very poor. It is also notably more susceptible to bacterial leaf blight and seems to have broader leaves and floppy tassels (*flta1*?).

(*o5 gl1*)^Hi27 3:1 – opaque 5 endosperm, glossy seedling. Location: *o5* = 7L-64, *gl1* = 7L-66. Origin: MGC 91-2340 (Stock 710E *o5 gl1*). Hawaii stock represents 6 BCs to Hi27 over 12 cycles of breeding (largely paired self and backcross). Seeds and seedlings generally resemble those described by discoverer Don Robertson (1967). Don recorded seeds to be opaque and pale yellow and often collapsed, and the seedlings to be virescent and often white, as characteristic of carotenoid involvement (cf. stock *lw1*^Hi27). In this background of hard-flint Hi27, the *o5/o5* kernels are best described as "white-capped," not always wholly opaque to transmitted light (cf. our stock of *os1*^Hi27). The kernels are very pale in color. They are very prone to fusarium rot and often fail to germinate in Hawaii, and generally produce yellow-green seedlings. Don's extensive work on this type of mutant started when he studied with E.G. Anderson at Cal Tech. I spent a wonderful summer in 1948 with him and people such as Ed Coe, Earl Patterson, Charlie Burnham, and Edgar Anderson in E.G. Ander-

son's "Bikini-bomb" corn fields.

***Og1*^Hi27 – old gold plant color.** Location: *Og1* = 10S-42. MGC X03B. Origin: MGC *Og1* stock crossed with an ABPl stock (1967). This Hawaii stock represents 8 BCs to Hi27 over 29 cycles of breeding. Expression is similar to that recorded by discoverer E.W. Lindstrom (1935), with leaf blades having variously-broadened yellowish stripes that can be highly variable and occasionally difficult to see. Plants are normal in vigor, height, maturity, and color (Fig. 120).

***Og1 B1*^Hi27 – old gold and booster plant color.** Locations: *Og1* = 10S-42, *B1* = 2S-49.3. Origin: MGC *Og1* stock crossed with an ABPl stock (1967). This Hawaii stock represents 8 BCs to Hi27 over 32 cycles of breeding, obviously selected with a horticulturist's eye. Old gold expression is striking against the deep purple color of stems, sheaths, husks, and cobs. Plants are often attenuated slightly in height as in all of our *B1* stocks and tend to be a bit late in maturity and color. Tassels are compact and erect but with branch number similar to Hi27. As in all of our *B1* lines, the anthers and cobs are red but silks are colorless (Fig. 121).

***os1*^Hi27 1:0, 3:1 – opaque crown.** Location: *os1* = 2S. MGC 220F. Origin: Xiao Wu Lu, doctoral student (1992-1996) obtained this stock evidently from discoverers C. Soave and F. Salamini (1981). The authors also called it "opaque endosperm small germ", thus "o" plus "s". The Hawaii stock represents 6 BCs to Hi27 over 18 cycles of breeding. Testcrosses excluded its identity with *o1* or *o2*, but not *o5*. Plants are normal in vigor, height, maturity, and color. The *os1* kernels actually appear like dent segregants among normal Hi27 flints and have a soft-starch crown that can dent slightly. The germ does not seem small, and germination and seedling development is normal. Unlike our floury versions of Hi27 (especially *o2*, *o5*, and *fl2*) this stock doesn't seem prone to fusarium kernel rots. In this hard flint background it is tempting to call this gene "white cap" but unlike *Wc1* (*white cap 1*) it is fully recessive (Figs. 122 & 123).

***oy1*^Hi27 1:0, 3:1 – oil yellow plant color.** Location: *oy1* = 10S-33.76. MGC X01A. Origin: MGC 69-372 self. Hawaii stock represents 7 BCs to Hi27 over 23 cycles of breeding. Expression is similar to that recorded by discoverer E.G. Anderson (1951), with seedlings that are very weak and yellowish. They become increasingly green later and can be sibbed in Hawaii's summer months. Flowering is delayed 7 to 10 days, leaves are variously narrowed, plants a bit dwarfed in height, and obviously struggling for energy. Tassel branching is reduced about 50% (to 4-5 branches). Stinard (MNL 83:52) provides evidence that this gene and *Og1* might in fact be allelic (Fig. 124).

***p1-rw*^Hi27 – pericarp color.** Location: *p1* = 1S-69.58. Origin: This line segregated from the *dbcb1 p1-rw* stock, which traces to *p1-vv*^Hi27 (and highly mutable). This represents at least a dozen BCs to Hi27 over > 23 cycles of breeding. Plants are normal in vigor, height, and maturity. Pericarps are red, cobs white, and anthers also white (unlike our *p1-rr* and *p1-wr* stocks). White anthers (versus bronze in Hi27) seem to occur in all our *p1-rw* stocks; e.g., (*dbcb1 p1-rw*) (Fig. 125).

***p1-vv*^Hi27 – pericarp cob color.** Location: *p1* = 1S-69.58. MGC 107F. Origin: Derived from a 1969 MGC *p1-vv* 1:1 stock (field data not recorded). Hawaii stock involved 10 BCs to Hi27 over 29 cycles of breeding. Homozygotes have presented immense variability that is evidently to be expected from this *Ac*-containing mutant. Variability has been seen in vigor, height, width of leaf width, striping of leaves, and shape of cob. Ears are commonly weak and segregate defective endosperms and there is a wide range in variegation of pericarp. Cobs are white, silks are red, and anthers bronze, as in Hi27. Mutants for double cob (*dbcb1*^Hi27) and narrow leaf (*nl3*^Hi27) arose from this germplasm, and a japonica-like stripe also has appeared (Figs. 126 & 127).

***p1-wr*^Hi27 – cob color.** Location: *p1* = 1S-69.58. MGC 107I. Origin: Segregated in Hi27 (which has bronze anthers) in 1991 as red anthers (91-1314 sib). Two additional BCs were made to Hi27 over 13 cycles of breeding. Plants are normal in vigor, height, leaf width, and maturity, but uniquely red-anthered (versus bronze in Hi27 and colorless in *p1-rw*). Homozygotes revealed that the red anthers were always associated with the reddish soft floral parts of the cob typical of allele *p1-wr*. This stock has red glumes and outer tissues of cob that are apparent at late sweet-corn stage. However, unlike l*c2 p1-rr*^Hi27 (that has red pith), the pith of both cob and stalk in *p1-wr* is white. The allele *p1-wr* is also found in stocks *blo1, dbcb1, el1*, and *Og1 B1*, not to mention many Corn Belt dents (Figs. 128-130).

***pm1*^Hi27 – pale midrib.** Location: *pm1* = 3L-77. MGC 307C. Origin: MGC 74-545 self (a stock with *cr1 ts4* and *pm1*). Stock involved 7 BCs to Hi27 over 35 cycles of breeding. As noted by discoverer R.A. Brink the *pm1* plants are dwarfed (20%) and have broadened and chlorotic midbribs at base of leaves. They are very delayed (up to 15 days) to flower and the ears are small ears and kernels are small and irregular. This mutant rarely germinates well and appears to be unusually susceptible to fusarium as a seedling rot. The gene is located in the region of *Mv1* locus (~3L-78), and my stock might be segregating susceptibility (Figs. 131 & 132).

***Pn1*^Hi27 1:1, 3:1 – papyrescent glumes.** Location: *Pn1* = 7L-?. MGC 714A. Origin: MGC 69-617Pn x M14/W23. Hawaii stock involved 10 BCs to Hi27 over 20 cycles of breeding. As recorded by discoverer Walt Galinat, his *Pn1* heterozygotes had enlarged, papery glumes covering the kernels and enlarged glumes in tassel. In Hi27 background the *Pn1* tassels are indistinguishable from Hi27 and long glumes on ears are usually evident only at the base. *Pn1*/+ plants tend to be delayed a few days in silking but are otherwise similar to Hi27. In 3:1

progenies the homozygotes are silkless and the stock is best maintained as a 1:1 (Figs. 133 & 134).

***po1-ms6*^Hi27 1:1 – polymitotic male-sterile**. Location: *po1* = 6S-17. MGC 612BA. Origin: MGC 89-1697 x 1696, received as *ms6*. Stock was derived from 6 BCs to Hi27 over 17 cycles of breeding. Another stock was developed from the same origin and labeled *ms1* incorrectly; after 6 BCs during 16 cycles of breeding it was crossed with the *ms6*^Hi27 and they proved to be identical and were pooled and correctly labeled *po1-ms6*^Hi27. Expression is similar to that of fellow Cornellian George Beadle's original description (1932) of polymitotic, with complete male sterility and partial female sterility. Plants are of normal height and vigor. I cherish memories of teosinte-like corn seedstocks shared with me by George even after he became President of the University of Chicago (Fig. 135).

***py1*^Hi27 1:0, 1:1 – pygmy plant.** Location: *py1* = 6L-77.75. MGC 612I (as "tan-py") Origin: MGC 61-2004 x 1998 (a stock with *py1 p1-rr* and *sm1*). Hawaii stock involved 7 BCs to Hi27 over 25 cycles of breeding. Plants are dwarfed to about 40% of normal and leaves are narrow and streaked. Anthers are red (unlike Hi27, bronze, but it is clearly also *p1-ww*). Plants are delayed about a week to flower and have high anthesis-silk interval. Homozygous seed is best obtained in summer under ideal growth conditions. Ears of *py1* homozygotes are very small (2") but seed quality is normal. This is probably the *py1-tan1* ("tangled") allele that alters growth patterns throughout the plant, but I respect the original name given by A.D. Suttle in 1924 (a Cornellian). (Figs. 137, 138).

***R1 C1*^Hi27 – blue kernels.** Locations: *R1* = 10L-95.25, *C1* = 9S-16.18. MGC X17E. Origin: MGC stock *a1 A2 C1 R1 Dt1* (field number not recorded), received in 1967. Stock was derived from 6 BCs to Hi27 over 40 cycles of breeding. This pure line is characterized by mottled blue kernels distinct from the "clean blue" kernels of our *C1*^Hi27 stock. I assume the *R1* allele here to be of different origin than that of Hi27 (which is *Cl-I R1*). During breeding we recorded high kernel diversity (marbling, mottling) suggesting presence of mutators. Plant height is reduced ~20% (cf. *R1-nj C1*), anthers are red, and silks are green (as noted in most *C1* stocks).

***R1 B1 C1*^Hi27 – blue kernels, purple plant.** Locations: *R1* = 10L-95.25, *B1* = 2S-49.3, *C1* = 9S-16.18. MGC M341B. Origin: See *R1 C1*^Hi27, which was segregating "sun-red" stalks early in the conversions to *C1* and *R1*. In cycle 35 of breeding it segregated *A1 B1 Pl1* plants with purple stalks and husks and typically somewhat elongated thin stems. The pure line is also characterized by mottled blue kernels.

***R1-nj C1*^Hi27 – navajo-red kernels**. Locations: *R1* = 10L-95.25, *C1* = 9S-16. MGC X17D. Origin: MGC stock Trisome 10. Stock was derived from7 BCs to Hi27 over 30 cycles of breeding. Kernels of the pure line are typical in appearance, capped with crowns of red-purple aleurone (purplish in this background). Our stock has always been dwarfed ~20% compared with Hi27 or other stocks, as noted also for our *R1 C1* stock (above), which implies a linked QTL for plant height. (Figs. 139 & 140).

***R1-nj y1 C1*^Hi27 – navajo-red kernels, white endosperm**. Locations: *R1* = 10L-95.25, *y1* = 6S-30, *C1* = 9S-16. Origin: Hybrids of Hi27 NILs *R1-nj C1* (above) and *y1*. Stock was derived from 3 cycles of breeding. Kernels are attractively white with purple crowns.

***ra2*^Hi27 – ramosa 2 tassel.** Location: 3S-32.76. MGC 308E. Origin: MGC 63-2564 sib (a stock with *lg2*, *pm1* and *ra2*). Stocks represent 6 BCs over 24 cycles of breeding. Tassel expression is typical with many (30+) erect and tightly-clustered, largely-secondary branches that are greatly shortened. Ears are often tipped with a miniature tassel and grain yields are poor with missing kernels and irregular kernel placement. Plants tend to be tall (8.5 ft., 15% above Hi27) and attenuated and ears are high (4 ft.) on stalk. Anthers are red (Hi27 has bronze anthers). (Figs. 141-143).

***rf4*^Hi27 (*cms1 C*) – fertility restorer for *C* cytoplasm.** Location: 8S-4.0. MGC Z031G. Origin: *Wf9* (C cytoplasm), BC8. Hawaii stock represents 6 BCs over 24 cycles of breeding. It is fully male sterile, completely restored by allele *Rf4*, and otherwise normal in appearance and yield. (Fig. 144).

***Rf4*^Hi27 (*cms1 C*) – fertility restorer for *C* cytoplasm**. Location: 8S-4.0. Origin; Fertility segregated in a hybrid of *rf4*^Hi27 and *lg1*^Hi27 (on 4S) in a study of NIL heterosis (MNL 81:17, 2007). Homozgyous *Rf4* line was derived by selfing or sibbing 5 cycles and eliminating *lg1* gene, with appropriate testcrossing. Two stocks are maintained, both with small tassels and small ears on small plants; one tends to vivipary, i.e., both are strangely distinguishable from Hi27.

***Rg1*^Hi27 1:1 – ragged plant.** Location: 3S-69.0. MGC315C. Origin: MGC *Rg1 Tp1* stock (1967). Hawaii stock represents 15 BCs over 21 cycles of breeding. Expression is typical of that described by discoverer R.A. Brink (1931), with plants dwarfed ~25%. Leaves are severely slashed, shortened and narrower, and tend to be parallel to the ground. Expression is extreme in cooler weather. Homozyous *Rg1/Rg1* plants are even more gross and sterile. (Figs. 145-147).

***sh1 wx1 flta1*^Hi27 – shrunken waxy kernels, floppy tassel.** Locations: *sh1* 9S-20.08, *wx1* 9S-47.93, *flta1* = 9S. MGC 909D. Origin: MGC 63-2696 sib (*sh1 wx1* segregating *C1*). Hawaii stock represents 6 BCs over 27 cycles of breeding. It is similar to other NILs of this region of chromosome 9 in having the floppy-tassel gene. It has occasionally segregated "square-stalk," with opposite leaves and ears. Nice ears and kernels are well-filled and basically look like a dent in the Hi27 (flint) background.

(*sh2 a1*)^Hi27 – shrunken 2. Location: *sh2* and *a1* = 3L-174.75. MGC 333D. Origin: MGC 63-2665-6/2666-2,

a stock noted to be *a1 sh2* with *B1, C1, Dt1*, *Pl1* and *R1* (presumed to have reference allele *sh2-R*). Stock was derived from 6 BCs to Hi27 over the course of 27 cycles of breeding. No segregants were verified to have the two loci separated. This is an unusually tall NIL (linked gene?) that is highly susceptible to fusarium kernel and seedling rot (pleiotropic effect). In the Hi27 background it is a very green plant with colorless silks, anthers, and tassels. (Fig. 148).

(*sh2 a1*) *sl1*^Hi27 – shrunken 2, slashed leaves. Locations: *sh2* and *a1* = 3L-174.75, *sl1* = 7L-84.0. MGC 705BB. Origin: MGC 69-612-2 selfed, a stock with *o2*, *gl1*, and *sl1* (slashed, a gene my father Harvey discovered); the stock was recorded not to be segregating *ra1* or *v5* that are presumed to have been in its pedigree. In progenies from my first BC to Hi27, the slashed plants in 1973 segregated wrinkled kernels. These were recovered in BC3 in 1990 and 4 subsequent BCs were followed by selection for the uniquely green plants (*a1*) and associated *sh2* seeds. Breeding involved a total of 28 cycles. Expression of the slashed leaves is environmentally sensitive with gross slashes in cool winters and minor slits in summer. Like all *sh2* stocks, it has trouble with fusarium. See Figure 149 for *sl1*^Hi27.

(*sh2 a1*) *y1*^Hi27 – shrunken 2, anthocyaninless, white. Location: *sh2* and *a1* = 3L-174.75; *y1* = 6S-30.13. Origin: Hybrid of (*sh1 a1*)^Hi27 x y1^Hi27, followed by 3 cycles of selfing and sibbing. Unlike the *sh2* parent, this is normal in height. Seeds germinate poorly due to fusarium seedling rot. It is a green plant with colorless silks, anthers, and tassels. Kernels are a "dirty white," unlike (*bt1 a2*) *y1*^Hi27, that is definitely bronze-kerneled.

***si1*^Hi27 1:1, 3:1 – silky ear.** Location: 6S-69.0. MGC 604F (as "*ms-si*"). Origin: MGC 64-13 x 10, referred to as *ms-si* ("male-sterile silky"). Hawaii stocks represent 6 BCs over 29 cycles of breeding. Silky mutants are highly male sterile in summer and essentially impossible to self. Ear expression is inconsistent with ears variably silky. Double-cobs have been common, along with other abnormalities. (Fig. 150).

(*sk1 fl1*)^Hi27 1:1 – silkless ear, floury kernels. Locations: *sk1* = 2S-57.0, *fl1* = 2S-75.7. MGC 214JA. Origin: MGC 64-2472 x 2465 (*lg1 gl2 b1 v4* segregating *sk1* and *fl1*). Hawaii stock represents 6 BCs over 25 cycles of breeding. Stock is similar to those described by D.F. Jones (1925) with no silks on ears. Floury kernels are typical in phenotype. Tassels are spikes with a single branch. (Figs. 151-153).

***sky1 lfl1*^Hi27 – skinny plant, leaf-flecked leaves.** Locations: Not known. Origin: MGC 74-545-2 self (a stock segregating *ts4*, *pm1* and *lg1*). Hawaii stock represents 7 BCs over 25 cycles of breeding. The genes *lg1*, *pm1* and *ts4* were eliminated early in backcrossing. The *sky1* NIL is characterized by normal ligules but thin stalks of normal height. The *sky1* plants have a somewhat more erect leaf habit. It is a double mutant with *lfl1*, heavily-flecked leaves but seasonal in expression. (Fig. 154).

***sl1*^Hi27 – slashed leaves.** Location: 7L-84.0. MGC 705BA. Origin: MGC 69-612 self (a stock carrying *o2* and *gl1*). Hawaii stock represents 7 BCs over 22 cycles of breeding. It is similar to plants described by my father (Hayes and Brewbaker, Am Nat 62, 1928) with slashes that enlarge with leaf expansion. However, leaves are generally normal in length and width. Plants are of normal height but can be reduced in our low-light winter season. (Fig. 155).

***sr1*^Hi27 – striate leaves.** Location: 1S-39.25. MGC 101C. Origin: MGC 65-201 sib. Hawaii stock represents 8 BCs over 26 cycles of breeding. The mutant is strikingly striped with both white and pale-green stripes, as reported by discoverer Art Brunson (with whom I later worked in Philippines 1954). Often identifiable in 15 days. Our NIL also shows early virescence and is delayed at least a week to flowering, but is normal in plant height. Sub-lines occur with red and with colorless anthers and with red cobs (*p1-wr*). (Figs. 156-159).

***su1*^Hi27 – sugary endosperm.** Location: 4S-63.55. MGC 407D. Origin: UH hybrid H68, a hybrid of Purdue's P39 and my AA8 (from Hawaiian Sugar). My stock represents 7 BCs over 27 cycles of breeding. Otherwise resembling Hi27 it tends to segregate poor tassels and bear kernels that are variously wrinkled and "pseudo-starchy." Also see (*la1 fl2 su1*)^Hi27, that also has pseudo-starchy kernels (backed by *fl2*). (Fig. 160).

***su1 lfl1*^Hi27 (*Tu1 JLB1*) – sugary endosperm, leaf fleck.** Locations: *su1* = 4S-63.55, *lfl1* = ?. MGC 411AA. Origin: Segregated among selfs from 5th backcross of a Tunicate stock (*Tu1* at 4S-118) from MGC 61F-607 x 801. Hawaii stock represents 3 additional BCs over 20 additional cycles of breeding. Validated to be *su1*, this stock has unusually pseudostarchy kernels and other unique traits. It has the leaf-freckle (cf. *lfl1*^Hi27) that is different from reported *les* (lesion-mimic) mutants. It is weakly rooted and often leans in the field. This also occurs in tropical varieties like Hawaiian Sugar that segregate plants resembling asr (absence of seminar roots). Plants often show fuzzy sheath similar to Tavcar's "hairy sheath." Tasssels have excessive number of branches (22). This host of traits confirms that many genes (and mutators) are often carried under the cloak of dominant mutants like Tunicate. While our *Tu1*^Hi27 1:1 stock is quite normal, the homozygous *Tu1/Tu1* is grossly vegetative and basically sterile. This linkage drag eliminates selfs that might reveal linked recessive alleles like *su1* or *la1* that were in the original MGC stock. See also our *la1 fl2 su1* and *Tu1* stocks. (Fig. 161).

tan1 (see *py1*^Hi27).

***tb1*^Hi27 1:1 – teosinte-branched.** Location: *tb1* = 1L-220.7. Origin: MGC 117D. Hawaii's NIL is a "work in progress" that now involves 4 BC cycles. In this background the gene is semi-dominant with heterozygotes

showing no tillers but long flag leaves that are very similar to *gt1* homozygotes (although the *gt1/gt1* plants produce tillers also). The *tb1* homozygotes appear to be female sterile, highly tillering and with long flag leaves. Tests are in progress for allelism with *gt1* and with *tlr1*. (Figs. 162 & 163).

***Tlr1*^Hi27 1:2:1 – tillering.** Location: *Tlr1* = 1L-?. Origin: MGC 95-212 self. Hawaii's NIL involved 6 BCs to Hi27 over 11 cycles of breeding. Mutants appear much as noted by (abundant-gene discoverer) Gerry Neuffer and colleagues (1987). The gene appears to be variably co-dominant with the heterozygotes producing no tillers but having long flag leaves and normal ears. However, the homozygous *tlr1/tlr1* plants are grossly vegetative (similar to *tb1*) with long flag leaves, tassel-tipped or tassel-replaced ears (often with no kernels), and spike-like tassels. Suspected to be allelic or close to *tb1* and *gt1* loci. (Figs. 164-167).

***Tp1*^Hi27 1:1, 3:1 – teopod.** Location: 7L-76. MGC 711A. Origin; Both sublines came from MGC 61F-607x807-1. The 1:1 stock is in BC11 after 18 cycles of breeding and the 3:1 is in BC9. Heterozygotes are identical in the two stocks, with unbranched tassels, prolificacy, large ears and the characteristic long glumes especially around basal kernels similar to those described by discoverer E.W. Lindstrom in 1925. Homozogyous *Tp1/Tp1* plants have no tassels and are highly tillered, grassy, and very prolific with long husk covers. (Figs. 168-170).

***ts2*^Hi27 1:1, 3:1 – tassel seed 2.** Location: *ts2* = 1S-69.52. MGC 106BA. Origin: MGC 60-1019 sib (a stock carrying chromosome 1 genes *br1, f1* and *bm2*). Stock involved 6 BCs to Hi27 over 25 cycles of breeding, eliminating the linked loci. Mutants appear much as noted by discoverer R.A. Emerson (1920), with tassels elongate and seedy. When tassels are removed the ears can be pollinated but are shoepeg and poorly seedy, so no attempt was made to create a pure line. Plant height is reduced ~25%, and tassels attract common smut (that is rare in Hawaii). (Figs. 171 & 172).

***ts4*^Hi27 3:1 – tassel seed 4.** Location: *ts4* = 3L-78. MGC 308A. Origin: MGC 59-1148 sib (a stock carrying chromosome 3 genes *cr1* and *na1*). Stock involved 7 BCs to Hi27 over 23 cycles of breeding, eliminating the linked loci. Mutants appear much as noted by discoverer L.F. Phipps (1928), with very compact tassels having pistillate and staminate flowers. When tassels are removed the ears can be pollinated but are shoepeg with secondary florets and are poorly seedy, so a 1:1 line was challenging and no attempt was made to create a homozygous line. Plant heights were slightly reduced, and the stock is unusually tall and late to maturity. It is probable that this stock is a double mutant with recessive allele mv for susceptibility to Maize Mosaic Virus (locus also suspected to be 3L-78), since Hi27 and all other NILs were bred to be homozygous *Mv1*. (Fig. 173).

***Ts5*^Hi27 1:1, 3:1 – tassel seed 5.** Location: *Ts5* = 4S-48. MGC 402D. Origin: MGC 63-2781 x 2790 (a stock carrying chromosome 4 gene *su1*). Stock involved 10 BCs to Hi27 over 24 cycles of breeding, eliminating linked sugary locus. Mutants appear identical to those of discoverer R.A. Emerson (1932), with large tassels sparsely silky on heterozygotes. Ears also have irregular rows with secondary florets and are poorly seedy. It's possible a pure line could be created, since mutant plant height of homozygotes is reduced only ~10%, although silkiness of tassels is exaggerated greatly. (Figs. 174 & 175).

***Ts6*^Hi27 1:1 – tassel seed 6.** Location: *Ts6* = 1L-260.04. MGC 119E. Origin: MGC 90-893 x 888. Stock involved 9 BCs to Hi27 over 12 cycles of breeding. Mutant heterozygotes appear identical to those of discoverer N.H. Nickerson (1955), with extremely compressed tassels that are sparsely silky or seedy. Ears also have irregular rows with secondary florets and are poorly seedy. Plant heights of heterozgyotes are greatly reduced ~30%. (Figs. 176-181).

***Tu1*^Hi27 1:1, 3:1 – tunicate.** Location: 4L-118. MGC 416A. Both 1:1 and 3:1 stocks have as origin MGC 59-1320-22 x 1326-3, a stock having *Tu1* together with *la1* and *su1* (short arm of 4) and *gl3* (4L-138). The 1:1 stock is in BC15 after 19 cycles of selection. Heterozygotes are classical in appearance and have ears with coarse glumes covering all kernels, a natural showpiece at our field days. Tassels have long glumes and condensed-appearing central spike, usually but not always easily distinguished. Homozygotes are dwarfed with no ears but a single complex branched tassel with long glumes and occasional kernels. See also *su1 lfl1*^Hi27 (*Tu1 JLB1*), a sugary line with freckled leaves, and our lazy stocks also having this genetic origin. (Figs. 182-184).

***v4 dbcb1*^Hi27 – virescent 4 plant, double-cob.** Locations: *v4* = 2L-87, *dbcb1* = 1?. Origin: 63-2370 x 2369 (chromosome 2 stock with linked *lg1 gl2 b1 fl1* and *v4*). This normal-flint stock was extracted from the (*v4 fl1*)^Hi27 in 2009. It is notable for strong brace roots, and segregates dwarfs, and was notably double-cobbed. This stock does not have the highly branched tassels (*Brta1*) observed in other NILs, e.g. (*fl1 brta1 v4*)^Hi27. See images in (*v4 fl1*) below.

(*v4 fl1*)^Hi27 – virescent 4 plant, floury endosperm. Locations: *v4* = 2L-87, *fl1* = 2L75.7. MGC 215G. Origin: 63-2370 x 2369 (chromosome 2 stock with linked *lg1 gl2 b1 fl1* and *v4*). Stock involved 6 BCs to Hi27 over 29 cycles of breeding. Mutants resemble those described by discoverer Milislav Demerec (1924) and are pale green almost immediately on emergence, and symptoms persist up until flowering. Unlike *opaque2* seeds (e.g., *v5 o2*) the *floury1* kernels are less subject to rot by fusarium. This stock does not have the highly branched tassels (*brta1*) observed in related NILs such

as (*fl1 brta1 v4*)^Hi27, derived from the same source. (Figs. 185 & 186).

(*v5 o2*)^Hi27 – virescent 5 plant, opaque 2 endosperm. Locations: *v5* = 7S-37, 7S-30.6. MGC 703AA. Origin: 64-258 x 267 (stock with linked *o2, v5, ra1* and *gl1*). Stock involved 8 BCs to Hi27 over 34 cycles of breeding. Mutants resemble those described by discoverer Milislav Demerec (1924), whom I mistakenly asked at Cold Spring Harbor in 1950 about the progress of his corn research (he'd done none since grad school). As in other stocks the *opaque2* seeds are always more subject to rot by fusarium, and dry kernels often have a brownish crown that appears to be rot-induced. Young seedlings are pale yellow. (Figs. 187-189).

***Vg1*^Hi27 1:1, 3:1 – vestigial glumes.** Location: *Vg1* = 1L-173. MGC 114D. Origin: MGC 74-550, from an innovative Walt Galinat, who tried to incorporate this into sweet corn breeding. The stock is in BC13 after 19 cycles of selection. Heterozygotes are classical in appearance as described by discoverer George Sprague (1932). They have very short glumes in tassel and cob and are a showpiece at our field days. Heterozygotes produce some pollen in good seasons, but homozygotes appear entirely sterile. Heterozygotes are dwarfed ~15%, but otherwise similar to Hi27. (Figs. 190-192).

***vp5*^Hi27 3:1 – viviparous kernels.** Location: *vp5* = 1S-40.25. MGC 103D. Origin: MGC 65-503 self. Hawaii stock is in BC15 after 19 cycles of selection. This stock is classical in appearance (Don Robertson, 1952) with 1/4 segregating carotenoid-deficient pale yellow kernels and albino seedlings. Heterozygous plants are normal in maturity and height, but have red anthers; ears segregate whitish kernels that might become viviparous late in maturity. (Fig. 193).

***w3-y11*^Hi27** – Location: 2L-122. Origin: MGC 96-6422 self. This was described by breeder George Sprague (1987) as a mutant with pale yellow kernels and green seedlings, and our stock mimics his description. The Hawaii stock is in BC6 (paired self and BC) after 11 cycles of selection and resembles Hi27 in appearance. Recent studies at the Maize Coop have shown this to be an allele of the locus *w3*, originally described by M. Demerec (1923) and E.W. Lindstrom (1924) as a white kernel mutant that tends to be viviparous and that produces lethal white seedlings. The *y11* allele has been verified in our sweet corns with Caribbean origin and appears to be rather common. Freshly dried mature kernels are a dark pale yellow that is distinguishably darker than *y8*. (Fig. 194).

***ws3*^Hi27 1:0, 3:1 – white sheath 3 plant.** Location: *ws3* = 2S-2.0. MGC 201FA. Origin: MGC 66-274 self (a chromosome 2 stock with linked *ws3, lg1, gl2* and *b1*). Hawaii stock is in BC8 after 41 cycles of selection that eliminated linked loci from MGC. The *ws3* stock was well described by discoverer Marcus Rhoades (1939) as having white leaf sheath and culms, whitish husks, and dwarfed plants that are slow to flower. Marcus asked me in 1950 about something I'd told him at Cornell but that I'd forgotten! He was a remarkable scholar with incredible intellect and memory. Our NIL emerges as a virescent seedling that is most striking in the field. Plants green up during growth but are dwarfed and very late to flower. It's actually easier to create the pure line than to provide a 1:1 population, as nearby tall plants shade it out. (Figs. 195-198).

(*wx1 flta1*)^Hi27 – waxy kernels, floppy tassels. Locations: *wx1* = 9S-47.93; *flta1* = 9?. MGC 915A. Origin: MGC 70-1000-3 x 999-3, a stock with allele *wx1-a* (Argentina). Hawaii stock was derived from 6 BCs to Hi27 followed by selfing over 23 cycles of breeding. Homozygous *wx1* lines are immediately identified as having unusually lax tassel branches, labeled "floppy tassel" (Brewbaker and Yu, MNL, 2009). Trait is inherited as a tightly linked single gene interpreted as co-dominant, with intermediate expression in heterozygotes. During our breeding of "sticky" (waxy or glutinous) commercial vegetable corns for the Asian market, we found that the floppy tassel characterized Asian waxies and could be used to identify many waxy genotypes (cf. *ae1 wx1*, *bk2 wx1*, *sh1 wx1*). One NIL that has normal tassels is *C1 sh1 bz1 wx1*^Hi27.

(*wx1 flta1*) *y1*^Hi27 – waxy kernels, floppy tassels, white endosperm. Locations: *wx1* = 9S-47.93; *flta1* = 9?; *y1* = 6L-30.13. Origin: hybrid of (*wx1 flta1*)^Hi27 x *y1*^Hi27. Hawaii stock was simply bred from F2 of this hybrid. Plants and ears mimic the waxy parent, with its floppy tassels. The kernels are unusually chalky-white at base of ears but become more vitreous like the *y1*^Hi27 seeds toward tips of the ears.

***y1*^Hi27 – white kernel.** Location: 6L-30.13. MGC 602C. Origin: MGC 87-65 sib. While rediscovering Mendel, Carl Correns (1901) identified *y1*, a locus that usually provides pure white kernels but occasionally pale-yellow ones. The Hawaii stock is pure white and in BC6 after 18 cycles of selection and is classical in appearance. Plants appear identical to Hi27. (Figs. 199 & 200).

***y1*^Hi27 (Guam)– white kernel, Guam.** Location: 6L-30.13. MGC 604AA. Origin: Guam Corn, a large-kerneled white variety well known in Guam, obtained in 1967. Verified in crosses to be *y1/y1*. The Hawaii stock is white or very pale yellow (dry kernels) and is in BC7 after 33 cycles of selection. Plants appear identical to *y1*^Hi27, but anthers are red and ears have tiny red glumes (*Pl1* is at 6L-52.88). (Fig. 199).

***y8*^Hi27 – pale yellow kernel.** Location: 7S-34. MGC 707FA. Origin: MGC 96-532 self (707F *y8 gl1*). Merle Jenkins (1947) identified *y8* as a pale yellow mutant, and our stock mimics his description. The Hawaii stock is in BC6 after 15 cycles of selection and resembles Hi27 in appearance. It's not clear whether the stock carries *gl1* (7S-66). Freshly dried mature kernels are a very pale yellow, and testcrosses with *y1* and *y11* were

distinguishing. (Fig. 199).

***y11*^Hi27 – pale yellow kernel.** See *w3-y11*^Hi27.

***yg2*^Hi27 3:1 – yellow green plant.** Location: 9S-minus 3. MGC 924C. Origin: MGC 63-327 sib (a chromosome 9 stock with *c1 sh1 wx1* and *bz1*). Merle Jenkins (1927) identified *yg2* as a pale yellow dwarf mutant, and our stock mimics his description. The Hawaii stock is in BC7 after 23 cycles of selection that eliminated other mutants. Plants remain pale yellow-green from emergence and are dwarfed (20%) and extremely late to flower. Homozygous lines could be created only with difficulty. (Figs. 202-205).

***zb232*^Hi27 – zebra leaf 232.** Location: Unknown. Origin: Inbred G232, a recombinant inbred in Hawaii's Set G (from Hi31 x Hi58 = derived B68 x Ki14). The Hawaii stock is in BC6 after 17 cycles of selection. This is a typical zebra pattern with whitish blotches as stripes on leaves, but it's seasonally variable, best seen in hot sunny days, and assumed to involve carotenoid damage. Bands are yellowish and resemble *zb1* (Milislav Demerec, 1921). Allelism tests continue. (Figs. 206 & 207).

***zbc1*^Hi27 – zebra leaf Caribbean.** Location: Unknown. Origin: Caribbean composite (1968, Thailand). The Hawaii stock is in BC6 after 29 cycles of selection A typical zebra pattern showing best in cool weather, unlike *zb232*, with whitish blotches as stripes on leaves after about 6 weeks. It cannot be seen during Hawaii's warm summers (as also noted for loci like *zb4* and *zb6*), and is assumed to involve carotenoid damage. Allelism tests ruled out *zb7*. (Fig. 208).

***zn1*^Hi27 – zebra necrotic leaf.** Location: 10L-44.0. MGC X10F. Origin: MGC *zn1* stock, 1970. The Hawaii stock is in BC7 after 34 cycles of selection. Described by S. Horovitz in 1947, this zebra pattern involves necrosis that can weaken the plants. However our pure line is late in maturity but otherwise is surprisingly healthy, with large zebra-necrotic blotches between leaf veins that tend to be terminal on leaves. It did once show some smut (rare in Hawaii), as have our *gt1* lines (Figs. 209-211).

***zn*-cm1*^Hi27 – zebra necrotic CM207 leaf.** Location: Unknown. Origin: Indian inbred CM207, derived out of Fla3H94. Discovered in 1995, the Hawaii stock is in BC6 after 19 cycles of selection. This resembles *zn1*^Hi27 but is very late in development and much less severely necrotic. Neither gene is actually very zebra-like (striped) in this background. Plants resemble Hi27 but kernels are unusually denty. Allelism tests with *zn1* and *zn2* have not been completed. Image is with *zn1* to left and *zu-cm1* to right (Fig. 212).

Translocation 4-6b. Location: 4S-80, 6L-16 (1970). Origin: MGC T4-6b. MGC2032F. This Hawaii stock has 14 BCs in 17 cycles of breeding. Segregation of defective kernels in the backcrossed ears of plants heterozygous for the translocation was approximately 1:1 in all crosses.

Translocation 4-10f. Location: 4L-90, 10L-14 (1970). Origin: MGC T4-10f. MGC3045A. This Hawaii stock has 13 BCs in 17 cycles of breeding. Segregation is ~1:1 of ears with defective kernels.

Translocation 9-10a. Location: Unknown. MGC2088B. Origin: MGC 71-B14A x 1805-5Tr. This Hawaii stock has 13 BCs in 15 cycles of breeding. Segregation is ~1:1 of ears with defective kernels.

Figure 1. Hi27 10.

Figure 2. *a1* left, *A1* right.

Figure 3. *ad1*.

Figure 4. *ad1* 10-1122b.

Figure 5. *ae1 segregating wx1* and fusarium 10-55.

Figure 6. *ae1* (ear segregating *ae1 wx1 ae1wx1*).

Figure 7. *ae1* ears segr *wx1*.

Figure 8. *B1*.

Figure 9. *B1* border.

Figure 10. *B1 Pl1 p1-rw.*

Figure 11. *B1* ears and husks.

Figure 12. *B1 C1 bz1* 10-250.

Figure 13. *ba1* right, *Ba1* left.

Figure 14. *bd1*.

Figure 15. *Bif1* left, *bif1* right.

Figure 16. *bk2 wx1*.

Figure 17. *bk2* .

Figure 18. *bk2* fragile husks.

Figure 19. *blo1* 10-233.

Figure 20. *bm1 ae1* ears to left.

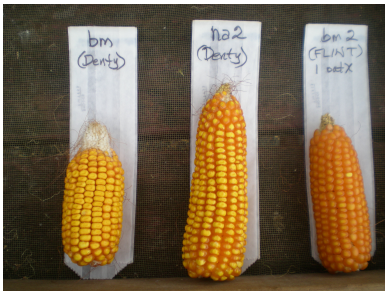


Figure 21. *bm1 ae1*.

Figure 22. *bm1 ae1* 10-235 segregating red anthers.

Figure 23. *bm2*.

Figure 24. *bm2 gt1*.

Figure 25. *bm4*.

Figure 26. *br1 f1* versus *d1*.

Figure 27. *br1 f1*.

Figure 28. *br1 f1* 10-240 rootless.

Figure 29. *br2* left, *br1* right 10-243.

Figure 30. *br3* 10-1008.

Figure 31. *Brta1*.

Figure 32. *brta1* (normal Hi27).

Figure 33. *brta1 dbcb1* 10-1134.

Figure 34. *bst1*.

Figure 35. *bt1 a2* 10-247.

Figure 36. *bt1* kernel rot.

Figure 37. *bt1 a2*.

Figure 38. *bt1* 3:1.

Figure 39. *bt1* segregating *su1*.

Figure 40. *bt1 a2* + *sh2 a1* 10-65.

Figure 41. *bv1* 10-991.

Figure 42. *C1* (*R1 Pr1*).

Figure 44. *C1 sh1 bz1 wx1*.

Figure 45. *Cg1*

Figure 46. *Ch1* ears.

Figure 47. *Cg1* plants.

Figure 48. *Cg1* ears.

Figure 49. *Ch1*.

Figure 50. *Ch1* cobs.

Figure 51. *cr1* 10-255.

Figure 52. *cr1* plants and Nora.

Figure 54. *ct1* 10-356 green tassel.

Figure 55. *d1*.

Figure 53. *cr1* leaf.

Figure 56. *d1* anther ear.

Figure 57. *d1* and rust.

Figure 58. *d1* and southern rust.

Figure 59. *dbcb1*.

Figure 60. *dbcb1* .

Figure 61. *dbcb1 p1-vv*.

Figure 62. *el1* 3:1 .

Figure 63. *el1* 3:1 20DAP 10-264.

Figure 64. *et1* 3:1.

Figure 65. *et1* 3:1; *C1* .

Figure 66. *f1*.

Figure 67. *f1* 10-267.

Figure 68. *fl1*.

Figure 69. *fl1* kernels.

Figure 70. *fl2* 3:1.

Figure 71. *flta1.*

Figure 72. *g1* 10-271.

Figure 73. *g1* left, *g1 li1* right 10-271.

Figure 74. *g1 li1* 10-272.

Figure 75. *gs1* 10-276.

Figure 76. *gt1.*

Figure 77. *gt1* ears, homozygous on top.

Figure 78. *gt1* seedling.

Figure 79. *gt1* hybrid.

Figure 80. *h1* left, *ij1* right 10-277.

Figure 81. *ij1* 10-278.

Figure 82. *ij1* 10-278 row.

Figure 83. *in1 C1* blue corn.

Figure 84. *j1*.

Figure 85. *j1* 11-192.

Figure 86. *j1* and Nora.

Figure 87. *j1*

Figure 88. *j2* left. *j1* right.

Figure 89. *j1* .

Figure 90. *Kn1* leaf 10-283.

Figure 91. *la1* in summer.

Figure 92. *la1* in winter.

Figure 93. *lc2*.

Figure 94. *lc2* 10-578.

Figure 95. *lc2* 11-205.

Figure 96. *lc2* closeup.

Figure 97. *lc2* leaves.

Figure 98. *lfl1*.

Figure 99. *lfl1* leaf.

Figure 100. *lg1*.

Figure 101. *lg1 gl2*.

Figure 102. *lg2*.

Figure 103. *lg2* ear.

Figure 104. *Lg3*.

Figure 105. *li1* leaves.

Figure 106. *li1* closeup.

Figure 107. *li1* ears with no rows.

Figure 108. *lw1* white seedlings.

Figure 109. *ms8* 1:1, *j1 j1* 10-295.

Figure 110. *ms8 j1* tassel 10-295.

Figure 111. *ms10* 10-296.

Figure 112. *na1*.

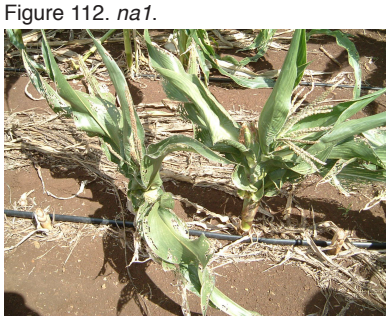


Figure 113. *na1, br1, d1, br2.*

Figure 114. *na1* ears.

Figure 115. *na2*.

Figure 116. *na2* ears.

Figure 117. *nl3*.

Figure 118. *o2* ear.

Figure 120. *Og1*.

Figure 121. *Og1 B1* 10-364.

Figure 122. *os1* left, Hi27 right.

Figure 123. *os1* 3:1 10-77.

Figure 124. *oy1*.

Figure 125. *p1-rw* ears.

Figure 126. *p1-vv*.

Figure 127. *p1-vv dbcb1*.

Figure 128. *p1-wr*.

Figure 129. *p1-wr* 10-313 young ear.

Figure 130. *p1-ww, Ch1, p1-vv, p1-wr*.

Figure 131. *pm1* plants.

Figure 132. *pm1* leaves.

Figure 133. *Pn1* ear.

Figure 134. *Pn1* heterozygous 10-79.

Figure 135. *po1-ms6* 10-294.

Figure 137. *py1* with wild type behind.

Figure 138. *py1* heterozygous with flags, *py1* in back 10-317.

Figure 139. *R1-nj C1*.

Figure 140. *R1-nj* dwarf 10-326, *R1 B1* in back.

Figure 141. *ra2.*

Figure 142. *ra2* ears with tassel-tip.

Figure 143. *ra2* ears, tassel-tipped.

Figure 144. *rf4 C* cytoplasm 10-324.

Figure 145. *Rg1.*

Figure 146. *Rg1* 10-327.

Figure 147. *Rg1* plants.

Figure 148. *sh2* 1:1.

Figure 149. *sh2 sl1* 10-331.

Figure 150. *si1* 10-332.

Figure 151. *sk1.*

Figure 152. *sk1 fl1* 10-333.

Figure 153. *sk1* left *Sk1* right.

Figure 154. *sky1 lfl1* 10-334.

Figure 155. *sl1*

Figure 156. *sr1.*

Figure 157. *sr1* row 10-336.

Figure 158. *sr1* strong expression.

Figure 159. *sr1* variation.

Figure 160. *su1* 3:1.

Figure 161. *su1-jlb3* with leaf fleck.

Figure 162. *tb1* 11-289.

Figure 163. *tb1* heterozygous tiller.

Figure 164. *tlr1* homozygous.

Figure 165. *tlr1* homozygous.

Fig 166. *tlr1* heterozygous.

Figure 167. *tlr1* x *gt1* 10-987.

Figure 168. *Tp1* homozygous

Figure 170. *Tp1* heterozygous tassel.

Figure 171. *ts2* 10-343.

Figure 172. *ts2* ear and tassel.

Figure 173. *ts4.*

Figure 174. *Ts5* heterozygous 10-346.

Figure 175. *Ts5* homozygous.

Figure 176. *Ts6.*

Figure 177. *Ts6* .

Figure 178. *Ts6* ears and tassels.

Figure 179. *Ts6* heterozygous 10-347.

Figure 180. *Ts6* showing dwarfism.

Figure 181. *Ts6* foreground.

Figure 182. *Tu1* homozygous and heterozygous.

Figure 183. *Tu1* ears.

Figure 184. *Tu1* homozygous.

Figure 185. *v4* left, wildtype right.

Figure 186. *v4* 2 weeks.

Figure 187. *v5 o2.*

Figure 188. *v5 o2* young seedlings.

Figure 189. *v5 o2* with brown caps.

Figure 190. *Vg1.*

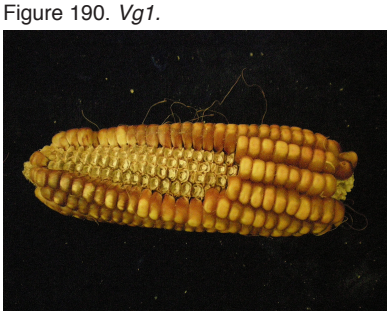


Figure 191. *Vg1* also.

Figure 192. *Vg1* het 10-354.

Figure 193. *vp5* segregating (white k).

Figure 194. *w3-y11* 3:1.

Figure 195. *ws3* 10-358.

Figure 196. *ws3* mature.

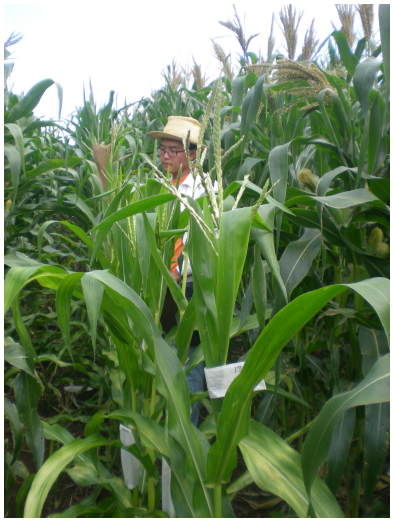


Figure 197. *ws3* 1 month 10-357.

Figure 198. *ws3* right, wildtype left.

Figure 199. *y1, y1-Guam, y8, y11, Y1*.

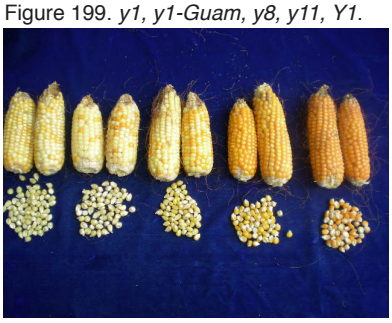


Figure 200. *y1* segr *bt1* 10-484.

Figure 202. *yg2 10-365* 1 to 1.

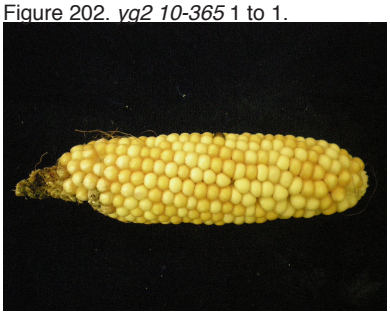


Figure 203. *yg2* 10-365.

Figure 204. *yg2* and normal *sdl1* 10-365.

Figure 205. *yg2* very young *sdl1.*

Figure 206. *zb232*.

Figure 207. *zb*-232* summer.

Figure 208. *zbc1* 09-2311.

Figure 209. *zn1* 11-258.

Figure 210. *zn1* seedlings 10-368.

Figure 211. *zn1* mature.

Figure 212. *zn*-cm1* 10-369 right, *zn1* left.

JOHNSTON, IOWA
Pioneer Hi-Bred, a DuPont company

Circadian regulation of maize transcriptomes in *B73* and *Mo17* inbreds and their reciprocal hybrids

--Hayes, KR; Beatty, M; Meng, X; Simmons, CR; Habben, JE; Danilevskaya, ON

Circadian rhythms have been shown in nearly all forms of life, ranging from algae to plants to mammals. These rhythms include oscillations of gene expression. We recently surveyed the maize transcriptome for diurnal rhythmicity and found >23% of all leaf measured transcripts displaying diurnal regulation under field conditions at the R1 stage of development (Hayes et al., PLoS ONE 5:e12887, 2010). This diurnal study involved environmental light and temperature variation and thus was different from classical "circadian" studies, which typically use constant conditions of light and temperature.

A molecular clock model was recently proposed that links heterosis to the improved or better-tuned circadian clock in Arabidopsis hybrids (Ni et al., Nature 457:327–331, 2009). To assess this hypothesis in maize and examine classical circadian transcription, leaf tissues of inbred lines *B73* and *Mo17*, along with their reciprocal hybrids (BxM and MxB), were assayed under constant conditions over three days by microarray mRNA profiling.

Briefly, plants were grown to the V3 stage under normal greenhouse conditions and then transferred to a growth chamber with constant light and temperature (25º C). After 24 hours of acclimation, leaves from three plants per genotype were collected every four hours for 72 hours. Additionally, *B73* whole-root tissue was collected at each time point. Messenger RNA was prepared from each genotype's time point and assayed using an Agilent microarray representing ~43.000 ESTs. The data were normalized via the Quantile Normalization method and assessed for rhythmicity via the GeneTS package in the R Statistical Programming Language as previously described (Hayes et al., PLoS ONE 5:e12887, 2010).

The leaf results indicate that overall the number of detected circadian cycling transcripts was lower than in leaves from the field diurnal study (Hayes et al., PLoS ONE 5:e12887, 2010), which might be due to the absence in the controlled environment of other environmental cues that reinforce the diurnal pattern (Table 1). Mo17 leaf tissue displayed slightly lower numbers of cycling genes and lower amplitude waves than *B73*, which might in part be due to probe affinity as the oligos on the microarray were designed largely according to the *B73* genome. There is evidence of a slight attenuation or "run down" of the cycling pattern because the median peak and trough values decreased each successive day. However, the third day median peak and trough values were still approximately two-fold different, which indicates good persistence of circadian rhythms (Table 1 and Figures 1-2). No significant numbers of cycling transcripts were detected in roots.

Both reciprocal hybrids display markedly lower numbers of cycling transcripts than the parents (Table 1). The possibility exists for allelic offsets in peak times to cause broader or lower amplitude waves that by additive interference diminish those scored as cycling in hybrids. To resolve this issue, shorter time period resolution and allelic specific expression should be used. Among those cycling transcripts that do appear to cycle in hybrids, transcripts tend to show an additive pattern between both inbreds (Fig. 1). In many cases the hybrids' diurnal patterns could be mimicked by taking the arithmetic means of the signal from the parent inbreds (Fig. 1). In other words the amplitudes of the hybrid cycling genes correspond to the mid-parent values. The mid-parent values of cycling transcripts, and the reduced overall number of cycling transcripts, both indicate an additive pattern likely prevails in hybrids.

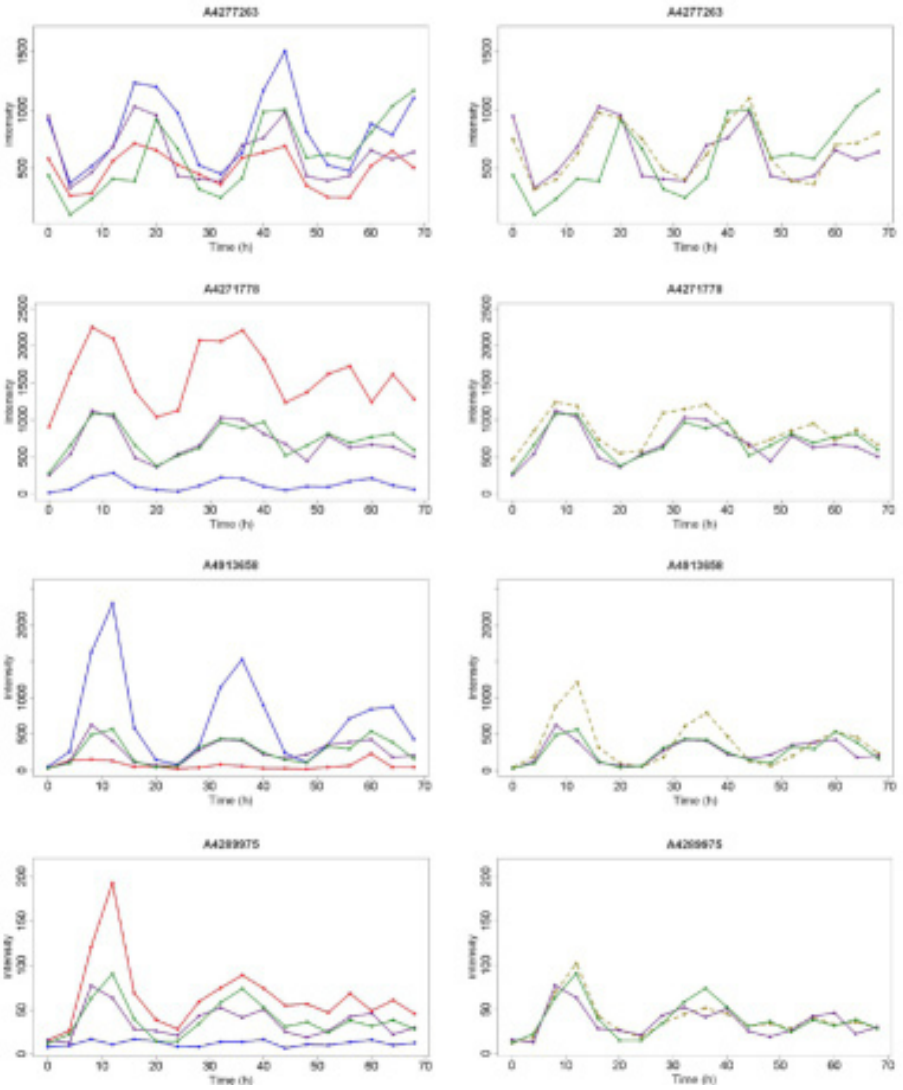
Table 1. Number of diurnally cycling genes and their median amplitude at 30% false discovery rate.

Genotypes	Number of cycling genes	Median amplitude (fold)
B73	2191	2.44
Mo17	1549	1.74
BxM	465	1.92
MxB	203	1.97

Maize core clock oscillators such as *ZmCCA1* and *ZmTOC1b* (Fig. 2) did not show consistent increased amplitude patterns in the reciprocal hybrids as predicted by the molecular clock model of heterosis for Arabidopsis (Chen, Trends Plant Sci 15(2):57-71). Rather, the circadian clock oscillators also appeared to more closely match the mid-parent value in hybrids as observed for the other circadian cycling transcripts.

These results do not support the model for the circadian machinery having a major impact on heterosis in maize. The results also indicate that rhythmic gene expression patterns appear to be mostly additive and do not show epistatic interactions. These results are consistent with other studies that showed additive gene expression as the major pattern observed in RNA profiling experiments of maize hybrids (Guo et al., Theor Appl Genet 113(5):831-45; Springer and Stupar, Genome Res 17(3):264-275).

Figure 1. Four examples of additive rhythmicity of reciprocal hybrid circadian cycling transcripts. Inbred and hybrid gene expressions are shown B73 (blue), Mo17 (red), MxB (green), and BxM (purple). Calculated mid-parent values in hybrids are gold-dotted lines. The probe ID on the Agilent microarray is written on the top of each graph.



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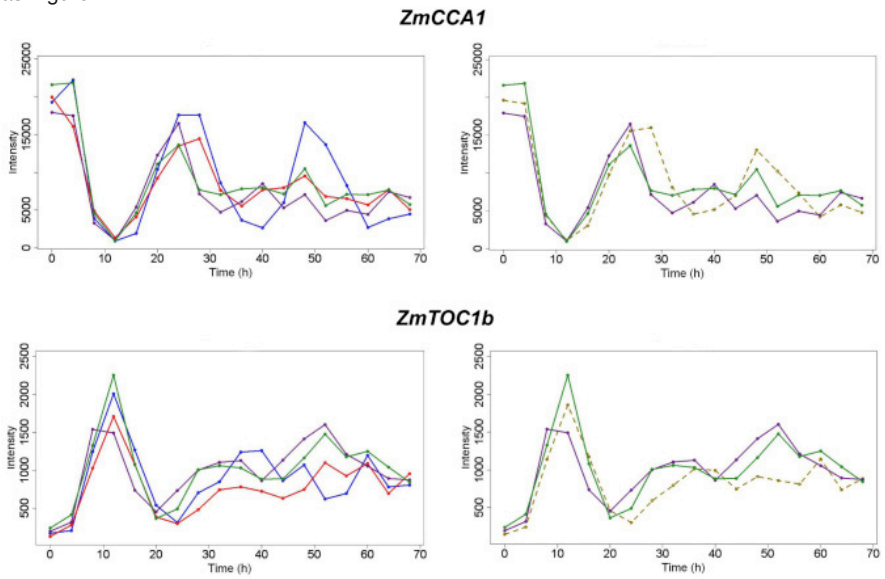
Baby corn (*Zea mays* L.): A means of crop diversification under temperate conditions of Kashmir.

--Najeeb, S; Rather, AG; Sheikh, FA; Ahangar, MA; Teli, NA

Kashmir comprises a major portion of the temperate area of India situated in its northern-most part and covers an altitude range from 4500 to 9000 feet above mean sea level (amsl). Main constraints to the development of agriculture are the short growing season and the remoteness of the region, which restricts the agriculture to a few cereal and vegetable crops. In the past fifteen years baby corn has emerged worldwide as a high-value crop because of its high nutritive value, exotic taste and most importantly, demand for it by foreign tourists. Baby corn (*Zea mays*) refers to whole, entirely edible cobs of immature corn that are harvested just before fertilization at the silk emergence stage (Galinat, Proc Northeast Corn Improvement Conf, 40:22-27, 1985). Kashmir's low night temperatures allow the accumulation of more hexoses (glucose and fructose) and reduce its conversion to starches, thus adding flavor and taste. Harvesting at such an early stage avoids biotic and abiotic stresses, particularly *turcicum* leaf blight and drought. The crop can be harvested for several months subject to the manipulation of sowing dates, or in nursery beds it can be raised in seven-day interval and then transplanted continuously (Miles and Shaffner, Baby Corn Research Re-

Figure 2. Circadian expression of the core clock oscillators *ZmCCA1* and *ZmTOC1b* in inbreds and reciprocal hybrids. The color lines are same as Figure 1.



port, Wash State U Coop Ext, p8. 1999).

The High Altitude Maize Research Sub-Station, Sagam (7500 feet amsl), has undergone serious efforts to develop the most promising and suitable varieties (cultivars) highly adopted under such conditions for baby corn production. Many varieties of maize (composite and hybrids) were evaluated for baby corn production. Materials that are continuously being received from CIMMYT (Mexico) and Directorate of Maize Research (India) were assessed for baby corn production using the typical characteristics of the international standard. The main features that are given attention include prolificacy (2 to 3 ears per plant), yellow-colored cobs with neatly arranged rows of immature seeds, tender cobs 7 cm to 10 cm in length, sweetness, little chaffiness, and a pleasant flavor so as to fetch a premier price in the market. After a thorough assessment, the two composites PS-78 and PS-79 and one single-cross hybrid, I114-2 x I178-1, were identified as the best choices for Kashmir. The two composites belong to a specialty corn group (sweet corn) and the hybrid is normal yellow maize. The relative comparison of important characteristics of the above-mentioned varieties identified for baby corn production is given in the Table 1. Other economic characteristics like

Table 1. Relative comparison of different morpho-agronomic and quality characters of the varieties identified for baby corn production (mean over years: 2005, 2006& 2007).

Quality characters	Varieties		
	PS-78	PS-79	I-114-2 x I-178-2
Days* to 50% tasseling	60	64	67
Days* to 50% silking	62	66	70
Days* to first picking	62	67	70
Cob colour	Yellow	Yellow	Cream yellow
Cob taste	Sweet	Sweet	Moderately sweet
Average weight of the cob with husk (g) [fresh]	60.5	65.7	70.6
Average weight of the cob (g) [dehusked, fresh]	30.4	33.6	35.7
Average length of the cob (cm)	8.0	12.3	11.5
Average diameter of the cob(cm)	2.10	1.90	2.20
Average height of the plant (cm)	180	205	230
Cob yield tons/ha [fresh]	10.04	10.83	12.04
Fresh fodder yield tons/ha	43.00	52.00	58.00
Biological yield tons/ha (one week after harvesting)	14.06	17.05	19.06
Benefit/cost ratio	2.70	2.75	2.80

*Days after sowing

biomass yield and benefit-cost ratio are also presented (mean of 2006, 2007 and 2008). The trial has indicated that an average of 500q (50 tons) of green fodder can be obtained per hectare besides the cob yield of 115 q/ha to 120 q/ha (11.5 tons/ha to 12 tons/ha). This indicates that yield of baby corn in the region is approximately 1.5 times higher than that obtained in Thailand (8 tons/ha).

Fresh baby corn (sweet corn) is highly perishable in hot weather. For every 5° C increase in pulp temperature, sugar breakdown to starch doubles, meaning that baby corn can completely lose flavor in a short period of time. Hence, it's suggested that growers cultivate baby corn in areas near a main market or tourist places. It also can be cultivated in other places provided there are assured transport facilities for lifting and carrying the baby corn to consumption markets.

Popularization of sweet corn (*Zea mays* L. *Saccharata*) to Boost the Socioeconomic Conditions in the Sher-e-Kashmir State

--Najeeb, S; Sheikh, FA; Ahangar, MA; Teli, NA

Sweet corn is one of the most popular vegetables in the United States, Canada, and Australia. It is becoming popular in India and other Asian countries, and creating export markets in the fresh and processing sectors. In the state of Jammu and Kashmir maize is grown on 310000 hectares (0.31 million ha). Because of low maize returns per unit area, growers are quickly shifting to specialty corn production, which gives better returns and opens opportunities for employment generation. Of various specialty corns, sweet corn has substantial market potential and has great genetic variability and scope to improve its nutritive value. This has potential not only in domestic markets but also in the international market.

Table 1. Relative comparison of various traits for the sweet corn varieties
evaluated under temperate conditions of Kashmir.

Quality traits	Varieties		
	PS-78	Madhuri	Almora
Days to 50% tasseling	60	75	78
Days to 50% silking	63	78	82
Days to maturity	120	135	136
Plant height (cm)	180	220	225
Cob height (cm)	95	115	115
Colour of the cob	Yellow	Yellow	Yellow
Taste	Super sweet	Super sweet	Sweet
No. of cobs/ plant	2	2	1-2
Fodder quality	Medium	Very good	Good
Cob yield (fresh) q/ha	90	130	120
Cob yield at 15% moisture level	30	50	45
Fodder yield	70	145	140
Harvest index	0.3	0.25	0.23
B/C ratio	2.3	2.8	2.7

Additionally, quality fodders (on the basis of sweetness) derived after harvest can be sold as cattle feed and bring handsome additional income to the corn farmers.

Sweet corn varieties are grown worldwide, and both yellow and white kernels are suitable for fresh market and processing. We report on varieties suitable for the state of Jammu and Kashmir, notably, the four composites PS-78 (also known as Mishri Makai), Madhuri, Priya, and Almora, These have been tested, evaluated, and analyzed for various traits suitable for sweet corn growing PS-78, which is a native sweet corn that is well-adapted to the temperate conditions of Kashmir. The variety is early-maturing and has the super sweetness trait. The main problem with the variety is its low yield and proneness to *turcicum* leaf blight (TLB). The other three varieties were collected from Almora. A comparison of the varieties for various characters is given in Table 1. A breeding program for developing new varieties with combinations of the desired traits such as high yield, earliness, tolerance to various biotic (cutworm and TLB) and abiotic stresses, and super sweetness is in progress.

Sweet corn is a crop that shows increasing potential. Exports by the fresh and processing sectors are developing, especially in Asian marketplaces where sweet corn popularity amongst consumers is increasing. It is an attractive crop for growers under temperate conditions because cultural requirements are similar to normal maize in that the crop grows quickly and all processing operations can be fully or partially mechanized. By improving management and adopting innovative means such as integrated pest management on the farm, productivity can increase. Increased planting densities and improved soil management options show promise, and adoption by producers is expected to increase. Additionally, the processing sector has taken a leading role in important initiatives aimed at becoming more internationally competitive and opening opportunities for temperate agriculture known for quality crop production.

Quality Protein Maize Variety (QPM-1) Way Out for Better Health and Economy for Temperate Conditions of Kashmir

--Rather, AG; Najeeb, S; Sheikh, FA; Ahangar, MA; Teli, NA

The most important nutritional improvement of maize is the development of quality protein maize (QPM), which is based on the mid-1960s discovery of a mutation in the *opaque2* gene (Mertz, Bates, and Nelson, Mutant gene that changes protein compositions and increases lysine content of maize endosperm, Science, 145:279-280, 1964). The mutation enhances levels of lysine and tryptophan in the endosperm protein. Through interdisciplinary research involving breeders, biochemists, and other scientists, researchers at CIMMYT and elsewhere have steadily developed what we now call QPM (Vasal,

Srinivasan, Pandey, Gonzalez, Crossa, and Beck, Heterosis and combining ability of CIMMYTs quality protein maize germplasm II, Subtropical Journal of Crop Science, 1993). QPM maize retains the *o2* mutation but has modifier genes to overcome agronomic constraints of the original material.

The Jammu and Kashmir state has one of the largest cereal-crop farming areas in India, of which, maize is grown on about 0.32 million ha. (Directorate of Statistics and Economics, Govt of Jammu and Kashmir, India, 2009). It is the chief source of livelihood for many people in the hilly tracts of the state. Demand for meat and poultry has thrust a further importance for maize. Because of the area's recurring droughts, maize is the only alternative to paddy cultivation. Efforts were started in the 2005 Kharif season (rainy season) to develop QPM varieties adapted to the high-altitude, temperate conditions of Kashmir, which is situated at 7500 feet above mean sea level (amsl).

Many QPM hybrids, lines, and composites have been evaluated, and most of the lines did not fulfill requirements of the area. In 2009 our composite QPM development program resulted in the PS-98 variety (Shalimar Maize Composite-5; also known as QPM-1 or first SKUAST (Kashmir) QPM variety), one of the first QPM varieties developed for the temperate conditions of Kashmir and 1650 feet amsl to 2500 feet amsl. The six QPM populations (Table 1) used as basic material for the development of PS-98 were selected on the basis of uniformity in maturity, plant and cob characteristics, plant status, and grain texture and color.

The variety should be released by the end of the 2010 Kharif season after it fulfills some prerequisite formalities. QPM-1 matures early and has good grain and plant characteristics, hard endosperm, an expected protein profile, and it is tolerant to various biotic and abiotic stresses. The cob is conical, semiflint with a cap, and creamy white in color. The other characteristics in comparison with standard check C-15 are given in Tables 2-6. The photographs of the green stand and dehusked cobs are given in Figures 1 & 2.

Table1: Lines/populations used for development of composite QPM-1 (PS 98).

S. No	Name of the entry	Source	Year of collection
1	SO3HI HQ	CIMMYT, Mexico	2004
2	SO3HI WQ	CIMMYT, Mexico	2004
3	SO1HLWQ1	DMR, New Delhi	2004
4	SO1HLWQ2	DMR, New Delhi	2004
5	SO2HLWQ1	DMR, New Delhi	2004
6	SO2HLWQ2	DMR, New Delhi	2004

Figure 1. QPM-1 at RRSS Larnoo (Kharif 2008).

Table 2: Grain quality traits of QPM-1 (PS 98) viz-à-viz check C 15.

S. No	Cultivars	Protein (%)	Trypto-phan (%)	Ly-sine (%)	Sug-ar (%)	Starch (%)	Oil (%)
1	QPM-1 (PS 98)	8.96	0.66	2.95	4.48	64.12	4.78
2	C15 (Check)	10.75	0.55	2.02	3.85	69.72	4.70

Table 3: Average yield performance of QPM-1 (PS 98) in station trials over years and locations (2007-2009).

S. No.	Cultivar	Grain yield (q ha^{-1})	% superiority over check
1	QPM-1 (PS 98)	57.78	7.45
2	C 15 (check)	53.77	

Table 4: Screening of QPM-1 (PS 98) against reaction to major diseases.

Cultivar	*Turcicum* leaf blight (%)	Common rust (%)
QPM-1 (PS 98)	17.5 (2)	12.3 (2)
C 15 (check)	27.3 (3)	19.5 (2)

Figures in parentheses indicate disease score. 1-5 scale was used for scoring the disease reaction. The cultivars were evaluated during Kharif 2008 & 2009.

Table 5: Screening of QPM-1 (PS 98) against the infestation of major insect pests.

Cultivar	Percentage of plants damaged			
	Stem borer	Blue beetle	Cut worm	Aphid
QPM-1 (PS 98)	9.7 (1)	9.5 (1)	27.3 (3)	7.2 (1)
C 15 (check)	14.4 (2)	12.9 (2)	32.9 (3)	11.6 (2)

Figures in parentheses indicate pest score 1-5 scale was used for scoring the infestation of insect pests.

Table 6: Benefit: cost ratio analysis (ha^{-1}) of QPM-1 (PS 98).

Variety	Cost of cultivation	Returns	Benefit	Ratio (Benefit/ cost)

QPM-1 is under seed production at various isolations in order to ensure availability of the seed to fulfill 30% of the initial demand. If possible in the future, the winter nursery facilities at DRR will be used for further multiplications of the seed. The extension and informal education regarding the new variety has been given due priority and farmers show willingness on their part to grow the variety and to replace their non-QPM conventional varieties.

CIMMYT cooperating cell and DMR are highly acknowledged for providing maize materials regularly through different trials for mountain agriculture.

Strategies to produce farmers own seed of open pollinated varieties (OPVs) of maize

--Rather, AG; Wani, AS; Najeeb, S; Sheikh, FA; Ahangar, MA

Maize varieties might be either hybrids or open pollinated (OPV). Though hybrids are inherently high yielding (World Maize Facts and Trends, Maize Seed Industries, Emerging Role of Public and Private Sectors, CIMMYT, Mexico, 1994), OPVs have the advantage of stable performance compared to hybrids in low-yielding and stressful environments (Quality Protein Maize, National Academy Press, Washington, D.C., 1988). Moreover, the seed of OPVs can be saved for subsequent planting. Because maize is a highly cross-pollinated crop, standards for minimum isolation have been developed for seed production that depend on class of seed and field size.

Maize, which occupies about 0.1 million hectares in Kashmir, is the Jammu and Kashmir state's second most important crop after rice. The crop is largely OPVs and cultivated under rainfed conditions. Among many factors responsible for low productivity of the crop, seed quality has been recognized one of the crucial elements and can enhance productivity up to 20%. More than 85% of farmers use their own saved seed. We report some precautionary measures to support seed production in OPVs so that farmers can use their own saved seed for several seasons without substantial loss of yield potential or good agronomic character. The seed production is relatively simple and inexpensive for resource-poor farmers

Choice of cultivar and seed source: Farmers in Kashmir should choose cultivars according to their ecological requirements, either valley or high altitude. The certified seed of a particular cultivar should be used to produce farmers' own saved seed.

Cultural practices: Agronomic practices are generally similar to those for producing a grain crop. However, careful consideration must be given to land preparation and fertilizer or manure application to produce uniform growing conditions in the seed production plot. This facilitates the identification of off-types during rouging operation. Plant 10% to 15% more seed than desired at harvest to compensate for reduced germination and seedling mortality caused by pest attacks from cutworms, etc. If there are provisions for irrigation, two might be given: one at tasseling and the grain filling stage each.

Field layout: In one acre of land, plant 9 kg to 10 kg of the desired OPV seed in lines uniformly drawn 60 cm apart. If line sowing is not possible in the entire area, seed can be broadcast in the non-selected area. It is highly desirable if 2 to 3 farmers, having their fields adjacent to each other, join to produce the seed of the same variety in a bigger plot of at least one hectare.

At leaf stage four, thin out excess plants maintaining a plant-to-plant distance of 20 cm. Emphasis should be given to the removal of plants that do not resemble the variety based on color of leaves and stem, height, vigor, etc. In the middle of the field, select 150 sq m (15 m x 10 m) area where slightly wider spacing (25 cm) is maintained between plants. This means there are 25 rows that are 10 m long, and each has about 40 plants instead of 100 after thinning. The wider spacing ensures good seed set and development, allows full expression of plant types, and aids the identification of off-types. When a hill is missing, allow two plants to grow in the next hill. Before pollen shed, detassel plants in the area described above. In the 17 central rows excluding five plants from each side of the row (500 plants), henceforth called seed plot, detassel 40% (200) plants before pollen shed. Detassel atypical plants based on plant and ear height, leaf and stem color, pubescence, disease and insect damage etc. Very tall and very dwarf plants as well as plants falling outside the range of maturity should be eliminated. Allow the crop to mature.

Harvesting and seed preparation for storage: Harvest the crop carefully by hand when seed moisture is between 16% and 20% (dry husk stage). Harvest seed plots first to avoid any chances of mixture and be careful not to harvest the rejected, detassled plants. Spread these 300 ears on a tarpaulin or on a clean, dry concrete surface and check for ear and grain characteristics (ear size and kernel color and texture) after dehusking. Select 250 to 275 true-type ears free from disease and insect damage. Sun dry selected ears on a clean and dry surface. Do not heap ears during sun drying. Turn ears frequently for uniform drying. Shelling is done when the seeds reach about 14% moisture content. To test, simply shell an ear and mix 100 kernels with one gram of common salt. If the salt feels moist after five minutes, ears need to be dried further, but if salt remains dry, shelling can begin. Shelling by hand minimizes seed damage. The best seed is obtained from the middle of the ear so the outermost 2 cm of seed from the tip and bottom might not be included.

Sun dry the seed until the moisture content reach-

es 12% (when seed under teeth is broken with a sharp sound) for safe storage. Low seed moisture increases the viability and storability of the seed. Ensure that seeds are turned frequently during sun drying to prevent "sun burn" damage to the embryo. The shelled seed might be cleaned (winnowed) and any chipped and diseased seed be removed by hand. A quantity of 18 kg to 20 kg seed is obtained from selected ears. Treat the seed with Thiram at a rate of 2 g Thiram per 1 kg of seed. Ensure that seed is uniformly treated. Store in gunny bags in a cool, dry place away from rodents.

During the next season, half of the seed should be sown and the other half should be retained to meet any eventuality. The process is repeated for three seasons before procuring fresh certified seed.

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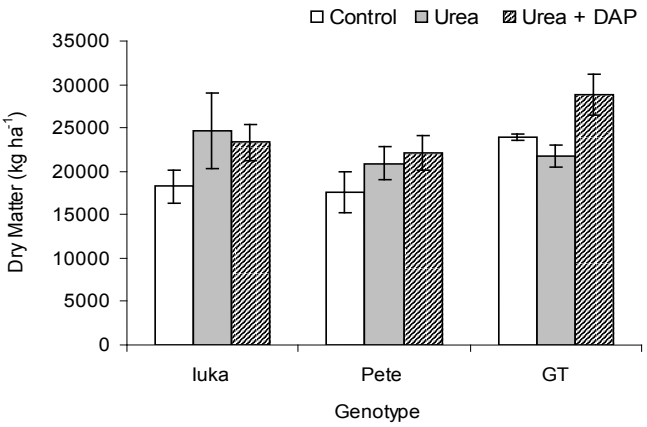
Nitrogen plus phosphorus fertilization increases forage yield of *Tripsacum dactyloides* (L.) L. (Eastern gamagrass)

--Garcia, MD; Huarte, RH; Pesqueira, J

*T. dactyloides (*Eastern gamagrass), a close relative of maize, is a warm-season, perennial forage grass with high productivity and tolerance to several soil adversities. The aim of this study was to compare tiller number dynamics and forage yield of three *T. dactyloides* genotypes unfertilized or with the addition of nitrogen and nitrogen plus phosphorus, during the second year of crop production.

Genotypes used in this study were diploid cultivars Iuka and Pete, kindly supplied by Dr. Maria Haytt (Iowa State University) and a tetraploid genotype (GT) from CIMMYT (Mexico). The research was conducted at the Instituto Fitotécnico de Santa Catalina, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Llavallol, Buenos Aires (34º 48' S, 48º 31' W). Planting was made on June 14, 2007 (to fulfill the period of low temperatures required to alleviate caryopsis dormancy). Field trials were established on a typic argiudoll soil that showed 32 g/kg organic matter and a pH of 6. Seeds were planted approximately 3 cm to 4 cm deep in rows 0.7 m apart, and spaced 0.2 m apart in the row. Weeds were controlled mechanically during the post-emergence period. Plants were fertilized on November 20, 2009. During the growth period the accumulated rainfall (September 15, 2009, to April 6, 2010) was 971.9 mm, and the mean temperature was 20.3º C. Gamagrass was cut by hand to 0.2 m stubble. Plants were cut whenever they attained a height of 0.8 m. Four harvests were carried out during the growing season: on November 20 and December 18, 2009, and February 15 and April 6, 2010. Emerged tillers were counted on October 16 and December 14, 2009, and March 16, 2010. Trials were conducted in a randomized complete block design with a factorial arrangement of treatments in three replicates. Factors included genotype (3 levels) and fertilization treatment (3 levels). Fertilization treatments were control (without fertilization), urea (217.39 kg ha^{-1}) and diammonium phosphate (DAP) + urea (200 kg ha^{-1} + 140.46 kg ha^{-1}, respectively). The last two treatments provided equal doses of nitrogen each (100 kg ha^{-1}). Genotype was the main plot treatment (plot size: 6.3 m by 8 m), and fertilization was the subplot treatment (plot size: 2.1 m by 8 m). The data were subjected to analysis of variance, and significant differences among the means and treatments were compared using a Tuck-

Figure 1. Forage yield (dry matter) of three *Tripsacum dactyloides* genotypes unfertilized (control) or fertilized with N (217.39 kg ha^{-1} urea) and N+P (200 kg ha^{-1} DAP + 140.46 kg ha^{-1} urea).



Vertical bars indicate the mean ± SE.

ey test at a level of 5% using the Infostat software package (InfoStat, 2008. InfoStat Group, FCA, Universidad Nacional de Córdoba, Argentina).

Tillering (measured as the number of tillers per plant) was not affected by the genotype but varied during the growing season. Interaction between genotype and date was not significant. The number of tillers per plant (mean ± SE) decreased ~50% from October to December (from 53,7 ± 2,94 to 27,3 ± 1,26, respectively), and then it remains stable up to March 16 (26,7 ± 1,42). Both factors, genotype and fertilization, affected tillering and total forage yield (expressed as dry matter, or DM). Genotype x fertilization interaction was not significant for both parameters. Tillering (media ± EEM) of GT (25 ± 0.9) was lower than that of the cultivars Iuka and Pete (34 ± 2.32 y 32 ± 2.04, respectively). The number of tillers per plant increased significantly in urea fertilized plants (34 ± 3.02) compared to control ones (27 ± 0.93). Urea+DAP fertilized plants showed intermediate values (29 ± 1.31).

Urea + DAP treatment induced significant increased of DM compared to the control one (mean ± SE, in kg ha^{-1}) (24757 ± 1497 *vs.* 19931 ± 1321). Urea fertilized plants showed intermediate values (22098 ± 1798). GT genotype and cv. Pete showed the higher (24836 ± 1306) and the lower (20213 ± 1241) DM production, respectively. DM produced by cv. Iuka showed intermediate values (22461 ± 1535) (Fig. 1).

These results show the potential of this species, especially the tetraploid genotype, in terms of high biomass production. Further studies, mainly its performance under more restrictive soil conditions as well as its nutritional quality, are necessary to determine the usefulness of this species as a forage resource for the area.

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An endosperm enzyme catalyzes the formation of phosphotriester and phosphodiester bonding complex between nucleic acids with altering their structures

--Pan, D

A newly discovered enzyme that can catalyze the alteration of the structure of nucleic acid through the formation of phosphotriester and phosphodiester bonding complex was partially purrified from maize developing endosperms. Maize developing endosperms after 22 days of pollination from W64A line were used for this study. The purification process combined the following sequential steps: 15% to 35% ammonium sulfate fractionation, DEAE-Cellulose anion exchange column chromatogaphy and Sephadex G 150 gel filtration. A routine 50 mM of Tris-HCL buffer, pH 7.5 was used for preparation of enzyme extract, ammonium sulfate fractionation, DEAE-cellulose anion exchange column chriomatography and Sephadex G 150 gel filtration. However, on DEAE-cellulose anion exchange column chromatography step, the enzyme was stepwise eluted out from the column by 50 mM, 100 mM, 200 mM of sodium chloride included in the Tris-HCL buffer, respectively. The enzyme activities were found in both 50 mM and 100 mM eluents. Enzyme activity was monitored by changing OD at 260 nm as a result of the formation of a complex of either phosphotriester and phosphodiester bonding between nucleic acids (Fig.1).

The optimum pH value for the enzyme activity is in 50 mM acetate buffer at pH 5.4. The enzyme is widely distributed in nature ranging from biological tissues to viral particles including barley mosaic virus, southern bean mosaic virus, poliovirus, influenza virus, and many others. The broad presence of this enzyme in the biological kingdom suggests that the enzyme is an evolution significant protein. A variety of short chain length nucleotides and poly nucleotides including polyU, polyC, and polyA had been tested as substrates for demonstrating the catalysis of enzyme reaction. However, the minimum

Figure 1. Changing spectra of the reaction products as a function of time. DNA (only), Enzyme (only).

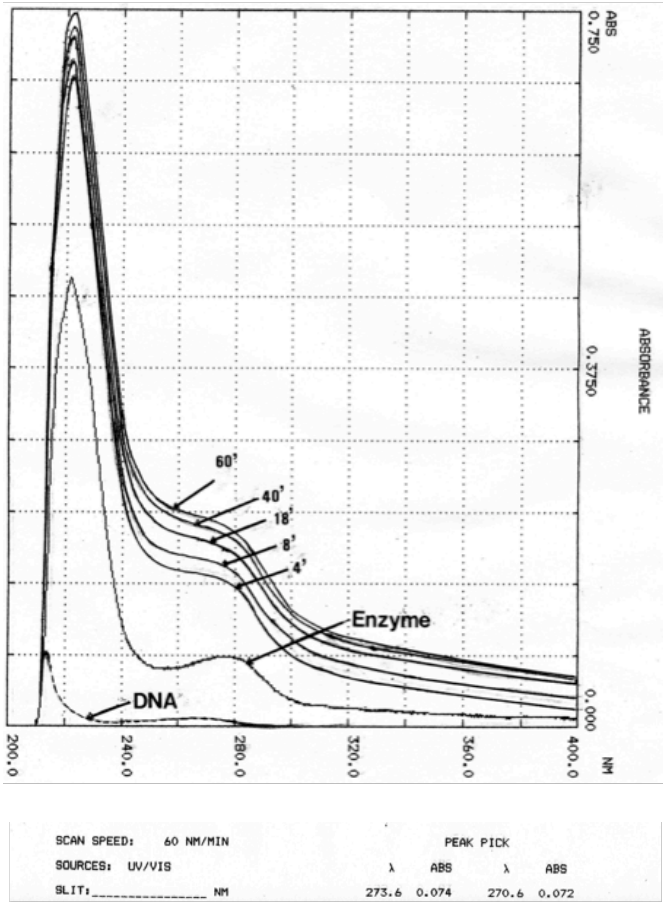
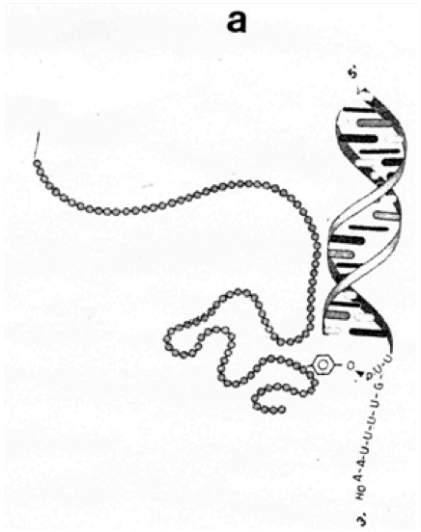


Figure 2a. Enzyme-nucleic acid complex via phosphotriester bonding.



requirement of a 3 to 4 nucleotide chain length in order to initiate the enzyme action suggests that a single phos-

Figure 2b. Enzyme-nucleic acid complex via phosphodiester bonding.

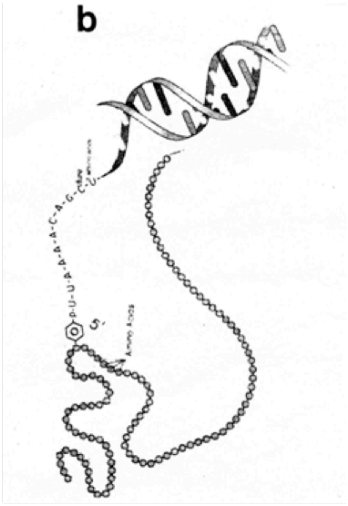
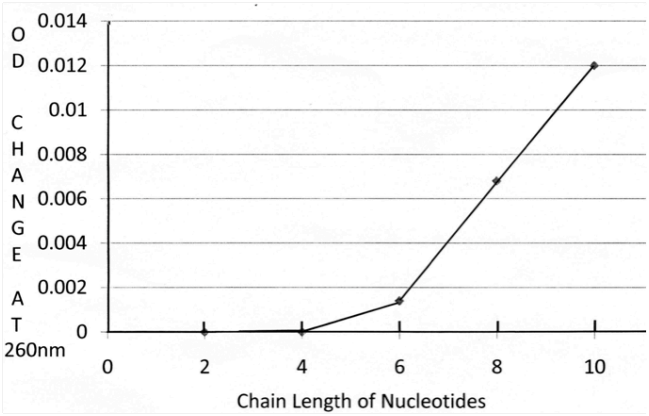


Fig. 3. Minimum requirement of nucleotide chain length (number of nucleotide Units).



phate group between two nucleotides could not be used to link with other phosphate group from other nucleotide through ester bonding (Figs. 2a, 2b, & 3).

It was found that the enzyme can exist in both monomer (24,000 KD) and dimer (50,000 KD) forms on Sephadex G 150 gel filtration. Both enzyme forms can catalyze the reaction. However, it could not rule out that the enzyme can re-associate into dimer from the monomer form in the reaction mixture before catalysis. The data of 1% agarose gel electrophoresis in 50 mM Tris-HCL buffer at pH 7.5 of the enzyme reaction products from either with long or short chain length of nucleotides as substrate consistently showed a very larger molecular form staying at the origin loading well without migration (data not shown). This together with the preliminary results of electron microscopy indicate that the enzyme can carry out the linking of multi-nucleotides together through either phosphotriester and/or phosphodiester bonding and the formation of a much larger molecular structure (aggregated form). Preliminary study also showed the presence of this high molecular nucleic acid complex, although the detail of structures is not known at the present time, in maize endosperms and Arabiadopsis leaves. A simple overall enzyme reaction mechanism can be depicted as follows:

Nucleotides + Enzyme <---------->
Enzyme-Nucleotide(s) complex (Fig. 2)

nEnzyme-Nucleotide(s) <---------->
nEnzyme + (Nucleotide(s)-Nucleotide(s))$_n$
Complex through phosphotriester & -diester bondings

This investigation was carried out in the late Dr. Oliver E. Nelson's laboratory in the Department of Genetics on and off for almost two decades with his continuing support and encouragement during a memorable relationship between him and the author. Understanding the detailed molecular structure of the enzyme reaction products with different chain lengths of nucleotides as substrates is under investigation.

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Isolation and preliminary characterization of a new maize low phytic acid 1 allele

--Badone, FC; Lago, C; Bucci, A; Cassani, E; Pilu, R

Phytic acid, also known as myo-inositol 1-, 2-, 3-, 4-, 5-, and 6-phosphate (IP6), is the major storage form of phosphorous in plants is mainly accumulated in pollen and seeds (up to 4% to 5% of dry weight). In the maize kernel 80% of phytic acid is localized in the scutellum and the remaining 20% is in the aleuronic layer. During germination, phytic acid is idrolized by phytases. Both phytic acid and the cations that it's able to bond are poorly bio-available for monogastric animals due to their lack of phytase activity. One strategy to solve this problem is to isolate cereal mutants to be able to accumulate low level of phytic P and a high level of free phosphate in the seeds.

We obtained a mutant population by the EMS (ethyl methanesulfonate) seed treatment and approximately 300 M2 families were screened using the molybdate staining method for free phosphate.

We found one *low phytic acid 1* mutant (named *lpa1-7*). The 3:1 segregation ratio of *lpa1-7* that was observed in the F2 generation indicated a monogenic recessive defect (Table 1).

The *lpa1-7* mutation causes approximately a ten fold increase in the amount of free phosphate (Fig. 1) and a

Figure 1. Mature dry seeds of the indicated genotypes were assayed for seed phytic acid P expressed as P concentrations (atomic wt = 31).

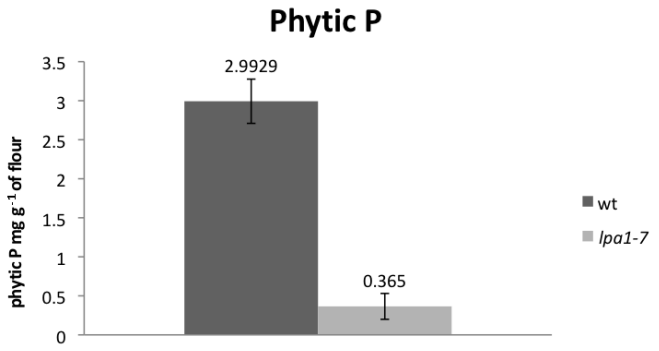
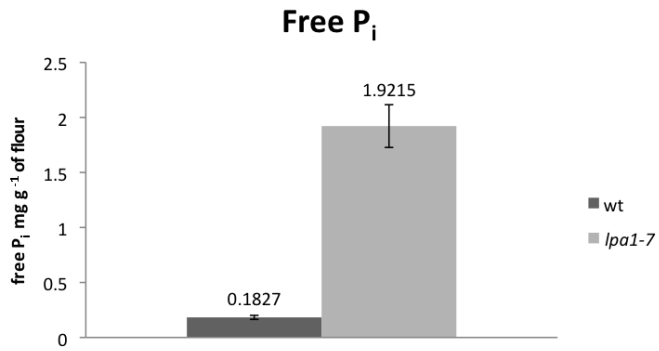


Figure 2. Mature dry seeds of the indicated genotypes were assayed for free inorganic P expressed as P concentrations (atomic wt = 31).



reduction of about 80 % of phytic acid (Fig. 2).

The presence of this new allele in homozygous condition is lethal. Germination could be partially restored by embryo-rescue. Embryos from mature seeds cultured in an MS medium grew slower than the wild type and some

Table 1. Segregation of +/*lpa1-7* phenotypes observed in the F2 progenies obtained by selfing.

	Genetic test	Segregation		χ^2	*p*
		wt	mutant		
+/*lpa1-7*	F2	159	51	0.057	0.8113

defective seedlings were observed. Mutant embryos displayed a reduction in dimension and alteration in the alignment of the shoot and root primordia.

The relationship of our low phytic acid mutation with the previously identified low phytic acid maize mutations was tested. It's known that the *lpa1* mutant is the only low phytic acid mutant exhibiting more than 60% of reduction of phytic acid. The rate of reduction shown by *lpa1-7* acid suggested that it could be an *lpa1* allele. The mutants *lpa1, lpa2* and *lpa1-7* were crossed inter se in all pairwise combinations to assay their pattern of complementation. The results obtained showed that the lpa1 mutant failed to complement *lpa1-7*, which suggests an

Table 2. Complementation test among *lpa1*, *lpa2*, and *lpa1-7*.

	lpa1	*lpa2*	*lpa1-7*
lpa1	-	+	-
lpa2		-	+
lpa1-7			-

allelic nature (Table 2).

Genetic analysis of this mutation, as well as its biochemical characterization, are under way.

NAIROBI, KENYA
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Improvement in inheritance of somatic embryogenesis and plantlet regeneration in tropical maize lines from friable callus

--Muoma, J; Ombori, O; Machuka, J

Recalcitrance to regeneration and transformation of tropical maize has slowed down the potential research in improvement of variety, quality, and capability of tropical maize to withstand abiotic and biotic stresses. The Plant Transformation Laboratory (PTL) at Kenyatta University in Kenya has developed a regeneration system for tropical maize inbred lines important to Kenyan breeders by using 1 mm to 2 mm immature zygotic embryos as explants. This system has proved to be highly genotype-dependent. In this study, inbred, single-cross hybrid and backcross generations were developed from crossing maize inbred CML216 with commercially important inbred maize line TL08. When immature zygotic embryos from the resulting generations were cultured on the N6 medium as previously used by Frame et al., (2002) genetic effects had an influence on somatic embryogenesis and plant regeneration. Additive gene effects were more important in the crosses than dominant gene effects and caused a 50% increase in somatic embryogenesis when maternal inbred was CML216 and TL08 as the paternal (Table 1) and up to 33.3% increase in the regeneration frequency (Table 2) in single cross hybrids relative to inbreds. In backcross generations of the four crosses, maternal and/or paternal effects were significant in the frequency of somatic embryos formed by the F1 three weeks after culture as well as in the frequency of plants regenerated per embryo, nine weeks after culture. Analysis of genetic variances suggests that crosses with CML216 as maternal donor with TL08 as pollen donor had up to 64% increase in somatic embryogenesis. Regeneration in all experiments was independent of the

Table 1. Callus induction from immature embryos of two tropical inbred lines TL08 and CML216 and their single cross hybrid and back cross generation.

Genotype	Number of embryos cultured	Calli forming somatic embryos	Somatic embryo induction frequency (%)
CML216	200	194	97
TL08	200	81	40
CML216 x TL08	150	96	64
TL08 X CML216	190	97	51
(CML216 x TL08) X CML216	120	91	76
(TL08 X CML216) X CML216	100	73	73
(CML216 x TL08) X TL08	250	118	47
(TL08 X CML216) X TL08	150	66	44

Table 2. Regeneration of tropical inbred lines TL08 and CML216 and their single cross hybrid and back cross generation.

Genotype	Calli forming somatic embryos	Regenerants	Regeneration frequency (%)	Acclimatized plants	Regeneration efficiency (%)
CML216	90	50	55	22	24
TL08	50	38	76	8	15
CML216 x TL08	45	44	99	27	60
TL08 X CML216	60	46	76	38	63
(CML216 x TL08) X CML216	55	40	73	34	61
(TL08 X CML216) X CML216	60	35	58	26	44
(CML216 x TL08) X TL08	40	37	92	13	32
(TL08 X CML216) X TL08	75	61	81	39	55

crossing pattern with the inbred poor rooting pattern resulting in poor acclimatization of the regenerants. TL08 and CML216 had low regeneration frequency, 15% and 24% respectively (Table 2). Hybrid vigor was exhibited by the high regeneration efficiency of between 60% and 63%, and 55% and 61% of the regenerated plants in case of the single cross hybrid and the back cross generation respectively with the exception of (CML216 x TL08) X TL08 and (TL08 X CML216) X CML216, which had a regeneration efficiency of 32% and 44%, respectively (Table 2). The experiments were done with CML216 as control for the F1 as it had consistently shown a high percentage of somatic embryogenesis of up to 97%.

In conclusion, the results from the two tropical inbred lines and their crosses indicated that regeneration is genetically controlled by nuclear genes in maize. Segregation for somatic embryogenesis in the cross between CML216 and recalcitrant but commercially important inbred TL08 could be accounted for by a small number of genes as a large proportion of genotypic variation for the formation of type 1 and type 2 callus might be due to additive gene effects. The effective understanding of inheritance of somatic embryogenesis of tropical maize is a very important process for any future work on maize transformation. This will provide a sure way of deciding which tropical maize lines can be used for gene transfer with maximum success. The current transformation frequencies of 50% can be further improved to 75% as a result of 50% increase in the number of immature zygotic embryos forming somatic embryos. At PTL, previous transformation efficiencies of 5-10% can equally be improved to up to 15%. Overall these results will provide a sure way to regenerate Agrobacterium mediated transformed events with a high number of putative transformants.

OTTOWA, ONTARIO, CANADA
Eastern Cereal and Oilseed Research Centre

Expression of different *Rp* genes to common rust of corn in Ottawa, Ontario

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

Twenty-eight H95 background inbred lines with different *Rp* resistance genes for common rust of corn (*Puccinia sorghi*), and resistant inbred B108 and B113; four inbred lines (H95, A679, CO348, and CO4428) with

different level of resistances were evaluated for the expression of resistance in our common rust nursery at the Eastern Cereal and Oilseed Research Centre (ECORC) of Agriculture and Agri-Food Canada (AAFC) in Ottawa, Ontario, in 2002 and 2010. Each genotype was planted in a one-row plot with single row plots of 15 plants. To achieve good infection levels plants are inoculated twice: once at the 6-8 leaf stage and again at the 10-12 leaf stage. Two ml of urediniospores suspension (2.5 ×105 spores/ml) are injected into the whorl (plant tissues are not damaged) of each plant each time by using a graduated, 10 ml, self-refilling, automatic vaccinator attached to a 2.5 L backpack container (Nasco Co., Fort Akinson, WI). If there was no rain after inoculation, the plots were irrigated for 10-15 minutes every day to provide a suitable environment for the disease to develop.

P. sorghi is an obligatory parasite, so the pathogen must be kept alive and cannot be stored in a dormant state as with the other pathogens outlined in this guide. Sweet corn, which is quite susceptible to common rust, is a useful overwintering host when planted in the greenhouse. Urediniospores can survive up to six months in the refrigerator (4° C to 6° C), so from January to mid-June, urediniospores are collected from greenhouse-grown sweet corn plants. Prior to inoculation, all the collected spores are mixed together and divided into two batches for inoculation at two stages of plant development. Before inoculation, the spores are suspended in sterilized water with 1 ml of 0.5% Tween 20 added to every 500 ml of water to aid followed by agitation (stirring) for 15 minutes. The suspension is then filtered through two layers of cheesecloth to discard any big particles or plant tissues and diluted with sterilized water to 2.5 ×105 spores/ml.

At the soft dough stage about 3 weeks after silk emergence, plants are rated for specific resistance (based on pustule type) and general resistance (based on disease severity). Six pustule types (Table 1) are classified as: I = immune, no infection symptoms; HR = hypersensitive response, yellowish or brownish pin-point fleck symptoms; R = resistant, yellowish or brown necrotic borders, no typical pustules and urediniospores; MR = moderately resistant, small pustules covered by the host cuticle, no broken-out urediniospores; MS = moderately susceptible, some pustules and urediniospores broken out of the leaf cuticle; and S = susceptible, large pustules, mostly link together and broken out with lots of urediniospores. Disease severity is evaluated by using a rating scale based on the percentage of leaf area with visible symptoms where 1= no symptoms, 2= < 1%, 3= 1% to 5%, 4= 6% to 20%, 5= 21% to 50%, 6= > 50% of leaf area with symptoms, and 7= plant is dead. It can be difficult to rate the percentage of leaf area infected; a more simple and practical scale to use is: 1= no symptoms; 2= a few lesions; 3= many lesions, but they are not linked together; 4= a lot of lesions, some linked together to form a necrotic (dead) area; 5= necrotic areas linked together and a few leaf tips are dead; 6= 50% of the leaf

Table 1. Common rust of corn screening results of inbred lines with different resistance in 2002 and 2010 in Ottawa, Ontario, Canada.

Genotypes	2002		2010	
	Rating	Pustule types*	Rating	Pustule types*
H95	5	S		
H95(*Rp1-A*)	3	MR/R	3	R
H95(*Rp1-B*)	2	R	2	R
H95(*Rp1-C*)	3	HR/R	3	R
H95(*Rp1-D*)	3	R/MR	3	R/MR
H95(*Rp1-Dj4*)	2	HR/R	2	HR
H95(*Rp1-FJ6q*)	2	R	2	MR/MS
H95(*Rp1-J*)	4	MR	3	MS
H95(*Rp1-Jc13a*)	2	I/HR	2	I/HR
H95(*Rp1-JFC1*)	2	HR	2	R
H95(*Rp1-K*)	2	HR	2	HR
H95(*Rp1-Kr1-J6*)	3	HR		
H95(*Rp1-Kr1-J92*)	3	MR/MS	3	MR/MS
H95(*Rp1-Kr3*)	2	R	2	R
H95(*Rp1-Kr4*)	2	R	2	R
H95(*Rp1-M*)	3	MR/MS	3	MR
H95(*Rp3-A*)	2	I/HR	1	I
H95(*Rp4-A*)	4	MS	4	MR/MS
H95(*Rp4-B*)	3	MR	3	MR/MS
H95(*Rp5*)	4	MR	2	R
H95(*Rp5-D*)	4	MS/S	3	MS/S
H95(*Rp6*)	2	HR		
H95(*Rp7*)	5	MS	4	MS
H95(*Rp8-A*)	4	MS	3	MR/MS
H95(*Rp-G15c*)	2	I/HR	1	I
H95(*Rp-G5*)	2	I/HR	1	I
H95(*Rp-G5JCa*)	2	I/HR	1	I
H95(*Rp-G6J1*)	2	I/HR	1	I
H95(*Rp-GFJ*)	2	I/HR	1	I
B108			3	R
B113	3	R	2	R
A679	5	S	4	S
CO348	5	S	5	S
CO428	4	S	3	S

* I = immune, no infection symptoms; HR = hypersensitive response, yellowish or brownish pin-point fleck symptoms; R = resistant, yellowish or brown necrotic borders, no typical pustules and urediniospores; MR = moderately resistant, small pustules covered by the host cuticle, no broken-out urediniospores; MS = moderately susceptible, some pustules and urediniospores broken out of the leaf cuticle; and S = susceptible, large pustules, mostly link together and broken out with lots of urediniospores.

tips are dead; and 7= most of the leaves are dead.

H95 serial of *Rp* lines were donated from the Kansas State University to AAFC in 2002. Most lines were uniform in agronomy traits such as plant height, ear height, tassel shape, silk color, etc. However, the *Rp* lines are somewhat different than H95 lines maybe because of not enough generations of backcrossing with H95. The *Rp* line's resistance to common rust also showed some segregation (Table 1). The most resistant plants from each Rp line were selfed for future breeding use. Their resistance had some changes in 2010 (Table 1).

H95(*Rp3-A*), H95(*Rp-G15c*), H95(*Rp-G5*), H95(*Rp-G5JCa*), H95(*Rp-G6J1*), and H95(*Rp-GFJ*) were immune lines that had very few and minor hypersensitive responses. H95(*Rp1-B*), H95(*Rp1-Dj4*), H95(*Rp1-Jc13a*), H95(*Rp1-JFC1*), H95(*Rp1-K*), H95(*Rp1-Kr3*), H95(*Rp1-Kr4*), H95(*Rp5*), and B113 showed excellent HR or R pustule type and disease severity rating 2; H95(*Rp1-A*), H95(*Rp1-C*), H95(*Rp1-D*), H95(*Rp1-M*), and B108 showed R or MR pustule types and disease severity rating 3; H95(*Rp1-J*), H95(*Rp1-Kr1-J92*), H95(*Rp4-A*), H95(*Rp4-B*), H95(*Rp5-D*), H95(*Rp7*), H95(*Rp8-A*), and CO428 showed MS or S pustule types, however, they still have some resistance because of less percentage of leaf area infected.

Rp-*G5, Rp-G5JCa, Rp-G15c, Rp-G6J1, Rp1-Jc13a,* and *Rp1-K* were used in AAFC corn program, backcrossed to CO325, CM105, and CO388. Early maturity resistance lines to common rust will be released in 2011 and 2012.

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Mapping candidate genes for *Fusarium* ear rot resistance

--Maschietto, V; Lanubile, A; Marocco, A.

Fusarium ear rot is one of the most important diseases of maize because Fusarium verticillioides produces mycotoxins fumonisins. Resistance to Fusarium ear rot is polygenic with nearly complete dominance or overdominance of resistance alleles. The availability of molecular markers associated to resistance genes could be a successful strategy to select lines resistant to F. verticillioides. Mapping of quantitative trait loci (QTLs) provides a powerful method to understand the genetic relationships between correlated traits.

We attempt to use a genetical-genomics strategy to localize candidate genes for resistance to F. verticillioides on the high-density molecular map.

Resistant (CO441; R) and susceptible (CO354; S) genotypes were selected and crossed to obtain F2 and F3 mapping populations. In previous works we found a high level of variability for the response to F. verticillioides infection between the two susceptible and resistant maize lines selected (Lanubile et al., J Plant Physiol 167:1398-1406, 2010; Lanubile et al., World Mycotox J 4:43-51, 2011). Similar functional categories of genes were involved in the response to infection in the two genotypes. In the resistant line, the defence-related genes assayed were transcribed at a high level before infection and provided basic defence to the fungus. In the susceptible line, the same genes were induced from a basal level and responded specifically to pathogen infection. The expression level of eleven genes involved in plant defence were validated by qPCR (Table 1). The candidate genes are physically mapped and the polymorphisms relative to 27 maize genotypes are available in http://www.panzea.org. About 70 primers were designed on the candidate sequences and SNPs will be identified between CO441 and CO354 parents.

In addition to SNP markers, 217 SSRs were selected, according to their chromosomic positions on the reference map (http://www.maizegdb.org), for genotyping F2 individuals. About 50%, or 110 SSRs, that were evenly distributed on the ten chromosomes became polymorphic (Table 1). Furthermore, 200 F3 progenies will be phenotyped for the response to artificial infection and fumonisin contamination.

Table 1. Number of polymorphic SSR markers and selected candidate genes.

Chromosome	SSRs	Candidate genes
1	12	Pathogenesis related protein 5 WRKY DNA-binding protein 1
2	12	-
3	6	Chitinase Pathogenesis related protein 6 Respiratory burst oxidase
4	11	-
5	13	MYB-like DNA binding protein
6	13	-
7	11	Pathogenesis related protein 1 Thaumatin-like protein
8	12	Wound-induced protease inhibitor
9	13	-
10	7	β-glucosidase Peroxidase 1

REGENSBURG, GERMANY
University of Regensburg

Growing maize using LED lighting

--Gahrtz, M; Coman, N; Ihringer, J; Dresselhaus, T

Growing maize year-round in greenhouses requires supplemental lighting. Because maize requires high light for adequate growth and yield, high intensity discharge (HID) lamps (high pressure sodium vapor and/or metal halide lamps) are commonly used. The drawback of these lamps is the high level of heat generated mainly by infrared light. This makes it difficult to maintain constant temperature profiles, especially in summer, and is a cause of heat stress during pollen development. The vast amount of heat produced by these lamps might not only be problematic for the plants; it also is a waste of energy and causes unnecessary CO2 emissions and energy costs. An alternative to HID lamps is light emitting diode- (LED-) based lighting systems. It has been shown that current LED lighting systems used for greenhouse illumination can cut energy costs by around 40%.

We wanted to investigate whether LED lighting is suitable and eventually superior to HID lamps in sustaining proper growth and development of maize in growth rooms and whether it is possible to use it as supplemental greenhouse lighting for maize.

In a growth chamber, 288 red LEDs and 144 blue LEDs (Golden Dragon Plus: Hyper Red, 660 nm and Deep Blue, 450 nm, Osram Opto Semiconductors, Regensburg, Germany) were mounted at the ceiling at a height of 2.45 m on an area of 4 m2. The overall power mounted per square meter was 205 W. In a control chamber HID illumination metal halide lamps (Philips HPI-T Plus, 400W) and high-pressure sodium vapor lamps (Philips SON-T Agro, 400W) were installed alternately with a total power of 523 W/m2. The respective spectra emitted from each type of lighting are shown in Figure 1. Both rooms were run at long-day conditions (16 hours light, 8 hours dark) and were set to 25° C during the day and night. In each chamber we grew six plants of maize inbred line H99 from germination to maturity. The photosynthetic active radiation in both rooms is given in `v and was found to be 1.35 times higher in the HID chamber than the LED chamber.

Figure 1. Lighting spectra of LED and HID growth chambers measured with the spectroradiometer Specbos 1211 (JETI Technische Instrumente GmbH, Jena, Germany).

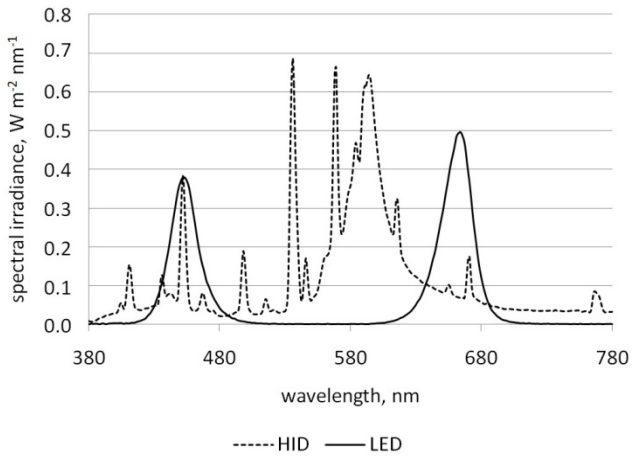


Figure 2. Vegetative growth and development of H99 maize inbred plants grown under LED and conventional HID lighting. The height of the uppermost leaf collar was measured as an indicator of plant height on the day when it appeared from the leaf sheath of the next lower leaf.

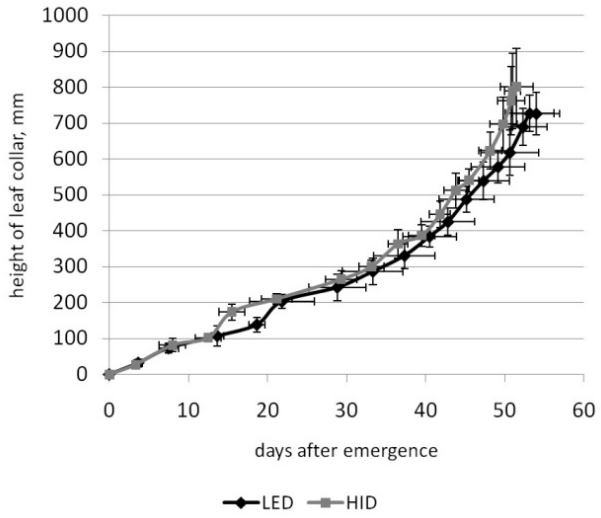


Figure 3. Phenotypes of H99 maize inbred plants grown in soil either under LED lighting or conventional HID lighting at 27, 49 and 69 days after emergence (dae) respectively.

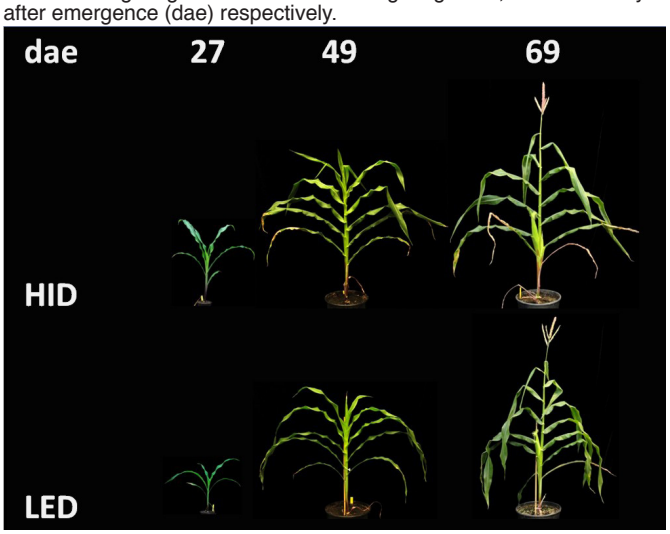


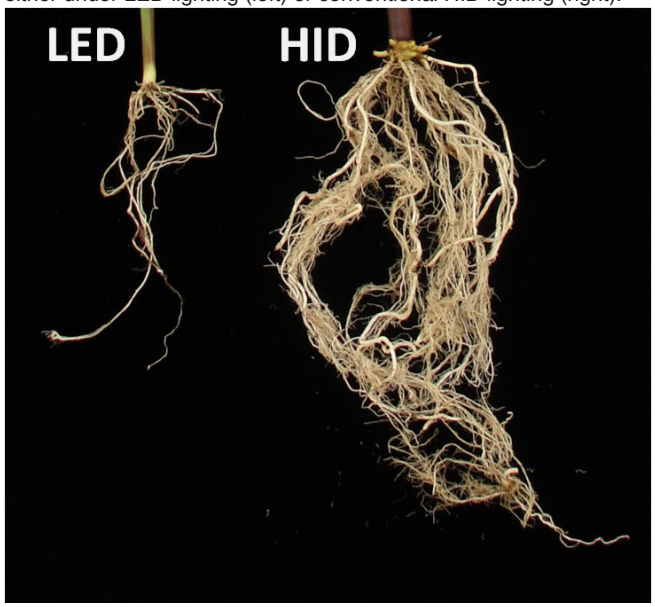
Table 1. Photosynthetic active radiation (PAR) in LED and HID growth chambers, respectively, and phenotypical aspects with respect to apparent photosynthesis rate, number of leaves, flowering time, plant height, and above-ground dry matter (biomass).

	PAR* (μmol m^{-2} s^{-1})	Photosynthesis** (μmol CO_2 m^{-2} s^{-1})	Number of leaves	Flowering time (days to silking)	Plant height (cm)	Biomass (g)
LED	212	10.04 ± 0.06	16.5 ± 0.5	58.3 ± 3.4	115 ± 5.7	79.2 ± 10.7
HID	287	15.16 ± 0,46	16.5 ± 0.5	56.3 ± 2.9	129 ± 8.8	93.3 ± 20.6

*Photosynthetic active radiation (PAR) at the height of 1 m
**Apparent photosynthesis rate at the uppermost expanded leaf, measured 50 days after germination around noon with the porometer LC*pro*+ (ADC Bioscientific Ltd., Great Amwell, UK). Since HID illuminated plants were on average 12 cm taller at the time of measurement, PAR intensity was up to 1.7 times greater at the leaf surface of HID-illuminated plants.

As expected, the apparent photosynthesis rate was higher (1.5 times) in the HID chamber. The HID plants were slightly taller (1.18 times) and produced slightly more above ground dry matter (1.12 times). Nevertheless, developmental aspects, like number of leaves or flowering time were unaltered (Table 1). As shown in Figure 2 and 3, the growth characteristics of the plants from both growth chambers are very similar. Nevertheless we found strongly reduced root growth in 4-week-old plants from the LED growth room (Fig. 4). However, adult plants of the HID and LED growth chambers had similar root penetration of the soil (data not shown). The lower photosynthesis rate of plants under LED illumination compared to HID illuminated plants is very likely responsible for the slight reduction in growth performance (height, biomass) and is a result of reduced light intensity. We therefore assume that the reduced growth performance might vanish or change into better performance under optimized light intensities and might play a minor or no role in a greenhouse, where lighting is only supplemental. Therefore, we conclude that a LED-based lighting system can be a very suitable means to support the growth of maize plants in a greenhouse, without the risk of overheating the greenhouse, better pollen performance, and with reduced energy costs. The LED lighting did not change developmental aspects of the maize inbred line H99. However, from these initial experiments we cannot conclude that other inbred lines will perform similar. Therefore we plan to test other maize inbred lines under the same and optimized conditions involving the usage of LEDs of different wavelengths.

Figure 4. Root phenotype of 28 day old maize plants grown in soil either under LED lighting (left) or conventional HID lighting (right).



SARATOV, RUSSIA
Saratov State University

Embryological peculiarities of tetraploid parthenogenetic maize forms

--Kolesova, AY; Tyrnov, VS

Apomixis is manifested, as a rule, in polyploids, so we started the work of producing analogues of the parthenogenetic lines. We investigated the tetraploid maize form, which is produced by pollinating the tetraploid form KrP-1 with the diploid parthenogenetic line AT-1. Line AT-1 is characterized by autonomous embryo- and endospermogenesis, and as result, the plants of this line form with high-frequency polyembryos and haploids. Producing the tetraploid parthenogenetic line became possible because sporadical unreduced male gametes formed at line AT-1, which lead to the development of tetraploid plants after diploid eggs of tetraploids were fertilized.

We carried out the analysis of 2800 embryo sacs (ES) of 18 plants. To do so we fixed isolated ears in 10 days after the stigma appeared. Additional elements (nuclei and cells) formed in the egg apparatus of ES or the autonomous development of embryos was noted in 16 plants. The frequency of ES development with additional elements in the egg apparatus varied from 0.5% to 5.9%. We discovered ES with four to 10 cells in the egg apparatus and ESs with one or rarely two embryos. Embryos were bicellular or globular and contained up to 92 cells. The eggs or synergids in 6 ES with a three-cellular egg apparatus contained one to four

additional nuclei.

ES with disturbed differentiation of egg apparatus were found in 13 plants. In the main the ES had a sinergid-like egg (~13.2%), in which the nucleus occupies a basal position. We found ES with egg-like synergid (~2.3%) and ES with undifferentiated egg apparatus. In six plants ES with 3, 4 and 6 polar nuclei were observed. Autonomous development of endosperm was found in four plants (~3.0%).

Antipodal complex cells in the ovules of all plants grew. Large, growing cells reaching up to two-thirds the size of the ES were always adjoined antipodes. The number of ES with growing cells was between 1.14% and 49.4%. Usually one to two cells were growing, rarely more (up to nine). The large cells contain more often one or two nuclei, the cells with three to 12 or more nuclei also have been observed (up to 66).

The analysis shows that the produced tetraploid form is characterized by autonomous development of embryo and endosperm. Atypical ES are like in many respects the ES of initial diploid parthenogenetic line AT-1.

Mitotic activity stimulation in apical root meristems of maize lines and hybrids under the influence of a low-frequency magnetic field

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

For various agricultural crops the effect of mitotic activity (MA) stimulation in apical root meristems of seedlings under the influence of a low-frequency magnetic field (MF) is established. It is shown that for different species various levels of MA stimulation can be observed. We already marked a stimulating effect of a low-frequency MF with the certain parameters in maize apical root meristems (Belyachenko et al., MNL 84:44, 2010).

Because different intraspecific units, including varieties and lines, are used in agricultural practice, comparison of intraspecific forms reactions to MF influence is necessary. The purpose of the given work consists in research of alternating MF action on maize lines and hybrids. The following parameters of MF were applied: frequency of 6 Hz, induction of 25 mT and 1 hour exposure.

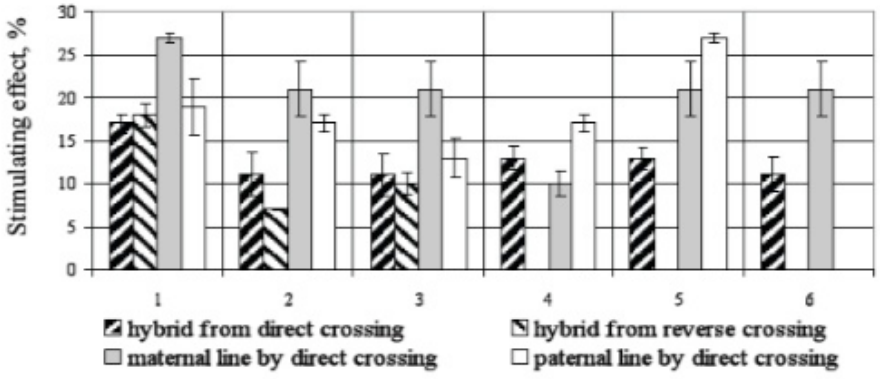
Dry maize seeds were exposed to MF influence. Root tips 1 cm to 1.5 cm long were fixed for cytological analysis. Amounts of cells at different stages of a cellular cycle were estimated on temporary acetocarminic squash preparations. In each of three repeatabilities it has been analysed not less than 3000 cells and mitotic index values were calculated.

Various maize lines and also hybrids from their reciprocal crossings were investigated (Fig. 1). Used lines: MT (Mangelsdorf's tester), AT-3, SPEM (Saratov purple embryonic marker), HDL-1 (haploid derivative line 1), PPf (purple precocious fasciated), Kr 703 (Krasnodar 703), PP-16 (purple precocious 16). In total 6 lines and 9 hybrids were explored.

It is shown, that lines can differ authentically among themselves on a level of MA stimulation under MF influence. It is possible to note that most often for hybrids the lower level of stimulating effect, in comparison with initial lines, is characteristic. Hybrids from reciprocal crossings only in one of three compared cases authentically differed among themselves on a level of MA stimulation.

MA stimulation under MF influence inheres in all lines and hybrids; however, its levels between parental lines and their hybrids can differ more than twofold. The variation of stimulation levels in plants with intraspecific genetic differences defines interest to more detailed comparison of plants with the distinct genetic constitution on their response to MF influence.

Figure 1. Stimulating effect of alternating MF on maize lines and hybrids MA in apical root meristems of seedlings (direct crossings:
1 – MT AT-3; 2 – SPEM HDL -1; 3 – SPEM PPf; 4 – Kr 703 HDL-1; 5 – SPEM MT; 6 – SPEM PP-16).



The new technology of maize breeding by parthenogenesis

--Tyrnov, VS; Smolkina, YV

With our current work we reveal a series of regularities of parthenogenesis in plants, which includes its occurrence in two forms: induced and hereditable (controlled by a parent form genotype).

A technology of haploids production on this basis induced reduced parthenogenesis with frequencies up to 3% to 12%, which was developed in our previous work.

Currently, haploid production technology with frequencies up to 50% to 100% is worked out. Alongside with haploids homozygous autodiploids, that is, lines without additional diploidization, arises up to 5% to 8% (more often 2% to 3%). An additional advantage of the given technology is that genetic factors of parthenogenesis are introduced into selection material and increases opportunities of diploid or polyploidy apomictic maize form development. Various elements of this technology have been previously published:

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- Embryogenetic regularities of haploidy in plants. Tyrnov VS, Proc of the XI Intl Symp Embryology and Seed Reproduction, p. 576-577, St. Petersburg: Nauka, 1992.
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- Single fertilization and haploinduction problem in maize. Enaleeva NH, Tyrnov VS, Selivanova LP, and Zavalishina AN, Proc Academy of Sciences of the USSR, 353-3:405-407, 1997.
- Haploidy in plants: terminology and classification (in Russian). Tyrnov VS, Saratov:Ed., p. 44, Saratov Univ, 2005.
- Reproductive biology, genetics and breeding (in Russian). Tyrnov VS, Haploidy and apomixes, Proc Conf Saratov:Ed., p. 32-46, Saratov Univ, 2002.
- Methods of haploids diagnostics in flowering plants (Study guide; in Russian). Tyrnov VS Saratov:Ed., p. 28, Saratov Univ, 2003.
- Possibilites of diagnosis of parthenogenesis by culture in vitro of unpollinated ovaries. Alatortseva TA and Tyrnov VS, MNL 71:75, 1997.
- Producing of parthenogenetic forms of maize. Tyrnov VS, MNL 71: 73-74, 1997.
- Cytological manifestation of apomixis in AT-1 plants of corn. Enaleeva NK and Tyrnov VS, MNL 71: 74, 1997.
- Development of seeds with haploid embryo on haploid plants of parthenogenetic line. Tyrnov VS, MNL 71:74-75, 1997.
- The occurrence of haploid on the second ears of parthenogenesis lines. Smolkina YV and Tyrnov VS, MNL 73:79-80, 1999.
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Incidence of male fertility in haploid elite dent maize germplasm

--Geiger, H; Schönleben, M

In vivo haploid induction presently is a widely used tool in maize research and breeding (Geiger, Handbook of Maize: Genetics and Genomics 641-657, 2009). However, virtually all haploid plants are male sterile and therefore cannot be selfed. In contrast many haploids display a certain degree of female fertility if pollinated by diploids (Chalyk, Euphytica 79:13-18, 1994; Geiger et al., MNL 80:28-29, 2006). To fully restore male and female fertility, the chromosome set of haploids needs to be doubled by mitosis-inhibiting substances like colchicine. However, artificial chromosome doubling is laborious and colchicine is extremely toxic. Spontaneous chromosome doubling occurs only at a rate of 1% to 10% (Chase, Iowa Acad Sci Proc 56:113-115, 1949; Beckert, Biotechnology in Agriculture and Forestry, 201-213, 1994). In the present newsletter we are reporting about two experiments in which we determined the male fertility of haploids in a genetically broad collection of actual elite maize breeding materials. One experiment was conducted in the field

(Exp. I) and one in the greenhouse (Exp. II).

Experiment I: Materials were composed of 23 haploid (H) lines which had been development from 22 dent doubled haploid (DH) lines and one flint DH-line by in vivo haploid induction. The H-lines were as uniform as their parental DH lines but smaller and less vigorous. All H-lines had been preselected for female fertility (Geiger et al., 2006). The experiment was laid out in a randomized block design with two replicates on single-row plots of 2.5 m length at Stuttgart-Hohenheim in 2007 (Fig. 1). Up to 20 seeds were drilled per row. Ten lines could not be grown in the second replicate due to lack of seed. No particular stress occurred during the growing season. Male fertility was assessed on a 1-4 scale defined as follows:

Figure 1. Haploid lines in the field compared with a vigorous hybrid; Exp. I, Hohenheim 2007.

Figure 2. Detail of a tassel of a haploid plant with anther score 2; Greenhouse, Hohenheim, 2007.

Score 1: One or very few extruding anthers, unbranched tassel.

Score 2: Several extruding anthers at the central spike; few lateral branches but without extruding anthers (Fig. 2).

Score 3: Multiple extruding anthers at the central spike and at most branches but no pollen release visible when touching the anthers.

Score 4: Many pollen-shedding anthers at all branches.

To avoid uncontrolled pollination, all ears were bagged before anthesis. Selfing was tried whenever a plant displayed any degree of male fertility. For this purpose, we bagged the tassel in the morning and pollinated the respective plant about four hours later. Before removing the bag from the tassel, the bagged tassel was carefully shaken to excite pollen release. No attempts were made to squeeze out pollen grains from closed anthers.

Experiment II: Materials consisted of H-plants developed by in vivo haploid induction from each of 70 single, three-way, double, or multiple crosses between inbred lines of the dent gene pools Iowa Stiff Stalk, Iodent, Lancaster, Longdent, and Danube. The crosses represented actual elite dent breeding material. Six H-plants per cross (420 in total) were raised in a greenhouse at Hohenheim in 10-liter pots during the summer in 2007. The experiment was laid out as a lattice design with six replicates. Forty-nine plants were removed before anthesis because they looked like heterozygous diploids. Optimal maize growing conditions (temperature, water supply, light, fertilizer) prevailed throughout the season. This was reflected in a faster growth and a taller and more vigorous stature than in the field. Male fertility assessment and selfing were conducted as in Exp. I.

To confirm haploidy, one plant per H-line in Exp. I and all plants in Exp. II were analyzed by flow cytometry (PARTEC instrument CA-II) For this purpose, tissue was taken from the youngest leaf in the 4-leaf stage. A second analysis was conducted during anthesis of all greenhouse plants with anther score 4. Tissue was taken from the top leaf. Microsatellite markers *phi011, phi072, umc1153, umc1887, phi032,* and *phi041*, located on chromosomes 1, 4, 5, 6, 9, and 10, respectively (http://www.maizegdb.org), were used to check the DH offspring of each successfully selfed H-plant for genetic uniformity. DNA extraction and marker assessment were

Table 1. Number of successfully selfed plants and means across these plants for anther score, number of kernels per ear, and plant height in nine haploid dent lines; Exp. I, Hohenheim 2007.

	Number of plants		Means across successfully selfed plants		
Line	Total	Successfully selfed	Anther score (1 - 4)[a]	Kernels per ear (No.)	Plant height (cm)
20	19	10	3,6	8,5	92
2	11	1	1,6	2,0	93
8	12	1	2,8	2,0	92
14	10	2	1,3	2,0	63
22	23	1	-[b]	2,0	92
1	10	2	1,0	1,0	66
7	11	1	1,0	1,0	87
18	19	2	1,1	1,0	93
21	11	2	1,0	1,0	109

[a] For score definitions see text.
[b] Missing value.

Table 2. Anther score, number of kernels per ear, and plant height of successfully selfed haploid plants yielding at least five kernels per ear; Exp.II, Hohenheim 2007.

Plant (No.)	Origin[a]	Anther score (1 - 4)[b]	Kernels per ear (No.)	Plant height (cm)
1	Da/La	3	23	162
2	Io/La	3	16	172
3	Ld/SS	3	11	160
4	Ld/SS	4	9	133
5	Ld/Io	4	8	173
6	Ld/Io	2	6	113
7	NN/SS	1	6	138
8	La	2	6	183
9	SS/Ld	2	6	125
10	Io/La	1	5	123
11	Io/La	3	5	151
12	Io/La	1	5	180

[a] Da = Danube, Io = Iodent, La = Lancaster, Ld = Longdent, SS = Iowa Stiff Stalk,
NN = Unknown.
[b] For score definitions see text.

performed according to standard protocols. An ALFexpress sequencer was used for visualizing the marker genotypes.

Results: In the field, 412 plants displaying the typical morphology of haploids were inspected for extruding anthers. About half of them (212 plants) conformed to anther scores 1, 2, or 3. They were found in 10 of the 23 tested H-lines. We attempted to self these plants repeatedly over two or three successive days. Twenty-nine of them (7% of all 412 plants) belonging to 9 lines set one or more seeds per ear (Table 1). By far the highest seed set occurred in line 20, in which 10 out of 19 plants were successfully selfed. Seed set ranged among these plants from 1 to 12 (85 seeds in total). In the other 8 lines only 1 to 2 plants per line set seed. A moderate correlation existed between anther score and seed set ($r = 0.56$, $P < 0.01$).

In the greenhouse, 248 out of 371 inspected haploid plants displayed some degree of male fertility. In all these cases, selfing was attempted in the same way as in the field experiment. Twenty-seven plants (7.3% of all tested plants) showed seed set. With three of these plants, the number of kernels per ear amounted to 23, 16, and 11, respectively, and 9 plants had 5 to 9 seeds per ear (Table 2, Figs. 3 & 4). The successfully selfed plants originated from 21 parental crosses comprising all tested gene pool

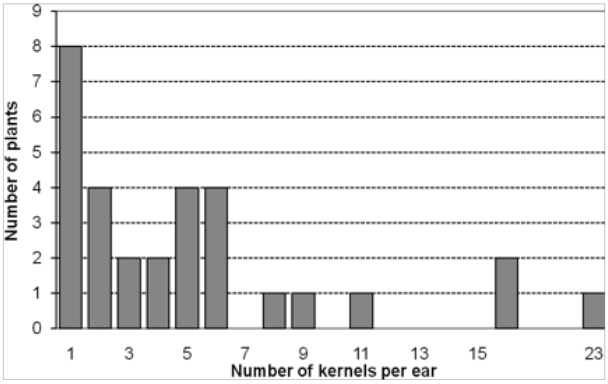

Figure 3. Frequency distribution of the number of kernels per ear obtained from the successfully selfed plants in Exp.II, greenhouse, Hohenheim 2007.

combinations. The correlation between anther score and seed set was only weak ($r = 0.30$, $P < 0.01$).
All visually classified haploids were confirmed by flow cytometry, and in Exp. II molecular marker analysis attested the genetic uniformity of all progenies resulting from self-pollination.

Results confirm the pioneering work of Chase (Heterosis, 1952) and later reports of Zabirova et al. (MNL 67:67, 1993), Chalyk (1994) and others. Beyond that, our flow cytometry and molecular marker studies for the first time proved that the selfed plants actually were haploid and gave rise to genetically uniform progenies. It remains unclear whether the functional male and female gametes arose by meiotic restitution (Ramana and Jacobsen, Euphytica 133:3-18, 2003) or by chromosome doubling due to endomitosis in meristematic cells of the reproductive tissue leading to diploid sectors in the gametophyte (Chase, 1952).

In both of our experiments, we found significant genetic variation for male fertility and selfing success in haploids. Recurrent selection (RS) for these characteristics therefore seems promising. This is supported by results of Zabirova et al., (1993) who obtained a remark-

able increase of male fertility after four generations of selection for male fertility in a Russian dent population. Thus breeders might not need to go back to accessions from the world collection to improve their elite gene pools in this regard.

Figure 4. Ears of two selfed haploid plants (No. 1 & 2 in Table 2); Exp. II, Hohenheim, 2007.

In conclusion, our studies indicate that in the long run breeders might be able to improve the degree of male fertility in haploids to an extent that would allow them to eventually abolish the laborious and hazardous artificial chromosome doubling step of the present maize DH technology.

The authors are indebted to the seed companies KWS Seed AG and Limagrain Europe for providing the haploid seeds used in Experiments I and II, respectively. Furthermore, we acknowledge the careful technical assistance of J. Jesse (field), H. Brandt and S. Spiess (greenhouse), M. Takacs (marker lab), and M.D. Braun (flow cytometry).

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A *Wc1* allele isolated from the maize variety Caragua
--Stinard, PS

OE White (Amer Jour Bot 4:396-406, 1917) isolated a dominant white endosperm mutant that he named “A” from the maize variety Zea Caragua. Emerson, Beadle, and Fraser (Cornell Univ Agric Exp Stn Memoir 180:1-83, 1935) list a dominant white endosperm mutant called “Wh” and cite both White (1917) and Hayes (Am Nat 67:75, 1933) as references in their gene list. Emerson, Beadle, and Fraser present linkage data of Hayes (1933 and unpublished) that places Wh on chromosome 7 (33% recombination with *gl1* and 40% recombination with *sl1*). However, Hayes (1933) does not describe the origin of *Wh* or cite White (1917) as a source, so the relationship between *A* of White and *Wh* of Hayes is unknown, despite the Emerson, Beadle, and Fraser citations.

If the linkage data of Hayes is correct, there exists the possibility of two independent dominant white endosperm loci in maize, *A/Wh* on chromosome 7, and *Wc1* on chromosome 9. Because stocks of *A* and *Wh* are no longer in existence, and because Hayes does not cite the source of *Wh*, the only remaining possibility for reisolating this gene is to examine accessions of Zea Caragua. For this purpose, the only accession of Caragua maize available from the North Central Regional Plant Introduction Station (PI 485411 - *Zea mays subsp. mays* - Chile 311 - Santiago, Chile), an accession with white endosperm, was obtained, and crosses were made to a *Wc1* stock. If the Caragua line carries a dominant white endosperm mutant independent of *Wc1*, then 3:1 segregation for dominant white to yellow should be observed when the F1 is crossed by a yellow endosperm line. We performed this cross last summer. Of a total of 5245 progeny kernels obtained from this cross, all were dominant white capped—no yellow kernels were observed. On the basis of this test, we conclude that the dominant white from Caragua maps to the same location as *Wc1* with a separation of less than 0.038 +/- 0.027 centiMorgans and is undoubtedly allelic to *Wc1* if not identical to it. We have named the *Wc1* allele isolated from PI 485411 *Wc1-Caragua*. We have not disproved the existence of a second dominant white endosperm in maize, but it seems unlikely at this point.

Allelism testing of miscellaneous stocks in Maize Coop phenotype only collection

--Stinard, PS; Jackson, JD

This report summarizes recent allele testing of miscellaneous stocks characterized by phenotype only in the Maize Genetics Cooperation Stock Center [MGCSC] collection. Crosses were made between known heterozygotes where possible. Plants from the *indeterminate1, lazy1,* and *ramosa* crosses were scored in the field at maturity. Kernels were scored on the ear for the *etched1* and viviparous crosses. Kernel samples from the *etched1* crosses were planted in sand benches to confirm virescent phenotype. New designations have been assigned to these alleles. These stocks have been increased and placed on our stock list. It is expected that with further sorting and allelism testing of mutations characterized by phenotype only, additional alleles of characterized mutants will be discovered and placed in the main collection.

Data from the *etched1* and viviparous crosses were compiled by Janet Day Jackson before her sudden and untimely death (Table 1).

Table 1. Data from the *etched1* and *viviparous* crosses.

Previous designation	Tested against	New designation	MGCSC stock #
id-PI245132*	*id1*	*id1-PI245132*	132A
la-N2333B*	*la1*	*la1-N2333B*	403E
la-03HI-B73GN-119*	*la1*	*la1-03HI-B73GN-119*	403F
ra-JD*	*ra1; ra2; ra3*	*ra3-JD*	725B
wl-N122*	*et1*	*et1-N122*	307F
su-N748A*	*et1*	*et1-N748A*	307G
et-8616*	*et1*	*et1-8616*	307H
et-73-766-1*	*et1*	*et1-73-766-1*	307I
sml-et-85-3522-29*	*et1*	*et1-85-3522-29*	307J
pale-y-89-1313-3*	*vp9*	*vp9-89-1313-3*	722K
w-vp-85-3304-13*	*vp2*	*vp2-85-3304-13*	513FA
y-vp-1999-059-10*	*vp2*	*vp2-1999-059-10*	513FB

Inbred W23 carries a heritable factor that allows dominant expression of *ws*-N947C*

--Stinard, PS

During the course of propagating the reportedly recessive white-sheath mutant *ws*-N947C* (Maize Genetics Cooperation Stock Center stock number 4508K), we outcrossed green heterozygous *ws*-N947C* plants to various nonmutant standard lines to increase vigor. When F1's were planted in our selfing blocks for increase, we noted that outcrosses to hybrids made with W23 as one of the inbred parents segregated for white sheath plants. Outcrosses to standards not carrying W23, such as the inbred *B73*, produced only green plants. To study this phenomenon further we outcrossed green heterozygous *ws*-N947C* plants to both inbred W23 and to *B73*. Again, only outcrosses to W23 segregated for white sheath plants in the F1 (Mutant seedlings can be observed in sand-bench plantings, but expression improves as the plants mature, so field plantings are preferable for scoring for this mutant.)

To further characterize the interaction between W23 and *ws*-N947C*, white sheath plants from F1 crosses with W23 were crossed both as male and female parents with W23 and *B73*. Regardless of the direction of the cross, crosses of mutant plants to W23 segregated approximately 1:1 for green and white sheath plants, and all crosses with *B73* were green. To carry the analysis one generation further, male and outcrosses of white sheath plants to *B73* were reciprocally crossed with W23, and the progeny were tested in both sand bench and field plantings. About a quarter of the crosses produced white sheath progeny regardless of whether the initial F1 cross with *B73* was made as a male or a female. The segregation ratios observed approached a 3:1 ratio of green to white sheath plants (actual data: *B73* as male parent in F1: 65 green : 22 white sheath; *B73* as female parent in F1: 56 green : 24 white sheath).

Two conclusions can be drawn from these data: i) W23 carries a single genetic factor, which in the homozygous condition, allows *ws*-N947C* to express in a dominant manner as a heterozygote; ii) The factor in W23 that allows dominant expression of *ws*-N947C* is nuclear and not cytoplasmic because *ws*-N947C* can express dominantly in either *B73* or W23 cytoplasm. We do not yet know whether the factor from W23 is necessary for *ws*-N947C* expression (i.e., Will homozygous *ws*-N947C* plants produce a mutant phenotype in the absence of the W23 factor?) In order to address this question *ws*-N947C* will be further crossed into *B73* and outcrossed to W23 during each generation of introgression to determine whether it is possible to obtain selfs that segregate for recessive *ws*-N947C* yet produce only green plants in outcrosses to W23.

Linkage tests of *waxy1* marked T3-9 reciprocal translocations

--Jackson, JD; Stinard, PS

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

Additional translocation stocks will be tested as time allows.

We report here the positive results of two- and three-point linkage tests for two of these translocations: *wx1 T3-9(034-11)* (Tables 1-3) and *wx1 T3-9g* (Tables 4 & 5). The linkage tests were set up as modified backcrosses as indicated. Findley source of *wx1 T3-9g* showed no linkage with its appropriate markers and was discarded. The data reported here were compiled by Janet Day Jackson before her sudden and untimely death.

Table 1. *wx1 T3-9(034-11)* (3L.46; 9S.36), E.G. Anderson source #1.

Two-point linkage data for *gl6 - wx1 T3-9(034-11)*. Testcross: *gl6 wx1* X [*Gl6 wx1 T3-9(034-11)* X *gl6 Wx1 N*].

Region	Phenotype	No.	Totals
0	Wx gl	391	
	wx Gl	471	862
1	Wx Gl	76	
	wx gl	90	166
Total			1028

% recombination *gl6 – wx1* = 16.1 +/- 1.1%

Table 2. *wx1 T3-9(034-11)* (3L.46; 9S.36), E.G. Anderson source #1.

Three-point linkage data for *gl6 – lg2 - wx1 T3-9(034-11)*.
Testcross: [*Gl6 Lg2 wx1 T3-9(034-11)* X *gl6 lg2 Wx1 N*] X *gl6 lg2 wx1 N.*

Region	Phenotype	No.	Totals
0	gl lg Wx	183	
	Gl Lg wx	189	372
1	gl Lg wx	71	
	Gl lg Wx	68	139
2	Gl Lg Wx	24	
	gl lg wx	32	56
1 + 2	gl Lg Wx	3	
	Gl lg wx	16	19
Total			586

% recombination *gl6 – lg2* = 27.0 +/- 1.8
% recombination *lg2 – wx1* = 12.8 +/- 1.4
% recombination *gl6 – wx1* = 39.8 +/- 2.0

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Table 3. *wx1 T3-9(034-11)* (3L.46; 9S.36), E.G. Anderson source #2.

Note: This source of *wx1 T3-9(034-11)* was maintained independently of the source tested in Tables 1 & 2 due to a pedigree error labeling it as *T8-9(034-11)*.

Three-point linkage data for *gl6 – lg2 - wx1 T3-9(034-11)*.
Testcross: [*Gl6 Lg2 wx1 T3-9(034-11)* X *gl6 lg2 Wx1 N*] X *gl6 lg2 wx1 N.*

Region	Phenotype	No.	Totals
0	gl lg Wx	557	
	Gl Lg wx	580	1137
1	gl Lg wx	73	
	Gl lg Wx	63	136
2	Gl Lg Wx	48	
	gl lg wx	53	101
1 + 2	gl Lg Wx	0	
	Gl lg wx	10	10
Total			1384

% recombination *gl6 – lg2* = 10.5 +/- 0.8
% recombination *lg2 – wx1* = 8.0 +/- 0.7
% recombination *gl6 – wx1* = 18.6 +/- 1.0

Table 4. *wx1 T3-9g(F-24)* (3L.40; 9L.14), Robertson source. Three-point point linkage data for *gl6 - lg2 - wx1 T3-9g*. Testcross: [*Gl6 Lg2 wx1 T3-9g* X *gl6 lg2 Wx1 N*] X *gl6 lg2 wx1 N.*

Region	Phenotype	No.	Totals
0	gl lg Wx	69	
	Gl Lg wx	49	118
1	gl Lg wx	4	
	Gl lg Wx	2	6
2	Gl Lg Wx	13	
	gl lg wx	2	15
1 + 2	gl Lg Wx	0	
	Gl lg wx	3	3
Total			142

% recombination *gl6 – lg2* = 6.3 +/- 2.0
% recombination *lg2 – wx1* = 12.7 +/- 2.8
% recombination *gl6 – wx1* = 19.0 +/- 3.3

Table 5. *wx1 T3-9g(F-24)* (3L.40; 9L.14), Robertson source. Two-point linkage data for *lg2 - wx1 T3-9g*. Testcross: [*Lg2 wx1* T3-9g X *lg2 Wx1* N] X *lg2 wx1* N.

Region	Phenotype	No.	Totals
0	Wx lg	400	
	wx Lg	394	794
1	Wx Lg	56	
	wx lg	17	73
Total			867

% recombination lg2 – *wx1* = 8.4 +/- 0.9

New alleles of *enr1* and *enr2*

--Stinard, PS

Stinard (MNL 81:33-35, 2007) describes two *r1* haplotype-specific enhancers of aleurone color isolated from an accession of *R1-r(Venezuela459#16039)* from the Brink *r1* haplotype collection donated to the Maize Genetics Stock Center by Jerry Kermicle. The enhancers were linked, with a separation of 25.2 centiMorgans. One enhancer, *Enr*-459A*, expressed equally well in male and female outcrosses with susceptible *r1* haplotypes, and the other enhancer, *Enr*-459B*, expressed only in female outcrosses. Chromosomal location was unknown, and their relation to the characterized enhancer loci *enr1* and *enr2* (Stinard et al., J Hered 100:217-228, 2009) was not known. Stinard et al. (2009) found *enr1* and *enr2* to be separated by 23.4 centiMorgans in direct linkage tests and expression patterns of *Enr1* and *Enr2* alleles match that of *Enr*-459A* and *Enr*-459B*, respectively. Linkage tests of *Enr*-459A* and *Enr*-459B* were subsequently performed with *enr1* and *enr2* and the results are reported here.

Because the phenotype of *Enr*-459A* most closely matches that of *Enr1* alleles with respect to good expression in both male and female outcrosses, it was mapped only with respect to *enr1*. A homozygous *r1-g(Stadler) Enr*-459A* line was crossed to a homozygous *r1-g(Stadler) Enr1-628* line, and F1 plants were outcrossed as males to responsive homozygous *enr1 R1-r(Venezuela559-PI302355)* testers. The parental classes and the double factor recombinant class would be expected to have full color aleurones, and the recombinant class lacking both factors would be expected to have pale aleurones. Kernels from these crosses were scored for aleurone color, and exceptional pale or colorless kernels were subsequently planted and the resulting plants selfed and outcrossed to *enr1 R1-r(Venezuela559-PI302355)* testers to confirm genotypes. Of 13 exceptional kernels obtained from a population of 5166 kernels, only 7 could be tested and all were found to be parental types. Therefore, no recombinants were found from an effective population of 2872 kernels, indicating a separation of less than 0.07 +/- 0.05 centiMorgans. We conclude that *Enr*-459A* and *Enr1-628* map to the same location and are most likely allelic. *Enr*-459A* has been renamed *Enr1-459A*.

The female-only expression of *Enr*-459B* matches that of other *Enr2* alleles, and therefore *Enr*-459B* was mapped only with respect to *enr2*. A homozygous *Enr*-459B R1-r(Venezuela559-PI302355)* line was crossed to a homozygous *Enr2-694 R1-r(Venezuela559-PI302355)* line, and F1 plants were crossed as females by homozygous *enr2 R1-r(Venezuela559-PI302355)* testers. The parental classes and the double factor recombinant class would be expected to have full color aleurones, and the recombinant class lacking both factors would be expected to have pale aleurones. Kernels from these crosses were scored for aleurone color, exceptionally pale kernels were subsequently planted, and the resulting plants were crossed as females by *enr2 R1-r(Venezuela559-PI302355)* testers to confirm genotypes. Of three exceptional pale kernels obtained from a population of 3712 kernels, only two could be tested and both were found to be parental types. Therefore, no recombinants were found from an effective population of 2475 kernels, which indicates a separation of less than 0.08 +/- 0.06 centiMorgans. We conclude that *Enr*-459B* and *Enr2-694* map to the same location and are most likely allelic. *Enr*-459B* has been renamed *Enr2-459B*.

Two-point linkage data for *wx1*- and *Wx1*- marked translocations in the Maize Coop's collection: Ten years of data collected and compiled by Janet Day Jackson (1958-2010)

--Jackson, JD; Stinard, PS; Zimmerman, SA; Sachs, MM

This report summarizes the results of linkage tests between translocation breakpoints (*T*) and *wx1* for *wx1*-(Table 1) and *Wx1*-marked (Table 2) translocations in the Maize Genetics Cooperation Stock Center's collection. Special attention was given to those translocations for which *T – wx1* recombination data had not previously been reported. See Anderson (Genetics 23:307-313, 1938), Patterson (MNL 26:8-12, 1952), Patterson (MNL 32:54-66, 1958), Anderson et al. (MNL 39:106-109,1965), Jackson (MNL 74:70, 2000), Jackson et al. (MNL 75:68-71, 2001), Jackson et al. (MNL 76:65-67, 2002), and Jackson et al. (MNL 76:67-68, 2002) for previous reports of *T – wx1* recombination data.

Linkage crosses were set up as indicated in Tables 1 & 2. Kernels from test cross ears were separated into starchy and waxy classes, planted in our summer crossing nurseries, and the resulting plants were self-pollinated. Selfed ears were scored for heterozygous (Wx) versus homozygous (wx) *wx1*, and for normal (N) versus semisterile (¥) seed set. Values and standard errors for the percent recombination between *wx1* and the translocation breakpoints were calculated according to the method of Coe (The Maize Handbook, Freeling and Walbot (eds.), 189-197, 1994).

In theory, linkage values should be the same for a given translocation regardless of genetic background (M14 or W23) or whether the translocation is marked with *wx1* or *Wx1*. In many instances this is the case; however, there are some exceptions. In some instances, the genetic background could have an effect (Patterson EB, 1952. Ph.D. Dissertation. California Institute of Technology, Pasadena, CA). In other instances, it is possible that a planting error resulted in the substitution of one translocation for another during propagation. Where

Table 1. Two-point linkage data for *wx1*-marked translocations in the Stock Center's collection. The inbred conversion used in the linkage tests is indicated (M14, W23, or combined M14 and W23 data). Testcross: *wx1 N1 X1* [*Wx1 N1 X1 wx1 T1*].

Translocation	Breakpoints	Parentals		Recombinants		Totals	% recombination *T – wx1*
		Wx N	wx ¥	Wx ¥	wx N		
wx1 T1-9c ^W23	9L.22 1S.48	442	448	39	30	959	7.2 +/- 0.8
wx1 T1-9(5622) combined data	9L.10 1L.10	331	252	48	36	667	12.6 +/- 1.3
wx1 T3-9(8562) ^W23	9L.22 3L.65	527	516	23	16	1082	3.6 +/- 0.6
wx1 T5-9(022-11) ^M14	9L.27 5S.30	111	115	5	5	236	4.2 +/- 1.3
wx1 T5-9c ^W23	9L.10 5S.07	96	98	5	5	204	4.9 +/- 1.5
wx1 T5-9d ^M14	9L.10 5L.14	70	73	2	6	151	5.3 +/- 1.8
wx1 T5-9d ^W23	9L.10 5L.14	104	108	7	8	227	6.6 +/- 1.6
wx1 T6-9e ^W23	9L.24 6L.18	171	169	25	13	378	10.1 +/- 1.5
wx1 T8-9d ^M14	9S.16 8L.09	42	42	4	0	88	4.5 +/- 2.2
wx1 T8-9d ^W23	9S.16 8L.09	209	196	8	4	417	2.9 +/- 0.8
wx1 T9-10(059-10) ^W23	9S.13 10S.40	166	125	6	5	302	3.6 +/- 1.1

Table 2. Two-point linkage data for *Wx1*-marked translocations in the Stock Center's collection. The inbred conversion used in the linkage tests is indicated (M14, W23, or combined M14 and W23 data). Testcross: *wx1 N1 X1* [*wx1 N1 X1 Wx1 T1*].

Translocation	Breakpoints	Parentals		Recombinants		Totals	% recombination *T – wx1*
		Wx ¥	wx N	Wx N	wx ¥		
Wx1 T3-9c ^W23	9S.20å 3S.15	243	200	8	8	459	3.5 +/- 0.9
Wx1 T5-9c ^M14	9L.10 5S.07	484	467	10	40	1001	5.0 +/- 0.7
Wx1 T5-9c ^W23	9L.10 5S.07	348	372	44	49	813	11.4 +/- 1.1
Wx1 T6-9b ^W23	9S.37 6L.10	475	475	15	13	978	2.9 +/- 0.5
Wx1 T7-9(4363) ^M14	9.ctr 7.ctr	906	854	18	23	1801	2.3 +/- 0.4
Wx1 T9-10(8630) ^M14	9S.28 10L.37	388	406	9	19	822	3.4 +/- 0.6
Wx1 T9-10(8630) ^W23	9S.28 10L.37	232	201	6	3	442	2.0 +/- 0.7

possible, linkage tests have been conducted with genetic markers on the relevant chromosome arms to at least confirm the chromosomes involved in the translocations. See the many MNL reports of Janet Day Jackson et al. (MNL 72:79-81, 1998; MNL 72:81-82, 1998; MNL 73:86-88, 1999; MNL 73:88-89, 1999; MNL 74:67, 2000; MNL 74:67-69, 2000; MNL 75:67, 2001; MNL 75:68-71, 2001; MNL 76:65-67, 2002; MNL 76:67-68, 2002; MNL 77:79, 2003; MNL 77:80, 2003; MNL 78:65-66, 2004; MNL 79:47, 2005; MNL 79:48, 2005; MNL 80:29, 2006; MNL 80:30, 2006; MNL 84: In press) for summaries of these data.

Which seed color gene of *r1* responds to inhibitors (*Inr*) and enhancers (*Enr*) of aleurone color?

--Stinard, PS

Stinard and Sachs (J Hered 93:421-428, 2002) and Stinard et al., (J Hered 100:217-228, 2009) recently reported on *r1* haplotype-specific inhibitors and enhancers of aleurone color. Genetic analysis of the *R1-r(sd2)* (*spotted dilute*) haplotype led Stinard et al., (2009) to conclude that one of the seed color genes (*S1* or *S2*) of the seed color (*S*) subcomplex of *R1-r(sd2)* contains a *dSpm* insert, and the other seed color gene responds to dominant alleles at inhibitory (*Inr*) or enhancing (*Enr*) loci (see Figure 4 of Stinard et al., 2009, for a proposed model). However, it was unclear which seed color gene carries the *dSpm* insert and which gene responds to *Inr* and *Enr*. Intragenic recombination experiments were designed in order to answer this question.

Intragenic recombination has been used a means of dissecting the structure and function of the genic components of the compound *r1* locus (See Kermicle in Plant transposable elements, 81-89, 1988, for a review.) The *S* subcomplex of *R1-r(Std)*, the presumed progenitor of *R1-r(sd2)*, carries duplicate genes, *S1* and *S2*, which are arranged as inverted repeats flanking a promoter region (Walker et al., Embo J 14:2350-2363, 1995). Both *S1* and *S2* are expressed in the aleurone, so a mutation in one of the *S* genes of *R1-r(Std)* would not be expected to give a visible phenotype. However,

intragenic recombination knocking out the *S2* gene followed by genetic analysis of the derivatives can help answer the question of which gene in *R1-r(sd2)* carries a *dSpm* insert and which one responds to inhibitors and enhancers. For these experiments, the *r1-sc:m3* allele was chosen to recombine with *S2*. *r1-sc:m3* carries a *Ds* insertion in the simplex *Sc* (aleurone *Self-color*) gene, which is homologous to and in direct orientation with respect to *S2* on chromosome 10 (Kermicle, Science 208:1457-1459, 1980; Walker et al., 1995). Recombination between *S2* and *r1-sc:m3* proximal to the *Ds* insertion point would result in the transfer of the *Ds* element to *S2*, and knock it out and at the same time as placing it under control of *Ac,* should *Ac* be present in the genome. Experiments were devised to discriminate between the following two possibilities:

Possibility 1: *S1* responds to *Inr* and *Enr* alleles. *r1-sc:m3* was crossed to *R1-r(Std)* (genotype *S1 S2*). The F1 was crossed by *r1-g Enr1-Fcu Inr1*. If *Ds* is transferred to *S2* by intragenic recombination, and if *S1* responds to the mutable enhancer allele *Enr1-Fcu* and the inhibitory *Inr1* allele, then the derivatives carrying *s2::Ds* with *S1* would be expected to show aleurone color sectoring on a pale background in the presence of *Enr1-Fcu* and *Inr1*. Nonrecombinants would have full aleurone color due to the presence of the uninhibited, nonmutant *S2* gene. This cross was performed in 2008, and out of a total of 10,288 scorable kernels (with aleurone color), 11 mottled exceptions were obtained. Subsequent testing of the exceptions revealed that the mottling was not heritable in 10 of the exceptions—they were actually parental *R1-r(Std)* types. One exception did not germinate. Therefore, from an effective population of 9,353 kernels, no visible recombinants were obtained. These negative results could mean that either the population size was not sufficient to isolate the desired recombinants, or that *S1* does not respond to *Enr* and *Inr* alleles.

Possibility 2: *S2* responds to *Inr* and *Enr* alleles. *r1-sc:m3* was crossed to *R1-r(sd2)*. The F1 was crossed by *r1 inr1 Spm*. If *Ds* is transferred to *S2* by intragenic recombination, and if *S1* of *R1-r(sd2)* carries a *dSpm* insert, then the derivatives carrying *s2::Ds* with *s1::dSpm* would be expected to show typical small revertant sectors (*S1 s2::Ds*) on a colorless (*s1::dSpm s2::Ds*) background (no *Ac* present). Nonrecombinants (*s1::dSpm S2*) would have full aleurone color since an inhibitory *Inr1* allele was absent from this cross. This cross was performed in 2009, and out of a total of 8,740 scorable kernels (with aleurone color), 3 mutable exceptions were obtained, for a frequency of 3.4×10^{-4}. Subsequent testing of the exceptions revealed that all three were heritable. Two of the exceptions also responded to *Ac*, which is what would be expected if *Ds* was transferred to *S2* by intragenic recombination (*s1::dSpm s2::Ds)*; one exception did not respond to *Ac*. The non-responder to *Ac* could represent a spontaneous mutation in *S2* (*s1::dSpm s2*) rather than a recombination event. The frequency of *Spm Ac* double responders was 2.3×10^{-4}.

The recovery of the expected and heritable events from the latter intragenic recombination experiment demonstrates that it is the *S2* gene that responds to *Inr* and *Enr* alleles, and the *S1* gene of *R1-r(sd2)* carries a *dSpm* insert. The frequency of recombination between *Sc* and *S2* (2.3×10^{-4}) is comparable with that observed between other components of the *r1* gene complex (e. g. 4.49×10^{-4} between the *P1* (*Plant color*) gene and *r1-sc:m3* restoring kernel color (Dooner and Kermicle, Genetics 113:135-143, 1986), and 3.93×10^{-4} and 3.68×10^{-4} for recombination between *P1* and *S2* in *R1-r(Std)* resulting in deletion of sequences between *P1* and *S2* (Dooner and Kermicle, Genetics 67:427-436, 1971).

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Characterization of two regulators of aleurone mottling—Summary of research initiated at the University of Wisconsin

--Goncalves-Butruille, M; Stinard, PS; Sachs, MM; Kermicle, JL

Kernel pigmentation in certain accessions of open-pollinated varieties from the Four Corners region of southwest United States is exceptional in that mottled aleurone is true breeding. However, F1 kernels resulting from outcrosses with various stocks, including *r1* testers, are fully colored. A provisional test of inheritance was performed by crossing two such collections, Osage and Kokoma, to *R1-sc:124* in W22 background. F2 kernel progeny gave a 63:1 ratio of full color to dark and light mottled as expected for a three gene difference (Table 1; see Stinard et al., MNL, this issue, for companion article). This outcome would result if the accessions carried three recessive factors relative to the *R1-sc:124* stock: two regulators of mottling, provisionally termed *mot1* and *mot2*, and a responsive *r1* haplotype. The *r1* haplotype in these accessions proved to be of the *R1-d* class, which is subject to dilution by dominant modifiers (Stinard and Sachs, J Hered 93:421-428, 2002).

An *r1-g mot1 mot2* tester was developed by backcrossing the Hopi mottled variants for four generations to a W22 inbred conversion of *r1-g(Stadler)*, a colorless seed and plant haplotype. The tester was selfed and crossed during each generation of introgression to a true breeding mottled stock to confirm the presence of the mot factors.

Twelve *R1-d* haplotypes isolated from geographic locations spread across western North America and the genetic stock *R1-d*(Arapaho) were chosen to further

characterize interaction between the *R1-d* class of haplotypes and the *mot* factors. The 13 *R1-d* stocks, each carried in W22 background, were crossed to the *r1-g mot1 mot2* tester to make an F1. These F1s (genotype *R1-d r1-g; Mot1 mot1; Mot2 mot2*) were then reciprocally crossed to the *r1-g mot1 mot2* tester. In backcrosses with the F1 as female, six of the haplotypes showed a ratio of 4 colorless (cl) to 3 dark mottled (DMT) to 1 light mottled (LMT) expected if three factors were segregating (Table 2). Only 3 ears out of 28 crosses had chi-square values showing deviation from this ratio. The remaining seven haplotypes did not segregate for an obvious light mottled class when the *R1-d* haplotype-carrying parent was used as a female, which indicates a lack of sensitivity of the *R1-d* haplotype to the *mot* factors in female backcrosses. These crosses showed a 1:1 segregation for colorless to dark mottled (Table 3). Only 3 ears out of 34 had chi-square tests that showed significant deviation from a 1:1 ratio. However, the male backcrosses of all twelve haplotypes showed sensitivity of the *R1-d* haplotype to the mot factors.

Table 1. Kernel counts from F2 ears of the cross of W22 *R1-sc:124* X Hopi mottled accessions.
DMT = full colored and dark mottled. LMT = light mottled. Chi-square for 63 DMT : 1 LMT.

Source	R1-d haplotype	DMT	LMT	Chi-square	Significance
GB 871-1	Osage	452	9	0.455	NS
GB 871-2	Osage	570	11	0.413	NS
GB 872-1	Kokoma	446	6	0.162	NS
GB 872-2	Kokoma	456	6	0.209	NS
GB 872-3	Kokoma	454	11	1.950	NS
GB 872-4	Kokoma	446	10	1.179	NS
GB 872-5	Kokoma	448	8	0.109	NS
GB 872-6	Kokoma	533	10	0.275	NS

Since we know from the male backcrosses that these *R1-d* haplotypes are capable of responding to the mot factors, imprinting or a dosage effect is indicated to have caused stronger expression of the aleurone color of these *R1-d* accessions when passed through the female in the presence of the mot factors. We call this the "strong imprinting response." On the other hand, the *R1-d* haplotypes responding to the mot factors when passed through the female can be said to have a null or weak response to imprinting on the female gametophyte, i.e. a "weak imprinting response." Although dosage effects have not been completely ruled out, we believe imprinting differences are involved due to the effects of the mot factors on *R1-r*(standard) in classic imprinting experiments (data not shown). Therefore, we tentatively conclude that *R1-d* haplotype-specific imprinting responses in interaction with the mot factors are responsible for the differences observed in the female backcrosses.

In all crosses where *R1-d* is inherited through the male gametophyte (cross: [*r1-g mot1 mot2*] X [*R1-d r1-g; Mot1 mot1; Mot2 mot2*]), a wider variance in the color classes is observed than when *R1-d* is inherited through the female. A distinct medium mottling (MMT) class appears, and the dark mottled class is not as strong as when the *R1-d* haplotype is inherited from the female parent. This indicates that the "weak imprinting response" *R1-d* haplotypes do have a slight response to imprinting rather than a completely null response. Thus the mot factors can be used to differentiate between *R1-d* haplotypes that respond strongly and weakly to imprinting.

We hypothesize that one of the mot factors, arbitrarily named *mot2* on this basis, has a stronger effect on seed color than the other. The expected distribution of classes should be 4 cl (*r1-g r1-g*) : 2 DMT (*R1-d r1-g; Mot1 mot1; Mot2 mot2* or *R1-d r1-g; mot1 mot1; Mot2 mot2*) : 1 MMT (*R1-d r1-g; Mot1 mot1; mot2 mot2*) : 1 LMT (*R1-d r1-g; mot1 mot1; mot2 mot2*) if *mot2* has a stronger effect (Fig. 1). The chi-square tests for this ratio were non-significant for all but 4 out of 72 ears (data not shown). So we conclude that *Mot2* has a stronger effect on aleurone color than *Mot1*. Both imprinting-sensitive and insensitive *R1-d* haplotypes show light mottling of the aleurone when inherited through the male gametophyte in the presence of both mot factors, but full color when the wild type Mot alleles are present. This must be due to a compensation effect by the wild type Mot alleles that intensifies color (or prevents color reduction) even in the absence of imprinting, when *R1-d* is passed through the male gametophyte. At the same time, this shows that the segregation ratio of 1:1 for the *R1-d* imprinting-sensitive haplotypes when *R1-d* is passed through the female (Table 3) is not due to them not being able to respond to the mot factors, but rather imprinting provides an alternative route to increasing *r1* gene expression.

Another interesting aspect of the mot factors' effect on these geographic and tribal *R1-d* haplotypes is the differential effect on seed and seedling colors. The *R1-d* class of *r1* haplotypes typically shows strong pigmentation of the aleurone and also of the scutellum and germinating roots. We observed that medium mottled kernels from the male testcross, [*r1-g mot1 mot2*] X [*R1-d r1-g; Mot1 mot1; Mot2 mot2*], had colored scutella and produced seedling root color when germinated, but the light mottled kernels had colorless scutella and produced seedlings with green roots (Fig. 2). We conclude that only one of the factors is needed to produce color in the scutellum and roots. This factor does not cause strong seed color and therefore must be mot1, since mot2 was designated as the factor causing stronger seed color. We hypothesize that a color class distribution of both seed and seedling should be as follows: 1 LMT/Green : 1 MMT/Red : 1 DMT/Green : 1DMT/Red, where Green and Red refer to plant colors, if two mot factors

Table 2. Kernel counts of female backcrosses of *R1-d* haplotypes showing "weak imprinting response" (light mottled kernels segregating). Cross: [*R1-d r1-g*; *Mot1 mot2*; *Mot2 mot2*] X [*r1-g mot1 mot2*]. cl = colorless kernels. Chi-square for 4 cl : 3 DMT : 1 LMT. Abbreviaitons: AZ Ariizona, NM New Mexico, Canada CA, Chi Chi-square; S Significanc

Source	R1-d haplotype	PI number	cl	DMT	LMT	Chi	S.
GB 645	AZ-1	PI213729	206	186	54	3.45	NS
GB 645	AZ-1	PI213729	202	161	39	3.22	NS
GB 645	AZ-1	PI213729	233	186	41	5.83	NS
GB 645	AZ-1	PI213729	147	102	29	1.46	NS
GB 645	AZ-1	PI213729	172	141	48	0.82	NS
GB 652	AZ-2	PI213738	207	158	65	2.72	NS
GB 652	AZ-2	PI213738	244	195	113	32.46	P< .001
GB 652	AZ-2	PI213738	171	142	45	0.806	NS
GB 652	AZ-2	PI213738	178	143	36	2.29	NS
GB 651	CA	PI214199	188	256	69	39.71	P< .001
GB 651	CA	PI214199	115	96	21	3.15	NS
GB 651	CA	PI214199	194	144	53	0.41	NS
GB 651	CA	PI214199	185	136	47	0.06	NS
GB 651	CA	PI214199	228	148	52	1.93	NS
GB 656	NM-3	PI218150	166	111	41	0.92	NS
GB 656	NM-3	PI218150	136	98	23	3.06	NS
GB 656	NM-3	PI218150	160	130	50	1.96	NS
GB 656	NM-3	PI218150	138	114	38	0.68	NS
GB 656	NM-3	PI218150	158	142	44	2.47	NS
GB 658	NM-5	PI218169	190	144	57	1.55	NS
GB 658	NM-5	PI218169	121	130	34	8.32	P <.05
GB 658	NM-5	PI218169	121	80	38	3.26	NS
GB 658	NM-5	PI218169	210	153	58	0.70	NS
GB 658	NM-5	PI218169	206	172	58	1.33	NS
GB 659	NM-6	PI218173	160	115	50	2.59	NS
GB 659	NM-6	PI218173	146	114	52	5.051	NS
GB 659	NM-6	PI218173	156	97	38	2.178	NS
GB 659	NM-6	PI218173	168	130	40	0.209	NS

Table 3. Kernel counts of female backcrosses of *R1-d* haplotypes showing "strong imprinting response" (no light mottled kernels segregating). Cross: [*R1-d r1-g*; *Mot1 mot2*; *Mot2 mot2*] X [*r1-g mot1 mot2*]. Chi-square for 1 cl : 1 DMT. (The few LMT kernels not included in chi-square tests.) Abbreviations: IA Iowa; ND North Dakota; OK Oklahoma; SD South Dakota; WA Washington; A Arapaho; Chi Chi-Square; S Significance

Source	R1-d haplotype	PI number	cl	DMT	LMT	Chi	S
GB 648	IA	PI217411	186	192	0	0.10	NS
GB 648	IA	PI217411	263	215	2	4.82	P< .05
GB 648	IA	PI217411	214	177		3.50	NS
GB 648	IA	PI217411	183	161	2	1.41	NS
GB 648	IA	PI217411	231	216		0.50	NS
GB653	ND	PI213807	168	162	1	0.11	NS
GB653	ND	PI213807	192	172		1.10	NS
GB653	ND	PI213807	151	130	1	1.57	NS
GB653	ND	PI213807	152	129		1.88	NS
GB653	ND	PI213807	108	110		0.02	NS
GB 647	OK	PI213756	133	131		0.02	NS
GB 647	OK	PI213756	280	287		0.09	NS
GB 647	OK	PI213756	186	179		0.13	NS
GB 647	OK	PI213756	238	230	2	0.14	NS
GB 649	SD 1	PI213779	194	179	2	0.60	NS
GB 649	SD 1	PI213779	120	122		0.02	NS
GB 649	SD 1	PI213779	209	221	3	0.34	NS
GB 649	SD 1	PI213779	233	273	2	3.16	NS
GB650	WA-1	PI217488	143	129		0.72	NS
GB650	WA-1	PI217488	198	235	8	3.16	NS
GB650	WA-1	PI217488	218	246	1	1.69	NS
GB650	WA-1	PI217488	217	238	1	0.97	NS
GB650	WA-1	PI217488	279	261	2	0.6	NS
GB660	WA-2	PI217489	256	229	5	1.50	NS
GB660	WA-2	PI217489	168	151	3	0.91	NS
GB660	WA-2	PI217489	148	200	3	7.77	P< .01
GB660	WA-2	PI217489	192	187		0.07	NS
GB660	WA-2	PI217489	92	124		4.74	P< .05
GB 873	A		240	206		2.59	NS
GB 873	A		291	278	2	0.30	NS
GB 873	A		225	230		0.06	NS
GB 873	A		229	226		0.02	NS
GB 873	A		256	260	3	0.03	NS
GB 873	A		219	230		0.27	NS

are segregating but these two mot factors have different effects on plant color. These classes would correspond to: 1 LMT/Green = *R1-d r1-g; mot1 mot1; mot2 mot2*; 1 MMT/Red = *R1-d r1-g; Mot1 mot1; mot2 mot2*; 1 DMT/Green = *R1-d r1-g; mot1 mot1; Mot2 mot2*; and 1 DMT/Red = *R1-d r1-g; Mot1 mot1; Mot2 mot2.* This ratio was generally observed when kernels were germinated and plant colors were scored (Table 4). A few seedlings in two unexpected classes were also observed: LMT/Red and MMT/Green. One possible explanation for these unexpected classes is heterofertilization; other possibilities include kernel color misclassifications and the presence of other as yet uncharacterized modifiers. Chi-square tests of counts of seedlings grown from colored kernels of male testcrosses of four *R1-d* accessions, with two

Figure 1. Male backcross of a heterozygous *R1-d*:*Arapaho r1-g Mot1 mot1 Mot2 mot2* plant to an *r1-g mot1 mot2* tester. Colored kernels are in three classes: dark mottled, medium mottled, and light mottled in a 2:1:1 ratio. Seedlings grown from medium mottled kernels were red, half of the seedlings grown from dark mottled kernels were red, and seedlings grown from light mottled kernels were green, indicating that the weak kernel mottling factor, *Mot1*, is responsible for induction of typical *R1-d* seedling color. Other *R1-d* geographic alleles showed variation in seedling pigmentation in response to the mottling factors.

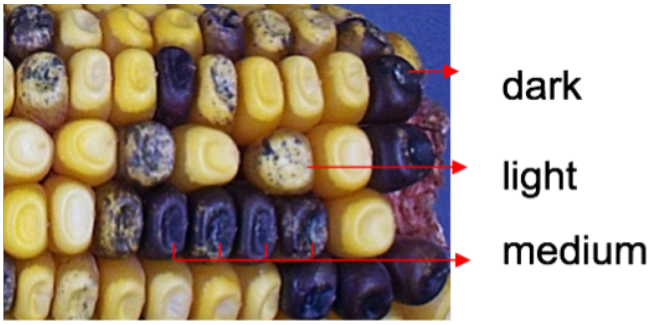
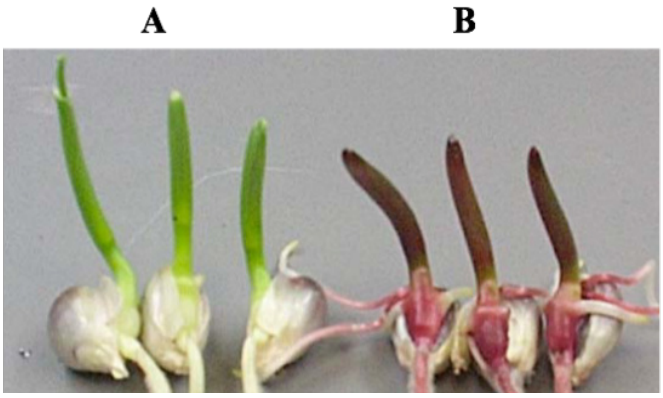


Figure 2. Seedling pigmentation phenotypes illustrating interactions between *R1-d* haplotypes and mottling factors *mot1* and *mot2*. (A) Green seedlings grown from *R1-d*:*Arapaho mot1 mot2* kernels. (B) Red seedlings grown from *R1-d*:*Arapaho Mot1 Mot2* kernels.



ears each, showed no deviation from the expected ratios for five out of the eight ears (Table 4).

In summary, the true-breeding mottled phenotype observed in Southwestern Native American accessions of maize results from the interaction of a permissive *R1-d* haplotype with two mottling factors, mot1 and mot2. For mottling to occur, the *R1-d* haplotype must be homozygous, or heterozygous with a colorless *r1* haplotype, and *mot1* and *mot2* must be homozygous. The *R1-d* haplotypes studied can be grouped into two classes based on phenotype in the presence of mot1 and mot2 when crossed reciprocally with *r1-g mot1 mot2* testers:

Table 4. Seedling phenotypes for colored kernels from test crosses: [*r1-g mot1 mot2*] X [*R1-d r1-g*; *Mot1 mot1*; *Mot2 mot2*]. Chi-square for 1 LMT/Green [L/G]: 1 MMT/Red [M/R]: 1 DMT/Green{D/G] : 1 DMT/Red [D/R]. The few seedlings in unexpected classes were not included in chi-square calculations. Abbreviations: A Arapaho; CA Canada; NM new Mexico; WA Washinigton; Imp Imprinting; st strong; wk weak; Ge germination; Chi Chi-square; S Significance

haplotype; PI no.	Imp	L /G	M /R	D /G	D /R	L /R	M /G	No Ge	Chi	S
A	st	63	53	56	64	5	12	2	1.46	NS
A	st	57	50	87	62	2	8	2	12.16	P< .01
CA; PI214199	wk	28	41	37	56	4	10	2	10.10	P< .01
CA; PI214199	wk	38	39	56	49		3	5	4.86	NS
NM-4; PI218157	wk	50	42	39	70	1	10		11.64	P< .01
NM-4; PI218157	wk	50	51	64	43	1	10	3	4.42	NS
WA-1; PI217488	st	53	37	45	33	3	5	1	5.62	NS
WA-1; PI217488	st	58	64	76	70	6	16	2	2.687	NS

i) Weak imprinting response *R1-d* haplotypes produce light mottled kernels in the presence of mot1 and mot2 regardless of whether the *R1-d* haplotype is transmitted through the male or female gametophyte. These are the *R1-d* haplotypes present in true-breeding light mottled lines, and ii) Strong imprinting response *R1-d* haplotypes produce dark mottled kernels when transmitted through the female, but light mottled kernels when

Table 5. Summary of *R1-d* haplotypes used in studies of *mot* factors and their origin (see Van Der Walt and Brink, Geographic distribution of paramutable and paramutagenic *R* alleles in maize. Genetics 61:677-695, 1969) and pattern of imprintability in combination with homozygous *mot1 mot2*.

R1-d haplotype	PI number	Imprintability
Arizona-1[1]	PI213729	weak
Arizona-2[1]	PI213738	weak
New Mexico-2	PI218143	weak
New Mexico-3	PI218150	weak
New Mexico-4	PI218157	weak
New Mexico-5	PI218169	weak
New Mexico-6	PI218173	weak
Canada[1]	PI214199	weak
New Mexico-1[1]	PI218170	strong
Oklahoma	PI213756	strong
Iowa	PI217411	strong
South Dakota-1[1]	PI213779	strong
South Dakota-2	PI213787	strong
North Dakota	PI213807	strong
Washington-1	PI217489	strong
Washington-2	PI217488	strong
Arapaho		strong

[1]Haplotypes analyzed molecularly by Walker and Panavas (Structural features and methylation patterns associated with paramutation at the *r1* locus of *Zea mays*. Genetics 159:1201-1215, 2001).

Table 6. Statistical summary of genotyping of parental and F1 samples with Maize50 Illumina plex.
P1 *Rsc:124 Mot1 Mot2 W22; P2 R1*-Navajo Robin's Egg Corn *mot1 mot2; F1 R1-sc:124*/Navajo Robin's Egg Corn

Genotype markers	P1	P2	F1	Pool 732-9DK	Pool 732-9Mot	Pool 732-5DK	Pool 732-5Mot	Pool 732-4DK	Pool 732-4Mot	Pool 732-2DK	Pool 732-2Mot	Pool 732-1DK	Pool 732-1Mot
Hetero (%)	1%	7%	36%	28%	23%	31%	18%	32%	25%	32%	23%	29%	23%
Homo markers (No.)	48752	45164	31495	32684	35329	32027	37325	31880	33865	31812	34786	32278	35070
Hetero markers (No.)	318	3378	17356	12884	10373	14147	8045	15061	11203	15183	10506	13114	10626
Not scored (No.)	6056	6584	6275	9558	9424	8952	9756	8185	10058	8131	9834	9734	9430

transmitted through the male in the presence of *mot1* and *mot2*. Table 5 summarizes the origins of the various *R1-d* haplotypes studied and their pattern of imprintability observed in combination with homozygous *mot1 mot2*. Note that an imprinting effect rather than an endosperm dosage effect was inferred on the basis of tests made using *R1-r*(standard). More direct imprinting tests using *R1-d*(Arapaho) are in progress.

Four of the *R1-d* haplotypes characterized for imprinting response, two strong responders and two weak responders, were analyzed molecularly (Table 5) by Walker and Panavas (Genetics 159:1201-1215, 2001). No differences were observed between the two types at the gross structural level—all four haplotypes showed the same structural features typical of *R1-d* haplotypes: a *q* gene, an intact *S2* gene, and a truncated *S1* gene

R1-d haplotype and the *Mot1* allele and homozygous for *mot2*—this interaction is most evident when *R1-d* is transmitted through the male. These differential interactions are the basis for distinguishing between the two mot factors.

We used a bulk segregant analysis in an attempt to map the three factors involved in the aleurone mottling and seedling color effects. Seeds from 5 F2 ears of Navajo Robin's Egg Corn (NREC) crossed to *R1-sc:124* showing the 63:1 ratio of full color to mottled were germinated, plus one plant from each parental and one from F1 seed. Leaf discs from two to five of the F3 plants from each color class were pooled to produce a bulked DNA sample. The MaizeSNP50 Illumina Corn Chip was used for genotyping. Table 6 shows some statistics on data resulting from the analysis. We expected that polymor-

Table 7. Looking at segregation patterns across multiple loci, one can define a region of confidence and a smaller region within the region of confidence where the presence of the locus most likely is. The *r1* locus is between positions 139,028,499 and position 139,122,292 on chromosome 10. Therefore, the other two locations should correspond to mottling factors *mot1* and *mot2,* which were not distinguished in this analysis.

	Chr 3[a]		Chr 4[a]		Chr 10[a]	
Start of region of confidence	11,924,509		32,233		66,503,927	
Start of region most likely	21,279,187	~13 cM[b] - centromere	32,233	~7 cM[b]	136,523,828	~13 cM[b]
End of region most likely	102,621,860		2,072,691		141,117,052	
End of region of confidence	102,621,860		4,139,968		142,144,176	

[a] Bp position of markers on the physical assembly B73 RefGen_v2 sequence (see www.maizegdb.org, www.maizesequence.org for details).
[b] Approximated genetic interval based on flanking marker information (data not shown).
Illumina MaizeSNP50 marker list and bp coordinates can be found at www.illumina.com/support/literature.ilmn.

missing 5' noncoding sequences. The molecular basis for the difference in imprinting responses remains a question.

The *mot* factors themselves have differential effects on intensity of aleurone and plant color produced by all *R1-d* haplotypes studied. Seedlings grown from kernels carrying an *R1-d* haplotype and homozygous or heterozygous for the *Mot1* allele produce plant color regardless of *mot2* genotype; homozygous *mot1* seedlings are green regardless of *mot2* genotype. Kernels carrying an *R1-d* haplotype and the *Mot2* allele and homozygous for *mot1* are more darkly mottled than kernels carrying an

phic markers between bulked samples will be genetically linked to the color factors. Because these three factors act as recessive genes, we expect the colored pool to more likely show heterozygous calls for the markers in the linked region while the mottled pooled samples show homozygous calls, the same calls as for the mottling parent NREC. By comparing the 5 paired pools, F1 and 2 parental lines using excel filters, we found three regions linked to the mottling effect as expected. The *r1* gene position was confirmed by this analysis (Table 7) and is contained in the smaller interval detected on chromosome 10. The locations of *mot1* and *mot2* were

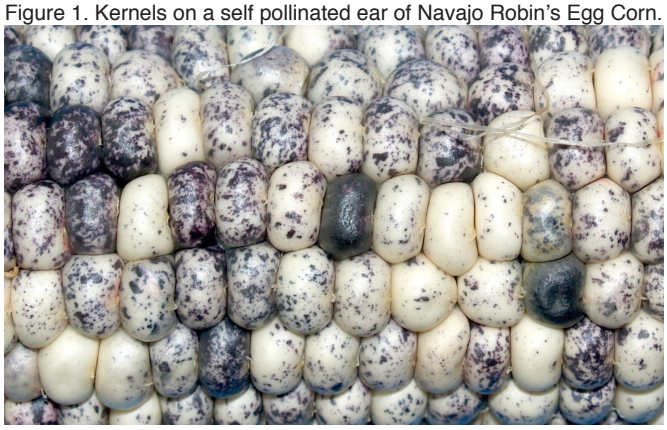
narrowed to two other segments on chromosomes 3 and 4. Which factor is located on which chromosome is not known, since this population was not large enough to allow their phenotypic discrimination. Their physical positions on chromosome 3 and chromosome 4 are shown in Table 7. Exact genetic positions are not provided, but the approximate size of each genetic interval is suggested.

Three-factor inheritance of aleurone color speckling in Navajo Robin's Egg and Hopi Speckled open pollinated varieties of maize—summary of research performed in Urbana, Illinois.

--Stinard, PS; Goncalves-Butruille, M; Kermicle, JL; Sachs, MM

Various systems of aleurone color mutability in South American maize land races have been isolated and characterized. Stippling produced by *R1 st* alleles has been identified in Andean land races (Brink RA, unpublished; Williams, Variability of the R stippled gene in maize. Ph.D. Thesis, University of Wisconsin, Madison, 1972); sectoring induced by mutable alleles of the *Enr1 r1* haplotype-specific aleurone color enhancer has been identified in northern South American land races (Stinard, Kermicle, and Sachs. J Hered 100:217-228, 2009; Gonella and Peterson, Genetics 85:629-645, 1977); and aleurone color marbling conditioned by an *R1 mb* allele has been identified in Pisccorunto maize from Peru (http://www.maizegdb.org/cgi-bin/displayvarrecord.cgi?id=9017398). Although exhaustive surveys have not been conducted, these previously described systems of mutability have not been reported in native North American maize land races. Nevertheless, there are North American land races with systems of variegated aleurone color that have not previously been characterized. We describe here the characterization of one such system: three factor inheritance for aleurone color speckling found in two North American open pollinated varieties of tribal maize (see Goncalves-Butruille M et al., this MNL, for companion article).

Figure 1. Kernels on a self pollinated ear of Navajo Robin's Egg Corn.



Seeds of the open pollinated variety Navajo Robin's Egg Corn (NREC) with purple aleurone color speckling on colorless background (Fig. 1) were obtained from Abundant Life Seed Foundation, Port Townsend, Washington. The sectors of speckling on kernels of NREC do not have well-defined borders but are more diffuse and reminiscent of *r1* mottling or the endosperm blotching of *Pl1 Bh1*. Crosses of NREC to the open pollinated variety Hopi Speckled Maize obtained from Native Seed Search, Tucson, Arizona produced speckled kernels in both the F1 and F2, which indicates that the speckling is due to the same system. Initial crosses of NREC to aleurone color testers for *a1, a2, c1, c2,* and *r1* produced full-colored kernels, indicating complementation. F2's of NREC with the Stock Center's full colored aleurone (*A1 C1 R1*) standard produced a very low frequency of speckled kernels, approximating a 63:1 ratio of full color to speckled. To further characterize the nature of NREC speckling and determine the number of genetic factors involved, test crosses were performed as follows: NREC was crossed to a stock carrying the nonparamutagenic self-colored *R1-sc:124* allele (and all other genes needed for aleurone color) in a W22 background, and the F1 was backcrossed by NREC to generate test cross ears. Kernel counts from the test cross ears indicated 7:1 segregation of full color to speckled aleurone (Table 1). All deviations from 7:1 were nonsignificant at the 0.10 level.

Table 1. Counts of full color (Cl) and speckled (spk) kernels from ears of the test cross: [*R1-sc:124* X NREC] X NREC.

Female parent	Cl	spk	7:1 $\chi 2$
2003P-139-1	231	35	0.105
2003P-139-2	331	44	0.202
2003P-139-3	255	30	1.015
2003P-139-4	241	39	0.522
2003P-139-5	312	44	0.006
2003P-139-7	363	50	0.058
2003P-139-8	269	44	0.694
2003P-139-9	337	48	0.000
2003P-139-10	288	52	2.427
Totals	2627	386	0.267

We hypothesized that the 7:1 test cross ratios observed were due to the independent assortment of three recessive triplicate factors, i.e. kernels need to be homozygous for all three factors in order to produce the speckled aleurone observed in the NREC line. In order to test this hypothesis, full colored kernels from the test cross ears were planted, and the resulting plants were crossed again by NREC. Kernels from these ears

Table 2. Counts of full color (Cl) and speckled (spk) kernels from ears of the test cross: [[*R1-sc:124* X NREC] X NREC] X NREC.

Female parent	Cl	spk	1:1 χ2	3:1 χ2	7:1 χ2
2004-2705-1	257	80	92.964	0.286[1]	38.919
2004-2705-2	352	54	218.729	29.639	0.238[1]
2004-2705-3	289	98	94.266	0.022[1]	58.180
2004-2705-4	217	85	57.695	1.594[1]	67.589
2004-2705-5	172	23	113.851	18.135	0.089[1]
2004-2705-6	358	106	136.862	1.149[1]	45.399
2004-2705-7	409	142	129.381	0.175[1]	88.728
2004-2705-8	266	104	70.930	1.906[1]	82.411
2004-2706-1	184	178	0.099[1]	112.799	445.084
2004-2706-2	180	179	0.003[1]	118.337	458.149
2004-2706-3	149	22	94.322	13.429	0.021[1]
2004-2706-4	149	136	0.593[1]	78.457	323.213
2004-2706-6	254	89	79.373	0.164[1]	56.710
2004-2706-7	180	27	113.087	15.783	0.0559[1]
2004-2706-8	319	108	104.265	0.020[1]	63.891
2004-2707-1	236	209	1.638[1]	114.518	483.316
2004-2707-2	311	113	92.462	0.616[1]	77.628
2004-2707-3	298	92	108.810	0.414[1]	43.852
2004-2707-4	197	66	65.251	0.001[1]	38.145
2004-2707-5	245	96	65.106	1.807[1]	76.384
2004-2707-6	168	168	0.000[1]	112.000	432.000
2004-2707-7	213	204	0.194[1]	127.259	505.730
2004-2707-8	227	218	0.182[1]	136.576	541.702
2004-2707-9	239	216	1.163[1]	122.550	508.800
2004-2707-10	355	122	113.813	0.085[1]	74.574
2004-2707-12	366	127	115.864	0.152[1]	79.261
2004-2808-1	87	91	0.090[1]	64.787	242.777
2004-2808-2	443	69	273.195	36.260	0.446[1]
2004-2808-3	243	249	0.073[1]	172.098	653.310
2004-2808-4	254	241	0.341[1]	148.122	592.638
2004-2808-5	284	38	187.938	29.917	0.144[1]
2004-2808-6	233	69	89.060	0.746[1]	29.565
2004-2808-8	182	79	40.648	3.863[3]	75.337
2004-2808-9	131	37	52.595	0.794[1]	13.932

were scored for full color versus speckled. If the system involves independently-assorting triplicate factors, we would expect to obtain 7:1, 3:1, and 1:1 ratios for full color to speckled on these second generation back cross ears. The results are presented in Table 2. Of 66 ears, 63 gave chi-square values that didn't differ significantly from 7:1, 3:1, or 1:1 ratios. One ear gave a 3:1 chi-square significant at the .10 level, one ear gave a 3:1 chi-square significant at the .05 level, and one ear gave a 7:1 chi-square significant at the .01 level. Given the population size, such deviations would not

Table 2 continued.

Female parent	Cl	spk	1:1 χ2	3:1 χ2	7:1 χ2
2004-2808-11	231	210	1.000[1]	120.333	497.286
2004-2709-1	146	165	1.161[1]	130.548	467.653
2004-2709-2	277	99	84.266	0.355[1]	65.751
2004-2709-4	307	109	94.240	0.321[1]	71.407
2004-2709-5	303	86	121.051	1.735[1]	32.832
2004-2709-7	178	157	1.316[1]	85.422	361.723
2004-2709-8	197	225	1.858[1]	180.477	642.818
2004-2709-9	291	85	112.862	1.149[1]	35.112
2004-2710-1	186	186	0.000[1]	124.000	478.286
2004-2710-2	130	120	0.400[1]	70.533	288.057
2004-2710-4	223	83	64.052	0.736[1]	59.834
2004-2710-5	231	238	0.104[1]	165.806	627.239
2004-2710-6	217	84	58.767	1.357[1]	65.326
2004-2710-8	218	231	0.376[1]	167.502	622.717
2004-2711-2	279	93	93.000	0.000[1]	53.143
2004-2711-3	437	162	126.252	1.336[1]	115.862
2004-2711-5	314	41	209.941	34.254	0.293[1]
2004-2711-7	456	64	295.508	44.677	0.018[1]
2004-2711-8	288	45	177.324	23.432	0.313[1]
2004-2711-9	214	76	65.669	0.225[1]	49.815
2004-2711-10	284	103	84.654	0.538[1]	70.494
2004-2711-11	180	29	109.096	13.794	0.362[1]
2004-2711-12	421	58	275.092	42.456	0.067[1]
2004-2712-1	287	104	85.650	0.533[1]	71.056
2004-2712-2	162	56	51.541	0.055[1]	34.666
2004-2712-3	270	42	166.615	22.154	0.264[1]
2004-2712-5	324	118	96.009	0.679[1]	81.449
2004-2712-6	285	102	86.535	0.380[1]	67.937
2004-2712-7	125	134	0.313[1]	98.750	364.572
2004-2712-8	105	48	21.235	3.314[2]	49.824
2004-2712-9	264	20	209.634	48.845	7.734[4]
2004-2712-10	354	121	114.293	0.057[1]	73.097

[1] $p > 0.1$ (NS)
[2] $p < 0.1$
[3] $p < 0.05$
[4] $p < 0.01$
$p < 0.001$ (no highlight or superscript)

be unexpected. Furthermore, based on independent assortment, the number of ears with 7:1, 3:1, and 1:1 ratios respectively would be expected to occur in a ratio of 1:3:3. Our observed number of ears matching these ratios (13, 33, and 20) did not differ significantly from the 1:3:3 ratio (chi-square = 4.57). Thus, the data from the second-generation back cross ears match what would be expected for three independently assorting triplicate factors.

In order to further characterize the NREC factors,

crosses of NREC were made to various aleurone color tester lines, which yielded interesting results. As mentioned above, crosses of NREC to the Stock Center's *r1* tester (in M14/W22 background) produced full colored kernels. Crosses of NREC to *r1* introgressed into W23 also produced full colored kernels. However, crosses of NREC to *r1* introgressed into Oh43 produced speckled kernels. This cross was repeated using an independent *r1 wx1* Oh43 conversion, also producing speckled kernels in the F1. From these results we deduced that the r1 locus is likely one of the factors involved in the speckling phenomenon, and that Oh43 is homozygous for the other two speckling factors, but M14, W22, and W23 are not.

Test crosses of NREC to an Oh43 conversion of *R1-g* produced 1:1 ratios of full colored to speckled kernels (1428 full color : 1384 speckled, 1:1 chi-square = 0.688, NS). This confirms that Oh43 is homozygous recessive for two of the speckling factors, and the 1:1 segregation is due to segregation at the *r1* locus (*R1-g* vs. the *R1* allele present in NREC, *R1-NREC*). Even though *R1-NREC* behaves as a dominant for aleurone color in crosses to the Stock Center's *r1* tester in the absence of speckling factors, it apparently acts as a recessive relative to *R1-g* and *R1-sc:124* with respect to response to NREC speckling factors.

Test crosses of NREC to a W23 conversion of *R1-r* produced 3:1 ratios of full colored to speckled kernels (746 full color : 266 speckled, 3:1 chi-square = 0.891, NS). Test crosses of NREC to a W23 conversion of *R1-Randolph* produced a 1:1 ratio of full color to speckled kernels (1499 full color : 1570 speckled, 1:1 chi-square = 1.642, NS). Since the difference between these two stocks is the *R1* allele and not the genetic background, we conclude that the W23 background is homozygous recessive for one speckling factor, and what differentiates between the 3:1 segregation and the 1:1 segregation is the R1 allele. In other words, *R1-Randolph* is susceptible to NREC speckling, but *R1-r* is not.

From these data, we conclude that speckling in NREC requires three factors: a permissive (e.g., *R1-NREC* or *R1-Randolph*) allele at the *r1* locus, and homozygous recessive factors at two other independent loci. Thus the genotype of *r1*^Oh43 is *r1 r1 fac1 fac1 fac2 fac2*, where *fac1* and *fac2* represent recessive alleles at the two independent speckling loci. NREC is *R1* permissive *R1* permissive *fac1 fac1 fac2 fac2*. *R1-sc:124* ^W22 is *R1* nonpermissive *R1* nonpermissive *Fac1 Fac1 Fac2 Fac2*; *R1-g* ^Oh43 is R1 nonpermissive *R1* nonpermissive *fac1 fac1 fac2 fac2*; *R1-r* ^W23 is *R1* nonpermissive *R1* nonpermissive *fac1 fac1 Fac2 Fac2*; and *R1-Randolph* ^W23 is *R1* permissive *R1* permissive *fac1 fac1 Fac2 Fac2*.

Jerry Kermicle initially referred to a similar speckling phenomenon as "Four Corners mottling" because it was identified in varieties of speckled maize from Native American open pollinated varieties from the Four Corners region of the southwestern United States. Studies in Wisconsin (see Goncalves-Butruille M et al., this MNL, for companion article) found that this speckling system requires a permissive *r1* allele (the strongest effect being found among certain *R1-d* haplotypes, though certain other haplotypes show a weaker effect) and two recessive factors named *mot1* and *mot2* for mottling factors. Tests of allelism were performed in Urbana and revealed the NREC system to be identical to the Four Corners mottling system. Separate *mot1* and *mot2* testers from Wisconsin were used to show that the Maize Coop's W23 lines are homozygous recessive for mot1. We all concur that the two independent factors should be called *mot1* and *mot2*.

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Constitution of microarray platforms for the analysis of the maize mRNA and small RNA transcriptome

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The completion of the maize *B73* inbred line genome sequencing project (Schnable et al., Science 326: 1112-1115, 2009; http://www.maizesequence.org) makes possible an implementation of genome-wide approaches to investigate several aspects of the maize genome activity. Using the database from the maize sequencing project we have developed custom microarray-based platforms to be employed as tools to analyze maize mRNA and small RNA (sRNA) transcriptome. Both arrays are based on the Combimatrix technology (http://www.customarrayinc.com). This microarray platform contains 35-40 mers oligos synthesized *in situ* using a semiconductor-based electrochemical-synthesis process, employs single channel Cy5 hybridization, and allows for array stripping and reusing.

The maize mRNA array was prepared using the Combimatrix CustomArray 90K (90,000 oligos). The filtered set of cDNA sequences (ZmB73_4a.53 version) released on March 2010 from the B73 Maize Genome Sequencing Consortium and the OligoArray 2.1 free software (Rouillard et al., Nucl Acid Res 31:3057-3062, 2003; http://berry.engin.umich.edu/oligoarray2_1) were used to design oligo probes. A specificity check for oligo cross-hybridization against all other genes and for unique genome position was applied. The result was a maize mRNA array containing 45,000 probes, which included two replicates for each selected target. These probes represent 51,109 transcripts and 30,190 protein-encoding genes that account for 96% and 93% of the filtered cDNAs set and related genes identified by the

Maize Genome Consortium (Schnable et al., Science 326:1112-1115, 2009). Information from the Maize Genome Consortium initiative also associated many of the mRNA probes with a Gene Ontology (GO) and Interpro annotation.

The maize sRNA array was prepared using the Combimatrix CustomArray 4X2K (4 arrays for each slide, and each array containing 2,240 oligos). For each sRNA probe two additional probes were also designed that contained mismatches and were used to maximally destabilize hybridization. Comparing the signals between the sRNA probe and its related probes with the mismatches allowed hybridization specificity to be detected. Three replicates for both match and mismatch probes were included; therefore, each sRNA is represented by nine probes. Accordingly, the 4X2K sRNA array represents 204 sRNA maize sequences. The tRNA and U6 sequences were also included as positive controls, while anti-sense probes representing abundant mRNAs were the negative controls for degraded mRNA. The sRNA sequences were selected to represent mainly microRNAs (miRNAs). The probes selection criteria were as follows: i) sRNAs encoded by different loci, but with identical sequence or with a single nucleotide polymorphism in the 5'- or 3'-end, were represented by only one probe, due to impossibility to distinguish differences by array hybridization; ii) all maize miRNA sequences from miRBase database release 14.0 (http://www.mirbase.org) were included; iii) all monocotyledonous miRNAs, distinct from maize miRNAs, with at least one predicted putative target into the maize genome (see below) were included; and iv) 78 sRNAs derived from high-throughput maize sRNA sequencing (Wang et al., Plant Cell 21:1053-1069, 2009), distinct to miRBase maize miRNAs and exhibiting a predicted ability to form short hairpin structure (shRNA), were selected as putative new miRNAs. Specifically, both monocotyledonous miRNAs and maize shRNAs were chosen by applying bioinformatics analysis to identify their putative targets according the method employed by Zhang et al., (PLoS Genetics 5:e716, 2009) and using the *B73* genome sequence as a database. This method allows us to enclose in the array the shRNAs that have as putative targets the exon or intron sequence of both a low (less than five) and a high (from five to approximately one hundred) number of genes. In some cases these shRNAs target MITE-like repeats located within the same gene. The shRNAs might, therefore, represent new maize miRNAs or new type of sRNAs, exhibiting a mix of miRNA and short interfering sRNA (siRNAs) features, thus representing possible ancestor of miRNAs.

Both maize mRNA and miRNA custom arrays described above were employed as a tool to analyze transcriptome change in response to abiotic stresses (Altana et al., MNL, this issue) and are available for scientific community for investigation of gene regulation.

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Identification of cold stress and sulfate starvation induced microRNAs in maize roots

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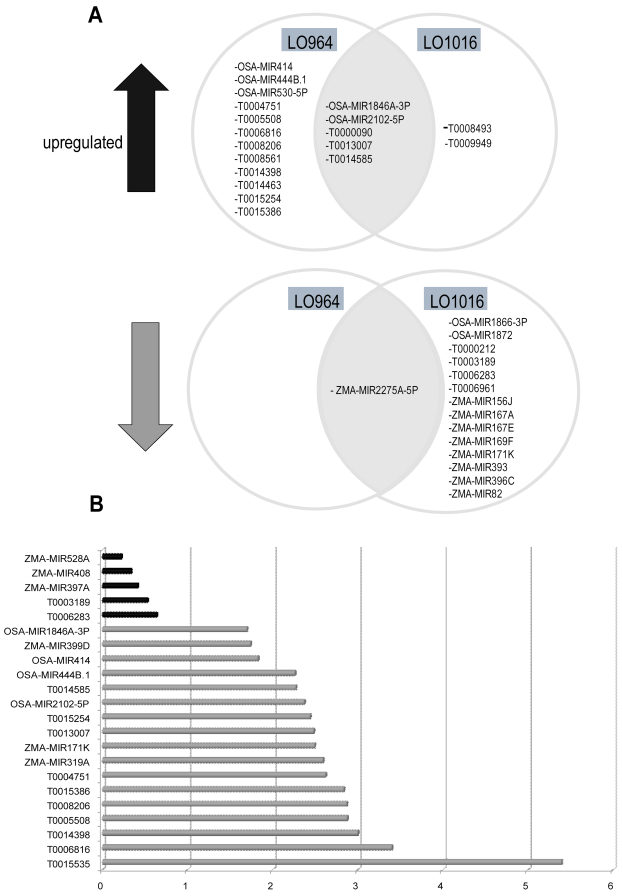
The maize root system plays an essential role in mediating plant interaction with environmental stimuli. It has been shown that epigenetic mechanisms and small RNAs (sRNAs) are involved in mediating transcriptome changes induced by various environmental stresses. In particular, microRNAs (miRNAs) act in negatively regulating the mRNA level of target genes (Sunkar et al, Trends in Plant Science 12: 301-308, 2007; Zhang et al., PLoS Genetics 5: e716, 2009). To analyze the contribution of miRNAs regulation of gene transcription in maize roots and in response to abiotic stresses we used the microarray platforms previously described (Altana et al., MNL, this issue). In particular, we analyzed changes in the miRNA root transcriptome induced by sulfate starvation and cold stress.

For the investigation regarding the sulfate starvation we employed two maize inbred lines: Lo964 and Lo1016, which are known to differ for root traits (Sanguineti et al., Maydica 43: 211-216, 1998) with the aim to analyze the genotype influence on the sulfate starvation mediated modification of miRNAs population. The protocol for sulfate starvation was established in hydroponically grown plants, by concomitantly monitoring sulfate uptake rate (in roots and in leaf) and root architectural parameters. The selected protocol (13 d-old plants grown 48 h without sulfate: hereinafter named: "-S" and with 500 μM sulfate: hereinafter named "+S) allowed to achieve sulfate starvation without significantly altering the growth rate of +S vs –S plants. The RNA was extracted by the primary root because the primary root diameter was one of the traits that significantly differ between Lo964 and Lo1016 lines. Three biological replicates were used for extraction of sRNA enriched fraction from each sample and Cy5 labeled RNA were employed for Combimatrix CustomArray 4X2K hybridization. Results showed that 18 and 22 miRNAs were differentially expressed in Lo964-S vs Lo964+S and in Lo1016-S vs Lo1016+S, respectively (a 1.5 fold change, between match probes in different sample types and between match/mismatch

probes within the same sample type, was considered). Six and 18 miRNAs exhibited different abundance when the comparison was between Lo964+S vs Lo1016+S and Lo964-S vs Lo1016-S. Six miRNAs were commonly affected (5 up- and 1 down-regulated) by sulfate starvation in both lines (Figure 1a). These observations indicate that the major number of miRNAs changes in response to sulfate starvation than to genotype. However, because 17 of the 18 sulfate induced miRNAs are up-regulated in Lo964, whereas most of the miRNAs were down-regulated in Lo1016 (Figure 1a), a possible genotype-dependent miRNA transcriptome response to sulfate starvation might occur. The 6 miRNAs differently expressed in both lines might be instead part of a genotype-independent conserved sRNA-related mechanism, activated by sulfate starvation to modulate gene expression.

Figure 1a and 1b. miRNAs differently expressed in maize roots in response to sulfate starvation and cold-stress. miRNAs up- and down-regulated (+/- 1.5 fold change) in primary root of two different inbred lines grown under sulfate starvation are reported in A, but in B the miRNAs with change in expression level after cold-stress treatment of apical root tips are indicated. The nomenclature adopted for miRNAs is the one from the miRBase, with addition of shRNA sequences (e.g. T0004751, see Altana et al., MNL, this issue) that are named according to the nomenclature of reads from high-throughput sequencing reported by Wang et al. (Plant Cell 21:1053-1069, 2009).



To investigate miRNA response to cold stress, hydroponically grown plants were subjected to a cycle of six cold-pulses (13-d old plants were submitted to 6 days of growth at 25° C for 14 h followed by 10 h where the temperature dropped to 4° C). Apical root tips were collected after that both cold stressed (CS) and not stressed (NS) samples were grown at 25° C for one day. Three biological replicates were used for each sample and the same approach described for sulfate starvation was used for miRNA microarray hybridization and data analysis. The results are illustrated in Figure 1b (22 miRNAs differentially expressed in CS vs NS). The mRNA transcriptome was also assessed for CS vs NS samples (false discovery rate < 0.05; only differences with a fold change > +/- 2 were considered). Because we know the predicted target genes of the differentially expressed miRNAs (Altana et al., MNL, this issue) and because miRNAs are expected to negatively regulated expression of their targets, we searched for mRNA probes exhibiting a negative correlation with changes in the level of cold-induced miRNA expression. Seventeen miRNAs up-regulated and 5 miRNAs down-regulated by cold stress negatively correlated with the signal of 30 and 7 mRNA probes, respectively. The analysis of Gene ontology (GO) terms for genes represented by these mRNA probes indicated that specific GO categories were enriched within predicted miRNA targets (phosphate transmembrane transport, glycerol metabolism, and microtubule-based movement), thus providing information about possible specific functions for the miRNA-mediated gene regulation in response to cold stress.

III. A MOMENT IN HISTORY

Reproduced in the following six pages are two letters, dated Jan. 20, 1927 and Feb. 14, 1927, to L. J. Stadler from Barbara McClintock, who was then a student at Cornell. The originals are archived in the papers of Lewis John Stadler at *The* State Historical Society of Missouri. Addressed to "My dear L. J. S.," with whom she had become acquainted in 1925-1926 while Stadler was at Cornell on an NRC fellowship (Kass, 2005, MNL 79:52), they have a distinctly collegial flavor. McClintock was about to complete her exam for the Ph.D. (see the penultimate paragraph in the January letter) and expresses a desire to "have time to do what work I wish to instead of studying what I ought to study." Stadler had completed the Ph.D. five years earlier at the University of Missouri, and began developing x-ray experiments in 1923. His first report on x-ray effects was in 1926 in a Missouri Agricultural Experiment Station bulletin.

At the start, McClintock expresses appreciation to Stadler for offering materials from his x-ray experiments for her to study. I have not found a copy of Stadler's letter to McClintock in which he initiates their propitious collaboration.

Meticulous details of technique, of microphotography limitations, of genetic analysis of triploids, and some planning of shared work are included.

Her research with Stadler's materials, which McClintock acknowledges heartily in her subsequent publications, led to and set the backdrop for her insights on the breakage-fusion-bridge cycle; the relation between genes and chromosomal deficiencies; the occurrence and behavior of ring chromosomes; the behavior of reciprocal translocations and inversions; non-homologous pairing; the telomere ("healed ends") concept; and the relation of the nucleolus to the chromosome.

These letters are reproduced with permission from Peter McKinley, Barbara McClintock's nephew, through the good graces of Cold Spring Harbor Laboratory Archives.

--Ed Coe

Jan. 20, 1927.

My dear L. J. S.,

Much thanks for your letter stating your intentions. I am much pleased with the prospects of having some material like that to work with, especially since I hope to see irregularities of permanent value come forth. About when will the material be coming off? It will be this spring, will it not? That is, the preliminary trials will be.

As to the method of working on material. The situation is this. I have been able to make some permanent smears directly from the Belling's smears. Enclosed is a specimen, that is, a picture of such figure which have come from slides made last summer but the pictures were just recently taken. They are not as clear as they can be but the photographic apparatus is not in correct, but right here in my table. The floor shakes so that the boundaries are fuzzy. I took many photos from the slides instead of making drawings. The slides are now 7-8 months old & there is no change in their appearance, they are even better than fresh as the eye is clearer, more contrast between eye & chromosomes.

The pictures were taken with the acid & fixed so the cyst. in some cases was extruded. I have repeated the methods with Haidenhain's & it worked perfectly there. I will send you some slides when they are made. (I am trying to get my degree & in the next few months!). The method is something as follows — crude, I know, but when I have some time this spring I can perfect it. The slides are never fixed before only a weak sol. is used & the cysts carmin + acetic acid is drawn off before the cover slip is sealed. Then, coat with rubber (or some thing else), put in warm atmosphere (not paraffin oven — too warm) & reseal edges so that there will be some slow evaporation. After that, carefully remove the cover (with point & xylol) & let some alcohol (70% - abs) run under the cover, slowly, they remove cover from slide. The sporocysts will stick to either the cover or the slide. They (cover glass or new slide) slide + cover run, then abs. + xylol & mount in balsam. I have ever obtained some sporocysts by running the coverglass & the slide into acid alc. To remove some of the excess carmin. As the pictures show, the slides are still good after several months, and there is no change visible in them yet. I hope I can get the methods to work absolutely - for that means time. It worked so far.

Since that is the case I want to find out whether you can put them (anthers) into weak aceto-carmine (i.e. 45% aceto + varying amt of carmine) + ship them as is letting me dissect them after they arrive. I will try keeping some anthers in carmine sol. for 3 days (or 4) then trying to make perm. smears. I think it will work, but will try several methods + let you know.

I would be very anxious to do it but am sorry we have to work at such distances.

Your method is interesting - esp. Tilley proposition. I am rather hopeful for the prospect of polyploid formation even if not for mutations (I don't know here). In the triploid Zea I found 7 factors showing triploid inheritance + worked out the genetic constitution to be

A? a bbb pl pl pl Tu Tu tu su su su Cc c R r r
A?
Pr Pr Pr y y y Ra Ra Ra J J J Lg Lg Lg. So far I have triploid inheritance for Tu, C (as selection against gametes with extra chro makes the expected 1:1 ratio modified nearer a 7:2) This modification is striking in the Tu Tu tu ratio in the 2 crosses, [selection of 10 chr 8] d. x triploid found 2:1 ratio or [this distinct - selection against extra chr. ♀ gametes] triploid x diploid here out of 10 individuals 8 were Tu + 2 were tu when 5:1 expected

I have a ~~5~~ 1/5 chance of locating a def-chr. with a def. factor or vice-versa which chance I will take this spring.

If you don't mind, I am going to send you my thesis to read that you might pick holes in it before parts of it are published.

Right now & for the next month I am working hard to get ready for my exam. I'll be so glad when I have time to do what work I wish to do instead of studying what I ought to study. (right now it is invertebrate Zool.) However, right now I am trying to acquire gripp - running & temp etc. Here's hoping it will disappear shortly. (sounds like an English-Japanese letter. How you read on?)

This letter is choppy, with much I - the gripp is getting the best of my English & its formation, at least that's the excuse.

Let me know how soon things will start moving so we can make plans very definitely. My best wishes to you & the family -

Sincerely

Barbara McC.

Feb. 14, 1927.

My dear L. J. S.

I am sorry not to have written you sooner concerning the seedling material. Grippe and its consequences have kept me from feeling well until just a few days ago.

Probably the most satisfactory fixer would be Allen's Modified Bouin's. You could mail the root or stem tips to me directly in this sol. It has been found not to injure the material when kept in it over a long period of time.

As for the chromosomes - I have tried several mixtures but, as preparation for my exams. I 2 courses to assist in have taken much time I am not certain what would be best. I can get right at it as soon as my exam is over, March 5. Certainly very little seems to have sunk in my cranium so that I may not get thru especially since reviewing is such a deadly task to which I seem to have totally succumbed.

I forgot to mention the formula of the Allen's sol. -

15 cc. sat. aqueous sol. picric acid
5 cc. formalin
1 cc. acetic acid

} just before using add to this

.3 gms (in crystals, dry) chromic acid
.4 gms urea crystals

Since we are anxious to see the effect of x-rays on the tissues and on the material any time and often if there. I rather imagine you will produce some polyploid sectors of the plant besides the irregular chr. behavior. You will know when to collect material - i.e., intervals of so that much etc. for your purpose. Possibly several stages running from very shortly afterwards to from 5 to 15 days after & then to the sporocytes. It would be well to know what type of a recovery, if one is made, take place within the tissues.

Anyway, just send on the material, labeled, & we'll run it thru.

I'll send on the corn data - trisomic inheritance - later for I may be all wrong, anyway, altho. up till now it does not seem so.

Sincerely,

Barbara McC.

maize gene review
maizegenereview.org

These reviews are an extension of the summaries and images provided by many cooperators for publication in the *Mutants of Maize*, 1997, eds M. Gerald Neuffer, Edward H. Coe and Susan R. Wessler , Cold Spring Harbor, NY, and which were included in the MaizeGDB, prior to publication in hard copy. Unlike the Newsletter notes, the maize gene reviews may be freely cited without prior permission of authors. The initial submissions were published in the vol 83 of this Newsletter. Data from each review is parsed for inclusion in MaizeGDB, and the reviews are highlighted at MaizeGDB locus and person (author) pages, to acknowledge expert contributions to the MaizeGDB. We thank these first submitters for being very generous with their time in participating in this fledgling project. Specifically these are: Alice Barkan (*caf1, caf2, crp1, crs1, crs2, crs4, csy1, ppr2, ppr4, ppr5, ppr10, rnc1, tha1, tha4, tha8, why1*) David Braun (*tdy1*), George Chuck (*bd1, ts4, ts6*), Erik Vollbrecht and Sarah Hake (*ra2*), Rachel Wang et al (*afd1, sgo1*).

We encourage unsolicited submissions. Authors should supply concise summaries of their favorite genes, with images of mutant phenotypes, preferably previously unpublished. We are especially interested in key alleles, regulation, gene products, pathways (metabolic, development), evidence for map locations, and any other key information about the locus. Submissions may be in a text format of author's choosing, or approximate the formats used in the following submissions. Reviews will be edited by Newsletter with author review of final copy. Updates may be added from time to time, similar to the process for the Online Mendelian Inheritance of Man, but where initial authors will be consulted regarding major updates. If you would like to contribute, send your reviews to Mary Schaefer, schaefferm_a_t_ missouri.edu.

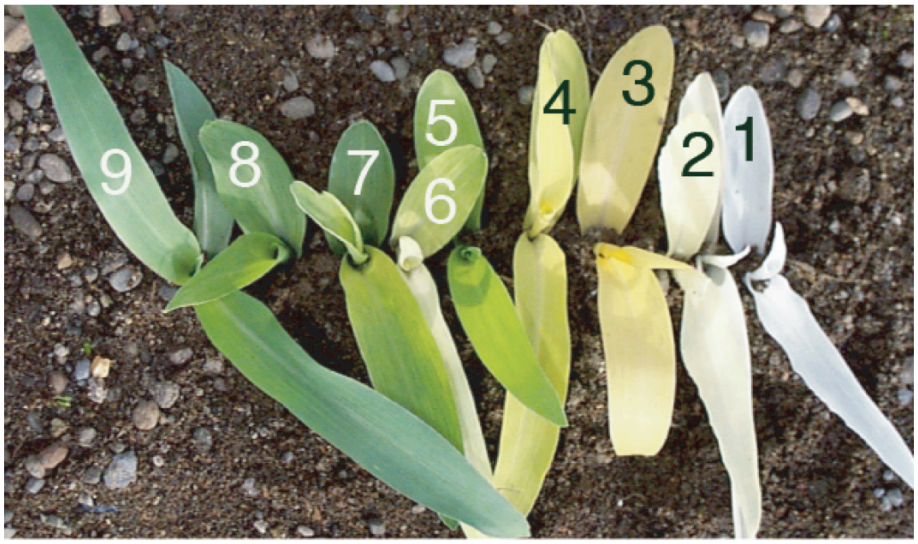
New gene reviews in following pages, ordered by author's last name and gene symbol:

Barkan, Alice
- *crp4 chloroplast RNA processing 4*
- *pet2 photosynthetic electron transport 2*
- *ptac12 plastid transcriptionally active chromosome 12 homolog*
- *tha5 thylakoid assembly 5*

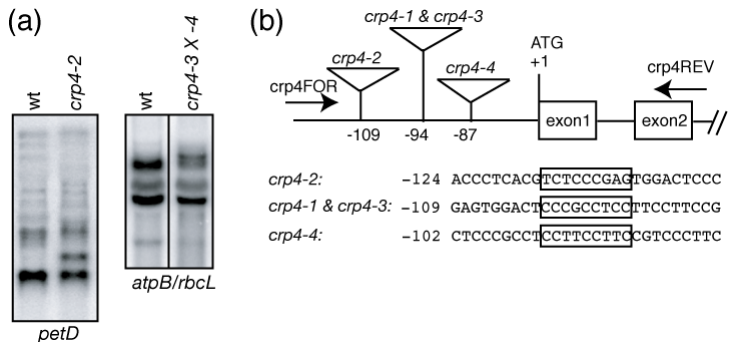
Consonni, Gabriella
- *emp4 empty pericarp 4*

Frey, Monika
- *bx1 benzoxazinless 1*
- *bx2 benzoxazinless 2*
- *bx3 benzoxazinless 3*
- *bx4 benzoxazinless 4*
- *bx5 benzoxazinoid synthesis 5*
- *bx6 benzoxazinoid synthesis 6*
- *bx7 benzoxazinoid synthesis 7*
- *bx8 benzoxazinoid synthesis 8*
- *bx9 benzoxazinoid synthesis 9*

Author Alice Barkan, University of Oregon, Eugene, OR 97403-121- USA
Name *crp4 chloroplast RNA processing4* Synonyms: TIDP3337
Chromosome 7S bin 7.01 **Gene Model** GRMZM2G377761
Function chloroplast RNA processing and turnover
Image mutant seedlings are pale green. See #5 on seedling color scale below.



Summary Encodes a polynucleotide phosphorylase involved in chloroplast RNA processing and turnover. The gene has been identified by *Mutator* insertion (Williams-Carrier et al 2010.)
First reported Williams-Carrier et al 2010.
Key Alleles Four *Mu* induced alleles have been described. These are weak alleles and condition pale green seedlings containing aberrant chloroplast transcripts that are extended at their 3' ends.



Figure, taken from Williams-Carrier et al (2010) Plant J with permission.
(a) RNA gel blots showing examples of aberrant chloroplast RNAs in *crp4* mutants.
(b) Summary of *Mu* insertion sites. Two alleles had insertions at identical positions. The sequences of the target site duplications are shown.

Map Location: Initially located by alignment to the B73_v2 reference genome sequence (Williams-Carrier et al 2010). This region overlaps a genetic mapping probe: INDEL TIPD3337 (Liu et al 2009.)
Gene Product polynucleotide phosphorylase, (aka polyribonucleotide nucleotidyltransferase) EC 2.7.7.8. A 3'→5' exoribonuclease involved in chloroplast RNA processing and turnover. Details

about the enzyme activity and chloroplast localization are from *Arabidopsis* (Walter et al 2002; Marchive et al 2009; Germain et al 2011).

Orthologs rice Os07g07310; Arabidopsis AT3G03710

References

Germain A, Herlich S, Larom S, Kim SH. Schuster G, Stern DB (2011) Mutational analysis of Arabidopsis chloroplast polynucleotide phosphorylase reveals roles for both RNase PH core domains in polyadenylation, RNA 3'-end maturation and intron degradation. **Plant J** 67:381-394.

Liu S, Yeh CT, Ji T, Ying K, Wu H, Tang HM, Fu Y, Nettleton D, Schnable PS Mu transposon insertion sites and meiotic recombination events co-localize with epigenetic marks for open chromatin across the maize genome (2009) **PLoS Genet** 5:e1000733

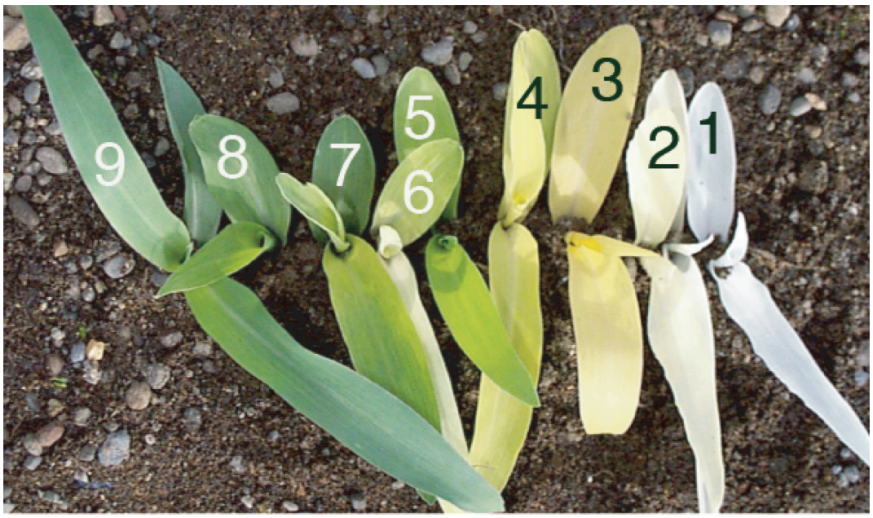
Marchive C, Yehudai-Resheff S, Germain A, Fei Z, Jiang X, Judkins J, Wu H, Fernie AR, Fait A, Stern DB (2009) Abnormal physiological and molecular mutant phenotypes link chloroplast polynucleotide phosphorylase to the phosphorus deprivation response in Arabidopsis. **Plant Physiol** 151, 905-924.

Walter M, Kilian J, Kudla J (2002) PNPase activity determines the efficiency of mRNA 3'-end processing, the degradation of tRNA and the extent of polyadenylation in chloroplasts. **EMBO J** 21: 6905-6914.

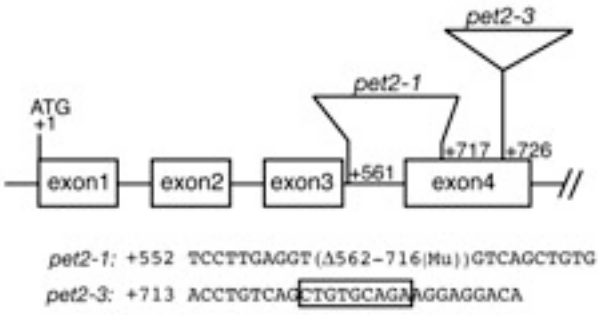
Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde RA, Coalter R, Barkan A (2010) Use of Illumina-HTS to identify transposon insertions underlying mutant phenotypes in high-copy *Mutator* lines of maize. **Plant J** 63:167-177.

Links: MaizeGDB: crp4 **|**

Author Alice Barkan, University of Oregon, Eugene, OR 97403-1210 USA
Name *pet2 photosynthetic electron transport2* Synonyms: Zmhcf208, TIDP5737, MAGI_114073,
Chromosome 1 bin 1.04 **Gene Model** GRMZM2G087063
Function: Chloroplast cytochrome b6f assembly
Image: Mutant seedlings are pale green. See #5 on seedling color scale below.



Summary Maize ortholog of the CCB2 protein required for covalent attachment of heme to cytochrome b6 in *Chlamydomonas* (Kuras et al 1997). See also *Arabidopsis* HCF208 (Lyska et al 2007). Maize mutant seedlings lack the cytochrome b6f complex. Cloned by *Mu* tagging.
Defining reports: Voelker and Barkan 1995; Williams-Carrier et al 2010
Key Alleles: Two *Mu*-induced alleles have been described. These are null alleles, and condition pale green, non-photosynthetic seedlings lacking the chloroplast cytochrome b6f complex. The positions of *Mu* insertions in *pet2-1* and *pet2-3* are diagrammed below. The insertion in *pet2-1* was accompanied by a deletion of flanking genomic sequence. The box marks the target duplication in pet2-3.



This figure is taken from Williams-Carrier et al, Plant J, 2010, with permission.
Map Location: Originally from corresponding gene model on B73 reference genome v2 (Williams-Carrier 2010). Mapping probe: the *pet2* gene model aligns with InDel TIDP5737 on the ISU IBM 2007 map (Liu et al 2009).

Gene Product A CCB2 protein homolog and required for assembly of cytochrome b6f in thylakoids. In other species, CCB proteins are involved in cofactor assembly on complex C subunit B of photosynthetic membranes (Kuras et al 1997; Lysak et al 2007; Saint-Marcouz et al 2009.

Orthlologs Os10g37840 (*Oryza sativa*); AT5g52110 (*Arabidopsis*)

References

de Vitry C, Desbois A, Redeker V, Zito F, Wollman FA (2004) Biochemical and spectroscopic characterization of the covalent binding of heme to cytochrome b6. **Biochemistry** 43:3956-3968.

Lyska D, Paradies S, Meierhoff K, Westhoff P (2007) HCF208, a homolog of Chlamydomonas CCB2, is required for accumulation of native cytochrome b6 in Arabidopsis thaliana. **Plant Cell Physiol**, **48**, 1737-1746.

Kuras R, de Vitry C, Choquet Y, Girard-Bascou J, Culler D, Buschlen S, Merchant S, Wollman FA (1997) Molecular genetic identification of a pathway for heme binding to cytochrome b6. **J Biol Chem** 272: 32427-32435.

Liu S, Yeh CT, Ji T, Ying K, Wu H, Tang HM, Fu Y, Nettleton D, Schnable PS (2009) Mu transposon insertion sites and meiotic recombination events co-localize with epigenetic marks for open chromatin across the maize genome. **PLoS Genet** 5:e1000733

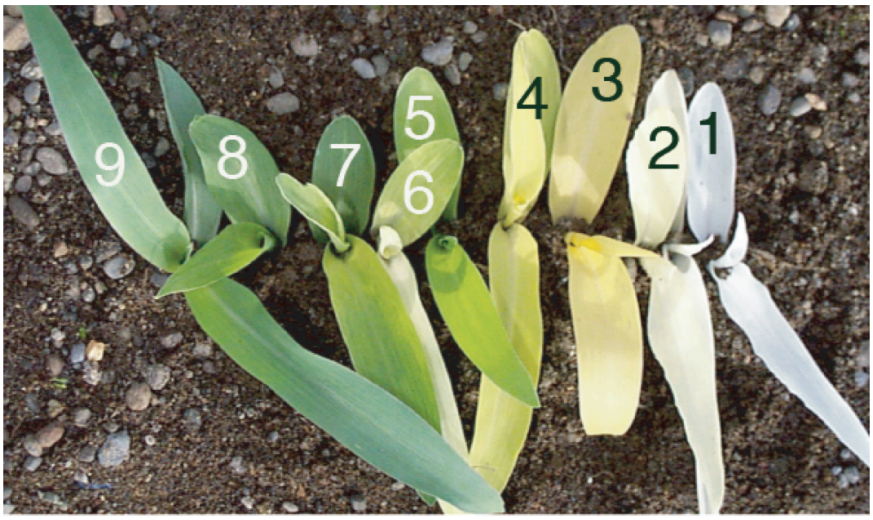
Saint-Marcoux D, Wollman FA, de Vitry C (2009) Biogenesis of cytochrome b6 in photosynthetic membranes. **J Cell Biol** 185:1195-207.

Voelker R, Barkan A (1995) Nuclear genes required for post-translational steps in the biogenesis of the chloroplast cytochrome *b6/f* complex. **Mol Gen Genet** 249: 507-514.

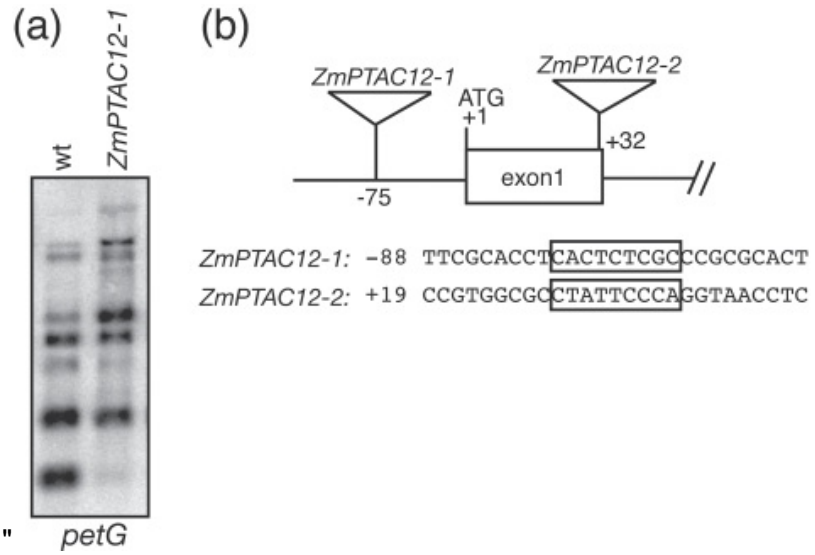
Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde RA, Coalter R, Barkan A (2010) Use of Illumina-HTS to identify transposon insertions underlying mutant phenotypes in high-copy *Mutator* lines of maize. **Plant J**, 63:167-177.

Links: MaizeGDB pet2 **|** NCBI LOC100194324 | Uniprot B4FIA3

Author Alice Barkan University of Oregon, Eugene, OR 97403-1210 USA
Name *ptac12 plastid transcriptionally active chromosome12 homolog* Synonyms: CL60164_1b, ZmPTAC12, crp5
Chromosome 10 bin 10.03 **Gene Model** GRMZM2G005938
Function chloroplast transcription
Image mutants are pale yellow green (#4 on color scale)



Summary Cloned by *Mu*-tagging and shown to have aberrant chloroplast transcript populations. The gene is homologous to PTAC12 gene characterized in *Arabidopsis*.
First reported Williams-Carrier et al. 2010.
Key Alleles *ptac12-1::Mu* and *ptac12-2::Mu*, with *Mu* inserts just upstream of the first exon and within the first exon respectively. Both are pale yellow green (see above image) and have aberrant chloroplast transcript populations (see below)



This figure is taken from Williams-Carrier et al. (2010) Plant J, with permission. (a) RNA gel blot showing chloroplast *petG* RNAs in the *ptac12-1* mutant.; (b) *Mu* insertion sites in *ptac12* mutant alleles. The sequences of the target site duplications are boxed in the sequence below.

Map Location: Based on gene model GRMZM2G300408 position on the v2 of B73 reference genome. Mapping probes: Overgo (physical map) CL60164_1 (Gardiner et al 2004)

Gene Product Homolog **of** PTAC12 (*Arabidopsis*). In *Arabiodopsis*, PTAC12 (AT3G59040) is plastid localized and required for plastid gene expression (Pfalz et al 2006.)

Orthologs: AT2G34640 (*Arabidopsis*); Os01g0769900 *(Oryza sativa*)

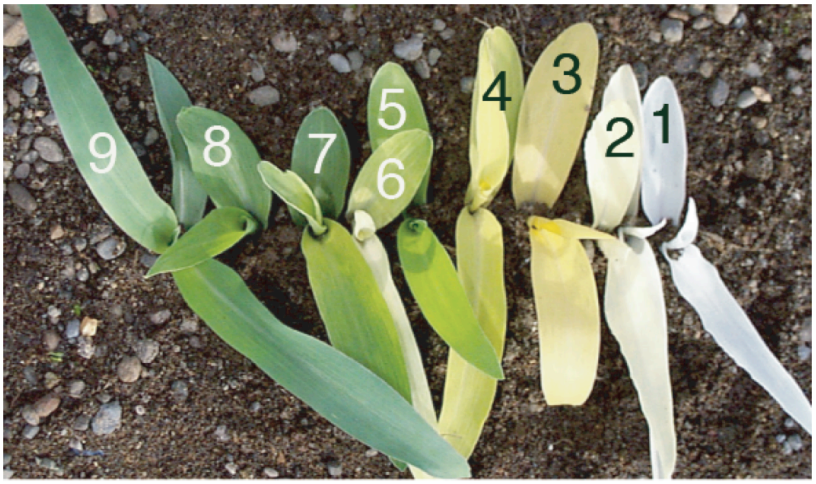
References

Pfalz J, Liere K, Kandlbinder A, Dietz KJ, Oelmuller R (2006) pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. **Plant Cell** 18:176-197.

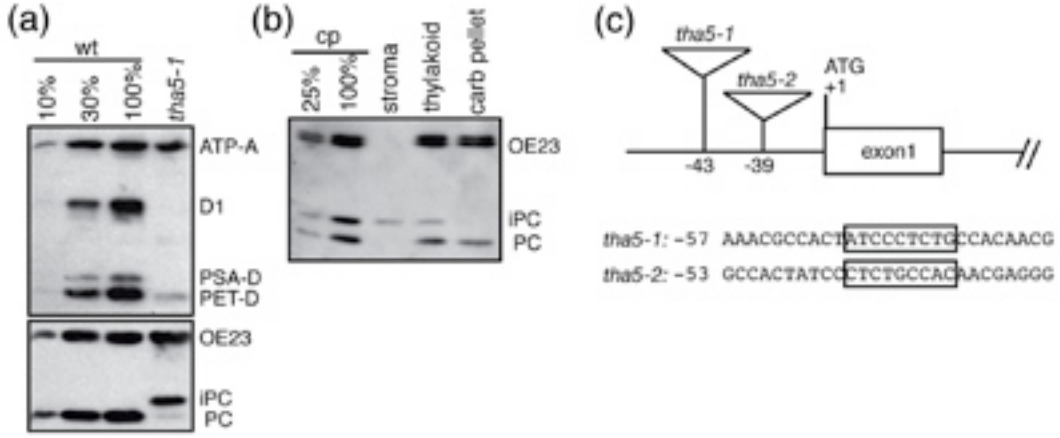
Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde RA, Coalter R, Barkan A. (2010) Use of Illumina-HTS to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize**. Plant J**, 63:167-177.

Links: MGDB ptac12 | NCBI LOC100501872 | UniProt C4J6N2

Authors Alice Barkan University of Oregon, Eugene, OR 97403-1210 USA
Name ***tha5 thylakoid assembly5***
Chromosome 10 bin 10.03 Gene Model GRMZM2G300408
Function. Translocation of proteins across the thylakoids.
Image mutant seedlings are pale green. See #5 on seedling color scale below.



Summary: Encodes cpSecE, involved in the cpSec pathway for the translocation of proteins across the thylakoid membrane. Two *Mu*-induced alleles have been described. *tha5* mutants are pale green and seedling lethal. They accumulate reduced levels of photosystem I, photosystem II, and the cytochrome b6f complex, and increased levels of the stromal intermediate of plastocyanin. These properties phenocopy those of *tha1* mutants, which lack cpSecA.
First reported: Williams-Carrier et al. 2010.
Key Alleles Two Mu-induced alleles have been described: *tha5-1::Mu* and *tha5-2::Mu*. Both have *Mu* insertions just upstream of the first exon (c).



The above figure was taken from Williams-Carrier et al (2010) Plant J, with permission. (a) The thylakoid protein profile of *tha5* mutants. The top shows an immunoblot of leaf proteins probed with antibodies to subunits of photosystem II (D1), photosystem I (PSA-D), the cytochrome b6f complex (PET-D) and the thylakoid ATP synthase (ATP-A). The bottom panel shows a duplicate blot probed with antibodies to OE23 and plastocyanin (PC); these proteins are targeted to the thylakoid lumen via the cpTAT and the cpSec

pathway, respectively. This protein profile is similar to that of *tha1* mutants, which have a *Mu* insertion in the gene encoding cpSecA. (b) *tha5* mutants have a defect in the translocation of plastocyanin across the thylakoid membrane. Stromal and thylakoid fractions were obtained from chloroplasts (cp) isolated from *tha5-1* mutants. An aliquot of the thylakoid fraction was incubated with carbonate to remove extrinsic proteins on the stromal face, and then pelleted to recover membranes. The results show that iPC accumulates outside the thylakoid lumen, demonstrating a defect in its translocation across the membrane (c) Summary of *Mu* insertion sites. The sequences of the target site duplications are shown above.

Map Location From alignment of cDNA BT018882.1|54653663.

Gene Product cpSecE, part of the cpSec pathway for thylakoid protein translocation. Inferrred from sequence similarity and mutant phenotypes.

Reference

Williams-Carrier R, Stiffler N, Belcher S, Kroeger T, Stern DB, Monde RA, Coalter R, Barkan A.(2010) Use of Illumina sequencing to identify transposon insertions underlying mutant phenotypes in high-copy Mutator lines of maize. **Plant J.** 63:167-177.

Links: MGDB tha5 | NCBI LOC100304099 | UniProt B6U410

Author Gabriella Consonni Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
Name *emp4 empty pericarp4* Synonyms: *abs*-7065, aborted seed7065*
Chromosome 1L bin 1.10 **Gene Model** GRMZM2G092198
Function seed development, mitochondrial transcript accumulation, PPR protein

Image ear segregating *emp4/emp4* kernels 20 days after pollination

S**ummary** *emp4* is a recessive lethal mutation resulting from a *Mutator* element (*Mu3*) insertion in the gene. The *emp4* phenotype is similar to other *emp* (*empty pericarp*) mutants, a class of *dek* mutants with the most severe reduction in kernel development (Dolfini et al 2007). *emp4* mutant caryopses are macroscopically recognizable as early as 10 days after pollination (DAP), because of their pale, translucent, and collapsed appearance. At later stages the comparison of wild-type and mutant sibling kernels shows a reduction in the size of the mutant caryopsis, which is surrounded by a loose pericarp. *emp4* seeds display severe morphological abnormalities. Differentiation of transfer cells in the nutrient-importing basal endosperm is very irregular in *emp4* mutant endosperms; cells are smaller, vacuolated, and lack defined cell wall ingrowths. The epidermal aleurone layer is discontinuous and contains many irregularly shaped cells. *emp4* RNA accumulates in a range of tissues, including immature embryos and endosperms, leaves, roots, stems, and ovaries. *emp4* is predicted to encode a 614–amino acid mitochondrial-localized protein that is highly homologous with the PPR class of proteins. Expression data indicate that EMP4 regulates transcript accumulation of a small group of mitochondrial genes, namely *rps2A*, *rps2B*, *rps3*/*rpl16*, and *mttb* (*orfX*) (Gutierrez-Marcos et al 2007).

First reported Giulini A et al 1998
Key Alleles: *emp4-1*::Mu3, recessive; *Mu3* insertion in the 5' end of the transcribed region
Map Location: maps to chromosome 1L by B-A chromosome translocations; between *umc140* and *umc106* using the LHRF inter-mated recombinant inbred mapping panel (Gutierrez-Marcos et al 2007)
Mapping probes: RFLP: "emp4 probe 1", a 401-bp PstII-MluI restriction fragment obtained from the genomic DNA flanking the *Mu3* insertion of *emp4-1::Mu3* (Gutierrez-Marcos et al 2007),
Gene Product pentatricopeptide repeat protein, PPR. Localized in the mitochrondrion; regulates transcript accumulation of a small group of mitochondrial genes, namely *rps2A*, *rps2B*, *rps3*/*rpl16*, and *mttb* (*orfX*, Gutierrez-Marcos et al 2007).

Orthologs Os03g51840 (*Oryza sativa*); AT3G49730, At5g65820 (*Arabidopsis*)

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Links: MaizeGDB emp4 | NCBI DQ291135

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Name ***bx1 benzoxazinless 1*** Synonyms: PCO064449, TRPA, trp1, umc1022, umi7

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G085381

Function biosynthesis of benzoxazinoids, insect resistance.

Summary First described by Hamilton in 1964 as a mutant sensitive to the herbicide atrazine and lacking benzoxazinoids (less than 1% of the wild type level). Mutants reduce the resistance to first generation European corn borer that is conferred by benzoxazinoids. Molecular characterization reveals that BX1 is a homologue to the alpha-subunit of tryptophan synthase (TSA) (Frey et al 1997; Melanson et al 1997) and catalyzes the first step in synthesis of endogenous benzoxinoid pesticides.

Image Plants synthesizing benzoxinoids have pale blue color when crushed and treated with FeCl3 solution. **A.** primary roots, segregating *bx1*/*bx1* and *Bx1*/*bx1* (blue). **B.** seedlings (left) *bx1bx1*, (right) *Bx1* *Images courtesy MaizeGDB.*

First reported Hamilton 1964

Features of the Bx-genes All *bx*-genes are tightly linked, a feature that is uncommon for plant genes of a biosynthetic pathways. Especially, genes encoding the different enzymatic functions BX1, BX2 and BX8 are found within about 50 kb. Results from wheat and rye indicate that the cluster is an ancient feature (Nomura et al 2003). In wheat the cluster is split into two parts. The wheat genes *Bx1* and *Bx2* are located in close proximity on chromosome 4 and wheat *Bx3*, *Bx4* and *Bx5* map to the short arm of chromosome 5; an additional *Bx3* copy was detected on the long arm of chromosome 5B (Nomura et al 2003). Recently, additional biosynthetic clusters have been detected in other plants for other biosynthetic pathway and this organization might be common in plants (Osbourn 2010).

Key Alleles The reference allele has a deletion of about 900 bp, located at the 5'-terminus and comprising sequences upstream of the transcription start site and the first exon. A second mutant allele is given by a *Mu*-insertion in the fourth exon (Frey et al 1997). Diversity analysis has been performed for 281 lines and indicates *Bx1* is responsible for much of the natural variation in DIMBOA synthesis (Butron et al 2010).

Map Location Maps to short arm of chromosome 4 using chromosome A-B translocations. (Simcox and Weber 1985); closely linked to other genes in the benzoxazinoid synthesis pathway [*bx2*, *bx3*, *bx4*, *bx5* Frey et al. 1995, 1997); 2490 bp from *bx2* (Frey et al 1997); between *umc123* and *agrc94* on 4S (Melanson et al 1997). Mapping probes: SSR p-umc1022 (Sharopova et al 2002); Overgo (physical map probe) PCO06449 (Gardiner et al 2004).

Gene Product Indole-3-glycerol phosphate lyase, chloroplast, EC 4.1.2.8 . The X-ray structure of BX1 has been resolved and compared with bacterial TSA (tryptophan synthase alpha subunit, Kulik et al 2005).

Homologs Three homologs have been reported in maize: *tsa1* on chromosome 7 (TSA, Frey et al 1997; Melanson et al 1997), *Igl1* on chromosome1 (Frey et al 2000), with a tightly linked "TSAlike", aka tsah1). TSA, and BX1 catalyze the same reaction, the cleavage of indole-3-glycerol phosphate to yield indole, but with different efficiencies (Frey et al 1997). BX1 has been wrongly annotated as maize TSA (ZmTSA; Kramer and Koziel 1995). Maize TSA has been characterized recently (Kriechbaumer et al 2008). IGL delivers volatile indole in tritrophic interaction (Frey et al 2000). The function of TSAlike is unknown.

DIMBOA biosynthetic pathway Benzoxazinoids or cyclic hydroxamic acids are defense chemicals that confer broad resistance against insects, pathogenic fungi and bacteria and have allelophatic function (Niemeyer 1988; Sicker et al 2000). Benzoxazinoids are found in a multitude of species of the family *Poaceae* (*Gramineae*), including the major agricultural crops maize, wheat and rye. Benzoxazinoids are not detected in *Oryza* sp. *Avena* sp., *Brachypodium distachyon* and *Hordeum vulgare*. Outside the grasses, benzoxazinoids are found sporadically in single species of the *Lamiaceae*, *Plantaginaceae*, *Acanthaceae* and *Ranunculaceae* (Frey et al 2009). In maize, genes *bx1* through *bx9* are sufficient to synthesize the 2-O-beta-glucoside of the benzoxazinoid DIMBOA [2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one]. Importantly, a correlation between benzoxazinoid content and control of the European corn borer (*Ostrinia nubilalis*) was detected (Klun et al 1970). High benzoxazinoid contents are restricted to young plants, hence control of the European corn borer is limited to the first brood. Benzoxazinoids are preformed defense chemicals that are synthesized prior to the challenge by herbivores or pests. For storage the reactivity is reduced by glucosylation. The intracellular storage organelle is the vacuole. In maize, specific beta-glucosidases (GLU1, GLU2) are located in the plastid (Czjzek et al 2000). Upon disintegration of the cell, the glucosidases bioactivate the benzoxazinoid glucosides by hydrolysis. See Figure 1 below.

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Links MGDB: bx1 **|** NCBI: bx1 **|** UniProt: P42390

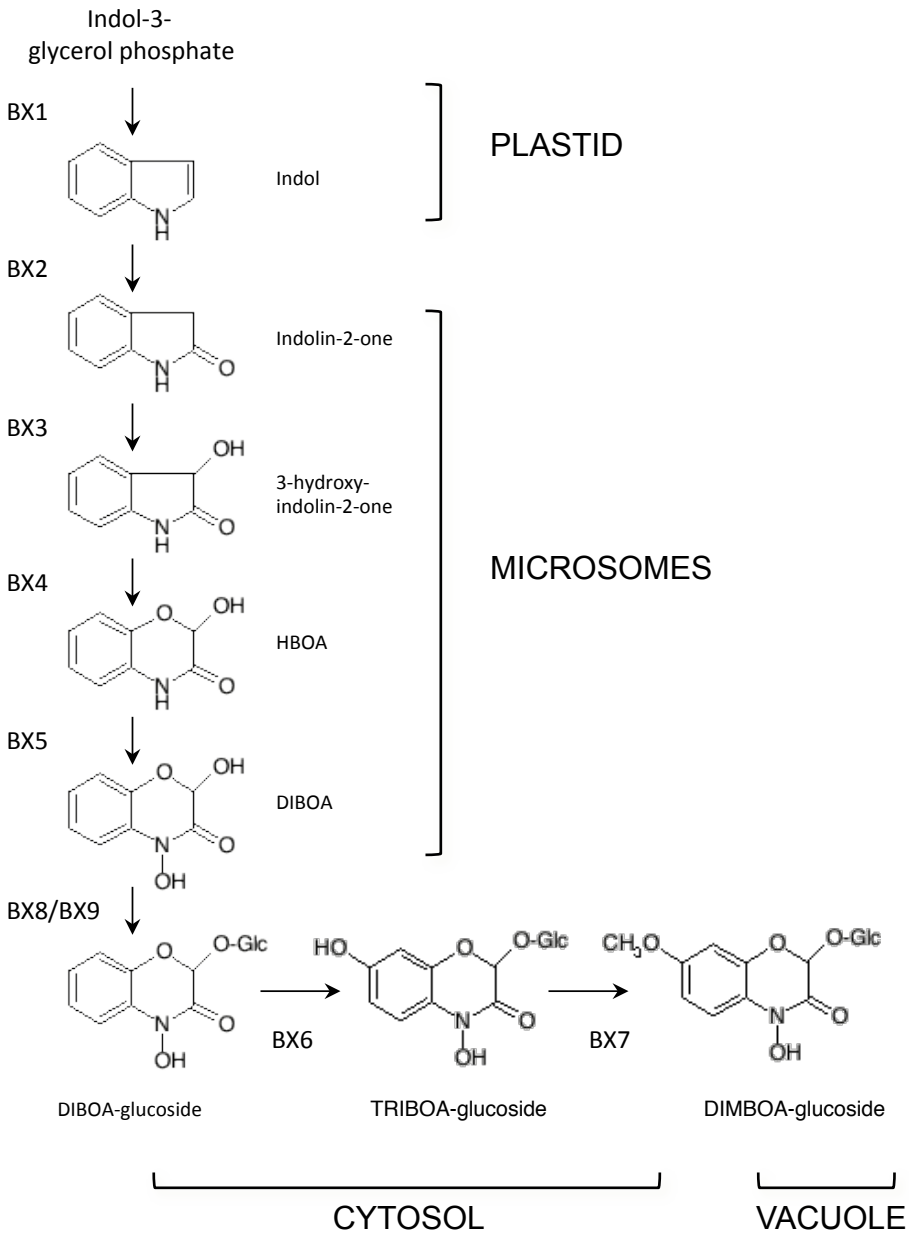

Figure 1: Benzoxazinoid biosynthesis in maize. Enzymes, biosynthetic intermediates and subcellular location of the pathway are indicated.

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Name ***bx2 benzoxazinless2*** Synonyms: CYP71C4, CYPzm4, cyp5, mpik8, umc1164, umc1682, CL1575_1

Chromosome 4 bin 4.01 Gene Model GRMZM2G085661

Function biosynthesis of benzoxazinoids; insect resistance.

Summary Encodes one of four cytochrome P450 monooxygenases that introduce specifically and consecutively four oxygen atoms in the conversion of indole to DIBOA. All four genes are closely related and are grouped into one P450-subfamily, CYP71C. Each share one short (70 to 149 bp) intron at analogous positions; a second intron position is shared by *bx3* and *bx5* (143 bp and 124 bp; Frey et al 1995). *bx2* is one of the *bx*-genes that are tightly linked, a feature that has been considered uncommon for plant genes of a given biosynthetic pathway. See also *bx1*, Frey et al 1997, 2007.

First reported Frey et al 1995

Key Alleles No mutant alleles are described in the literature. Candidate *Ds* insertion mutants related to GenBank accessions FI849846, FI84971 are available (MaizeGDB genome browser; Vollbrecht et al 2010).

Map Location Closely linked to other genes in the benzoxazinoid synthesis pathway [*bx1*, *bx3*, *bx4*, *bx5*, *bx8*]; 2490 bp from *bx1* (Frey et al 1995,1997, 2009). Mapping probes: RFLP sed32.1(mpik8); SSRs umc1164, umc1682 (Sharopova et al 2000); Overgo (physical map probe) CL1575_1 (Gardiner et al 2004).

Gene Product indole-2-monooxgenase, aka indole,NAD(P)H:oxygen oxidoreductase (2-hydroxylating), EC 1.14.13.137 (pending review), member of the P450 CYP71C family, localized in the endoplasmic reticulum, and specifically transfers a molecular oxygen atom to position 2 of indole (Glawischning et al 1997, 1999; Frey et al 1997).

DIMBOA biosynthetic pathway: see *bx1*

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Links: MGDB: bx2 | NCBI: bx2 | UniProt: Q43257

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Name: ***bx3 benzoxazinless 3*** Synonyms: CYP71C2, CYPzm2, cyp3, mpik6, umc1017, CL1574_1, gpm290, gnp_QAE32a05

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G167549

Function biosynthesis of benzoxazinoids; insect resistance in young seedlings

Summary Encodes one of four cytochrome P450 monooxygenases that introduce specifically and consecutively four oxygen atoms in synthesis of DIBOA from indole. All four genes are closely related and are grouped into one P450-subfamily, CYP71C. Each share one short (70 to 149 bp) intron at analogous positions, a second intron position is shared by *Bx3* and *Bx5* (143 bp and 124 bp). (Frey et al 1995) *Bx3* is one of the tightly linked *Bx*-genes. See also *Bx1,* Frey et al 1997, 2009.

Images see *bx1*

First reported Frey et al 1995

Key Alleles *bx3-m:M*u is benzoxazinless (Frey et al 1997). *Mu* is inserted into the second exon, corresponding to position 1260 of sequence accession Y11404.

Map Location Closely linked to other genes in the benzoxazinoid synthesis pathway [*bx1*, *bx2*, *bx4*, *bx5*, *bx8*]; 2490 bp from *bx1* (Frey et al 1995,1997,2009). Mapping probes: SSR umc1017 (Sharopova et al 2002); RFLP gnp_QAE32a05 (Falque et al 2005) ; overgo (physical map) CL1574_1 (Gardiner et al 2004).

Gene Product indolin-2-one monooxgenase, aka indolin-2-one,NAD(P)H:oxygen oxidoreductase (3-hydroxylating), EC 1.14.13.138 (pending review). A P450 CYP71C family enzyme that is localized in the endoplasmic reticulum. It specifically transfers a molecular oxygen atom to position 3 of indolin-2-one (Glawischning et al 1997, 1999; Frey et al 1997).

DIMBOA biosynthetic pathway *see bx1*

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Links MGDB: bx3 | NCBI: LOC100502029 [includes RFLP gnp_QAE32a05 sequence] | UniProt: Q43255 [assigned to mRNA X81829]

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Name ***bx4 benzoxazinless 4*** Synonyms: CYP71C1, CYPzm1, cyp2, mpik5A, mpik5B, agrc94, pco117584, PHM1184, PZA00136, TIDP9229

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G172491

Function biosynthesis of benzoxazinoids; insect resistance

Summary One of four cytochrome P450 monooxygenases that introduce specifically and consecutively four oxygen atoms in the conversion of indole to DIBOA. All four genes are closely related and are grouped into one P450-subfamily, CYP71C. Each share one short (70 to 149 bp) intron at analogous positions, a second intron position is shared by *bx3* and *bx5* (143 bp and 124 bp; Frey et al 1995). *bx4* is tightly linked to other *bx*-genes, a phenomenon that has been considered uncommon for plant genes of a given biosynthetic pathway. See also *bx1*, Frey et al 1997, 1999.

First reported Frey et al 1995

Key Alleles No mutants are known.

Map Location Closely linked to other genes in the benzoxazinoid synthesis pathway (*bx1*, *bx2*, *bx3*, *bx5*, *bx8*; Frey et al 1995,1997, 2009). Mapping probes: sequenced RFLPs agrc94, cyp71c1, mpik5; NAM SNP PHM1184-26 (McMullen et al 2009); INDEL TIDP9229 (Fu et al 2006); overgo (physical map) PCO117584 (Gardiner et al 2004).

Gene Product 3-hydroxy-indolin-2-one hydroxylase (P450), aka 3-hydroxyindolin-2-one,NAD(P)H:oxygen oxidoreductase (2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one-forming), EC 1.14.13.139 (pending review), a cytochrome P450 71C family enzyme, localized in the endoplasmic reticulum. It specifically transfers a molecular oxygen atom into the ring at carbons 3 and 3a to form 2-hydroxy-1,4-benzoxazin-3-one (HBOA; Glawischning et al 1997, 1999; Frey et al 1997; Spiteller et al 2001.)

DIMBOA biosynthetic pathway see *bx1*.

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Links: MGDB: bx4 | NCBI: CYP71C1 | UniProt: Q43250

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Name *bx5 benzoxazinoid synthesis 5* Synonyms: CYP71C3, CYPzm3, cyp4, mpik7, pco142958, umc1276

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G063756

Function: biosynthesis of benzoxazinoids; insect resistance in young seedlings

Summary: Encodes one of four cytochrome P450 monooxygenases that introduce specifically and consecutively four oxygen atoms in the conversion of indole to DIBOA. All four genes are closely related and are grouped into one P450-subfamily, CYP71C. Each share one short (70 to 149 bp) intron at analogous positions, a second intron position is shared by *bx3* and *bx5* (143 bp and 124 bp; Frey et al 1995). *bx5* is tightly linked to other *bx*-genes, a phenomenon that has been considered uncommon for plant genes of a given biosynthetic pathway. See also *bx1*, Frey et al 1997, 2009.

First reported: Frey et al. 1995

Key Alleles No mutants are known. Natural diversity has been described (Butron et al 2010).

Map Location Closely linked to other genes in the benzoxazinoid synthesis pathway (*bx1*, *bx2*, *bx3*, *bx4*, *bx8*; Frey et al 1995, 1997, 2009). Mapping probes: RFLPs mpik7 (sed7.1); SSR umc1276 (Sharopova et al 2002); overgo (physical map) PCO142958 (Gardiner et al 2004).

Gene Product 2-hydroxy-1,4-benzoxazin-3-one monoxygenase, aka 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one,NAD(P)H:oxygen oxidoreductase (*N*-hydroxylating), EC 1.14.13.140 (pending review), a cytochrome P450 71C3 enzyme that is localized in the endoplasmic reticulum. It specifically transfers a molecular oxygen atom onto the ring N of HBOA to form 2,4,-dihydroxy-1,4-benzoxan-3-one (DIBOA; Bailey and Larson 1991; Glawischning et al 1997, 1999; Frey et al 1997; Spiteller et al 2001).

DIMBOA biosynthetic pathway see *bx1*

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Links: MGDB: bx5 | NCBI: LOC100275822 (cDNA EU958243 alignment to B73_V2 genome sequence) | UniProt: P93703

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Name ***bx6 benzoxazinoid synthesis 6*** Synonyms: umc1232

Chromosome 4 bin 4.01 Gene Model is not found in B73 v2 reference genome sequence (see also Butron et al 2011)

Function biosynthesis of benzoxazinoids; insect resistance

Summary Functionally cloned as the 2-oxoglutarate dependent monooxygenase in DIMBOA synthesis. *Mu*-insertion mutants are available; instead of DIMBOA-glucoside, these mutants contain DIBOA-glucoside. Popcorn varieties have reduced *bx6* transcript levels and are enriched in DIBOA-glucoside compared to other maize lines (Frey et al 2003). Gene *bx6* is one of several *bx*-genes that are tightly linked, a phenomenon that has been considered uncommon for plant genes of a given biosynthetic pathway. See also *bx1*, Frey et al 1997, 2009.

First reported: Frey et al. 2003

Key Alleles Four *Mutator* insertional mutants have been described, all within the single *bx6* intron (Frey et al 2003). Mutants do not synthesize DIMBOA glucoside; instead DIBOA glucoside is the main benzoxazinoid that accumulates.

Map Location Closely linked to other genes in the benzoxazinoid synthesis pathway, and 7 cM distal to *bx3*, *bx4* (Frey et al 2003). Mapping probes: RFLP, the cDNA corresponding to sequence AF540907 (Frey et al 2003); SSR umc1232 (Sharopova et al 2002).

Gene Product 2,4-dihydroxy-1,4-benzoxazin-3-one-glucoside dioxygenase, aka 2-oxoglutarate dependent monooxygenase, EC 1.14.20.2 (pending review). Localized in the cytosol; introduces a hydroxyl-group, from molecular oxygen, at position C-7 of DIBOA-glucoside to generate TRIBOA-glucoside. It requires glucosylation of the substrate and does not take the aglucon as substrate (Glawischning et al 1997, Frey et al 2003, Jonczyk et al 2008)

DIMBOA biosynthetic pathway see *bx1*

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Links: MGDB: bx6 | NCBI: bx6 | UniProt: Q84TC2

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Name ***bx7 benzoxazinoid synthesis 7*** Synonyms: AY106107,IDP478, PCO065953, PCO088803a, PZA00115, umc2039

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G441753

Function benzoxazinoids synthesis; insect resistance

Summary Functionally cloned and demonstrated to encode a cyotosolic O-methyltransferase specifically involved in the DIMBOA biosynthetic pathway. Expression is highest in young seedlings (Jonczyk et al 2008). Loosely linked to the *Bx* gene cluster on chromosome 4S. See also *Bx1*, Frey et al 1997, 2009.

First reported Jonczyk et al 2008

Key Alleles Mutants are currently unavailable (Jonczyk et al 2008). No significant QTL near *bx7* were found in recombinant inbred lines (RIL) from crosses between B73 and inbreds CML52, CML322, IL14H, M37W, MS71, NC350, Oh43 and Tx303 (Butron et al 2010).

Map Location The *bx7* gene is on chromosome arm 4S, 30-40 cM proximal to the tight *bx* gene cluster. Mapping employed PCR probes and a BNL recombinant inbred mapping panel, CM37 x T232 (Jonczyk et al 2008). Other mapping probes: SSR umc2039 (Sharopova et al 2002); INDEL IDP47 (Fu et al 2006); overgo (physical map) PCO065952 (Gardiner et al 2004).

Gene Product TRIBOA-glucoside O-methyl transferase, EC 2.1.1.241. Localized in the cytosol; specifically O-methylates TRIBOA-glucoside; and has no catalytic activity toward phenylpropanoids and flavonoids (Jonczyk et al 2008).

DIMBOA biosynthetic pathway see *bx1*

References

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Links MGDB: bx7 NCBI: bx7 UniProt: B1P123

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Name ***bx8 benzoxazinoid synthesis 8*** Synonyms: gnp_QBS13f04, PCO085820, gpm562

Chromosome 4 bin 4.01 **Gene Model** GRMZM2G085054

Function benzoxazinoid synthesis; insect resistance

Summary Cloned from cDNAs corresponding to peptide sequences of the purified enzyme, a family 1 plant secondary metabolic pathway UDP-glucosyl transferase. Two similar enzymes have been described, BX8 and BX9. Both the BX8 and BX9 enzymes specifically O-glucosylate DIBOA and DIMBOA. Glucosylated benzoxinoids have reduced toxicity and are the main benzoxinoids in young maize seedlings. In the endogenous biosynthetic pathway, the substrate is DIBOA. However, glucosylation of exogenous DIMBOA in allelopathic interaction can be achieved by the enzyme. Both genes share one short (119 bp and 94 bp) intron at analogous positions (von Rad et al 2001). The *bx8* locus is included in the *bx* gene cluster on chromosome 4S. Such clustering had been considered uncommon for plant genes of a given biosynthetic pathway. See also *bx1*, Frey et al 1997, 2009.

First reported von Rad et al 2001

Key Alleles Mutants are currently unavailable (Jonczyk et al 2008).

Map Location Tightly linked to *bx1*, using as RFLP probe, the 5' end of cDNA AF331854; no recombinants are found with *bx1* and *bx2* using the CM37 x T232 recombinant mapping panel (von Rad et al 2011). Other mapping probes: RFLP gnp_QBS13f08 (gpm562, Falque et al 2005); overgo (physical map) PCO085820 (Gardiner et al 2004).

Gene Product UDP-glucose:2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one 2-D-glucosyltransferase, EC 2.4.1.202. Localized in the cytosol; DIMBOA and DIBOA are preferred substrates; there is no activity with indole acetic acid or quercitin; and very low activity for HMBOA or HBOA substrates (von Rad et al 2001). See also Bailey and Larson 1989.

DIMBOA biosynthetic pathway see *bx1*

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Links MGDB: bx8 **|** NCBI: bx7 **|** UniProt: B6TT6

Author Monika Frey Lehrstuhl für Genetik, Technische Universität München, 85354 Freising, Germany

Name ***bx9 benzoxazinoid synthesis 9*** Synonyms: gnp_QBL10f08, PCO129924, gpm524, PZA00484

Chromosome 4 bin 1.06 **Gene model** GRMZM2G085054

Function benzoxazinoid glucosylation; insect resistance

Summary Encodes a family 1 plant secondary metabolic pathway UDP-glucosyl transferase. Both BX8 and BX9 enzymes specifically O-glucosylate DIBOA and DIMBOA. Glucosylated benzoxinoids have reduced toxicity and are the main benzoxinoids in young maize seedlings. In the endogenous biosynthetic pathway, the substrate is DIBOA. However, the enzyme can achieve glucosylation of exogenous DIMBOA in allelopathic interaction. Both genes share one short (119 bp and 94 bp) intron at analogous positions. Loci were cloned from cDNAs corresponding to peptide sequences of the purified enzyme (von Rad et al 2001). Unlike *bx8*, *bx9* is not included in the *bx* gene cluster on chromosome 4S. See also Frey et al 1997, 2009.

First reported: von Rad et al 2001.

Key Alleles Mutants are currently unavailable (Joncyk et al 2008).

Map Location Mapped using the CM37xT232 RI population (von Rad et al 2001) and the IBM population (Falque et al 2005). RFLP mapping probes: cDNA AF331855; gnp_QBL10f08 (gpm524); overgo (physical map) PCO129924 (Gardiner et al 2004.

Gene Product UDP-glucose:2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one 2-D-glucosyltransferase, EC 2.4.1.202; cytosolic. Substrate specificity is similar the BX8, with some differences: BX9 is less efficient than BX8; and also glucosylates HMBO somewhat more efficiently than DIBOA (von Rad et al 2001). See also Bailey and Larson 1989.

DIMBOA biosynthetic pathway see *bx1*.

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Links: MGDB: bx9 | NCBI: bx9 | UniProt: B4G072

Maize Genetics Cooperation • Stock Center

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8,801 seed samples have been supplied in response to 300 requests for 2010. These include 108 requests received from 24 foreign countries. Popular stock requests include the NAM RIL populations, Hi-II lines, *ig1* lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, UniformMu sequence indexed stocks, other transposable element lines, and Maize Inflorescence Project EMS lines.

Approximately 5.7 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Optimal spring weather allowed us to plant our crossing nurseries in a timely manner. Our first crossing nursery planted on April 29 had excellent stands. However, seedling predation by redwing blackbirds reduced the stands in our second crossing nursery planted on May 25. This appears to be becoming a perennial problem caused by lack of rainfall between planting and seedling emergence. Birds find it relatively easy to pull seedlings out of the soft soil bed. We may have to routinely irrigate if there is no rainfall prior to seedling emergence in order to harden up the seedbed and make it more difficult for the birds to uproot the seedlings with attached kernel. Fortunately, despite the reduced stands in our second planting this year, there were sufficient plants for an adequate increase in most instances. During June, above normal rainfall patterns were established resulting in repeated flooding and standing water in portions of our fields. This affected our observation blocks more than our crossing blocks, and little material will need to be replanted next year. Warm temperatures and low plant stress following planting resulted in an earlier than normal pollination season and excellent yields.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of Alice Barkan (photosynthetic mutants), James Brewbaker (Hi27 near-isogenic mutant lines), Karen Cone (plant and kernel color lines), Ryan Douglas (*rgd2-R*), Patrice Dubois (*elm1-ref*, *phyB1*, and *phyB2* lines), Giuseppe Gavazzi (*a1-eap*), Inna Golubovskaya (meiotic mutants), Jerry Kermicle (various *r1* and *ga2* alleles), John Woodward (*thi2-blk1*), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

2. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We identified additional alleles of *lazy1, indeterminate1, viviparous2, viviparous9*, and *etched1*. In 2011, we plan to continue testing additional members of the pale endosperm class of mutants if space and manpower are available. In this manner, we hope to incorporate more stocks from our vast collection of unplaced uncharacterized (phenotype-only) mutants into the main collection.

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

4. We are continuing to characterize the *Enr* (*Fcu*) system of *r1* aleurone color enhancers as well as other factors that affect expression of *r1*. We are characterizing additional alleles of

Enr1 and other *r1* aleurone color enhancers. We are also trying to recover instances of the lapsed *y5* locus from PI accessions of orange endosperm tropical flints.

6. Fresh increases were made of several older A-A translocation stocks. The outcrosses will be grown in 2011 Observation to confirm by seed set which ones actually carry the translocation. We continued checking translocations received from W. R. Findley and Don Robertson marked with *wx*1 to confirm the chromosome arms involved. For those where we found no linkage, all sources were discarded.

7. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: http://www.maizegdb.org/MIP/) were grown again this summer. Approximately 300 families of M2 materials that were produced in 2007 were grown to increase seed supplies and recover previously observed mutations; this also included previously phenotyped families that had limited seed supplies. In addition, 1,306 families of 2008 and 2009 EMS seed increase materials were grown for adult plant observation and 595 families were screened in sand benches for seedling traits; the materials observed include mainly mutated B73 and Mo17 inbred lines, B73xMo17 hybrid, and various other inbred lines.

We grew a winter crop of 0.5 acres during the 2009/2010 growing season at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico. Heavy rainfall following pollination resulted in many moldy ears and a less than normal harvest. We did not plant a winter nursery in Puerto Rico this year. Critical plantings of a limited number of stocks were made in our greenhouse facilities this winter instead.

We have received 2,450 additional UniformMu sequence indexed lines produced by the Construction of Comprehensive Sequence Indexed Transposon Resources for Maize project (http://www.maizegdb.org/documentation/uniformmu/). We presently have 3,665 of these stocks.

Our IT Specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our collection. The tools are also used for our internal database (*e.g.,* inventory, pedigrees and requests). Currently, a more advanced search tool is being written in order to allow more flexibility in locating specific items in our inventory. Improvements have been made to the pedigree input tool in order to fix some problems with certain types of entries. Planning for a tool or improvements to current tools is underway for harvest information management as well as other work to improve the process used to generate tags for use during harvest. Importing data from MaizeGDB into our local database has been streamlined. We worked closely with MaizeGDB to insure that all communications and tools continued to work after MaizeGDB's migration to a new database- and web-hosting arrangement. Maintenance continues on our web site (http://www.uiuc.edu/ph/www/maize).

We mourn the recent and sudden loss of our colleague and friend Janet Day Jackson. Janet worked at The Maize Genetics Cooperation - Stock Center since 1989. Janet began her career as a maize geneticist in 1980, working with John Laughnan and Susan Gabay-Laughnan. Janet will be profoundly missed.

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

MAIZE GENETICS COOPERATION STOCK CENTER -- ADDITIONS TO OUR CATALOG OF STOCKS SINCE MNL83

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

Chromosome 1 Markers

111GA rs2-twd
112L rth3-3
112M rgd2-R::Mu5
125G hcf31-N1268B
132A id1-PI245132
132B bif2-04HI-A632xOh43GN-105
132C bif2-03HI-B73xMo17GN-556
132D bif2-03IL-A619TR-503

Chromosome 2 Markers

219AA B1-Peru; A1 A2 C1 C2 Pl1-Rhoades r1-g

Chromosome 3 Markers

301H g2-bsd1-s1
301I a1-eap
305E rnc1-2::Mu1
305F rnc1-3::Mu3
305H thi2-blk
307D lg2-03IL-A619TR-358
307E lg2-03IL-A619TR-767
316AA ts4 ^Hi27
333F vp1-Pookie

Chromosome 4 Markers

402H bt2-H2328
403F la1-03HI-B73GN-119
406E la1-05HI-RnjxW22GN-333
406F la1-MTM4659
411AA su1-JLB3
412I ppr5-1::Mu1

Chromosome 5 Markers

511D A2 Bt1 Ga2-w pr1
511E a2 Ga2-w
511L Ga2-s(mexicana)
511M Ga2-s(parviglumis)
513G bm1 ae1
513I A2 Ae1-5180::Mu1

Chromosome 6 Markers

617A pl1-0 c1-EMS Sh1 B1-S R1-r
617B pl1-0 C1-1162 Sh1
617C pl1-W22 B1-b C1 pr1 R1-r
617D pl1-987 B1-b C1 pr1 R1-r
617E pl1-987 B1-S C1-1162 Anr1 P1-wrb
617F pl1-987 B1-S C1-1170 anr1 p1-www
617G pl1-987 B1-S C1-1170 anr1 P1-wrb
617H why1-1::MuDR
617J ppr10-1::MuDR
617K ppr10-2::MuDR

Chromosome 7 Markers

725B ra3-JD

Chromosome 8 Markers

804AA v21-N25
811I wtf1-3::Mu1

Chromosome 9 Markers

919JA bz1-Mum9; MuDR ^W22 (UniformMu)
938A Bf1-DR044-3
938B Bf1-DR046-1
938C Bf1-DR046-2
938D Bf1-DR047-1
938E Bf1-DR047-4
938F Bf1-DR047-9
938G Bf1-DR047-11
938H Bf1-DR048-10
938I Bf1-DR049-2
938J Bf1-DR049-5
938K Bf1-DR050-5
938L Bf1-DR544-3
938M Bf1-DR5334-8

Chromosome 10 Markers

X07CG y9-87-2422-14
X12I DfK10(I) R1
X17EA R1-r; A1 A2 C1 C2 ^W22 (UniformMu recurrent parent)
X237C R1-nj:st(n361)
X237D r1-g(n8580)::Ds Nj-loss isolate 1
X237E g1 r1-g(n8580)::Ds Nj-loss isolate 2
X237F R1-g(n8580)-R1
X237G R1-g(n8580)-R2
X237H R1-g(n8580)-R3
X237I R1-g(n8580)-R4
X237J R1-d:pale-m9
X237K R1-d:pale-m10
X237L R1-d:pale-m22
X237M R1-d:pale-m23
X237N R1-d:pale-m34
X237O R1-g(Harinoso de Ocho)
X237P r1-r:n46
X237Q g1 r1-r:n35
X237R g1 r1-r:n101
X237S r1-r:n156
X237T r1-r:N1-3-1
X237U r1-r:N1-3-2
X237V r1-r:N1-3-3
X237W r1-r:N1-3-4
X237Y R1-g:9
X335A R1-g:12

X335B R1-g:13
X335C R1-g:14
X335D g1 R1-g:m40 Mst1
X335E g1 R1-g:m41 Mst1
X335F g1 R1-g:m47 Mst1
X335G R1-nj:S(isolate 1)
X335H R1-nj:S(isolate 2)
X335I g1 R1-Sc:P(g1005)
X335J g1 R1-Sc:P(g1006)
X335K R1-S:P(g1502)
X335L R1-S:P(g1503)
X335M r1-g:de128
X335N r1-g:de137
X335O r1-g:de139
X335P r1-g:de142
X335Q r1-sc:m1::Ds
X335R r1-sc:m9::Ds
X335S R1-sc:m3-RevJ2355
X335T Ds-10L2 R1-sc:124; No Ac
X335U Ds-10L4 R1-sc:124; No Ac
X335V R1-g:1:lc1(del-g425)
X335W R1-g:1:lc1(del-g427)
X335X R1-st Lc1
X335Y R1-st(del)lc1:g327
X335Z R1-st(del)lc1:g301
X336A R1-st(del)lc1:g308
X336B R1-st(del)lc1:g307
X336C R1-sc(del)lc1
X34K y9-84-5275-14
X37E Enr1-628 r1-g(Stadler)
X37F Enr1-ZC r1-g(Stadler)
X37G enr1-m594 Enr2-6117a R1-r(Venezuela559-PI302355)
X37H Enr2-694 R1-r(Venezuela559-PI302355)
X37I Enr3-594 R1-r(Venezuela559-PI302355)

Unplaced Genes
U740M crs4-2::Mu
U740N ppr4-1::Mu9
U940H pun1-bsd3
U940I cks1-O
U940J ffm1-O

Multiple Genes
M242K Skb1 Pl1-Bh c1 Sh1 wx1-m7::Ac7
M242L Skb1 Pl1-Bh c1 Sh1 Wx1 B1-S
M242M skb1 Pl1-Bh c1 sh1 Wx1 B1-S
M242N Pl1-Bh c1 sh1 B1-S
M242O Spb1 Pl1-Bh c1 B1-S
M242P spb1 Pl1-Bh c1 Sh1 B1-S
M641H gl6 lg2 wx1

Rare Isozyme
3004-002 Pgm1-1; Pgd2-10
3004-042 Mdh1-n; Mdh2-n; Mdh3-17.3
3004-050 Hex2-.5
3004-058 Mdh1-n; Mdh2-n; Mdh4-8
3004-067 Mdh4-8.5; mmm1-1; Pgm1-18
3004-081 Mdh1-.65; Mdh2-n; Mdh3-n; Mdh4-n; Mdh5-n
3004-107 R35 check line

Toolkit
T0940FA Muk

B-A Translocations (Others)
935A TB-9Sb(8)
935B TB-9Sb(9)
935C TB-9Sb(10)
935D TB-9Sb(11)
935E TB-9Sb(12)
935F TB-9Sb-1854
935G TB-9Sb-Dp9
935H IsoB9-9 isochromosome (1)
935I IsoB9-9 isochromosome (2)
935J IsoB9-9 isochromosome (3)
935K IsoB9-9 isochromosome (6)
935L IsoB9-9 isochromosome (7)
935M IsoB9-9 isochromosome (8)
935N IsoB9-9 isochromosome (9)
935O IsoB9-9 isochromosome (10)
935P IsoB9-9 isochromosome (13)

Stocks Characterized Only by Phenotype

adherent leaf
3610P ad*-03HI-B73GN-145

adherent tassel
3610Q ad*-03IL-A619TR-935

albescent
6008N al*-07MO-B73xMo17GN-345

albino seedling
4307R w*-04HI-Oh43xA632GN-39
6111K w*-04HI-A632xOh43GN-7

barren inflorescence
3611N bif*-03IL-A619TR-287
3611O bif*-03HI-B73xMo17GN-1055

bleached leaf
3712L zn*-N2403A

brachytic plant
5502H br*-03IL-A619TR-135

brittle endosperm
5810F bt*-06HI-B73GN-38
5810G bt*-07IL-B73GN-14

brown kernel

5810C bnk*-07IL-B73GN-80
5810D bnk*-07IL-B73GN-107
5810E bnk*-07IL-B73GN-137
6402G bnk*-07MO-B73xMo17GN-62

brown midrib
5803N bm*-03IL-A619TR-458

colored leaf
5812K lc*-04HI-A632xOh43GN-16

crinkled leaf
4106I cr*-03HI-B73xMo17GN-1036

defective kernel
3704J de*-N1090A
5810T dek*-04HI-A632xOh43GN-13
5811J dek*-06HI-B73GN-19
5812G dek*-04HI-A632GN-40
5812H dek*-03HI-A619xB73GN-49
5812I dek*-04HI-A632GN-36
5812J dek*-04HI-A632xOh43GN-9
5812K dek*-04HI-A632xOh43GN-16
5812L dek*-07MO-B73xMo17GN-54
6402G dek*-07MO-B73xMo17GN-62

dwarf plant
4407E d*-N2383B
5506A d*-03HI-B73GN-47
5506B d*-03HI-B73GN-71
5506C d*-03HI-B73xMo17GN-47
5506D d*-03HI-B73xMo17GN-185
5506E d*-04HI-A632xOh43GN-347
5506F d*-04MO-A619xB73GN-37
5506G d*-04MO-A619xB73GN-239
5506H d*-03HI-B73xMo17GN-423
5506I d*-04HI-A632xOh43GN-347
5506J d*-07IL-B73GN-371
5506K d*-07MO-B73xMo17GN-20
5506L d*-07MO-B73xMo17GN-54
5506M d*-07MO-B73xMo17GN-380

early flowering
4008H Erl*-N1699

floury endosperm
5810K fl-dcr*-04HI-A632GN-12
5810L fl*-04HI-A632GN-47
5810M fl*-06HI-B73GN-52
5810N fl*-06HI-Mo17GN-11
5810O fl*-07IL-B73GN-29
5810P fl*-07IL-B73GN-41
5810Q fl*-07IL-B73GN-88

glassy endosperm
5810A ae*-06HI-Mo17GN-18
5810B ae*-04HI-A632GN-48
5812C ae*-07MO-B73xMo17GN-52
5812D ae*-07MO-B73xMo17GN-55
5812E ae*-07MO-B73xMo17GN-57
5812F ae*-07MO-B73xMo17GN-1

glossy leaf
5411E gl*-04HI-A632xOh43GN-78
5411F gl*-04HI-A632xOh43GN-184
5411G gl*-04HI-Oh43xA632GN-242
5411H gl*-04MO-A619xB73GN-239
5411I gl*-04MO-A619xB73GN-399
5411J gl*-04MO-B73GN-239
5411K gl*-04HI-Mo17xA632GN-169
5411L gl*-03HI-B73xMo17GN-1190
5412A gl*-07MO-B73xMo17GN-218

golden plant
4009E g*-N2332
4009O g*-03HI-B73xMo17GN-1201

green striped leaf
4009P gs*-04HI-A63GN-45
6005K gs*-3-8(4872-2)

lesion
3909K Les*-07IL-Mo17GN-1
3909L Les*-07IL-Mo17GN-3
3909M les*-03IL-A619TR-135
3909N les*-03IL-A619TR-516
3909O les*-03IL-A619TR-481

loose pericarp
5810H lsp*-06HI-B73GN-11
5810I lsp*-06HI-B73GN-44
5810J lsp*-06HI-B73GN-46

luteus yellow seedling
6111K l*-04HI-A632xOh43GN-7
6111L l*-04HI-Oh43xA632GN-73

many tillers
4209K tlr*-03IL-A619TR-249

miniature kernel
5811P mn*-07IL-B73GN-5
5811Q mn*-07IL-B73GN-9
5811R mn*-07IL-B73GN-18
5811S mn*-07IL-B73GN-55
5811T mn*-07IL-B73GN-84
5811U mn*-07IL-B73GN-117
5811V mn*-07IL-B73GN-131

necrotic leaf
6002Q nec*-03HI-A619xB73GN-142
6002R nec*-04HI-A632GN-18

6002S nec*-04MO-A619xB73GN-48

oil yellow plant
3811Q oy*-04HI-A619xB73GN-49
3811R oy*-03IL-A619TR-251
3811S oy*-03IL-A619TR-343
3811T oy*-03IL-A619TR-970
3811U oy*-04HI-Mo17GN-56
3811V oy*-03HI-A619xB73GN-186

pale green plant
6103O pg*-04HI-A632xOh43GN-9
6103P pg*-04MO-A619xB73GN-37

pale green seedling
6103Q pg*-07IL-B73GN-149
6103R pg*-07IL-Mo17GN-53

pale yellow endosperm
5703N y*-06HI-Mo17GN-6
5703O y*-06HI-B73GN-40
5703P y*-06HI-B73GN-41
5703Q y*-07IL-B73GN-103

piebald leaf
6103I pb*-04HI-A632GN-9
6103J pb*-07IL-B73GN-348

pigmy plant
4408A Py*-N1444

red auricle
5809A rau*-6522

shrunken kernel
5710M sh*-87-2496-21
5811J sh*-06HI-B73GN-19
5811K sh*-06HI-B73GN-25
5811L sh*-06HI-B73GN-43
5811M sh*-07IL-B73GN-3
5811N sh*-07IL-B73GN-65
5811O sh*-07IL-B73GN-118
5812B sh*-03HI-B73xMo17GN-549

small plant
4401H na3-N156A
4406N d*-N2362A

speckled leaf
5808 spc*-04HI-A632xOh43GN-57

spotted leaf
4107X spt*-04HI-A632GN-53
4107Y spt*-04HI-Mo17xA632GN-98
4107Z spt*-04HI-Mo17xA632GN-44

staminate ear tip
3408C staminant tip*-N1967B

striate leaf
3709B sr*-N1477A

sugary kernel
5811A su*-04HI-A632GN-10
5811B su*-04HI-A632GN-28
5811C su*-04HI-A632TR-12
5811D su*-04HI-Oh43xA632GN-4
5811E su*-06HI-B73GN-26
5811F su*-06HI-B73GN-53
5811G su*-06HI-Mo17GN-27
5811H su*-07IL-B73GN-66
5811I su*-07IL-B73GN-141
5812A su*-07IL-Mo17GN-4
5812M su*-07IL-Mo17GN-5

tassel seed
5807N ts*-03HI-B73GN-381
5807O ts*-03HI-B73GN-230

unbranched tassel
4012X ub*-03IL-A619TR-59

virescent seedling
6206O v*-07IL-B73GN-20

viviparous kernel
5904A y-vp*-07IL-B73GN-20
5904B y-vp*-07IL-B73GN-33
5904C y-vp*-07IL-B73GN-36
5904D vp*-03IL-A619TR-48
5904E vp*-03IL-A619TR-172
5904F vp*-03ILA619TR-189
5904G vp*-03IL-A619TR-202
5904H vp*-04HI-A632GN-1
5904I vp*-04HI-A632TR-16
5904J vp*-07IL-B73GN-19
5904K vp*-07IL-B73GN-25
5904L vp*-07IL-B73GN-27
5904M vp*-07IL-B73GN-39
5904N vp*-07IL-B73GN-44
5904O y-vp*-04HI-Oh43xA632GN-251
6402A vp*-04HI-A619xB73GN-26
6402B vp*-04HI-A619xB73GN-52
6402C vp*-04HI-A619xB73GN-60
6402D vp*-04MO-B73xMo17GN-3
6402E vp*-04MO-A619xB73GN-361
6402F vp*-07MO-B73xMo17GN-60
6402G vp*-07MO-B73xMo17GN-62
6402H vp*-07MO-B73xMo17GN-64
6402I vp*-07MO-B73xMo17GN-66
6402J y vp*-07IL-Mo17GN-7
6402K y vp*-07IL-Mo17GN-34

6402L y vp*-07MO-B73xMo17GN-59
6402M y vp*-07MO-B73xMo17GN-61
6402N y vp*-07MO-B73xMo17GN-63

white stripe leaf
4210M wst*-N529A
4211P Wst*-07IL-B73GN-1

white stripe leaf (japonica-like)
6006H j*-03HI-B73GN-291

white striped seedling
4211Q wst*-07MO-B73xMo17GN-252

wrinkled kernel
5810R wr*-07IL-B73GN-130
5810S wr*-03IL-A619TR-207

yellow green leaf
4307O yg*-04HI-A632GN-126
4307P yg*-04HI-A632GN-207
4307Q yg*-04HI-A632xOh43GN-211
4307R yg*-04HI-Oh43xA632GN-39
4307S yg*-04HI-A619xB73GN-122
4307T yg*-04MO-A619xB73GN-357
4309G Yg*-N2449
4309L yg*-04MO-A619XB73GN-122

yellow stripe leaf
3812H ys*-N2000
3812K ys*-N2300
6003K ys*-03HI-B73xMo17GN-1123
6003L ys*-03HI-B73GN-182

zebra necrotic leaf
3712L zn*-N2403A

zebra striped seedling
3712N zb*-03IL-A619TR-1124
3712O yg-zb*-03HI-A619xB73GN-156

VI. MAIZE GENETICS AND GENOMICS DATABASE -- 2011
www.maizegdb.org

Genome annotation.
Please see the Maize Executive Committee Report, this issue.

Site redesign. At the 2011 Maize Meeting, we announced a 2-year plan for a full website redesign. A beta release is planned for the March 2012 Maize Meeting, with final release slated for the March 2013 Maize Meeting. Design targets are:
(1) operational - handling large scale data such as multiple sequenced genomes, and diverse genotypes, easily integrating with off-site data, shifting toward greater interoperability, and acting more like a data portal
(2) functional - speeding up page load time, developing improved visual tools, integrating existing third-party solutions, providing better and new data integration
(3) cosmetic - improving navigation, bringing hierarchal structure to the site, creating new visuals (logo, icons, buttons), replacing the color scheme, and creating more intuitive page organization.
In addition, the underlying machine infrastructure has been upgraded to make the site more disaster-proof, robust, and flexible, and to improve queries and website performance. To accommodate ongoing needs of the community, MaizeGDB will continue to take in new data, provide interfaces to new data when needed, and update the database on a regular basis (usually the first Tuesday of the month). The challenges of a redesign require that the MaizeGDB website undergo an interface freeze in July 2011. As stated, the database will continue to be updated, but functional changes to the interface will stop until the completion of the new design. Please continue to provide feedback to our site. Bug fixes and urgent updates will still be provided, but any new functional features will be incorporated into the new site directly. A link to learn more about the interface redesign is posted on the in the lower right corner of http://www.maizegdb.org.

Tool development and new datatypes
Sequence searching tools developed as part of the NSF-funded POPcorn project, (http://popcorn.maizegdb.org) have been integrated into MaizeGDB. These include major improvement in sequence searching using BLAST, whereby a user is now able to simultaneously search multiple datasets distributed among multiple websites, and can also use BLAST to retrieve relevant genetic stocks (e.g., UniformMu stock or other stocks). Outcomes of the POPcorn project are now being incorporated directly into the MaizeGDB redesign. For example, the POPcorn database of maize projects and resources will continue to be maintained for searching, but will be better integrated into persons/organization data records at MaizeGDB as well as via the 'search all' utility. The POPcorn project involved collaboration with cooperators at the University of South Dakota, Ohio State University, the Dana -Farber Cancer Research Institute, and the University of Florida.
The ZeaAlign BLAST tool (http://zealign.maizegdb.org) enables mapping large-scale sets of multiple sequences to the B73 reference genome assembly simultaneously. Outputs from the tool are formatted for use with GBrowse, making ZeAlign a great tool for preparing your data for inclusion in the MaizeGDB Genome Browser (http://gbrowse.maizegdb.org).
Gene Expression Data. The community is generating enormous amounts of high quality gene expression data, using both microarray and RNA-seq approaches. Some of these data are now available at MaizeGDB as pre-computed tracks via the MaizeGDB Genome Browser. Examples include Tom Brutnell's 6 leaf expression tracks; raw gene expression data computed for early versions of the maize gene models (BAC-based and v1 sequence); gene expression data for v2 gene models, a B73 gene atlas for some 60 tissues from Shawn Kaeppler's group; and expression data derived from micro-dissected shoot apical meristems from Mike Scanlon). The data supplied by the Kaeppler group were aligned to the B73 RefGen_v1 gene models. These mappings were updated by MaizeGDB to B73 RefGen_v2 in collaboration with PLEXdb with data analysis and displays deployed to indicate normalized expression of Nimblegen probes relative to the gene models as well as the expression of individual probes aligned anywhere on the genome. Plant ontology associations have been provided to the Plant Ontology project. Persons wishing to contribute this sort of data to MaizeGDB are encouraged to contact Carolyn Lawrence prior to the time they submit research proposals or manuscripts.
Genome Browser Enhancements. The MaizeGDB Genome Browser has added special glyphs to represent gene expression data. Current data represented in this manner include the Maize Gene Atlas for B73 involving 60 tissues (Sekhon et al 2011 Plant J 66:553-563) and shoot apical meristems (6 stages) from the Scanlon group

(publication in press). The glyph was adopted from FlyBase, which uses the same genome browser software, GBrowse, as MaizeGDB.

Functional Genomics Display Tools. Through generous support from the National Corn Growers, MaizeGDB will be implementing several functional genomics applications that will allow access to pre-computed, large scale data sets which have been projected on to various pathways. While still in its early planning stages, it is likely that MapMan (Thimm et al 2004 Plant J 37:914-939; Usadel et al 2009 Plant Cell Environ 32:1211-1229); and the electronic Fluorescent Pictograph browser (Winter et al 2007 PLoS One 8:e718) will be utilized to create an environment where MaizeGDB users can explore large gene expression (microarray based and RNA-Seq) data sets to compare and contrast pathways with respect to various treatments and tissues.

Metabolic Pathways. In collaboration with Gramene, we have made available a PathwayTools suite for maize, called MaizeCyc (http://maizecyc.maizegdb.org/). This tool provides access to metabolic pathways. The pathways are largely pre-computed, with some curation for maize function. It relies on various pipelines for gene model function prediction, and also on the Plant Metabolics Network, which has been heavily curated by TAIR and PMN staff, so that the pathways represent plant metabolism well. The software for the pathway visualization and curation are those developed by Peter Karp at the SRI (Karp et al. 2010 Brief Bioinform 11:40-79). We will continue to curate MaizeCyc, and have begun collaborating with the Plant Metabolics Network. We encourage persons expert in pathways to review and contribute to this project.

Curation tools. We have implemented a local installation of Textpresso (Muller et al 2004 PLoS Biol 2:e309), a text-mining software package, to improve curation of data from the literature into MaizeGDB. Improved by our inclusion of lexica for maize terms along with standards (Gene Ontology and Plant Ontology terms), our use of Textpresso promises to increase the number of high-quality datasets going into the MaizeGDB. In this project we have consulted with curators at TAIR who have been using this resource for some time as an aid to manually curate the literature.

Publications 2011

(1) Cannon EKS, Birkett SM, Braun BL, Kodavali S, Jennewein DM, Yilmaz AY, Antonescu V, Antonescu C, Harper LC, Gardiner JM, Schaeffer ML, Campbell DA, Andorf CM, Destri Andorf D, Lisch, DR, Koch KE, McCarty D, Quackenbush J, Grotewold E, Lushbough CM, Sen TZ, Lawrence CJ (2011) POPcorn: an online resource providing access to distributed and diverse maize project data. *Int J Plant Genet* (in press)

(2) Green JM, Harnsomburana J, Schaeffer ML, Lawrence CJ, **S**hyu CR (2011) Multi-source and notlogy-based retrieval engine for maize mutant phenotypes. *Database* 2011:bar007 *doi:10.1093/database/bar012.*

(3) Lawrence, CJ (2011) MaizeGDB – Past, present, and future. *Maydica* 56:1-3.

(4) Harper, LC, Schaeffer ML, Thistle J, Gardiner JM, Andorf CM, Campbell DA, Cannon EKS, Braun BL, Birkett SM, Lawrence CH, Sen TZ (2011) The MaizeGDB genome browser tutorial: one example of database outreach to biologists via video. *Database* 2011:bar016 *doi:10.1093/database/bar016.*

(5) Schaeffer ML, Harper LC, Gardiner JM, Andorf CM, Campbell DA, Cannon EKS, Sen TZ, Lawrence CJ (2011) MaizeGDB: curation and outreach go hand-in-hand. *Database* 2011:bar022 *doi:10.1093/database/bar022* .

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

VII MAIZE GENETICS EXECUTIVE COMMITTEE

Maize Genetics Executive Committee Meeting -- March 18, 2011

In attendance: Tom Brutnell (chair), Pat Schnable, Bill Tracy, Sue Wessler, Ed Buckler, Carolyn Lawrence, Brent Buckner and two new members, Nathan Springer and Marja Timmermans. Guests: Jack Okamuro from USDA-ARS, Rick Vierling from NCGA, and Karen Cone from NSF

OPEN SESSION

NCGA (Vierling)

There was much discussion about growers' potential dissatisfaction with apparent lack of translation of discovery science to application. Clearly, a large part of this is communication. The maize community should think of ways of being more proactive with the growers – perhaps a "Maize Genetics Blog" on the NCGA web site or a link to a blog at MaizeGDB to inform of how major breakthoughs are having impact. A set of one pagers of newsworthy maize stories was suggested as one mechanism that could be used to emphasize important discoveries for the popular press, members of Congress of web posts. There was also discussion of performing growouts of NAM populations in farmers' fields as test plots for phenotyping. Vierling mentioned a potential to partner with NCGA for yield trials and that the USFRA (US Farmers and Ranchers Alliance) could be involved. This would be one way to engage farmers directly with basic science.

USDA (Okamuro)

Many ARS scientists are in National Program 301 (Plant genetic resources, genomics, and genetic improvement), which is in its 5th year cycle and about to start a customer/stakeholder workshop. Input is desired for the current needs, issues, and challenges in the field. University partners and stakeholders are welcome to contribute. For maize, ARS supports genetics, genomics, databases, and computational tool development. Thus far for FY2011, a ~$140M cut has been absorbed. The stock center is not affected thus far. For maintenance of B73 genome, there was an update at PAG and there was another update at the maize meeting. The MaizeGDB Working Group reports on this topic were received by the ARS National Program Staff and NPS will also be willing to take guidance from the MGEC on B73 genome stewardship.

NSF (Cone)

Cone emphasized that what NSF wants to fund is compatible with the maize genetics research community and there are new funding opportunities. For example, G2P is a new collaboration with USDA NIFA to have new direction in phenomics and there will be a St. Louis workshop on phenomics coming soon. Rob Last and Tim Close are serving to organize this event. There is an emphasis on international collaboration including a solicitation on metabolomics with Japan's equivalent of the Office of International Science and Engineering (OISE) to serve as a "new collaborator incubator". The NSF OISE's new administrative director is Machi Dilworth. BREAD also emphasizes international collaboration.

Other topics discussed with NSF, USDA, and the Corn Growers:

Need to store large datasets

NCBI SRA is closing except to human datasets (this policy has subsequently been reversed, but it is not clear how long SRA will be open to plant scientists). Buckler, Lawrence, and iPlant are open to making changes for large dataset storage and access and are trying to work on this together.

There was concern that PGRP will lose its impact in IOS. In 2012 there was no line item. Vierling mentioned that NCGA and growers want to see PGRP continued. (See "Sept 2011 Meeting with NSF representatives" for a more detailed discussion on this point; follows this report)

Discussion on the 2010 meeting with NSF

Buckler, Brutnell, Walbot, Lawrence met with Diane Okamuro and Jane Silverthorne. Main points were (1) that we need new genome assembly and build mechanisms and that the data should be hosted and stored, (2) community annotation is needed, (3) basic, single-investigator granting opportunities are not apparent, and (4) help for funding the Maize Genetics Cooperation - Stock Center are needed. (see also 2011 Meeting with NSF representatives)

Sequencing projects at BGI in China

There is growing sequence capacity at BGI in China and many in the community are beginning to use this resource. However, sequence access and visualization do not seem to be priorities of BGI. Although data should be provided to GenBank prior to publication, it is not clear that publishers are enforcing this rule. Reviewers should be more insistent on this point. US collaborators with BGI need to get a copy of the sequence and RELEASE IT. Authors should communicate to editors, talk to reviewers, and alert the community that this is an issue. It was noted that this should not be an issue because people want to publish their papers and the publishers have the ability to enforce this rule. They just need to be more diligent.

Plant Summit planned for end of September by ASPB

It was noted that a Plant Science Research Summit is planned to bring attention to plant science and begin a dialog aimed at increasing funding for plant science research. (members of the MGEC in attendance at the Plant Science Summit (URL: www.aspb.org/plantsummit) included Bennetzen, Brutnell, Buckler, Buckner, Lawrence, Schnable)

Stock Center

The Stock Center experienced the untimely death of Janet Day Jackson. Marty is currently putting in the paperwork to allow him to refill the position but, at the time of this report (Nov 2011), has been unable to do so.

- Submitted by Tom Brutnell on behalf of the MGEC Nov 2011.

MGEC-NSF meeting -- Sep 2010

On Sept. 8, 2010 four members of the MGEC (Brutnell, Buckler, Lawrence, and Walbot) met with Jane Silverthorne and Diane Jofuku Okamuro to discuss the concerns of and opportunities for the maize genetics community. Issues listed below emerged from a "committee of the whole" meeting during the March 2010 annual maize genetics meeting. A summary of the points raised with NSF (bulleted), discussions, and action items are given below.

1. Need for maize genome annotation, genome assembly, new genome builds, and current progress on annotation

- Current genome assemblies – B73 RefGen_v2 has been available at MaizeGDB since May 2010 and the associated annotation file is due soon.

- Plans for future genome assemblies and annotations are unknown, however, sequencing is ongoing. Thus, continued assembly and annotation of the maize genome sequence as well as display of data (including SNP's and expression data, especially RNA-seq) are of the highest priority.

- Community annotation of maize genome was discussed at the 2010 Annual Maize Genetics Conference and some efforts are now underway in collaboration with iPlant to facilitate this process.

Discussion:

We discussed the need for coordination among annotators and the importance of maintaining a high quality sequence for the community. DNA subway (an iPlant tool) may be implemented for this project, but would need to be modified for this task.

Jane suggested that there are programs at NSF that might support these activities. One such program is Advances in Biological Informatics (ABI) (http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=5444&org=DBI&from=home). For bringing together a multinational team for annotation, the Research Coordination Network (RCN) program may provide another option. She encouraged the community to learn more about the RCN program. An excellent example of a PGRP-supported RCN is the RCN: Epigenomics of Plants International Consortium (EPIC) (see: (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0925071). She also strongly suggested that, as always, best practice is to contact the cognizant Program Director for guidance before submitting a proposal.

The RFA for Plant Genome also has language that specifically addresses the need for annotation:

"Four kinds of activity will be supported in FY 2011: (1) Genome-Enabled Plant Research (GEPR) awards to tackle major unanswered questions in plant biology on a genome-wide scale; (2) Transferring Research from Model Systems (TRMS) awards to apply basic biological findings made using model systems to studying the basic biology of plants of economic importance; (3) Tools and Resources for Plant Genome Research (TRPGR) awards to support development of novel technologies and analysis tools to enable discovery in plant genomics; and (4) **Improving Plant Genome Annotation (IPGA) to improve existing tools or develop new tools for improved annotation of the genomes of plants of economic importance.**

Action items:

Develop a "Maize Genome Annotation Consortium" to perform structural and functional annotation of the maize genome and interface with MaizeGDB. Personnel at TAIR or other community databases should be brought in as advisors and participants.

Carolyn Lawrence has taken the lead on this issue.

2. The role of iPlant/informatics in shaping maize genomics research

- A number of excellent informatics tools exist for *A. thaliana* (e.g., eFP browser, ATTED), but are not currently available to researchers for work with the maize genome
- New sequencing technologies are likely to be more widely and rapidly adopted for maize, tomato, and legume research communities than for *A. thaliana* or rice as no common transcriptome profiling platform exists for maize.

Discussion:

To encourage the development of informatics tools, we suggested a focus area for the Plant Genome Research Program (PGRP) (e.g., the recent Heterosis Challenge focus area) on the theme of "crop plant informatics tools".

Jane mentioned that a US-EC taskforce has been established to generate dialogue between the EU and US on bioinformatic tool development. See http://ec.europa.eu/research/biotechnology/ec-us/index_en.html

The maize community sees a great opportunity to coordinate informatics tools for maize that transcend national boundaries. Although discussions are underway with EC colleagues, nothing immediate is planned for joint funding opportunities. However, Jane mentioned that EU Framework 7 proposals are

due the same time as Plant Genome proposals thus coordination between the groups is possible.

We also discussed the vast amount of sequencing data that is coming out of China – largely at BGI -- and how little is being done to coordinate sequencing projects with Chinese colleagues. The MGEC has one member from China, and the community is interested in worldwide coordination. NSF-OISE has a Beijing office (see: http://www.nsf.gov/news/news_summ.jsp?cntn_id=107006); the former director of that office William Chang is now based in Washington DC and new director Alexander DeAngelis has recently started. Thus, it would be prudent to start dialog with this office.

In the long term sequencing is a commodity but informatics and QC are needed.

Jane also pointed to the value of leveraging existing tools for *A. thaliana* and other species in bringing maize informatic tools up to par quickly.

Action items:

Contact the NSF Head of the NSF Overseas Office in Beijing to discuss coordinating maize sequencing project between US/BGI. We should begin a dialogue with colleagues at BGI where there is a strong interest in maize research. Buckler has been involved in some of these discussions already and perhaps Jinsheng Lai (a member of the MGEC) could help facilitate these interactions with other members of the MGEC and BGI to discuss common needs in informatics and visualization.

As discussed above, the development of an RCN in maize bioinformatics may be a very effective way of coordinating these activities between US, European and Asian bioinformatics communities. Lawrence, Buckler, Brutnell and other volunteers from the MGEC could spearhead this initiative.

3. **Value of strong basic and applied science funding streams through Plant Genome and single investigator grants**

- Much left to discover in taxon-specific gene functions and in regulation of acclimation (arguably more important than adaptation for short term crop sustainability).

- Fewer than 25% of genes have biological support for function – thus need to support functional genomics and exploratory research.

- The shift in AFRI target areas has made it very difficult for many single

investigator genomics-enabled projects to be funded. While large-scale field operations cannot be done this way, a single lab can sequence and analyze a genome or add another important trait to a larger study already funded.

Discussion:

Jane was quick to point out that NSF does not support applied research. The Basic Research to Enable Agricultural Development (BREAD; see: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503285&org=BIO) does support projects with a focus on smallholder agriculture in developing countries, but the focus is on basic research at an early concept stage rather than downstream applications.

Diane emphasized that single investigator grants are supported by the PGRP and that the program welcomes these applications. She also noted that single investigator grants are funded at rates that are very similar to multi-investigator grants; however, only a handful of single investigator grant proposals are received each cycle. The program was recently reviewed by a Committee of Visitors (COV) team (see: http://www.nsf.gov/od/oia/activities/cov/covs.jsp), and their report will be online by November.

Jane also mentioned that many projects to address taxon-specific questions should go through core programs for review with a possible co-review from the PGRP, if appropriate.

Action items:

This report will be circulated to the MGEC and posted on MaizeGDB to inform the community that we should be more aggressive in applying for single investigator grants.

4. Postdoctoral/graduate student fellowships

- Need infusion of new skills to sustain research in new directions and recruiting postdocs into plant science is a very efficient mechanism that was used for molecular biology and now for mathematics and physical sciences (see: http://www.nsf.gov/pubs/2010/nsf10587/nsf10587.htm)

  Jane mentioned that the NPGI has identified Plant Breeding as an area in greatest need for training new students and that plans are in the works via the NPGI to address this issue.

5. The maize stock center: community needs and mechanisms for funding

- Acknowledge that long-term support of Stock Center is an ARS responsibility. Nevertheless, the lack of funding is threatening to impact of several NSF-funded projects through limiting the distribution of materials
- Marty Sachs anticipates receiving 3,000 to 5,000 additional stocks/yr. mostly from NSF-funded projects
- As part of National Plant Germplasm System, Marty cannot charge for seed distribution.

Discussion:

It would greatly help NSF if funds for distribution and propagation of stocks were explicitly stated/requested. This could be either by Marty directly, or Marty could request that the groups developing the stocks do the initial increases using NSF funds before depositing stocks with the Stock Center.

Action items:

Bill Tracy has offered to draft a letter that will be sent to USDA representatives stating the maize community's strong support for the Stock Center from the MGEC.

Sept. 9, 2011 MGEC meeting with NSF Program directors

Meeting notes as provided by Tom Brutnell, Chair, Maize Genetics Executive Committee and posted at MaizeGDB shortly thereafter

Bill Tracy, Ed Buckler and I met with Diane Okamuro, program director for the Plant Genome Research Program at the Awardees meeting on Sept. 9, 2011 to discuss several items that members of the Maize Genetics Executive Committee have brought to our attention related to the Plant Genome Research Program.

Below is a summary of our discussion.

1. Continued support for the Plant Genome Research Program.

Discussion topic: As you know, much of the research conducted by the maize research community is funded by the PGRP. Thus, we were concerned to hear that the funding for the PGRP program within IOS may be getting reduced. Could you please clarify if this is indeed the case and if so, how the funds are being distributed? What can the maize community do to help support this program?

Diane: Moving the PGRP to IOS was a good decision. The newly appointed Assistant Director for BIO, Dr. John Wingfield, is a strong proponent of the PGRP and wants to leverage the success of this program within NSF. As are all programs here at NSF, the PGRP program is very efficient (maintains a very low overhead rate) and delivers high impact science.

The PGRP has funded single investigator grants and would encourage submission of additional single investigator and CAREER applications. I would also like to point out that we have a new call for postdoctoral fellowship applications and anticipate awarding 15 grants of $189,000/ 3 yrs in FY 2012.

2. Peer review of PGRP grants

The conflicts of interest (COI) list of large collaborative grants associated with the PGRP, are often several pages long. This is often due to multi-author publications (e.g., the maize genome sequencing paper) where there is little if any direct interaction among co-authors. This is problematic when some of the best qualified reviewers are also co-authors. Is it possible to redefine a conflict of interest for PGRP to ensure that the most qualified reviewers are will be permitted to review proposals? Perhaps it would be possible to implement a narrower definition of COI, e.g., such as a funded collaboration or co-author within the previous 24 months?

Diane: We recognize that with large genome-sequencing consortium papers, many coauthors of publications have little direct interaction, so it is important to list as conflicts only those co-authors with whom you have a direct interaction. It is also important for applicants to suggest reviewers that you are not in conflict with to help ensure the most knowledgeable reviewers are selected. In regards to panel selections, we seek representation from all members of the research community. This includes scientists from international and R01 institutions as well as small colleges. We also aim to have a balanced panel with respect to male and female panelists and scientists from underrepresented groups. Finally, we generally allow only one scientist from a given institution/university and no more than two from any one state per competition to serve on a panel.

3. Maize genome annotation and assembly.

The maize community continues to ask for improvements to assembly and annotation and we're encouraged that resubmission of the RCN submitted by Lawrence was suggested by program staff. Are there other funding mechanisms available for maize genome annotation and assembly?

Diane: Yes, I encouraged Carolyn to resubmit the RCN with an emphasis on getting community input and establishing best practices. The RCN will also support international collaborations and these are encouraged.

4. Role of iPlant in helping drive informatics tool development.

Several in the community have engaged with iPlant on informatics tool development and outreach. However, it is unclear if iPlant will continue to receive funding giving pause to some investigators to commit strongly to developing tools through an iPlant interface. Could you advise PI's on how to best interface with the iPlant Collaborative.

Diane: Members of the maize community should continue to utilize iPlant infrastructure where appropriate.

5. Maize stock center

As USDA budgets for germplasm maintenance are dwindling, there is increasing concern that the maize stock center will struggle to meet demand. We realize that funding the stock center is under the purview of USDA but are there ways to support seed stock maintenance and distribution?

Diane: NSF is willing to support funding seed stock increases or storage costs if they are directly relevant to Plant Genome Research projects, such as generating a mutant collection or propagating accessions used in a PGRP-supported project for community access. Applicants should discuss with PGRP and with Marty regarding what mechanism for funding is most appropriate and be sure to budget appropriately.

This newsletter shares current research on genetics, cytogenetics, molecular biology, and genomics of maize. Information is shared by Cooperators with the understanding that it will not be used in publications without their specific consent. The maize gene reviews are an exception to this practice.

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

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SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

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

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

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

MaizeGDB needs Cooperators (this means you) to:

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