MAIZE GENETICS COOPERATION

NEWSLETTER

64

March 1, 1990

Department of Agronomy
and
U.S. Department of Agriculture
University of Missouri
Columbia, Missouri

This issue is dedicated to

George W. Beadle

and

Herschel L. Roman

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I. FOREWORD

The working research information here is shared with the understanding that each item is unpublished and is not to be cited in publications without specific consent of the author. The Maize Genetics Cooperation News Letter, in common with other research newsletters, "is particularly suitable for information not usually suitable for scientific journals: continuing updating of gene symbols and nomenclature, location of mutants, strains and stocks, . . . Short technical notes, short research findings, observations, new ideas and compilations of data . . . " (H. V. Wyatt, 1986). Sharing these things here, we contribute to the advancement of biology and to the power of shared technical knowledge.

Information here is in the form of "notes" and is not "published" in the sense used by some refereed journals, which require that there has been no prior publication. Because cooperators have reported experiencing some difficulties with journals on this, I would like to offer the following suggestions to ensure against misunderstanding of our News Letter:

1) In publications, refer to MNL notes in the text, never in the bibliography. Specify "unpublished data", or "personal communication" (i.e., with the colleague's consent). The volume and page numbers might be given, as an aid to the reader.
2) Emphasize, in preparing your MNL notes, brief technical notes, updates, mutants, mapping data, and the like. Avoid presenting comprehensive material and analyses that are better directed to formal publication.
3) Never refer to MNL notes as "published".
4) If challenged, forward these comments as a statement of the purpose, intent, and policy of the cooperators who contribute to this News Letter.

Because so many cooperators helpfully supply their material in electronic form, we are able to concentrate more now on updating, summarization, and compilations. Both Zealand 1990 and the Symbol Index reflect this concentration.

Gifts to the Endowment Fund for support of the News Letter total over $63,000. Please see the listing, in the front of this issue, of donors whose generosity has made this total. We are all grateful for the support of our colleagues and of organizations with which we have common interests. Part of our support also comes from the National Science Foundation, from the Integrated Mapping Project.

A warm acknowledgement for advice and ideas is given to my colleagues, Shiaoman Chao, Diego Gonzalez de Leon, and Dave Hoisington. Dave originated the foundation for the refined gene list and linkage maps in this issue. Diego's help this year with computer trickery, copy refinement, and composition was invaluable. Their participation makes the editing work feasible and efficient.

Shirley Kowalewski not only cajoled the word-processor, edited the copy, and screened the year's literature, but also gave special creative advice at critical moments. Mary Ann Steyaert booked addresses and subscriptions through the year, and artfully prepared the mockup. Randall Grogan swiftly and efficiently set up our computer system. Denis Hancock lashed our computers when needed. Chris Browne kept research materials going and helped with vital tasks. Rosalind Richards ensured that office and secretarial needs were efficiently met. Ray Baxter, Ann Blakey, Jack Gardiner, Chang-deok Han, Susan Melia-Hancock, Masumi Katsuta, Elizabeth Lee, and Madhavi Reddy helped with proofing of copy and with library work. At University Printing Services, Yvonne Ball and Dale Kennedy and their staff again efficiently and carefully made sure that the job was done promptly and well.

Included in this issue are systematic organelle maps, once again generously volunteered by Steven Rodermel and Lawrence Bogorad and by Christiane Fauron. Their contributions are gratefully acknowledged.

For submission of notes for the next issue (Number 65, 1991), please see details inside the back cover.

If you wish to subscribe to this News Letter please use the form in the back of this issue. Gifts to the Endowment Fund, toward our goal of $100,000, will be very much appreciated.

Ed Coe

ALBANY, CALIFORNIA
Plant Gene Expression Center, USDA-ARS

Amplification of sequences flanking transposable elements by inverse polymerase chain reaction

--David J. Earp, Brenda Lowe, Sarah Hake and Barbara Baker

We have utilized an inverse polymerase chain reaction (IPCR) methodology (Triglia et al., Nucl. Acids Res. 16:8186, 1988) to amplify sequences flanking transposable elements in transgenic tobacco and in maize. A line of tobacco containing a single, stable *Ac* element (*Ac-18*, deleted by 4 bp at the 3' terminus) at the *Nt-1* locus (Hehl, R., and Baker, B., Mol. Gen. Genet. 217:53-59, 1989) was used as a model to develop the procedures discussed below. Figure A shows a protocol allowing amplification of sequences flanking both ends of the *Ac-18* element. PCR primers utilized for this were selected such that one primer (from the group designated A primers) was homologous to the non-coding strand of *Ac*, allowing polymerase extension out of the element through the 5' end, and the second primer (from the B primers) was homologous to the coding strand at the 3' end of *Ac*. PCR amplification of approximately 4 µg of the illustrated template DNA (35 cycles of 94 C, 1 min., 55 C, 2 min. and 72 C, 3 min.) yielded ca. 1 µg of a single 970 bp product. This was cloned and used as a probe to verify the identity of the material as *Nt-1* sequence.

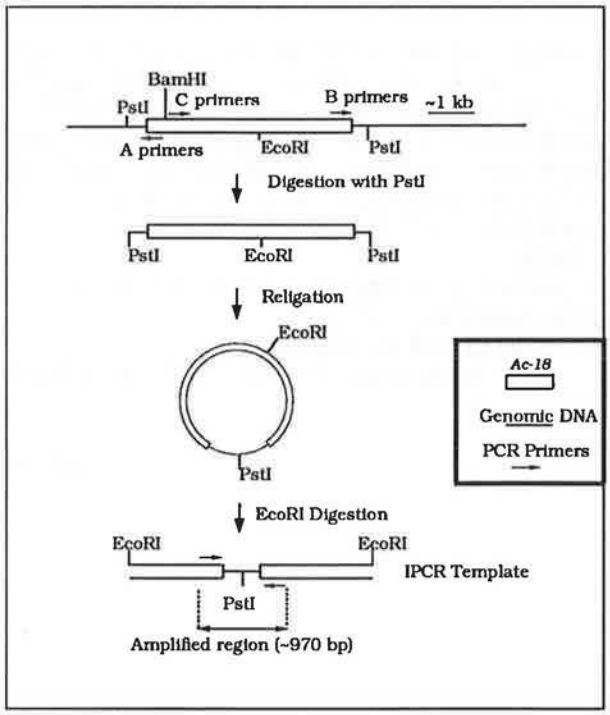

Figure A. Schematic representation of IPCR template generation from genomic tobacco DNA carrying *Ac-18* inserted at the *Nt-1* locus. For amplification of sequences flanking both ends of the element, genomic DNA was digested with *Pst* I, ligated at a DNA concentration of 2 µg/ml and then linearised with *Eco*RI to yield the illustrated template. Primers not to scale.

The number of enzymes suitable for the initial cut in the above IPCR scheme is restricted to those which do not cleave within the *Ac* element and is further reduced to a sub-group of these with sites in the flanking genomic DNA relatively close to each end of the inserted element. In general application, where often the goal is to obtain a probe for the target region, amplification of sequences flanking only one end is sufficient. This enables the utilization of the many frequent-cutting enzymes with sites within the element for the initial digestion. This notion was used to generate 'short-cut' IPCR templates from the *Nt-1*::*Ac-18* locus. Primers from locations A and C were utilised in this procedure. Template was produced by initial digestion with enzymes cleaving within the genomic DNA and 3' of the C primers within the element followed by religation and linearization with *Bam*HI. Intermolecular ligation in the template generation protocol, producing various chimeric templates, was found to be a potential problem when frequent cutting enzymes were utilized for the initial digestion (the frequency of intermolecular ligation increasing with the concentration of ligatable ends). This was overcome by the use of a methylation sensitive enzyme (*Eco*RII) for the first cleavage (which resulted in frequent digestion within and around the element while leaving much of the genomic DNA in relatively large pieces).

Subsequently we utilized this methodology to amplify and clone sequences flanking an *Spm* element in a line of maize derived from a *c2-m1* line. A specific *Spm* insertion, which was characterized as a 10.2 kb *Sal*I fragment hybridizing to an *Spm* derived probe, was selected as a target for amplification. Primers were designed to prime synthesis out of the 5' end of the element (primer L1, Figure B) from the region immediately 3' of the *Sal*I site at position 276 (this *Sal*I site is unmethylated in active elements, Banks et al., Genes Dev. 2, 1364-1380, 1988) and from the

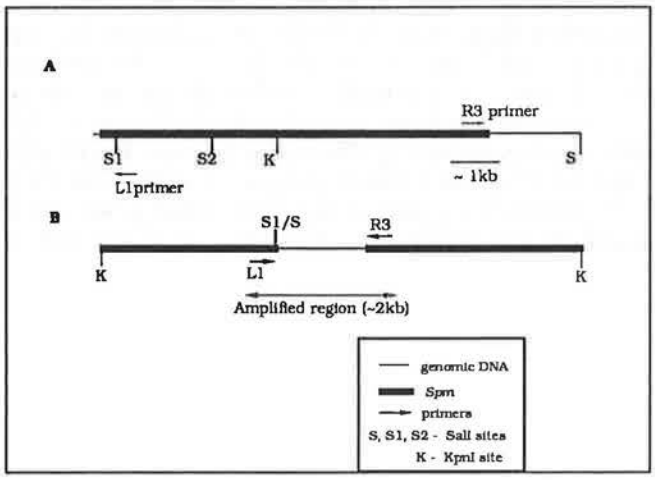

Figure B. Schematic representation of IPCR template generated from genomic maize DNA.

(A) Partial restriction map of *Spm* showing locations of PCR primers. The genomic *Sal*I site 3' of the *Spm* element selected as a target for IPCR is also indicated.
(B) IPCR template derived from the selected *Spm* shown in (**A**) by ligation of *Sal*I digested size fractionated DNA and subsequent linearisation with *Kpn*I. Primers not to scale.

3' end of the element (primer R3). Genomic DNA was size fractionated to enrich for this 10.2 kb *SalI* fragment, and IPCR template generated as illustrated in Figure B. Amplification yielded a product of ca. 2 kb which hybridised to the 10.2 kb band and to an additional band of 2.0 kb in lines heterozygous for the *Spm* insertion. The IPCR product was used as a probe to map the location of this element to position 112 on chromosome 4L (B. Burr, personal communication).

The method should provide considerable advantages in obtaining element flanking sequences compared with conventional lambda cloning and screening. IPCR amplification of genomic sequences flanking transposed elements will facilitate the subsequent isolation of wild type genes in tagging experiments and will also generate RFLP probes for use in genome mapping.

Hypermaize : A Hypercard program for recording and analyzing crosses

--Brian I. Osborne and Julie L. Mathern

We have used the Macintosh program Hypercard to construct a "stack" to help the maize geneticist store and analyze data amassed over generations of crosses. The appearance of the "cards" in this specifically tailored stack, called "Hypermaize", resembles the crossing card normally taken into the field. However, the accessibility of Hypercard's programming language Hypertalk has enabled us to add useful features that are activated by clicking on buttons. These features include: 1) The stack will search for and display all instances a plant is used as a parent, or solely as male or female parent. Clicking on the field containing the results of a search takes you to the appropriate card; 2) The stack will enact case-sensitive genotype searches and display the result of the search. A subsequent option allows the searcher to eliminate "found" plants on the basis of genotype (e.g., in order to find all plants that are *Adh/adh* but not *sh/sh*); 3) The stack will print all cards, or a specified subset; 4) Genotypes may be entered by clicking on words in a field, making data entry fast and consistent; 5) Clicking on a button directs the stack to draw a family tree of a plant that extends back as far as 6 generations.

Hypermaize functions on all Macintosh computers tested, from the Mac Plus up. However, all the features of the stack are enabled only on machines with 2 or more Mb.'s of RAM. The stack uses the XCMD's SysEnvirons and Strings (STaK X), PopUpMenu (101 Scripts and Buttons), PrintField and FindInField (Dartmouth XCMD's 3.1) and was made with Hypercard version 1.2.2. The stack comes with a Hypercard tutorial on its use. For a copy send a sturdy, self-addressed, stamped envelope and formatted floppy disk to: Dr. Brian Osborne, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

Towards transposon tagging of the TMV resistance gene *N* using Activator in transgenic tobacco

--Reinhard Hehl and Barbara Baker

Utilization of the maize controlling element Activator (*Ac*) as an insertional mutagen in heterologous plant species is facilitated by comprehensive understanding of its molecular genetic properties in novel genomic settings. Our results, summarized below, indicate that *Ac* can be effectively employed as an insertional mutagen in tobacco as well as other dicot species.

1) *Ac* maintains the genetic capacity to transpose and *trans*-activate *Ds* transposition at a high frequency for at least five tobacco generations. 2) *Ac* is transcribed and internal element sequences remain unmethylated. 3) The mechanism of *Ac* integration in maize and tobacco is similar, conserving *Ac* structure and function. 4) *Ac* transposes adjacent to unique and low copy genomic sequences and alters transcription of a unique target DNA, suggesting that it has transposed into a gene. 5) Increasing copies of *Ac* correlate with an increased frequency of *Ds trans*-activation.

We are currently employing *Ac* in an attempt to tag the dominant Tobacco Mosaic Virus (TMV) resistance gene *N* in transgenic tobacco. The *N* gene mediates a hypersensitive response (HSR) upon virus infection and restricts virus spread throughout the plant. We have introduced *Ac* into *Nicotiana tabacum* cv. Samsun NN, homozygous for *N*, and crossed *NN* plants bearing transposing *Ac* to *N. tabacum* cv. SRI (*nn*). *Ac* insertion into the single copy of *N* of the F1 population should lead to loss of HSR and to systemic TMV infection. Large F1 populations have been screened for loss of HSR as well as systemic virus infection by applying conditions lethal to seedlings expressing *N*. Screening of two F1 populations of 12,000 and 27,000, harboring transposing *Ac*, produced 2 and 28 systemically infected plants respectively. Surprisingly, several of these F1 plants display patches of necrotic lesions resembling HSR on mature leaves. This phenotype was not observed in the control population. The possibility that this phenotype correlates with transposon induced somatic instability of the *N* locus is under investigation.

Intragenic recombinants of Knotted

--Sarah Hake, Bruce Veit and Erik Vollbrecht

One of our strategies for determining the structure of the Knotted (*Kn1*) locus is to isolate intragenic recombinants. The *Kn1* mutation is defined by the dominant alleles (presently 7) that all map near *Adh1* on 1L and alter leaf development. The alteration in normal leaf development is centered around the lateral veins; outpockets or knots occur along lateral veins, and ligule fringe is found displaced from its normal position into the leaf blade along the lateral veins. Heterozygotes are much milder in their phenotype than homozygotes and are generally taller. Some of the *Kn1* mutations differ dramatically in their phenotype, particularly *Kn1-O* and *Kn1-N*. *Kn1-O* is the original mutation found in 1924 (Bryan and Sass, J. Hered. 32:343, 1941). It is characterized by severe alteration to the ligule, and a tendency for knots to occur on older leaves. Our analysis has shown that *Kn1-O* is caused by a tandem duplication of 17 kb (Veit, Vollbrecht and Hake, submitted). *Kn1-N* was isolated by Dr. Neuffer (Univ. of Missouri) in an *Ac-Ds* background. All *Kn1-N* leaves tend to be

knotted, particularly the juvenile leaves. Ligule displacement is not as common. Our Southern analysis indicates that this locus is identical to wildtype with the exception of a 500 base pair insertion that is within the 17 kb region duplicated in *Kn1-O*. These two *Kn1* alleles are linked to different *Adh1* alleles.

Plants that were doubly heterozygous for *Kn1-N Adh1-S* and *Kn1-O Adh1-F6* (Figure 1a) were crossed to normal plants that were marked with a different *Adh1* allele. The majority of the 2700 plants screened appeared to be either *Kn1-O* or *Kn1-N* heterozygotes. However, 2 plants were found that were normal and 3 plants were found that were extremely knotted. The normal plants were crossed and progeny obtained. We determined whether recombination had occurred in the derivatives with the flanking markers *UMC107* and *Adh1*. *UMC107* is proximal to *Kn1* by 7 map units (Mathern and Hake, MNL 63:2, 1989); the polymorphism we have designated (*U*) is linked to *Kn1-O*, (*L*) is linked to *Kn1-N*. *Adh1* is distal to *Kn1* by 1 map unit. One normal derivative appears to be a contaminant by an assortment of markers. The other normal derivative does not carry the tandem duplication of *Kn1-O* nor the insertion of *Kn1-N*. It carries *Adh1-F6* and *UMC107(L)*. Therefore, it is likely to have arisen following unequal crossing over in Figure 1b. The knotted plants were too severe to obtain a cross, but DNA was isolated and analyzed. The DNA of one of the severe plants appears similar to *Kn1-N* and may be due to an unlinked gene increasing the severity of *Kn1-N* or a small DNA alteration at *Kn1-N*. The DNA from the other 2 severely knotted plants appears similar to both *Kn1-O* and to *Kn1-N*. The tandem duplication characteristic of *Kn1-O* is present as well as the insertion that has come to characterize the *Kn1-N* mutation. Both derivatives carry the proximal marker of the *Kn1-O* chromosome, *UMC107(U)*, and the distal marker of *Kn1-N*, *Adh1-S*. Thus it appears, as shown in Figure 1c, that recombination has occurred, replacing the distal repeat of *Kn1-O* with similar DNA from *Kn1-N*.

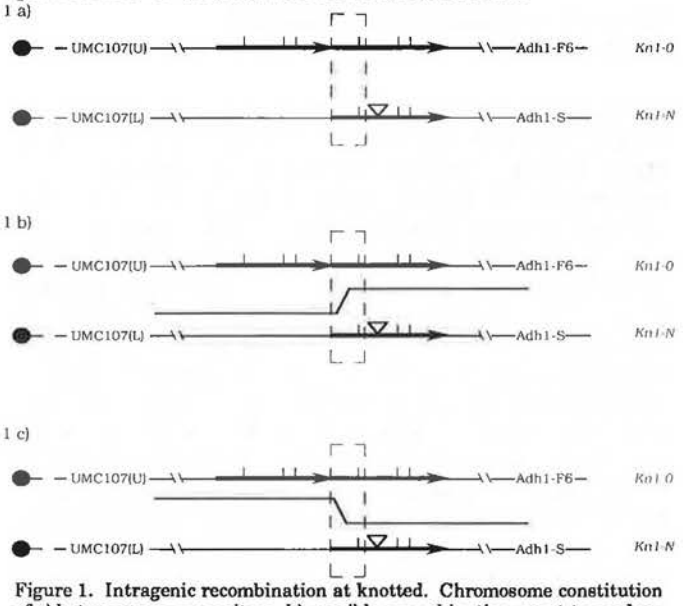

Figure 1. Intragenic recombination at knotted. Chromosome constitution of a) heterozygous progenitors, b) possible recombination event to produce normal derivative, c) possible recombination event to produce severe Knotted derivative. Dashed box delimits recombination region.

We do not know the cause of the *Kn1-N* mutation as we do with *Kn1-O* or *Kn1-2F11*, which result from a tandem duplication and an insertion of *Ds2*, respectively. The results of this recombination delimit the lesion that causes *Kn1-N* to the right of the recombination event in Figure 1c. It suggests that the 500 bp insertion may be the cause of the *Kn1-N* mutation but with such few numbers and without the reciprocal recombinant, it is impossible to say. Similar to the Bar locus in *Drosophila*, the *Kn1-O* locus undergoes reversion at a high rate (1 in 3000). We have isolated derivatives from homozygous *Kn1-O* plants that have gained an extra copy of the tandem duplication and are more severely knotted, and have a number of derivatives that have lost the duplication and are normal. The rate of recombination with the *Kn1-O/Kn1-N* double heterozygotes is equally high, if not higher.

Ac influences expression of the dominant *Ds2* insertion mutation *Kn1-2F11*

--Erik Vollbrecht and Sarah Hake

Kn1-2F11 is one of the dominant Knotted (*Kn1*) mutations that map near *Adh1* on the long arm of chromosome 1. We cloned *Kn1-2F11* using *Ds2* as a transposon tag (Hake, Vollbrecht and Freeling, EMBO J. 8:15-22, 1989). The mutation is caused by the insertion of a *Ds2* element, and revertants in which *Ds2* has excised are normal. Recent data indicate that the *Ds2* element lies within the *Kn1* transcription unit (B. Veit and E. Vollbrecht, unpublished).

Dominant mutants at *Kn1* (7 have been identified) are characterized by abnormal lateral vein development which results in protruding, hollow, finger-like pockets of tissue referred to as knots. Additionally, the Knotted mutation conditions ectopic and/or displaced formation of the ligule, a tissue which normally demarcates the leaf blade-sheath boundary. A specific, mild phenotype is associated with the *Kn1-2F11* mutation. Typically, only the first one to three seedling leaves are affected by the formation of small knots, and ligule displacement is rare. The penetrance of the mutation varies and we have observed values from zero (no expression) to one (all individuals heterozygous for the mutation express the mutant phenotype). The expressivity of the mutation is also variable, but within the progeny of a given cross affected individuals usually express the mutation to similar degrees.

We suggested previously that variation in penetrance of *Kn1-2F11* was correlated with the segregation of *Ac* or a gene closely linked to the *Ac* in our lines. The *Ac* element in our lines is on 9S, about 20 map units from *Wx* (K. Dawe, pers. comm.), and will be referred to as *Ac-2F11*. We have begun a genetic analysis to examine unlinked genes affecting *Kn1-2F11*, and the initial results of that analysis are reported here.

To test the effect of *Ac* on *Kn1-2F11* expression, we used a two step crossing scheme. In the initial cross, an individual homozygous for the dominant mutation (*Kn1-2F11/Kn1-2F11, ac/ac*) was used as a male to many different *Ac*-containing females (*kn/kn*, *Ac/Ac* or *Ac/ac*). As controls, males used in the initial cross were selfed and crossed

onto the *bz2-m* tester line. We used standard *Ac* elements located on 9S (*wx-m7::Ac*, *wx-m9::Ac*, *bz1-m2::Ac*, *Ac-2F11*) and 10L (*r-njm::Ac*), and *Ac2* elements on 8L in one (referred to as "*Ac2*") or two closely linked (referred to as "*Ac2Ac2*") copies. We then selected progeny of the initial cross which were heterozygous for *Ac* and for *Kn1-2F11* and backcrossed these individuals to the *Ac* line. The kernels from both crosses were separated by *Ac* dosage and planted to screen the progeny for the presence of knots. These data were used to calculate the penetrance (p) of *Kn1-2F11* in each genotype.

The genetic analysis from progeny screens for two different males shows that *Ac* acts to increase the penetrance of the *Kn1-2F11* mutation (Table 1). After crossing onto the no-*Ac*, *bz2-m* tester stock, both *Kn1-2F11* lines show infrequent expression of the mutant phenotype. Line 36 shows knots only rarely after selfing (p=0.03), while the progeny of male 38 selfed exhibit higher penetrance (p=0.21). After crosses to *Ac* lines, however, penetrance is substantially higher in plus-*Ac* plants than in their no-*Ac* siblings. In general, the 38 line retains a higher "baseline" penetrance through additional crosses, and *Ac* acts to increase *Kn1-2F11* penetrance above that baseline.

Table 1. Penetrance of *Kn1-2F11* in progeny containing variable *Ac* dosage.

		MALE #36 (*Kn1-2F11* line)		MALE #38 (*Kn1-2F11* line)	
FEMALE	Progeny Ac Genotype	*Kn1* Penetrance Initial Cross	*Kn1* Penetrance Backcross	*Kn1* Penetrance Initial Cross	*Kn1* Penetrance Backcross
Controls					
bz2m	-/-	0.01	-	0.03	-
Self	-/-	0.03	-	0.21	-
Ac lines					
Ac2	-/-	-	-	-	-
	Ac/-	0.07	-	0.02	0.00
	Ac/Ac	-	-	-	0.52
Ac2Ac2	-/-	0.04	-	0.14	-
	Ac/-	0.10	-	0.25	-
	Ac/Ac	-	-	-	-
r-njm::Ac	-/-	0.04	-	0.27	-
	Ac/-	0.77	0.90	0.80	1.00
	Ac/Ac	-	0.88	-	0.52
wxm7::Ac	-/-	0.07	-	0.35	-
	Ac/-	0.61	0.42	0.77	1.00
	Ac/Ac	-	0.54	-	0.82
wxm9::Ac	-/-	0.00	-	0.00	-
	Ac/-	0.54	0.00	0.30	0.50
	Ac/Ac	-	0.12	-	0.52
bz1m2::Ac	-/-	-	-	-	-
	Ac/-	0.04	0.19	0.21	0.14
	Ac/Ac	-	0.15	-	0.20
Ac-2F11	-/-	0.00	-	0.76	-
	Ac/-	0.20	0.00	0.97	0.82
	Ac/Ac	-	0.00	-	0.82

The results from the five standard *Ac* elements used in this study can be discussed in three groups according to penetrance scores from the initial cross. The *Ac* elements at *r-njm::Ac* and *wx-m7::Ac* both greatly increase the frequency of occurrence of knots. For the initial cross of each line, the penetrance in the plus-*Ac* seedlings is much higher than the baseline score in the no-*Ac* class (difference ranges from 0.42 to 0.73). In the backcross progeny, penetrance remains high and is similar for plants containing one or two doses of the element. As a second group, the *Ac* elements at *wx-m9::Ac* and *Ac-2F11* are comparatively weaker in the initial crosses, as the penetrance difference between the plus-*Ac* and no-*Ac* classes ranges from 0.20 to 0.54. In the backcrosses to the *wx-m9::Ac* and *Ac-2F11* lines, penetrance remains high or increases slightly in the presence of *Ac* for line 38. The line 36 backcrosses appear to uncover suppression, although the *Ac-2F11* data may represent reversion of *Kn1-2F11* by excision of *Ds2*. Finally, the crosses to the *bz1-m2::Ac* line give peculiar results. Contrary to the results seen with other *Ac* elements, none of the crosses in Table 1 show an increase in penetrance segregating with *bz1-m2::Ac*. Backcrosses to a homozygous *Kn1-2F11*, no-*Ac* line, however, show a striking increase in penetrance in the plus-*Ac* class over no-*Ac* siblings (difference = 0.4, data not shown). Either suppression has been relieved by two generations of crossing away from the *bz1-m2::Ac* line or the effect of this particular *Ac* element is only evident when *Kn1-2F11* is homozygous.

The data also show that one and two doses of standard *Ac* in the plant affect penetrance similarly, but that the mutation is sensitive to *Ac2* dosage. *Ac2* is an anomalous *Ac* element that shows additive dosage on frequency of transposition events (Rhoades and Dempsey, MNL 57:14-17, 1983). Three (or more) doses of *Ac2* are required to see *bz2-m* spots in the endosperm, while four doses are necessary to induce chromosome breaks at *Ds*. As indicated in Table 1, one dose of *Ac2* has little or no effect on *Kn1-2F11*. In fact, in all initial crosses to the *Ac2* line *Kn1-2F11* showed low penetrance ($p \leq 0.07$). In contrast, two doses of *Ac2* in the plant increases penetrance of the mutation. This is seen in plants homozygous for *Ac2* or heterozygous for a chromosome containing two closely linked *Ac2* elements. The crosses to the *Ac2* lines may uncover suppression, but in this case the enhancer effect of 2 doses of *Ac2* confers expression of the mutant phenotype despite that suppression. Crosses are in progress to test for any combinatorial effects with *Ac2* and standard *Ac*.

We are interested in the mechanism by which *Ac* influences expression of *Kn1-2F11*. Several points exclude somatic excision of *Ds2* as the cause of knot formation. For example, plants carrying the mutation but lacking any active *Ac* elements are capable of expressing the mutant phenotype. In plants which do carry an active *Ac* element, individuals may be normal although *Ds2* excision occurs, evident at both the *bz2-m* locus (plant and kernel sectors) and the *Kn1-2F11* locus (Southern data). Furthermore, while *Ac* shows a dosage effect on the timing and frequency of *Ds* excision events, we see no dosage for standard-*Ac* enhancement of *Kn1-2F11*. Although *Ds2* is inserted within transcribed sequences, we have not detected transcript differences between mutant and wild type at the level of Northern blots.

The crossing scheme we used defines trans-acting factors influencing *Kn1-2F11* expression. While the data show that *Ac* is a dominant enhancer of *Kn1-2F11*, they also suggest that some background suppression effects can preclude the *Ac* effect. We are analyzing the background effects observed thus far to determine whether any of them segregate as single genes. Furthermore, we are investigating whether changes in the ability of *Kn1-2F11* to respond to *Ac* are correlated with changes in methylation of the *Ds2* insertion at the *Kn1-2F11* locus.

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Transactivation of the anthocyanin pathway structural genes with wild-type and altered *C1* proteins

--Stephen A. Goff, Michael E. Fromm and Karen C. Cone

Complementation of c1 *aleurones and embryos:* High-velocity microprojectile bombardment was used to deliver the *C1* gene or a CaMV 35S promoter-driven *C1* cDNA construct into intact *c1 R-scm* aleurones in an attempt to transactivate the anthocyanin biosynthetic pathway. In aleurones, the introduction of the *C1* genomic clone or the expressed *C1* cDNA resulted in purple, anthocyanin-producing cells. This observation confirms the identity of these clones as coding for functional *C1* gene products.

Transactivation of the Bz1 *promoter following transfer of the* C1 *gene into aleurones or embryos*: A reporter construct using the firefly luciferase coding region under control of the *Bz1* structural gene promoter was used to test for transactivation by the *C1* gene product. As an internal control for the efficiency of gene transfer, a plasmid containing the alcohol dehydrogenase-1 promoter driving the chloramphenicol acetyltransferase (CAT) coding region, was included in each DNA sample used for bombardment. The values of *C1* transactivation of *Bz1*-luciferase are therefore expressed as a ratio of luciferase enzyme activity to the CAT enzymatic activity generated from the internal control plasmid. Co-bombardment of the *Bz1*-luciferase reporter construct with the *C1* gene or the expressed *C1* cDNA resulted in an 85 to 300-fold induction of the *Bz1* promoter relative to tissues receiving the reporter plasmids alone (Table I).

Table I

c1 R-scm	Embryos		Aleurones	
Plasmids	Luc/CAT ± SEM	Induction ± SEM	Luc/CAT ± SEM	Induction ± SEM
pMF6/pUC p*Bz1*Luc p*Adh*CAT	0.049 ± 0.007 n=12	1.00 ± 0.014	0.040 ± 0.005 n=12	1.00 ± 0.14
p35S *C1* p*Bz1*Luc p*Adh*CAT	4.18 ± 0.94 n=12	85 ± 19	3.64 ± 0.68 n=9	91 ± 17
p$C1_{gen}$ p*Bz1*Luc p*Adh*CAT	16.6 ± 3.87 n=6	339 ± 79	13.8 ± 5.02 n=6	346 ± 125
p*C1*GAL10 p*Bz1*Luc p*Adh*CAT	N.D.	N.D.	4.6 ± 0.90 n=5	114 ± 23
p*C1*fs2 p*Bz1*Luc p*Adh*CAT	0.168 ± 0.015 n=3	3.44 ± 0.30	0.035 ± 0.002 n=3	0.72 ± 0.04

Plasmid Definitions:

pUC = pUC19.
pMF6 = CaMV 35S Expression Vector without Insert.
p*Bz1*Luc = *Bz1* promoter driving luciferase.
p35S *C1* = *C1* cDNA under 35S promoter control.
p*C1*gen = *C1* genomic clone.
p*C1*GAL10 = Fusion of GAL4 acidic domain at amino acid 258 of *C1*.
p*C1*fs2 = Frameshift of *C1* at amino acid 258.
p*Adh*CAT = *Adh1* promoter driving Chloamphenicol Acetyltransferase.

Complementation and transactivation with C1-*GAL4 fusion proteins*: To obtain more definitive evidence that the product of the *C1* gene functions as a transcriptional activator, we have replaced the acidic region of *C1* with the acidic domain of the yeast transcriptional activator GAL4, and tested for complementation of the anthocyanin pathway in *c1* aleurones. Three CaMV 35S-driven fusion protein plasmids were constructed with varying lengths of the *C1* protein fused to the C-terminal acidic domain of GAL4. These fusions were made at amino acids 117, 144, and 258 of the intact 273 amino acid *C1* protein. Each of these constructs was delivered to *c1 R-scm* aleurones and resulted in the generation of pigmented cells, however placement of the GAL4 acidic domain immediately adjacent to the putative DNA-binding region (at amino acid 117 of *C1*) resulted in only weakly pigmented cells. The largest *C1*-GAL4 fusion (at amino acid 258 of *C1*) was also tested for its ability to activate the *Bz1* promoter. A 115 fold induction of *Bz1*-luciferase was observed in *c1 R-scm* aleurones co-bombarded with this *C1*-GAL4 fusion construct (Table I). A frameshift placed in the *C1* acidic region (amino acid 258) renders the altered *C1* protein unable to transactivate the *Bz1* promoter (Table I). These results demonstrate that the *C1* carboxy-terminal acidic region can be replaced by an acidic domain from a known transcriptional activator and result in a functional regulatory protein.

A frameshift mutant of C1 *inhibits transactivation of the* Bz1 *promoter*: A frameshift in the carboxy-terminal acidic region of the *C1* cDNA coding sequence (amino acid 258) was constructed in vitro in an attempt to mimic the effects of the dominant inhibitor allele of *C1* designated *C-I*. The product of this construct did not complement *c1* aleurones nor transactivate the *Bz1* promoter. At 1 to 1 ratios of wild-type *C1* and this *C1* frameshift construct, an 80% reduction of transactivation was observed following gene delivery, and at higher ratios, inhibition of transactivation approached 95%. Thus it is possible to mimic the effects of *C-I* and generate a dominant inhibitor of *C1* with a frameshift in the coding region which disrupts the acidic nature of the *C1* carboxyl-terminus.

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Transactivation of anthocyanin structural gene promoters following transfer of the *B-Peru* or *B-I* gene into maize tissues

--Stephen A. Goff, Michael E. Fromm, Karen C. Cone and Vicki L. Chandler

High-velocity microprojectiles were used to transfer the *B-Peru* or *B-I* genes into intact *C1 r b* aleurones or embryos to determine the ability of the *B* gene products to transactivate the anthocyanin biosynthetic pathway. In aleurones, introduction of the intact *B-Peru* gene or constructs expressing either the *B-Peru* or *B-I* cDNA from the

CaMV 35S promoter resulted in purple, anthocyanin-producing cells. These results confirm that the *B-Peru* and *B-I* clones code for functional regulatory proteins as well as establish a system for the analysis of their gene products. In addition, these results demonstrate that the product of the *B-I* gene can functionally substitute for *B-Peru* as well as *R*. Together with the observation that no *B* RNA is observed in *B-I* aleurones (P. Radicella and V. Chandler, unpublished observations), these results suggest that the differences in tissue-specific anthocyanin production conferred by *B-Peru* and *B-I* result from differential expression of these *B* alleles in various tissues.

Reporter genes using the firefly luciferase coding region and the promoter of either the *Bz1* or *A1* structural genes were also tested for transactivation by the *B-Peru* or *B-I* genes in *C1 r b* aleurones or embryos. As an internal control for the efficiency of gene transfer a plasmid containing the *Adh1* promoter driving the chloramphenicol acetyltransferase (CAT) coding region was included in each DNA sample used for bombardment. The values of *B-Peru* or *B-I* transactivation of *Bz1*- or *A1*-luciferase are therefore expressed as a ratio of luciferase enzymatic activity to the CAT enzymatic activity generated from the internal control plasmid. Co-bombardment of the *Bz1*-luciferase reporter construct with the *B-Peru* gene, or an expressed *B-Peru* or *B-I* cDNA resulted in an 80 to 130-fold increase in the ratio of luciferase to CAT activity relative to tissues receiving the reporter plasmids alone. Aleurones and embryos receiving the *A1*-luciferase reporter plasmid and a *B-I* expression plasmid contained a 15 to 50 fold higher ratio of luciferase to CAT activity than did tissues receiving only the reporter plasmid alone. The magnitude of induction of the *A1* promoter appears lower than that of the *Bz1* promoter due to higher basal activity of the *A1* promoter. The induced levels of luciferase/CAT from the *A1* and *Bz1* promoters are comparable.

In an attempt to extend the above described system to more easily obtained tissues, we tested for transactivation of the anthocyanin pathway in embryogenic callus cells and BMS suspension culture cells. Following delivery of either the *B-Peru* genomic, or an expressed *B-Peru* or *B-I* cDNA, no pigmented cells were observed. Since this embryogenic callus was derived from *r c1* embryos, we determined the effect of delivering an expressed *C1* cDNA with each of the *B* plasmids. This combination results in the activation of the anthocyanin biosynthetic pathway and pigmented cells were observed. Unlike either aleurones, embryos, or embryogenic callus cells, BMS suspension culture cells were not observed to contain pigmented cells regardless of the construct or combination of constructs delivered. These results demonstrate that the expression of both the *B-Peru* (or *B-I*) and *C1* genes is required to activate the anthocyanin biosynthetic pathway in *r c1* embryogenic callus.

Transactivation of the *Bz1* or *A1* promoters was also examined in embryogenic callus and BMS suspension culture cells. The *B-Peru* genomic or expressed *B-Peru* or *B-I* cDNAs alone were insufficient to transactivate either the *Bz1* or *A1* promoters. Transactivation of the *Bz1*-luciferase or *A1*-luciferase constructs required cotransformation of one of the *B-Peru* or *B-I* genes with an expressed *C1* cDNA. In embryogenic callus a 30 to 190 fold transactivation of the *Bz1* promoter and a 15 fold transactivation of the *A1* promoter was observed. Thus embryogenic callus appears similar to aleurones and embryos in this transactivation assay. In BMS suspension culture cells however, only a 2 to 10 fold transactivation of the *Bz1* promoter was observed. This low level induction of the *Bz1* promoter is probably too weak an induction to be observed using the anthocyanin pigmentation assay.

The activation of the *Bz1* and *A1* promoters or the genes for the entire anthocyanin biosynthetic pathway in embryogenic callus tissue indicates that this system is a valid alternative to the time-consuming process of isolating aleurones and/or embryos.

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c2-m85-2 gives rise to lethal derivatives

--Peter A. Peterson

This mutable gives a very early pattern variegation. Though it contains a *Cy* element, the relationship to *Cy* has not been demonstrated. In crosses of the heterozygote *C2/c2* x *c2/c2* a relatively high frequency of colorless kernels arises, which would support independent control, but the segregating pattern does not support this. A unique feature of this allele is the colorless kernels arising from a self. In the self of *C2/c2-m*, the colorless kernels when planted are unable to proceed beyond the seedling stages: pale green seedlings appear which soon die. It appears that the mutation to colorless gives rise to a deficient form of the *c2* allele which when homozygous, lacks a necessary function that leads to death of the seedling. Possibly, this is an essential component of the *c2* locus or an adjacent region that leads to lethality following excision (loss of mutability).

c-m888104 - a mutable allele under 2-element control

--Peter A. Peterson

In a segregating population arising from the self of *c-m/c sh wx*, colorless shrunken and colorless round were selected from kernels segregating for variegated round, colorless round and colorless shrunken. In the cross of colorless round x numbered colorless shrunken, the following segregating progeny was observed.

Colored	Spotted	Colorless
11	119	195

This would indicate that the colorless round is a responsive allele responding to a separable element carried in the colorless shrunken kernels. Since this mutable arose in a *Cy*-containing plot, the initial tests were for a *Cy* relation. These tests exclude *Cy* as the functional element triggering mutability.

_a-m877515_ - a likely _a-rcy_ allele with a low mutability pattern

--Peter A. Peterson

The mutability occurs in the absence of *En*, and *Cy* is implicated. This is based on the absence of *En* in 10/10 cases of *a* mutability and the presence of *Cy* in 6/6 cases of *a* mutability. A reconstruction test has not been completed. The mutability pattern is a very low type (very late, infrequent spots). This is surprising because in an associated test, this same *Cy* gives a very strong effect (the standard high frequency, late spots) on *bz-rcy*. This would indicate that the receptor elements are responding differently to the same *Cy*, either because of a size difference or a positional alteration.

***En* (Active) in inbred 4Co63**

--Peter A. Peterson

In extensive screening of numerous populations, only *Uq* and *Mrh* have generally been found. In an accidental crossing with a W23/4Co63 hybrid, every ear showed mutability when crossed with *c-m(r)*. In order to distinguish which of the two lines carried the *En*, *c-m(r)* was crossed to the individual inbreds. Every plant (from 4Co63) (9/9 tested) carried *En* and in the homozygous condition. This is surprising since even with *Uq* or *Mrh*, rarely do all the plants have the element and even more rarely, in the homozygous condition.

***c-836956* - a deficiency that is not male transmitted**

--Peter A. Peterson

That a deficiency is not male transmitted is no surprise. This arose from a *C-I* allele where changes in *C-I* were isolated. The female transmission is slightly disturbed. Where a linked marker (*Bz*) is expected in 50% of the progeny, this deficiency appears approximately 38-45% of the time. Many embryos do not develop a seed. It is ambivalent. The deficiency has a variable penetrance.

c-m897133

--Peter A. Peterson

Both *Uq* and *Mut* are implicated but additional tests are necessary. In the only test cross available (*c-m Sh Wx/c sh wx* x *c sh wx*) the progeny segregated as follows:

	Round			Shrunken	
Cl	Spotted	cl	Cl	sp	cl
1	57	11	0	3	68

Such a ratio would suggest either 3-4 elements or autonomous mutability with a high frequency of change to colorless

c-m897143

--Peter A. Peterson

This allele appears to be autonomously mutable. The following are segregating ratios from a test cross (*c-m Sh Wx/c sh wx* x *c sh wx*):

	Round			Shrunken	
Cl	Spotted	cl	Cl	sp	cl
6	220	21	0	3	177
1	37	4	0	1	36

Unidirectional cross-incompatibility in maize - a new system

--Abdul Rashid and Peter A. Peterson

A heritable unidirectional cross-incompatibility was observed in 1975 (Sukhapinda and Peterson, Can. J. Genet. Cytol. 25:270, 1983). The nature of this incompatibility is that a specific male genotype is "largely" rejected by a specific female genotype.

The incompatible female is *a-m(pa-pu)/a sh2* while the male parent in this incompatible pair is *a et/a et*. A partial seed set (less than 25 seeds per ear) is observed in this cross but in reciprocal crosses the seed setting is normal. The genetics of this partial cross-incompatibility was studied and the results indicate that three independently segregating recessive genes are involved. One gene pair named *cif* (cross-incompatible female) is expressed in the female and when in the homozygous recessive state, the plant is an incompatible female. When used with the appropriate male the other two genes *cim1* (cross-incompatible male) and *cim2* are expressed in the male. When both of the *cim* genes are in the homozygous recessive state, the result is a partial seed set. The putative genotype of the incompatible cross is *cif/cif* x *cim1/cim1, cim2/cim2*.

Normal seed setting is dominant over reduced seed setting and this incompatibility is under the control of a sporophytic system of incompatibility. It has also been observed that the rejection of incompatible pollen occurs before fertilization. The mode of rejection of incompatible pollen is under study.

***ba3* (formerly designated *ba*-861059b*) - a new barren-stalk locus**

--Yong-Bao Pan and Peter A. Peterson

ba-861059* (*ba**) is a barren-stalk mutant derived from the progenies of a WSMV infected maize plant (MNL 62:4). It is a single recessive gene. Its relation to the three previously described barren-stalk mutant genes, *ba1*, *ba2* and *baf* (Annu. Rev. Genet. 22:352), has been tested. The results are summarized as follows:

Cross type	No. of crosses	Progeny per cross tested
Ba/ba** x *ba1/ba1*	1	13
Ba1/ba1 x *ba*/ba**	1	14
Ba2/ba2 x *Ba*/ba**	1	24
Ba2/ba2 x *ba*/ba**	3	13
Ba/ba** x *ba2/ba2*	5	13-14
Baf/baf x *Ba*/ba**	1	22
Baf/baf x *ba*/ba**	2	13
Ba/ba** x *Baf/baf*	4	13-14

That none of the designated crosses yielded any barren-stalk progeny provides genetic evidence that *ba** is not allelic to *ba1*, *ba2* or *baf* and represents a new independent locus influencing the barren-stalk trait. We conclude that a

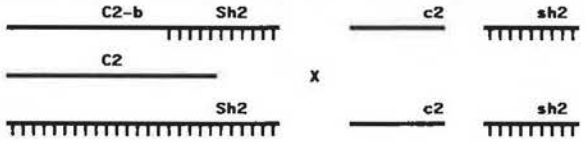
mutation at any of these 4 loci could cause a barren-stalk phenotype and that these 4 (possibly more) genes act in different steps in a pathway leading eventually to a barren-stalk phenotype. We designate *ba*-861059b* as *ba3*.

C2-b857246 and two mutable derivatives: *c2-m881058P* and *c2-m881058Y* and control of their variegated phenotypes

--Michael G. Muszynski and Peter A. Peterson

A variegated (colored to colorless) kernel, *C2-b857246*, was rescued from a 1984 *c2* isolation plot (*C2/C2* x *c2/c2*) containing many elements including *Cy, En, Uq, Ac* and *Dt* (MNL 60:2). This variegated phenotype was heritable upon outcrossing to *c2/c2* testers and segregated 1/4 colored:1/4 variegated:1/2 colorless as a male. When used as a female, the 2 doses of *C2* make identification of the variegated kernels more difficult and therefore that class is usually less than 25% and the colored class greater than 25% by the same amount, on a per ear basis. The phenotype and inheritance seem to suggest that the mutability does not reside at *C2* but that the *C2* locus and perhaps other genes on 4L were being lost via a chromosomal breakage event.

This was proven by linking *C2* to 3L carrying *Sh2* with a translocation and the heterozygote was then crossed by and on a *c2 sh2* tester. All the colorless sectors on the variegated kernels from the outcross ears were also *sh2* indicating the loss of both markers.



Therefore, this mutant is designated *C2-b* for *C2* breaker. System tests indicate that this mutant does contain many *Cy* and *En* but neither show a correlation with the occurrence of breakage. This breakage may be under control of a previously undefined element.

Two mutable derivatives have been isolated from this mutant.

c2-m881058P: An exceptional spotted kernel (2-5 a-b) was found on an ear segregating colored, variegated and colorless. The kernel (*c2-m/c2*) was selfed and outcrossed several times to *c2/c2* testers. The spotting phenotype was heritable and segregated 45 spotted:19 colorless from the self and 3 spotted:5 colorless from the outcrosses. This mutant allele was designated *c2-m881058P* and seems to be controlled by two independently segregating functional elements. The spotted kernels from the outcrossed ears segregated for three distinct phenotypes: 1 high (6-8 aa-d):1 low (2-6 aa-b):1 extreme low (1-3 aa-a). It may be that each of the two independent regulators causes a different spotting pattern in this *c2-m* allele and both regulators acting together elicit a third spotting phenotype. This can be verified by further testing.

c2-m881058Y: Another exceptional spotted kernel (5 b-c) was found on an ear segregating colored and colorless from *C2-b857246*. This mutant was found to be heritable, designated *c2-m881058Y* and also seems to be under control of two independently segregating regulators. The spotting phenotype is heavy (5-7 a-c) with occasional (1%-14%) low (1-4 aa-a) spotted kernels appearing. This lower spotting phenotype may be due to a change of state of the *c2-m* allele or to changes to one or the other or both independent regulators.

A study of the relation of *c2-m881058P* and *c2-m881058Y* to each other and to *C2-b857246* is in progress. Also, studies on the cause and heritability of the lower spotting patterns and system tests are in progress.

c2-m884259Y - a mutable allele under two-factor control

--Michael G. Muszynski and Peter A. Peterson

The *c2-m884259Y* allele originated as an exception from *c2-m826040* (MNL 57:2). The mutability of this new allele seems to be under control of an independent factor. Without the factor the allele is pale; with the factor, the allele shows a pale coloration with spots or colorless with spots (2-5 a-c). Selfed ears originating from *c2-m/c2* kernels segregate 3/8 pale:3/8 spotted:2/8 colorless. When spotted kernels from the selfed ear are used as a male on *c2/c2* testers, the progeny ears segregate 1 pale:1 colorless or all pale without any spots. These data indicate that the factor controlling mutability is not male transmissible and may be linked to a gametophyte factor or a pollen lethal gene. Tests of this hypothesis and of system relations are underway.

C-I-b836024 - a chromosomal breaker linked to *C-I*

--Michael G. Muszynski and Peter A. Peterson

A highly variegated kernel (2-6 a-d) was isolated from a *C-I* isolation plot (*C-I/C-I* x *C/C*) containing *En* (MNL 58:2). Crosses to various testers (Line C - a full color line, *a1-o wx, c2, C sh bz wx, r-g* and others) always showed the same segregation of 1 colored:1 variegated. This variegated phenotype is linked to *sh1* and is likely autonomously controlled. Because of its dominant inheritance and linkage, this mutant was designated as an unstable *C-I* allele. The variegated phenotype of this mutant is most likely colorless to colored, but variable in timing. Within the larger colored sectors (loss of *C-I*) of *C-I-b sh Bz Wx/C sh bz wx* kernels, *bz* sectors can be identified and subtending these *bz* sectors are smaller *wx* sectors. The phenotypic pattern suggests that *C-I* is being lost via a chromosomal breakage event and the BBF cycle is causing further loss of more proximal markers. Therefore this mutant has been designated *C-I-b* for *C-I* breaker. Mapping of the breaker and systems tests are currently underway.

Promoter activity, ARS function, dnaA binding sites and transposition of the activator (*Ac*) element

--James H. Zhou and Alan G. Atherly

Our previous observations showed that the end(s) of *Ac* exhibit: (i) prokaryotic promoter activity when fused to a promoter-less gene encoding chloramphenicol acetyl-

transferase (CAT) in *E. coli*, (ii) dnaA protein-binding repeats (TTATACACA), overlapped with the promoter-like repeats, and (iii) functional DNA autonomous replication sequences (ARS) when introduced into yeast cells. We now report construction of a set of defective *Ac* elements with internal replacements and subterminal deletions that we used to analyze the function of the terminal sequences of *Ac*. To test functionality each of the defective *Ac* elements was inserted into the *Xba*I site, in both orientations, between a CaMV 35S promoter and a GUS reporter gene in the *Agrobacterium* binary vector pZA3 (Zhou and Atherly, Plant Cell Reports, 1989, in press), and then co-transferred with a normal *Ac*-carrying plasmid p*ZAc*20 into tobacco cells via T-DNA-mediated transformation. Expression of GUS activity was used to detect excision of the defective *Ac* elements. We found that a minimal 3'-terminal sequence of 95 bp, plus a short internal sequence between bp 3630 and 3644 (or 188 bp without any internal sequence) was required for transposition of the artificially defective *Ac* elements.

The deletion of the distal putative ARS on the 3'-end gave an approximate three-fold decrease in transposition frequency, and deletion of both the proximal and distal ARS's gave no transposition, suggesting the putative ARS's may be required for high efficiency of the transposition. Another very interesting finding was that the 3'-terminal sequence was found to be highly homologous to the region of DNA replication of maize strip virus (Dellaporta, 1989, personal communication), suggesting the origin of DNA replication at the 3'-end of *Ac* element may be needed for transposition.

Deletions of the dnaA protein-binding sequences in the 3'-end gave a read-through phenotype when in the same orientation with respect to the GUS gene, but not in the reverse orientation. This observation is analogous to that of prokaryotes, in which the DNA protein binding sites function as a terminator of transcription when present in one strand of the DNA double helix (Trends in Genetics 5:319-321, 1989). A surprising observation is that a short internal sequence between bps 3630-3644 was found to be a terminator that prevented read-through when present in the reverse orientation with respect to the GUS gene. But, both terminators were inactivated when an active *Ac* element was present in *trans*. However, one termination sequence (when in the reverse orientation) showed more *Ac*-dependence than the other. An analogous observation was made in the maize *R* locus where an *Ac* element was inserted into the regulatory sequence (Dellaporta, 1989, personal communication). We theorize that this may be due to the relaxation of the DNA double helix when the product of the *Ac* element interacts with the subterminal sequence of the defective *Ac* element.

We speculate from these data that the functional sequences observed within the terminal sequences of *Ac* (promoters; dnaA binding sequences, which also function as terminators in plants; and ARS's), that have evolved from three very divergent organisms (*E. coli*, yeast, and plants) play analogous functions in each of these organisms. Also, promoter activity identified at both ends of the element may correlate with transposition, and dnaA protein-binding sites, as well as ARS's may correlate with regulation of transposition frequency.

Revertants of the putative Mutator-induced dominant amylose-extender allele, *Ae-5180*

--Philip S. Stinard

The availability of germinal revertants of transposable element induced mutations aids in the cloning and characterization of the mutant and wild type alleles. This report describes our preliminary efforts to obtain revertant alleles of the putative Mutator-induced dominant amylose-extender allele, *Ae-5180*. Our approach was to cross *Ae-5180* to germinally active Mutator lines, sib the F1's to achieve homozygosity for *Ae-5180* while maintaining Mutator activity, backcross the homozygous *Ae-5180* Mutator plants by homozygous *ae* testers, and select the starchy (nonmutant) *Ae-5180* to *Ae-Rev* revertants.

During the summer of 1987, germinally inactive stocks of *Ae-5180* were crossed to germinally active *Mu2* lines. The F1's were sib-pollinated in 1988, and mutant kernels were planted in winter 1988-89. Of the plants growing from these kernels, two thirds would be expected to be heterozygous for *Ae-5180* (*Ae-5180 Ae*), and one third homozygous (*Ae-5180 Ae-5180*). These plants were pollinated by pollen from homozygous standard *ae* (*ae ae*) plants. The results of these crosses are presented in Table 1. Of 17 crosses made, only 6 had ratios of nonmutant to

Table 1. Counts of nonmutant and mutant kernels on ears of [(*Mu2/Ae-5180*) X (*Mu2/Ae-5180*)] X *ae ae*.

Female Parent	Nonmutant Kernels	Mutant Kernels	% Mutant Kernels	1:1 Chi-sqr
88-89-8591-1	218	109	33.3	36.33***
8591-2	191	79	29.3	46.46***
8591-3	91	44	32.6	16.36***
8591-4	200	89	30.8	42.63***
8591-5	234	142	37.8	22.51***
8591-6	205	0	0.0	-----
8591-7	97	49	33.6	15.78***
8591-8	244	25	9.3	-----
88-89-8592-1	139	108	43.7	3.89*
8592-2	100	110	52.4	0.48
8592-3	0	298	100.0	-----
8592-4	4	374	98.9	-----
8592-5	107	119	52.7	0.64
8592-6	96	104	52.0	0.32
8592-7	146	129	46.9	1.05
8592-8	153	172	52.9	1.11
8592-9	190	186	49.5	0.04

* 1:1 chi-square significant at p = 0.05 level
*** 1:1 chi-square significant at p = 0.001 level

mutant kernels that did not differ significantly from the 1:1 ratio expected of heterozygous plants. Only one ear was completely mutant. Seven ears deviated from a 1:1 ratio, having a surplus of nonmutant kernels. The surplus of nonmutant kernels on these ears could be due to a transmission anomaly (although this has rarely been seen in female outcrosses of *Ae-5180* in a non-*Mu* background) or a high reversion rate of *Ae-5180* in heterozygous (or even homozygous) *Ae-5180* plants. Of the remaining ears, one had only nonmutant kernels (possibly the result of a heterofertilization event in the previous generation or an early reversion event encompassing the entire ear in a heterozygous *Ae-5180* plant), one was nonmutant with an ear sector of mutant kernels, and one was mutant with a few nonmutant kernels. Of the 17 ears, the latter two provide the greatest evidence for reversion of *Ae-5180*. Ear

88-89-8591-8, mapped in Figure 1 (left), is predominantly nonmutant with an ear sector of 25 mutant kernels. This most likely represents an early reversion encompassing most of the ear of a heterozygous *Ae-5180* plant. Ear 88-89-8592-4 (Figure 1, right), is mostly mutant, with four putative revertant kernels. Two of the revertants are in a sector, and the other two occur separately on the ear.

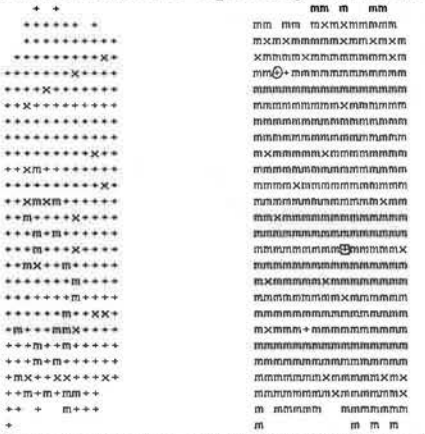


Figure 1. Ear maps of sectored ears 88-89-8591-8 (left) and 88-89-8592-4 (right). Revertant kernels from 88-89-8592-4 that were planted under numbers 89-3035.0-1 and 89-3035.1-1 are circled and boxed, respectively. Symbols: + nonmutant kernel, m mutant kernel, x aborted kernel or ovule.

Two of the putative revertant kernels from ear 88-89-8592-4 (of the presumable genotype *Ae-rev ae*) were planted in summer of 1989 under the field numbers 89-3035.0-1 (revertant from two-seeded sector, see Figure 1) and 89-3035.1-1 (single revertant). The resultant plants were backcrossed by *ae* testers, and the ears scored for nonmutant (*Ae-rev ae*) and mutant (*ae ae*) kernels (Table 2). The ears from both plants had 1:1 ratios of nonmutant to mutant kernels indicating that the two reversions were heritable.

Table 2. Counts of nonmutant and mutant kernels on ears of *Ae-rev ae* X *ae ae*.

Female Parent	Nonmutant Kernels	Mutant Kernels	% Mutant Kernels	1:1 Chi-sqr
89-3035.0-1	135	156	53.6	1.52 (N. S.)
3035.1-1	169	173	50.6	0.05 (N. S.)

N. S. 1:1 chi-square not significant at p = 0.10 level

We are currently in the process of developing stocks of homozygous *Ae-5180* in Mutator and non-Mutator backgrounds for isolation plots to check the reversion rate of *Ae-5180* in these two backgrounds. We are also crossing *Ae-5180* into *Spm* and *Ac-Ds* stocks in order to determine whether reversion of *Ae-5180* is triggered by either of these two controlling elements.

Putative Mutator-induced etched alleles

--Philip S. Stinard

In the course of our studies of kernel mutants with sugary/translucent phenotype arising from Mutator populations, we found several independent mutants which produced virescent seedlings when mutant kernels were planted in the sandbench (nonmutant kernels gave rise to green seedlings). A close examination of the mutant kernels revealed that some, but not all, of them had pitted or etched endosperms. Phenotypes ranged from smooth glassy shrunken to plump translucent, and within these types, the etching varied from none to severe. Heterozygous mutant plants were crossed with etched testers, and the mutants proved to be allelic, with good expression of etched in the F1 kernels. The mutants are summarized in Table 1. Mutant and nonmutant kernels from the testcross ears were planted in the sandbench, and the resulting seedlings scored for virescence. Nonmutant kernels gave rise to green seedlings as expected, and mutant kernels produced virescent seedlings. In at least one testcross for each mutant, one or more of the virescent seedlings were mutable, suggesting that all five mutants are transposable element induced.

Table 1. Etched alleles arising from Mutator stocks.

Mutant Designation	Former Designation	Source	Source Description
et-Mu3328	su-sh*-3328	83-3328-46@	Mu2 2 X O.C.
et-Mu5079	su-sh*-5079	84-5079-24@	Stand/wx-Mu2
et-Mu2424	su-sh*-2424	87-2424-3@	c sh bz wx/Mu2
et-Mu2457	et*-Mu2457	87-2457-32@	Mu12 O. C.
et-Mu24	ae*-Mu24	88-8121.0-1	y1 wx gl1/Mu2 o.p.

The sugary/translucent (with or without etching) phenotype of etched kernels has not been reported previously, but this may be due more to the genetic background of the standard etched stocks than to the unique nature of our mutants. Our etched mutants arose in dent stocks. When crossed into the dent background of our purple aleurone stocks, the standard etched allele also produces a sugary/translucent phenotype. This phenotype may not be expressed as well in the flint background in which many genetic stocks are maintained.

Recombinational removal of linked Mutator elements from lines carrying Mutator-induced mutants in preparation for molecular analysis

–Philip S. Stinard

Two problems frequently encountered in the use of segregation analyses to identify *Mu* element tagged genes are the large number of different types of *Mu* elements that could conceivably be inserted in the gene, and the presence of *Mu* elements that may be closely linked to the tagged gene, but which are not themselves inserted in the gene. The first problem can be approached by using a *Mu* end probe, or successively probing segregation analyses with probes homologous to the unique portions of different *Mu* elements (providing that the mutant is caused by a known *Mu* element insert). It is the second problem that I address in this report.

The copy number of *Mu* elements in the line carrying the tagged gene can be reduced by selecting plants in which transposition of *Mu* elements has ceased, and outcrossing these plants to non-*Mu* standard lines for several successive generations. The number of unlinked elements should be approximately halved each generation, and an occasional linked *Mu* element may be removed by recombination. However, it is possible to greatly enrich for the removal of linked elements by selecting for recombinational events. To do this, one can choose non-*Mu*-containing genetic stocks that carry identifiable markers (e.g. genetic mutations, RFLP's, isozymes, translocations) flanking either side of the wild type counterpart of the tagged

gene. F1's are made between the marker stock and the tagged gene stock, followed by the appropriate backcross and identification of plants carrying crossovers between the marker and the tagged gene. Barring the occurrence of a second crossover distal to the marker, all linked *Mu* elements distal to the marker will be removed, and the probability of the removal of linked *Mu* elements from the interval between the tagged gene and the marker will be increased. The crosses can be set up to select crossovers between the tagged gene and flanking markers on each side as single crossover events in successive generations of backcrossing, or as double crossover events in a single generation of backcrossing. The latter strategy has the disadvantage of possible chiasma interference limiting the proximity of the crossover events to the tagged gene.

How useful is this method? If the linked marker is 10 map units from the tagged gene, one can cut by a factor of 10 the number of plants analyzed in a segregation analysis where one is trying to determine with a reasonable degree of certainty whether a specific *Mu* element is inserted at the locus, or whether it is merely linked. If the marker is 2 (or x, where x < 50) map units from the gene, the savings is a factor of 50 (or 100/x). The closer the markers are to the tagged gene, the better. It is also important to obtain crossovers on each side of the gene since one cannot know in advance on which side of the tagged gene linked *Mu* elements may be.

A slightly different method can be used to select for the removal of unlinked elements on other chromosomes in order to decrease total copy number. The line carrying the tagged gene and in which transposition has ceased is crossed to a non-*Mu* genetic stock carrying markers on chromosomes different from the one carrying the tagged gene. Backcrosses are made, and segregants carrying the markers plus the tagged gene are selected. The greater the number of different markers selected, the greater will be the *Mu* copy number reduction. A vigorous Mangelsdorf tester would work well, unless the phenotype of the tagged mutant is masked by one of the Mangelsdorf tester genes.

Although this report concerns *Mu* elements, a similar approach may be taken for any other transposable element system for which linked elements and copy number are a problem.

Three-point linkage data for *pr-Dap-v2* and *pr-v12-Dap* on 5L

--Philip S. Stinard and Donald S. Robertson

We report the results of three-point linkage tests for *pr-Dap-v2* (Table 1) and *pr-v12-Dap* (Table 2). The linkage tests were set up as modified backcrosses as indicated in Tables 1 and 2. Kernels from the backcross ears were planted in the field, the resulting plants selfed, and the selfed ears scored for *pr* and Dappled (*Dap* = dominant aleurone mosaic, *dap* = wild type solid aleurone color). Approximately 50 kernels from each selfed ear were grown to seedling stage in a cold sandbench (18 C) and the seedlings scored for *v2* and *v12*. The following linkage relationships were established: *pr*-28.7-*Dap*-14.7-*v2* and *pr*-5.6-*v12*-24.2-*Dap*. These data are consistent with the

Table 1. Three-point linkage data for *pr-Dap--v2*.

Testcross: (Pr Dap V2 / pr dap v2) X Pr Pr dap dap V2 V2

Reg.	Phenotype	No.	Totals
0	pr + v2	107	
	+ Dap +	87	194
1	pr Dap +	53	
	+ + v2	38	91
2	pr + +	17	
	+ Dap v2	27	44
1+2	pr Dap v2	3	
	+ + +	2	5

% recombination pr -- Dap = 28.7 ± 2.5

% recombination Dap -- v2 = 14.7 ± 1.9

Table 2. Three-point linkage data for *pr-v12-Dap*.

Testcross: (Pr V12 Dap / pr v12 dap) X Pr Pr V12 V12 dap dap

Reg.	Phenotype	No.	Totals
0	pr v12 +	57	
	+ + Dap	57	114
1	pr + Dap	4	
	+ v12 +	4	8
2	pr v12 Dap	16	
	+ + +	22	38
1+2	pr + +	0	
	+ v12 Dap	1	1

% recombination pr -- v12 = 5.6 ± 1.8

% recombination v12 -- Dap = 24.2 ± 3.4

known linkage relationships between *pr*, *v12*, and *v2*, and would place Dappled approximately 29 cM distal to *pr* on the linkage map of chromosome 5, very close to *Got2*. The data also suggest the placement of *v2* 43 cM distal to *pr*, which would move *v2* to a new map position of 110 from its previous position of 107. The suggested map revision is:

Dap

--*pr*----------*Got2*----------*v2*--

67 96 110

New dull and sugary-2 mutants from Mutator populations

--Philip S. Stinard

We are continuing to characterize new kernel mutants with sugary-translucent phenotype arising from our Mutator selfing blocks and other *Mu* sources. Over forty independent mutants are in the process of allele testing and mapping. Alleles of *ae*, *du*, *su2*, and *et* have been identified. The alleles of *ae* have been described in previous reports (MNL 62:14; MNL 63:8-9). The *et* alleles are described elsewhere in this News Letter. This report summarizes the *du* and *su2* alleles which we have identified (Table 1). The mutants *du-Mu1* and *du-Mu3* share a common progenitor *Mu2* plant (86-8319-4), yet they arose independently in two different generations of outcrossing, suggesting, perhaps, a "hot spot" of Mutator activity on one of the homologues of chromosome 10 originating from the *Mu2* parent.

Table 1. New *du* and *su2* mutants from Mutator populations.

Mutant Designation	Former Designation	Source	Source Description
du-Mu1	du*-2197	87-2197-9@	B73 Mu Loss/Mu2
du-Mu2	du*-2339	87-2339-2@	Mu16 per ae
du-Mu3	du*-2649	87-88-2649-11@	(B73 Mu Loss/Mu2) O.C.
du-Mu4	su-sh*-2370	87-2370-20@	Mu1/A632
su2-Mu1	su-sh*-3389	80-3389-4@	Mu Striped O.C. (from M. Freeling)

Location of a putative regulator of Mutator-induced mutability of *a1* on chromosome 2

--Donald S. Robertson and Philip S. Stinard

In a soon to be published paper in Developmental Genetics, we present extensive evidence that in some stocks of *a1-Mum2* and *a1-Mum3*, a regulator element of the somatic mutability of these mutable alleles may be present. If a regulator is involved, it should be possible to map it. In an endeavor to accomplish this, mutable kernels were planted from ears of the cross *a1-Mum/a1 sh2* x *a1 sh2*, in which half of the plump kernels were mutable and half were stable. If a single regulator gene is present, as the 1:1 ratio would suggest, the mutable seeds will be heterozygous for the *a1-Mum* allele and the regulator. These plants were crossed to an extensive series of waxy (*wx*) marked chromosome nine translocations. The F1 plants from these crosses were outcrossed as males to an *a1 a1 wx wx* tester stock. If linkage is present between *wx* and a regulator of mutability there should be a surplus of mutable kernels in the starchy (*Wx*) class and a deficiency of mutable kernels in the *wx* class. Table 1 shows the results of crosses of *a1-Mum3* with *wx* T2-9d. Cross number 1 was made in the '88-'89 winter nursery. The results were suggestive of linkage but the classes are not balanced

Table 1. Results of testcrosses of *Wx a1-Mum3 R*/wx* T2-9d-* plants with *a1 a1 wx wx* - -.

Cross No.	Parental		Crossover			
	Wx mutable	*wx* stable	*Wx* stable	*wx* mutable	Totals	% c.o.
#1						
88-89-9522-6	32	56	28	4		
8522-7						26.67
Totals	88		32	120		
#2						
89-6253-2	90	92	63	2		
5253-3						26.32
Totals	182		65		247	
#3						
89-6255-6	78	83	8	16		
5254-4						14.91
Totals	161		24		185	

*R = Symbol for putative regulator, - = absence of regulator.

(the imbalance is significant at the 1% level). The imbalance is due to an excess of stable kernels in both the *Wx* and *wx* classes. Methylation of the *Mu* element inserted at a locus is known to result in loss of mutability. If methylation of the *Mu* element was occurring in some kernels of this cross, the observed excess of stable kernels would be expected. (It also is possible that methylation of the putative regulator could result in a loss of its function and as a consequence somatic mutability would cease.) These results were encouraging enough to make additional testcrosses in the 1989 summer nursery (the second and third crosses, Table 1). The second cross has balanced parental classes but a large excess of stables in the *Wx* crossover class. This is not the only imbalance in this cross since the combined *Wx* classes make up 61.94 percent of the progeny (these imbalances are significant at the 1% level.). It has been frequently observed that in heterozygous plants the *wx* allele shows reduced transmission through the male. A combination of some *Mu* element modification and reduced *wx* transmission could explain the results of the second cross.

Cross number three has statistically balanced classes and gives a value of 14.91 percent recombination between *wx* and the putative regulator. Because the breakpoint of T2-9d in chromosome two is 2L.83, and because the regulator did not show linkage with other *wx* translocations, these data would suggest that in this *a1-Mum2* stock there is one regulator (autonomous element?) in the long arm of chromosome two.

Additional information on Mutator-induced deletions involving the *yg2* locus

--Donald S. Robertson and Philip S. Stinard

Robertson and Stinard (Genetics 115:353-361, 1987) described 12 deletions in the short arm of chromosome nine involving the *yg2* locus. Additional deletions of this region, which when homozygous result in the *wd* phenotype, were described by us in 1988 (MNL 62:24-25). The data from these reports demonstrated that in Mutator stocks, deletions can occur that range in size from small fully male and female transmissible deletions, to larger ones that are fully female transmissible but are not transmitted through the male, to the largest ones that have no male transmission and reduced female transmission.

This last summer, we selected four of the largest deletions reported in our 1987 paper (116-10, 104-7, 117-5, 107-1) to test against *sh1*, *bz1* and *wx* to determine if any of these loci were within the deleted regions. Plants known to be heterozygous for the deletions were tested. To produce these, plants of the genotype *Mu-del/+* were pollinated by *yg2* plants. The yellow-green plants from these crosses are deletion heterozygotes. The original *Mu-del/+* stock as well as the *yg2* male parents carried, but were not homozygous for, *sh1*, *bz1*, and *wx*. The constitution of the *c1* alleles was unknown in these stocks. Thus, we could not test for this locus with these deletions. Non-shrunken, starchy kernels with purple aleurone or colorless aleurone from the above crosses were sown and the resulting plants reciprocally crossed with a *C1 C1 sh1 sh1 bz1 bz1 wx wx* tester stock (Table 1). Because none of the deletions are male transmissible, the predominance of bronze-shrunken kernels when *Mu-del/+* stocks are crossed as males indicates that *sh1* and *bz1* were carried on the non-deleted homologue. The heterozygous deletion parent plants must have been of the genotype *Mu-del (C1) Sh1 Bz1 Wx/(C1) sh1 bz1 wx*. The occurrence of a few purple kernels in the outcrosses of all these deletion stocks is expected if the proximal ends of the deletions fall short of the *bz1* region and if a crossover has occurred between the deletion and the *bz1* locus. Thus, the deletions do not involve the *bz1* locus. The observation of crossovers in the *bz1-wx* region confirms this conclusion. Because all the deletions also have crossovers in the *c1-sh1* region, the *sh1* locus also is not involved in these deletions. The possibility exists, however, that the *c1* locus might be involved for three of these deletions (104-7, 117-5, 107-1). The latest map distance for the *c1-sh1* region is 3 centiMorgans and between *c1* and *bz1* is 5 cM. Three of the deletions map within less than 1.5

cM of *bz1*. On the basis of mapping data alone these three deletions would be expected to have included the *sh1* locus. But from the data above it is known that this is not so. Thus, heterozygosity for these deletions must be reducing crossing over in this region. If this is so, it probably is not wise to speculate, on the basis of crossover data alone, whether or not the *c1* locus is involved in these deletions. The fourth deletion (116-10) probably falls far short of the *c1* locus because of the observed 7% crossing over between the deletion and *bz1*.

Table 1. Results from crosses of plants of the genotype *Mu--del* (*C* or *c*) *Sh Bz Wx*/+ (*C* or *c*) *sh bz wx* with *C sh bz wx*.

Cross	Phenotypic classes in the male outcross progeny: purple	sh	bz	C.O. in the bz to wx region: yes or no	C.O. in the c to sh region: yes or no	Predominant phenotypic classes found on ears of heterozygous deletion parent*
Deletion 104-7						
89-6102-2 5102-2	2	198		yes	yes	Pl, sh-bz
89-6108-8 5102-3	5	359		yes	no	Pl, sh-bz
89-6112-3 5102-5	3	107		yes	no	Pl, sh-bz
89-6107-6 5103-3	1	49		yes	no	Pl, sh-bz
89-6105-3 5104-5	3	238		yes	no	
Total 14 + 951 = 965, % Bz = 1.45%						
Deletion 116-10						
89-6110-3	16	214		yes	yes	Pl, sh-bz
Total 16 + 214 = 230, % Bz = 6.96%						
Deletion 117-5						
89-6111-4 5111-1	1	138		yes	no	Pl, bz (homo Sh)
89-6111-5 5111-2	0	118		yes	no	Pl, sh-bz
89-6106-7 5111-3	2	209		yes	yes	Pl, sh-bz
89-6107-2 5112-2	3	123		yes	no	Pl, sh-bz
89-6111-1 5112-3	5	261		yes?	?	Pl, sh-bz (homo Wx)
89-6113-1 5113-1	2	125		yes	no	Pl, sh-bz
89-6110-2 5113-4	6	367		yes	yes	Pl, sh-bz
Total 19 + 1,341 = 1,360, % Bz = 1.40%						
Deletion 107-1						
89-6103-4 5104-1	0	128		yes	no	Pl, sh-bz
89-6102-1 5104-3	1	259		yes	no	Pl, sh-bz
89-6105-1 5104-4	0	223		yes	no	Pl, sh-bz
89-6116-2 5105-1	3	377		yes	yes	Pl, sh-bz
89-6102-3 5105-5	0	131		yes	no	Pl, sh-bz
Total 4 + 1,118 = 1,122, % Bz 0.36%						

*Pl - purple plump (*Sh*) kernels, *sh-bz* = double recessive shrunken bronze. Rare crossovers in the *sh-bz* region were observed. Kernels were not classified for waxy.

On the basis of previous tests (Genetics 115:353-361, 1987), deletion 116-10 was suggested to be the smallest of the four deletions involved in this report, while 107-1 was thought to be the largest. The crossover data in Table 1 confirm the previous conclusions. The crossover data indicate that the other two deletions are intermediate in size and probably are about the same length.

Tests of Mutator-induced events involving the *Bf1* locus on the long arm of chromosome 9

--Donald S. Robertson and Philip S. Stinard

In the 1988 News Letter, we described test results that suggested that the Mutator system was capable of inducing deletions in the region of the *Bf1* locus (MNL 62:25-26). Plants heterozygous for the putative deletions showed reduced male transmission of the deleted homologue through the pollen and full or near full transmission through the female.

Thirteen of these putative deletion stocks were tested to determine if they uncovered the *bm4* locus, which is distal to *Bf1*. *Bf1* seedlings (putative genotype = *Bf1-Mu(del)/Bf1*) from the *Bf1-Mu(del)*/+ x *Bf1 Bf1* crosses reported in the 1988 News Letter (Tables 1 & 2) were transplanted to the field, and the mature plants were pollinated by pollen from mature *bm4* plants. If a putative deletion extends as far as the *bm4* locus, brown midrib plants should be observed in the progeny of the cross in approximately the frequency reported for *Bf1* seedlings in the female crosses in the 1988 article.

Only two of the 13 putative deletions gave results that suggested that *bm4* might be uncovered by the deletion (i.e., 044-4, 046-6, Table 1). However, the frequency of *bm4* plants is much below the expected frequency. Of the 9 crosses involving plants heterozygous for the putative 044-4 deletion, two did not segregate for *bm4* plants. The percentage of *bm4* plants in the 7 progeny that did segregate was 15.7%, which is considerably less than the 37.7% female transmission observed in the 1988 tests. The heterozygous deletion 044-4 plant had semisterile pollen in which the abortive grains were partly filled with starch. Pollen for 15 of the 18 segregating *bm4* plants was scored

Table 1. The results of tests to determine if putative *Mu*-induced 9L deletions uncover *bm4*. Testcross: *Bf1-Mu(del)*/+ x *bm4 bm4*.

Deletion	Results of previous tests on Bf1 transmission (MNL 62:25-26, 1988)*: % Bf through ♀	% Bf through ♂	1989 tests: No. of crosses scored	Total No. of plants scored	No. of bm4 plants	% bm4 plants
548-1	52.7	30.8	11	164	0	-
547-6	56.7	32.9	11	184	0	-
044-4	37.7	2.0	9	146	18	12.3
[No. of crosses seg. bm4 = 7 (N=115), % bm4 in the seg. crosses = 15.7%]						
544-5	49.3	30.6	10	167	0	-
048-3	59.3	28.4	7	103	0	-
544-9	41.5	25.0	12	205	0	-
546-5	46.8	32.0	9	162	0	-
045-3	36.8	25.3	1	15	0	-
050-8	33.0	15.8	8	128	0	-
046-6	46.1	27.0	5	77	1	1.3
[No. of crosses seg. bm4 = 1 (N=15), % bm4 in the seg. cross = 6.7%]						
045-8	51.9	40.0	6	103	0	-
047-7	52.8	14.3	5	85	0	-
547-5	48.4	31.2	4	72	0	-

*Testcross: Bf1-Mu(del)/+ x Bf1 Bf1

and each had semisterile pollen with abortive partly filled pollen grains. In addition, all segregating brown midrib plants were shorter than their green siblings. Thus, the *bm4* plants would seem to be heterozygous for a deletion that involves the *Bf1* and *bm4* loci. But why the reduced frequency of the deletion plants in these crosses? Perhaps the hypoploid *Bf1 bm4* condition is not fully viable. If so there should be a reduced stand in these 9 progenies. From each cross 20 kernels were planted. For the 9 crosses of deletion 044-4 scored, the following numbers of plants were observed with the number of *bm4* indicated in parentheses: 12(1), 15 (2), 14, 15 (1), 19 (2), 17 (4), 19 (5), 18 (3), 17. If all the missing plants are assumed to be *Bf1 bm4* hypoploids that did not survive (an assumption that very likely is not true), the frequency of hypoploids (heterozygous deletion plants) would be 28.9%, a percentage of transmission 8.8% less than the 37.7% transmission frequency observed in 1988 when plants carrying this deletion were crossed as females. However, because some of the plants that did not survive in these families probably were normal, 28.9% is likely an over-estimate of the frequency of hypoploid *Bf1 bm4* kernels produced by this deletion.

In the five families of deletion 046-6 grown, only one short *bm4* plant was found (1.30%). If it is again assumed that the kernels that did not produce plants were hypoploid for a *Bf1 bm4* deletion, the percentage of *Bf1-Mu(del)/bm4* kernels would be 24.0%, which is far short of 46.1% female transmission frequency of this deletion in the 1988 tests.

Why the lower than expected frequency of *bm4* plants in the crosses with these two deletions? Perhaps failure of all *Bf1-Mu(del)/bm4* kernels to germinate or the inability of plants of that genotype to survive can explain the deficiency for deletion 044-4. In this case, the deficiency could be due to chance alone. In the case of deletion 046-6, however, such an explanation seems unlikely. Perhaps the one *bm4* plant observed was due to a mutation of the wild type allele in what would otherwise have been a *+/bm4* sibling, or a loss of the chromosome 9 homologue with the wild type allele, or at least a segment of it involving the allele in such a plant. The *bm4* plant had normal pollen, which is not expected if this plant was monosomic for chromosome 9 or heterozygous for a deletion involving *Bf1* and *bm4*. Previous work with reciprocal translocations has shown that plants heterozygous for terminal *Bf1-bm4* deletions as a result of the functioning of duplicate-deficient elements of reciprocal translocations in eggs have semi-sterile pollen, with the abortive grains being partly filled (similar to that observed for deletion 044-4). Thus, it is unlikely that the *bm4* plant observed in the crosses of deletion 046-6 is due to hypoploidy for this deletion.

In summary, out of 13 putative deletions involving *Bf1*, only one seems to involve the *bm4* locus. Thus most of the remaining 12 are probably not terminal deletions. The 044-4 deletion could be terminal, but just because it seems to include the *bm4* locus does not necessarily mean that it does.

In 1988, 10 kernels each from crosses of 100 different putative deletion events involving the *Bf1* locus were sown. In each cross, half of the kernels would be expected to be of the putative genotype *Bf1-Mu(del)/+* and half *Bf1/+*. All plants were self-pollinated and scored for the segregation of *Bf1* seedlings. The *Bf1/+* plants would segregate 3 normal to 1 *Bf1* seedlings, while heterozygous deletion plants, if they exist, might have a reduced *Bf1* frequency if the deletion is not completely selected against through the pollen, or no *Bf1* seedlings at all if there is very rare or no male transmission. Some of the *Mu*-induced *Bf1* events are likely to be *Bf1* mutations. In families with such mutations, all plants would segregate 3 wildtype:1 *Bf1*. Some of the seedlings selected from the original screening as ones that carried *Mu*-induced events at the *Bf1* locus were of doubtful classification. They had phenotypes such as weak zebra patterns of blue fluorescence or sectors of blue fluorescent tissue. If the phenotypes of such plants were not due to genetic events involving the *Bf1* gene (pseudo *Bf1* event), some of the outcross plants that were self-pollinated also would not segregate for *Bf1*. In summary, all families would be expected to have half of the selfed progenies segregating 3:1 for *Bf1* seedlings. For the other half of the selfed progenies there would be expected to be families which segregated 3:1 for *Bf1* (putative small deletions or *Bf1* mutations), less than 25% *Bf1* seedlings but not 0% (putative deletions with poor pollen transmission), or no *Bf1* seedlings segregating (putative deletions not transmitted through the pollen, or pseudo *Bf1* events). Table 2 summarizes the results of these tests.

Table 2. Summary of the results of the selfing tests of stocks carrying putative *Mu*-induced deletions of the *Bf1* region of chromosome 9.*

Families according to *Bf* segregations					
10-20%	<10% >0%	<10% & 0%	0%	Low %	All 25%
23	4	61	4	6	2

*Most families had some selfed ears that segregated in 3:1 ratios (note 6 exceptions, next to last entry).

Last summer, 10 kernels from each of 38 selfed ears that did not segregate for *Bf1* seedlings were sown and the resulting plants reciprocally crossed to *Bf1 Bf1* stocks (Table 3). Fifteen of the original *Bf1* events turned out not to be transmitted (i.e., no *Bf1* seedlings were observed in these tests). Some of these were expected because the classification of the original *Bf1* seedling was doubtful (e.g., pseudo *Bf1*). Most, however, were originally classified as good *Bf1* seedlings. The question is why *Bf1* is now lost. At least three explanations are possible: 1) The original mutational event was an early somatic event that did not involve the ear lineage. 2) Methylation of the element at the *Bf1* locus has occurred and the mutant phenotype is not observed (See Martienssen et al., EMBO J. 8:1633-1639, 1989, for a similar phenomenon with a *Mu*-induced mutant of *hcf-106*.) 3) The transmission or viability of the original mutant is so poor that it has been lost. Twelve of the mutants have equal frequencies of *Bf1* seedlings in the male and female outcrosses. Only three of these have 1:1 ratios (047-8, 049-2, 5333-9). The rest have equal male and female transmission frequencies but with less than 50% *Bf1* seedlings (e.g., 049-5 with a *Bf1* frequency of 39.92% in the female cross and 39.72% in the male cross). There is

no indication of deletions being involved in these lines. These mutant alleles, however, do seem to either have lowered male and female transmission or are partially lethal in zygote or embryo. Three mutants (046-2, 049-4, 544-3) had good 1:1 ratios when crossed as females and significantly less than 1:1 ratios when crossed as males. Yet the frequencies of *Bf1* seedlings in female and male crosses were not significantly different. A very small deletion may be involved in these. Eight of the mutants have frequencies of *Bf1* seedlings in the male cross that are significantly less than 50% *Bf1* and also have frequencies of *Bf1* seedlings in the male cross that are significantly less than those in the female crosses. These are likely candidates for being deletions. Three of these (045-1, 049-7, 545-6) seem to have reduced female transmission as well because the frequencies of *Bf1* seedlings in the female crosses are significantly less than 50%. These three could be larger deletions that are reduced in both male and female transmission, while the remaining 5 are smaller deletions with only reduced male transmission.

Table 3. Reciprocal crosses of plants from the selfed progeny of heterozygous plants with putative *Mu*-induced *Bf1* deletions.

1989 Family No.	Putative Deletion	Seedling phenotype[a] ♀ +	♀ Bf1	♀ % Bf1[a]	♂ +	♂ Bf	♂ % Bf1	Contingency X^2_{2df}
5001	044-1	All	0	0.0	All	0	0.0	
5002	044-2	78	45	36.85**	42	14	25.0**	1.8425
5003	044-3	64	22	25.58**	125	29	18.83**	1.1263
5004	044-5	All	0	0.00	All	0	0.00	
5005	044-7	All	0	0.00	All	0	0.00	
5006	044-8	All	0	0.00	All	0	0.00	
5007	045-1	73	47	39.17**	63	9	12.50*	14.2252**
5008	045-2	All	0	0.00	All	0	0.00	
5009	046-2	104	97	48.26	66	40	37.74**	2.6986
5010	046-8	All	0	0.00	All	0	0.00	
5011	046-9	8	8	50.00**	46	11	19.30**	4.6257*
5012	047-1	131	103	44.02**	181	19	9.5**	60.9708**
5013	047-4	All	0	0.00	All	0	0.00	
5014	047-8	16	15	48.39	23	24	51.06	0.0535
5015	047-9	All	0	0.00	All	0	0.00	
5016	047-11	All	0	0.00	All	0	0.00	
5017	048-1	All	0	0.00	All	0	0.00	
5018	048-6	74	66	47.14**	125	0	0.00**	94.9675**
5019	048-10	72	45	38.46**	108	50	31.65**	1.0961
5020	049-1	All	0	0.00	All	0	0.00	
5021	049-2	82	55	40.15	46	41	47.13**	0.7949
5022	049-4	102	88	46.32**	46	27	36.99**	1.8835
5023	049-5	96	62	39.92**	85	56	39.72**	0.0012**
5024	049-7	98	73	42.69**	135	18	11.76**	36.7195**
5025	050-4	46	21	31.34**	237	74	23.79**	1.2925
5026	050-1	All	0	0.00	All	0	0.00	
5027	050-5	75	57	43.18**	31	21	40.38**	0.0324
5028	050-7	41	21	33.87	97	42	33.56	0.1234
5029	5333-9	11	11	50.00	21	15	41.67	0.3829
5030	5334-7	All	0	0.00	All	0	0.00	
5031	544-1	All	0	0.00	All	0	0.00	
5032	544-2	All	0	0.00	All	0	0.00	
5033	544-3	56	59	51.30**	96	71	42.52**	1.7784
5034	544-4	124	84	40.38**	227	113	33.24**	2.5626
5035	544-7	81	85	51.12**	146	38	20.65**	34.4126**
5036	545-6	104	74	41.57**	161	29	15.26**	30.2710**
5037	545-7	89	78	46.71**	132	26	16.46**	32.7674**
5038	048-7	67	17	20.24**	67	12	15.19**	0.4062

*Significant difference at the 5% level.
**Significant difference at the 1% level.

[a]Under seedling phenotypes the significant X^2 difference give a measure of the significance of the deviation from the 1:1 ratio for +:Bf1 seedlings in each cross. The contingency X^2 measures the significance of the difference between the male and female ratios in each set of reciprocal crosses.

One interesting fact should not be lost sight of in these tests. The material grown for these reciprocal tests came from selfed ears, and the seedling tests of the kernels from these ears did not segregate for *Bf1* seedlings. Yet in many of the reciprocal crosses to homozygous *Bf1* stocks, significant numbers of *Bf1* seedlings are observed. In all families where *Bf1* seedlings were observed, some of the reciprocal crosses gave no *Bf1* seedlings, as would be expected because the plants that were selfed were heterozygous for the wildtype allele at the *Bf1* locus. In all the reciprocal tests only one was found that had all *Bf1* seedlings expected of a homozygous, *Bf1-Mu* plant. Thus, in spite of the fact that the reciprocal tests indicate, for most of the crosses in Table 3, that *Bf1* is readily transmissible through both male and female, homozygous *Bf1* parental plants are not or are only very rarely observed. Why? For those cases where there are indications that a deletion is involved, it is very likely the deletion is lethal in the homozygous condition. In the other cases, the *Mu*-induced events involving the *Bf1* region of the chromosome might result in zygotic or kernel lethals.

Some anomalies associated with Mutator-induced events involving the *Bf1* locus

--Donald S. Robertson and Philip S. Stinard

Some of the results reported in the previous paper are suggestive that Mutator-induced events involving the *Bf1* locus can produce unexpected phenomena (e.g., disappearing mutants). In this report several additional anomalies will be described.

In the 1988 News Letter (MNL 62:25-26), we reported that some putative *Mu*-induced deletion heterozygotes (*Bf1-Mu(del)/+*), when pollinated by TB-9Lc, produced a few *Bf1* seedlings, which were assumed to be homozygous for the putative deleted segment but hypoploid for the rest of the translocated long arm of chromosome nine. These plants were shorter than normal plants, had narrow leaves, were late maturing and had poor tassel development. Some did not extrude anthers and those that did produced little, if any, viable pollen. *Bf1* seedlings from the cross of the same putative deletion stocks by TB-9Lc(male) were transplanted to the field last summer. *Bf1* seedlings from nine other putative *Mu*-induced deletion stocks pollinated by TB-9Lc also were transplanted. In addition, *Bf1* seedlings from the cross of *Bf1 Bf1 Bm4 Bm4* and *Bf1 Bf1 bm4 bm4* plants by TB-9Lc heterozygotes were transplanted as controls. The former *Bf1* stock was brought to Iowa State University from the California Institute of Technology in 1957 and the latter stock was obtained from the Maize Genetic Cooperation a few years ago. All of the putative deletion TB hypoploids showed the characteristics described above. However, the hypoploid controls from the I.S.U. *Bf1* stock also had the same phenotype. Thus, these traits are a result of hypoploidy per se in the I.S.U. genetic background and have little or nothing to do with the possibility that the putative *Bf1-Mu(del)* crosses were possibly homozygous for a deleted segment involving the *Bf1* locus. The hypoploids from the cross of the Coop *Bf1 Bf1 bm4 bm4* stock had much broader leaves and were taller than the I.S.U. hypoploids. They, however, had sterile pollen and brown midribs. Thus, genetic background has a strong influence on the hypoploid phenotype of TB-9Lc.

The TB cross of one putative deletion (546-5) produced two putative *Bf1-Mu(del)* hypoploid plants. Both of these were smaller than other *Bf1-Mu(del)* hypoploids, but all other characteristics were the same except these plants had brown midribs. There were putative hypoploid *Bf1-Mu(del)* plants from three other TB-9Lc crosses of the

same *Bf1-Mu(del)* grown (8 plants total) and none of these had brown midribs. This putative deletion was also tested for its ability to uncover the *bm4* locus (See previous report). Plants from eight crosses with *bm4* stocks were grown, totaling 144 plants. None of these had brown midribs.

A second anomaly was found in analyzing the results from the reciprocal crosses of progeny plants from selfs of *Bf1-Mu(del)/+* plants that did not yield any blue fluorescent seedlings (See previous report for details).

Before considering the anomalous results, a brief reminder of the phenotype of *Bf1* mutants is in order. *Bf1* is expressed as a recessive trait in the seedling. Only *Bf1 Bf1* seedlings are blue fluorescent. However, anthers will fluoresce blue when only one *Bf1* allele is present. Thus, in the tassel, *Bf1* expression is dominant.

All plants of the selfed progenies that were reciprocally crossed in the previous report, were scored for anther fluorescence. In most of these progenies, there were plants with blue fluorescent anthers and ones without. In most families, reciprocal crosses to *Bf1 Bf1* plants of those without fluorescent anthers did not segregate for blue fluorescent seedlings, while those with fluorescent anthers segregated for fluorescent seedlings. However, for three families involving progeny of the independent putative deletions 047-4, 047-9 and 047-11, plants with blue fluorescent anthers did not segregate for blue fluorescent seedlings. For 047-4 there were 6 plants tested, for 047-9, 2 plants and for 047-11, 4 plants. There was one exception for the latter stock. One plant gave no fluorescent seedlings when the fluorescent anther plant was crossed as a female but produced 2 very weak and small fluorescent seedlings when crossed as a male.

It seems that one or more of the *Mu*-induced *Bf1* events has generated a mutant allele that only expresses the dominant anther phenotype. Yet that can not be the whole story, for this mutant was first selected as a blue fluorescent seedling from an isolation plot in which the female plants were + + *Mu* and the pollen source was *Bf1 Bf1*. Thus, the original plants of these stocks were of the genotype *Bf1-Mu(del)/Bf1*, and fluoresced blue as seedlings. These plants were crossed as females to a standard (+ +) line, and it was the progenies of these crosses that were self-pollinated, and from which the ears that did not segregate blue fluorescent seedlings were selected. Thus, these mutants originally expressed fluorescence in the seedling but have lost this ability, while retaining the ability to produce anther fluorescence. Could this be a phenomenon caused by the methylation of the *Mu* element at this locus similar to that observed by Martienssen et al. for the *Mu*-induced *hcf106* mutant (EMBO J. 8:1633-1639, 1989)? Perhaps the *Mu* elements in these mutants are differentially methylated in the seedling and anthers. The seedlings might have methylated elements and thus the wild type gene expression, while in the anther the elements might be unmethylated, resulting in the mutant phenotype. There is the remote possibility that the observed anther fluorescence is not due to the presence of *Bf1* but rather to *bf2*, which would explain the lack of fluorescence in the seedlings of the reciprocal crosses. However, many, but not all, of the original *Bf1-Mu/Bf1* plants isolated from the progeny of the isolation plot were crossed with *bf2 bf2* to make sure that this locus was not involved. Seventy-one plants were tested and none carried *bf2*. The 047-9 stock was one of those that tested negative with *bf2*. Mutants 047-4 and 047-11 were not tested but will be this summer. However, it is very unlikely that they carry *bf2* in light of the 71 negative tests of plants from this isolation plot. Also, because the plants with the blue fluorescent anthers in these three selfed progenies came from selfed ears that did not segregate blue fluorescent seedlings, it is very unlikely that *bf2* is involved because *bf2 bf2* seedlings would be blue fluorescent.

The third anomaly associated with *Mu*-induced alterations involving the *Bf1* locus involves the selfed progeny of another *Bf1-Mu(del)/+* plant (putative deletion 544-3). The seedlings of a selfed ear did not segregate for blue fluorescent seedlings. Ten mature plants were obtained from this selfed ear. Eight of these plants did not have fluorescent anthers, one had one anther with 2 fluorescent spots, and one had many anthers with fluorescent spots. Four of the plants with no fluorescence in the anthers, when reciprocally outcrossed to *Bf1 Bf1* plants, did not give fluorescent seedlings. Three plants without fluorescent anthers gave 50% fluorescent seedlings when crossed as both male and female. One plant without fluorescent anthers was outcrossed only as a male and gave only fluorescent seedlings. The plant with one anther with two fluorescent spots gave 50% fluorescent seedlings in reciprocal crosses, while the plant with many fluorescent spotted anthers produced only fluorescent seedlings in crosses in both directions. This situation seems to be just the opposite of the previous anomaly. The 544-3 allele seems to be one that only expresses in the seedling and weakly or not at all in the anthers. The anther expression would suggest that this mutant allele is mutable, in some plants at least. In others, mutability may not be expressed because of methylation. There is one last important aspect of this mutant to consider. The progeny of the self from which these plants were grown did not segregate for fluorescent seedlings! Yet the original isolate (*Bf1-Mu/Bf1*) was a fluorescent seedling and the reciprocal crosses of these tests gave fluorescent seedlings, which also are of the genotype *Bf1-Mu/Bf1*. This would suggest that the *Bf1-Mu* allele might be lethal when homozygous, yet two of the plants from the progeny of the self when outcrossed to *Bf1 Bf1* gave only *Bf1* seedlings, suggesting that homozygous *Bf1-Mu* plants were outcrossed. Thus, it seems likely that this particular *Bf1-Mu* allele does not give fluorescent seedlings when homozygous but does when heterozygous with the standard *Bf1* allele.

A test to separate housekeeping mutants from those unique to kernel development

--Donald S. Robertson and Mike Scanlon

We have been trying to map and clone Mutator-induced defective kernel mutants (*dek*'s). These mutants undoubtedly are of two classes: 1) Those that result from the mutation of housekeeping genes essential in all stages

of development, and 2) those that affect some step unique to kernel development but not essential to complete other stages of the life cycle.

One phase of our mapping work involved crossing the *dek* stocks by the series of B-A translocations. One of the *dek*'s placed in these tests was *dek*-1339*. The defective endosperm of this mutant is always underdeveloped. Sometimes the kernel can be almost an empty pericarp without a germ, while some kernels can be about one-third the size of normal. The endosperm of the latter mutant kernels is opaque, and etched. Occasionally the larger *dek* kernel will have a small germ. Some of the homozygous mutant kernels may be germless because the defective endosperm does not develop sufficiently to support embryo development.

This mutant was placed to the long arm of chromosome 10 with TB-10L19. The cross producing the positive test was +/*dek* x +/TB. Some of the plump kernels from this cross should have *dek*/- (hypoploid) germs, if the *dek* mutation does not affect a housekeeping locus and if its gene product is not essential for embryo development. Seventeen plants from plump kernels were scored for pollen sterility. Seven had semisterile pollen, expected of hypoploid plants. All semisterile ears were self-pollinated and scored for the presence of the *dek* phenotype. Half of the semisterile plants are expected to carry only the wild type allele and indeed three did. If a hypoploid plant is *dek*/-, only *dek* kernels would be expected. The other four semisterile plants gave ears completely devoid of any kernels. If a single dose of the *dek* allele will not permit kernel development, such ears would be expected. An alternative explanation for such empty ears would be that the *dek* mutants depend on the presence of normal kernels to augment the partial development seen in the *dek*'s on segregating ears. This could be by some manner of cross feeding by the adjacent normal kernels or could be due to cob changes necessary for kernel development that the *dek* kernels can not mobilize. The latter seems a likely explanation since the cobs were quite small and non-lignified.

The hypoploid test for kernel specific *dek*'s can only be utilized for those B-A translocations that produce hypoploid plants with sufficient vigor to permit self-pollination. Thus, probably most of the shorter translocations involving chromosomes 4 and shorter chromosomes will work, while most of the balance of the B-A translocations would not be usable in such tests.

Support for a hypothesis to explain the coincident activation of *Ac* and the origin of a *P-RR* allele

--Patrick S. Schnable

We have previously reported (MNL 60:5) on an *Ac* activation event in a single kernel which coincided with the origin of a *P-RR* allele. We hypothesized that a *P-WR* or *P-WW* allele present in our lines consists of a *P-RR* allele inactivated by the insertion of an *Ac* element which is itself inactive. Activation and subsequent transposition of the resident *Ac* element restored the *P-RR* allele to its functional mode. Linkage of the newly activated *Ac* to the *P* locus would support this hypothesis.

The plant derived from the kernel exhibiting *Ac* activation was crossed as shown. From this cross 135 colorless

C-I Bz Ds/C-I Bz Ds P-WR/P-WR X *C bz-m4/C bz P-RR Ac/P-WR*

(no *Ac*) and 156 variegated (with *Ac*) kernels were obtained. This is not significantly different than the 1:1 ratio expected if a single *Ac* element is segregating. To test the linkage of this *Ac* to the *P* locus plants from kernels with and without *Ac* were grown to maturity at which point the ears were scored for the presence of colored pericarp. 63 of 65 plants with *Ac* carried the *P-RR* allele. In contrast only 1 of 29 plants that did not receive *Ac* carried the *P-RR* allele. This yields a recombination value between *P-RR* and *Ac* of 3.2 cM with a standard error of 1.7. This tight linkage between the newly arisen *P-RR* allele and the newly activated *Ac* element supports our original hypothesis.

Mutator-tagging of developmental and physiological traits

--Brad Hedges and Patrick S. Schnable

Two approaches were taken to tagging genes affecting developmental and physiological pathways with *Mu* elements. Both approaches involved screening selfed families for the appearance of novel mutations. The selfed families used in the first approach were derived from plants carrying an active Mutator system and were a gift from Philip Stinard and Donald Robertson. The selfed families used in the second approach were derived from germinal revertants from the *bz-rcy824333y* state. In both approaches 15 to 30 kernels from each selfed family were field grown to maturity. All individuals within each family were screened for visible mutations several times during the growing season.

A large and diverse collection of putative mutations was obtained. As would be expected the most frequent aberrant phenotypic class consisted of small, weak plants. However, the collection also includes phosphorus and nitrogen deficiency mimics, disease mimics or susceptibilities, male and/or female steriles, and alterations in tassel morphology and placement (e.g., the inclusion of pistillate flowers, reduced and absent tassels); ear placement, number and morphology (e.g., the inclusion of staminate flowers, club or fasciated ears, silky and reduced ears); tiller number; leaf morphology and placement; and gross plant morphology (e.g., dwarfs and brachytics). Approximately 150 of these are being tested for heritability. Some are included in a crossing/selection program to reduce *Mu* element copy number.

Three new mutable alleles from a *Cy*-containing population

--Patrick S. Schnable

In an effort to trap *Cy* at a cloned gene two *a-m* alleles (*a-m895216* and *a-m895259*) and one *c-m* (*c-m895227*) have been isolated. Each of the gametes which gave rise to these mutations carried *Cy*.

Anther production by *Cg*-855621y*

--Patrick S. Schnable

Plants carrying the dominant *Cg*-855621y* mutation, which was isolated from *rcy/Cy* stocks, do not usually produce tassels or anthers under summer conditions in Ames. This summer for the first time we observed an anther on a plant grown in our Ames nursery. No anthers were produced under winter greenhouse conditions in Koln, West Germany. However, under winter conditions in Hawaii this mutant does sometimes produce an occasional anther.

Molecular evidence for *Mu*-element transposition in plants regenerated from an active Mutator callus line

--Martha James and Joan Stadler

Mutator activity was assessed in plants regenerated from an embryogenic callus line derived from the F1 of an A188/*Mu2* cross (88-646-8C). This line was determined to have an active Mutator system based on the generation of new mutants in outcross progeny of the *Mu2* parent, and by the lack of *Mu*-element modification in callus DNA. Regenerant progeny from this line displayed Mutator activity as well. Genetic studies showed that new mutations continued to arise in R3 and R4 plants and molecular analyses confirmed these findings. *Hin*dIII hybridization profiles of DNA from eight R2 plants revealed *Mu*-homologous restriction fragments in tissues of six plants which were not present in callus DNA. These novel fragments undoubtedly represent transpositions of *Mu* elements to new sites in the genomes of the regenerant progeny. Thus, molecular evidence substantiates the genetic findings that Mutator activity can be retained through a tissue culture phase.

The maintenance of Mutator activity during and after tissue culture may have interesting applications in the generation of useful variants. Linkage of mutagenesis with somaclonal variation has previously been explored with the incorporation of mutagenic agents in the culture medium. The high mutation rate seen in the Mutator system (30-50X the spontaneous level) and the ability of Mutator to generate mutants by insertion at virtually any maize locus could make this system a desirable tool for increasing culture-induced variation.

***Mu* elements as molecular markers for genomic stability in culture**

--Martha James and Joan Stadler

Somaclonal variation in plants regenerated from tissue culture is known to stem, in part, from chromosome instability. Reports have suggested that this instability, which can result in breakage and/or rearrangements, occurs in late-replicating DNA (heterochromatin) (Lee and Phillips, Annu. Rev. Plant Physiol. Plant Mol. Biol. 39:413-437, 1988). Little is known, however, about the effects of tissue culture on euchromatic, or active, regions of the genome.

Mu elements are present in approximately 10-70 copies in Mutator lines of maize. They have been shown to reside primarily in active regions of the genome and to exhibit a gene-specific insertion preference (Alleman and Freeling, Genetics 112:107-119, 1986; Bennetzen et al., UCLA Symp. Mol. Biol. 62:183-204, 1987). Thus, *Mu* elements may serve as useful molecular markers for those active portions of the genome in which they are found. We have used the internal *Mlu*I fragment of the *Mu1* element as a hybridization probe in Southern analyses of DNA from Mutator embryogenic callus tissues. The six callus lines examined were derived either from the F1's of H99/*Mu32* crosses or the self-pollinations of a *bz-Mum8* plant. Each of these lines contained approximately 20-40 *Mu* elements that were modified, as determined by the failure of the restriction enzyme *Hin*fI to cleave the DNA at sites located within *Mu*-element inverted repeats. The hybridization profiles of each line obtained by restriction digestion with various enzymes that cut outside *Mu1* and hybridization with the *Mu1* probe revealed restriction fragment uniformity and stability over approximately one year in culture (10-60 weeks). This stability suggests that gross rearrangements of regions of the genome occupied by *Mu* elements did not occur in culture. Because *Mu* elements are associated with active regions of DNA, stability of the active, or genic, portion of the genome during culture is implied.

Tissue-specific differences in *Mu*-element modification and restriction fragment profiles in plants regenerated from Mutator embryogenic callus lines

--Martha James and Joan Stadler

As part of a molecular analysis of Mutator activity in plants regenerated from tissue culture, comparisons were made of *Mu*-element modification and *Mu*-homologous restriction fragments among various plant tissues. Surprisingly, tissue-specific variations in both *Hin*fI-site modification and restriction fragment position were seen.

Mu-element modification was assessed by the failure of the restriction enzyme *Hin*fI to cleave DNA at sites located within *Mu*-element terminal inverted repeats. Southern analysis of *Hin*fI digests of DNA from H99/Mutator callus line 657-2B and various tissues of primary regenerants (R0) of this line revealed differences in *Mu*-element modification, with *Mu* elements in the immature cob of one R0 substantially undermethylated compared to those in husk tissue from the same plant. Primary regenerants of a *bz-Mum8* callus line also showed tissue-specific modification differences, although these differences were slight. For example, a *Mu*-homologous restriction fragment of approximately 1.3 kb, probably representing an unmodified *Mu* element, was present in DNA from immature cob and young shoot tissues of two primary regenerants. This fragment was absent in DNA from leaf tissues of both plants; however, a novel higher-molecular-weight fragment was present in these leaf DNA's.

Tissue-specific differences in *Mu*-element modification have at least four possible explanations. First, it is possible that regenerated plants in which variation was seen were of multicellular origin. Second, uncharacterized tissue- or stage-specific factors could have been responsible for pro-

tecting at least some of the *Mu* elements from modification by general cellular methylases. Third, position effects could have influenced *Mu*-element methylation in certain tissues. If *Mu* elements were adjacent to regions of DNA that were hypomethylated in specific tissues, these elements could have remained hypomethylated as well. Fourth, the lower level of *Mu*-element modification seen in specific tissues could have been due to the immaturity of those tissues. A mathematical model which predicts an increase in genomic methylation with tissue maturity lends support to this argument (V. Walbot, personal communication).

Southern analysis was also used to compare DNA from various tissues of R1 plants (first-generation regenerant progeny of H99/*Mu2* callus line 657-2B) that had been digested with restriction enzymes that cut outside *Mu1* (*Eco*RI, *Hin*dIII, and *Xba*I). Three of ten R1 plants examined showed restriction fragment polymorphisms among tissues. In each of two plants derived from the same primary regenerant (88-141-1 and 88-176-6), restriction fragments which were present in DNA from immature cob tissue were absent in DNA from leaf or husk tissue. In one of these plants (88-176-6), the reverse was also true. In a third plant (88-140-7), derived from a different primary regenerant, a restriction fragment that was present in DNA from immature cob and husk tissues was missing in DNA from the leaf. In each of these cases, all restriction fragments seen in the R1 plants were also present in the parental R0 plants. Thus, the observed polymorphisms were considered indicative of the absence of one or more *Mu* elements in a particular tissue relative to other tissues.

It is unlikely that restriction fragment differences among tissues can be traced to somaclonal variation. Although it is known that tissue culture can induce chromosomal rearrangements and deletions that could be reflected as altered restriction fragments, tissue-specific differences such as these have not been reported. We are exploring the possibility that these differences represent tissue-specific *Mu*-element excisions. Further studies of both *Mu*-element modification changes and restriction fragment polymorphisms among tissues are planned using plants which have not gone through a tissue culture phase to help eliminate either somaclonal variation or genetic chimerism as contributing factors.

Effectiveness of two restriction enzymes for detecting RFLPs in the Iowa Stiff Stalk Synthetic (BSSS) maize population

--E.A. Lee, M. Lee, K.R. Lamkey and W.L. Woodman

BSSS progenitor and derived inbred lines were examined at 58 RFLP loci with single digests of *Eco*RI and *Hin*dIII. In all groups of inbreds, a higher percentage of clones detected polymorphisms when tested with *Hin*dIII digests (Table 1). Also, the average size of the restriction fragments detected with *Hin*dIII seems to be lower molecular weight than those observed with *Eco*RI digests, suggesting a higher frequency of *Hin*dIII recognition sequences in BSSS germplasm.

Table 1. Percentage of polymorphic clones summed over inbred groups and restriction enzymes.

Inbred Group	Restriction Enzyme *Eco*RI	*Hin*dIII
Progenitors	90.4%	92.7%
Elites	75.0	89.1
Random	74.5	89.1

RFLP analysis of isogenic lines B14 and B14A

--E.A. Lee, M. Lee, and K.R. Lamkey

B14 and B14A are isogenic lines distinguished from each other by the greater degree of resistance to corn leaf rust (*Puccinia sorghi*) for B14A. B14A was developed through eight generations of backcrossing and selection for rust resistance using B14 and Cuzco as recurrent and donor parents, respectively. The objective was to transfer an allele for rust resistance at the *Rp1* locus from Cuzco to B14 and recover the agronomically desirable features of B14 (Russell, Crop Sci 5:95-96, 1965). Morphologically, B14 and B14A appear to be identical. Theoretically, B14A should contain 99.8% of the nuclear genome of B14.

The two lines were examined for RFLPs with 58 mapped clones in combination with three restriction enzymes, *Eco*RI, *Hin*dIII, and *Eco*RV. With *Eco*RI digests, 98.3% of the clones detected identical banding patterns for B14 and B14A; however, only 87.9% of the clones revealed identical patterns with *Hin*dIII digests of the two inbreds. Similar observations have been reported for other pairs of inbred lines isogenic for rust resistance (Beckman and Weck, MNL 62:107, 1988).

Nine RFLP clones detected polymorphisms between the two inbreds (Table 1). The clones have been mapped to chromosome one (3 clones), three (2), six (2), seven (1), and ten (1). The *Rp1* locus has been mapped to the short arm of chromosome ten (Russell and Hooker, Crop Sci 2:477-480, 1965); however, RFLP clones used in the study did not detect differences between B14 and B14A in that region.

Table 1. RFLP clone-enzyme combinations that detected differences between B14 and B14A.

Enzyme	Locus	Chromosome arm
*Hin*dIII	*UMC084*	1L
*Hin*dIII	*UMC106*	1L
*Hin*dIII	*UMC011*	1S
*Eco*RV	*UMC017*	3L
*Eco*RI	*UMC050*	3S
*Hin*dIII	*UMC046*	6L
*Hin*dIII	*UMC085*	6S
*Hin*dIII	*UMC012*	7S
*Hin*dIII	*UMC064*	10L

The presence of donor parent (or non-recurrent parent) *Hin*dIII RFLP patterns at a level greater than theoretical expectations may be attributable to several factors. The unexpected RFLP patterns could be linked to donor chromosome segments selected during backcrossing; possibly, they contain genes influencing the response to rust infection or the segments contain genes which enhance the agronomic performance of B14. Russell and Hooker (Crop Sci. 5:95-96, 1965) reported significant differences

for grain yield between B14 and B14A single-cross hybrids in rust-free environments. Mutations in B14 or B14A seem to be an unlikely source of the variation given the number of clones detecting polymorphisms. Pollen or seed contamination cannot be eliminated as sources of the variation until the donor parent has been examined for polymorphisms.

On the origin of the inbred line B52

--M. Lee, W.A. Russell, A.E. Melchinger, and W.L. Woodman

Inbred line B52 has attracted considerable attention by maize breeding programs because of its high level of resistance to sheath collar feeding and stalk tunnelling by second generation larvae of European corn borer (*Ostrinia nubilalis* H.). There have been numerous requests regarding the source population or origin of this line; unfortunately, such records do not exist. In this report, we would like to present what we do know about relationships to other inbred lines.

In 1952 Dr. G.F. Sprague obtained 382 lines of corn that were at various stages of selfing from a private breeder (Milton Robinson?) in southern Iowa. The source population(s) of the lines has not been established and we do not have further information concerning their source. These materials were grown in the nursery in 1952 at Ames, IA.

Inbred B52 was recognized in the S3 generation of selfing as having a vigorous plant with relatively good ear size and good grain. Evidently, its pollen shed at that time was not recognized as a problem. In particular, it was noted for an extremely hard stalk. In its first hybrid performance tests, it was not outstanding but must have been good enough so that it was not discarded. In Russell's 1958 field book, B52 was labeled for discard because of poor topcross (hybrid) performance but this was marked out and it was designated B52. Fortunately, in the same summer, the line was included in a test at Ankeny, IA (USDA Corn Insects Laboratory) where there was an evaluation for second generation European corn borer. It had the lowest count for stalk and shank cavities, which ranged from 0 to 8.4. Artificial infestation, 3 egg masses per plant, had been made during pollen-shedding stage of plant development. Evaluations in following years continued to show this high level of resistance for B52: at infestation levels of 600-700 larvae per plant, B52 has averaged 10-15 inches of stalk tunnelling compared to 30-35 inches for inbreds Mo17 and B73.

B52 has been examined for RFLP patterns with 82 mapped clones. In cluster and principal component analyses of the RFLP data, B52 has been more closely associated with Lancaster inbreds, particularly Oh43 types. RFLP mapping studies have been initiated to identify the B52 chromosome segments contributing to corn borer resistance and to facilitate transfer of these segments into elite inbred backgrounds.

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Developmental basis for the origin of polystichy

-- Marshall Sundberg and John Doebley

Although there is no consensus concerning the origin of the maize ear, there has been general agreement that the polystichous arrangement of the spikelet pairs, in both the ear and central tassel spike, was derived from the distichous condition via "twisting" of distichously arranged primordia. Such "twisting" has been viewed as a response to spatial constraints imposed by extreme telescoping of the primary inflorescence axis. This interpretation, first proposed by Collins (J. Agric. Res. 17:127-135, 1919), has been adopted by many subsequent authors. Collins' hypothesis was derived from observations of maize-teosinte hybrids. Based on ontogenetic studies of the early development of Argentine popcorn inflorescences, we propose an alternative mechanism for the evolutionary switch from the distichous to polystichous arrangement of spikelet pairs.

An examination of more than 50 developing tassels and ears of Argentine pop revealed that, in the majority of cases, early primordia are produced in two ranks by the apical meristem of the inflorescence. Figure 1 illustrates a young tassel primordium oriented such that one rank of initials (lateral primordia) is in face view on the distal end

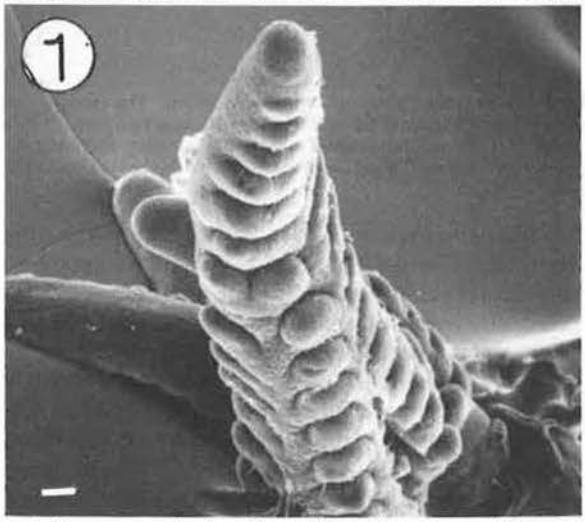

Figure 1. A young tassel primordium of Argentine popcorn. The specimen is oriented such that one rank of initials on the distal end of the axis is in face view. These initials are distichously arranged as would be found in teosinte or lateral branches of the maize tassel. Bar = 50 μm.

of the axis. These initials enlarge laterally, then bifurcate to form paired primordia. Such bifurcation is clearly visible near the middle of the axis in this figure. However, rather than initiating a pair of spikelets (one sessile, one pedicellate) as would be expected in teosinte inflorescences or the lateral tassel branches of maize, these paired primordia bifurcate a second time. The result of this second bifurcation is seen clearly in Figure 2, which illustrates an

ear primordium at a slightly later stage of development. Above the middle of this inflorescence, primordia are in two ranks; however, below the midpoint, there are four ranks (8 rows) of spikelet primordia as a result of the second bifurcation. Two of these ranks are visible and two additional ranks are out of view behind the axis. Thus, as a result of the second bifurcation, an inflorescence meristem that initially produced only two ranks of primordia (i. e., distichous) becomes one with four ranks of spikelet primordia (i. e., polystichous).

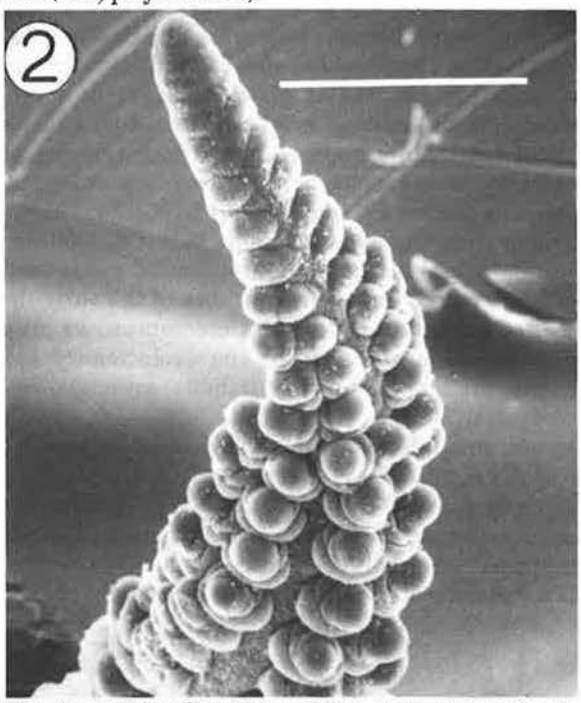

Figure 2. An ear primordium of Argentine popcorn. The specimen is oriented such that the plane of the initial bifurcation is in face view. The older (proximal) initials have undergone a second bifurcation resulting in four ranks of spikelet pairs. Two of these ranks are hidden behind the axis. Bar = 500μm.

It is also apparent in Figure 2 (although perhaps most clearly to the trained eye) that the ranks of spikelet primordia are not exactly opposite each other along the upper portion of the axis, but are shifted toward the abaxial side of the developing ear. Even at the stage when the inflorescences first become eight rowed, they are bilaterally symmetrical with the rows shifted toward the abaxial side of the axis (not shown). However, subsequent enlargement of the spikelets displaces the rows into a radially symmetric arrangement as illustrated by the polar view of a slightly older central tassel spike (Fig. 3). These observations are consistent with the derivation of the maize ear and central tassel spike from distichous, bilaterally symmetrical inflorescences such as those of teosinte.

From these observations, we have formulated a model for the origin of polystichy in maize. This model takes into account the fact that virtually all of the Andropogonoid grasses (the group to which maize belongs) have distichously arranged sessile-pedicellate pairs of spikelets. As such, the inflorescences of this enormous group of grasses must share a common developmental program by which the apical meristem first produces distichously arranged

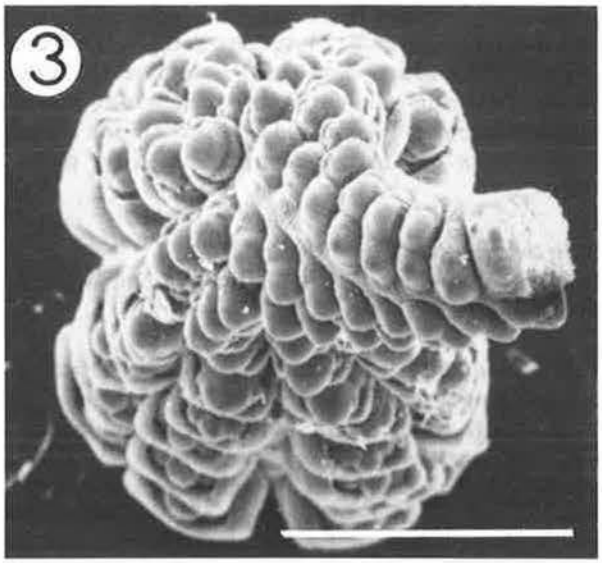

Figure 3. A polar view of a developing central tassel spike of Argentine popcorn. This specimen shows how enlargement of the spikelets displaces the rows into a radially symmetrical arrangement. Bar = 500 μm.

primordia, each of which bifurcates to produce paired (sessile-pedicellate) spikelet primordia. In this framework, the origin of polystichy in maize can be seen as a change in the timing of the developmental program controlling bifurcation of the initials. If the execution of this program is simply modified (prolonged) to allow one additional cycle of bifurcation, a four-ranked, eight-rowed inflorescence is produced, this the most primitive condition in cultivated maize. This is apparently what happens in Argentine pop. Thus, "twisting" as suggested by Collins would not be involved in the origin of polystichy, which is consistent with the fact that we see no evidence for twisting in the development of Argentine pop inflorescences. Other modifications of the program controlling bifurcation would be required to produce 10-, 12- or 14-rowed inflorescences. Ontogenetic analyses of maize varieties with 10- or higher-rowed inflorescences should provide insights into the nature of these modifications. Additional studies should also clarify the developmental basis of the production of inflorescence branches, which are also the products of bifurcation of the developing inflorescence primordia and thus also occur in pairs. We are currently exploring these issues and testing the validity of our model for the origin of polystichy.

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A potential novel approach for transformation: DUT (dessication-uptake technique). Evidence of transient expression in embryogenic structures and regenerated plants

---E. Lupotto and M. C. Lusardi

Genetic transformation in maize is one of the fundamental requirements for the direct introduction of desirable traits into this crop species. Although genetic trans-

formation of this plant has been addressed with several strategies, there is a general lack of effective systems to routinely transform this species. Direct injection into pro-embryogenic structures and the particle bombardment device, successfully used for transformation of *Brassica*, soybean and other crop species, are generally based on the availability of highly embryogenic tissues. Recently, it has also been reported that dried mature zygotic embryos of wheat can absorb and transiently express a reporter gene, by direct intake of the supplied DNA, thus apparently not needing a physical injection into them (Topfer et al., Plant Cell 1:133-139, 1989).

Our approach to transformation is essentially based on the use, as acceptor system, of highly embryogenic callus tissue of type 1 (compact and nodular), obtained from a particular F2 segregating population, which we refer to as WA1. This callus type is also characteristically produced in other monocots such as *Pennisetum*, *Panicum* and *Sorghum*, and its peculiarity relies on the continuous proliferation through embryogenesis at the upper surface. When 2,4-D is removed from the culture medium and the embryogenic tissue fragmented into 1 mm^2 pieces, in the first 10 days it develops further into embryogenic structures. Subsequently, these structures can be divided again in single germinating somatic embryos and regenerated into complete plantlets. Although the efficiency of this last step varies greatly depending on various factors of "maturation" of the tissues during transfer, it is possible to raise the efficiency of regeneration up to 30-60% of the isolated somatic embryos by selecting an appropriate timing of the regeneration step. The embryogenic structures in an early phase of development can be desiccated until 20-25% of their initial fresh weight and, when rehydrated and cultured again, they can be induced to proliferate and regenerate. WA1 plantlets are recovered with the same efficiency as reported for fresh non-desiccated tissue.

By using the materials previously described we have developed a possible method of transformation, herein referred to as DUT (desiccation-uptake technique of transformation). Although various plasmids carrying different reporter genes were used, most of the work was done with the plasmid pCGN778, kindly provided by Calgene, Davis, USA, containing a NPT-II chimaeric (neomycin phosphotransferase-II) reporter gene. The expression of the NPT-II gene was tested in callus and plant tissues either after SDS-PAGE electrophoresis of the extracts assayed according to Reiss (Reiss et al., Gene 30:211-218, 1984), or through the protein dot-blot assay performed according to McDonnell (McDonnell et al., Plant Mol. Biol. Rep. 5:380-386, 1987). In the case of dot-blot assays, we included as an additional step an incubation in 0.1% Proteinase-K, 1% SDS, at 65 C for 45 min for a better cleaning of the false positive signals. DUT was performed in a series of seventeen independent experiments each one consisting of 150-200 mg initial fresh weight of tissues to be treated, with 30-50 ug plasmid DNA in 50 mM NaCl, 50 mM Tris HCl pH 7.8. DUT was performed by a desiccation (about 4 hrs depending on the amount of tissue at 27 ± 2 C, constant flow rate 0.45 $m \cdot sec^{-1}$) followed by uptake (2 hrs at 27 ± 2 C). Rehydrated calli were incubated for complete recovery 48 hrs in non-selective medium and subsequently transferred to selective and regenerative conditions. At this level, concentrations as low as 20 ug/ml of kanamycin were effective. In about 15 days complete regeneration of plantlets was obtained; the leaves of these were white (NPT-II negative), green (NPT-II positive) and striped, thus showing a chimaeric composition of the tissues (NPT-II positive or negative respectively in green and white sectors till the 6th leaf stage). Callus tissue after DUT, if propagated on 2,4-D medium in the presence of kanamycin, was NPT-II positive for at least one month. Resistance, detected as NPT-II activity as well as capacity of healthy growth on kanamycin, was then lost, thus revealing the transient nature of the transformation at the callus level. Some of the plants putatively transformed (Rt0) have been grown to maturity, selfed or crossed. Rt1 and Rt2 progenies were screened for NPT-II activity. Green tissue of the Rt0 plants was NPT-II positive. Four Rt0 plants were considered and among these plants two (RK4 and RK7) were green and two (RK5A and RK5B) were variegated at various levels. The first two plants were barren with vital pollen which was used to pollinate B79 ears. RK5A was a plant not bearing a tassel and was pollinated with normal A69Y pollen, while the RK5B plant was a fertile complete plant and was selfed. Table 1 reviews the situation observed. Various seed sets were obtained from the crosses. For each Rt0 some Rt1 seeds were considered and seeded for the screening of Rt1 plants. The strongest signal was given by the two plants from RK5A. Eighteen out of 28 plants of RK5B were positive, and none from the other three progenies screened. When Rt2 progenies of the NPT-II positive Rt1 plants were tested in vitro for germination on kanamycin (50 and 100 ug/ml) on MS medium as isolated mature embryos, they showed different degrees of resistance at the seedling level which, however, ended in a complete discoloration of the seedlings in vitro of the next leaves when potted into soil. These results are indicative of the transient expression of the gene which might have been inserted and expressed in a first period and then excised and lost, or even replicated as plasmid DNA molecules independently from the genomic DNA and subsequently lost. Although several parameters have still to be optimized and molecular analysis on the genomic DNA of the plants performed, these results might be indicative of the possible use of this approach for maize transformation.

Table 1 : Putatively transformed Rt0 plants and their progenies. Situation.

Rt0	Features	Operation	Seeds Rt1 n°	Plants Rt1 considered
RK4	- completely green - male plant not bearing ear - efficient pollen	on B79 ♀ A on B79 ♀ B	235 62	10 15
RK7	- completely green - male plant not bearing ear - efficient pollen	on B79 ♀	43	3
RK5A	- female plant - variegated	x A69Y ♂	7	2
RK5B	- complete plant - variegated	⊗	57	28

Tissue culture, characterization and evaluation of in vitro salt tolerance in Arizona 8601

--E. Lupotto, M. C. Lusardi and F. Locatelli

It is still rather controversial whether through tissue culture selection in the presence of salt, it is possible to regenerate individuals effectively salt tolerant in the environment, when grown as normal plants in the field. Although it is quite easy to select and isolate salt tolerant cell cultures in vitro and characterize them as effectively stable and tolerant, it is, however, not fully clear if there are common parameters which may be indicative of the selected material in vitro in respect to the behaviour in planta. A study performed on several *Medicago* species showed indeed that the most salt tolerant species, *M. marina*, capable of growing in salty environments, displays in vitro the most sensitive behaviour (McCoy, Plant Cell Rep. 6:31-34, 1987).

During our work on the isolation and characterization of salt tolerant embryogenic cultures of maize (Lupotto et al., MNL 62:30-31, 1988; MNL 63:31, 1989), the identification of parameters indicative of salt tolerance, both in vitro and in planta, was considered essential for the development of the work. Therefore, tissue culture studies on the behaviour of a maize germplasm derived for growth in salty soils was undertaken. Arizona 8601 (Day, Crop Sci. 27:1096, 1987) released by the Arizona Agricultural Experiment Station, Tucson, USA, can be grown in soils containing up to 6000-7000 ppm TDS (total soluble salts) and irrigated with saline water (2000-4000 ppm TDS): it represented a useful material for comparisons against salt-selected and salt-sensitive maize. Arizona 8601 can be established as embryogenic callus cultures of type 1 in vitro (Fig. 1a, b). By the use of N6I medium (Lupotto and Lusardi, Maydica 33:163-177, 1988) a callus induction frequency of about 70% of the explanted immature embryos (10-11 DAP) was obtained. Embryogenic callus cultures could easily be established and propagated on N6P medium with high efficiency (91.3% on the derived embryogenic cell lines). Growth of the callus cultures was differentially affected by the presence of 86 mM NaCl in N67P medium, a level representing the LD_{50} at which our STSC (salt tolerant somaclones) were originally derived from the salt sensitive material. Ten different cell lines were tested for their response to the presence of 86 mM NaCl in the medium, and the alterations recorded as loss/gain % in fresh weight after a 21 day subculture: this varied between -35 and +27% of the control on salt devoid medium. Comparisons were made with other salt sensitive genotypes (Fig. 2). Regenerative capability, expressed as total regenerated plantlets from 1 g fresh weight tissue, was unaffected by the presence of salt (18±6.5). The sensitivity of Arizona 8601 to increasing amounts of NaCl in the medium was tested in comparison to salt sensitive genotypes (W64A and A188) and salt selected clones (STSC10, 20 and 21), capable of growth on 129 mM NaCl. The LD_{50} for Arizona 8601 cell lines ranged between 171 and 250 mM NaCl comparable to STSC10 (158 mM), STSC20 and STSC21 (200 mM NaCl), whereas LD_{50} was around 86 mM for the salt sensitive material used for the subsequent isolation of the three STSCs.

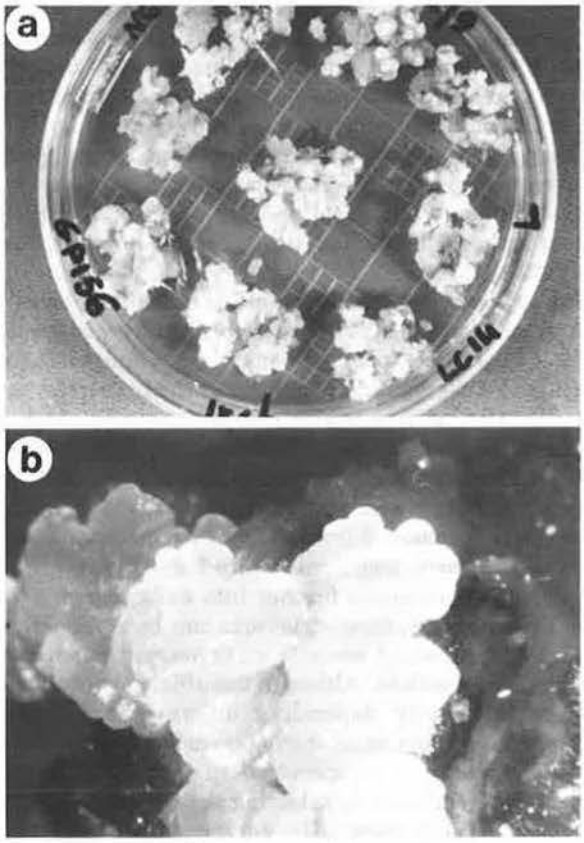

Figure 1. Embryogenic callus cultures of Arizona 8601. a) Propagation on N6P medium, growth of the tissues at the end of a 21 day subculture; b) early stages of somatic embryos at the callus surface (48 X).

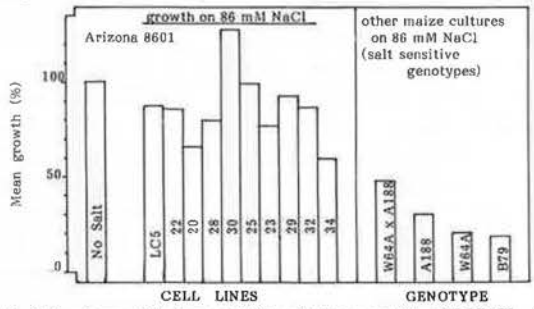

Figure 2. Behaviour of Arizona 8601 cell lines on 86 mM NaCl. Growth expressed as % of the control grown on salt devoid medium. Comparison is made with other maize salt sensitive genotypes.

It was also interesting to investigate whether the specific polypeptides detected in the STSCs grown in the presence of NaCl in the medium were also expressed in Arizona 8601. Callus cultures of Arizona 8601 were characterized by a protein pattern, analyzed through SDS-polyacrylamide gel electrophoresis, very similar to the pattern detected in the STSCs (strong increase of bands at 24-26 kDa), different from the pattern of salt sensitive material. The presence of augmented amounts of NaCl in the medium led to an overproduction of the b26 band with a corresponding decrease of a group of protein bands between 20 and 17 kDa. The same pattern was observed for the three STSCs considered. Interestingly, the permanence of Arizona 8601 on salt led to a gradual regression of

the b26 polypeptide to the normal level, after 4 month subculture in vitro, whilst the STSCs retained this overproduction. This behaviour might be indicative of a different pathway of regulation of Arizona 8601 in respect to the STSCs in response to the salt stress.

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Molecular analysis of the *Bg-rbg* transposable element system

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The *Bg-rbg* transposable element system consists of both autonomous (*Bg*) and nonautonomous (*rbg*) members. The autonomous *Bg* elements are able to transpose on their own, whereas *rbg* elements can transpose only when an active *Bg* element is present in the same cell.

In earlier work of our laboratory a newly isolated unstable mutation of the waxy locus (*wx-m32::Bg*) was reported to be caused by the insertion of an autonomous *Bg* element into the waxy gene (Motto et al., Maydica 34:107, 1989). In order to isolate this element, a genomic library in lambda EMBL4 vector was prepared using plant DNA extracted from heterozygous A69Y *wx/wx-m32* plants. The screening of the library with two different pairs, one derived from the central part of the waxy gene and the other derived from the *rbg* receptor element, has permitted the identification of a clone containing the complete autonomous *Bg* element.

Detailed restriction endonuclease mapping indicated that the *Bg* autonomous element has a length of about 5.0 kb and is inserted in an intron, between exon 12 and exon 13, in the C-terminal part of the waxy gene. The DNA sequence of the *Bg* element was partly determined and revealed a number of interesting features. The *Bg* element appears to generate an 8-bp duplication of the target site upon integration and contains 17 bp imperfect inverted repeats (CAGGGAAAACTTTATCG----CGATAGAGTAAA CCCTG) at its termini. The inverted repeat starts with the bases CA, like a number of other elements, and exhibits some further homology to these elements. At 815 bp from the 5' end of the element we have found an ATG codon that, considering its surrounding sequence, is consistent with consensus sequence for the translation start reported for plant genes. The ATG codon is followed by an ORF with a length of 736 bp. The end of this ORF coincides with a sequence canonical for a splice-junction site. The rest of the *Bg* element sequence following this site contains 3 major ORFs, interrupted with obvious non-coding regions. A number of putative splice-junction sites are also present in this part of the sequence suggesting the existence of one or more introns in the coding region of the element. The *Bg* sequence located upstream of the presumed translation start site at bp 815 does not contain any motifs in common to most plant promoters. Particularly, it lacks a recognizable TATA box. However, an interesting aspect of the *Bg* element can be revealed if we consider the G+C content of this part of the sequence. A low percentage of G+C (approximately 40%) is found in the first 120 bp of the sequence. Following these 120 bp, a region of 700 bp with a very high percentage (approximately 80%) of G+C can be found. The rest of the *Bg* sequence starting from the ATG codon at position 815 has a G+C content of approximately 40%. Because it has been reported that a number of mammalian housekeeping genes lack TATA boxes and have instead GC-rich sequences preceding the transcription start sites, the presence of a similar region in the *Bg* element appears of great interest.

A number of findings indicate several similarities between the *Bg* element and the *Ac* transposable element of maize. Both elements generate 8 bp duplications at the target sites. As is the case for the *Ac* element *Bg* lacks a recognizable TATA box or any other motif common to most plant promoters. Furthermore, both the *Bg* element and the *Ac* element contain a region with a high content in G+C upstream of the translation start site. This region might act as a promoter causing a weak expression on a basal level of the *Bg* element.

Preliminary sequence data obtained for the *rbg* element indicated not more than 75% of homology between this element and the autonomous one. Furthermore, a comparison of the restriction maps of these elements indicated the presence of a 700 bp deletion in the 5' part of the *rbg* element. This deletion involves part of the GC-rich region present in the autonomous *Bg* element and extends beyond the ATG codon at position 815 of these elements.

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Analysis of in vivo and in vitro grown endosperms of high and low protein strains

--C. Balconi, E. Rizzi, L. Manzocchi[1], C. Soave[2] and M. Motto

Illinois High Protein (IHP) and Illinois Low Protein (ILP) plants differ drastically in their ability to accumulate proteins and starch in the grain. In previous work of our laboratory we have reported that in the two strains the partitioning of assimilates between the vegetative organ and the grains was different: IHP translocates nitrogen (N) much better than sucrose while the reverse was found to be true for ILP. Furthermore, it was also evident that protein and starch content of the kernels of the two strains used was strictly dependent on the genotype of the mother plant as indicated by the data obtained from reciprocal crosses of IHP and ILP plants.

To verify this hypothesis we have examined the effect on dry weight, total N content and electrophoretic patterns

of total proteins of immature endosperms of IHP and ILP grown for 5, 8, 12 days on a solid medium containing a different ratio of sucrose to asparagine content. Furthermore, these results were compared with those obtained on plants of the same genotypes grown in the field (Fig. 1).

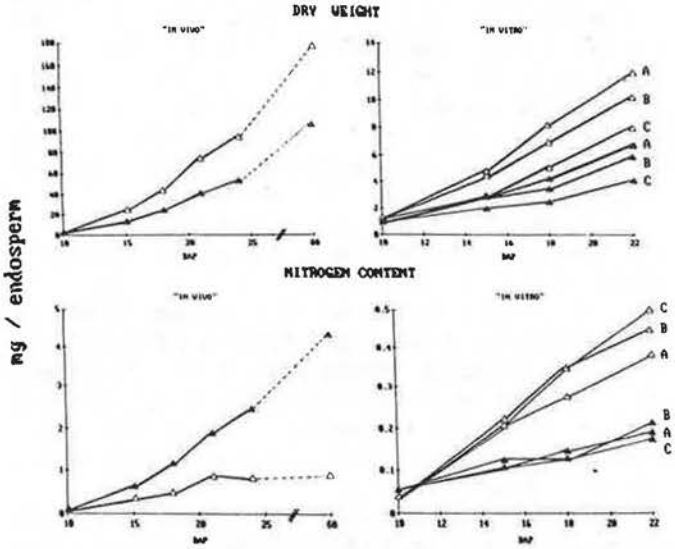

Figure 1. Dry weight and N content of IHP (▲-----▲) and ILP (Δ-----Δ) endosperms grown in vivo and in vitro. All media contained 0.4 mg/l thiamine, 100 ug/l inositol, salts as described in Nitsch and Nitsch (1969) and 8 g/l agar. A, B, C culture media contained various concentrations of sucrose (30, 20 and 10 g/l, respectively) and asparagine (2, 3, 4 g/l, respectively).

The ILP strain cultured in vitro accumulated a greater amount of dry matter per endosperm than the IHP strain, maintaining the same behaviour observed in vivo. The accumulation of total N per endosperm followed a different trend when the in vivo and the in vitro conditions were compared. In vivo IHP endosperms were more efficient than ILP in accumulating N; the reverse was observed in vitro: here the ILP strain, at all stages of development considered and on all culture media, was always capable of accumulating higher amounts of N per endosperm than the IHP strain. IHP endosperms, during development in vivo and in vitro, synthesized both the 22 kDa and the 20 kDa fractions of zeins. While ILP endosperms grown in vivo were not able to accumulate the 22 kDa fraction of zeins, the same endosperms, under in vitro culture, were able to restore the synthesis of the 22 kDa zein fraction. Our data suggest that the expression in endosperm of the IHP and ILP phenotype is controlled by the N metabolites supplied to the developing kernels.

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Maize meiotic mutants used to study the control of microtubule distribution in higher plants

--Chris J. Staiger and W. Zacheus Cande

Maize microsporogenesis proceeds through a well-defined developmental sequence and has the potential to be a model system for studying the changes in microtubule distribution and function that occur during meiosis and plant development. Microsporocytes are superb cells for cytology and, by selecting for male sterile mutants, it is possible to obtain mutants defective in meiosis. By using immunocytological and genetic techniques to study the cytoskeleton we are investigating basic questions critical for understanding microtubule function in all cells. In particular, we are interested in what controls the changes in microtubule distribution during meiosis and plant development.

Microtubule distribution during wild type sporogenesis has been characterized using indirect immunofluorescence microscopy (Dev. Biol., in press). Briefly, sporocytes from the inbred lines A344, B73, F86, and 1S2P were extruded from cut anthers and fixed in a microtubule stabilizing buffer. A short digestion with wall degrading enzymes made these cells permeable to antibodies. Microtubules were stained using a monoclonal antibody against sea urchin tubulin and a secondary antibody coupled to fluorescein. Using such methods, microtubule arrays can be visualized from early prophase I stages until pollen wall formation begins around the young microsporocyte. Modification of these procedures will be necessary to obtain adequate staining of microtubules arrays during mitotic pollen divisions and pollen development.

During normal meiosis, microtubule arrays undergo predictable temporal and spatial changes. Cytoplasmic microtubule arrays usually emanate from the nuclear envelope and radiate towards the cortex during prophase, interphase and telophase of both meiotic divisions. Immediately preceding and after meiotic divisions, microtubules are found to be associated predominantly with the nucleus. It has been postulated that a site closely associated with the nuclear envelope serves as a microtubule organizing center (MTOC). Meiotic spindles of both divisions have highly focused poles and a specific orientation within the sporocytes and in the anther locule. Cytokinesis occurs after both meiotic divisions and is accomplished by a typical phragmoplast that is initiated in the spindle midzone during late anaphase and telophase. The parallel arrays of phragmoplast microtubules propagate centrifugally forming a ring around the newly formed cell plate. Cytokinesis is always completed before the next division ensues. An isobilateral tetrad of microspores is the ultimate product of this controlled pattern of meiotic divisions.

Examination of several existing maize meiotic mutants suggests that abnormal meiosis can be correlated with disruption of the cytoskeleton (Dev. Biol., in press: J. Cell Sci, submitted). Mutants we have examined include *dv, ms28, ms43, Mei025 (Mei1), and ms17.* The meiotic mutant *dv* is defective in the transition from a prophase microtubule array to a metaphase spindle. Instead of converging to form focused poles, the metaphase spindle poles remain diffuse as in prometaphase. This defect correlates with several abnormalities in subsequent developmental events. These results suggest that *dv* is a mutation that affects MTOC organization (Dev. Biol., in press). Breakdown of the anaphase spindle and failure to progress through the cell cycle are characteristic of the mutant *ms43* . We speculate

that this disruption could be caused by a defective spindle structural component, possibly one that mediates lateral interaction between microtubules. Or more likely, *ms43* $^{+}$ is a cell cycle gene necessary for progression through meiosis. A dominant mutant, *Mei025*, has super-condensed metaphase chromosomes that cannot be separated to daughter cells. However, cytoplasmic radial microtubule arrays characteristic of interphase reform, but are not associated with typical nuclei. Analysis of the mutant *ms28* lends further evidence to the hypothesis of Golubovskaya and coworkers (Adv. Genet. 26:149-192) that abnormal anaphase chromosome segregation and partial or complete failure of cytokinesis is due to altered microtubule dynamics. We have observed spindles with an unusual proliferation of astral microtubules, as well as spindle-shaped phragmoplasts that remain in the cytoplasm as late as the second meiotic division. Interestingly, examination of *ms17* showed many of the same abnormalities observed in *ms28*. Most notable are the phragmoplast arrays which fail to propagate and remain in the cytoplasm until division II. However, *ms17* exhibits other abnormalities suggesting it is either a stronger allele or a different gene than *ms28*. Based on the similar meiotic behavior, and the fact that both have been mapped to chromosome 1S, we suspect that these male sterile mutations are allelic. Genetic tests of this are currently underway, but we would be interested to learn of any other attempts to check allelism between these mutations.

Ultimately we would like to analyze meiosis and the cytoskeleton at the molecular level. We have generated and partially characterized several meiotic mutants induced by *Mu* mutagenesis. During the summer of 1987, we examined 2000 *Mu* F2 families (20 plants each) for segregation of a male sterile phenotype. Sixty-six families contained plants with defective anther exsertion, shriveled anthers, and either no pollen shed or greater than 10% of the pollen shriveled. Twenty plants for each of these families were regrown in the field during the summer of 1988. Sporocyte samples were collected from each plant, and examined for meiotic defects using temporary DAPI stained preparations. Seventeen families, representing 9 *Mu* F1 lines, contained plants with meiotic abnormalities. Sterile plants from each of these lines were outcrossed using B73 as the pollen parent. Heritability of these putative ms lesions was examined by selfing presumed ms/+ plants and examining the progeny for meiotic defects. Of the 9 original classes, 1 class failed to segregate any meiotic disruption and 2 others were too incompletely expressed for further study. Furthermore, 2 classes of meiotic lesions reduced female sterility to the extent that outcrosses of sterile plants are impossible. Analysis of heterozygous sibs is being pursued to determine if these two classes are heritable. The remaining four classes segregate meiotic, male sterile lesions as simple monogenic recessive traits. The most interesting of these is similar in phenotype to *ms17*. Although we have not yet examined microtubule distribution in this new mutant, the earliest detectable defects, based on chromosome behavior, are observed during metaphase I. This mutant, designated *mms23**, is unable to align its chromosomes normally on a metaphase plate, and often forms multiple spindles. The end-product of meiosis is typically polyads of microspores, but other products are possible. Allelism tests between this mutant and *ms17* are in progress.

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Proteolytic activity in coleoptile extracts causes artifactual ß-glucosidase multiplicity

--Asim Esen and Cumhur Cokmus

Maize ß-glucosidases (ß-D-glucoside glucohydrolase, EC 3.2.1.21) isolated from inbred lines display multiple electrophoretic variants. These variants appear in a time-dependent manner during storage, most notably at acidic pHs (below pH 6.0) and in the presence of a reducing agent. The enzyme was extracted from the coleoptiles of 6-day-old seedlings with a variety of aqueous buffers and assayed for activity. Zymograms were obtained by incubating the gels with the synthetic substrate 6-bromo-2-naphthyl ß-D-glucopyranoside after electrophoresis. The pHs of the crude enzyme preparations (extracts) were adjusted to vary from 3.0 to 10.0, and the preparations were incubated at different temperatures (-30, 4, 25, and 37 C) for varying lengths of time in the presence and absence of the reducing agent 2-mercaptoethanol (2-ME) and assayed for activity and changes in zymogram patterns. The results showed that the enzyme lost activity at higher temperatures (25 and 37 C) and at pHs below 4 and above 9. Similarly, the number of charge variants resolved by electrophoresis increased during incubation, especially at higher temperatures. These novel variants had increased anodal mobilities, and could be resolved into 3 to 5 distinct bands after incubation at pHs between 4 and 6 (Fig. 1a, lanes 1-6). When polypeptide profiles of the extracts were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after the above-mentioned treatments, it was observed that the staining intensity of individual polypeptides decreased and there was a complete loss of some polypeptides at pHs between 4 and 6 in a time-dependent manner under reducing conditions, especially after incubation at 25 and 37 C (Fig. 1b, lanes 1-7). An example of the results is shown in Fig. 1. Figure 1a shows the changes in ß-glucosidase zymograms of the coleoptile extract from inbred K55 made with 25 mM sodium acetate buffer (pH 5.0) containing 35 mM 2-ME. The extract (final pH 5.2) was incubated at two different temperatures (25 and 37 C) for 6, 12, and 24 hours, respectively. It is evident that zones (bands) of ß-glucosidase activity increase in number, and novel anodal bands appear as cathodal bands disappear or decrease in intensity in a time- and temperature-dependent manner (Fig. 1a, lanes 2-6). When the pH of the same extract was adjusted to 7.0 before treatments, the shifts from cathodic to anodic zones of activity slowed substantially and the anodic shift produced mostly a diffuse zone of activity instead of distinct bands (Fig. 1a, lanes 9-14). At both pHs, the highest enzyme activity and the least anodal shift in zymograms was

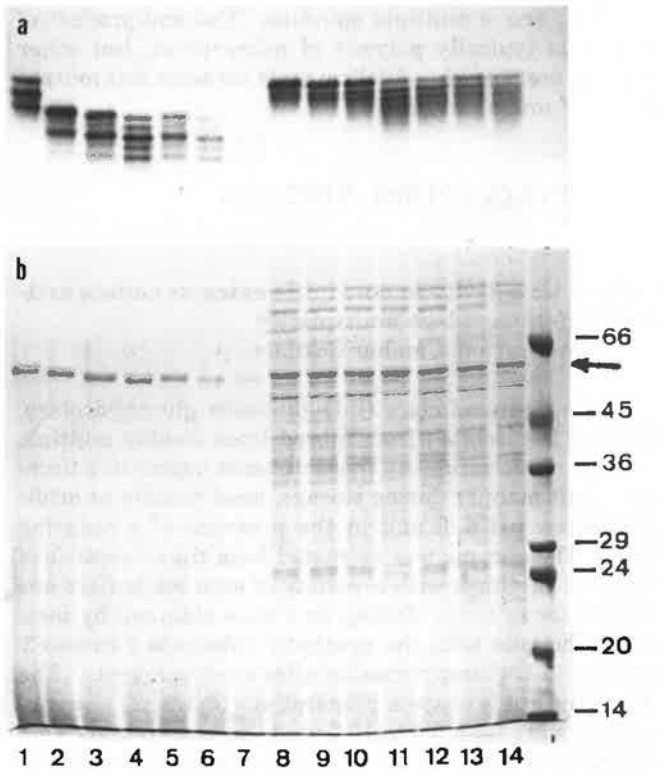

Figure 1. Time-course of the changes in ß-glucosidase zymograms (a) and polypeptide patterns (b) after extraction with 25 mM Na-Acetate, pH 5 / 35 mM 2-ME. The extract (pH 5.2) was incubated at 25 and 37 C for 6, 12, and 24 hrs. Lane 1, 4 C control (24 hrs); lanes 2-4, after 6, 12, and 24 hrs, respectively, at 25 C; lanes 5-7, after 6, 12, and 24 hrs, respectively, at 37 C. Lanes 8-14, after the pH of the extract was adjusted from 5.2 to 7. Lane 8, 4 C control (24 hrs); lanes 9-11, after 6, 12, and 24 hrs, respectively, at 25 C; lanes 12-14, after 6, 12, and 24 hrs, respectively, at 37 C. The last lane (unnumbered) in b includes marker proteins ranging in size from 14 to 66 kD. The arrow on right points to the 60 kD polypeptide (ß-glucosidase monomer).

in the samples stored at 4 C for 24 hours (Fig. 1a, lanes 1 and 8). Figure 1b shows the changes in polypeptide profiles of the same samples used to develop the zymograms in Figure 1a. It is apparent that the cause of the changes in zymograms at pH 5.2 is proteolysis because the size and intensity of the ß-glucosidase polypeptide (60 kD band, arrow) decreases in a time- and temperature-dependent manner (Fig. 1b, lanes 2-7). Moreover, essentially complete proteolysis of polypeptides other than the ß-glucosidase monomer is evident from (1) the absence of any bands in lanes 1-7 (Fig. 1b), and (2) the presence of a diffuse, low molecular weight Coomassie blue stained zone at the anodic end (bottom) of the gel. In contrast, little or no proteolysis is evident in samples whose pH was adjusted to 7.0 (Fig. 1b, lanes 8-11), except some slight proteolysis at 37 C (Fig. 1b, lanes 12-14). We were able to inhibit the proteinase active at acidic pHs with antipain, leupeptin and the alkylating agent iodoacetic acid, but not the one active at neutral or alkaline pHs. Based on these data, it was concluded that maize ß-glucosidase multiplicity observed in zymograms of inbred lines is an artifact of proteolysis. An SH-proteinase active at acidic pHs and requiring a reducing agent (e.g., 2-ME) for activation was primarily responsible for artifactual ß-glucosidase multiplicity. Another class (undefined) of proteolytic activity was found to be responsible for producing charge multiplicity above pH 6; but this multiplicity appeared mostly as a broad and diffuse zone instead of distinct bands. Using buffers of neutral or alkaline pH or excluding reducing agents from the extraction medium and performing all the operations at 0-4 C appear to be effective strategies against the SH-proteinase.

Null alleles at the *Glu* locus code for ß-glucosidase activity and immunoreactive protein

--Asim Esen and Cumhur Cokmus

Maize ß-glucosidase (ß-D-glucoside glucohydrolase, EC 3.2.1.21) was extracted from coleoptiles of fifteen genotypes (three normals, 10 nulls, and two hybrids) in two fractions, the buffer-soluble and the buffer-insoluble (extracted with SDS-sample buffer). The enzyme activity was measured spectrophotometrically in the soluble fraction and also studied on zymograms after native gel electrophoresis and isoelectric focusing. The enzyme was purified from a normal genotype by ion exchange chromatography and preparative electrophoresis. Antisera were raised in four rabbits, and the soluble and the insoluble extracts of each genotype were analyzed for a cross-reacting material by ELISA and immunoblotting. The results showed that extracts from both the normal and the null genotypes had ß-glucosidase activity, and the activity measured spectrophotometrically was two to ten-fold higher in normals than in nulls (Table 1). Zymograms (not shown) of the null genotypes were devoid of distinct bands that were present in those of normals and hybrids from

Table 1. ß-glucosidase activity in coleoptile extracts of selected maize lines and hybrids.

	Genotype		ß-Glu Activity	
Line	Literature[a]	This study	Specific[b]	Relative[c]
F6	N*	n	0.54	8.3
CG10	N*	+	0.80	12.3
OH51A	N*	+	3.11	47.0
CO125	N*	n	0.60	9.2
CH586-12	nd	+	3.41	52.1
CH592-46	nd	n	0.64	9.8
CH593-32	nd	n	0.65	9.9
CH701-30	N	n	0.74	11.3
MICH77-6	N	+	1.12	17.1
CI.90A	N	n	0.67	10.3
OH7B	1	+	6.54	100.0
OH7BxH95	1/N	+ /n	3.12	47.8
H95	N	n	0.85	13.0
H99xH95	6/N	+ /n	1.44	22.0
H99	6	+	4.97	76.0

C.V = 0.6-3.1%

[a] Stuber and Goodman, 1982 (MNL 56:127).

[b] μM pNP/hr/mg protein

[c] expressed as percent of the line with highest activity (e.g., OH7B)

N or n = null; N* = segregating for a null allele at the *Glu* locus

+ = normal; nd = no data; 1 and 6, allelic designations based on mobility towards the anode.

crosses between normals and nulls. Zymograms of both the normal and the null genotypes had a diffuse, smeared zone of activity at the cathodic end of native gels. A cross-reacting antigen was present in extracts of both genotypes when assayed by ELISA and a 60 kD polypeptide (ß-glucosidase monomer, Figs. 1a and 1c, arrow) was detected by four different monospecific ß-glucosidase antisera on

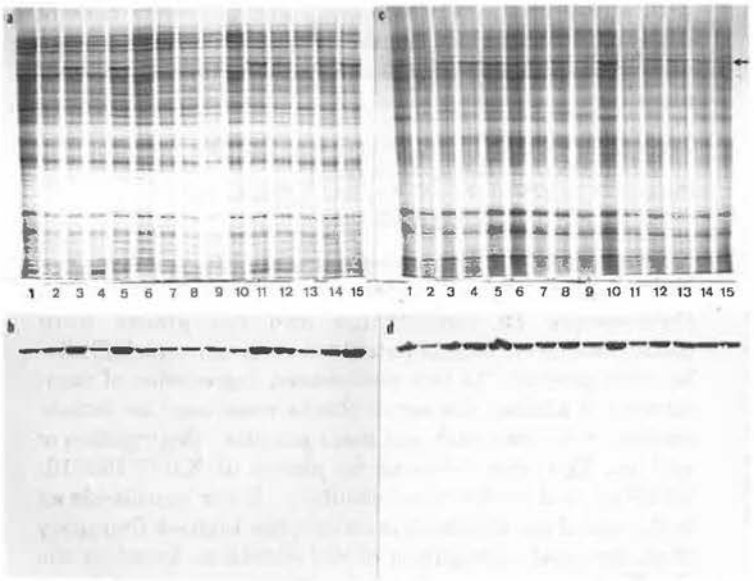

Figure 1. **a.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 12%T, 4%C) profiles of coleoptile extracts (soluble protein). **b.** Immunoblot of **a** developed with antiserum (R681) to ß-glucosidase. **c.** SDS-PAGE profiles of coleoptile pellet extracts (insoluble protein, extracted with SDS-sample buffer). **d.** Immunoblot of **c** developed with antiserum (R681) to ß-glucosidase. In both gels and blots, the cathode is at top. 1, F6; 2, CG10; 3, OH51A; 4, CO125; 5, CH586-12; 6, CH592-46; 7, CH593-32; 8, CH701-30; 9, MICH77-6; 10, CI.90A; 11, OH7B; 12, OH7BxH95; 13, H95; 14, H99xH95; 15, H99. Note the presence of an immunoreactive 60 kD polypeptide (ß-glucosidase monomer) in both null and normal genotypes and its increased amount in the insoluble fraction from null genotypes.

Western blots by immunostaining (Figs. 1b and 1d). Moreover, six out of seven null genotypes had a larger amount of their 60 kD polypeptide in the insoluble fraction (Fig. 1d) than in the soluble fraction (Fig. 1b). These data show that both the null and the normal genotypes have similar amounts of the enzyme protein, but the enzyme occurs mostly as in soluble or poorly soluble polymers in nulls and does not enter the gel; thus, it is not detected by zymogram techniques. The monogenic inheritance reported for the null alleles of the *Glu* locus is likely to be for a factor encoded by another locus which affects directly or indirectly the solubility of the enzyme by increasing its polymerization into large quaternary structures.

ß-glucosidase zymograms in denaturing (SDS) gels

--Asim Esen and Gunay Gungor

We performed a systematic search for concentrations of protein denaturing agents that inactivate maize ß-glucosidase activity. In the course of these studies, it was discovered that the enzyme retained about 50% of its activity after treatment with or in the presence of the anionic detergent sodium dodecyl sulfate (SDS), a potent denaturant, when used at or around 1% concentrations. When coleoptile extracts or purified enzyme preparations were incubated in the presence of SDS concentrations varying from 0 to 1.6%, electrophoresed through 6-8% polyacrylamide gels and stained for activity, zymogram patterns similar to those of untreated samples were obtained (Fig. 1a). However, the intensity of enzyme bands decreased as SDS concentration increased to 0.8% or higher. In view of these

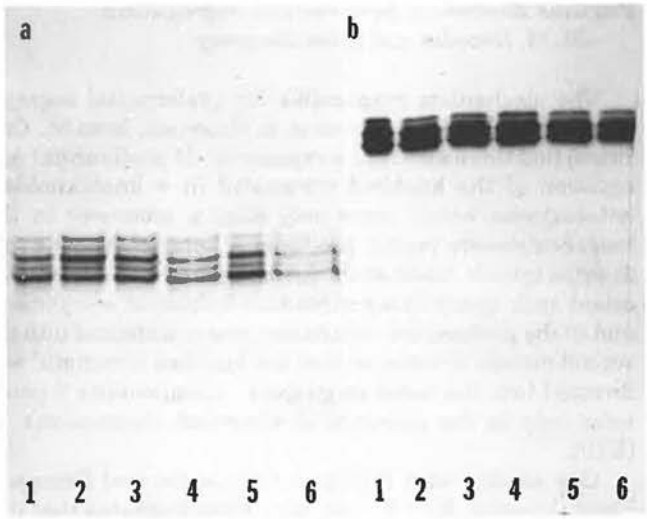

Figure 1. ß-glucosidase zymograms of a maize coleoptile extract after treatment with SDS prior to electrophoresis. **a**, 6% nondenaturing (native) gel. **b**, 10% denaturing (SDS) gel. The enzyme was extracted with 25 mM NaAc buffer, pH 5.0, containing 35 mM 2-mercaptoethanol. Lane 1, control (no SDS added). Lanes 2-6, after adding SDS to a final concentration of 0.1 to 1.6%. 2, 0.1%; 3, 0.2%; 4, 0.4%; 5,0.8%; 6, 1.6%.

results, controls and SDS-treated samples were subjected to standard SDS-PAGE through 10-12% gels using the method of Laemmli and the resulting gels were stained for enzyme activity. Surprisingly, a zone of activity including 1 to 6 bands, depending on sample age and buffer pH and composition, in the 60 kD region of the gel was obtained (Fig. 1b). Figure 1 shows representative zymograms obtained when the control and SDS-treated samples were electrophoresed through a 6% native (Fig. 1a) and a 10% SDS gel (Fig. 1b). Multiple ß-glucosidase bands are visible in both zymograms. In this case, the source of the heterogeneity is thought to be artifactual due to the activity of an endogenous SH-proteinase because it occurred independently of SDS treatment and the samples were handled under conditions (e.g., extraction and storage in a pH 5 buffer containing 2-mercaptoethanol) promoting the activity of such proteinase prior to SDS treatment and electrophoresis. In subsequent experiments (results not shown), a single band was obtained when the sample was not exposed to the reducing agent. Furthermore, it was shown that the zymograms of the maize inbreds classified as null at the *Glu* locus contained ß-glucosidase bands after their coleoptile extracts were treated with SDS. These results suggest that the 60 kD polypeptide, the ß-glucosidase monomer, shows full enzymatic activity and, thus, the in vivo form of the functional enzyme is a monomer, not dimer as assumed on the basis of zymogram patterns of hybrid genotypes. In addition, the results clearly show that the maize inbreds that are classified as null for *Glu* encoded ß-glucosidase activity are not null. The enzyme of these so-called null genotypes appears to occur as large aggregates that fail to enter the gel and cannot be detected by standard zymogram techniques.

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Further studies on preferential segregation

--M. M. Rhoades and Ellen Dempsey

The mechanism responsible for preferential segregation proposed in 1952 (Rhoades, in Heterosis, Iowa St. Coll. Press) had three essential components: 1) preferential segregation of the knobbed chromatid in a knob/knobless heterozygote would occur only after a crossover in the knob-centromere region produced a heteromorphic dyad; 2) extra spindle fibers at the knob region were necessary to orient such dyads in a nonrandom fashion at anaphase I; and 3) the preferential orientation was maintained into the second meiotic division so that the knobbed chromatid was directed into the basal megaspore. Components 2 and 3 exist only in the presence of abnormal chromosome 10 (K10).

Our studies with K10-type I (Rhoades and Dempsey, Plant Genetics, Alan R. Liss, Inc., 1985) indicated that this chromosome differs from normal chromosome 10 (N10) by the possession of a mainly euchromatic internal segment of unknown origin (the differential segment) and a large heterochromatic knob, as well as by the inversion and transposition of a distal stretch of 10L. We found neocentric activity in plants with terminally deficient K10 chromosomes, which retained the differential segment of K10-type I but had lost the terminal knob and varying portions of the subterminal inverted region--i.e., neocentric activity was possible in the absence of the K10 knob. We also studied the same Df K10 plants genetically by following the female transmission of the *yg2* allele, closely linked to a large terminal knob (K9l) vs. small (K9s) on the short arm of chromosome 9 in K9l *yg2*/K9s *Yg2* heterozygotes. There was no preferential segregation of the *yg2* allele, suggesting that the K10 knob was necessary for this phenomenon. While the differential segment sans the K10 knob induced neocentric activity at knobs in heterologous chromosomes, no preferential segregation of these knobs resulted. It seemed possible that the K10 knob was involved in component 3--i.e., maintenance of preferential orientation through the second meiotic division.

If this idea is correct, the K10 knob plays a role after the end of the first meiotic division. If its activity is cell-limited, segregation of the K10 knob into only one of the prophase II daughter cells would result in random segregation of heterologous knob-knobless dyads at the second division in the other daughter cell. One might at first expect to find a reduction in preferential segregation frequencies in K10/N10 plants as compared with K10/K10 homozygotes because of the higher frequency of prophase II cells without K10 in the former group. While there is a slight decrease in K10/N10 vs. K10/K10 plants, the difference is small, probably because the occurrence of a crossover in the long arm of almost every chromosome 10 bivalent would give prophase II cells both of which had one K10 chromatid. To circumvent this difficulty, we utilized trisomics of K10 N10 N10 constitution, in which the long arm of the K10 chromosome seldom undergoes crossing over with either of the N10 homologs and prophase II cells without any K10 knob should be frequent. Two crosses were made differing only in the marker genes on chromosome 10:

Cross A: K10 *R-st*/N10 *R-nj*/N10 *R*; K9s *Yg2*/K9s *Yg2*
X N10 *R*/N10 *R*; K9l *yg2*/K9l *yg2*
Cross B: K10 *R*/N10 *R-nj*/N10 *r*; K9s *Yg2*/K9s *Yg2*
X N10 *R*/N10 *R*; K9l *yg2*/K9l *yg2*

Progeny of the two crosses are all K9l *yg2*/K9s *Yg2* heterozygotes. Sporocytes were collected to determine the chromosome 10 constitution and the plants were testcrossed on *r/r* female parents to ascertain which *R* alleles were present. To test preferential segregation of chromosome 9 alleles, the same plants were used as female parents in crosses with *yg2* male parents. Segregation of *yg2* vs. *Yg2* was followed in plants of K10/N10/N10, K10/N10, and N10/N10 constitution. If our hypothesis as to the role of the K10 knob is correct, the highest frequency of preferential segregation of *yg2* should be found in the K10/N10 disomics, a reduced frequency is expected in the K10/N10/N10 trisomics and a random segregation of 1:1 should occur in the N10/N10 disomics.

Table 1. Frequencies of preferential segregation and recombination found in female testcrosses of disomic and trisomic progeny of Cross A and Cross B

	Total	% *yg*	Recombination percents		
			yg-bz	*bz-wx*	Total
Cross A, 2n + 1 (K10/N10/N10)	2602	56.0	17.5	21.3	38.8
Cross B, 2n + 1 (K10/N10/N10)	2033	55.4	17.2	21.4	38.6
Cross A, 2n (K10/N10)	1192	60.0	12.5	23.4	35.9
Cross B, 2n (K10/N10)	921	59.4	16.0	22.5	38.5
Cross A & B, 2n (N10/N10)	2203	52.1	11.5	19.2	30.7

Table 1 shows the frequencies of preferential segregation found with trisomic and disomic progeny of Cross A and Cross B. Similar values were observed with populations from two crosses. As expected, the highest percentages of *yg2* were found in testcrosses of K10/N10 female parents, somewhat lower percents occurred in K10/N10/N10 trisomic plants and the lowest values were in the N10/N10 plants. The N10/N10 data came mainly from progeny of Cross A; these plants surprisingly showed a slight excess of *yg2* when 50% *yg2* was expected. The reason for the excess of *yg2* is not clear. In other respects, the data are understandable and both sets (Cross A and B) are in agreement with the predicted outcome. If it is assumed that all of the daughter cells following the first meiotic division in K10/N10 plants contain a K10 chromatid and that 60% is the basic rate of preferential segregation of the K9l chromosome in such cells, the reduction to 55.4-56% *yg* observed in K10/N10/N10 plants would indicate that in a high proportion of the meiotic divisions (80-90%) one daughter cell receives no K10 chromosome.

Cytological counts bearing on the meiotic segregation in K10/N10/N10 plants are available (Rhoades, in Heterosis, 1952). At diakinesis, 60% of the cells possessed a trivalent configuration and in 90% of these the K10 chromosome was joined to a N10 chromosome by a chiasma in

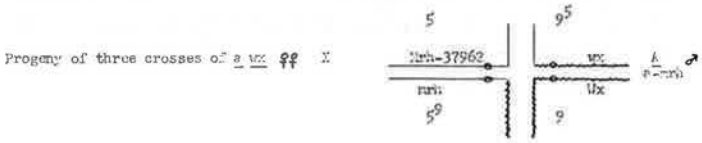
the short arm and had no chiasma in the long arm. In the 40% of cells where a bivalent + univalent situation was found for chromosome 10, the univalent was the K10 chromosome in 83% of the cells. In these cells (90% of the trivalent group and 83% of the bivalent + univalent group), the K10 chromosome would pass intact to one pole or the other since there is no opportunity for a crossover in the long arm of 10 resulting in formation of a heteromorphic chromosome 10 dyad. These data are reinforced by similar observations on two K10/N10/N10 plants from the Cross A progeny. 65.9% of 387 diakinesis cells had a trivalent association and 34.1% had a bivalent + univalent situation. Within the latter group, the K10 chromosome was the univalent in 114 (86.2%) of the cells and N10 was the univalent in only 18 (13.8%). A high percentage of resulting segregations should have no K10 chromosome at one of the poles. When daughter cells without K10 undergo the second meiotic division in megasporogenesis, no preferential orientation occurs and the genetic data indicate that the overall rate of preferential segregation is reduced. We conclude that the K10 knob must be present in the second division daughter cells to allow completion of the process resulting in preferential segregation.

Recombination was followed in the chromosome 9 bivalents in the genetic study (Table 1). Total recombination in the *wx-yg2* segment of 9S did not vary greatly in disomic and trisomic plants with K10, although the K10/N10 plants from Cross A progeny had a different distribution of crossovers in the two regions studied. Thus, heteromorphic chromosome 9 dyads were present in K10/N10 disomics and K10/N10/N10 trisomics in similar frequencies. According to the hypothesis explaining preferential segregation, such dyads are required if preferential segregation is to occur. The difference in preferential segregation of *yg2* observed in the two groups cannot be ascribed to a lower amount of crossing over in the trisomic 10 group. Incidentally, in the K10/N10 and K10/N10/N10 plants, recombination frequencies in both *yg2-bz* and *bz-wx* regions were higher than in the N10/N10 controls. This is consistent with the secondary role of the K10 chromosome in promoting recombination. Since crossing over occurs in prophase of the first meiotic division before segregation has taken place, no difference is expected in the frequency of recombination in the K10/N10 and K10/N10/N10 plants.

Evidence for location of *Mrh-37962* on chromosome 5

--Ellen Dempsey and M. M. Rhoades

In our recent publication (Shepherd, Rhoades and Dempsey, Devel. Genet., 1989) we described a second occurrence of *Mrh* (possibly by transposition). The new allele was tentatively placed on chromosome 5. The new *Mrh* (*Mrh-37962*) was found in the progeny of a selfed plant of *a-mrh Sh2/a sh2*; *Mrh/mrh* constitution. The *sh2* kernels were all dotless, but the *Sh2* kernels were of three types: high-dot, low-dot, and dotless. The unexpected "low-dot" phenotype was shown to be the result of segregation of a second *Mrh*, while the high-dot kernels possessed the original *Mrh* on chromosome 9 or both *Mrh* factors. Plants with *Mrh-37962* were crossed with the *wx*-translocation series and the F1 progenies testcrossed with *a wx* testers to determine its chromosomal location. The F1 plants were selfed and used as male parents in the testcross. Close linkage of *wx* and *Mrh-37962* was observed in only one of the F1's, that involving translocation 5-9c (breakpoints 5S.07-9L.10). The data presented in the table came from three testcrosses of the same male parent.



	colored Wx	colored wx	colorless plus dots Wx(0)	colorless plus dots wx(x)	colorless no dots Wx(x)	colorless no dots wx(0)
Cross 1	59	34	21	0	28	36
Cross 2	29	21	11	0	19	23
Cross 3	53	43	14	0	20	37

The colorless seeds all have one dose of the *a-mrh* responding allele and should show dots when *Mrh-37962* is present. The new *Mrh* specifies a low rate of somatic mutation to *A* and, when introduced by the male parent and present in one dose, *Mrh-37962* is often unexpressed. The colorless kernels without dots were therefore grown and tested for presence of *Mrh-37962*. Most of the *Wx* kernels proved to have *Mrh-37962* while most of the *wx* kernels did not (noncrossover (0) classes). The revised data for the colorless kernels gave the following totals: 85 *Wx* with dots, 1 *wx* with dots, 3 *Wx* without dots, and 60 *wx* without dots. It is clear that *Mrh-37962* is closely linked to *Wx*. The progeny plants were scored for pollen sterility and this permitted identification of the point of exchange in the four crossover plants. Three of them (including the *wx* dotted individual) were crossovers between the *wx* gene and the translocation breakpoint. The fourth was either a crossover in the *Mrh*-breakpoint region or a loss of the *Mrh-37962* controlling element. Several additional male testcrosses of the same type were made. Since these have not been completely analyzed for unexpressed *Mrh* alleles, only data from the positive dotted group are presented. Among the dotted kernels, 291 were *Wx* and 16 were *wx*. Again, close linkage between *Wx* and *Mrh-37962* was observed, indicating location of *Mrh-37962* on chromosome 5.

The expected 1:1 ratio of *Wx*:*wx* was not found in the above crosses. The deficiency in the *wx* class may be due to presence of a gametophyte factor on chromosome 5, closely linked to *wx* because of translocation heterozygosity. Chromosome 5 is known to harbor such factors and they often complicate the ratios for genes on chromosome 5 when transmitted through the male parent. We encountered this difficulty in self-pollinations to establish linkage of *Mrh-37962* with the *bt* gene near the centromere of chromosome 5. Two self-pollinations of *A/a-mrh*; *Bt Mrh-37962/Bt mrh* plants gave 8.6% and 6.8% *bt* when 25% was expected. However, among the *a-mrh* kernels showing dots, 177 were *Bt* and only 2 were *bt* in phenotype. The percentage of *bt* in the group known to possess *Mrh-37962* was only 1.1%. Another indirect indication that

Mrh-37962 is on chromosome 5 came from an examination of 41 selfed ears in family 41069. Twenty of these were produced by *Bt mrh/bt mrh* individuals and 21 were from *Bt Mrh-37962/bt mrh* plants. Among the first group the percentage of *bt* was 12.7%; among the second group 3.8% *bt* was found. We conclude the *Bt Mrh-37962* and the *Bt mrh* chromosomes differed in the type of gametophyte factor present, with the stronger factor located on the *Bt-Mrh-37962* chromosome. There was some variability in both groups, with a range of 3.7%-22.2% in the dotless group and 1.1%-10.4% in the group exhibiting dots. This may be due to crossovers between *Mrh-37962* and the gametophyte factor.

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Genomic diversity detected by transposable element probes is correlated with heterosis

--L. E. Talbert, W. F. Tracy and J. Gerdes

Although the transposable elements *Mu*, *Spm*, and *Ac* are detectably active only in certain maize stocks, multiple sequences hybridize to probes for the elements in all stocks. Sequences that hybridize to *Spm* and *Ac* tend to be deletion derivatives of the intact elements (Doring and Starlinger, Annu. Rev. Genet. 20:175-200, 1986). There is no evidence for activity of the *Mu* hybridizing sequences. In fact, a *Mu4* element was shown to be in the same genomic location in diverse *Zea* taxa, suggesting an ancient and stable insertion (Talbert et al., J. Mol. Evol. 29:28-39, 1989).

Approximately 20-40 separate hybridizing bands may be visualized in all maize lines on Southern blots using probes for *Mu*, *Ac*, and *Spm*. Assuming that these hybridizing sequences are not actively transposing, this suggest that a single Southern hybridization with a transposable element probe may be used to monitor genomic variation at several genomic segments. Heterosis in maize is correlated with genomic diversity of parents. Thus, we were interested to determine whether genomic diversity of parents as measured by transposable element probes is correlated with heterosis of F1 progeny.

Initial experiments suggested that genomic diversity as monitored by transposable element probes may be too great among unrelated maize lines to make meaningful comparisons regarding relative diversity. Thus, we monitored seven sublines of the maize inbred line P39 for transposable element variation. A difference among parents was measured as the presence of a hybridizing band in one subline not found in the other line. A total of 56 hybridizing bands were observed on *Eco*RI/*Hin*dIII-digested DNA from the seven sublines using *Mu*, *Ac*, and *Spm*. Differences among the 21 possible pairwise comparisons ranged from 0-16, with an average number of differences of 7.6 between sublines.

The seven sublines were crossed in a diallel fashion to provide 21 F1 hybrids. These were yield tested in Wisconsin in 1988. Parental diversity of parents as measured by the three transposable element probes was positively correlated with ten-ear weight (Table 1). Thus, parents that showed the greatest genomic diversity tended to produce the highest yielding F1 progeny. These results suggest that probes for the maize transposable elements may be efficient tools for monitoring useful genetic variation among closely related maize lines.

Table 1. Correlation between transposable element differences among parents and yield of F1 progeny for seven P39 sublines. Yield was measured as dry weight of ears from ten plants per plot in ten reps in Wisconsin in 1988.

Mu	*Ac*	*Spm*	Total
0.67	0.65	0.56	0.70

Previous work has shown that probes for *Mu* and *Spm* clearly delineate the *Zea* and *Tripsacum* genomes, as hybridizing sequences were observed in *Zea* but not *Tripsacum*. This fact has been used to determine that *T. andersonii* is a natural hybrid between *Tripsacum* and *Zea* (Talbert and Doebley, MNL 63:45, 1989; Talbert et al., Amer. J. Bot., 1990, in press). Thus, transposable elements are not only interesting in their own right, but may also be exploited to assess relatedness for either taxonomic or plant breeding purposes.

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Confocal light microscopy: A new tool for maize research

--P. C. Cheng and D. R. Pareddy

The intention of this article is to point out the recently developed confocal light microscopy and to suggest its potential applications in maize research. In addition, some technical hints and hardware improvements are discussed to assist maize researchers to set up their confocal systems.

The principle of confocal microscopy is that objects in the focal plane of the objective lens are illuminated by a point source and the light reflected or emitted by the specimen is seen by a point detector (Figure 1). The key element of confocal microscopic design is a small, diffraction limited aperture positioned at the secondary focus of the objective lens in the reflected light path. The size of this aperture is such that the virtual image at the sample is of the same order of size as the focal spot. In other words, a diffraction limited illumination spot is formed on the sample. In practice, the point source and the point detector are obtained by placing apertures between a light source, detector and objective lens. Confocal imaging is achieved when the system is aligned precisely so that rays from the source aperture pass through the viewing aperture. Rays that emerge from objects out of the focal plane are not focused at the viewing aperture and are thus blocked from reaching the detector. The result is a high contrast image of a small portion of the specimen at the focal plane. To see

an entire field, a means must be derived to scan either the specimen or the illumination and detector. At the present time, biological confocal microscopes can be subdivided into two categories: (1) Multi-beam scanning (e.g. Tandem scanning [A. Boyde, 1985, Proc. of RMS, 20(3):131-139] and Single-sided scanning confocal microscopes [P. C. Cheng et al., 1989, Proc. EMSA, 136-137]) and (2) Single-beam scanning (e.g. Laser scanning confocal microscope). Figure 1 shows the principle of a confocal microscope. A 50/50 beam splitter replaces the dichroic beam splitter and barrier filter when the microscope is to be operated in epi-reflective mode.

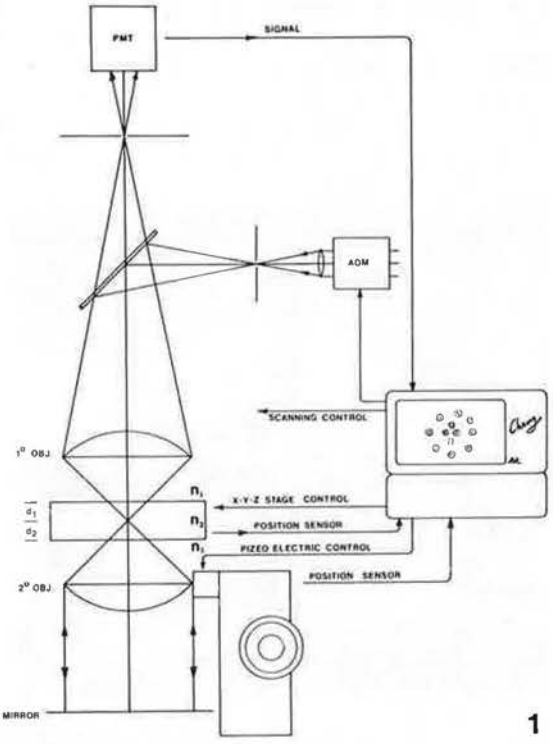
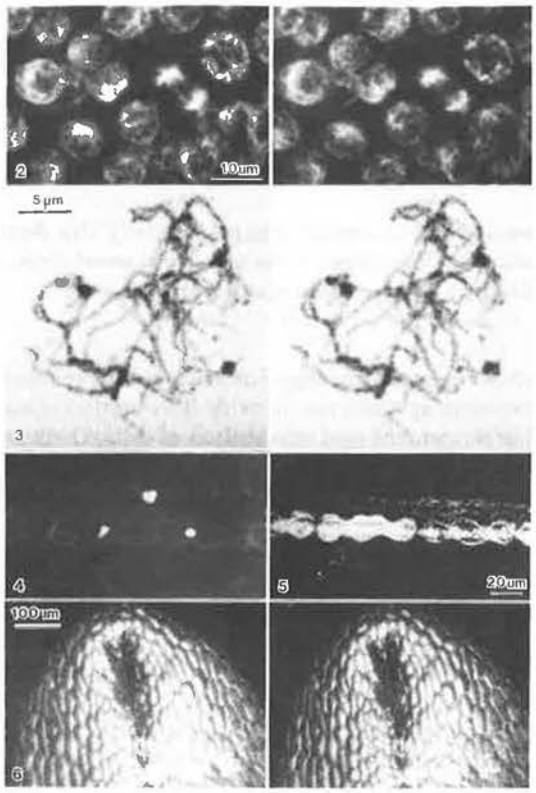


Figure 1.

The confocal microscope has the advantage over conventional, field illumination microscopes in that it enables the contrast capabilities, not available in the conventional system, to be exploited. One of the prime advantages is the capability of optical section tomography, opening the possibility of noninvasive imaging of 3D structures with exceptional contrast and the ability to rapidly acquire and reconstruct the 3D information. Confocal microscopy can be expected to have a considerable impact on studies of chromosomal arrangement, cellular structure and tissue organization.

The following are some examples of the potential applications of confocal light microscopy in maize research. For practical reasons and simplicity in demonstrating the applications, a commercially available (BioRad MRC-500) laser scanning confocal microscope is used in our discussion. One of the potential applications of confocal microscopy is the study of tissue organization. Figure 2 shows a 3D image pair of the nuclei of a developing tapetum layer in a maize anther. The image was obtained from a whole mount specimen by focusing through three layers of cells (epidermis, endothecium and middle layer). Figure 3 shows a 3D image pair of chromosomes at pachytene stage of meiosis. Both Figures 2 and 3 were obtained from intact anthers which were fixed in 1:3 acetic acid-EtOH fixative, stained with Feulgen reaction, dehydrated in EtOH and cleared in methyl salicylate (to improve the optical properties of the specimen [Cheng and Summers, 1990, In: The Handbook of Biological Confocal Microscopy, ed. J. Pawley, Plenum Press]). Figure 4 shows a confocal fluorescent image of the nuclei of silica cells of maize leaf (Feulgen stained). All three images were obtained in epi-fluorescent mode by using the 514nm line of Ar ion laser as the excitation wavelength. In order to improve the fluorescent signal, four Al-coated folding mirrors in the scanning system were replaced by high reflective dielectric mirrors.



Figures 2-6.

One of the major difficulties we have encountered in confocal fluorescent imaging is photo-bleaching of the fluorochromes. This is particularly problematic when numerous high resolution optically sectioned images are required. In order to further improve the signal strength and reduce photo-bleaching, we are currently working on two major hardware implementations: (1) design and construct an auto-focus, folded optics confocal trans-illumination system (TIS, optics placed below the specimen in Figure 1). Instead of operating in trans-illumination mode, the TIS can be used to increase the excitation intensity and improve the strength of the fluorescent signal (by increasing detector solid angle); and (2) an acousto-optic modulator (AOM) will be installed in the illuminating beam to minimize photo bleaching resulting from the retracing cy-

cle of the scanning laser beam. The AOM turns off the laser beam during the retracing cycle of the two scanning mirrors, eliminating all the non-image producing exposure to the laser beam.

In addition to epi-fluorescent mode, confocal microscopy operated in reflective (back scatter) mode can also be very useful in maize research. For instance, Figure 5 illustrates a back scattered confocal image of the silica deposition within dumb-bell shaped silica cells. The reflective image was obtained simultaneously with the fluorescent image shown in Figure 4. Furthermore, reflective confocal microscopy can be used to study the surface of a specimen (both fresh or fixed and dehydrated tissue). A typical example of a reflective confocal image which shows the surface features of a critical point-dried coleoptile is illustrated in Figure 6.

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The use of confocal microscopy to study the developmental morphology of shoot apical meristems: a procedure to prepare the specimens

--V. R. Bommineni[1] and P. C. Cheng

Confocal light microscopy associated with modern image processing systems can provide information about the spatial arrangement and orientation of cells, their nuclei, and their division patterns within a developing organ, such as a shoot apical meristem. Data obtained from confocal microscopy may also be used to obtain information about changes in the metric relationships of cells during development (such as the initiation of leaf primordia) or following exposure to different metabolic conditions.

We have developed a procedure to prepare the cells in the apical meristem to permit high resolution confocal microscopy.

1. Dissect the meristems and fix in 3:1 (EtOH: acetic acid) fixative for overnight.

2. The tissue is then stained in Feulgen for 20 min. (follow the maize root tip procedure described by Chen, C. C., Can. J. Genet. Cytol. 11: 752-754, 1969).

3. Then the tissue is washed in bleaching solution (which contains 1ml concentrated HCl, 0.4g potassium metabisulfite or sodium metabisulfite and up to 100ml of distilled water) for several hours to overnight with three changes.

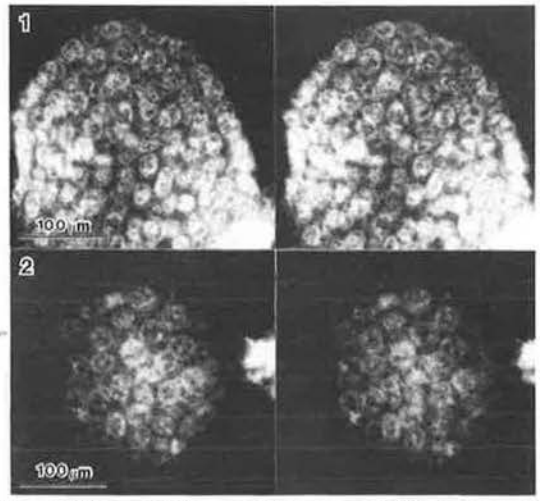
4. Proceed with gradual ethanol dehydration and clear first with 1:1 absolute ethanol: methyl salicylate for 1-2 hours.

5. Clear the specimens in methyl salicylate and store them in refrigerator until use.

6. Mount the specimen in a specially made specimen holder or in a small chamber on a slide (made of a ring of vacuum grease on a microscope slide), add cover slip and gently press the cover glass so that the specimen just touches the cover slip (do not crush!).

7. A modified BioRad MRC-500 laser scanning confocal system attached to a Leitz Orthoplan microscope equipped with a Nikon Fluor 40X objective is used. BioRad GHS filter pack (Excitation filter 514 DF 10 and dichroic beam splitter DR 540LP) is used. Therefore, the 514nm green line of the Ar ion laser is used as the excitation wavelength. An OG550 barrier filter (560nm long wavelength pass) is used for the detector. The resulting optical sections can be projected at different viewing angles to obtain 3D stereopairs on a computer.

A stereo viewer is required to view Figure 1 and 2 which illustrates reconstructed three dimensional views of the apical meristem prepared from a 72 hr imbibed mature seed of waxy (*wx wx*) genotype. Figure 1 illustrates a 20um thick median longitudinal optical section, whereas Figure 2 illustrates 20um deep cells from a top view of the meristem. Note the clear view of the L1 tunica layer (protoderm) in Figure 1 and the systematic and organized pattern of cells in Figure 2.



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Allometric genetics: mapping of gametophyte gene *Ga10*

--Luiz Torres de Miranda and Luiz Eugenio Coelho de Miranda

In MNL 49:71-73, J. Gonella and P. A. Peterson reported linkages of *A2*, *Bt* and *Ga10* but did not calculate the recombination values of *Ga10* with the other marker genes. Since there are data between *A2* and *Bt* with and without the presence of *Ga10* it occurred to us that it is possible to calculate linkages with *Ga10*.

The values of *A2 Bt* linkage can be calculated using the formula in MNL 61:32 and calculating the αs and βs from their Table 1, $46.4^{1+\alpha+\beta} = 79.7$ etc. to $2.8^{1-\alpha-\beta} = 0.4$ from a to d taken from data with and without the presence of the gametophyte factor. This gives a value of p = 0.048 near the 0.061 reported for data without *Ga10*.

We propose the model by least squares.

Ga	p(1+α)	(1-p)(1+α)
ga	(1-p)(1-α)	p(1-α)

As a and c are indistinguishable

$$\left\{[p(1+\alpha)+(1-p)(1-\alpha)] - \frac{a+c}{n}\right\}^2 - \left\{[p(1-\alpha) + (1-p)(1+\alpha)] - \frac{b+d}{n}\right\}^2$$

After deriving and simplification the formula reduces to $\alpha(2p-1)+\frac{1}{2}\left(\frac{b+d}{n} - \frac{a+c}{n}\right) = 0$. Making the same formulation for Bt $\alpha(2q-1)+\frac{1}{2}\left(\frac{b'+d'}{n} - \frac{a'+c'}{n}\right) = 0$ and when q=p+0.048 is the solution giving Ga10 A2 0.088 Ga10 Bt 0.136 and α=0.862.

Mapping pairs of complementary or duplicated loci in the same chromosome

--Luiz Eugenio Coelho de Miranda, Luiz Torres de Miranda, Omar Villela, Sylmar Denucci and Toshio Igue

In one of our commercial selfed lines which we call IAC B we observed that by taking the husks off and uncovering the kernels after the green corn stage, a reddish pericarp color develops that is visible the next day. At maturity it tends to disappear. This line's origin is PD(MS)6x[Tehua x Tx 303(3)](3), the recurrent parent being the once commercial line Texas 303, USA. Two Tuxpan lines, 2 Cateto lines, 1 ETO Colombian line, 1 Yellow Tuxpeno San Luis Potosi line, and more than fifty plants of the ample synthetics IAC Maya, IAC 1 and a double topcross IAC Maya latente pollinated by a (Cateto x ETO) single cross were tested for this character, and no color appeared in these entries. In the IAC Maya latente the color appeared in 27% of the plants. As the color did not appear in the previous double topcross the factor is recessive. From 3000 plants of IAC 1 latente which were subjected to natural field frost (-3.7 C in the grass in a nearby installation) only 25 survived to set seed, and from these 5 had the color, that is 20%. The absence of the factor in our original varieties and its intermediate gamete frequencies in the latente versions leads to the conclusion that the factor is linked to the latente supergene introduced, as described in MNL 56:28-30, into our original varieties using as source Michoacan 21 Comp. 1-104.

On the other hand, based on our first rainbow estimate of latente linkage, *fl1* seemed to be one possible marker for it. By one cross and four backcrosses we obtained the floury-1 versions of 2 Tuxpan lines, 2 Cateto lines, 1 ETO flint line and 1 San Luis Potosi line, derived from the same source as in MNL 61:27-29. These floury versions were crossed to a latente source and used as recurrent parents. In this material we husked the tips of the ears. In the SLP backcrosses there was a segregation for color, so color acted as dominant. It appeared in all its progenies, which were plants selected by means of a Steady State Porometer Licor-1600 apparatus in the previous year. The plants were under severe atmospheric stress by heat and very low relative humidity. The data are presented in Table 1 including also kernel row numbers and the analysis. An

Table 1. Factorial presentation of collected data from back-crosses of recurrent line floury which was crossed to a latente source. Two by two tables, of observed, models who fit the data, expected values calculated by parameters fitted to data by least squares iteratively, by the logarithmic maximum likelihood solution, values of χ^2 p and q values Fℓ1 Krn12, Fℓ1 Krn2 and Fℓ1p2, Fℓ1 p2. Krn is Tr, Fℓ1 normal fℓ1 floury endosperm C colorless pericarp, c colored reddish pericarp on sunlight exposed kernels removing the husks after green corn stage. Sample size n=87. See bottom note.

													C	c					
Fℓ	+	+	+	+	-	-	-	-				Krn	19	9					
C	+	+	-	-	+	+	-	-				krn	39	20					
Krn	+	-	+	-	+	-	+	-									Chi		
	8	13	1	4	11	26	8	16									χ^2		
	Observed						Krn	Espec	krn			Calculated		devi-					cM
	Krn		krn				q	ted	(1-q)			values		ations	p%				values
Fℓ	9		17		=	p	pq		p(1-q)		x 87	8.7	17.7	0.09	p=30.0±10.6		Krn12	Fℓ	34.7
fℓ	19		42			(1-p)	(1-p)q		(1-p)(1-q)			19.5	41.4		q=32.2±10.6		Krn2	Fℓ	37.9
	C		c				C		c										
							1-q		q										
Fℓ	21		5		=	p	p(1-q)		pq		x 87	17.5	8.7	3.32	p=30.0±10.4		p2	fℓ	34.7
fℓ	37		24			1-p	(1-p)	(1-q)	q(1-p)			40.7	20.1		q=33.3±11.0		ℓtp	Fℓ	39.5

Note: An unspecified Fℓ1 Krn linkage can be calculated diretly subtrating the Krn C 2x2 table from the Fℓ C one. The sum of the absolute values of the difference divided by n would give a value of p as 13.8±8.0. With the values in cM in the last rows by differences Krn2 ℓtp is 39.5-37.9=1.6, Krn12 p2 is 34.7-34.7=0.0. Krn2 Fℓ1 distance 37.9 taken from 68 position of fℓ1 puts it in position 30.1 near the position 22 as reported in MNL 61:27-29. So our personal working map reads 20 ℓtp - 22 Krn2 - 30 ℓte - 35 Lsc - 49 B - 68 fℓ1 - 103 Krn12 - 103 p2. Probably the distance fℓ Krn12 is overestimated.

unspecified linkage *Krn Fl1* is readily visible comparing the factorials *Krn* x *C* with *Fl* x *C* (*C* = uncolored) but the data can not be explained by only one pair of alleles. In *Fl C* factorial one sees many more colored (39%) in the *fl1* line than in the *Fl1* (19%) and again the variation can not be explained by only one pair of alleles. The models which explain the data are presented in Table 1, giving nearly perfect fit for *Krn Fl1* and a reasonable one for *Fl1 C*. The sum of the probabilities in each 2x2 factorial is one. To correct it to the population size n=87, we can multiply them by 87 or correct the calculated values in the end. In the case of *Fl Krn* for example, if *Fl1 Krn2* (see MNL 61:27-29) linkage is q, and *Fl Krn12* linkage is p, the joint probability of *Fl1* bringing both together is (1-p)(1-q), and so on. Taking the logarithm likelihood expression including a, b, c and d, differentiating in relation to p and q, and multiplying by minus one to simplify we arrive at

$$\frac{\partial L}{\partial p} = -\frac{a}{p} - \frac{b}{p} + \frac{c}{1-p} + \frac{d}{1-p} \quad \therefore \; p = \frac{a+b}{a+b+c+d}$$

$$\frac{\partial L}{\partial q} = -\frac{a}{q} - \frac{c}{q} + \frac{b}{1-q} + \frac{d}{1-q} \quad \therefore \; q = \frac{a+c}{a+b+c+d}$$

and p=30.0±10 and q=32.2±10, the errors being calculated by Fisher's indirect method (see MNL 62:37-38), with survival equal to 1 for all entries, a very rough estimation, we guess. The inclusion of allometric coefficients did not improve the results.

For *Fl1* and colour we did *Fl1 ltp* recombination value (*ltp* = latente p color) equal q and *Fl1 p2* (*p2* because it is similar to existent *p1*) as p, and *p2 fl1* is p=30.0±10 and q=33.3±11.

In maize x teosinte studies kernel row numbers should be counted and this model applied.

The high frequency, at least in our reported work of these types of situations, suggests that in QTL x RFLP programmes these models should be included in the computer in order not to lose important results.

The systematic occurrence of flavone glucoside factors linked to *Krn* suggests that these blocks may be the triggers for a system of horizontal resistance to pests (see MNL 60:33-34).

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The effect of high sugar content media on callus proliferation and shoot regeneration

----Y. C. Ting and Stephen Schneider

In the summer of 1989 a study of the effect of high sucrose-content media on callus proliferation and shoot differentiation was carried out. The following five callus lines were employed: 81-B5, 88-S5, 87-S4, 85-N1, Cm17-3-54. All of these lines derived from anther culture and they were maintained for at least one year by continuous subculturing. Except for line 81-B5, which was recently cytologically identified to be polyploid, their genomic constitutions were not scrutinized. The morphology of line 81-B5 was creamy and rough-surfaced, while that of the others was green-yellow and compact-surfaced. The media employed were MS and N6 to which 8 g sucrose, 2 mg 6BA, and 2mg NAA were added per liter. There were five dishes (replicates) of callus of each line for each treatment (medium). In each dish, there were 25 pieces of callus and they were about the same in size. Their diameter and weight were not accurately determined. After the calluses had been on the media for four weeks they were examined under a binocular. It was found that lines 88-S5, 87-S4, 85-N1, and Cm17-3-54 had abundant proliferation and embryogenesis. Certain embryogenic areas showed shoot differentiation and recovery of plantlets. However, for line 81-B5 no evidence of embryogenesis was observed even though the proliferation was generally adequate. In view of the above, it seems clear that the in vitro traits such as growth and regeneration of maize callus lines are governed by genotypic background.

Kernel viability of selfed R0 plants

--Y. C. Ting

In the summer of 1988, 43 kernels were obtained from the R0 plants (first generation plants from anther culture) of maize strain 88BC-6. All of these kernels were grown in a vermiculite mixture last spring. However, I found later that only three of them grew into fully developed plants. I was very disappointed. Among the other kernels, five germinated but when the hypocotyls reached about 2 centimeters in length, they stopped growth. Their shoots were never recovered. The remainder never germinated. In view of this, it seems likely that an *Ac*-like element was activated during the differentiation of the R0 plants. This element might become inserted into a certain region of a chromosome and subsequently cause lethal embryos. In consequence of this, the embryos might not grow. On the other hand, if the element was somehow inactivated, the embryos might develop into regular plants. Since obtaining a stable progeny is the prerequisite for the application of plant tissue culture in crop improvement, it is significant to investigate the mechanism of low kernel viability of the R0 plants.

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Mu1-associated *hm1* alleles

--Guri Johal and Steven P. Briggs

A collection of *hm1* mutants from a Mutator background (Briggs, Curr. Topics Plant Biochem. Physiol. 6:59-67, 1987) was assayed for co-segregation of the mutant alleles with *Mu1* homologous sequences. The mutant alleles were characterized by hybridization with the Pioneer RFLP probes PIO200644 and PIO200044, which are 5 cM proximal and distal, respectively, to *hm1*. Both probes could distinguish the alleles in our material, restricting our error in classification to double crossovers (estimated to occur in less than 1% of the progeny). For *Mu1* hybridizations, the DNA was cut with either *Sst*I, *Bam*HI, or

*Hin*dIII, none of which cut *Mu1*. Southern blots were probed with the *Ava*I-*Bst*NI fragment of *Mu1*, isolated from pAB-5 (pAB-5 was obtained from Loverine Taylor). Analysis was simplified by outcrossing progeny in which the Mutator system was inactive, to reduce the number of *Mu1*-homologous fragments. Two mutants were identified in which a unique *Mu1*-homologous fragment co-segregated with the mutant *hm1* allele. No recombination between the co-segregating *Mu1*-homologous fragments and the *hm1*-flanking RFLP probes has been observed in studies of 74 progeny for the *hm1-6561* allele and 91 for the *hm1-10621* allele. Our tentative conclusion is that these co-segregating fragments are *Mu1* insertions in the *Hm1* gene.

Placement of *hm1* on the Pioneer RFLP map

--Guri Johal and Steven P. Briggs

The Pioneer probes PIO200644 and PIO200044 were hybridized to Southern blots of progeny from the cross K61/Pr1 x K61 where K61 is homozygous *hm1 hm2* and Pr1 is homozygous *Hm1 hm2*. The enzymes *Hin*dIII and *Sac*I detected useful polymorphisms. Each plant was inoculated with *Cochliobolus* (*Helminthosporium*) *carbonum* race 1, isolate SB111 and scored for susceptibility. Out of 60 progeny, 6 resulted from single recombination events; there were no double crossovers. Three crossovers occurred distal to *hm1* while the other 3 were proximal. Mapping in larger populations from other crosses indicates that PIO200644 is 9.4 cM proximal to PIO200044 (David Grant, personal communication). Our results indicate that *hm1* is 5 cM distal to PIO200644 and 5 cM proximal to PIO200044.

Isolation of cDNA clones homologous to the *P*-gene flanking regions

--Erich Grotewold and Thomas Peterson

The *P* locus is involved in the synthesis of the phlobaphene-like red pigment found in mature cob glumes and pericarps. The *P* gene comprises a 6.6 kb transcribed region which produces 5 RNAs from the same DNA strand (Lechelt et al., Mol. Gen. Genet. 219:225, 1989). The transcribed region is flanked by two 5.8 kbp homologous repeats in direct orientation; the significance of the repeats in the expression of *P* is not yet known. This report concerns the isolation of cDNA clones homologous to the direct repeats.

During a search for cDNA clones from the *P* gene, several clones homologous to the direct repeats were recovered. An oligo dT-primed cDNA library was constructed using mRNA purified from fully pigmented pericarps (*P-rr*), and 1.8 million independent clones were screened using a 664 bp genomic DNA fragment which codes for the 3' end of the *P*-specific messenger RNAs. This genomic fragment is included within the repeat located at the 3' end of the *P*-gene. In addition to several *P* cDNA clones, we rescued six clones up to 850 bp long coded by the opposite strand from which *P* is transcribed. The 3' end of the transcripts represented by these clones would be 10 bp from the 3' end of the most extended *P* cDNA clones found. The six clones could be divided into two families depending upon the length of their homology with the direct repeat genomic sequence: clones in family I contain 343 bp identical to the direct repeat, while family II contains 393 bp identical to the direct repeat. Beyond the region of identity with the direct repeat sequence, the sequences of the six clones were completely unrelated to the *P* genomic sequence.

From where were these clones transcribed? To answer this, we made a 650 bp probe from the 3' end of one of these clones and used it to hybridize Southern blots of the 34 kbp of the *P* locus previously cloned; hybridization was found only over the 343 bp region homologous to both flanking repeats. Hybridizations of the probe to genomic Southern blots showed homology to at least five other sequences in the maize genome. The same hybridizing fragments were detected using the 664 bp *P* genomic sequence outside the *P* locus. A Genbank search showed no significant homology of the cDNA clones to any known sequence.

When used as a probe on RNA blots, the 650 bp probe from the clone failed to show any signal under conditions in which the *P* transcripts are readily seen. Only very weak signals were seen when a high specific activity single strand probe was used. Therefore, the abundance of these transcripts must be much lower than the *P* transcripts.

We don't yet know the significance, if any, of these transcripts for expression of *P*. However, our results resemble in some ways those obtained for the *Bz2* locus by Schmitz, Theres and Starlinger (MNL 63:60, 1989). In the *Bz2* case, the "anti-sense" transcripts are about 100 times less abundant than the *Bz2* message; however, the "anti-sense" transcripts partially overlap with the *Bz2* transcript.

We thank Susan Allan for technical assistance.

***Ac* transpositions reflected as twin sectors on *P-ovov* ears**

--Prasanna Athma and Thomas Peterson

We have been studying a variegated *P* allele derived from *P-vv* termed *P-ovov*. *P-ovov* specifies orange variegated pericarp and cob, and resulted from excision of *Ac* from *P-vv*, transposition 161 bp towards the 5' end of the *P* gene, and reinsertion in the opposite orientation. In order to study new *P* locus mutants derived from *P-ovov*, we examined a large number of ears produced on plants of *P-ovov/P-ww* or *P-ovov/P-wr* genotype. Among 22,916 progeny ears, 13 ears had recognizable twin sectors on otherwise orange variegated ears. The twin sectors were of several types: red twinned with light orange variegated; red twinned with white; light orange variegated twinned with white; and light orange variegated twinned with dark orange variegated. Kernels from the twin sectors were sown and DNA was prepared from the resulting plants for Southern analysis. We used a combination of probes to detect insertions of *Ac* or other alterations within a 26 kb region of the *P* locus. We recovered progeny carrying the alleles derived from both cotwins of 12 of the 13 twin

sectors. The derivative alleles were grouped into three classes based on their molecular structure:

Class I (2 cases): In these cases, a sector of light orange variegated pericarp is twinned with red in one case and with dark orange variegated in another case. In both cases the light orange variegated sector contains two copies of *Ac* at the *P* locus: one at the original site as in the progenitor *P-ovov* allele, and one at a different site nearby. The red or dark orange variegated cotwin contained only the single transposed *Ac*, in the same genomic restriction fragment as in its twin. These cases appear to have resulted by excision of *Ac* from its original site after replication of the donor locus and insertion into an unreplicated target site. Thus, these cases are analogous to the Type I twin sectors derived from *P-vv*; Type I twins contain *Ac* activity in the red sector (Greenblatt and Brink, Genetics 47:489, 1962; Chen, Greenblatt and Dellaporta, Genetics 117:109-116, 1987).

Class II (5 cases): In these five cases, red pericarp is twinned with either light orange variegated, light variegated, or white pericarp. In all five cases *Ac* was not detected within the red sector. The light orange variegated, light variegated, and white twin sectors contained *Ac* at the original *P-ovov* site, and a transposed *Ac* elsewhere within the region covered by our probes. These cases may have resulted from *Ac* excision from one chromatid after replication of the donor locus and insertion into the target site that has already replicated.

Class III (5 cases): In these cases, white pericarp is twinned with either light orange variegated or red pericarp. In this class, *Ac* transposition appears to be accompanied by deletions or other chromosomal rearrangements at the *P* locus.

Although the structures of *P-ovov* and *P-vv* are quite similar, twin sectors occur at an apparently much lower frequency with *P-ovov* than with *P-vv*. With *P-vv*, sectors of red pericarp are twinned with light variegated pericarp. The red pericarp derives from excision of *Ac* from *P-vv*, while the light variegated pericarp results from the dosage effect of a transposed *Ac* on the timing of sectoring of the *P-vv* allele. In *P-ovov*, the effect of a transposed *Ac* outside the *P* locus may also decrease the frequency of revertant red sectors, but the change is probably not noticeable against the dark orange background conditioned by *P-ovov*. The Class I and Class II twin sectors studied here involve *Ac* transpositions to other sites within the *P* locus. Such intralocus transpositions occur much less frequently than transpositions to sites outside the *P* locus.

Thanks to Susan Allan for excellent technical assistance.

Intragenic recombination at *A1*

--Jennifer Brown and V. Sundaresan

Since *A1* function is required for production of red and purple anthocyanin pigments in the maize plant and kernel, intragenic recombination events between mutant alleles of *A1* restoring *A1* expression can be scored easily. *a1-Mum2*, a *Mu*-induced mutable allele which contains a *Mu1* transposon at nucleotide-100 relative to the *A1* transcription start site (O'Reilly et al., EMBO J. 4:877-882, 1985), and *a1*, a *Dt* induced mutable allele which contains an *rDt* transposon at +1077 (Brown et al., Mol. Gen. Genet. 215:239-244, 1989), were tested for recombination. Neither *a1-Mum2* nor *a1* give *A1* progeny when either allele is homozygous. From crosses of *a1-Mum2/a1* plants with *a1/a1* plants, 11 of 23,477 kernels which had restored *A1* were found. The ratio of genetic to molecular distance is 0.1 cM/kb in this case. McClintock had previously reported a similar genetic result using *a1-m2*, which contains a *Spm* insertion at -100 (Masson et al., Genetics 117:117-137, 1987), and *a1*. Seventeen out of 70,039 kernels from testcrossed *a1m-2/a1* plants were restored for *A1* (McClintock, MNL 39:42-45, 1965). Since the distance between insertions is 1.2 kb, this gives a recombinational distance of 0.05 cM/kb.

When the recombination distance of 0.05-0.1 cM/kb at *A1* is compared to the overall genome value of 3.0×10^{-4} cM/kb (1200 cM per genome - Coe et al., in O'Brien, Genetic Maps, Cold Spring Harbor Lab., pp. 491-507, 1983; and 3×10^9 bp - Galbraith et al., Science 220:1049-1151, 1983) it seems that recombination rates at *A1* are 30-60 fold higher. Similar results have been previously reported for recombination at the *Bz1* locus by Dooner (Genetics 113:1021-1036, 1968) who found a recombination distance of 0.07 cM/kb within the *Bz1* gene. Our data lend further support to the idea that the larger genomes contain highly recombinogenic regions--perhaps the structural genes--interspersed with non-recombinogenic regions (Thuriaux, Nature 268:460-462, 1977).

Synthesis of cytosine methylated DNAs in vitro

--J. Colasanti and V. Sundaresan

In our studies of the effects of transposon activity on DNA methylation it was necessary to devise a simple method to synthesize cytosine methylated DNA. We have found that this can be done by PCR in which 5-methyl-dCTP is substituted for dCTP (Colasanti and Sundaresan, 1990, submitted). The reaction conditions are much the same as for regular PCR except that the extension time had to be increased to 5' for a 1 kb fragment as the enzyme operates less efficiently with the methylated nucleotide, and the yields decrease significantly for amplification of larger fragments. Using this method we could demonstrate that cytosine-methylated DNA is completely resistant to the restriction enzyme *Hin*fI at the concentrations normally used to cut genomic DNA, i.e. 10-100 fold enzyme excess. This would account for the observed resistance to *Hin*fI digestion of the *Mu1* elements in inactive-*Mu* lines (Chandler and Walbot, PNAS 83:1761771, 1986). We find that *Hin*fI at 15000 fold excess will completely digest cytosine methylated DNA, but use of such vast excess of enzyme is impractical in most genomic digests.

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A new *Mu*-induced suppressible mutation

--J. Colasanti, M. Freeling[1] and V. Sundaresan

Recently a *Mu*-induced mutation at the *hcf106* locus has been described which appears to be suppressed when *Mu*-activity is lost (Martienssen et al., EMBO J. 8:1633-1639, 1989). This raises the possibility that other *Mu*-induced mutations may also be suppressible by *Mu* inactivation. We have now found that this appears to be the case with a dwarf mutation isolated in a *Mu* stock. This mutant (MF 6316-21) arose in a self of a *Mu* outcross ear and was observed to segregate at less than 1 in 4 in this ear. In subsequent generations of selfing, the mutation disappeared altogether. DNA from the later generations was analyzed and the *Mu1* elements were found to be methylated, suggesting that *Mu* activity was lost (Chandler and Walbot, PNAS 83:1761-1771, 1986; Bennetzen, MGG 208:45-51, 1987). During the winter of 1988, plants from this line were crossed to active *Mu* lines and the progeny were selfed this summer. The seeds from these selfs were planted. One out of 5 selfs screened so far have been found to segregate dwarf plants, at ratios of 1 in 4, suggesting that the mutation has been rescued by crossing to active *Mu* stocks. Therefore, the suppression phenomenon described for *hcf106* may be representative of a whole class of *Mu*-induced mutations.

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The frequency of *Ac* transposition from a beta-glucuronidase gene in tobacco varies between individual transformants

--Brian H. Taylor, Naoma S. Nelson and Chantel F. Scheuring

Previous studies of *Ac* transposition in dicotyledonous hosts such as tobacco and tomato have shown that there is a high degree of variability in the frequency of *Ac* transposition between individual transformants. For example, in the initial study of *Ac* transposition in tobacco, Baker et al. (PNAS 83:4844-4848, 1986) found evidence for transposition in 4 out of 9 transformed calli. Transposition was assayed by probing Southern blots of transformant DNA with probes corresponding to either the *Ac* element or to the donor DNA. Detection of transposed copies of *Ac* or empty donor sites indicated that transposition was occurring in the transformed tissue. Using a similar approach, Yoder et al. (Mol. Gen. Genet. 213:291-296, 1988) found that 9 out of 12 tomato plants carrying *Ac* exhibited detectable transposition, with some plants exhibiting evidence of multiple transpositions by the same *Ac*. Taylor et al. (Plant Mol. Biol. 13:109-118, 1989) examined 22 tobacco transformants containing *Ac* and found that 9 exhibited relatively high levels of activity, 11 were weakly active and 2 appeared to be completely inactive. While these results clearly indicate that different *Ac* "alleles" in transgenic plants can have very different transposition frequencies, a major limitation of this approach is that Southern hybridization techniques do not provide information on the distribution of the transposition events within the sampled tissue and are not sufficiently sensitive to detect very low levels of activity.

To circumvent these difficulties, a phenotypic assay for *Ac* transposition was developed using the beta-glucuronidase (GUS) gene of *E. coli* as a reporter gene. Recognition sequences for the restriction endonuclease *Bgl*II were inserted near each end of the *Ac* element isolated from the *P-vv* allele of maize by Peterson and Schwartz (MNL 60:36, 1986). The element was then inserted into a *Bam*HI site located in the untranslated leader of a 35S-GUS expression cassette in the *Agrobacterium* binary vector Bin19 (pBI121; Jefferson et al., EMBO J. 6:3901-3907, 1987). When the *Ac* element is present between the 35S promoter and the GUS coding region, the GUS gene is inactive. Excision of the *Ac* element during transposition, however, reunites the 35S promoter and the GUS coding region and the gene is expressed. This results in the appearance of clonally derived sectors of cells expressing GUS that can be detected using either a sensitive fluorometric assay or by histochemical staining with the indigogenic substrate X-gluc (ibid.). Initial studies with this assay performed in the Peacock laboratory in Australia have been reported (Finnegan et al., Plant Cell 1:757-764, 1989).

To assess the utility of this assay as a means of monitoring *Ac* transposition frequency, we generated a series of tobacco transformants containing the *Ac*-GUS gene. Of 26 plants tested, 21 exhibited transposition activity, as determined by the appearance of GUS-expressing blue sectors after staining with X-gluc. Southern analysis of these plants showed that all 21 active plants contained restriction fragments indicative of the complete *Ac*-GUS gene, whereas none of the remaining inactive plants carried a complete gene. Although it was possible to detect transposition in most tissue types, for convenience our initial assays were performed on hand-cut petiole sections 0.2 to 0.5 mm thick. GUS-positive sectors could be readily detected in the vascular bundle region, cortex and epidermis, however, the transposition frequencies in each of these tissue types were often not correlated. Sector sizes ranged from single cells to complete staining of a particular tissue type. No completely stained sections were obtained and sectors extending from one tissue type into another were rare. Activity levels ranged from extremely low (only one sector observed in over 50 sections) to nearly uniform (very high activity levels and large sectors encompassing entire tissues are difficult to distinguish). The overall distribution of plants into low, medium and high activity classifications is shown in Table 1. In general, the apparent activity levels in sections taken from different leaves on the same shoot or different shoots from the same plant were consistent, although occasional differences were noted. No correla-

tion between the copy number of *Ac* and the frequency of *Ac* transposition was observed. Many of the *Ac*-GUS plants discussed above have been in soil for more than a year. In that time a trend toward loss of sectoring in the vascular bundle and cortex region with continuing transposition in the epidermal tissue has been noted. One transformant exhibited this pattern initially; two plants that earlier showed sectoring in all tissues now show sectoring only in the epidermis and several appear to be in an intermediate stage, exhibiting clear zones encompassing most or all of the vascular bundle region in which no sectoring occurs. The reverse phenotype, sectoring in the vascular bundle and not in the epidermis, has also been observed. The numbers of plants exhibiting these phenotypes are indicated in Table 1. The nature of this apparent tissue specificity and the events leading to inactivation of the *Ac*-GUS gene in the vascular bundle and cortex are being studied. Initial analyses of epidermal and total leaf DNA from the plant that exhibited the tissue specific pattern originally have uncovered no detectable differences in restriction pattern or methylation state of the resident *Ac* element.

Table 1. Activity distribution of *Ac*-GUS tobacco transformants.

Activity level	Sectors/section	VB+/E+	VB+/E-	VB-/E+
Low	<10	3	1	1
Medium	10-30	3	-	-
High	>30-uniform	8	1	4

*VB = Vascular bundle region, E = Epidermis. + = GUS-positive sectors, - = no sectors.

The results discussed here indicate that the frequency of *Ac* transposition varies considerably between individual transformants, is not necessarily uniform throughout each plant and may change with time. The transposition frequency of *Ac* in dicots is likely to depend on a number of factors, including the level of transposase present, the methylation state of the *Ac* DNA and, perhaps, factors relating to the specific site of *Ac* insertion in a given transformant. Further elucidation of the influence of these factors on the frequency, timing and tissue specificity of *Ac* transposition will increase our understanding of *Ac* regulation and perhaps lead to ways of optimizing the *Ac* element for gene tagging. The *Ac*-GUS phenotypic assay provides a useful means of monitoring *Ac* element activity in transgenic plants and will also be used to assess the transposition activities of modified *Ac* elements.

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A reexamination of *Ac* transcript quantification

--Sylvia Schein, Reinhard Kunze and Peter Starlinger

In this note we report about recent improvements of the expression analysis of transposable element *Ac* in maize. The only known *Ac*-specific transcript is 3.5 kb long and encodes an open reading frame 807 amino acids long. Remarkably, multiple transcription initiation sites are scattered over about 100 bp and no TATA- or CAAT-boxes are found in the upstream sequence. The abundance of the *Ac*-mRNA was determined to be between 1 and $3x10^{-7}$ of the polyA-RNA (Kunze et al., EMBO J. 6:1555, 1987). Recent estimations show a 2-4 times higher amount depending on genotype and tissue. Assuming that maize cells contain similar amounts of messenger RNA per cell as the monocot *Tradescantia palludosa* (i.e. 0.16 pg, Willing and Mascarenhas, Plant Physiol. 75:865, 1984) we calculated that only one *Ac* transcript per every 20th to 100th cell is synthesized (Kunze, Ph.D. thesis). This low transcription rate could indicate that the transcription event is the rate limiting step for transposition of *Ac*. However, at least two observations are in contradiction with this interpretation:

1) A peculiarity among transposable elements is the "negative dosage effect" of *Ac*: with increasing *Ac* copies in the genome the reversion frequency drops. In plants homozygous for *Ac* about twice as much *Ac* transcript is synthesized as in plants carrying only one *Ac* (Kunze et al., EMBO J. 6:1555, 1987). If transcription initiation of *Ac* is a stochastic event, in the homozygotes twice as many cells should contain one *Ac* mRNA (i.e. one *Ac* mRNA per 10-50 cells). However, such a higher proportion of cells expressing *Ac* is inconsistent with a reduced transposition frequency.

2) In a recent paper it was reported that in *Ac*-free *wx-m9::Ds* plants the *Bam*HI site at the 5'-end of *Ds9* is modified in all cells. In the presence of an active *Ac* these *Ds9* elements are completely cleavable with *Bam*HI, which could be due to a production of the *Bam*HI site by the transposase (Schwartz, Proc. Natl. Acad. Sci. 86:2789, 1989). This interpretation implies that transposase must be present in every single cell, a prediction that is hard to explain with the low abundance of the *Ac* transcript.

Therefore, we reexamined the transcription rate of *Ac* by employing a modification of our quantification procedure: instead of comparing the transcript band-intensity with that of defined amounts of denatured *Ac*-DNA fragments, we used defined amounts of in vitro transcribed RNA from *Ac* fragments as concentration markers on the Northern blots. Unexpectedly, circa 50 times more RNA than DNA had to be applied to yield hybridization signals of equal intensities. This phenomenon was observed with four different *Ac*-probes. A control experiment with sucrose synthase RNA and DNA had the same result, demonstrating that the observed difference in hybridization signals between DNA and RNA is not an *Ac*-specific phenomenon. Since we believe that the in vitro transcribed RNA behaves more similar to the *Ac*-mRNA than DNA fragments, we have to raise our estimate of the abundance of the *Ac*-mRNA in the polyA fraction to ca. $7x10^{-5}$ or roughly one transcript per cell, respectively. In contrast to the former estimate, this value does not conflict with the above two observations.

Properties of *Ac* protein synthesized in *E. coli*

--Siegfried Feldmar, Reinhard Kunze and Peter Starlinger

The 3.5 kb long *Ac* transcript encodes a polypeptide 807

amino acids long, the ORFa protein, which is assumed to code for the *Ac* transposase. The ORFa coding sequence was cloned in a baculovirus expression vector (Hauser et al., Mol. Gen. Genet. 214:373, 1988). It was shown that the recombinant ORFa protein synthesized in insect cells binds specifically subterminal *Ac* element DNA sequences (Kunze and Starlinger, EMBO J. 8:3177, 1989).

To obtain an easy and fast method for expression and analysis of in vitro mutated ORFa protein derivatives we have cloned and expressed the ORFa sequence in *E. coli* using the T7 expression system (Rosenberg et al., Gene 56:125, 1987). The yield of soluble ORFa protein in *E. coli* extracts was too low to detect DNA-binding in gel retardation experiments. However, substantial amounts of ORFa protein accumulate in the pellet fraction after cell breakage and low speed centrifugation. These insoluble aggregates are predominantly composed of recombinant protein. After solubilization in 6 M guanidinium chloride the ORFa extracts were renatured by dilution to relatively low concentration of the denaturant.

We began to study DNA-binding properties of renatured protein extract in gel retardation experiments. These experiments were performed with an *Ac* fragment containing 180 bp from the *Ac* 5' end and an unrelated pUC19 fragment. A protein-DNA complex was formed upon incubation of the renatured ORFa extract with the *Ac* 5' fragment. No complex was observed after incubation with the pUC19 fragment. In competition experiments we have shown that the renatured ORFa protein binds specifically to the 5' end of the *Ac* element as described for the soluble ORFa protein synthesized in cultured insect cells. However, the electrophoretic mobility of the DNA-protein complex is altered. We have also observed a nuclease activity in the renatured extracts which is not yet investigated in more detail.

In conclusion, we have demonstrated that the DNA-binding activity of the ORFa protein synthesized in *E. coli* can be reactivated after denaturation in guanidinium chloride.

Nuclear factors bind to subterminal sequences of the transposable element *Ac*

--Heinz-Albert Becker, Reinhard Kunze and Peter Starlinger

DNA-protein interaction studies revealed that the protein encoded by the transposable element *Ac* can bind to AAACGG motifs in the subterminal DNA sequences, but not to the 11 bp inverted repeats (Kunze et al., EMBO J. 8:3177, 1989). It is known from some other transposons that host proteins are involved in transposition. Therefore we began to search for cellular factors binding to *Ac*. Here we report about additional DNA-protein interactions to those observed by the *Ac* encoded protein.

In electrophoretic mobility shift assays (EMSA) crude nuclear extracts from kernels were investigated for binding activities. 0.2 ng labeled DNA fragments and 2 µg pdIdC as unspecific competitor were incubated with 0.3 µg nuclear protein extract. Three fragments of the *Ac* ends form strong retarded complexes. Fragment 1 contains the 181 5'-terminal nucleotides of *Ac*, fragment 2 contains nucleotides 4195-4419, and fragment 3 consists of the 146 3'-terminal bases of *Ac*.

In homologous competition experiments complex formation of each fragment was strongly reduced by the inclusion of a ca. 10-fold excess of any of these three fragments. Heterologous competition for fragments 1 and 3 was done with two different fragments isolated from pUC19, including the polylinker into which fragments 1 and 3 were subcloned. These two fragments do not interfere with complex formation of fragment 1 and 3. Complex formation with fragment 2 was also not inhibited by the addition of a pUC19 fragment to the binding assay.

When fragment 1 was split at the *Ac* position 75 and both resulting fragments were tested, the fragment containing positions 76 to 181 was much more strongly retarded. Preliminary results from EMSAs with the isolated 11 bp inverted repeat indicate only a weak complex formation. However, no binding was observed with the isolated AAACGG hexamers which can be bound by the putative transposase.

For further characterisation of the observed protein-DNA interactions indirect DNase I footprints were made. After protein binding the incubation mix for the EMSAs was subjected for one minute to DNase I digestion. The reaction was stopped on ice. Separation was done on a low melting agarose gel. Two bands--free DNA and the retarded DNA, which should contain protected fragments--were excised. The DNA was eluted and separated on a sequence gel.

Fragments 1 and 3 both show two protected sequence regions. In each fragment they have a similar distance. We do not yet know if this similar spacing has a functional meaning. In fragment 1 the observed binding positions lie downstream of position 76. This result is in accordance with the observation of the EMSAs. The binding positions are overlapping with *Ac* regions containing the AAACGG motifs. So far no protection of the inverted repeats was detectable under indirect DNase I footprint assay conditions applied.

It has to be investigated if there is an interaction of the nuclear proteins with the *Ac* transposase and if an additional component of the nuclear extracts binds to the 11 bp inverted repeats of the *Ac* element.

DNA methylation of the *Ac* in transgenic tobacco plants

--Birgit Nelsen-Salz and Hans-Peter Doring

Transgenic tobacco plants carrying the complete *Ac* element from maize or an inactive deletion derivative of *Ac* were studied. Plants were transformed via direct DNA transfer methods or via *A. tumefaciens*. DNA of the transgenic plants was examined with a number of different restriction enzymes whose activity is sensitive for C methylation in their target sequence. Thirty CpGs and 26 CpXpGs were analysed up to now. In four different transgenic plants the *Ac* or *Ds* sequences remain completely unmethylated at those methylatable sites which were examined. There was one plant which showed par-

tial methylation at the *Xho*I site. It is interesting to note that the *Pvu*I site at the 5' end of the *Ac* sequence and at least one of three closely spaced *Hpa*II sites at the 3' end of the *Ac* sequence are cleaved and thus are unmodified in complete *Ac* sequences as well as in the internally deleted, inactive *Ac* element. This is different from what has been found for *Ac* or *Ds* elements in maize (Schwartz and Dennis, Mol. Gen. Genet. 205:476-482, 1986; Schwartz, Proc. Natl. Acad. Sci. USA 86:2789-2793, 1989). We conclude that the *Ac* sequence is not a good target for the de novo methylation activity of the tobacco methyltransferase. If the *Ac* sequence used for transformation is methylated at the *Eco*RII sites, this preimposed methylation pattern is not recovered in the transgenic plant. The methylated *Eco*RII sequences are not recognized by the methyltransferase which confers maintenance of methylation patterns.

Chromosome breakage at the *Ds*-induced *sh-m6233* allele

--Christian Korfhage and Hans-Peter Doring

Chromosome breakage at the *sh-m6233* allele was scored in the sporophyte and in the endosperm. Plants heterozygous for *Yg2 C sh-m6233, Ac/yg2 c sh* were examined for breakage events. Chromosome breakage at the *sh-m6233* allele would lead to the loss of the *Yg2* allele and thus to the appearance of *yg2* sectors. Most of the chromosome breaks occurred late in the development of the plant. Normal size *yg2* sectors were 1-2 mm wide and several mm long. Only rarely we observed sectors which comprise 1/8 or more of one leaf or of two or more successive leaves.

The *sh-m6233* allele is caused by the insertion of a double *Ds* structure consisting of two 2044 bp *Ds* elements in the first intron of the *sh* gene. We concluded from previous experiments (Doring et al., Mol. Gen. Genet. 219:299-305, 1989) that the double *Ds* structure is causing chromosome breakage. If the chromosome breaks at the position of the double *Ds* structure, it is expected that the DNA sequences located distally to *Ds* are lost whereas the sequences located proximally are still present.

We analysed the DNA of 17 independent *yg2* sectors. In 4 cases the hybridization to the 5' region of the *sh-m6233* allele was missing and the hybridization to the 3' region was still present. This finding strongly supports the notion that chromosomes can indeed break at the double *Ds* structure. This finding also determines the orientation of the *Sh* locus with respect to the centromere. The 5' end of the *Sh* gene points towards the telomere.

In the DNA of 8 other sectors we did not observe any change in the hybridization compared to non-sector DNA. Apparently, these sectors were caused by a break whose position is distal to the *sh-m6233* allele. In 3 sector DNAs we did not find any hybridization to the *sh-m6233* allele. Probably, these sectors were caused by a break which occurred proximal to the *sh-m6233* allele. In the remaining 2 cases we observed a distal chromosome break plus rearrangements of undetermined nature at the *sh-m6233* allele. It is suggestive that the distal and proximal chromosome breaks are the result of aberrant transposition attempts of the double *Ds* structure.

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Fluoroindole resistance of orange pericarp

--Allen D. Wright

Orange pericarp (*orp1 orp2*) accumulates indole (Wright, MNL 63:61, 1989), probably due to its inability to make tryptophan from indole. If this is the case, the mutant should be more resistant to indole analogs than its normal sibs. Normal plants are able to convert the less toxic 5-fluoroindole into the more toxic 5-fluorotryptophan (Widholm, Plant Physiol. 67:1101, 1981). The mutant, being unable to do so, should not be affected. Thus, mutant plants and normal sibs were grown for 12 days in 5 inch clay pots (1 plant/pot) containing moist gravel then given 0, 0.05 or 0.10 mM 5-fluoroindole in a Long Ashton nutrient solution. The solution was given twice daily by completely flooding the pots, then allowing to drain. The effect of fluoroindole was visually discernible in the normal, but not mutant plants as early as 7 days after the treatments were imposed. Plants were harvested for dry matter 14 days after imposition of the treatment. The fluoroindole effect on dry matter was evident in the normal sib, but no effect was discernible in the mutant. The data are consistent with what may be expected of a mutant defective in tryptophan synthase (B subunit) activity.

Phenotype	mM 5-fluoroindole	Dry weight (mg/plant ± SE, n=5)	(% of control)
Normal	0.00	522 ± 70	100
Normal	0.05	384 ± 54	73
Normal	0.10	315 ± 22	60
orp	0.00	117 ± 30	100
orp	0.05	126 ± 27	107
orp	0.10	113 ± 23	97

Note on EMS pre-meiotic mutagenesis

--Allen D. Wright

Reports of success using chemicals for gametophytic screening for traits such as alcohol dehydrogenase activity (Cheng and Freeling, MNL 50:11, 1976) and herbicide tolerance (Sari Gorla et al., MNL 63:98,1989) no doubt are due to pollen tube gene expression. For this reason, it may be desirable to mutagenize prior to meiosis, thus enabling subsequent chemical selection from a population of gametophytes having the mutation of the tube nucleus reflected in the sperm. The following study was an attempt to determine the treatment conditions required for optimal mutation frequency with pre-meiotic EMS treatment.

Plants of the inbred line A632, 51 to 58 days after planting, were used (cool weather early in the season accounts for the late treatment date). Tassel length at the time of each treatment was estimated by dissecting a similar plant (range: 7-51 mm, all before the reduction-division stage). Leaf tips (one or more leaves, usually starting with the

ninth leaf) were cut below the surface of the EMS solution (usually 0.1%) and left for various times (10 minutes to 168 hours). Fresh leaf cuts were made daily during the course of the treatment due to cell death near the cut edges. Concurrent studies with methylene blue solution indicated considerable daily variation in the rate of solution uptake with faster uptake occurring during warm, sunny days. Pollen from treated plants was crossed on *y su* testers and backcrossed to untreated A632. Unfortunately the testers had poor seed set in many cases, with an average of only 179 tester kernels per treated plant. In one case (ninth leaf, 0.1% EMS, 144 hours) a single *y Su* kernel was found out of 415 test kernels. In the other 13 treatments only *Y Su* kernels were observed. Samples from the backcrosses will be selfed to obtain a better estimate of mutation frequency and the *y Su* will be selfed to determine if the mutation was premeiotic. Further studies, with more controlled environmental conditions during the treatment period, are warranted.

Isolation of nucleic acids from pollen grains

--Gurjal Madhavi Reddy and Ed Coe

As a step towards developing a simple new method for analyzing the plant genome, we have developed a technique for isolation of nucleic acids from pollen grains, which contain a haploid genome. Thus the production of two types of pollen grains either by use of markers or by use of B-A translocations, followed by their separation and isolation of nucleic acids would allow us to analyse exactly half the number of chromosomes of the sporophyte. The isolation methods used for leaf tissue when directly used for isolation of nucleic acids from pollen grains yielded very little to no DNA. In the few successful instances of moderate yield, the crude extract contained starch, proteins and yellow pigments and was extremely resistant to further purification.

To obtain a more pure DNA preparation from pollen the extraction medium was supplemented with reagents capable of suppressing the precipitation of starch.
CTAB extraction buffer: 1% CTAB, 1M Tris-7.5, 5M NaCl, 1M EDTA-8.0, 0.001 mg/ml protease K, dH_20.
SDS extraction buffer: 20% SDS, 1M Tris-8.0, 5M NaCl, 1M EDTA-8.0, 0.001 mg/ml protease K, dH_20.
For 1000mg of pollen 10 ml of extraction buffer was used. Isolation was done using the following procedure.

1. Grind 1000 mg of fresh pollen grains with a mortar and pestle using liquid nitrogen, into fine powder.
2. Immediately after all the liquid nitrogen has evaporated add 5 ml of the extraction buffer and continue to grind and transfer into a 15 ml polypropylene tube. Add 5 ml of the extraction buffer to the mortar and transfer the remaining ground pollen into the polypropylene tube. Vortex briefly, or mix several times by inversion.
3. Incubate for 60 min with continuous gentle rocking at 60 C.
4. Remove tubes from oven, wait 4-5 minutes and add 4.5 ml phenol/chloroform/isoamyl (25:24:1). Rock gently to mix for 5 min.
5. Spin in table top centrifuge for 10 min.
6. Pour off the top aqueous layer into 15 ml polypropylene tubes. Add 4.5 ml chloroform/isoamyl (24:1) and rock gently for 5 min.
7. Spin in table top centrifuge for 10 min.
8. Pipette off top aqueous layer into 15 ml polypropylene tubes. Add 1.8 ml of NaCl and 1.5 ml of CTAB/NaCl solution and incubate at 65 C for 20-30 min.
9. Add 4.5 ml of chloroform/isoamyl and rock gently for 5 min.
10. Pipette off the top aqueous layer and add 5 ml of NH_4OAc. Put the 50 ml polypropylene tube containing the aqueous solution at -80 C for 10 min and then add 25 ml of cold EtOH. Mix by gentle rocking.
11. Remove nucleic acids with a glass rod (or centrifuge for 10 min and then decant and vacuum dry for 1-2 hrs) and dissolve in 500 ul of T.E. (10 mM Tris-8.0, 1 mM EDTA-8.0).

To get rid of the RNA and obtain only DNA the aqueous solution (in step 10) was treated with RNase A and RNase T1 for about 30 min before precipitation with EtOH. Approximately 0.4 ug/ul of DNA was obtained by these methods. Care was taken to isolate nucleic acids rapidly and to store the samples at 4 C to avoid denaturing of DNA.

Mechanical separation of aborted and normal pollen grains

--Gurjal Madhavi Reddy and Ed Coe

It is known from Bryan Kindiger's work (Kindiger, Beckett and Coe, manuscript in preparation) that heterozygous and homozygous B-A translocations produce two types of pollen grains - a starch-filled normal class and a smaller starchless aborted class that is different for each translocation. Separation of aborted and normal grains would pave the way for further work on the haploid genome. Separation of aborted pollen and normal pollen was done using a series of sieves with openings 297, 210, 177, 150, 125, 106, 88, 62 and 53 u. The pollen obtained from hypoploids as well as heterozygous B-A translocations, containing 50% and 20-25% aborted pollen grains respectively, was allowed to pass through the series of sieves placed in decreasing order of their pore size. Sifting was done by gently rocking the sieves allowing the pollen grains to separate on the basis of size.

It was observed that separation of aborted and normal pollen was better with pollen dried at room temperature than with vacuum dried pollen or fresh pollen. Separation of aborted and normal pollen is good for pollen that has been left at room temperature for 3-4 hrs after collection as the aborted starchless grains shrivel faster than the normal starch filled pollen grains, increasing the difference in size. Further removal of normal pollen from the fraction containing 85-90% aborted pollen obtained upon sifting was achieved by subjecting the pollen mixture to vibrations using a seed counter. The last fraction so collected contained 95% aborted pollen grains. Not all of the aborted pollen grains present in the original starting fraction were recovered by this method, but the separated fraction had 95-97%

aborted pollen grains, good enough separation to expect to get DNA for further analysis.

The NCS2 mutant mitochondria exert a pleiotropic effect upon chloroplast structure and function

--D. Roussell, K. Newton, T. Troyan, G. Bullerjahn, D. Miles, M. Lauer, and S. Pallardy

As previously reported (Thompson and Newton, MNL 1987), both the mitochondria and chloroplasts in the pale-green sectors on NCS2 leaves are structurally abnormal. Electron micrographs of cells from mutant sectors show reduced inner membranes (cristae) in mitochondria and reduced thylakoids and grana stacking in chloroplasts. Our initial hypothesis to explain the altered mitochondrion's pleiotropic effect upon the chloroplast was that photodamage had occurred. Yet, even plants grown under very low light conditions (4UE) showed evidence of striped sectors (pale and very pale-green) on the leaves. The chloroplasts have therefore probably not undergone photodamage, but have instead either developed abnormally or arrested at some stage of normal development.

Further characterization of these altered chloroplasts has concentrated on functional assays. Fluorescence induction kinetic analyses have identified events after PSII, PSI/electron transport, as abnormal in NCS2 sectors. Thylakoid protein profiles of the PSII complexes were similar to controls. However, the CP1 protein of the PSI complex was reduced in chloroplasts from the very pale-green sectors as compared to the adjacent fully green or normal controls. These results suggest that the PSI complex is altered in the chloroplasts of the NCS2 sectors. We have additionally determined that these pale green sectors fix CO2 less efficiently than adjacent fully green sectors on NCS2 leaves.

Mutability of *ij-ref*

--Ed Coe and Chang-deok Han

We have found high mutability of the reference allele of *ij* when particular background constitutions are brought together. The mutability is much higher (i.e., more frequent) than the occasional green sectors on iojap plants reported previously (Coe et al., Amer. J. Bot. 75:634, 1988). Some explanation of the requirements for recognizing mutability, and some prior observations pertinent to the new observations, may be desirable. Because the common iojap phenotype in many backgrounds includes white striping on the green leaf field, green sectors can be recognized only on plants that display a uniform pale field of expression, namely the pale leaf color termed "grainy" that is typical of iojap plants in the background of inbreds Tr and Ky21 (see Coe et al., 1988). In Tr background (but not Ky21), clonal green sectors are seen on 10% or more of plants, typically only one sector on a plant. In two cases in Tr, in fact, a green sector entered the tassel and back-mutations of *ij* to + have been recovered. In Ky21, where green sectors would be recognized, none have been seen. In another background in which green sectors might have been recognizable, Oh51a, most seedlings are pure white or nearly so and do not usually survive; if green sectors occur on Oh51a iojap seedlings, they are infrequent.

The high mutability appeared in experiments intended to study the inheritance of the background-dependent expression of iojap. Several backgrounds carrying iojap (+/*ij* x +/*ij*) were intercrossed and the F1 plants were backcrossed with parent stocks carrying *ij* (male parents either +/*ij* or *ij ij*). Surprisingly, among the iojap plants from backcrosses of Oh51a/Tr x Oh51a most of the segregating "grainy" plants showed high mutability. In leaves at the flowering stage hundreds of small sectors (1-10 cm long and 0.5-4 mm wide), were evident. Close study of F1 iojap plants of Oh51a/Tr and its reciprocal, Tr/Oh51a, also revealed mutability at a lower frequency. When the backcross had been made with Tr instead, low mutability was recognizable but confident classification was not possible.

We assume that in the cross and backcross there has been an activation of a mobile element, inasmuch as neither inbred background itself shows mutability. It is reasonable, in the absence of germinal products, to assume that the sectors are back mutations at the locus like those in Tr background, from which germinal reversions have been isolated. Tests of iojap plants for activation of receptors of *Ac* and *Spm*, carried out in 1980, were negative. Tests of these and other systems in the "activated" material have not yet been done.

Another proposal on the action of the *ij* gene and its consequences in phenotypic diversity

--Chang-deok Han and Edward H. Coe, Jr.

In spite of genetic evidence that a mobile element might reside in the *ij* locus (e.g., high rates of reversion), the striping pattern of the *ij* plants is highly non-clonal and diverse in a background-specific manner (Coe et al., Am. J. Bot. 75:634, 1988). The stripes per se might be explained by epigenetic changes (e.g., methylation or interaction with modifier genes) of the mobile element. Neither epigenetic change alone nor mutation explains well the way that the defective tissues are expressed in *ij* plants (see Coe et al., Corn and Corn Improvement, pp. 179-180, 1988). Especially, distribution of maternal-exception seedlings on the ear is highly polarized and position-dependent.

Further extensive studies on *ij* and maternal-exception seedlings grown even in darkness show that *ij*-affected plastids do contain the transcripts of the plastid genes, and the relative content of the plastid DNA is maintained at the normal level (Han et al., manuscript in preparation). Briefly, additional, new transcripts are detected, and some of the normal transcripts are not found or are barely detectable. The transcripts of different genes are present from barely detectable levels up to the normal level. Also, the transcripts from individual monocistronic or polycistronic genes are differentially accumulated. Our data strongly suggest that *ij*-affected plastids may undergo altered processing or stability. Surprisingly, *ij* and maternal-exception progeny show almost identical patterns and levels of the transcripts. Therefore, the aberrant pattern of the transcripts is not correctable (by +/*ij* constitution) in the subsequent generation. However, aberrations of the plas-

tid transcripts per se are not unique to the *ij* mutant because we found almost identical transcript patterns in the *w1* mutant (Han and Coe, manuscript in preparation).

Since we found that the *ij*-affected plastids still maintain transcriptional activity, we feel that it is necessary to offer another possible explanation for the diverse and non-clonal expression in *ij* plants. White stripes on leaves of *ij* plants are more frequently in the margins than in any other parts of the leaves. A hypothesis of 'ribosome-loss' (Walbot and Coe, 1979) might explain the 'position-dependent' phenotypic expression of the *ij* plant. Once the biogenesis of the plastid ribosomes is defective, ribosomes are limited. The ribosomes 'run out' during leaf development, especially at the regions of leaves where rapid cell division and expansion occur. The clonal analysis of maize leaves (Poethig, 1984) indicates that clonal sectors in the margins of leaves are much broader than in the middle of leaves. Therefore, it is likely that 'depletion' of ribosomes could be more severe in the margins than in any other parts of leaves. Phenotypic expression of *ij* is predominantly noticed in the margins of the leaves.

However, it is somewhat difficult to explain the diverse genetic background-dependent phenotypes of the *ij* mutant by the 'ribosome-loss' hypothesis alone. Based on the data on the transcripts of the *ij*-affected plastids, it is reasonable to argue that the 'ribosome-loss' event might be a consequence of alterations in the plastid transcripts. Therefore, the background-dependent expression of the *ij* phenotypes can be paraphrased in the following way: 'white plastids', a phenotypic marker of 'ribosome-loss', are generated via the alterations in the transcripts, in a genetic background-dependent manner. It is possible that there is a temporal difference between the immediate effect of the *ij* gene, whatever it might be, and the phenotypic defect ('white plastid'). This possibility already has been suggested by Coe et al. (Am. J. Bot., 1988), who postulated that "the *iojap* defects do not arise until after a leaf differentiates". Also, the temporal dependency of the *ij* defect might be an explanation for the positional distribution of the maternal-exception progeny on the ear of the female parent. The maternal-exception albino seedlings are almost exclusively located in the apical region of the ear, which matures at a different time than the basal region. This implies that the defect, probably not the immediate effect, of the *ij* mutant could be sensitive to a temporal program during plant development.

The simplest explanation for the complex phenotypic expression of the *ij* mutant during plant development is that the action of *ij* resides in the gate of the plastids that is required for communication between two genomic compartments, nucleus and plastid. Once the communicating devices of plastids are defective or defaulted, the plastids might no longer follow the developmental processes (e.g., cell lineage). However, we found that *ij*-affected plastids contain the mature sizes of the nuclear encoded photosynthetic proteins and still maintain the normal level of the genomic DNA. Therefore, the defective plastids still maintain the normal transport and processing of imported nuclear products. Taking into account the fact that the aberration in the pattern and level of the plastid transcripts is persistent in the subsequent generations, there is a possibility that a 'plastid gate' (e.g., signal transduction pathway) that is necessary for temporal and spatial expression of the plastid genomes could be altered by the *ij* mutant. The 'developmental kinetics' (i.e., temporal difference) from the action of the *ij* gene to 'white plastids' (phenotypic defect) could be determined by the sensitivity or efficiency of the plastids to receive and relay the developmental signal from the cytosol or nucleus, and even by the rate of the 'ribosome-loss' process via the alteration of the transcripts. The efficiency and activity of the 'downstream' processes could be different among the different genetic backgrounds. Also, the plastids in the tissues with the prolonged growth time (e.g., the tip of the ear) and in the leaf margins showing rapid cell division and elongation are more vulnerable to the defects of the mutant.

The hypothetical, even though highly plausible, pathway in which the *ij* gene might be involved could take a pivotal role in fine-tuning the developmental switch to determine photosynthetic tissue (mesophyll or bundle sheath cells) or nonphotosynthetic tissue (epidermal cells) during leaf development.

Severe reduction of plastid DNA is characteristic of *w2* mutant

--Chang-deok Han and Edward H. Coe, Jr.

The copy number of plastid genomes per cell increases dramatically during leaf formation and maturation of the leaf tissues (Lawrence and Possingham, Plant Physiol. 81:708, 1983; Miyamura et al., Protoplasma 133:66, 1986). It is still puzzling what is the significance and role of the multiplicity of plastid genomes in the expression and regulation of the plastid. A possible explanation for the multiplicity of the plastid genome has been proposed in terms of cellular demand for plastid ribosomes during leaf development (Bendich, BioEssays 6:279-282, 1987).

During studies on 'class II' albino mutants (Robertson, J. Hered. 1978), on which very little work has been done beyond the early studies by Lindstrom (1921) and Demerec (1924), we found evidence that the albinism of *w2* plants might be due to severe reduction of the plastid DNAs. The relative content of the plastid DNA was measured by Southern blot analysis (Figure). Total cellular DNAs were extracted from the seedlings grown under very dim white light. *Bam*HI-digested total cellular DNAs were probed at the same time with the nuclear rDNA, pGmr1, and 1.2 kb plastid *Bam*HI fragment containing *psa*B. The signal intensities of the autoradiogram, exposed without an intensifying screen, were measured with a densitometer. After normalization with the hybridized signals of the nuclear rDNA, we found that the relative content of the plastid DNAs of the mutant decreases 20 fold. The general patterns of the transcripts of the plastid genes in the *w2* + and *w2 l* mutants are relatively unaltered and the levels of the transcripts are uniformly reduced 10 - 20 fold. Since there is a good correlation between the levels of the transcripts and the relative content of plastid DNA, the limited expression of the *w2*-affected plastids might be due to the shortage of templates of the plastid

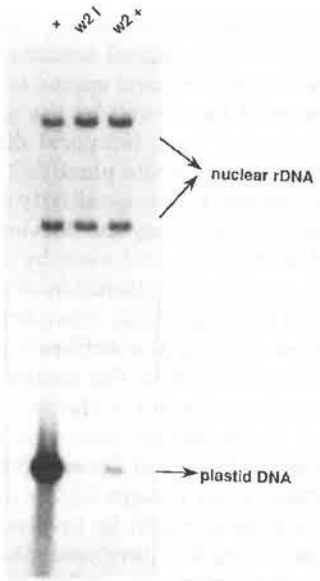

Figure. Southern analysis of the nuclear rDNAs and 1.2 kb plastid DNA containing *psa*B in *w2* + and *w2 l* seedlings. The seedlings were exposed to very dim light (0.01 umol/m^2.sec) for 1 day after 5 days darkness. Total cellular DNAs were extracted from the mutants (*w2* + and *w2 l*). 5 ug of the DNAs were loaded in each lane. '+' indicates the normal siblings. The probes for the nuclear rDNA and plastid DNA were hybridized at the same time.

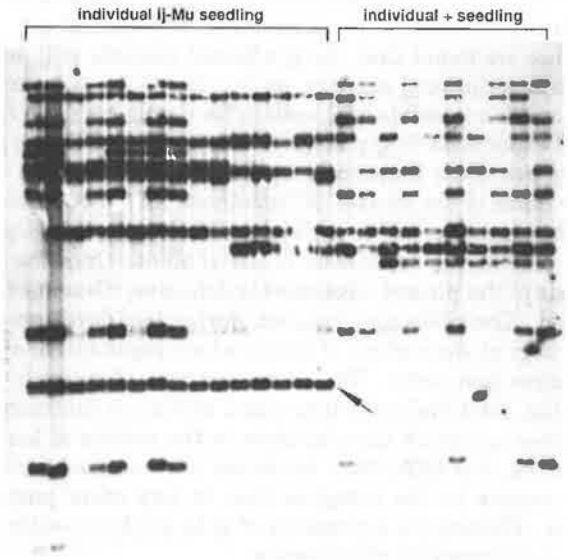

Figure. Southern analysis of the *ij-Mu*1* plants with *pA/B5* (internal sequence of *Mu1*). Around 6 ug of total cellular DNA from individual seedlings were digested with *Sst*I enzyme that does not recognize the DNA sequence of the *Mu1* transposon. The blot was probed with an internal DNA sequence of *Mu1*. A unique *Mu*-hybridizing band that is present only in *ij-Mu*1* plants is marked with an arrow.

genes. We propose that maintenance of a minimum number of plastid genomes is necessary or required for the expression of plastids. Current studies are directed to ask whether the severe reduction of the plastid DNA in the mutant is tissue-dependent or is due to failure of the plastid division. Also it is worthwhile examining whether *w2* also affects the level of mitochondrial DNA.

Progress on *Mu* tagging of *ij*

--Chang-deok Han and Edward H. Coe, Jr.

In the last MNL (63:65), we reported one *ij* mutant from Robertson's *Mu* background, which met the following genetic criteria: first, all the selfed progeny of the backcrosses of the *ij-Mu* plant to inbred Ky21 segregated for *ij* phenotype; secondly, the backcross progeny showed 1:1 segregation of *ij*-linked RFLP markers from the *Mu* and *ij-ref* parents; lastly, allelism tests confirmed that the new *ij* gene from *Mu* background is allelic to *ij-ref*.

To find a *Mu* element that cosegregates with the *ij-Mu* plants, we performed Southern blot hybridizations with an internal sequence (pA/B5; from Dr. Loverine Taylor) of *Mu1*. 47 *ij-Mu* and 19 normal plants from the selfed progeny after 2 consecutive backcrosses to inbred lines Ky21 and Mo17 were examined. In a preliminary experiment, DNA from each of 14 *ij* and 10 normal seedlings was digested with each of 5 different restriction enzymes that do not cut *Mu1*, *Eco*RI, *Hin*dIII, *Xba*I, *Sac*I, and *Sst*I (an isoschizomer of *Sac*I). *Eco*RI and *Hin*dIII generate 2 bands unique to *ij* seedlings. One *Mu*-hybridizing band cosegregated with the *ij* seedlings on Southern blots of *Sst*I, *Sac*I, or *Xba*I-digested genomic DNA. Using *Sst*I or *Sac*I, an additional 33 *ij* and 9 normal seedlings were examined. All 47 *ij*-Mu plants examined with the *Sst*I DNA blots contained the unique *Mu* fragment that was absent in all 19 normal plants (see Figure). Our data suggest that the DNA containing a *Mu* transposon might be located at or near the *ij* locus. The verification whether the *Mu* resides in the *ij* locus needs further characterization of the following materials: 1) genetic and molecular studies on another *ij-Mu* plant from the second screening, 2) molecular studies on germinal revertants of the *ij-ref* allele (Coe et al., 1988; see Coe and Han, MNL, this issue).

Suppressible expression of *ij-Mu*1* plants

--Chang-deok Han and Edward H. Coe, Jr.

The new *ij* mutant from Robertson's *Mu* background that we described in this issue shows unusual phenotypic expression during plant development. The '*ij*-like' stripes progressively disappear as the *ij-Mu*1* plants grow. Since there is precedent for such epigenetic switch of a *Mu* element in *hcf106* (Martienssen et al., EMBO J. 8:1633-1639, 1989), the gradual loss of the phenotypic expression of the *ij-Mu*1* mutant might be due to 'modifiable status' (e.g., methylation or interaction with modifier) of the resident *Mu* element.

A most interesting observation is that the stripes almost disappear in the 6th or 7th leaf in almost every mutant that manages to grow up to mature plants in the greenhouse. Taking into account the fact that the embryo of mature seeds already contains 5-6 leaf primordia, if, indeed, gradual loss of the phenotypic expression of the mutant were due to the epigenetic switch of the resident element, the epigenetic modification might be subject to the developmental processes of the plant. A relationship between developmental processes and such reversible genetic change in transposable elements already has been implied in the early work of McClintock on *Spm* elements and recently

has been characterized at the molecular level (for review see Fedoroff et al., BioEssays 10:139-144). Further characterization on the *ij-Mu*1* mutant should be followed up to verify the hypothesis.

"Preferred Set" of RFLP probes

--Jack Gardiner, Dave Hoisington, Ed Coe, Shiaoman Chao and Susan Melia-Hancock

A set of 120 public-domain probes has been selected, based upon clarity of definition of bands, demonstrated polymorphism, and spacing on the linkage map. This Preferred Set will provide a baseline series of probes that should aid and simplify characterization and mapping, and is the series we intend to use in a core map (Hoisington and Coe, Stadler Genetics Symposium, in press) against which defined-function probes, physical points, and other traits are placed. The refined verification and standardization of this set is now largely completed; probe distribution has been slowed while this process was carried through.

As part of the characterization and standardization of the probe set, we have generated a new F2 of Tx303 x CO159 and will map the Preferred Set with this F2. The F2 plants were selfed, and F3 pooled populations will be produced to derive an immortal F2.

The Integrated Mapping Project: Interval mapping of visibles with RFLPs

--Ed Coe, Dave Hoisington, Shiaoman Chao, Susan Melia-Hancock and Jack Gardiner

In the spring and summer of 1989, families segregating for about 125 visible factors (naked-eye polymorphisms, NEPs) have been grown, classified, and sampled for RFLP characterization. Selection of NEPs to be interval-mapped was made primarily as follows: (1) map location has been previously defined; (2) the utility of the factor as a marker is established; and (3) whenever possible a multiple-factor F2 family in cis arrangement was available from the Stock Center or other source (we greatly appreciate the advice, encouragement and help of Earl Patterson in making the selections and supplying seed of most of these materials from the Stock Center collection). Some additional viable seed or seedling traits have been included toward the potential of adding to the bank of useful markers. The accompanying list identifies factors that are currently projected for interval mapping, plus isozymes that we plan to incorporate as well. We hope to be able to interval-map a major part of this ambitious list. Our intention has been to make a first-priority mapping selection of NEPs, not to exclude anyone's favorite locus on assumption that someone else would map it.

Loci (NEPs and Isozymes) Projected for Mapping

Chromosome 1: *sr1 vp5 zb4 ms17 ts2 P1 dek1 nec2 as1 rs2 hm1 Amp2 br2 br1 Vg1 fl Mdh4 an1 id1 bz2 ad1 Kn1 Adh1 D8 gs1 Phi1 vp8 Ts6 bm2*

Chromosome 2: *ws3 al1 lg1 gl2 d5 B1 gs2 sk1 Les1 wt1 fl1 ts1 ba2 v4 w3 Ht1 Ch1 spt1 whp1*

Chromosome 3: *g2 E8 cr1 Cg1 d1 ra2 cl1 rt1 Lg3 Rg1 gl6 ys3 ts4 pm1 vp1 ig1 lg2 ba1 na1 a3 Mdh3 a1 sh2 et1 ga7 h1*

Chromosome 4: *Aco1 Ga1 Ts5 la1 fl2 st1 su1 bt2 zb6 gl4 Tu1 j2 gl3 o1 c2 dp1*

Chromosome 5: *Pgm2 Mdh5 am1 anl1 lu1 gl17 a2 vp2 ps1 bm1 bt1 v3 bv1 td1 ae1 pr1 gl8 lw2 ys1 v2 eg1 sh4*

Chromosome 6: *hcf26 Pgd1 po1 l11 rgd1 Enp1 w15 l12 y1 l10 si1 pb4 wi1 pg11 Dt2 Pl1 Bh1 su2 sm1 Pt1 Hex2 py1 w14 w1 Idh2 Mdh2*

Chromosome 7: *Hs1 o2 y8 in1 v5 vp9 ms7 o5 ra1 gl1 Tp1 sl1 ij1 bd1 Pn1*

Chromosome 8: *Bif1 ats1 fl3 gl18 nec1 pro1 v16 ms8 j1 v21 Idh1*

Chromosome 9: *Dt1 yg2 C1 sh1 bz1 l7 baf1 lo2 w11 wx1 d3 pg12 ar1 v1 ms2 gl15 bk2 v30 Wc1 Bf1 bm4 da1 Acp1*

Chromosome 10: *sr3 oy1 Og1 y9 zn1 du1 bf2 Glu1 nl1 li1 ms10 Tp2 g1 cm1 R1 Lc1 Mst1 l1 w2 o7 l13 sr2 ms11*

To maximize information and statistical accuracy with minimal laboratory effort and cost, it is preferable to carry out molecular probing on a selection of plants rather than on all members of the F2 (see Hoisington and Coe, Stadler Genet. Symp., in press). The interval mapping approach, in simplest terms, is to select the recessive class for the marker (the mutant if the trait is recessive, the normal if the trait is dominant) in an F2 segregating for the trait and for whatever RFLP loci along that chromosome inherently segregate in the family. RFLPs in the DNA of the recessive individuals are probed to ask which RFLP alleles have stayed together, compared to checks in the dominant class. The interval that has stayed together will have the highest probability of containing the marker. A particular efficiency of the F2 design is that each F2 plant characterized by the molecular probing carries two strands, either or both of which may show recombination between the marker and RFLP loci at increasingly distant points on the map: one plant characterized by probing means two strands tested.

We anticipate that interval mapping against the core map of RFLPs will be advanced during the spring of 1990, and that a considerable integration across the genome should be available by the next Newsletter.

The Integrated Mapping Project: Physical mapping

--Ed Coe, Dave Hoisington, Shiaoman Chao, Jack Beckett, Susan Melia-Hancock, Shirley Kowalewski and Jack Gardiner

Among the most powerful mapping methods are those that employ defined deficiencies or the equivalent to parse the genome or its parts into ordered segments. For example, monosomics generated by *r-x1*-induced non-disjunction in the embryo sac divisions were used in maize to locate genes and RFLP loci to chromosome (e.g., Helentjaris et al., PNAS 83:6035, 1986). B-A translocations generate deficiencies for part of a chromosome arm, and each breakpoint on the same arm allows mapping of genes into parsed segments, i.e., a locus will lie between one breakpoint and the next breakpoint on the arm (e.g., Lin, Genetics 100:475, 1982; Beckett, 1990, in press). Every defined deficiency provides a point of reference on the physical map. For physical mapping of RFLPs, we are produc-

ing and analyzing segmental deficiencies from each B-A translocation available. In addition, we are using a strategy involving the reciprocal translocations, described below (Hoisington and Coe, Stadler Genet. Symp., in press), to produce and analyze segmental deficiencies across the genome.

The approach might be called 'tertiary monosomy', as it employs *r-x1*-induced nondisjunction to generate losses of a translocated chromosome. The design is to cross the translocation stock onto *r-x1* stock, and to cross the F1 plants by recessive seedling or plant markers that are located on one of the translocated chromosomes. Selected recessive plants will include ones that have lost the translocated chromosome on which the test gene is located, and the translocated segment of the other chromosome. Diagrammatically, the following represents the F1 for a translocation between chromosomes 1 and 2, in which the gene *A*, affecting a plant trait, is near the end of one arm of chromosome 1:

```
1111o1111111111111A11   222222o22222222222      r/r-x1
1111o1111112222222      222222o22221111111A11
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This F1 will generate eggs deficient for *A* for either of two reasons. Either the whole chromosome 1 is lost, or a translocated chromosome bearing that segment of 1 translocated with 2. The progeny from crosses by *a* will express the recessive gene, and will be either the monosomic or the tertiary monosomic. When RFLP probing is done for loci on chromosomes 1 and 2, the deficiencies in each of the two chromosomes can be defined, not only parsing both chromosomes for the deficiency they generate but pinpointing the translocation breakpoint on each of the two chromosomes.

We are now screening a pilot study, using a series of 13 *wx* translocations crossed onto *r-x1* and testcrossed with *yg2* and *Bf1* (and several other markers on chromosome 9). Tertiary monosomics occur, albeit at a low rate (1 per 1000). Leaf samples are to be collected from them when they grow large enough, and RFLP probing will be carried out. An earlier pilot produced a small sample of cases, from which preliminary probings showed some of the expected events.

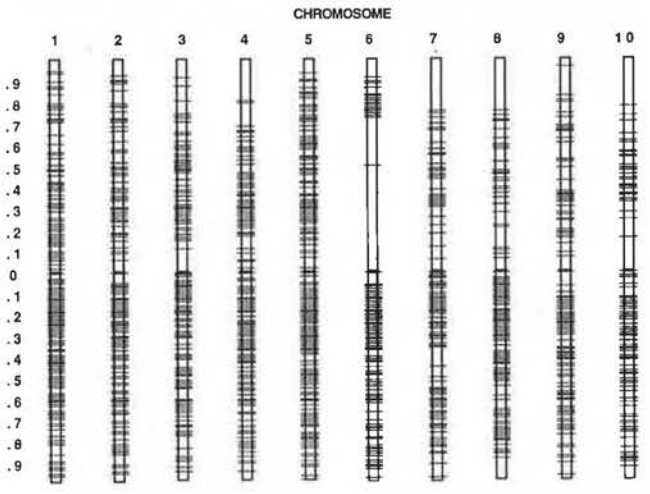

Figure. Breakpoints of the reciprocal translocations.

In the summer of 1989, we planted samples of all 879 of the translocations from the Stock Center (obtained with the enthusiastic help of Earl Patterson) and achieved crosses of over 800 onto *r-x1* stocks. As testcrossing and screening proceed, we hope to be able to define as many as possible of the deficiencies produced with these translocations. The accompanying figure shows the distribution of cytologically determined breakpoints across the genome. Whether 800 translocations (1600 breakpoints) can be defined, or only as few as 400 or 500, each translocation characterized will represent reproducible physical breakpoint locations on two chromosomes, at a wide distribution of physical locations.

The hypoploid frequency of progeny from crossing B-A translocations in four inbred backgrounds

--M. Katsuta and E.H. Coe

B-A translocations are a powerful tool to produce plants that have a chromosome arm deficiency. When a heterozygous B-A translocation pollinates a normal female, 25% hypoploid embryos with hyperploid endosperm and another 25% hyperploid embryos with hypoploid endosperms can be expected theoretically. However, Roman (1948) found that the frequency of hypoploid progeny shows a difference between endosperm and embryo, which he explained as due to preferential fertilization. Also, Carlson(1969) reported that the tendency for preferential fertilization depended on genetic constitution of the female-parent testers.

In this study we attempted to evaluate the effect of genetic background of B-A translocations on the frequency of hypoploid progeny. We used 17 B-A translocations which were backcrossed up to five times with four inbred lines, A619, A632, Mo17 and B73. They were crossed on appropriate testers in 1989, and the hypoploid frequency of endosperm and embryo was determined from the recessive characters of kernels or of seedlings on sandbench tests. Materials are shown in Table 1.

Table 1. List of B-A translocations and testers.

	Times backcrossed by inbreds[1]				Marker gene of tester	
B-A translocation	A619	A632	Mo17	B73	endosperm	embryo
TB-1La	BC5	BC5	BC5	BC5	*bz2*	*bz2*
TB-1Sb	BC5	BC5	BC5	BC5	*up5/ dek1*	---
TB-3La	BC5	BC5	BC5	BC5	*a1/sh2*	---
TB-3Sb	BC5	BC5	BC5	BC5	*c1*	*c1/d1*
TB-4Lc	BC5	BC5	BC5	BC5	*c2*	*c2*
TB-4Sa	F1	BC5	BC5	BC5	*su1*	---
TB-5La	BC5	BC5	BC4	BC5	*lw/pr*	*gl8*
TB-5Sc	BC5	BC5	BC5	BC5	*a2*	*a2*
TB-6Lc	BC5	BC5	BC5	BC4	*y1*	*l12*
TB-7Lb	BC5	BC5	BC5	BC5	*o5*	*gl1/o5*
TB-7Sc	BC1	BC1	BC1	BC1	*ux6/o2*	*u5*
TB-8Lc	BC5	BC5	BC5	BC5	*pro1*	---
TB-9Lc	BC5	BC5	BC5	BC5	*dek13*	*gl15/v1*
TB-9Sd	BC5	BC5	BC5	BC5	*bz1*	*bz1*
TB-10L19	BC5	BC5	BC5	BC5	---	*gl*
TB-10Sc	BC5	BC5	BC5	BC5	---	*y2*

[1]Heterozygous B-A translocations were backcrossed by these inbreds and have been maintained.

For each ear 50 kernels were used to estimate the hypoploid frequency in progeny. Hypoploid frequency was calculated from the number of kernels or seedlings that showed recessive characters. As shown in Figures 1-4,

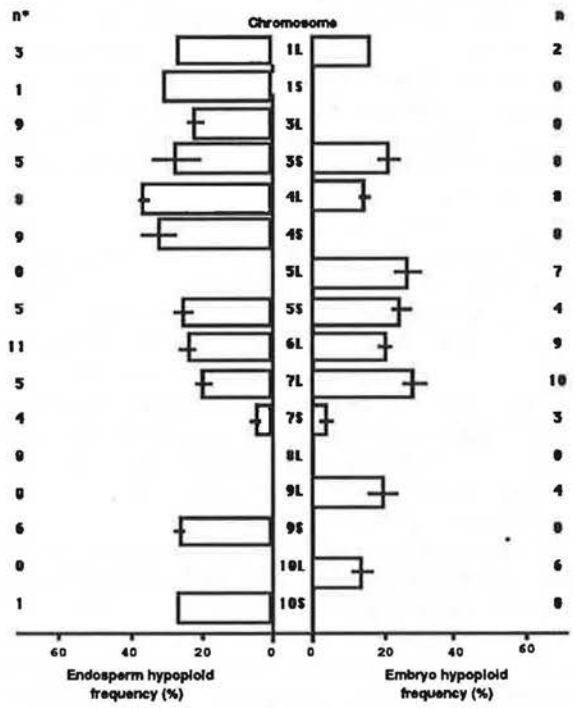

Fig.1 Hypoploid frequency of progeny from tester x TB(A619). The columns headed with n represent the number of ears examined. Data are shown as means of estimated hypoploid frequency with standard error.

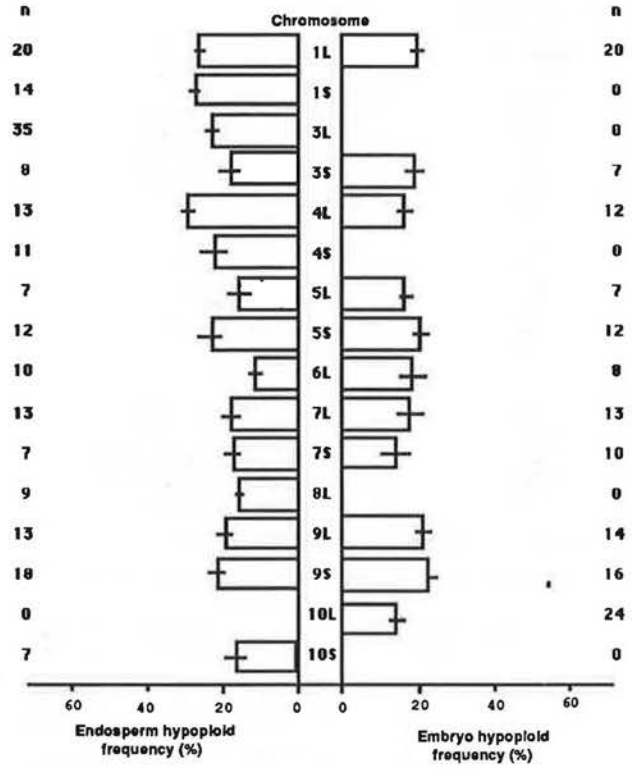

Fig.3 Hypoploid frequency of progeny from tester x TB(Mo17).

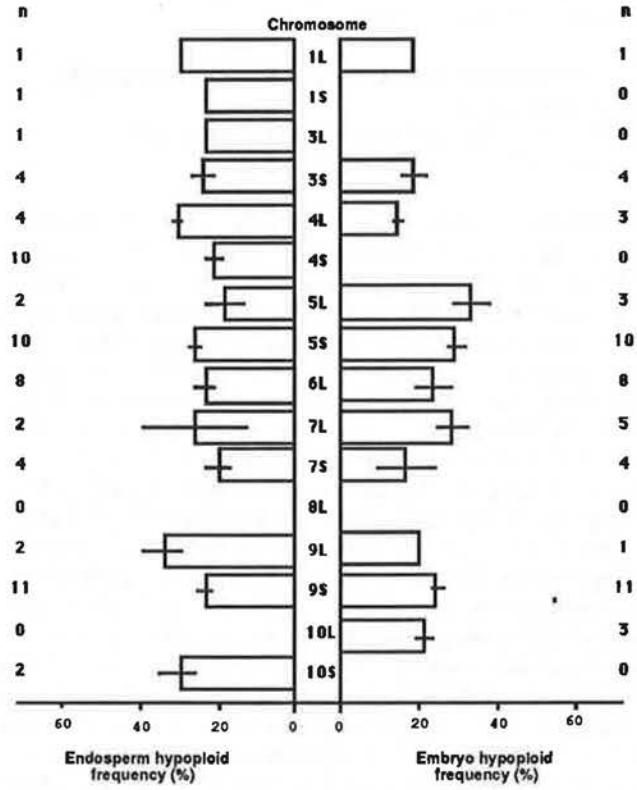

Fig.2 Hypoploid frequency of progeny from tester x TB(A632).

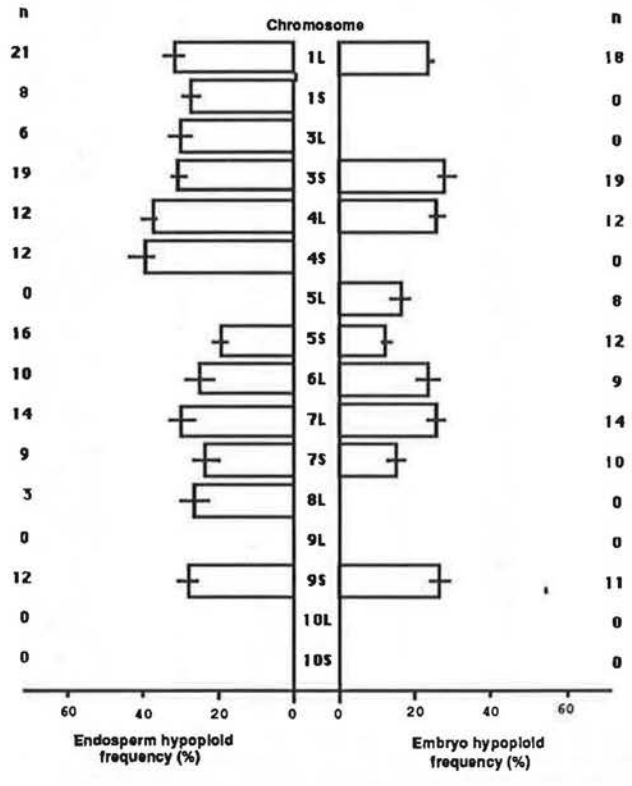

Fig.4 Hypoploid frequency of progeny from tester x TB(B73).

Table 2. List of testers.

Tester No.	Genotype and background
1L1	*bz2 R-r b pl* [W22b[1] x W23]
1L2	*bz2 R-r b pl* [W23 x W22b]
3S1	[B73 x A632] x (*d1* + / *d1 cl1*)
3S2	[Mo17 x A619] x (*d1* + / *d1 cl1*)
3S3	[A632xMo17] x (*d1* + / *d1 cl1*)
3S4	[B73 x A632] x (*d1* + / *d1 cl1*)
4L1	*c2 R-r b pl* [K55 x W23}
4L2	*c2 R-r b pl* [W22b x K55]
4L3	*c2 R-r b pl* [W23 x W22b]
5S1	*a2 R-r b pl* [W22b x K55]
5S2	*a2 R-r b pl* [W23 x K55]
5S3	*a2 R-r b pl* [K55 x W23]
5S4	*a2 R-r b pl* [W22b x W23]
6L1	*y1 l12* / *y1* +
7L3	[B73 x A619] x *o5 gl1*
7L4	[Mo17] x *o5 gl1*
7S2	([B73xMo17] x (+/*vp9*)) selfed
7S3	[B73 x Mo17] x *o2 v5*
9S1	*sh bz wx R-r/R-scm* [W23 x W22b]
9S2	*sh bz wx R-r/R-scm* [W22b x W23]
9S3	*bz R-r/R-scm* [W23 x W22b]

[1]W22b (p-*ww b* y *C R-scm2*) is a modified version of W22.

Table 3. Endosperm and embryo classification of progeny from tester X TB

TB background	Tester	Endosperm: Number of crosses	Endosperm: Percent[1] recessive	Embryo: Number of crosses	Embryo: Percent recessive
A619	3S1	2	13.0 ± 0.70	2	12.7 ± 3.07
	3S2	2	16.0 ± 5.65	2	12.5 ± 1.28
	4L1	2	26.4 ± 1.72	2	18.9 ± 0.74
	4L2	6	35.3 ± 1.53	6	11.8 ± 1.21**[2]
	5S2	3	24.0 ± 0.92	3	21.3 ± 2.72
	6L1	11	21.6 ± 1.35	9	9.6 ± 0.96
	7S2	4	2.3 ± 0.41	3	2.0 ± 0.46
A632	3S2	2	11.0 ± 3.53	2	11.4 ± 1.80
	4L2	3	30.0 ± 1.63	3	14.5 ± 1.74**
	5L1	2	18.3 ± 5.43	2	22.8 ± 7.45
	5S1	5	24.4 ± 2.61	5	30.7 ± 3.61
	5S2	5	27.6 ± 1.04	5	29.8 ± 2.50
	6L1	8	23.1 ± 2.39	8	12.1 ± 2.40
	7S2	4	10.0 ± 1.87	4	8.5 ± 3.89
	9S1	6	23.3 ± 1.67	6	25.3 ± 1.40
	9S2	5	23.2 ± 1.45	5	25.1 ± 3.70
Mo17	1L1	10	25.0 ± 1.18	10	19.8 ± 1.35**
	1L2	10	26.5 ± 2.70	10	19.8 ± 0.84*
	1S2	7	13.7 ± 1.68	7	9.5 ± 0.65*
	1S3	7	13.7 ± 1.87	6	13.1 ± 1.81
	3S3	5	10.2 ± 1.53	4	12.0 ± 2.16
	3S4	3	7.3 ± 1.44	3	6.3 ± 0.94
	4L2	7	30.0 ± 2.37	6	13.2 ± 1.28**
	4L3	6	28.1 ± 0.48	6	20.1 ± 2.63**
	5S3	6	22.0 ± 1.85	6	20.0 ± 2.09
	5S4	6	25.1 ± 2.20	6	22.0 ± 1.66
	6L1	10	11.8 ± 0.99	8	9.2 ± 1.42
	7L3	8	9.2 ± 1.26	8	7.6 ± 1.82
	7L4	5	8.4 ± 2.21	5	10.9 ± 1.4
	7S3	7	8.7 ± 0.67	10	7.4 ± 0.76
	9S1	10	21.7 ± 1.34	9	22.8 ± 1.98
	9S3	8	20.9 ± 2.30	7	23.2 ± 2.06
B73	1L1	10	29.5 ± 2.91	9	23.6 ± 1.99
	1L2	11	35.2 ± 2.92	9	25.2 ± 2.27**
	3S3	9	15.9 ± 1.08	9	13.7 ± 1.71
	3S4	10	14.9 ± 1.43	10	15.3 ± 1.44
	4L2	7	39.9 ± 2.94	7	25.5 ± 2.07**
	4L3	5	34.0 ± 1.45	5	27.1 ± 1.62**
	5S3	6	24.7 ± 3.17	5	15.7 ± 1.91*
	5S4	10	17.3 ± 2.69	7	10.2 ± 1.29*
	6L1	10	25.3 ± 2.86	9	12.4 ± 1.54
	7L3	5	12.5 ± 1.56	5	13.6 ± 1.56
	7L4	9	16.5 ± 2.16	9	13.1 ± 1.82
	7S3	9	12.6 ± 1.68	10	7.7 ± 1.12**
	9S1	6	29.0 ± 1.82	5	25.1 ± 1.27
	9S3	6	28.2 ± 1.84	6	27.7 ± 2.85

[1]Data are shown as means of recessive percentage with standard error.
[2]Recessive frequency between kernels and seedlings was examined by t-test. T-value for difference of recessive percent was significantly different at 0.05 level(*) or 0.01 level (**). Since the genotype of the tester for TB-6L*c* consists of a homozygous endosperm marker and heterozygous seedling marker, percentages of recessive seedlings were doubled and examined by the t-value.

hypoploid frequency varied depending on the background and the B-A translocation. We found a significantly lower hypoploid frequency for some B-A translocations with Mo17 background. It appears that the background of B-A translocations affects the transmission rate. Also, hypoploid frequency varied for each TB significantly. However, the cause of the difference between TB's is not clear, since testers were different for each TB.

TB-7Sc with A619 background showed extremely low hypoploid frequency. However, this TB was backcrossed only once with inbred A619 and the others, so that it is difficult to determine the effect of inbred background.

For several of the TB's, the hypoploid frequency was determined for both kernel and seedling characters. Carlson (1969) reported that the occurrence of preferential fertilization is dependent on testers. To analyze the tendency for preferential fertilization, hypoploid frequency was examined for each tester. Information on testers is shown in Table 2, and data are given in Table 3.

With TB-4Lc, preferential fertilization occurred without regard to inbred background. Although Carlson (1969) found that some testers generate equal fertilization, all of our testers showed preferential fertilization for this chromosome. However, the difference in recessive frequency between endosperm and embryo seemed to be altered depending on the tester. This might be more clear after additional investigations.

Preferential fertilization also occurred with TB-1La in Mo17 and B73 background, except for 1L1 tester when it was pollinated by B73 background. This indicates the possibility that tester and TB background generate the preferential fertilization together.

The orange pericarp mutant of maize is a tryptophan auxotroph

--Allen D. Wright, Karen C. Cone, Cynthia A. Moehlenkamp, and M. G. Neuffer

Orange pericarp is a seedling lethal mutant caused by the duplicate unlinked recessive loci, *orp1* and *orp2*. Several lines of evidence suggest that the mutant is a tryptophan auxotroph. Mutant plants benefit from tryptophan supplementation. Mutant seedlings accumulate indole and a glycoside of anthranilic acid. This phenotype would be expected if the mutant were deficient in tryptophan, which normally inhibits synthesis of anthranilate synthase by a feedback mechanism. Accumulation of indole implies that the last step in the tryptophan pathway (catalyzed by tryptophan synthase) is defective. In keeping with this interpretation, the mutant is more resistant to the indole analog 5-fluoroindole than normal plants, probably due to the inability of the mutant to convert 5-fluoroindole to the toxic product 5-fluorotryptophan (A. Wright, MNL, this issue). The tryptophan synthase complex is composed of two subunits A and B, both of which are required for the conversion of indole glycerol phosphate plus serine to tryptophan plus glyceraldehyde-3-phosphate. The function of the B subunit is to catalyze the condensation of serine and indole to form tryptophan. The accumulation of indole in the *orp* mutant suggests that the mutant is deficient in the

B subunit of tryptophan synthase (*trpB*) and furthermore that maize has duplicate genes for *trpB*.

Recently, the presence of two *trpB* synthase genes in the *Arabidopsis* genome was reported (PNAS 86:4604-4608, 1989). Using a heterologous hybridization probe from the *Arabidopsis* gene (kindly provided by Robert Last, Cornell University), we isolated a maize cDNA clone for *trpB*. Preliminary sequence analysis indicates that the clone shows about 40% identity at the amino acid level to the *Arabidopsis trpB* sequence. The maize cDNA hybridizes to two bands in restriction digests of maize genomic DNA, consistent with the notion that maize has two *trpB* genes.

To determine if the maize *trpB* genes are indeed *orp1* and *orp2*, we took two approaches. First, we used RFLP mapping to find out if the *trpB* genes map at the same locations as *orp1* and *orp2*. Second, we used molecular segregation analyses to ask if the orange pericarp phenotype segregates with the *trpB* RFLP's present in the *orp1 orp2* homozygote.

The map locations of the maize *trpB* genes were determined by RFLP mapping in the recombinant inbred family TxCM. DNAs were prepared from the members of the family, digested with a restriction enzyme that allowed us to distinguish all four parental alleles of *trpB* as discretely sized fragments. Hybridization patterns were scored and linkage was determined by comparison to the compiled data for the TxCM family (Ben Burr, Brookhaven National Laboratory). By RFLP analysis, *orp1* maps equidistant between BNL markers 15.45 and 7.20L at position 82 on chromosome 4L. This is in fairly good agreement with genetic mapping that placed *orp1* less than one map unit from *su* on 4S (J. Heredity 80:229-233, 1989). *orp1* is uncovered by TB-4Sa, but not by TB-4Lf (MNL 61:44-45). The linkage of *orp1* to molecular markers on 4L rather than on 4S seems to suggest that the placement of the centromere relative to these molecular markers may need to be re-evaluated. By RFLP analysis, *orp2* maps on 10S near the centromere. *orp2* maps at the same location as Pioneer marker 06003, i.e., at position 23. Genetic analysis of repulsion backcrosses had placed *orp2* near position 45 on 10L between *g1* and *r1*. This discrepancy may be due to suppression of recombination across the centromere between *orp2* and the markers on 10L. In summary, the RFLP mapping data are in reasonably good agreement with the genetic data and place the maize *trpB* genes at the same locations as *orp1* and *orp2*.

Molecular segregation analysis was performed on a population segregating for orange pericarp. The population was generated by backcrossing *orp1* and *orp2* into the inbred Mo17 background for two generations and then selfing to obtain an ear that segregated 15:1 normal: orange kernels. Plants were grown from 6 orange kernels and from 16 normal kernels. DNA was isolated from the individual plants, digested with a restriction enzyme that allowed all parental alleles to be distinguished, and hybridized with the maize *trpB* cDNA. If the orange pericarp phenotype is due to mutations in both *trpB* genes, then we should see a *trpB* hybridization pattern unique to the DNAs from plants grown from orange kernels. The results confirmed this expectation, i.e., a distinct RFLP pattern was present for all DNAs from orp plants and absent for DNAs from normal plants.

Taken together, the molecular data verify the identity of *orp1* and *orp2* as duplicate structural genes for the B subunit of tryptophan synthase. Furthermore, these results confirm *orp1 orp2* as a tryptophan auxotroph of maize.

Location and description of dominant dwarf mutants

--M. G. Neuffer

D-1452*: Dwarf; EMS induced; located on 1L, 4±2.3cM from *wx* T1-9(8389) breakpoint; linked to *D*-2023*, 13±3cM. Broad, dark green leaves. Medium size, andromonoecious type like *D8*. No GA response.

D-1591*: Semi-dwarf; EMS induced; located on 1L, 3.8±2.2cM from *wx* T1-9(8389) breakpoint; short plant 1/3 to 1/2 normal height. Leaves slightly darker and broader than normal. Occasional anthers in ear.

D-1991*: Semi-dwarf; NG induced; located on chromosome 3, 10±4cM from *wx* T3-9c breakpoint; short plant 1/2 to 3/4 normal height, leaves lighter green than normal and normal width. Not andromonoecious.

D-2023*: Dwarf; spontaneous origin (D. Hoisington); located on chromosome 1L, 5±2.4cM from *wx* T1-9(8389) breakpoint. Linked to *D*-1452*, 13±3cM. Very short andromonoecious dwarf type; no GA response; frequent tillers (could be due to linked factor for tillering).

D-2319*: Semi-dwarf; EMS induced; located on chromosome 5, 23±5cM from *wx* T5-9c breakpoint. Short plant with broad, dark green leaves, 1/2 normal height; not andromonoecious and no GA response.

Location, description and notes on other dominant mutants

--M. G. Neuffer

Wi-1614*: Wilted; EMS induced. Like *Wi2* but backcross of double heterozygote to normal gave 3/4 mutant progeny thus indicating no allelism and probably no linkage. This mutant is tentatively designated *Wi3*.

Gs-1439*: Green striped; EMS induced; located on chromosome 10, 4.2±4.1cM from *wx* T9-10b breakpoint. Also shows linkage with *R*, 23±3cM in a coupling backcross. Small green plant with lighter green, longitudinal stripes which wilt and become grayish under stress, like *gs1*. We are designating this mutant *Gs4*.

Rs-1606*: Rough sheath; EMS induced; located on chromosome 7, 1±1cM from *wx* T7-9(4363) breakpoint and 14±4cM from *wx* T7-9g breakpoint. Leaf sheaths on plants nearing flowering have distinct rough corrugated appearance caused by enlargement of the vascular bundles. Expression excellent in Missouri summer field but very poor in Hawaii winter field. Tentatively designated *Rs4*.

Rld-1441* and *-1990*: Rolled leaf; EMS and NG induced, respectively; not located after extensive T *wx* tests but double heterozygote crossed on normal produced 1/54

normal plants. The other 53 were mutant, suggesting that the two are not alleles but closely linked, 2±1.3cM. Contamination has not been ruled out.

Nl2: Narrow leaf (was *Rgd2, Rgd*-1445*); linked to *a2* in the order *Nl*-10-*a2*-3-*bt1*. The descriptive symbol and name previously reported were found to be inappropriate. The mutant phenotype is that of irregular development so that leaves, tassel, ear, etc. are imperfectly formed and sometimes irregularly placed. This distortion results in small, less vigorous plants. Some plants do resemble *rgd1* but the most common feature is that of narrow leaves, hence the name change to *Nl2*, narrow leaf.

Location and designation of recessive mutants

--M. G. Neuffer

idd-2286A*: Indeterminate dwarf; located on chromosome 1L, uncovered by TB-1La.

sbd1: Sunburned (was *wxl*-2292*); located on 6L, uncovered by TB-6a. Top surface of upper leaves develops a greyish waxy appearance as plant nears flowering. Underside remains bright green. If leaf is turned over and held in that position for two or more days the underside develops the same phenotype, indicating that the surface exposed to the sun may be getting sunburned.

dek7: New linkage data show *Ts5*-1.3-*dek7*-5.3-*su*.

Dominant male sterile

--Marc Albertsen and M.G. Neuffer

Ms41 and *Ms*-7255*, located on chromosome 4L, are not alleles but are linked. Because *Ms41* sometimes makes small amounts of viable pollen, it was possible to cross on *Ms*-7255* and obtain progeny, which when evaluated should be 1/4 double heterozygote. These individuals should be male sterile and when crossed as female by normal should give material which would provide a test for allelism. Such a test was made and the results proved that the two mutants are not alleles but are linked with 14±2cM between them. *Ms*-7255* is designated as *Ms44*.

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A second mitochondrial *cox2* mutation associated with an NCS phenotype

--M. Lauer, K. Newton, C. Knudsen, S. Gabay-Laughnan and J. Laughnan

The maternally inherited nonchromosomal stripe (NCS) mutants are characterized by variable poor growth, abnormal morphologies and leaf striping. We have suggested that the NCS phenotypes are due to lesions involving essential mitochondrial genes, that NCS plants carry both mutant and "normal" mtDNAs and that somatic segregation of the mixed organelles leads to sectors of defective and normal growth (Newton and Coe, PNAS 83:7363). Last year we reported that NCS5 plants carried a partially deleted version of the mitochondrial cytochrome oxidase subunit 2 gene. We have now found that a second NCS mutant, NCS6, which has a phenotype indistinguishable from that of NCS5, also carries a partially deleted *cox2* gene. The *cox2* rearrangement in NCS6 occurs at a site (within the first exon) different from that in NCS5 (within the intron)

The NCS6 mutant was found among plants descended from a fertile revertant of the RD (=R) cytoplasm, a member of the *cms-S* group. Like NCS5, the NCS6 leaf and plant stripes are yellow, and clonal sectors with aborted kernels are seen on ears from striped plants.

Mitochondrial DNA was isolated from NCS6 striped and from non-striped control plants (RD-revertant relatives). DNA blots were hybridized with several cloned gene probes. The only consistent striped/nonstriped difference involved the cytochrome oxidase subunit 2 gene (*cox2*). A 2.4 kb *Eco*RI fragment carrying the whole *cox2* gene (pZmE1; Fox and Leaver, Cell 26:315) hybridized to a 5.5 kb *Xho*I fragment in mtDNAs from control, nonstriped plants. However, in mtDNAs from the NCS6 plants, the amount of 5.5 kb *Xho*I hybridizing fragment was reduced and an additional 9.2 kb restriction fragment hybridized strongly. By restriction endonuclease analyses, we determined that the restriction map of a 9.2 kb *Xho*I mutant fragment diverged from that of the 5.5 kb fragment within the first exon of the *cox2* gene. The proximal third of the first exon and the 5' flanking region of the cox2 gene is not carried on the 9.2 kb *Xho*I fragment. A 2.65 kb *Xho*I/*Bam*HI restriction fragment from the 9.2 kb *Xho*I insert, outside of the region of homology with the *cox2* gene, was used as a probe onto DNA blots. This probe identified the 9.2 kb *Xho*I fragment in NCS6 mtDNA and an additional 4.6 kb *Xho*I band in both NCS6 and control DNA.

A comparison of the restriction maps of the three cloned *Xho*I fragments suggested that the molecular origin of this mutation was a recombination event. The two "parental" (4.6 kb and 5.5 kb) restriction fragments do not show detectable homology to each other under stringent hybridization conditions. However, subsequent sequencing of portions of the three clones showed that they all contain the same 31 base-pair sequence at the site of the rearrangement.

The partially deleted *cox2* gene in NCS6 plants does not appear to be expressed. Transcripts corresponding to the *cox2* gene are specifically reduced in NCS6 plants. The *cox2* protein is also present at reduced levels in mitochondrial preparations from NCS6 plants. This was shown by Western blot analyses using antibodies directed to a petunia *cox2* peptide sequence (Nivison and Hanson, The Plant Cell 1:1121).

Studies with NCS mutations suggest that they result from homologous recombination occurring between small repeats. The sizes of the repeats vary: 6 bp in NCS5 (Newton et al., The Plant Cell, in press, 1990), 12 bp in NCS3 (Hunt and Newton, MNL 63:70, 1989) and 31 bp in NCS6. The repeats associated with different NCS mutations do not appear to be related to one another.

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Products of *Mu* insertion and excision at the Bronze1 gene

--Anne Bagg Britt and Virginia Walbot

This laboratory has previously reported the size, orientation, and approximate location of the Mutator element insertions at the *bz-mu1* and *bz-mu2* alleles. The *bz-mu1* allele contains a 1.4 kb *Mu* element inserted near the 3' end of the bz1 intron, while the *bz-mu2* allele includes a 1.7 kb *Mu* insertion just 5' of the gene's *Xho*II site. Both insertions are oriented 5' to 3' (as the Mutator element sequence has always been written) with the 5' to 3' orientation of the bronze1 transcript. Here we report the exact sequence of the insertion site of both *bz-mu1* and *bz-mu2*. We also have sequenced the "empty site" at *bz* left behind after the somatic excision of *Mu1.4* from the *bz-mu1* allele in cob tissue. In addition, both sequencing and Southern blot analysis have shown that both the *bz-mu1* and *bz-mu2* alleles are derivatives of the *Bz-W22* allele sequenced by Ralston, English, and Dooner (Genetics 119:185, 1988).

Mu *insertion sites at* bz-mu1 *and* bz-mu2: Restriction digests and Southern blotting were used to determine the exact point of insertion of *bz-mu2*. The element has previously been mapped to within 50 bp (or less) 5' of the *Xho*II site (AGATCC) in the coding region of *Bz-W22*. Paradoxically, restriction with *Sau*3a, an enzyme that cuts within the *Xho*II site (recognizing GATC) revealed that the 180 bp *Sau*3a fragment 5' of (and bordering on) the *Xho*II site does not carry an insertion of any kind. Assuming that the *Mu2* element at this site, like the previously sequenced *Mu2* element, contains no *Sau*3a, sites, this apparent contradiction (that the element maps 5' of *Xho*II but not 5' of *Sau*3a,) can be resolved if we assume that the element has inserted within the *Xho*II site, precisely after the *Sau*3a site. Because *Mu* insertion generates a 9 bp repeat, the following sequence should exist at the *bz-mu2* allele: ...GCGGAGATC(*Mu2*)GCGGAGATCC... In this way the insertion of *Mu* regenerates the *Xho*II site 3' of the element, while maintaining the *Sau*3a site 5' (and 3') of the insertion site. We plan to confirm the sequence of the putative 9 bp repeats by cloning and sequencing.

In order to determine the points of insertion of the *Mu1* element of the *bz-mu1* allele, PCR was used to amplify the *bz* sequences bordering the insertion. The amplified fragments were then cloned and sequenced. The sequence of the 9 bp repeat, located just 3' of the bronze1 intron, is CAAACAGGG. Remarkably, this is the exact point of insertion of the *Mu7* element at the *bz*-rcy allele reported earlier this year by Schnable, Peterson, and Saedler (MGG 217:459, 1989). There is virtually no possibility that one of these mutable alleles was derived from the other: they carry *Mu* elements with homology only within the ~~200 bp TIRs, and they were isolated independently (*bz-mu1* at Stanford in the summer of 1982, *bz*-rcy at Iowa State) by laboratories that do not directly exchange materials. The significance of the isolation of two independent insertions at precisely the same site is unclear. The handful of Mutator insertion sites sequenced so far have displayed no homology at the 9 bp repeats. The possibility exists that *Mu* may have a tendency (but not an absolute requirement) for insertion at sites near to a certain sequence or secondary structure. Only the sequencing of more *Mu* insertion sites will reveal whether or not the selection of *Mu* insertion sites is totally random.

Sequence of Mu1 *somatic excision sites*: In order to determine the nature of the lesion left behind in the host chromosome after *Mu* excision, we have cloned a number of "empty sites" generated at the *bz-mu1* allele. DNA carrying such empty sites can be isolated from germinal revertants, and two excision products of *rcy:mu7* from *bz* generating phenotypically revertant plants (fully colored kernels) have been analyzed by Schnable et al. Unfortunately, this technique for the selection of excision products will yield only those products which generate alleles which cause phenotypic revertants. In order to sequence a wider spectrum of excision products, we used PCR to specifically amplify the products of *Mu1* excision from *bz* present in an immature cob from an active Mutator line homozygous at *bz-mu1*. If we assume that Mutator activity in cob tissue is roughly the same as that in aleurone (that is, perhaps 5% of all cells have experienced *Mu* excision from *bz*--Taylor and Walbot, Genetics 117: 297, 1987--and excision sectors are an average of 20 cells in size), a 2 mg DNA prep (with a haploid genome size of 3 pg) should contain about 20 copies each of 1.7 million independent excision events. Any primary PCR reaction, employing 0.4 μg of chromosomal DNA, should include, on average, 6800 molecules of "empty sites" at *bz*, most of which are derived from independent excision events.

We have found that the *Mu* element (perhaps due to its TIRs) is itself a very effective block to PCR amplification. Thus, using *bz* specific primers on both sides of the *Mu* insertion, specific amplification of excision products was achieved. Because the initial round of synthesis produced DNA in levels insufficient for cloning, a second round of synthesis (with one "nested" primer and one original primer) was undertaken to increase both the quantity and specificity of the products. These were then digested with *Hpa* II as depicted in Figure A, and cloned into pBSKS- for sequencing. The sequence of the excision products is presented below. The number of different excision products cloned and sequenced was not consistent with our estimate of the number of independent excision events per primary PCR reaction. Considering the frequency at which sequences were cloned in duplicate or triplicate (even from independent ligation reactions), either the number of independent excision events present in the original DNA prep was lower (by 3 orders of magnitude!) than that estimated above, and/or the number of independent excision events amplified by the primary PCR reaction was much lower than the initial number of independent products present in the 0.4 μg of chromosomal DNA added to the primary reaction. The presence of an excess of triplicates over duplicates strongly suggests that the different excision products are represented (at the end of the secondary PCR re-

Figure A1: Partial map of bz::mu1 showing position of 5' end of primers (→) and cloning sites.

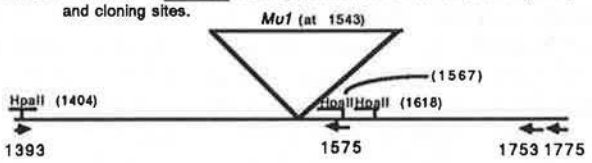
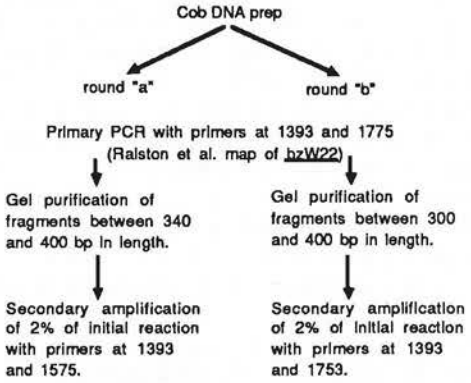
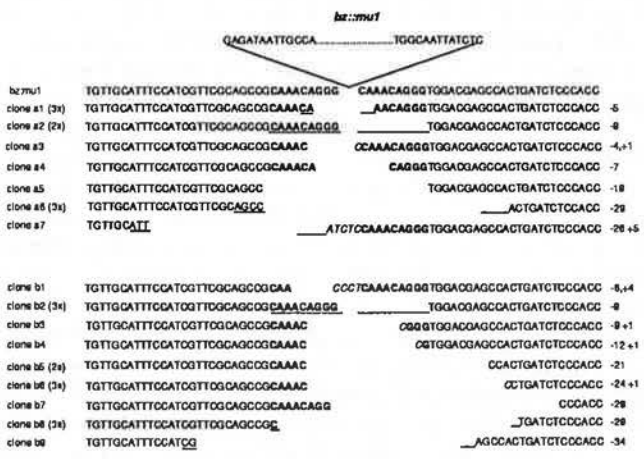


Figure A2: Synthesis and cloning of excision products from bz::mu1



Mutator excision products



action) in different frequencies. Such differential representation in the "pool" of DNAs for cloning might be the result of skewed amplification during the early primary PCR reactions, but it might also represent a tendency by Mutator to delete chromosomal sequences in a nonrandom fashion. In order to distinguish between these two possibilities, the entire protocol (both primary and secondary PCRs) was performed twice on the same cob DNA prep. The second trial produced a different set of clones, with the same excess of triplicates over duplicates. These results suggest that the skewed distribution of excision product types is due to the process of amplification by PCR, rather than to a nonrandom behavior of the *Mu* element. Clones from trial "a" vs trial "b" are identified as such. Presumably additional repeats of this protocol would continue to yield additional excision products.

If *Mu* were to excise precisely, removing only *Mu* sequences from the insertion site, two copies of the 9 bp repeat should be left behind. Of the sixteen different excision products sequenced, none were of this structure. The excision products we obtained can be divided into two broad categories. Simple imprecise deletions of *Mu* from *bz* are observed in eleven of the 16 sequenced products. In these products, as in the two sequenced by Schnable et al., the excision of *Mu* has resulted in the loss of *bz* sequences bordering the insertion site. There is no convincing evidence for a preference for any particular 5' or 3' endpoint for the deletion. In fact, in clone a7 the 3' deletion endpoint is 5 to 8 bp within the *Mu* element itself. Clone a3 might also be an example of a 3' deletion endpoint within *Mu*. On the other hand, a3 might also be the result of the same repair syntheses which produced the second category of excision products, the complex imprecise deletions. Clones b1, b3, b4, and b6 all carry sequences not present in the original *bz-mu1* allele, as well as deletions extending into *bz*. In three of these four cases the additional sequence is simply a C residue. In two clones this C residue unambiguously replaces an A in the original sequence (clones b3 and b6). In a third clone (b4) the C might replace either an A or a G. The other clone which carries additional sequences not present in the original *bz-mu1* allele is b1, which carries a CCCT insertion. While it is possible that this insertion, like the insertions of single C residues, is derived from some sort of error-prone DNA repair activity, this sequence is closely related to the 3' (AGGG) end of the 9 bp target site repeat. Either the "hairpin loop" model suggested by Coen, Carpenter, and Martin (Cell 47:285, 1986) or Saedler and Never's "strand switching" model (EMBO J. 4:585, 1985) for the generation of post-excision footprints would account for the generation of this 4 bp insertion.

The only clone isolated in both trials is the clone that restores the original sequence of the *Bz-W22* allele (a1 and b2). While it is possible that this sequence was amplified as a contaminant in the DNA prep (either from contaminating W22 DNA in the first round of cloning, or contaminating clone a1 in the second), the fact that this excision product appears at approximately the same frequency as other excision products makes this seem unlikely. This (and the other excision products) is not an artifact generated by PCR; extensive attempts at amplification with *Mu*-containing plasmid templates failed to generate this or any other excision sequences. It is possible that this sequence is produced more often upon excision than any other single excision product. Alternatively, this sequence might be generated (perhaps even in inactive lines) by homologous recombination between the 9 bp repeats, as suggested by Kloeckener-Gruissem and Freeling (MNL, 1989).

The sequences obtained so far of *Mu* insertion sites show that newly transposed *Mu* elements carry with them only *Mu* sequences; the chromosomal sequences from the previous insertion site are not carried along to the new target. In order to generate "clean" elements for transposition, the sequences immediately abutting the element must be nicked precisely at some time during the transposition

process. If *Mu* truly transposes replicatively, in the same fashion as the *Mu* phage, the first step to transposition should be the generation of single stranded nicks on each side of the element (one to each strand). Assuming that the same enzymes required for replicative transposition are also employed for excision, one would then expect that some excision products would still contain some Mutator sequences, as only one strand of *Mu*, on each side of the element, is specifically nicked. The structure of clone a7 supports this model, providing evidence that this nicking does not always occur on both strands of both sides of the element.

The amplification and cloning process limited the size and position of the deletions within the excision products (see Figure A). For clone set "a" the maximum clonable deletion size was limited to approximately 35 bp by the gel purification of the primary PCR reaction. The range of fragment sizes gel purified for clone set "b" was broader, and the nested primer for secondary synthesis set farther 3' of the insertion site, permitting the cloning of excision products carrying deletions of up to 75 bp. Even within these limits, the size and position of the deletion present in each of the excision products does not appear to be totally random. Smaller deletions occur more frequently than large ones; no deletion extended more than 26-29 bp 5' or 26 bp 3' of the insertion site. If the position of the deletion end points is truly random (i.e., entirely unrelated to the position of the *Mu* insertion) such a distribution might be expected for clones from set "a", but certainly a broader range of deletion sizes would be expected for clone set "b". The fact that deletion endpoints cluster near the point of *Mu* insertion supports the model suggested above–that the initial nicks are generated specifically at the point of insertion of *Mu*. The deletions might then be extended by exonucleolytic degradation.

This semirandom nature of the distribution of deletion endpoints in the excision products seems at first to suggest that the excision process of the *Mu* element includes no mechanism for the restoration of the integrity of the host chromosome—that the element simply hops out and leaves the two broken ends behind. If this were so, however, one would expect every chromosome which has experienced an excision event to be equivalent to a broken chromosome, such as those generated by gamma irradiation. This does not seem to be the case. Recent data presented by Rowland, Robertson, and Strommer (Genetics 122:205, 1989) have demonstrated that *Mu* activity, or at least activity in the female germline, does not frequently induce chromosome breakage. Our own unpublished data support these results. The sequenced footprints of other transposable elements of maize (with the exception of certain "chromosome breaking" elements) suggest that these elements behave similarly to *Mu*; excising imprecisely, and yet not causing chromosome breakage. Thus any model for transposable element excision/transposition must explain this ability to rejoin, however imprecisely, the 5' and 3' ends of the empty site.

DEFIANCE, OHIO
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Temperature programming of paramutant *R*-gene expression

--Bernard C. Mikula

Transposable elements offer the opportunity to witness genetic change through modulation of gene expression. A transposable element system which permits high frequency genetic changes directed at a specific allele becomes interesting from an evolutionary point of view since sources of heritable change which can serve evolutionary purposes have evaded experimental methods. Paramutation, a two element system explored by the late R. A. Brink and his students, generates a heterogeneity of *R*-gene expression which could model evolutionary mechanisms for directed, high-frequency (100%) change at the single gene level. This paramutation system has been shown to be subject to environmental modulation and becomes even more interesting when it is recalled that the paramutated *R*-gene can undergo incremental change from one generation to another. This system with its long-term, incremental memory makes it possible to search environmental and developmental variables for more efficient methods of programming gene expression.

Our efforts have been directed at the light and temperature environments during early development, the period tassels are determined. In the present experiment, seedlings were kept under continuous light for 21 days except for the two to four-day, 12 hr light and 12 hr dark treatments. Each set of seedlings was held either at 28 or 22 C during the 21-day treatment period. For the rest of the life cycle treated seedlings were transplanted and matured under field conditions. Other growth chamber conditions were those reported in previous MNL reports, Vols. 40-45. Our objective is to find allelic systems most responsive to our variables.

Table 1. Comparison of pigment expressions of paramutated *R*-alleles from plants grown at temperatures of 28 and 22 C during the first 21 days of seedling development. Several pollen samples were taken from the same plant on different days and are indicated as No. of ears scored. All testcrosses from a single tassel were scored and represented as an average.

R-g ex R R-lst				*R-g ex R R-st*			
No. ears scored	28 C *R-g* score	22 C *R-g* score	No. ears scored	No. ears scored	28 C *R-g* score	22 C *R-g* score	No. ears scored
4	0.7	2.5	1	3	0.4	1.8	6
3	0.9	2.7	1	3	1.6	1.8	2
4	1.3	4.6	1	3	1.8	2.4	3
3	1.4	5.2	5	2	1.8	2.5	5
2	1.4	5.2	3	2	1.8	2.9	5
3	1.5	7.0	6	5	2.0	3.2	3
1	1.7	7.3	1	2	2.3	3.6	3
3	1.9	7.5	1	3	2.5	3.8	4
5	1.9	8.4	2	4	2.5	4.1	3
4	2.0	8.7	2	4	2.6	5.0	6
2	2.0	8.9	2	3	4.1	5.1	3
3	2.9	9.4	4	3	4.6	5.2	4
4	3.0	10.1	1	2	6.3	5.6	2
2	4.8	10.1	5			5.9	2
Mean	2.0	7.0				5.9	2
						6.6	3
				Mean	2.6	4.1	

An *R-g* allele, obtained from Native Seeds/SEARCH, Tucson, AZ, was crossed as female to *R-st* and *R-lst* obtained from the Corn Coop. Testcrosses of these heterozygotes were made onto W23. Scoring of pigment from resulting paramutated *R* alleles was done by matching 50-kernel samples against a set of 20 standard kernels. Values for these 50-kernel samples are reported as ear means. Since we have reported significant variation from pollen samples of a single tassel, where possible, several pollinations over a seven day period were made and the average of all is reported.

Our results in Table 1 show that *R R-lst* heterozygotes produced pigment scores related to the temperature the seedlings received. Yet, the same *R* allele from the *R R-st* combination showed no such temperature relationship though treated under the same growth chamber conditions. We have no explanation for the difference; many variables remain to be tested at this early stage of development. It is quite possible that for the *R R-st* combination developmental timing is different and will require more careful attention.

Table 1. RFLP probes and alleles detected.

probe	rating	Alleles Oh43	W153R	B37	probe	rating	Alleles Oh43	W153R	B37
		chromosome 1					chromosome 6		
5.62	C	0	0	1	6.29	C	0	0	1
8.05	D	--	--	--	15.37	B	0	1	1
10.38	C	0	1	0	5.47	B	0	1	0
5.59	B	0	1	1			chromosome 7		
15.18	C	0	0	1	15.40	A	0	0	1
8.10	D	--	--	--	15.21	A	1	1	1
7.25	C	0	1	0	4.24	D	--	--	--
8.29	A	0	1	0	13.24	C	0	0	0
6.32	B	0	1	2	8.32	B	1	0	1
		chromosome 2			8.37	A	0	0	0
8.45	B	1	0	2	14.07	A	1	1	1
12.36	C	0	0	1	8.39	D	--	--	--
8.04	D	--	--	--	16.06	C	0	0	1
12.09	C	1	0	1	8.44	C	0	0	1
		chromosome 3					chromosome 8		
8.15	C	1	1	1	9.11	A	1	1	0
8.35	A	0	0	0	10.39	C	0	0	0
6.06	A	0	1	2	9.44	A	1	1	0
5.37	D	--	--	--	9.08	D	--	--	--
8.01	B	0	1	0	8.26	D	--	--	--
6.16	A	0	1	0	12.30	B	1	1	2
3.18	A	0	0	0	10.12U	D	--	--	--
		chromosome 4					chromosome 9		
5.46	D	--	--	--	3.06	B	0	0	0
15.27	C	0	0	0	5.10	A	0	0	0
15.45	B	0	0	1	7.13	B	0	0	1
7.65	A	0	1	2	5.04	D	--	--	--
10.05	A	0	1	2	8.17	B	0	1	2
5.67	A	0	0	0			chromosome 10		
		chromosome 5			10.17	D	--	--	--
8.33	D	--	--	--	3.04	D	--	--	--
6.25	A	0	0	0	10.13	D	--	--	--
7.56	A	0	0	0	7.49	D	--	--	--
5.02	D	--	--	--					
6.10	D	--	--	--					
6.22	C	0	0	0					
10.06	C	0	0	0					
7.43	C	0	0	0					
4.36	C	0	0	0					
7.71	C	0	0	0					
5.71	B	0	0	0					
5.40	C	0	0	0					
5.24	C	0	1	0					

Ratings: A = single clear band; B = several clear bands; C = bands obscured by high background; D = too faint or too much background to see.
Alleles: All A619 alleles are "0"; others are listed in increasing order.

DEKALB, ILLINOIS
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Comparison of maize RFLP probes

--M. A. Johns, C. A. Hennelly, A. P. Ryan and S. L. Morrow

In the course of looking for RFLP markers linked to various disease resistance genes, we have used many of the Brookhaven National Lab's probes on several common inbred lines. We have found that, in our hands, the probes vary in quality, and we are aware that other labs have had similar experiences. Although it is not possible to give a detailed description of all of our data, we give here a list of the probes we have tried, our estimate of the quality of the resulting bands, and whether the inbred we used showed any polymorphism.

In pilot studies, we found that *Eco*R1 gave the greatest number of polymorphisms (at least, of the reasonably priced enzymes), and all of the probes were tested on *Eco*R1-digested genomic DNA. Inserts were separated from the plasmid vector DNA by digestion of mini-prep plasmid DNA with *Pst*I, electrophoresis, and then electroelution of the insert bands. About 0.1 ug of insert DNA was labeled by random priming for each blot. We used Zeta-probe membrane (Bio-rad) and hybridized overnight at 42 C in a solution of 10% dextran sulfate, 50% formamide, 1% SDS, 3 x SSPE, 1x Denhardt's and 50 ug/ml denatured salmon sperm DNA. The blots were then washed in 6x SSPE, 0.25% SDS for 1 hr at room temperature, then in 1x SSPE, 1% SDS for 1 hr at 42 C, and finally in 0.1x SSPE, 1% SDS for 1 hr at 65 C. We have found that results are improved for some probes with a final wash at even higher stringency, in 0.05x SSPE, 0.15% SDS at 65 C. The high stringency wash is 13.4 mM Na^+; in comparison, we have successfully removed all of the probes using a 65 C wash that is 5.2 mM Na^+. Thus, we do not believe that further increases in the stringency of wash are likely to help much.

We examined several examples (disease-resistant conversion lines) of the inbreds A619, B37, Oh43, and W153R. Not counting differences specific to individual conversion lines, we found that A619 and Oh43 differed at 8 of the 51 scorable loci, reflecting the high percentage of Oh43 that went into A619 initially. All other pairs of lines differed at 17 to 25 loci. The data are shown in Table 1. The allele numbers are assigned so the A619 allele is always "0" and then each different allele is given a different number. The numbers do not reflect the position of the bands on the blot.

Potential pitfalls in mapping with recombinant-inbreds

--M.A. Johns

The recombinant-inbred (RI) mapping technique brought to maize by Burr et al. (Genetics 118:519-526, 1988) allows the rapid localization of almost any DNA

probe. Using this method, genes can be mapped with just a few Southern blots performed on DNA from pre-existing and readily available plant material. It is a major advance in maize gene mapping. However, after mapping more than 30 gel bands by this method, I realized that it is not always easy to pinpoint the chromosomal location of a probe. This problem is due to the limited number of RI lines available and thus to the limited number of recombinations which have occurred between any two points.

The first difficulty in RI mapping will only be mentioned briefly without a detailed analysis. At best, a new gene can be localized to an approximate position between two previously mapped loci, and quite frequently the new gene can only be mapped near another locus without knowing which side it is on. Because the number of recombinations in any region is so low, distances between loci often do not add linearly. Also, the determination of gene order in classical gene mapping depends on examining flanking markers. With RI mapping, there are numerous cases where several recombinations have occurred on a chromosome in any given line, and so the examination of nearby markers in the RI lines is not a reliable indication of gene order.

The second difficulty in RI mapping concerns what could be called "ectopic localization": unlinked loci can appear to be linked, and unknown genes can appear to be located in more than one region of the genome. It is this problem that I wish to address in more detail here.

The RI mapping scheme compares the allelic distribution of 205 loci (database from early 1988) distributed over the maize genome, using two independent families of recombinant-inbreds. The COXTx family has 48 RI's, and the TXCM family has 41 RI's. As explained by Burr et al., the RI method allows a direct calculation of an R value, which is related to recombination frequency by the formula, $r = R/(2-2R)$. Some of the lines show heterozygosity for some loci even after 7 generations of selfing: Burr et al. report a residual heterozygosity of 7.5%. The database does not contain all possible data points for every locus: 13.7% of the potential data points are missing. Most of these missing points are for probes which gave no usable data for one of the RI families, presumably due to lack of detectable polymorphisms.

I devised a computer program to compare the allelic distribution of each locus with that of every other mapped locus, except for the nearest 5 loci on either side. This exclusion eliminated most of the tightly linked loci. I found that, on the average, each locus was 0.481 R units (46.4 map units) away from every other locus, with a range of 0.434 to 0.512. That is, except for nearby loci, the RI method shows that every locus is essentially unlinked to the bulk of other loci. This result is exactly as expected.

However, the distance to the "nearest" locus on a different chromosome is quite variable, with an average of 0.317 R units (23 map units), and a range of 0.207 to 0.400 (13.0 to 33.3 map units). This means that, with the use of RI mapping, every locus is between 13 and 33 map units from another locus which is definitely unlinked. The lower number is especially significant, because in a normal mapping experiment, loci 13 map units apart are clearly linked. Also, some of the adjacent loci mapped by Burr et al. are more than 13 map units apart. Thus, it seems possible that an unknown locus mapped by the RI method could by chance appear to be located quite far from its actual location.

To take a specific example, the region between *8.05* and *Pl* on chromosome 1S is apparently close to a region on 7L between *7.61* and *8.37*. The closest approach is between *10.38* on 1S and *7.61* on 7L, which are separated by 0.207 R units (13.0 map units). In comparison, the loci flanking *10.38* on 1S are 9.4 and 1.8 map units away. It is clear that the loci on 1S and 7L have been properly located, because they are linked in a chain to previously mapped genes. However, an unknown gene could fall at an ambiguous position, equally close to loci on different chromosomes. This is especially true if the unknown gene falls in a relatively sparsely mapped area. It was not difficult for me to create an artificial set of data that was equally close to *7.61* and *10.38*, and more distant from every other locus. This example is by no means unique: many regions of the genome are apparently close to one another when mapped by the RI method.

After seeing that the RI mapping method produces apparent linkages between loci on different chromosomes, I decided to see how well random data sets could be mapped. These data sets were created by assigning the two parental alleles to the different RI lines at random. After a number of trials it became clear that random numbers for both the COXTx and TXCM families rarely produced any apparent linkages. That is, since the two families are independent, using both of them to map a locus is quite likely to yield a good, unique location.

However, not all probes will give polymorphic bands for both families: 52 of the 205 probes in the RI data base have data for only one family. Also, some probes, such as those from transposable elements, will not map to the same locations in both families. For these reasons, I attempted to map random data into the COXTx family only. To summarize the results, out of 709 random sets of data, 8 contained an R value of less than 0.25, and 2 of these had an R value of less than 0.225. Out of 296 trials, 25 had $R < 0.275$ for some locus, and out of 192 trials, 39 had an R of less than 0.30. The R value at which there is less than a 5% chance of getting random data to fit would seem to be between 0.275 and 0.25 (i.e. between 16.7 and 19.0 map units). The positions of these R value minima were randomly distributed in the genome. It can be seen that even random data, which might be produced as a result of wishful thinking applied to marginal data, can produce a "locus" for a probe.

This problem becomes even more acute for incomplete data sets. To address this issue, I created random data sets for the COXTx family that contained 10-50% missing data points, and compared them with the RI data base. As mentioned above, 1.1% of complete data sets contained an R value of less than 0.25. Using $R < 0.25$ as a criterion, I found that 1.8% of data sets with 10% of the data missing fit the criterion, 3.1% of data sets with 20% of the data missing fit, 8.2% of data sets with 30% missing fit, 17% of data sets with 40% missing fit, and 50% of data sets with

50% of their data missing fit the criterion of containing an R value of less than 0.25. Thus, small amounts of missing data do not seem likely to give false localizations, but the chance of getting a fit to a random location rises sharply as the amount of missing data increases. This problem is significantly eased if mapping can be performed with both RI families.

In conclusion, the RI method is an excellent method for quickly mapping a probe to a genomic position. However, since there are only a limited number of members in the RI families, certain problems arise which are not seen in standard genetic mapping. Specifically, there is a significant chance of mapping to an incorrect location, especially if there are no previously mapped loci near the unknown probe's apparent position. This problem is significantly increased when only one of the RI families is used, and is increased further if the data set is not substantially complete.

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Detection of RFLPs among strains of W22

--E.B. Godshalk and M. Lee

Inbred lines of maize exhibit various levels of genetic stability over generations of self-pollination (Bogenschutz and Russell, Euphytica 35:403-412, 1986). Inbred W22, released in 1948 by the University of Wisconsin, has been maintained at Iowa State University for at least 25 generations of self-pollination (strain W22-IA). Replicated comparisons of two strains of W22 (W22-IA and W22-WI, maintained at the University of Wisconsin) detected significant differences for 1000-kernel weight (243 vs. 199 g for W22-IA and W22-WI, respectively). W22-IA and W22-WI were not significantly different from each other for nine other traits; however, significant mid-parent heterosis values were detected for four traits (grain yield, kernel row number, cob diameter, and tassel branch number) in the W22-IA/W22-WI hybrid (Lamkey et al., Crop Sci 28:896-901, 1988).

The strains were evaluated for RFLPs with 29 mapped clones distributed throughout the genome. Five clones detected polymorphisms between the strains; four of the clones have been placed to chromosome ten.

The origin of the polymorphisms is unknown. Pollen contamination or seed mixture do not seem to be likely explanations given the overall morphological similarity of the strains. Residual heterozygosity in the original W22 could account for some or all of the observations. Whatever the cause(s), our observations for W22 strains and similar reports for inbred P39 (Gerdes and Tracy, MNL 63:93, 1988) suggest limitations (and opportunities) for identification of inbred lines with RFLPs.

EUGENE, OREGON
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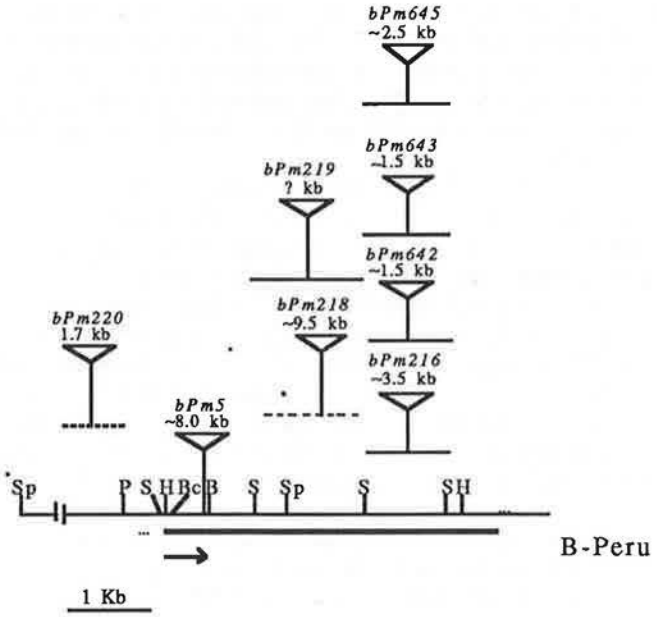
Mutator and *Spm* elements at the *B-Peru* locus

--Linda J. Harris, Garth I. Patterson, and Vicki L. Chandler

The *B-Peru* allele regulates anthocyanin synthesis in both kernel and plant tissues. We have used a stock of Robertson's Mutator to isolate four unstable and four stable (upon self-pollination) mutations in *B-Peru* (Chandler et al., MNL 62:56). Genetic and Southern blot analyses demonstrate that all eight mutants are the result of insertions within an ~5 kb region of the *B-Peru* gene. A map of the *B-Peru* gene and the insertion sites are shown below.



All eight insertions alter pigment synthesis in all kernel and plant tissues pigmented by *B-Peru*, suggesting that each insertion has disrupted a region of the gene required for expression in all tissues.

Two insertion alleles have been cloned using *B* sequences. *b-Perum5* contains an *Spm*-related element. Restriction mapping of the clone and genetic experiments suggest that it is an *Spm-w* element. Southern analysis of the *b-Perum220* allele suggested that there had been a complex rearrangement at the *B-Peru* locus, including a duplication of *B-Peru* sequences. The cloning of a portion of the *b-Perum220* allele revealed that a *Mu1.7* element had inserted upstream of the *B-Peru* coding region.

Genetic analysis of one other unstable allele, *b-Perum216*, is consistent with the presence of a *dSpm* element in the *B-Peru* gene. None of the four unstable alleles contained *Ac* activity. Genetic and molecular analysis of the remaining five insertion alleles is underway.

These results demonstrate that both Mutator and *Spm* elements were active in our stocks. Crosses examining the progenitors of these stocks showed that *Spm* activity

(identified by crosses to *wx-m8*, a *dSpm*) was present in the original Mutator stocks obtained from D. Robertson. No *Spm* activity was detected in our *B-Peru* and *b r-g* stocks. These results, combined with the low spontaneous mutation frequency observed in the progenitor *B-Peru* stock (less than 5 X 10^{-6}) (MNL 62:56), suggest that the source of the *Spm* activity was the Mutator stocks. Thus, when using Mutator in transposon tagging experiments, we recommend monitoring the mutagenesis stocks and isolated mutants for the activity of other transposable element families.

Expression of paramutable and paramutagenic alleles of the *B* locus

--Garth I. Patterson and Vicki L. Chandler

B is a regulator of the maize anthocyanin pathway. We have been studying paramutation, an unusual genetic phenomenon that occurs at the *B* locus. The phenomenon, as originally described by E. H. Coe, Jr., is briefly described here. The *B-I* allele gives intense color in many plant tissues. *B'*, a frequently isolated, spontaneous mutant of *B-I*, gives faint color in the same plant tissues colored by *B-I*. Paramutation occurs in a *B-I/B'* heterozygote. When this heterozygote is crossed to a *b* tester, 100% of the progeny are *B'/b*. This invariant change of *B-I* to *B'* is called paramutation.

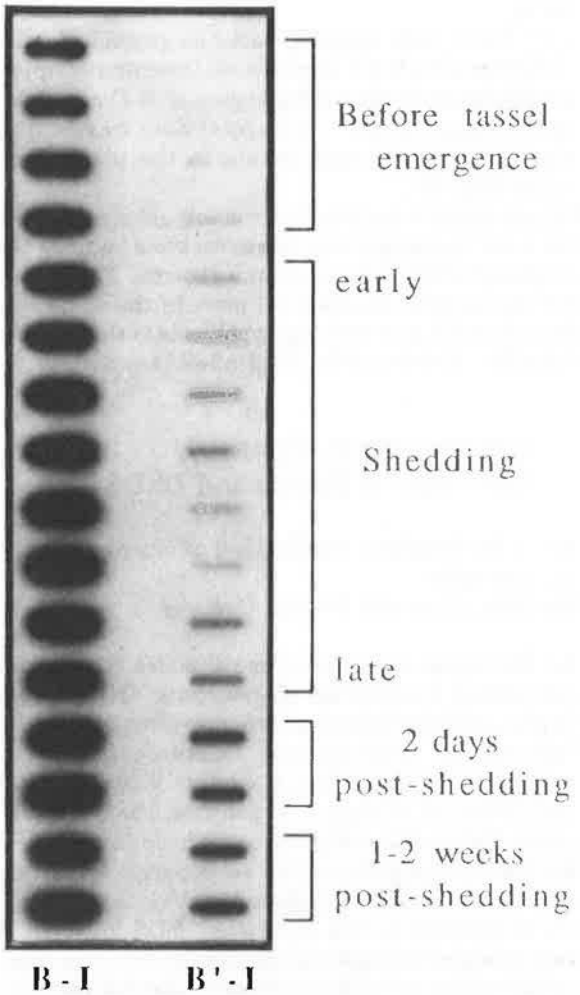


Figure. PolyA+ RNA was prepared from 7 *B-I* and 7 *B'* plants at various developmental stages. The RNA was slot-blotted and probed with a *B*-specific probe. Each sample was loaded in duplicate, and approximately 0.5 micrograms of RNA was loaded into each well. The developmental stage of the plants is indicated along the side.

Understanding the alteration in expression that leads to decreased pigmentation in *B'* plants might provide clues as to the nature of the genetic alteration that occurs in the *B-I/B'* heterozygote. As a first step, we have examined the difference in expression in *B-I* and *B'* plants. We isolated polyA+ RNA from husks of *B-I* and *B'* plants of various ages. These RNA samples were slot-blotted onto nitrocellulose and probed with a *B*-specific probe. We find that *B-I* plants have 20-100 fold more *B* mRNA than *B'* plants at each age examined, as shown in the figure. Northern blot analysis demonstrated that the *B* polyA+ transcripts are the same size in *B-I* and *B'* plants. These same *B-I* husks have accumulated 20-100 fold more anthocyanin pigment than *B'* husks. Pigment accumulation was measured by acid extraction and spectrophotometric determination of anthocyanin concentration. The same slot blot was stripped and reprobed with *A1* and *Bz1* probes (*A1* and *Bz1* are structural genes induced by *B* in husk tissue). The *B-I* husks also have much more mRNA from these loci. These results demonstrate that the difference in pigmentation and structural gene induction between *B-I* and *B'* correlates with a difference in mRNA accumulation. Experiments are in progress to determine if there are differences in transcription of the two alleles.

EUGENE, OREGON
University of Oregon
COLUMBIA, MISSOURI
University of Missouri

Characterization of two *Ds* mutants of *B-Peru*

-- J. K. Clark, V. L. Chandler, and M. G. Neuffer

Isolation and genetic analysis: The *B-Peru* allele of the *B* locus conditions purple color in the kernel and several plant tissues. Two *Ds* insertion mutants of *B-Peru* have been isolated and characterized: *b-mutable1* (*b-m1*) and *b-mutable dilute-2* (*b-md-2*). The *b-m1* and *b-md-2* alleles were found in material prepared for identification of transpositions of a chromosome breaking *Ds* into the short arm of chromosome 2 (MNL 60:55, 1986, 63:61, 1989). Ears of *b b, r-g r-g* were crossed by pollen from plants of the genotypes *P-vv* (*Ac*)/*P-wr B-Peru B-Peru, Ds-2 r-sc/r-sc* or *r-r*. The *Ds-2* segment of the long arm of chromosome 10 carried the self colored mutant *R-sc* from *R-st* and a chromosome breaking *Ds* element located between the centromere and the *R* locus. *Ds-2* was produced, characterized and designated by Dr. Jerry Kermicle who generously supplied the stock.

Ears from the above cross produced kernels which were all solid (slightly dilute) purple colored. They were examined for single kernel exceptions such as colored-colorless mosaics indicating repeated loss of the *B* locus which should occur if a *Ds* with chromosome breaking

properties were present somewhere near the *B-Peru* locus. Two were found and reported (MNL63:61, 1989).

Two other types of kernels were also found. The first was a colorless kernel with tiny colored dots; it was designated *b-m1*. The second kernel was pale purple with darker colored dots; it was designated as *b-md-2*. Subsequent tests showed that both kernels were mutable alleles of a color factor on chromosome 2S. Both map to the position of the *B* locus in 3-point linkage tests. Both *b-m1* and *b-md-2* were shown genetically to be *Ds* mutants, since neither activates the *Ds* at *C-I*, and both produce purple sectored kernels in the presence of the *Ac* at *P-vv* but not in its absence.

The amount of pigment in *b-md-2* mutant aleurones depends on the direction of the cross: kernels of the constitution *b-md-2/b/b, +Ac* show deeply colored sectors against a pale purple background, while in *b-md-2/b-md-2/b +Ac* kernels the background is so dark that the sectors cannot be distinguished. Homozygous *b-md-2* kernels lacking *Ac* show intense pigmentation that is visually indistinguishable from that of wild-type *B-Peru*. The color differences may indicate a dosage relationship; however, we have not done the appropriate experiments to determine whether the difference in color intensity among the various classes of kernels is due to dosage effects, or is the result of reduced expression in male versus female transmitted gametes, as was found for *R* mottling (J. Kermicle, Genetics 66:69-85, 1970).

Molecular analysis: *b-md-2*: Restriction mapping based on genomic Southern blots indicated that *b-md-2* contains a 400-base insertion, which was cloned and sequenced. This was accomplished by cloning a 3.5 kb *Spe*I fragment of *B-Peru* containing the insertion into the *Xba*I site of the lambda vector LongC (Stratagene), and subcloning a *Hin*dIII fragment from this clone into pTZ vectors (U.S. Biochemical) for restriction mapping and sequencing. The insertion in *b-md-2* proved to be a *Ds1* element of 400 bases. This *Ds1* contains the 11 bp perfect *Ac* inverted repeats and is flanked by an 8 bp target site duplication. This *Ds1* has 85-95% homology with the sequences of the 11 maize and *Tripsacum Ds1* elements that have been published (Sutton et al., Science 223:1265-1268, 1984; Wessler et al., EMBO J. 5:2427-2432, 1986; Schiefelbein et al., Genetics 120:767-777, 1988; Gerlach et al., J. Mol. Evol. 26:329-334, 1987). It shares with the maize *Ds101* element a 10 bp deletion not found in the other *Ds1* elements sequenced to date.

The *b-md-2* insertion is in the opposite orientation with regard to the direction of gene transcription as the *Ds1*'s in *Adh-Fm335* (Sutton et al., 1984), *wx-m1* (Wessler et al., 1986), and *bz-wm* (Schiefelbein et al., 1988). It is inserted in the 5' leader region of the *B-Peru* gene (Fig. 1). Preliminary RNaseA protection experiments with wild-type *B* alleles suggest that this region is in an intron (D. Turks, personal communication). The reduced level of pigmentation observed in the mutant may be due to a *B-Peru* message that is altered in either structure or stability. Two other *Ds1* insertions into the upstream regions of genes have been described; each was associated with a leaky phenotype as is *b-md-2*. A *Ds1* insertion 30 bases upstream of the TATA box in *bz1* is associated with a reduced steady state level of *bz1* mRNA and protein (Schiefelbein et al., 1988). At *Adh*, *Ds1* has inserted into the 5' leader where it is spliced out as an intron using a 5' donor site within the element and an acceptor site in immediately adjacent flanking DNA. The altered message is apparently reduced in stability (E.S. Dennis et al., Nucl. Acids Res. 16:3815-3828, 1988). Experiments are in progress to determine the nature of the interaction of *Ds1* and *B-Peru* in *b-md-2*.

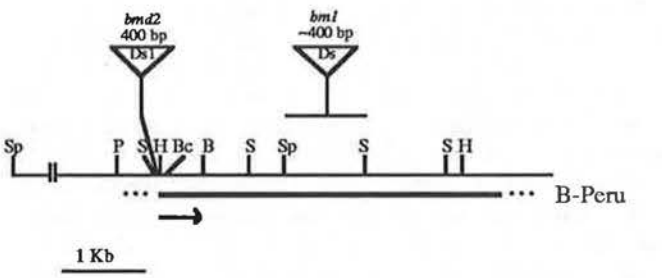


Figure 1. Restriction map of the *Ds* insertions in the *B-Peru* gene. The 2.2 kb message and direction of transcription are shown below the 4.3 kb genomic DNA. B = *Bam*HI, Bc = *Bcl* I, H = *Hin* dIII, P = *Pst* I, S = *Sac* I(*Sst* I), Sp = *Spe*I.

b-m1: Restriction mapping based on genomic Southern blots indicates that *b-m1* contains an insertion of approximately 400 bases in the coding region of *B-Peru*. This insertion is contained in a 1.0 kb *Spe*I/*Sac*I fragment that contains several exons and introns in the progenitor *B-Peru* allele (Fig. 1).

It is not possible to identify unambiguously the *Ds* element in *b-m1* using genomic Southern blots because of the large number of *Ds* elements in the genome. However, because of its size and because *b-m1* arose in the same experiments as *b-md-2*, our working hypothesis is that the *Ds* in *b-m1* is a *Ds1*. Cloning of the *b-m1* allele is underway.

GAINESVILLE, FLORIDA
University of Florida and USDA-ARS

Intracellular immunolocalization of Wx protein in endosperm cells

Yen-Ching Chen and Prem S. Chourey

The *Wx* locus on chromosome 9 codes for a starch-granule-bound nucleoside diphosphate (NDP) sugar-starch glucosyltransferase, which transfers glucose from UDP-glucose (or ADP-glucose) to starch (Nelson and Rines, Biochem. Biophys. Res. Commun. 9:297-300, 1962). Echt and Schwartz (Genetics 99:275-284, 1981) and Shure et al. (Cell 35:225-233, 1983) reported isolation of a 58 kD protein from starch grains of *Wx* endosperm. Both groups presented various lines of evidence to show that the 58 kD protein is encoded by the *Wx* locus. SDS Western blot analyses revealed a single polypeptide in *Wx* and none in some of the stable *wx* endosperm starch-bound protein extracts using the polyclonal antiserum raised against the Wx protein (Shure et al. 1983). We have used this monospecific Wx antiserum (kindly provided to us by Dr. Nina

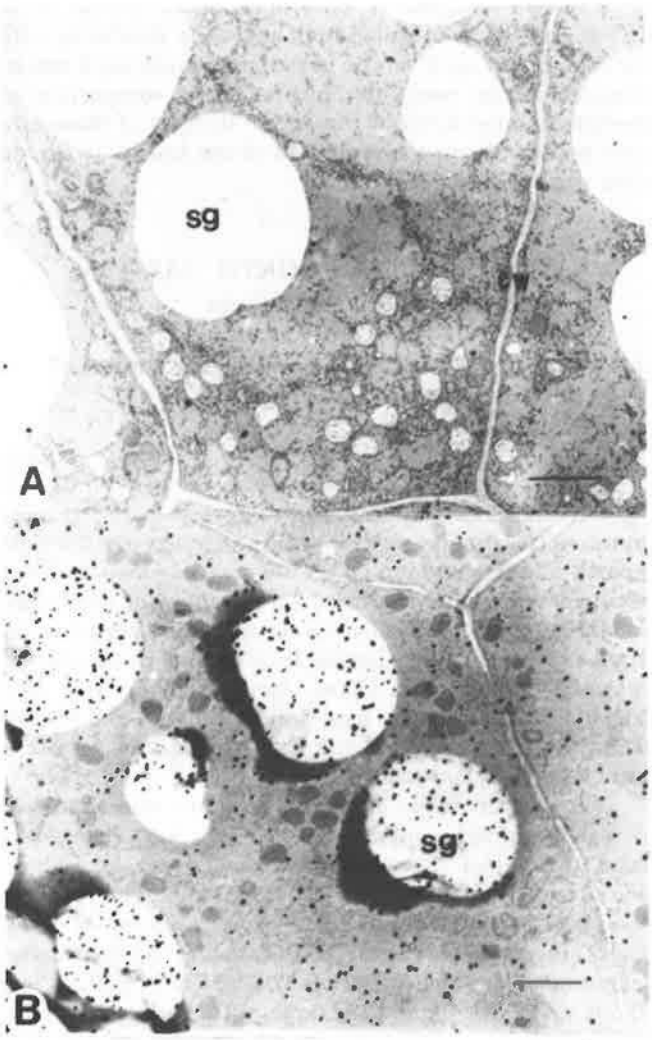


Figure 1. Immunocytological localization of Wx protein in endosperm cells. Sections stained with (A) preimmune serum (B) Wx antiserum. sg = starch grain. cw = cell wall. bar = 2μ.

Fedoroff) to localize the Wx protein in developing *Wx* endosperm cells through immuno-gold silver enhancement reactions.

The kernels at 15 days after pollination were fixed in cacodylate buffer (pH 7.2) containing 3% glutaraldehyde, 2% paraformaldehyde, 2.5% dimethyl sulfoxide and 0.5% acrolein. The thin sections of maize endosperm at crown region were treated with 1:200 diluted Wx antiserum, incubated with secondary antibody conjugated with 15 nm colloidal gold, and stained with silver enhancement reagents. As expected, the labeling as evidenced by the black dots was predominantly limited to the starch grains; background labeling over cytoplasm, organelles, and cell walls was very low (Fig. 1B). Immunostaining with a preimmune serum (1:200 dilution) gave low to undetectable levels of nonspecific staining (Fig. 1A).

Nuclear-mitochondrial interactions affecting transcription of mitochondrial open reading frames

--Torbert R. Rocheford* and Daryl R. Pring
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The circular mitochondrial genome of maize (570 kb) consists mainly of single copy sequences. For several maize mitochondrial genes, multiple mRNA transcript size classes are present. mRNA processing or multiple transcript initiation sites can contribute to the presence of multiple mRNA transcripts for an individual mitochondrial gene. Some mt mRNA processing events have been shown to be under nuclear control. We are reporting on the effect of nuclear genotype on the transcriptional patterns of the maize mitochondrial gene T-*urf13* and open reading frame 25. T-*urf13* is expressed only in T cytoplasm maize and is associated with T cytoplasmic male sterility. ORF25 is transcribed in all four major maize cytoplasms (N,C,S,T), and is cotranscribed with T-*urf13* in T cytoplasm.

Variation in T-*urf13*/ORF25 transcripts among inbred lines in T cytoplasm and ORF25 transcripts among inbred lines within N, C and S cytoplasms was detected. Northern analysis of genetic progenies developed from inbred lines associated with these transcriptional differences indicated presence or relative abundance of specific ORF25 transcripts was under nuclear control.

Transcriptional patterns of the T-*urf13*/ORF25 region include up to six transcripts. Our survey of diverse maize inbreds in T cytoplasm revealed absence of the generally detected 1538 nt transcript for the inbreds 33-16(T) and B14A(T). The absence of this transcript is accompanied with presence of novel transcripts of 1400 and 1475 nt, respectively. These two novel transcripts were not detected in all other inbred lines evaluated. Detection of the 1400 nt transcript and absence of the 1538 nt transcript in the F1 progeny of Wf9 (T) X 33-16(N) indicates presence of this transcript is under nuclear control.

To investigate the relationship of the T-*urf13*/ORF25 processing event encoded by *Rf1* and the effect associated with inbred 33-16, the line R213(N) *Rf1 Rf1 rf2 rf2* was crossed onto 33-16(T) *rf1 rf1 Rf2 Rf2*. The T-*urf13*/ORF25 transcriptional pattern of this F1 exhibited a 1400 nt transcript, and the *Rf1*-associated reduction in abundance of 2013, 1830, and 1785 nt transcripts and appearance of the 1571 nt *Rf1* specific transcript. Therefore the mRNA processing associated with *Rf1* appears independent of the event generating the 1400 nt transcript. Additional information was gained by evaluating fertility phenotypes and corresponding T-*urf13*/ORF25 transcript patterns of some stocks. Since inbred 33-16(T) (male sterile) and the F1 progeny of 33-16(T) X R213(N) (male fertile) both exhibit the novel 1400 nt transcript and not the 1538 nt transcript generally detected, T-*urf13*/ORF25 transcriptional patterns can therefore be variable in both sterile and fertile states.

Evaluation of ORF25 transcript patterns for the inbred W64A in N, C, and S cytoplasms revealed a difference in

the size of the most highly abundant transcript detected. This transcript migrated at 2200 nt in N cytoplasm and 2100 nt in C and S cytoplasms. Since the nuclear complement was essentially the same among the three cytoplasms, the transcript size difference is probably due to a mitochondrial configuration difference, possibly a 100 bp deletion or insertion. For each of these cytoplasms, this 2200/2100 nt ORF25 transcript varied considerably in abundance when associated with different inbred lines. For example, the 2100 nt transcript is not detected or is detected in very low abundance in RDW182BNRf(C). The F1 of RDW182BNRf(C) X A619(C) exhibits the highly abundant 2100 nt transcript. The other ORF25 transcripts (3400, 3200, 1350 nt) detected in the F1 did not change in abundance in comparison to RDW182BNRf(C). Reciprocal crosses of RDW182BNRf(C) X A619(C) both exhibited a highly abundant 2100 nt transcript, suggesting dominant gene action for control of the highly abundant form of the 2100 nt transcript.

A minor 2800 nt ORF25 transcript is present in some inbred lines, e.g. Wf9(C), that do not exhibit the highly abundant 2100 nt transcript. This 2800 nt transcript is not apparent in lines that exhibit the highly abundant 2100 nt transcript, e.g. W64A(C). The F1 progeny of Wf9 (C) X W64A(N) did not exhibit the 2800 nt transcript and did exhibit the 2100 nt transcript. This indicates the W64A genotype somehow modifies transcription of the 2800 nt transcript and there is a relationship between the absence of the 2800 nt transcript and presence of the 2100 nt transcript.

The ORF25 transcript pattern associated with inbred A188(N) is different from all other lines examined in N cytoplasm (e.g. 187-2) in that the largest mRNA species detected is 3100 nt and not the 3500 nt species generally detected. To examine whether this transcript size difference was due to mitochondrial configuration or due to nuclear effects, the cross A188(N) X 187-2(N) was performed. The ORF25 transcript pattern of the F1 progeny exhibited transcripts of both 3500 and 3100 nt, indicating nuclear control of the presence of these transcripts. Since the 3500 nt transcript is the largest species detected, this transcript may be a primary initiated transcript.

The tissue culture mutant A188(T7), which has lost T-*urf13*, displays an ORF25 transcript pattern that is identical to A188(N) (3100, 1700 & 1300 nt). The F1 progeny of A188(T7) X 187-2(N) exhibits an additional highly abundant 2100 nt transcript. This 2100 nt transcript is not present in lines with T-*urf13*. The deletion of T-*urf13* and the observation of the highly abundant 2100 nt transcript when 187-2(N) is crossed onto this deletion mutant, suggests that the mtDNA region associated with expression of the highly abundant 2100 nt transcript was present in T cytoplasm mt DNA. However, this region apparently was unable to contribute to presence of the highly abundant 2100 nt transcript. These data demonstrate the complex interactions of the nucleus and mitochondria during the process of mt DNA transcription. The T-*urf13* data are suggestive of additional nuclear controlled processing events for this gene. Collectively, the ORF25 data are consistent with modification of transcription by nuclear encoded gene products that enhance initiation of specific ORF25 transcripts.

Sequence analysis of ORF25 and the region 5' to ORF25 in B37(N) revealed high sequence similarity with the region 160 bp 5' to the presumed wheat *atp6* amino terminus leader sequence. Interestingly, comparison of predicted amino acids of the amino termini of these two open reading frames revealed 13 of the initial 18 amino acids were shared.

GRAND FORKS, NORTH DAKOTA
University of North Dakota

Induction of twin embryos by x-ray treatment of early proembryo stage embryos

--William F. Sheridan

An experiment was conducted with the goal of analyzing cellular autonomy of mutant expression of the *dek1* mutation (for results regarding this question see the next report). Plants grown in pots were used as females. They were crossed and later administered a dose of about 800 Roentgens of soft x-rays over a seven minute period using the x-ray machine in Curtis Hall at the University of Missouri-Columbia. I thank Jerry Neuffer and Dan England for growing the experimental plants and for use of the equipment and I thank Evelyn Bendbow for assistance in treating the ears.

Ears were treated at 50 hours, 72 hours, and 104 hours after pollination in mid-July 1988. Ears were harvested, dried, and tagged. They were then shelled and the embryo side of each kernel was visually examined. The frequencies of kernels with twin embryos for each set of treatments among the ears analyzed to date are: 50 hours after pollination, 4 out of 170 (2.3%); 72 hours after pollination, 39 out of 752 (5.2%); and 104 hours after pollination, 4 out of 197 (2.0%).

Under the summer growing conditions in Missouri fertilization occurs probably by 18 hours after pollination. At 50 hours after pollination the zygote has divided to produce a few-celled proembryo, by 72 hours after pollination the proembryo is still very small, consisting probably of no more than 16 cells. Even if fertilization occurred at 12 hours after pollination and the zygote divided 12 hours later to form two cells, and there was a doubling of embryo cell number every 12 hours thereafter, at 72 hours after pollination, embryo cells would be dividing to form a 32 cell embryo, including the suspensor. An examination of the data on maize embryogenesis reported by L. F. Randolph (1936) reveals that "four days after pollination the embryos contained ordinarily 10 to 24 cells" and his Figure 5B showing a radial longitudinal section through a proembryo fixed 5 days after pollination reveals that there are only a few additional cells. Furthermore, Randolph noted that "except for the localization of growth, primarily in the upper portion, the proembryo is wholly undifferentiated up to and including the seventh day" after pollination.

X-ray treatment of early developing maize embryos was performed by R. S. Poethig, E. H. Coe, and M. M. Johri (1986). They performed a histological analysis on their greenhouse-grown materials. Their embryos were slightly ahead in their rate of development but at 48-53 hours after pollination the early proembryo consisted of four cells, and at four days (96 hours) after pollination, their midproembryo appears about equivalent in development and cell number to the 5 day proembryo figured by Randolph.

The results presented in this report show a frequency of twin embryos of 5.2% when x-ray treatment was administered 72 hours after pollination. In contrast, Poethig, Coe, and Johri observed, following treatment of developing embryos with 1000R of soft x-rays at two, three, four and five days after pollination the following frequency of twin shoots: two days, 0.5%; three days 0.3%; four days, 6.7%; and five days, 6.2%. A high twinning rate did not occur until treatment was given at four days after pollination and in most of these twins, twinning occurred at the first node or above.

The results described in this report are of particular interest because they indicate that the very early proembryo of maize is comprised of cells that can regulate when cell death is inflicted by x-ray treatment. The data on embryos treated 72 hours after pollination are particularly persuasive. Although a morphological analysis of the embryos was not undertaken these embryos were undoubtedly at the early proembryo stage. Yet analysis of kernels of five different ears irradiated at 72 hours after pollination revealed a twin embryo frequency ranging from 4.2% to 5.8%. Of additional interest is the observation that nearly all of the twin embryos examined were complete twins and were fused only in the suspensor region if they were fused at all.

The presence of complete twin embryos indicates that the cells of the early proembryo surviving the x-ray treatment were able to sufficiently regulate that they could proceed with complete embryo formation. The x-ray treatment may have killed a cell (or small group of cells) occupying an apical position in the proembryo. The death of this cell (or cells) may have had the effect of microsurgery so as to split the proembryo so that each of the two surviving portions proceeded to form an embryo. This idea is congruent with the fact that frequently one of the embryos was larger than its twin. It is also conceivable that the effect of x-ray induced cell death may have been to release the proembryo cells from apical dominance. Twinning may normally be absent because of the very early establishment of apical dominance in the proembryo. These studies will be continued with the addition of structural studies to seek evidence for the occurrence, timing, and location of cell death in treated embryos.

Sectorial analysis of *dek1* mutant expression

--William F. Sheridan

The accompanying report on x-ray treatment of early proembryo stage embryos describes the timing and dosage of x-ray treatments used in an effort to induce *dek* mutant sectors in *+/dek1* embryos. Among the kernels analyzed to date a total of 534 were produced with pollen shed by plants heterozygous for the *dek1* mutation (*+/dek1*). Both the male and female parents were homozygous for all of the aleurone color factors. They were also homozygous for the *R-scm2* allele. A normal embryo carrying all of these factors (with at least one dose of the dominant allele at each locus) is pigmented with anthocyanin in the scutellum. The embryonic axis is yellowish-white except for a region of purple pigmentation at the tip of the coleoptile.

Each of the 534 kernels was examined visually (with an 8-power magnification hand lens when needed) in a search for colorless sectors since it is known (M. G. Neuffer and W. F. Sheridan, 1980) that *dek1* mutant embryos do not synthesize anthocyanin. The *dek1* mutation segregates in a normal three to one fashion on self-pollinated ears. Therefore about half or about 267 of the kernels examined should be heterozygous for the *dek1* mutation. In their study, Poethig, Coe, and Johri (1986) irradiated plants carrying the *R-sc* allele (which conditions anthocyanin synthesis only at the periphery of the scutellum, and is therefore less favorable material than that containing the *R-scm2* allele) in searching for colorless sectors against a colored scutellum background. These workers used x-rays to break chromosome arm 9S bearing the *C-I* allele, the loss of which allowed pigment formation to occur. Poethig and coworkers found six sectored embryos out of a total of 815 kernels examined of material given 500R of hard x-rays at 56 hours after pollination. This is a frequency of 0.7 percent. If the same frequency of sectors were expected in the *+/dek1* embryos then two embryos of the 267 presumed heterozygotes or two among the 534 total embryos examined should have displayed a colorless sector of *dek1* mutant tissue. No embryos have been found that contain colorless sectors. These results are limited by the small sample size but are suggestive either that mutant sectors cannot survive in the competition for nutrients during early kernel development or that the mutant sectors are not cell autonomous. It seems most likely that the former suggestion or small sample size applies to these results.

Embryo developmental profiles of *cp*-1379A*, *dscptd*-1425A*, and *dek17*: three embryo-lethal mutants

--Guy E. Farish and William F. Sheridan

The three embryo-lethal defective kernel (*dek*) mutations are defective in both their endosperm and embryo development. Standard histological and fresh dissection techniques were used to examine and compare mutant and normal kernels from the same ears at ages ranging from 5-6 days after pollination (dap) through kernel maturity. Embryo stages are those of E. C. Abbe and O. L. Stein (Amer. J. Bot. 41:285-293, 1954). Two of the mutants are affected early in embryo morphogenesis while the third, *dek17* on chromosome arm 3L, is capable of forming one or more leaf primordia but is unable to germinate.

The mutant *cp*-1379A*, also on 3L but not allelic to *dek17*, is blocked early in embryogenesis. By 12 dap, mutant embryos were lagging behind normal embryos on the same ear. Normal embryos were at a late transition stage while mutant embryos had not progressed beyond an early proembryo stage. At 18 dap mutant embryos reached a mid-transition stage and did not proceed beyond this with regard to formation of a coleoptilar ring or a shoot apical meristem, morphological features characteristic of the coleoptilar stage. Mutant embryos did become broadened so as to become uniformly shell-like in appearance. Because the mutant embryos lacked shoot meristems and leaf primordia they were unable to germinate at maturity.

The mutant *dscptd*-1425A*, located on chromosome arm 6L, is also disturbed early in embryogenesis. Mutant embryos lagged behind normal embryos in their development by 18 dap when normal embryos were at stage 1 while mutant embryos were at a mid-transition stage. By 32 dap normal embryos were at stage 4 while the mutant embryos varied in their progression so that they ranged from a late transition to an abnormal coleoptilar stage. None of the mutant embryos ever formed leaf primordia by kernel maturity and all were abnormal in appearance. Because the mutant embryos lacked leaf primordia they were unable to germinate at maturity.

Mutant *dek17* embryos could not be distinguished from normal embryos until 18 dap. At this age, mutant embryos were at an abnormal late transition stage and normal embryos were at the coleoptilar stage. Mutant embryos continued to develop at a much slower rate than normal embryos; at 45 dap mutant embryos had formed a shoot apical meristem, a root meristem, a coleoptile, and one leaf primordium whereas normal embryos were at stage 5 (5 leaf primordia formed). Despite the presence of at least one leaf primordium, mutant embryos were unable to germinate at kernel maturity. The pleiotropism of this mutation results in a collapsed endosperm, a much delayed but otherwise essentially normal embryo morphogenesis, and a blockage to the escape of the embryo from dormancy.

Listing of reciprocal translocation stocks being maintained by individual cooperators

--William F. Sheridan

One of the advantages of maize for biological research is the availability of a large number of reciprocal translocations. The collection originally compiled and characterized by Longley and Anderson contained about 1000 translocation stocks. About 85% of these are available from the Stock Center (see MNL 55:140, 1981). Some potentially useful translocations of the original collection that are not in the Stock Center collection may be in the genetic stock collections of individual cooperators. This note is a request that interested cooperators send me a listing of the reciprocal translocation stocks they are maintaining. I will compile a combined listing of these stocks and distribute the list to the contributors. In this way we may locate some translocations not available from the Stock Center and also provide a list of backup sources for the Stock Center collection. Send your list to me at Box 8238 University Station, Grand Forks, ND 58202.

Mapping of *dek31* on chromosome arm 4L

--William F. Sheridan

The embryo-lethal defective kernel mutation *dek31* (*ptd*-1130*) is located about 14 cM distal to the *gl4* locus on 4L. Last spring backcross kernels were scored for sugary (*su1*) and colorless (*c2*), planted in the greenhouse, scored for glossy (*gl4*) as seedlings and transplanted to the field and self-pollinated. The 361 selfed ears were scored for *dek31* segregation. Frequencies of each of the 16 classes of backcross progeny and the recombination frequencies for the four point linkage test are presented in Table 1.

Table 1. Linkage of *dek31* with *su1*, *gl4*, and *c2*.

Backcross: sul gl4 Dek31 c2/sul gl4 Dek31 c2 X Sul Gl4 dek31 C2/ sul gl4 Dek31 c2

		Progeny genotypes*	Frequency	
a)	P:	Su Gl4 dek 31 C2	91	
		su gl4 Dek 31 c2	38	129 = 35.7%
b)	COI:	Su gl4 Dek 31 c2	13	
		su Gl4 dek 31 C2	13	26 = 7.2%
c)	COII:	Su Gl4 Dek 31 c2	10	
		su gl4 dek 31 C2	8	18 = 5.0%
d)	COIII:	Su Gl4 dek 31 c2	46	
		su gl4 Dek 31 C2	76	122 = 33.8%
e)	COI & II:	Su gl4 dek 31 C2	3	
		su Gl4 Dek 31 c2	5	8 = 2.2%
f)	COI & III:	Su gl4 Dek 31 C2	28	
		su Gl4 dek 31 c2	6	34 = 9.4%
g)	COII & III:	Su Gl4 Dek 31 C2	14	
		su gl4 dek 31 c2	4	18 = 5.0%
h)	COI & II & III:	Su gl4 dek 31 c2	2	
		Su Gl4 Dek 31 C2	4	6 = 1.7%
Total			361	100%

% Recombination:

su-gl4 (b+e+f+h) = 20.5% ± 2.1%
gl4-dek31 (c+e+g+h) = 13.9% ± 1.8%
dek31-c2 (d+f+g+h) = 49.9% ± 2.6%

*Genotypes of maternally inherited chromosome 4; every progeny also contained a chromosome 4 inherited from the pollen parent and carrying sul gl4 Dek31 c2. Self pollination of the progeny allowed for scoring for dek31 in segregating ears.

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Codon bias in maize nuclear genes

--Wilbur H. Campbell

Considerable interest has recently centered on the codon bias of higher plant nuclear genes. This has partly come about because a much larger number of plant nuclear genes have been sequenced recently. But it has also been realized that there are significant differences between the codon bias of monocots and dicots. This is particularly striking when comparing some large genes which have recently been sequenced from maize, such as the cDNAs for nitrate and nitrite reductases, to their corresponding

dicot genes. The maize genes were found to be encoded with a much smaller set of codons than the dicot genes for these enzymes. Furthermore, the codon set used for the maize genes was narrowly biased toward the synonymous codons ending in G and C, while the dicot genes had little bias toward these G+C ending codons and in fact, used all codons for encoding the polypeptides. Murray et al. (Nucl. Acids Res. 17:477, 1989) described the codon usage in 207 plant genes and concluded that it differed between monocots and dicots. We (Campbell & Gowri, Plant Physiol. in press, 1989) have also analyzed codon usage in 100 monocot and 63 dicot genes, which included all data available in GenBank (Release 57) as well as many recently published sequences. Although our total number of genes analyzed appears smaller than the prior study, we included only one example for genes which are represented by gene families (i.e. Cab, RbcS, Zeins etc.) when the members of the family did not differ in codon bias. In addition, we defined a set of preferred codons for each gene by selecting those codons which accounted for 85% of the amino acids encoded in a gene's sequence. This allowed a more compact and perhaps, we hope, a more understandable presentation of codon bias data for a broader audience. In any case, while we also found that dicots and monocots differed in codon usage, our results showed that this difference was not a simple case of the dicots nuclear genes showing less preference for synonymous codons ending in G+C than the monocots. In fact, monocots appear to have two classes of genes with respect to codon usage: those with a strong preference for G+C ending codons and those with less preference for these synonymous codons. When all the data we collected were plotted with gene number versus G+C in the 3rd position of the codon, dicot genes were found to have a modal distribution positioned at about 45%, while monocot genes had a bimodal distribution with nodes at 50% and 95%. A similar plot for maize nuclear genes is presented in Figure 1.

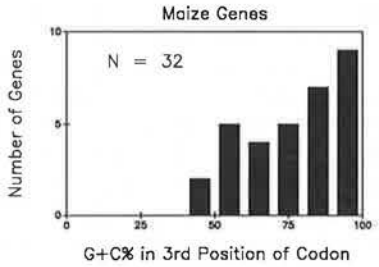

Figure 1. A plot of the number of maize genes versus the G+C percent in the third position of the codons of their respective coding sequences. A 5% G+C window was used. The data were taken from Gowri & Campbell, Plant Physiol. in press, 1989.

This difference in codon usage among maize nuclear genes is clearly illustrated when homologous genes are compared. For example, chloroplastic and cytosolic glyceraldehyde-3-phosphate dehydrogenase (MZEG3PD1 & MZEG3PD2) differ significantly in codon usage with MZEG3PD1 using a total of 39 codons for all amino acids encoded and a preferred set of 29 codons and MZEG3PD2 using a total of 51 codons and a preferred set of 40 codons. A similar difference in codon usage among the catalase genes is also found with MZECAT1 and MZECAT2 having a codon preference like MZEG3PD2, while MZECAT3 resembles MZEG3PD1 in codon usage pattern. The data available on nuclear genes of other monocots show a similar difference, but fewer examples are available to illustrate it using homologous genes. However, these differences in homologous genes are not found when the dicots are analyzed. Thus, it would appear that different mechanisms governing silent mutations in coding sequences of monocots and dicots have been operating during the evolution of these species.

We also noted in our review on codon usage in plant genes that the bimodal pattern of percentage of G+C in the 3rd position of codons for monocot nuclear genes was similar to the pattern observed for human genes (and perhaps other warm-blooded vertebrates). The bimodal distribution of codon usage in human genes has been explained by the finding that the human genome is a mosaic of A+T and G+C rich regions, which have been called isochores (Aota & Ikemura, Nucl. Acid Res. 14:6345, 1986). Wolf et al.(Nature 337:283, 1989) showed that A+T and G+C rich regions of mammalian genomes have different rates of mutation at silent sites, which may account in part for the existence of isochores in the genomes of these organisms. This leads to the question: do isochores exist in the nuclear genome of maize and does this account for the differences in codon usage among maize nuclear genes?

Bernardi and coworkers (Salinas et al., Nucl. Acids Res. 16:4269, 1988; Matassi et al., Nucl. Acids Res. 17:5273, 1989) have analyzed plant nuclear genomes to determine if isochores exist. Originally, they used buoyant density analysis of genomic DNA fragments to compare 3 dicots and 3 monocots, but more recently, they compared 5 dicots and 9 monocots. They concluded that isochores exist in all plant genomes, but that the usual distribution is toward a lower G+C content in the genomes of dicots as compared to monocots. However, the recent study showed a dicot (*Oenothera hookeri*) with a much higher and a monocot (*Allium cepa*) with a much lower G+C content. But their analysis of Poaceae indicates that all grasses have a high G+C content, including maize, and these species display evidence for isochores in their nuclear genomes. They suggest that the bimodal distribution of codon usage among monocot genes which have been sequenced is found because these genes come from different regions of their respective genomes with differences in G+C content. Thus, in the future as more maize genes are sequenced and mapped to their chromosomes, it should be possible to relate their G+C content and predict the isochore structure of the maize genome. However, the mechanism underlying the evolution of the maize genome into a mosaic structure of A+T and G+C rich regions is yet to be explained. Furthermore, it is not clear why some plant genomes have evolved toward a higher G+C content relative to others. Finally, it would appear that the differences in codon bias among maize nuclear genes, which probably reflects their genomic environment more than other features such as mRNA stability or translation efficiency, may have little physiological significance.

Characteristics of maize leaf cDNA clone Zm7gg

--G. Gowri, Björn Ingemarsson and Wilbur H. Campbell

In the process of screening a maize leaf cDNA library in lambda gt11 with the nitrate reductase antibody, we isolated and purified 4 positive clones. All these clones show very strong reaction with the antibody when the inserts are expressed as lacZ fusion proteins. All 4 clones were subcloned into pUC12 for further characterization. Two clones had identical restriction maps and one of them (Zmnr1) was sequenced and shown to be a partial clone for the NADH:nitrate reductase, from which the polypeptide had been partially sequenced. The third clone (Zm6) was sequenced and found to be a near full-length clone for chloroplastic glyceraldehyde-3-phosphate dehydrogenase by comparison to the sequence published for another cDNA clone of this enzyme. We have described these results (Gowri & Campbell, Plant Physiol. 90:792, 1989). However, the fourth clone (Zm7gg) has not been described.

Zm7gg contains an insert DNA of 0.9 kb. Since our nitrate reductase clone encoded only about two-thirds of the enzyme's sequence as compared to the full-length polypeptide deduced from clones of tobacco and *Arabidopsis*, we sequenced Zm7gg to determine if it encoded the rest of the maize enzyme. Unfortunately, the amino acid sequence deduced from the nucleotide sequence of Zm7gg does not match the amino acid sequence expected for the N-terminal region of nitrate reductase and does not match strongly to any sequence in the databases. However, the sequence of Zm7gg does have a polyA tail and appears to represent a mRNA expressed in maize leaves. A Northern blot of total RNA and polyA+RNA from nitrate-induced maize leaves was probed with Zm7gg and several bands were found to hybridize, which appeared at first to suggest that the probe was non-specifically hybridizing to rRNA despite the stringent conditions used for developing the blot. However, a Northern blot of nitrate-induced maize scutella RNA showed no hybridization to Zm7gg, which ruled out non-specific binding to rRNA as an explanation for the clone's binding to leaf RNA. In addition, a Northern blot of nitrate-induced root RNA probed with Zm7gg had a single RNA of 3 kb which weakly hybridized.

Since the deduced amino acid sequence of Zm7gg is very rich in proline residues, we compared it to the sequence of the proline-rich domain of CTF, a eukaryotic DNA-binding protein with affinity for the CCAAT-box (Santoro *et al.* Nature 334:218, 1988). We found a significant degree of homology between these sequences. Another DNA-binding protein found in HeLa cells and rat liver, NF1, also has a protein-rich domain with homology to the deduced amino acid sequence of Zm7gg. A comparison of the proline-rich regions of these three sequences is shown in Figure 1. Zm7gg, CTF and NF1 have 18, 19 and 23 proline residues in these proline-rich sequences, which yields from 16 to 24% proline. The consensus sequence derived from these three sequences, which is shown in Figure 1, indicates that 13 proline residues are shared. However, the degree of homology over the entire sequence of Zm7gg and the C-terminal half of CTF/NF1 is only about 18%, which indicates that Zm7gg is probably not similar in function to these DNA-binding proteins. But it does indicate that Zm7gg probably falls into the class of proline-rich domains commonly found in DNA-binding proteins. Most interestingly, the Northern blots of RNA from rat liver and other tissues resemble those we have found for Zm7gg, which have multiple transcripts hybridizing to the probe (Paonessa et al., EMBO J. 7:3115, 1988). The sizes of the transcripts found for corn leaf are: 3.5, 2.0 and 1.8 kb, with the middle band being the strongest.

```
Zm7gg      WPRPRPRRQLHEPDAQGAATERRRRRPPPPPHGRGAAPGRERPVHGVPPGGAGQVR
CTF-Pro    PPHLNPQDPLKDLVSLACD.......PASQQPGRL.....NGSGQ.LMPSHCLSAQ
NF1-Pro    AIRYHPQETLKEFVQLVCP.......DAGQQAGQVGFLNPNGSSQGKVHNPFLPTP
consensus  PR  PQ  LKE V L C        PA QQ GR      NGS QG  P   L

Zm7gg      QRQRVAHPGLQRSSSSLSVPHAQQSFPGVGIGVGDDDIYNATAAPLSVSAWRRRTTSP
CTF-Pro    ML.APPPPGLPR....LALP..PATKPATTS.EGG.....AT.SP.SYSP...PDTSP
NF1-Pro    MLPPPPPPPMARP...VPLPMPDTKPPTTSTEGG......AA.SPTSPT.YSTPSTSP
consensus  ML  PPPPGL R    L LP      P T   G      AT  P S S    P TSP
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Figure 1. A comparison of the proline-rich regions of Zm7gg and two mammalian DNA-binding proteins. Residues 82 to 195 are shown of the open-reading frame of Zm7gg. The proline-rich domains of CTF and NF1 are in the C-terminal half, while the DNA-binding domain, which is a highly positively charged alpha-helix region, is in the N-terminal half of CTF and NF1 (Mitchell and Tjian, Sci. 245:371, 1989).

More recently, we isolated a cDNA clone with some sequence homology to Zm7gg when using the same anti-nitrate reductase antibodies to screen a lambda gt11 library of corn roots, which had been induced with nitrate. However, this root clone, which is 1.5 kb in length, hybridizes to mRNAs of 4.5 and 3 kb in a Northern blot of polyA+RNA from nitrate-induced corn roots, but not from uninduced root RNA. Slot blots indicate that the transcripts, which hybridize to the new cDNA clone from maize roots, are increased in level by at least 3-fold after 1.5 hours of nitrate treatment of the roots. We are unable at this time to explain why antibodies for nitrate reductase pick up these clones when their inserts are expressed as lacZ fusion proteins in lambda gt11, nor do we have any clearer idea what this protein does in maize. We are doing more detailed analysis of the nucleotide sequence of the maize root clone, which we hope will assist in unraveling more about its function and perhaps shed light on its relationship to Zm7gg. If other investigators are interested in utilizing Zm7gg, we would be glad to share it.

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Anthocyanin synthesis during embryogenesis in vitro

--P. Suprasanna, K. V. Rao and G. M. Reddy

Callus cultures were established from immature embryos of Deccan Hybrid Macca (DHM-1) on MS medium

supplemented with 2 mg/l 2,4-D with a frequency of 38-40%. Callus was initiated within a week of inoculation and after three weeks, was subcultured onto MS maintenance media. The subcultured calli exhibited embryogenic and non-embryogenic sectors. Embryogenic sectors were separated from non-embryogenic calli. Upon transfer to MS regeneration media devoid of hormones, embryogenic clumps showed purple anthocyanin synthesis, followed by differentiation into embryoids. Spectral studies indicated that the purple pigment was cyanidin-3-glucoside (0.197 OD units/mg tissue). Anthocyanin was retained for 6-8 days after which embryoids became greenish in colour. DHM-1 genotype generally exhibits anthocyanin in plant parts, however, the specific gene(s) regulating the biosynthesis of anthocyanin are not clearly known. The present study suggests that association of anthocyanin synthesis may be exploited for studies on in vitro differentiation in maize.

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The translocation of bacterial vector plasmids into intact mitochondria of seedlings

--Jury M. Konstantinov, Vladimir A. Podsosonny and Galina N. Lucenko

The study of nucleic acid translocation into maize seedling mitochondria is recently getting much emphasis in terms of theoretical possibility of cloning recombinant DNA in mitochondria and chloroplasts and using the latter in experiments on genetic engineering of plants by fusion of protoplasts and subcellular fragments. This communication reports results from the study of translocation of pBR322 and pBR327 bacterial vector plasmids into isolated maize seedling mitochondria. To demonstrate the translocation of plasmid DNA into the inner space of organelles, the template activity of the pBR322 plasmid has also been studied as regards DNA synthesis in the genetic system of intact mitochondria.

The mitochondria isolated from 3-day-old etiolated seedlings of Krasnodarsky 303 TV hybrid (W64A x Sg25) by the conventional method of differential centrifugation were used in the experiments. The mitochondrial pellet was resuspended in the solution containing 68 mM sucrose, 20 mM Na_2HPO_4, 4 mM KCl, 20 mM sodium succinate, 0.5 mM ATP, 10 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7.4). To translocate the plasmid DNA into mitochondria, 4-8 μg DNA of the pBR322 or pBR327 plasmid was added to the suspension (8-10 mg protein per 1 ml) and the mixture was incubated on ice bath for 30 min. The plasmid DNA that failed to bind to mitochondrial membrane was removed by thrice centrifugation of mitochondria. To protect mitochondria from possible contamination with nuclear DNA and to remove the plasmid DNA adsorbed on the surface of the mitochondrial membrane, the organelles were treated with pancreatic DNase (100 μg/ml) obtained from Serva (FRG). In the experiments on hybridization the electrotransfer of DNA from agarose gel to nitrocellulose membrane (Schleicher and Schuell) was performed in the Trans-Blot Cell apparatus (Bio-Rad) in tris-Borate buffer. The membrane was prehybridized in the standard solution at 65 C for 4 h and then hybridized with DNA of the pBR327 plasmid labelled with ^{32}P GTP by means of nick-translation under the same temperature for 16 h. The nitrocellulose membrane was washed out and dried as described by Maniatis and exposed to X-ray film for some hours. The DNA was synthesized in mitochondria according to the method of Schegget and Borst (Biochim. Biophys. Acta 95:239-248, 1971) with the use of ^{32}P ATP (specific radioactivity is 111 PBq mol^{-1}). $^{32}PNTP$ that failed to incorporate into the mitochondrial material was removed by centrifugation. To isolate and partially purify the mitochondrial DNA, the slightly modified method of Kemble et al. (Genetics 95:451-458, 1980) was followed. The DNA of pBR322 and pBR327 plasmids was purified by the method of alkaline extraction (Birnboim and Doly, Nucl. Acid. Res. 7:1513-1523, 1979) followed by gel filtration of the preparation on a column with sepharose CL-4B (Pharmacia, Sweden). Protein concentration in the mitochondrial fraction was determined by the Lowry method using bovine serum albumin as a standard. Electrophoresis of nucleic acids was performed in 0.8% agarose gel (Pharmacia). For radioautography the gel was dried and exposed to X-ray film for some days.

Electrophoretic and Southern blot analyses of DNA of maize mitochondria incubated with the pBR327 bacterial plasmid vector and followed by DNase treatment showed that under the conditions indicated the plasmid DNA was effectively translocated into the inner space of the organelles (Fig. 1A, B). It should be noted that only certain physical forms of the plasmid DNA were able to penetrate

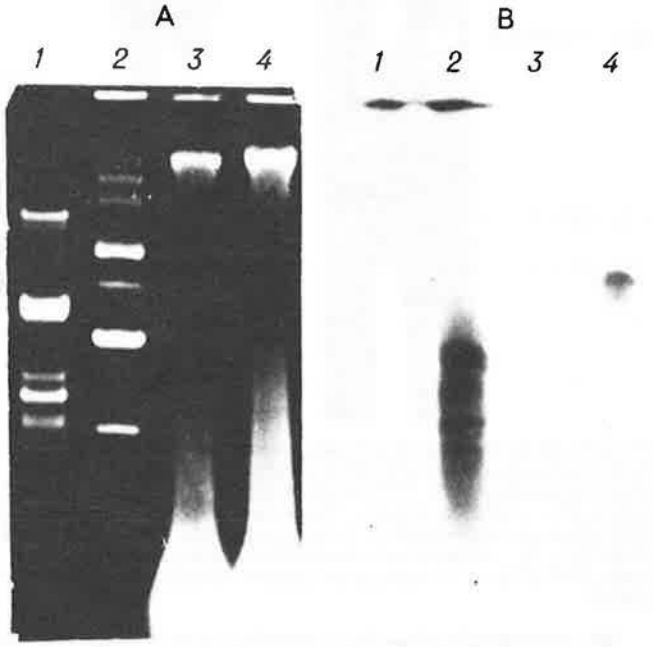

Fig. 1. Electrophoretic (A) and Southern blot hybridization (B) analyses of DNA in maize mitochondria incubated with pBR327 plasmid. 1, *Pst*I-fragments of λ phage DNA used as markers; 2, pBR327 plasmid DNA; 3, DNA of control mitochondria; 4, DNA of mitochondria incubated with pBR327 plasmid. B, Radioautography from hybridization of blot A with pBR327 plasmid labelled with ^{32}P in the reaction of nick-translation.

into the mitochondria. The illegible spot in the lower part of the gel in the radioautograph of the DNA from the treated mitochondria (Fig. 1B4) is likely to result from hybridization of the probe with degradation products of the plasmid DNA.

Figure 2 shows the results of radioautographic study of the DNA synthesis in isolated mitochondria incubated with DNA of the pBR322 bacterial plasmid. Several fractions of ^{32}P-labelled DNA were registered to appear during incubation of the plasmid-treated mitochondria under conditions favourable for DNA synthesis in organello. Electrophoretic mobility of these fractions corresponded to the high molecular DNA of mitochondria, the covalently closed circular form of the pBR322 plasmid and the fraction of plasmid-like mitochondrial DNA. The use of the bacterial plasmid as a template for DNA synthesis by mitochondrial DNA polymerase in intact organelles is further evidence of the effective translocation of the plasmid DNA into the inner mitochondrial space. Moreover, only the covalently closed circular form of the plasmid was determined in the mitochondria which seemed to indicate the primary translocation of this molecular form or its preferential involvement into mitochondrial DNA synthesis as compared to other plasmid forms (open, circle and linear molecule). Nevertheless, further study is needed to clarify the nature of DNA synthesis of the pBR322 vector plasmid in maize seedling mitochondria (replicative or reparative).

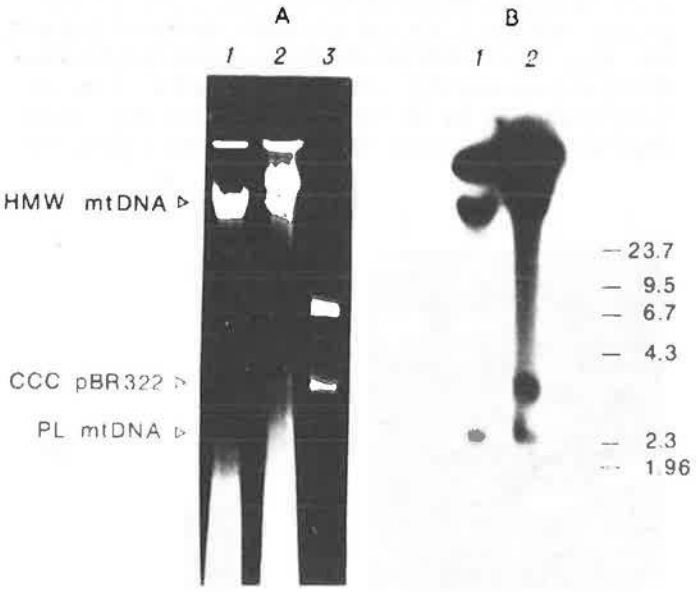

Figure 2. Electrophoretic (A) and radioautographic (B) analyses of DNA of control and incubated with pBR322 plasmid maize mitochondria. 1, DNA of control mitochondria; 2, DNA of mitochondria incubated with the plasmid; 3, DNA of pBR322 plasmid. The position of *Hin*dIII-fragments of DNA of λ phage used as markers is shown to the right. HMW mtDNA and PLmtDNA, high molecular and plasmid-like mitochondrial DNA, respectively; CCC pBR322, covalently closed circular form of bacterial pBR322 plasmid.

The data obtained on the possibility of the translocation of bacterial plasmids into isolated mitochondria, as well as the presence of the plasmid-like DNA set in the maize mitochondrial genome (Kemble and Bedbrook, Nature 284:565, 1980), suggest the existence of a specific mechanism of polynucleotide translocation in maize mitochondria.

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Possible free radical mechanism of appearance of somaclonal variations in maize and other plant species

--Yu. M. Konstantinov and A. S. Mashnenkov

Somaclonal variations which have been shown recently in many plant species appear in vitro mainly in callus, cell suspension and isolated protoplast cultures (P. J. Larkin, W. R. Scowcroft, Theor. Appl. Genet. 60:197-214, 1981). These somatic variations can result from cytological changes, point mutations and other less well-defined alterations to the nuclear and cytoplasmic (mitochondrial and chloroplast) genomes. A knowledge of the factors inducing somaclonal variations in plant cell culture is very important since to solve particular biotechnological problems, it is necessary for the cell population to be of high genetic homogeneity or on the contrary it is possible for the culture to be genetically unstable which serves in the latter case as a non-traditional source of economically valuable genotypes.

It is well known that oxygen radicals are generated in plant cells in vivo and in vitro as by-products of normal oxidative metabolism. Being highly reactive, these free radicals can attack cellular DNA directly (J. Imlay, S. Lin, Science 240:1302-1309, 1988). Alternatively, it has been reported that in complex biological systems oxygen radicals can cause DNA damage indirectly by initiating lipid peroxidation (LP) (A. Hruczkewycz, Biochem. Biophys. Res. Commun. 153:191-197, 1988). It is generally agreed that malondialdehyde produced as a peroxidation by-product is a potent cross-linking agent which can inactivate critical biomolecules including enzymes, nucleic acids, lipids (A. Tappel, Fed. Proc. 32:1870-1874, 1972). Besides, oxygen dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase has been reported (S. Farr et al., PNAS 83:8268-8272, 1986).

Oxidative stress of isolated plant cell and tissue cultures seems to be responsible for the appearance of somaclonal variations. The main reasons for the oxidative stress are obviously the following: 1) altered oxygen regime in plant cells in vitro as compared to in vivo; 2) the disturbance in vitro of the normal antioxidant status of plant cells specific for in vivo conditions and provided with the system of antioxidant defence of the whole organism; 3) the presence in the cell cultivation media in vitro of strong pro-oxidants capable of initiating the LP reactions.

The oxidative stress of plant cell cultures results in a sharp increase of free radical lipid peroxidation of membranes and the resulting products can serve as powerful mutagenic factors and cause various mutations and modi-

fications in the genetic system of plant cells including genomes of nuclei, mitochondria and chloroplasts.

The primary, secondary and final products of LP (free radicals including oxygen radicals, such components as conjugated diene, malondialdehyde and Schiff's bases) can be directly responsible for the plant genome instability during the oxidative stress. It should be emphasized that the LP initiation in certain membrane compartments of plant cells can cause spreading of the peroxidation to other organelles due to distant effect of LP products. When intracellular concentrations of natural antioxidants (α-tocopherol, thiol-containing compounds etc.) are reduced, LP products can induce structural rearrangements of DNA causing single- and double-strand breaks of polynucleotide chains and possibly other structural changes.

At the same time, LP products can greatly affect genetic processes by disturbing normal functions of enzyme systems providing different stages of realization and reproduction of genetic information (transcription, translation, replication) including the systems of repair and recombination of DNA. The enhanced level of transposition of mobile genetic elements can also indicate cell genome instability during LP reactions induced by the oxidative stress in culture systems.

It is well known that the level of DNA methylation determined by the activity of the enzyme system of methylases plays an important role in the regulation of gene expression. In this connection, certain changes in gene functioning in somaclonal variants appear to be determined by disturbances in the normal function of cell methylases affected by LP products.

In addition, a significant amount of resulting chromosomal and other mutations in cell cultures seems to be eliminated at the stage of plant regeneration and at the stage of gametogenesis in asexually and sexually propagated species, respectively.

The analysis of the composition of the most widely used plant tissue culture media argues in favour of the advanced free radical mechanism of the appearance of somaclonal variations. Thus, the Murashige and Skoog medium (T. Murashige, F. Skoog, Physiol. Plant. 15:473-497, 1962) and other plant tissue culture media (E. Linsmaier, F. Skoog, Physiol. Plant. 18:100-127, 1965; J. Nagy, P. Maliga, Z. Pflanzenphysiol. 78:453-455, 1976) contain Fe-EDTA complex (ca. 0.1 mM) with a strong pro-oxidant effect. Consequently, metabolic conditions of plant cells cultivated in artificial plant cell media (increased O_2 concentration, the presence of pro-oxidants and reducing equivalents) contribute to the initiation and proceeding of enzymic (NADH- or NADPH-dependent) and nonenzymic (ascorbate-dependent) lipid peroxidation.

Now the experiments to check the validity of the advanced hypothesis are being carried out using cell cultures of maize and other cereals.

In conclusion, significant changes in the oxygen regime of plant tissue and cells during their in vitro cultivation can obviously result in increase of the level of free radical oxidation of membrane lipids, whose products affect the genetic system of the cell and induce the formation of somaclonal variations.

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A new 100% earless recessive trait

--Daniel Nadel and Barry Nadel

A new 100% earless recessive trait has been discovered. In a breeding program for the development of new inbred field corn lines, an S4 inbred line was found that was 100% earless. This line was developed from four open-pollinated varieties: two Midwestern dents (Wilson Farm Reid Yellow Dent and Clarage) and two Southern dents (Yellow Tuxpan and Florida Laguna).

The genetic basis for the first barren stalk genes was reported in 1935 (Emerson et al.). Two independent genes were found, known as *ba1* and *ba2*. *ba1* is a barren stalk gene located on chromosome 3 (Emerson, 1935). It is monogenic and recessive. All barren plants are both earless and tasselless. A normal heterozygote when self-pollinated segregates 3 normals:1 barren stalk. *ba2* is a barren gene located on chromosome 2 (Emerson, 1935). It also is monogenic and recessive. All earless plants, however, produce normal tassels with viable pollen. A normal heterozygote crossed by an earless recessive homozygote produces a 1:1 ratio of normals to barren stalk (earless). The new earless trait produces an earless plant similar to *ba2* in appearance, with normal tassels and viable pollen. To verify the uniqueness of the new 100% earless trait, a genetic analysis was conducted comparing it with *ba1* and *ba2*. The results are shown here.

Table 1. Comparison of New 100% Earless trait with ba_1 and ba_2.

	ba_1	ba_2	New Earless	
1978:				
line:	108	112	102	106
	9N[a]/14E-[a]	13N/11E-	100%E-	2N/23E-
1979:	(108 × 112)	(108 × 106)	(112 × 102)	
F_1 Results:	100%N	100%N	100%N	
1980:	self	self	self	
F_2 Results:	10N/8ba_1 E- 7ba_2 E-	100% N	100% N	

[a] N = normal plants with ears, = E- = earless

The uniqueness of the new earless trait is clearly demonstrated by the fact that the F1s of all the crosses were normal. If the new earless trait was allelic to either *ba1* or *ba2*, when two heterozygotes were crossed some of the progeny would have to be homozygous recessive and therefore earless. However, all F1 progeny were normal, showing that all 3 earless traits are different from each other and give rise only to heterozygotes expressing the normal phenotype. The other unique feature of the new earless trait lies in its ability to produce 100% earless progeny.

The stalks of the new 100% earless lines were analyzed and were found to be high in sucrose, ranging from 18-21

Brix. This new 100% earless trait makes possible for the first time the economic utilization of maize for sucrose production and all of its derivatives (ethanol, sugar syrups, etc.) and for sweet earless corn forage.

The genetic basis of this trait is presently being worked out and will be reported in the near future.

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Carbon isotope discrimination among selected Pioneer Hi-Bred and public inbreds and hybrids

--Larry L. Tieszen[1] and David Grant

Plants discriminate against the heavy form of carbon, ^{13}C, during the process of photosynthesis. This discrimination is usually represented as a $\delta^{13}C$ value where:

$$\delta^{13}C = (R[sample]/R[standard] - 1) \times 1000$$
$$\text{and } R = {}^{13}CO_2/{}^{12}CO_2.$$

This discrimination is greater among C3 plants, where the δ value is often around -27‰ (parts/thousand), than among C4 plants, where it is often around -12‰

It is now firmly established that variation in ^{13}C discrimination in C3 plants depends on leaf intercellular carbon dioxide concentrations (Farquhar et al., Pl. Phys. 9:121, 1982; Farquhar et al., Annu. Rev. Pl. Phys., in press) and that the magnitude of the discrimination is related to water use efficiency and plant yield. This discrimination therefore holds promise as a surrogate for characteristics of importance in plant breeding. Selection strategies based on this measurement have been shown for wheat and barley (Hubick and Farquhar, Plant Cell Envir. 12:795, 1989), range grasses (Johnson et al., Crop Sci., in press), and other species. The factors which determine discrimination in C4 plants, for example maize, are more complicated (Farquhar, Aust. J. Pl. Phys. 10:205, 1983) and are less well documented. As an initial step in studying carbon discrimination in maize, we undertook a survey of selected cornbelt inbreds and hybrids to evaluate the range of discrimination in this germplasm pool.

Seeds were cleaned of cob material, ground to a fine powder and dried at 90 C. Approximately 1 mg samples were combusted in a Carlo Erba elemental analyzer, cryogenically purified for CO_2, and analyzed in an isotope ratio mass spectrometer (SIRA 10). The precision of this analytical technique is <0.1‰.

The results presented in Figure 1 show that these samples exhibited a range of $\delta^{13}C$ values ranging from -11.65 to -10.74‰. Although these values are quite positive for a C4 plant, they are in agreement with a mean of -10.99‰ previously obtained from a collection of CIMMYT cultivars. Those 33 cultivars were substantially more variable, however, ranging between -12.00 and -9.76‰. The smaller amount of variation among the Pioneer samples is probably due to 1) the limited number of samples analyzed

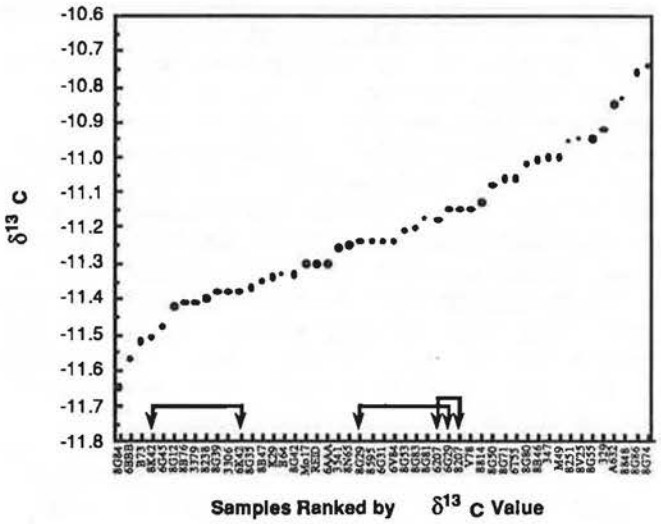

Figure 1. $\delta^{13}C$ values, in ‰, for seeds from 40 inbreds, 1 open pollinated variety (REID = Reid Yellow Dent) and 5 hybrids of maize grown under uniform conditions. Each point represents one analysis, except for the points shown as * which are the average of 3 analyses. These replicates agreed to within 0.2‰. Two independently grown samples each of 3 inbreds were analyzed and are indicated in the figure.

and 2) the fact that cornbelt germplasm represents only part of the worldwide maize germplasm pool.

Isotopic variation in maize should be determined by four main components: stomatal diffusion, hydration of CO_2 in cell sap, PEP carboxylation of HCO_3^-, and loss of CO_2 from the parenchymatous bundle sheath cells (Sasakawa et al., Pl. Phys. 90:582, 1989). Internal leaf CO_2 (C_i) should change as the balance between diffusion and carboxylation varies and this change should be reflected in subsequent $\delta^{13}C$ measurements. Sasakawa et al. provided evidence that supports this relationship as they found increasingly negative $\delta^{13}C$ values as carboxylation capacity increased in maize leaves. Theoretically, increased diffusion resistance and potentially higher water use efficiency should also result in less negative values. However, there are as yet no experimental data to evaluate this relationship.

These data indicate that this sample of cornbelt germplasm contains a relatively small fraction of the genetic variation for $\delta^{13}C$ present in maize. This suggests a similarly small variation in carboxylation activity and stomatal resistance in this germplasm. The larger range of $\delta^{13}C$ values found in the CIMMYT samples, however, indicates greater genetic variation in these physiological properties. This variation may, in the future, be used to develop selection strategies that will aid in the development of inbreds with improved water use efficiency.

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Distribution of C-banded bivalents in maize

--Maria del Carmen Molina, Lidia Poggio and Carlos A. Naranjo

Molina and Naranjo (Theor. Appl. Genet. 73:542-550,

1987) and Naranjo and Molina (MNL 61:62-63, 1987) have obtained cytological evidence supporting X=5 as a basic number of the genus *Zea*, and have suggested a hypothetical genomic constitution for the species and hybrids studied. These authors showed that in hexaploid hybrids (2n=30) forming 5 III + 5 II + 5I in meiosis, there is a tendency of A, C and B genomes to separate into trivalent, bivalent and univalent groups, respectively. Later, in several 2n=20 species of the genus, two spindles of five bivalents each have been observed in first metaphase (Molina, Naranjo and Poggio, MNL 62:74, 1988). Detailed studies in diplotene and diakinesis indicate also two groups of five bivalents. Moreover, in some of these cells, an asynchronous development of meiosis between groups was observed.

With the aim of analyzing the distribution of bivalents on the two spindles, C-banding with Giemsa was done (Giraldez et al., Z. Pflanzenzuchtg. 83:40-48, 1979). The material used was the Ever Green variety. In meiosis, this variety showed a total of five C-banded bivalents, and in all cells where double spindles were observed the distribution of the C-banded bivalents was two in one spindle and three in the other. The other two possible distributions (5-0 and 4-1) have never been observed.

These results suggest that 5-5 bivalent distribution in diakinesis and metaphase I is not random.

DNA content and heterochromatin in the genus *Zea*

--C. Tito, L. Poggio and C. A. Naranjo

New data about the 2C nuclear DNA content in seven taxa of the genus *Zea* are reported in the present work. The measurements were made by microdensitometry of Feulgen stained root tip telophase cells. The technique was done according to Poggio and Hunziker (J. Hered. 77:43-48, 1986). The results are summarized in Table 1 in which are included part of our previous data (Tito, Poggio and Naranjo, MNL 62:74-75, 1988).

An analysis of variance and comparisons between means using Scheffe's method on the overall data revealed significant differences between species of *Zea* and between lines and varieties of *Z. mays* ssp. *mays*. Lines or varieties with significant differences in total DNA content are indicated in Table 1.

Rayburn, Price, Smith and Gold (Amer. J. Bot. 72:1610-1617, 1985) and Laurie and Bennett (Heredity 55:307-313, 1985) have shown that there is a significant positive correlation between C band number, percent C band heterochromatin and genome size. The same correlation was observed in the accessions studied. Correspondence between the amount of C banded material in metaphase and in interphase nuclei has been observed as in other genera (Fig. 1).

Table 1. DNA content, C-band number and interval from germination to flowering.

TAXA	DNA CONTENT (2C) pg. $\bar{x}$ ± ES		DNA CONTENT PER BASIC GENOME pg.	C-BAND NUMBER IN METAPHASE AND INTERPHASE*	INTERVAL FROM GERMINATION TO FLOWERING (DAYS)
SECTION ZEA (2n=20)					
Z.mays ssp. mays					
Inbred lines					
104	5.863	0.048	1.466ab[1]	4	62
9063	6.157	0.070#	1.589a	11-13*	65
Z	6.758	0.040	1.689ce	8-10*	84
129	6.873	0.065	1.718cf	10	103
E	7.087	0.041	1.772df	8-10*	91
Open pollinated var.					
Ever Green	6.065	0.060	1.588ab	5	70
Colorado Klein	6.192	0.062	1.546a	6-7*	75
Z.mays ssp. mexicana					
Line 4024	6.789	0.073#	1.655cd	17-18*	170
SECTION LUXURIANTES					
Z.diploperennis(2n=20)	6.322	0.080#	1.590a	12-14*	190
Z. perennis (2n=40)	11.150	0.160#	1.419b	8-10*	200
Z. luxurians (2n=20)	8.939	0.029	2.239g	26	212

Seed source: Z. mays ssp. mays, line 104: maize 'C-tester' introduced in Argentina since 1933 from EEUU.; line 9063, commercial hybrid; line 129, single hybrid; line Z homocigous line orig. EEUU 'Multiple Dominante Dr Randolph 1977'; line E, homocigous line, with similar genotype of Z and plasmon of Z. mays ssp. mexicana (N°2587, 'Florida Variety' was introduced in 1941; Z. mays ssp. mexicana line 4024, orig. CIMMYT K-69-5. Z. luxurians, Univ. de Guadalajara.
(#): Tito, Poggio & Naranjo, MNL 62-74-75, 1988.
(1): equal letters indicate data with no significant differences.

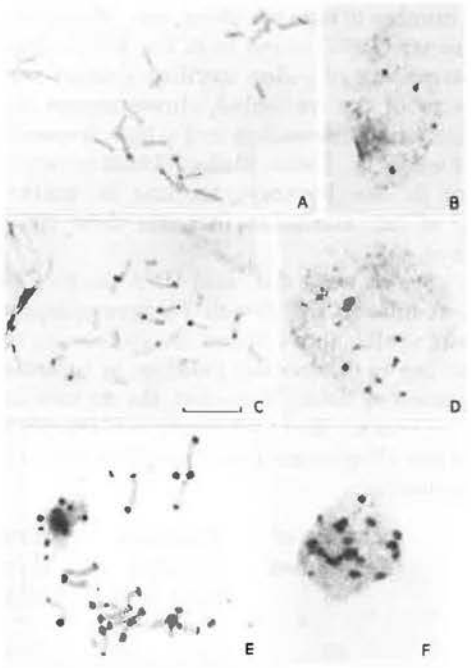

Figure 1. C-banding. A, C and E mitotic metaphases. B, D and F interphases (chromocenters). A and B = *Z. m.* ssp. *mays* line 104; C and D = *Z. m.* ssp. *mays* line 129; E and F = *Z. luxurians*. The scale represents 10 um, all with the same enlargement.

The E and Z lines let us analyze an interesting phenomenon. Mazoti (Mendeliana 8:45-52, 1987) established that the knobs of Z line increase their size and DNA content in the presence of one plasmon of *Z. mays* ssp. *mexicana* (this combination is named E line). The total content is higher in the mentioned E line (Table 1). Although the C band numbers in mitotic interphase nuclei are the same in both lines, the C^+ chromocenters are bigger in E line. Whether the results are due to an exceptional nucleus-cytoplasmic system or because of a more generalized phenomenon is still unknown.

The annual *Z. luxurians* has higher DNA content and C heterochromatic bands in comparison with other 2n=20

members of the genus (Table 1, Fig. 1). Similar results were obtained by Laurie and Bennett (ibid.).

Moreover, the results shown in Table 1 indicate a positive correlation between total DNA content and the interval from germination to flowering.

Meiotic behaviour and total DNA content in a homozygous line of maize with cytoplasm of *Z. mays* ssp. *mexicana*

--L. Poggio, C. Tito and C. Naranjo

A pure line of maize (Multiple Dominant of Dr. Randolph, No. 1877) (Z line) was used as male recurrent parent during 20 backcrosses onto *Z. mays* ssp. *mexicana* (Florida variety, Huixtla, Mexico), obtaining the E line. Both lines have the same homozygous genotype but different cytoplasm. Mazoti (1958) reported that the cytoplasm of ssp. *mexicana* was responsible for several inherited morphological and physiological characters such as precocity, number of ears per plant, etc. Moreover, Mazoti and Velazquez (1962) found that the E line presented a greater percentage of pollen sterility, greater variance in the diameter of the nucleolus, chromosomes in meiosis with stickiness manifestation and a high frequency of intercellular contacts. Later, Mazoti (1982) established that the knobs in the homozygous line of maize in the "plasmon" of ssp. *mexicana* increase their size and the DNA content.

In the present work the total DNA content was measured in both lines (E and Z) with Feulgen cytophotometry with the aim to elucidate whether the increase in the size of knobs was due to differential packing, or insertion or endoreduplication of DNA. Moreover, the meiotic behaviour of both lines was studied to determine if the partial male sterility of the alloplasmic line E could be due to chromosomal irregularities.

Line	Number of cells studied	Bivalents (range)	Univalents (range)
Z	96	9.22 ± 0.09 (6-9)	1.56 ± 0.18 (2-8)
E	98	8.09 ± 0.16 (2-9)	3.25 ± 0.33 (2-16)

Although Z does not have regular meiosis, it has a mean of univalents significantly lower than E. Moreover, in the E line several meiotic irregularities were observed: desynapsis, cytomixis, nuclear fusion, nucleolar bodies and pseudomultivalents. These abnormalities were only observed in some of the flowers of the panicle. This fact could indicate that the nucleus-cytoplasm interaction alters the canalization of development, although the origin of the disharmony is yet unknown.

Line	DNA content (2C) pg	Number of nuclei measured
Z	6.75 ± 0.04	155
E	7.08 ± 0.04	157

There is a quantitative change in the total DNA content, being significantly high in the E line. Moreover, the E line presents bigger knobs in pachytene, and bigger C+ bands in mitotic and meiotic metaphases when C-banding technique is applied. These results could indicate that the cytoplasm of ssp. *mexicana* produces nucleotypical changes in the nucleus of maize, increasing the highly repetitive DNA in the zone of the knobs. This could be affecting the regulatory system.

It can be concluded that the interaction between the nucleus of maize and the cytoplasm of *Z. mays* ssp. *mexicana* may induce a mechanism that generates rapid genome changes which will be manipulated to our advantage when more information about the nature of the phenomena described would be obtained.

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Regeneration in callus cultures and cytological analysis of regenerated plants

--Dina Garcia, Maria del C. Molina and Osvaldo Caso[1]

Many investigators have obtained maize plant regeneration by organogenesis or somatic embryogenesis in callus cultures initiated from immature embryos (Green and Phillips, Crop Sci. 15:417, 1975; Lu et al., Theor. Appl. Genet. 62:109, 1982; Tomes and Smith, Theor. Appl. Genet. 70:505, 1985; among others), but some inbreds, such as B73, showed a very low percentage of response.

The object of this work is to evaluate the plant regeneration capacity of maize cv. Colorado Klein cultured in vitro. This cultivar is often used in the IFSC to obtain hybrids with other species of the genus *Zea*. The method used in this work is an adaptation from that of Lowe et al. for B73 (Plant Sci. 41:125, 1985). Cytogenetic analysis will show any chromosomal change in regenerated plants.

Table 1. Relationship between embryo length and days after pollination. Plant material: immature embryos of Colorado Klein.

Days after pollination	Average embryo length (mm)
11	1.15
14	2.08
16	3.03

Embryos were aseptically excised and placed on the culture media with the plumule radical axis side in contact with the medium. Cultures were incubated at 30-32 C in darkness for 15 days. Cultures were transferred to maintenance media and were incubated with a 16 hour photoperiod (I=2500 Lx) and subcultured every 30 days. Tissues like leaves or roots were excised in each subculture. After 7 months in culture, callus was transferred to regeneration medium. Plantlets were subcultured to rooting medium. Cultures were incubated in the same environment as in maintenance. Callus which did not regenerate plants was discarded.

The embryos germinated from 1 to 3 days after isolation, but they did not continue their normal development.

Culture media:

	Initiation		Maintenance		Regeneration	Rooting
	M	N	M1	N1	N2	N3
Mineral salts	MS[1]	N6[2]	MS	N6	N6	N6
Vitamins	N6	N6	N6	N6	N6	N6
Asparagine[3]	150	150	150	150	150	150
2,4-D[3]	0.5	0.5	0.5/1	0.5/1	0	0
Kinetin[3]	0	0	0	0	2	0
Sucrose(%)	12	12	2	2	2	2
Activated charcoal(%)	0	0	0	0	0	0.1
Agar(%)	0.6	0.6	0.6	0.6	0.6	0.6
pH	5.8	5.8	5.8	5.8	5.8	5.8

(1) Murashige and Skoog, Physiol. Plantarum 15:473-497, 1962
(2) Chu et al., Sci. Sinica 18:659-668, 1975
(3) mg.L^{-1}

About 7 days after isolation, white to pale yellow callus arose from 1 to 2 mm embryo scutellum. Embryos larger than 2 mm gave callus which turned brown and died.

Structures like small leaves arose from green areas of callus initiated in N medium from 11-day-old embryos when these were transferred to N1 medium with 1 mg·L^{-1} 2,4-D. These structures arose especially near the end of each culture period. No regeneration was observed from callus initiated in M medium. Plantlets originated when the green areas with structures like leaves were transferred to N2 medium. No adventitious roots were observed to appear from plantlets in this medium. Adventitious roots arose from plantlets in N3 medium.

Six percent of plated embryos gave callus capable of plant regeneration. These calli still regenerate plants after 20 months in culture.

Cytogenetic analysis of 10 regenerated plants revealed that 7 of them had a somatic chromosome number of 2n=20. Three plants showed alterations in the chromosome number, one of them had 2n=21, another one had 2n=23 and the third had 2n=40, with characteristic tetraploid cells.

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Perennial teosinte introgressed population of maize: relation between protein and yield

--J. L. Magoja, I. G. Palacios and R. Burak

It is well known that in maize, as well as in other cereals, there exists an inverse relation between the protein content of kernels and yield. Even if the force of such association can be of greater or lesser intensity, according to the material involved, there always exists the interest of combining high yield with high protein content, or at least getting both characters to express in such a balance that might, in the end, condition the production of kernels of higher nutritive value at low cost.

As we have mentioned earlier (see MNL 62:80), perennial teosinte (*Zea perennis*) introgression in maize produces a positive effect in the protein level of the kernel which, added to the variability produced for this character, would allow selection of maize with a protein content higher than that of those cultivated at the moment.

With the aim of obtaining more information about the relationship between kernel protein content and yield, 108 S1 lines and 11 half sib families derived from the introgressed population were studied. Together with commercial hybrids (controls), they were part of a trial plot cultivated in Llavallol during the 1987/88 growing season.

The results obtained for protein content of the complete kernel in S1, half sib families and controls (21 data) are summarized in Table 1. The S1 have a significantly higher protein content than the controls and half sib families, and the latter also exceed the commercial hybrids significantly.

Table 1. Whole kernel protein content of S1, half sib families (HS) and commercial hybrids (CH).

	Protein(%)		
	Mean	SD	Range
S_1	12.2 a	1.3	8.8-14.9
HS	11.6 b	1.2	7.0-14.9
CH	10.3 c	1.0	8.7-12.2

Individual means within a column followed by different letters are significantly different at 1% level.

When the kernel protein content is correlated with a total of 28 evaluated characters of evolutive cycle, plant, ear, etc. within each type of progeny (S1 or half sib), or considering the total of both, only associations of statistical significance were detected for the characters shown in Table 2. The results obtained show an inverse association between yield and some of its components as regards protein content. It is important to point out that this relation is not significant when considered within the half sib families. This fact might seem contradictory with the data provided: why, if the protein content is associated with the yield components in a similar way, both in the S1 and the half sib, does the same not occur as regards yield?

Table 2. Correlation coefficients between some traits and whole kernel protein content (WKP).

	S_1	Half sib	Total
WKP vs.cob percentage	0.290**	0.300**	0.400**
uppermost ear weight	-0.287**	-0.210*	-0.367**
average ear weight	-0.337**	-0.270**	-0.450**
ear diameter	-0.295**	-0.247**	-0.330**
number of kernel rows	-0.293**	-0.315**	-0.298**
grain yield	-0.217**	NS	-0.230**

*:significant at 5% level,**:significant at 1% level

To answer this question we should explain that in the half sib families the principal yield component is prolificity (ears per plant). Since ears per plant is not significantly associated with protein, it results that protein is not significantly associated with yield.

This constitutes a matter of great importance, for it would possibly allow us to break the inverse relation between yield and protein, at least in this particular case, and within this introgressed maize, in half sib families.

Table 3. Whole kernel protein content and grain yield considering all families and the 37, 20 and 10 more yielding families of the whole (MYF).

	Protein (%)	Yield (kg/ha)
All families(219)	11.9 a	7,313 a
37 MYF(16.9%)	11.9 a	11,831 b
20 MYF(9.1%)	11.8 a	13,074 c
10 MYF(4.6%)	12.1 a	14,474 c

Individual means within a column followed by different letters are significantly different at 5% level.

With the purpose of verifying whether this lack of significant association still remained when selecting high yield progenies, the comparisons practised are shown in Table 3. As it is perfectly demonstrated, the average protein content of all the families (219), does not vary significantly from that of the 37, 20 or 10 higher yield families. This fact is illustrative enough as regards the possibilities that might derive from these materials when trying to find adequate combinations between protein content and grain yield.

More about maize introgressed with diploperennial teosinte germplasm

--G. Pischedda* and J. L. Magoja
*Fellow of CONICET

Together with the development of our improvement programmes using the wild germplasm, we are obtaining an amount of information related to these new materials, which is interesting to analyze. In this particular case, we intend to provide information about the experiences we have gathered in relation with the research work begun from a population of maize introgressed with diploperennial teosinte (*Z. diploperennis*) (see MNL 60:82 and 61:65).

As has been previously reported (see MNL 62:84), as a consequence of a cycle of recurrent selection we obtained S1 families which were evaluated for different characters. During the 1988/89 growing season, progenies of such S1 were cultivated in Pergamino (Province of Buenos Aires) as part of a trial with 2 repetitions.

On evaluating the mentioned progenies for different characters, we completed the necessary information to make heritability estimates on the basis of the parent-offspring regression.

The results of the evaluation of some important characters in the progenitors (S1 families) and their respective progenies are summarized in Table 1. These families have as general characteristics the peculiarity of producing tillers and of being prolific (2 to 5 ears per plant). The size of the average ears is generally small, though the depression caused by inbreeding must be taken into account.

Table 1. Relevant traits in parents and offspring of diploperennial teosinte introgressed maizes.

	Parent			Offspring		
Character	Mean±SD	Range	cv%	Mean±SD	Range	cv%
Plant height(cm)	188.4±16.0	160-215	8.4	188.5±19.5	138-220	10.3
Ear insertion height(cm)	91.0±15.9	55-120	17.5	81.7±10.9	45-103	13.3
Ears per plant	2.7± 0.6	2-4.1	21.2	3.0± 0.8	1.8-5.2	27.9
Ear lenght(cm)	12.4± 1.7	8.9-15.8	13.6	13.3± 2.1	8.6-17	16.0
Ear diameter(cm)	2.7± 0.4	1.9-3.5	15.6	3.0± 0.3	2.3-3.7	11.6
Average ear weight(g)	46.4±22.9	25-100	49.3	40.0±17.5	16-100	43.8
Number of kernel rows	10.1± 1.8	8-13.6	17.5	10.8± 1.2	8.7-13.2	11.2
Weight of 50 kernels(g)	8.7± 2.0	5.8-14.2	22.7	10.4± 1.8	7-14.9	17.2

As can be seen in Table 1, there exists an acceptable congruence between means of progenitors and progenies, but it must be particularly pointed out that, for most of the characters, there remains a similar variation from one generation to the other. The heritability estimates made (see Table 2) show from medium to low values, with the exception of prolificity (ears per plant), high heritability character for this case. On the other hand, the yield, represented in this case by ear weight per plant, is the character of lowest heritability.

Table 2. Heritability (h^2) of relevant traits.

Character	r	$h^2 = b$
Plant height	0.270	0.28 ± 0.20
Ear insertion height	0.580	0.35 ± 0.10
Ears per plant	0.611	0.86 ± 0.23
Ear lenght	0.304	0.38 ± 0.25
Ear diameter	0.605	0.48 ± 0.13
Number of kernel rows	0.550	0.36 ± 0.11
Weight of 50 kernels	0.632	0.52 ± 0.13
Ear weight per plant	0.330	0.19 ± 0.11

On analysing the phenotypic correlation between ear yield and other characters, contrary to what might be expected, the prolificity is not significantly associated with yield. Consequently, just as is documented in Table 3, the yield seems to be determined mainly by ear and kernel size and weight. These results are congruent with the heritability estimates for prolificity and yield: if the number of ears per plant were a main component of yield, the latter should have a high heritability, which unfortunately does not occur in practise.

Table 3. Phenotypic correlation coefficients (r) between ear weight per plant (IWP) and some other traits.

EWP vs.	plant height	0.702**
	ear insertion height	NS
	ears per plant	NS
	ear lenght	0.817**
	ear diameter	0.815**
	number of kernel rows	0.432**
	weight of 50 kernels	0.734**

**:significant at 1% level,NS:no signif.

This particular relation between prolificity and yield is absolutely different from the one we have found in the perennial teosinte introgressed maize (*Z. perennis*). Probably, the high prolificity of all the families tested conceals the true association type between ears per plant and yield.

If the calculated associations are correct, and feasible to be verified again, we could infer that, yield being conditioned by ear size and not by the number of ears, the high prolificity would produce a unitary and total reduction of the ear weight. Our experience so far, derived from the study of these particular materials, leads us to think that there is a point of balance between prolificity and yield which must be taken into account, in order to achieve further benefits when intending to improve the grain yield.

A possible relation between germinative energy and grain yield

--I. G. Palacios and J. L. Magoja

With the purpose of finding out whether there is any kind of association between the germinative energy and the grain yield, seeds from 15 full sib families derived from a perennial teosinte (*Zea perennis*) introgressed population of maize, and from a complete diallel cross among five of those families (the ones of high yield), were put to germinate in the darkness at 28 C for 6 days. The 15 families used represent in equal number (five), low, medium and high grain yields in the population. For each family or cross, 10 seeds per Petri dish were used and weighed at 24 hour intervals until the 6th germination day. The families and the crosses were part of a complete randomized design with two repetitions for each stage. Being the most relevant difference, we only consider herein the stage of six germination days.

Based on the fresh weight of each seedling (SFW) and the initial kernel weight (KW), the germinative energy (GE) was quantified by the formula: GE = SFW-KW/KW. The results obtained on the 6th germination day, for the full sib families classified according to their yield, are supplied in Table 1. As can be seen, the high yield families have significantly higher GE values than the low yield ones. The correlation between yield and GE at the 6th germination day is significant (r = 0.668**), indicating that in most cases, a high germinative energy is associated with a high yield.

Table 1. Grain yield and germinative energy means of full sib families.

Type of family	Yield (kg/ha)	Germinative energy
Low yield	3,027 ± 706	2.74 ± 0.43
Medium yield	5,897 ± 1,000	3.06 ± 0.82
High yield	10,985 ± 536	3.79 ± 0.82

In the crosses between families (see Table 2) a very variable and significant heterosis takes place in most cases. If the association between yield and germinative energy can be verified again after an analysis involving a greater number of families and their crossings, these quick and simple determinations could be of use to predict the combining ability for yield of this introgressed maize.

Table 2. Percent of heterosis (%H) for germinative energy.

Cross	Germinative energy P_1	MP	%H
3 x 4	4.76	3.48	36.8
3 x 5	5.09	3.23	57.4
3 x 6	5.99	2.71	120.8
3 x 7	4.76	4.11	15.9
4 x 5	4.94	3.47	42.2
4 x 6	6.61	2.95	124.0
4 x 7	5.08	4.35	16.9
5 x 6	3.60	2.70	33.2
5 x 7	4.28	4.10	4.5
6 x 7	6.04	3.58	68.8
Average	5.12	3.47	52.1

MP=mid parent; $\%H=(P_1-MP/MP).100$

Perennial teosinte introgressed population: seed mutants and mutation rates

--R. Burak and J. L. Magoja

In the course of our research work related to the development of wild germplasm introgressed populations of maize, the high frequency of spontaneous mutations easily recognized in the harvested ears caught our attention. With the purpose of documenting this unusual fact in a perennial teosinte (*Zea perennis*) introgressed population of maize, we evaluated seven seed traits considered deviations of the normal phenotype and conditioned by genes of simple inheritance: waxy, white, floury and sugary endosperm, defective kernel, red pericarp and aleurone colour.

In the original population (P0), cultivated in the 1986/87 growing season, these mutations were recognized in a sample of 812 plants (ears) obtained at random and by controlled pollination. In the following growing season (1987/88) the progenies of said ears were cultivated. After a generation in the derived population (P1), the mutant phenotypes were recognized in a random sample of 1,617 ears. On the basis of phenotypic frequencies observed, the genetic frequencies were estimated and from the latter, the respective mutation rates.

The results drawn (see Table 1), show that the mutation frequencies, as well as their respective rates, are very high, and can be considered exceptional for maize, probably associated with the wide variability generated by the introgression.

Table 1. Genetic frequency and mutation rate (μ) in the perennial teosinte introgressed population.

Phenotype	Gene	Genetic frequency P_0	Genetic frequency P_1	μ
Waxy endosperm	wx	2.5×10^{-3}	9.3×10^{-3}	6.8×10^{-3}
White endosperm	y_1	46.8×10^{-3}	128.0×10^{-3}	85.2×10^{-3}
Floury endosperm	fl	3.7×10^{-3}	55.0×10^{-3}	51.5×10^{-3}
Sugary endosperm	su	3.7×10^{-3}	19.8×10^{-3}	16.2×10^{-3}
Defective kernel	de	13.5×10^{-3}	55.7×10^{-3}	42.8×10^{-3}
Red pericarp	P^{rr}	4.9×10^{-3}	10.7×10^{-3}	5.8×10^{-3}
Aleurone color	A_1,A_2,C,R	31.7×10^{-3}	187.3×10^{-3}	160.7×10^{-3}

If one bears in mind that the mutations found in the seeds constitute only a part of those which occur in practise (others have not been considered or detected) and that such mutations had not been observed in the materials which originated the population, it can be said as a consequence, that the presence and high frequency of the same can be attributed, as other research has pointed out (Mangelsdorf, Cold Spring Harb. Symp. Quant. Biol. 23:409, 1958), to the mutagenic effect of the teosinte introgression in maize.

We ignore the mechanisms through which these mutations occur, and we have not conducted any research work to determine it. However, we acknowledge that the wild germplasm introgression in maize conditions a wide variability that is expressed in different ways: modifying traits of quantitative inheritance, or as in this case, producing or increasing the production of variants of simple inheritance.

Yield and yield components of full sib and half sib families derived from a perennial teosinte introgressed population

--R. Burak and J. L. Magoja

As part of a recurrent selection cycle from a perennial teosinte (*Z. perennis*) introgressed population of maize, during the 1987/88 growing season, full sib and half sib families derived from such populations were evaluated in Llavallol. These families and controls (commercial hybrids) were part of an augmented randomized complete block design cultivated at a 57,143 pl/ha density.

The average yield of the full sib families was of 6,912 ± 1,711 kg/ha (range: 1,811 to 15,471), while that of the half sib families was of 8,600 ± 2,500 kg/ha (range: 1,800 to 17,900).

When the yield was correlated with 27 other characters of agronomic importance, measured both in the full sib and the half sib families, it was found that yield was significantly associated with only 8 characters.

As is shown in Table 1, the yield seems to depend on the plant height, the prolificity and the size and weight of the ears and kernels.

Table 1. Phenotypic correlation coefficients between grain yield and some traits.

	Full sib	Half sib
Yield vs. plant height	0.410**	0.256**
ear per plant	0.452**	0.630**
cob percentage	-0.367**	-0.296**
uppermost ear weight	0.472**	0.265**
average ear weight	0.466**	0.390**
ear lenght	0.375**	0.222**
number of kernels/row	0.382**	0.275**
50 kernel weight	0.360**	0.238**

**:significant at 1% level

Even if the greater part of these families are prolific, this being the character which can be taken as the most evident sign of introgression, the number of ears per plant is not always the principal yield component. While it seems that in the half sib families the kernel yield mostly depends on the prolificity rather than on other characters, in the full sib families prolificity as well as size and weight of ears seem to have similar importance to condition the yield.

When studying those families with greater yield in depth, it could actually be proven that in some of such families, the high kernel yield could be attributed, basically, to the production of one (sometimes two) big and heavy ears. In most cases the high kernel yield of the families depends especially on the production of several (multiple) ears of acceptable size. If the biological efficiency can be measured through kernel production per plant under certain cultivation conditions, it is evident that within this introgressed maize there are different ways of achieving it: through prolific individuals (most cases) or through non-prolific. This fact is another element which contributes to document that, within the wide yield ranges, even in the positive extreme the variation persists (high yield due to one or several ears).

The genetic and/or physiological yield bases are not yet well known, thus being sometimes difficult to foresee advances in the yield improvement as a consequence of a certain kind of plant. In spite of that, in the particular case of this introgressed maize there still exists the hope of achieving the production of greater yields, maybe through the possibility of combining genotypes that condition high yield through different ways (with or without prolificity).

It cannot be said what might result from these combinations, but probably this kind of experience might lead to a better understanding of which factors condition a greater biological efficiency.

Effect of defective kernel mutants on isoenzymatic patterns

--I. G. Palacios and J. L. Magoja

Biochemical modifications of the seed may be conditioned or related to mutants which produce defective kernels. In certain cases, the greater or lesser biochemical alterations go together with the greater or lesser defectiveness of the kernels (see MNL 62:82).

In previous issues we have reported upon some of these mutants. One of them in particular (*de*-7601*), studied most extensively, conditions modification in the protein pattern of the endosperm, increases the lysine level, causes electrophoretic variants of the soluble proteins of germ and endosperm, and of the lipoproteins (see MNL 56:108). We have also said that the aforementioned mutant and others of defective kernel modify the content of free amino acids of the kernel (see MNL 58:120 and 62:820.

The study of the biochemical modifications conditioned by or related to these mutants offers the opportunity of better understanding the reason why the kernel is defective, and the lethal character, in most cases.

With the purpose of giving more information on these subjects related to the defective kernel mutants, we have begun research work destined to compare the isoenzymatic patterns, with the aim of verifying some kind of association with the defectiveness of the kernels. Defective kernels and their normal equivalents, corresponding to maize inbred lines, carriers of four mutants, were used to analyze the isoenzymatic patterns obtained by electrophoresis. These mutants are recessive genes of simple inheritance, three of which (*de*-7601*, *de*-7670* and *de*-7510*) condition kernels of very little weight and are lethal. The other (*de*-7547*) only reduces to half of normal, and the seed is viable. Out of each inbreeding line, 10 normal kernels and 10 defective ones have been taken at random and been individually tested. The seed extracts, obtained after undergoing 24 hour soaking in distilled water, were used in a starch gel. After the electrophoretic separation, the gel was horizontally cut in three layers which were stained for alcohol dehydrogenase (ADH), malic dehydrogenase (MDH) and esterases (EST).

The patterns obtained showed that: 1) with the exception of the *de*-7601* and *de*-7547* for MDH and *de*-7547* for ADH, the isoenzymatic pattern of the defective kernels is different from its normal equivalents; 2) mainly in the kernels carrying the mutants which condition greater de-

fectiveness, the differences are settled by the absence of bands, or less intensity of them, in the defective ones; and 3) in extreme cases, the defective kernels show very altered patterns as a consequence of not resolving in bands, and the activity zones are diffused in the gel.

With the purpose of quantifying these differences in order to verify whether the same are congruent with the greater or lesser defectiveness produced, that is, whether the greater differences correspond to more defective kernels, we have evaluated the similarity between the isoenzymatic patterns corresponding to defective kernels and their normal equivalents, by simple matching coefficients.

The results obtained for each of the systems and in average are shown in Table 1. If the defectiveness degree may be represented by the defective kernels' weight, expressed as a percentage of their normal equivalents (SW%N), it is easy to conclude from the data of Table 1 that the greater the defectiveness, the greater the differences between the isoenzymatic patterns of normal and defective kernels.

Table 1. Seed weight of defectives as percent of normals (SW%N) and simple matching coefficients (SMC).

		SMC			
Mutant	SW%N	EST	ADH	MDH	Average
de*-7670	9.0	0.46	0.00	0.38	0.28
de*-7510	17.7	0.69	0.88	0.27	0.61
de*-7601	28.3	0.64	0.84	1.00	0.83
de*-7547	45.0	0.89	1.00	1.00	0.96

In the case of the *de*-7547* mutant, which slightly reduces weight and keeps the viability of the kernel, the alteration is null for ADH and MDH, and little for EST. On the contrary, the other three mutations, which are lethal, condition greater differences, sometimes to an extreme degree, although without a specific tendency for isoenzymatic system. On average, there is a very good association between isoenzymatic differences and defectiveness.

According to the above mentioned and to what has been previously reported (see MNL 62:82) it can be stated, at least for the few mutants studied, that the degree of kernel defectiveness is significantly associated with the intensity of the biochemical modifications, in this case with greater or lesser alteration of the isoenzymatic patterns.

The analysis of the differences over a greater number of mutants will result in greater information, which will surely be used to settle the reason for kernel defectiveness.

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Stamens and lodicules of male sterile-silky (*ms-si* or *si1*) tassel spikelets are lemma-like structures

--D.W. Dales, R.I. Greyson and D.B. Walden

As reported in MNL 63:88, the male sterility of male sterile-silky (*ms-si*) results from failure in development of functional stamen locules. The extent of stamen and anther development varies and ranges from no development to structurally normal but non-functional. We report here further details on the extent of development of floral organs from mature tassel spikelets.

For each of the genotypes 1) homozygous recessive *ms-si*; 2) heterozygous *ms-si* (wild type, WT); and 3) Oh43, greater than three hundred spikelets from thirty or more nursery plants were analyzed at maturity. The following observations were made on the flowers at maturity:

1) Glumes, lemmas, paleas and gynoecium of the three genotypes were essentially normal.
2) Lodicules of Oh43 and wild type (WT) were similar, but those of *ms-si* resembled additional lemmas. This close resemblance made it difficult to distinguish (other than by position) the lemma-like lodicules from the true lemmas.
3) The six stamens (three in each spikelet's upper and lower florets) of the WT and Oh43 were similar and normal.
4) More than sixty percent of the *ms-si* spikelets examined possessed stamens with a planar dimension rather than the normal radial, and development to the mid-portion of the range mentioned previously.
5) Only the stamens of the upper *ms-si* floret developed to an abnormal state while those of the lower floret became aborted at an early stage. The abnormal stamens varied in appearance but in general resembled lemmas. Either the complete stamen or each locule (four in total for each stamen) had been modified to mimic a lemma. There were instances where any number (zero to four) of the locules were lemma-like while the others looked normal.
6) For those stamens from *ms-si* flowers in which the anther locules were modified, the connective tissue was intact and similar to those from Oh43 and WT flowers except that the connective tissue at the tip of *ms-si* stamens continued, in some instances, to expand or grow to various lengths (zero to five centimetres from the tip of the locules) to develop what we have termed the silk-like structure (SLS).

In summary, the lodicules and stamens of *ms-si* tassel spikelets resemble lemma-like structures. This recalls other species, i.e. barley and tomato, reports of staminal transformation into other floral parts (M. L. H. Kaul, Male sterility in higher plants, Springer-Verlag, 1988) and (V. G. Meyer, Bot. Rev. 32:165-218, 1966). Terms such as phylody, petaloidy and pistiloidy are used to describe these variations. For the *ms-si* phenotype, we have used "lemma-like" to describe these modifications.

Allometric growth studies indicate an early expression of *ms-si* (*si1*) on floral development of tassel spikelets.

--D.W. Dales, R.I. Greyson and D.B. Walden

Allometry is a method of comparing the constantly changing dimensions of two organs from the same individual even though their growth rates are constant (R. I. Greyson and V. K. Sawhney, Bot. Gaz. 133:184-190, 1972; G. G. Simpson, A. Roe and R. C. Lewontin, Quantitative Zoology, Harcourt Brace, 1960). In addition, the

relationship of growth rate between the measured organs can be shown. It can detect differences at the early stages of development which otherwise may not be noticeable. Reported here are allometric studies on the development of floral organs from *ms-si* and wild type (WT) tassel spikelets.

Five tassels of each genotype: 1) homozygous recessive *ms-si* and 2) heterozygous *ms-si* (WT), were harvested at random every third day from plants in the field nursery, beginning at the stage of spikelet primordia until anthesis (displayed by WT only). From the mid-portion (one third) of the main rachis, five spikelets (random mixture of sessile and pedicellate) were removed for measurements including length of outer glumes; lodicules and stamens (excluding any silk-like structures, SLS) of the upper and lower flowers of each spikelet. The mean length of individually measured organs for the five spikelets was calculated. The logarithm of mean length for either the lodicule or stamen was plotted against the logarithm of mean glume length and a regression line with coefficient of correlation calculated for the wild type and *ms-si* using the graphics program "Sigma Plot".

In Figure 1A, the slope of the regression line of upper floral stamen length measured against outer glume length for *ms-si* is slightly smaller compared to wild type. Although the differences between WT and *ms-si* may not be significantly different, additional comparisons of 1) stamen length to width measurements; 2) the length of silk-like structures, added to the plots, and 3) an examination for the development of pollen, may present significant differences as noted in our first contribution, this Newsletter.

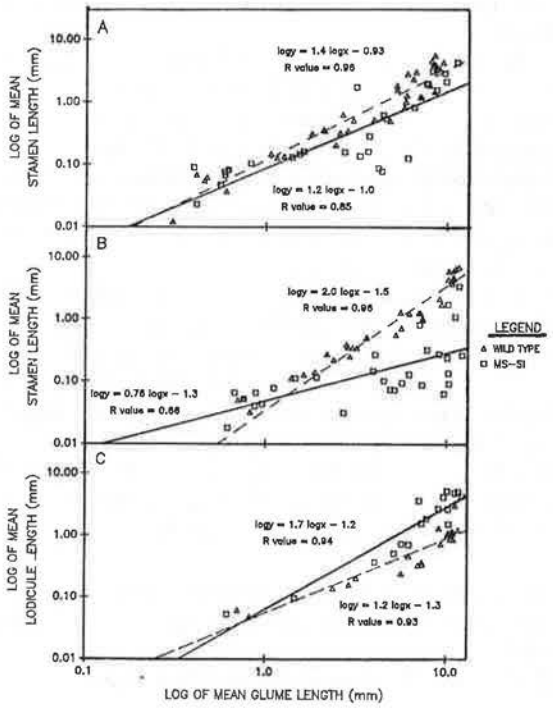

Figure 1. Log mean lengths of outer glumes; stamens (excluding silk-like structures, SLS) from upper (A) and lower (B) and lodicules from upper (C) flowers of spikelets from the mid 1/3 portion of the main rachis of *ms-si* (*ms-si/ms-si*) and wild type (*Ms-si/ms-si*) tassels at various stages of maturity. The mean was calculated for five replicates or spikelets from the same tassel. Linear regressions are plotted to display the relationship between glume length and stamen or lodicule length. Differences in floral development between *ms-si* and wild type are depicted by the regression lines. R represents the coefficient of correlation.

The decrease in slope of the regression line of *ms-si* in Figure 1B reflects the ultimate abortion of the lower flower stamens in the *ms-si* spikelet. This developmental effect resembles in part the selective abortion of the lower flower in the ear.

In Figure 1C the enhanced slope of the regression line for *ms-si* upper floral lodicule measured against the outer glume depicts the development of lemma-like lodicules that are longer in total length than WT lodicules. Observations made of lower spikelet florets replicate the results noted, in this letter, of the upper flower. If measurements of length to width were to be plotted, differences in the two genotypes would also be shown.

Examination of spikelets from the tip one third and base one third of the main tassel rachis replicated the outcome noted for the mid-portion.

The important point to note from the plots is that floral organ differences between *ms-si* and WT can be detected at early stages of development in young spikelets. This observation further documents the statement, that *ms-si* is expressed early in floral development (MNL 63:88).

In conclusion, *ms-si* influences floral development at the early stages, perhaps before or at the time of organ primordia initiation. For *ms-si* genotypes, in comparison to the wild type, the lower floral stamens abort and the lodicules are larger in length.

Shoot apical meristem culture of maize, wheat, barley and oat

--V. R. Bommineni and D. B. Walden

We have reported the successful recovery of plantlets from 14 days-after-pollination (DAP) immature and 72 h imbibed shoot apical meristems of maize (Bommineni et al., MNL 63:87-88; Plant Cell Tissue Organ Cult., 1989, in press). No limitations were observed (such as genotype specificity, recovery of fertile plants, etc.), with this culture procedure. However, the explanted meristems were treated with different stress-related conditions (physical or horticultural) and the percentage of recovery of plantlets compared to our earlier data.

A summary of the data is in Table 1. Among the genotypes tested, the percentage of plantlets recovered is lower

Table 1: Recovery of maize plantlets through shoot apical meristems exposed to environmental stress conditions in field and glasshouse nurseries.

Genotype	Number			Percentage	
	Meristems Explanted (2)	Plantlets to nursery (3)	Plantlets to maturity (4)	Plantlets (3/2)	To maturity (4/3)
Field:					
wx wx	347	97	94	28	97
Oh43	155	49	45	31	92
A188	183	65	49	35	75
Total	685	211	188	31	90
Glasshouse:					
wx wx	876	386	361	44	94
A188X W23	20	10	10	50	100
Total	896	396	371	44	94

than that in our previous data. Further, there is a decrease in plantlet recovery in the field nursery compared to the glasshouse nursery. For example, in waxy (*wx wx*) genotype, 28% of plantlets were recovered in the field nursery in comparison to 44% in the glasshouse nursery. Once the plantlets became established, there was no further loss of plants either in the field or glasshouse (Table 1). The data on percentage of plantlets grown to maturity (column 6 in Table 1) is similar to the recovery of plantlets in our earlier reports.

Other cereal shoot apical meristem culture: We also attempted to apply basic culture protocol (without any treatment) to immature shoot apical meristems of wheat, barley and oat.

Immature seeds of wheat, barley, and oat were collected from the field in the summer of 1989. The grains were surface sterilized in 10% 'Javex' for 30 min after removing the glumes. Other procedures, dissections, and medium composition were similar to maize (MNL 63:87-88) and the final size of these meristems ranged from 0.5-0.8 mm. After 3 weeks of culture, the plantlets with roots and leaves were transferred to small plastic nursery pots (2-3 plantlets per pot) in the glasshouse and grown in the same pot through flowering.

Table 2. Recovery of plantlets through shoot apical meristem culture from immature embryos of wheat, barley and oat.

Crop (cultivar)	Meristems	Number Plantlets	Plantlets	Percentage Plantlets	To maturity
	Explanted (2)	to nursery (3)	to maturity (4)	(3/2)	(4/3)
Wheat (Agusta)	130	94	87	72	93
Barley (Birka)	142	80	75	56	94
Oat (Donald)	124	103	95	83	92

A high percentage of plantlets were recovered in all three cereals (Table 2). However, a maximum percentage of plantlets were recovered in oat (83%) and a lower percentage of plantlets were recovered in barley (56%). Fertile seeds were recovered from all the plants which flowered.

Micro-propagation through axillary bud culture: an update with four different genotypes

--V. R. Bommineni, E. Banasikowska, and D. B. Walden

We have reported a summary of our axillary bud culture data (cv. Seneca-60) (Walden et al., Maydica 34: 263-275, 1989) derived from twelve original explant lines for up to 13 transfer generations. One of the original 12 explants has been maintained for 21 transfer generations (Table 1). We began recently to extend this system to important inbred lines and genetic stocks as extensive micropropagation of axillary buds permits clonal propagation of maize.

The original explant of each genotype was derived from a glasshouse grown, 20-30 day old seedling. The stalk of the seedling was surface sterilized in 20% 'Javex' for 20-30 min and rinsed three times with sterile distilled water. The sheaths of stalk were removed under the microscope in the culture hood to expose the shoot apical meristem. Each explant consisted of a shoot apical meristem with 5-6 nodes at the time of culture. The explant was placed in a small jar containing MS medium and other plant growth hormones (optimal) as reported in Table 1. Other incubator and growth conditions were same as in our earlier report (Bommineni et al., MNL 63: 87-88).

Table 1: Micro-propagation of maize through axillary bud culture from a single explant.

Genotype	Medium	Number of generations* from bud to bud	Total number of explants recovered into a plant	Propagation time (months)
Seneca-60	SI: MS + 3 mg KN, 1 mg IAA RI: BM + 5 mg NAA	21**	2653	23
A188	SI: MS + 3 mg BAP, 1 mg IAA RI: BM + 5 mg NAA	2	14	1
Oh43	SI: MS + 3 mg ZN, 1 mg IAA RI: BM	2	3	2
B73	SI: MS + 3 mg ZN, 1 mg IAA RI: BM + 5 mg NAA	2	5	1

* = every generation represents a single transfer to fresh medium followed by proliferation of buds
** = Line "D" in Walden *et al.*, Maydica 34:263-275, 1989
SI = shoot inducing medium; RI = root inducing medium
MS = MS medium with 100 mg/l inositol, 30 g/l sucrose, 120 mg/l adenine sulphate dihydrate, 170 mg/l monobasic sodium phosphate
BM = MS medium without adenine sulphate dihydrate and monobasic sodium phosphate
BAP = 6-benzylamniopurine, KN = kinetin, ZN = zeatin, IAA = indole-3-acetic acid, NAA = naphthalene acetic acid

Characterization of cDNAs encoding two different members of the low molecular weight heat shock protein family

--Ing Swie Goping, D. B. Walden and Burr G. Atkinson

Five-day-old maize seedlings (cv. Oh43) grown at 27 C and incubated at 42.5 C for one hour synthesize a characteristic set of heat-shock proteins (HSPs) of 108, 89, 84, 76, and 73 kDa, as well as an 18 kDa family containing at least 6 prominent isoelectric variants. Messenger RNAs, encoding some of these low molecular weight (LMW) HSPs, were hybrid-selected from lysates of heat-shocked radicles with a fragment from the putative coding region of a Black Mexican sweet corn HSP18 genomic subclone (BMS HSP18 provided by R. Sinibaldi; Dietrich et al., J. Cell Biol. 103:311a, 1986). Cell-free translations of the hybrid-selected RNAs reveal that the genomic fragment of the BMS HSP18 shows homology to a set of mRNAs which synthesize at least 4 different members, designated as 1, 3, 5 and 6, of the maize LMW HSP family (Atkinson et al., Genome, 1989, in press).

A cDNA library was constructed in λ Zap (Stratagene) from polyribosome-associated poly(A)$^+$ RNAs isolated from the radicles of heat-shocked five-day-old maize seedlings. The library was screened with a fragment from the BMS HSP18 gene and two clones, cMHS18-3 and cMHS18-9, were isolated from it. RNA transcripts, generated from cMHS18-3 and cMHS18-9, were translated in a cell-free system and their protein products were separated

on a two-dimensional gel (IEF/SDS-PAGE) electrophoretic system. The proteins synthesized by these RNAs show the same electrophoretic characteristics as those displayed by the LMW HSPs numbered 6 and 3, and are immunoreactive with anti-HSP18 antibodies generated against the 6 LMW HSPs of maize (Oh43).

Sequence analyses revealed that the cDNA cMHS18-3 is 982 nucleotides (nt) in length, and contains a putative open reading frame of 495 nt which is capable of encoding a polypeptide consisting of 164 amino acid residues (M_r = 17.8 kDa and pI = 5.1). The cDNA cMHS18-9 is 843 nucleotides in length, and contains a putative open reading frame of 486 nt which is capable of encoding a polypeptide consisting of 161 amino acid residues (M_r = 17.5 kDa and pI = 5.5). The two cDNA sequences share 21% and 32% homology in their 5'- and 3'- untranslated regions, respectively, and are 92% homologous in their putative coding region. The apparent conservation of the protein encoding nucleotide sequences and the marked differences in the untranslated regions suggest that the LMW HSPs numbered 3 and 6 are the products of a gene duplication event that has since undergone divergence. In fact, many of the apparent insertions evident in cMHS18-3 and cMHS18-9 are at sites of imperfect repeats, possibly created by the imperfect excision of a transposable element. Interestingly, the sequence of the first 194 nucleotides of the 5'-untranslated region of the clone cMHS18-3 is 97% complementary to the 3' region of RNA found in rice 25 S ribosomal RNA (rRNA) and shows equally high complementarity (74-95%) to the 25 S or 28 S rRNA of other organisms. The relevance of these observations is being explored.

Finally, comparisons of the amino acid sequences deduced from these clones reveal that some, albeit limited, homology exists with the class VI LMW HSPs of soybean (particularly in the carboxy terminus; 46% homology) while considerably less homology is evident among the LMW HSPs of other organisms. Despite these amino acid differences, inspection of Hopp-Woods hydrophilicity plots suggests that the LMW HSPs of maize and soybean contain highly conserved structural regions in their carboxy termini. Therefore, it appears that some of the LMW HSPs of maize (cv. Oh43), specifically those designated as number 3 and number 6, are the products of different but related genes and that these proteins appear to share some structural similarities with the LMW HSPs of other organisms.

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Genetic analysis of tolerance to low-phosphorus stress using RFLPs

--Robert Reiter, James Coors, Michael Sussman and Buck Gabelman

An understanding of the genetic nature underlying tolerance to low-phosphorus (low-P) stress would permit efficient development of tolerant plant strains. This study was initiated to identify the number of loci in a maize population segregating for tolerance to low-P stress. In addition, the approximate location and magnitude of effect of identified loci were established.

Seventy-seven restriction fragment length polymorphisms (RFLPs) were identified and scored in an F2 population derived from a cross between line NY821 (tolerant) and line H99 (intolerant). The F2 individuals were self-pollinated to produce F3 families. Ninety F3 families were grown in a sand-alumina system that simulated diffusion-limited, low-P soil conditions. The F3 families were evaluated for vegetative growth in two controlled-environment experiments. To identify quantitative trait loci (QTLs) underlying tolerance to low-P stress, the phenotypic performances of the F3 families were contrasted based on genotypic classification at each of the 77 RFLP loci.

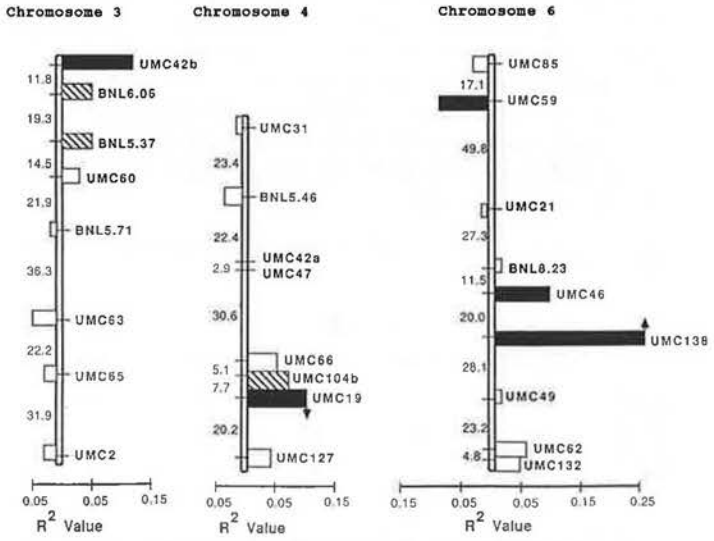


Figure 1. Location and R^2 values of individual marker loci for total dry weight under P-stress. A highly significant ($P \leq 0.01$) marker locus is represented by a solid bar, and a significant ($P \leq 0.05$) marker locus is represented by a hatched bar. Distances, in cM, between marker loci are indicated to the left of each chromosome. Bars lying to the right indicate the alleles with positive effects came from the tolerant parent, NY821. Bars lying to the left indicate the alleles with positive effects came from the intolerant parent, H99.

Six RFLP marker loci, UMC46, UMC42b, UMC138, UMC19, UMC117, and UMC59, were identified as being significantly associated ($P \leq 0.01$) with some aspect of performance under low-P stress. Four of these loci, UMC46, UMC42b, UMC138, and UMC19, were associated with shoot dry weight under low-P stress. Five loci, UMC46, UMC42b, UMC138, UMC117, and UMC59, were associated with root dry weight under low-P stress. Loci UMC46, UMC42b, UMC138, UMC19, and UMC59 were associated with total dry weight under low-P stress (Fig. 1). One marker locus, UMC138, accounted for 25% of the total phenotypic variation in total dry weight. Additive gene action was predominant for all the QTLs identified. Significant marker loci were located on four separate chromosomes representing five unlinked genomic regions. Two marker loci, UMC42b and UMC138, were significantly associated with an additive by additive epistatic in-

teraction. A multiple regression model including UMC42b, UMC138, and UMC19 and the significant epistatic interaction between UMC42b and UMC138 accounted for 46% of the total phenotypic variation for total dry weight.

Evidence implicating the *Spm* (*I-En*) family of transposable elements in the mosaic pericarp (*P-mo*) allele

--Oliver Nelson

Several years ago, a test of the possible involvement of the *Spm* (*I-En*) family of transposable elements in the *P-mo* allele of the *P* locus was initiated. In common with the extensively studied *P-vv* allele, *P-mo* conditions a variegated pericarp phenotype when homozygous or heterozygous with *P-ww*, but *P-mo* is known not to result from the insertion of an *Ac*(*Mp*) element in a *P-rr* allele as does *P-vv*. The investigation was started with three *P-mo* stocks provided by R. A. Brink. These stocks had been originally received by him from three different sources (Fontaine, St. John, and Weatherwax) and had been crossed and then backcrossed five times to the inbred 4Co63, which is *P-ww/P-ww* (white pericarp, white cob). 4Co63 is the same inbred that was used as a recurrent parent in investigations of the genetic basis of the variegated pericarp (*P-vv*) phenotype by R. A. Brink and his students. It should be noted that *P-mo/P-ww* plants have white cobs while *P-vv/P-ww* plants have variegated cobs. The backcross progeny from a plant showing the mosaic pericarp phenotype consists of nearly equal numbers of mosaic pericarp (*P-mo/P-ww*) and white pericarp (*P-ww/P-ww*) plants although there is an excess of *P-ww/P-ww* plants over the proportion expected on the assumption that there should be a 1:1 segregation.

Since the stocks that would allow an immediate answer to the question of whether these *P-mo/P-ww* accessions from Dr. Brink had *Spm* activity were not available, the question was posed by crossing these accessions by a stock that is *P-wr*, *bz-m13*, no *Spm*. The *bz-m13* allele resulted from the insertion of a defective *Spm* (*dSpm*) in a *Bz* allele, and it conditions a nonmutant phenotype in the absence of a transacting *Spm*. The F1 progenies were then crossed by a *C sh bz*, no *Spm* tester stock. Since the mosaic pericarp stocks are *Bz/Bz*, if they do not contain an active *Spm*, the resultant kernels on the testcross by the *C sh bz*, no *Spm* tester would all display the *Bz* phenotype. If the *P-mo/P-ww* accessions do contain one or more active *Spm*'s, then a certain proportion of the kernels would show variegation typical of *bz-m13* in the presence of the active *Spm*. For the Fontaine and Weatherwax accessions where mosaic pericarp plants were used as the parents in the original cross by *bz-m13*, 80% of the mosaic pericarp plants showed kernels with the variegation typical of *bz-m13* in the presence of *Spm*. No cross onto a mosaic pericarp plant of the St. John accession was made. The same proportion of *P-ww/P-ww* plants in these same progenies showed variegated *bz-m13* kernels. Thus nearly 3/4 of the plants in the F1 progeny had an active *Spm* whether or not they were mosaic pericarp. On the assumption that the production of a mosaic pericarp plants requires an active *Spm*, the mosaic pericarp plants that do not have *bz*-variegated kernels need to be explained. Such could result from contamination (i.e., the F1 plant being tested resulted from pollination by a *Bz* gamete, not a *bz-m13* gamete), from presetting of *P-mo* before being separated from *Spm* at meiosis, or from an excision event creating a stable *Bz* or *bz* taking place early enough in development that the entire ear is derived from this cell lineage.

Even if the parent plant in the backcross progeny were *P-ww/P-ww* rather than *P-mo/P-ww*, the majority of the F1 plants have an active *Spm*, and this is so for all three accessions. This observation as well as the presence of an *Spm* in most nonmosaic plants in the progeny of mosaic pericarp parents raised the question of whether the active *Spm*(s) were contributed by the recurrent parent, 4Co63. Crosses analogous to those described above but crossing the *bz-m13*, no *Spm* stock onto 4Co63 instead of *P-mo/P-ww* and pollinating the F1 by the *C sh bz*, no *Spm* tester showed that 4Co63 is not the source of the active *Spm*(s). Thus three accessions of mosaic pericarp backcrossed five times to a recurrent parent that lacks *Spm* with selection each generation for plants strongly expressing the mosaic phenotype have retained at least one and possibly two active *Spm*(s) that are not linked to the *P* locus.

A test has been made for the presence of a *dSpm* inserted at the *P* locus. This was done by pollinating the Weatherwax accession (five times backcrossed to 4Co63 and hence segregating *P-mo/P-ww* and *P-ww/P-ww* plants) by a *C sh bz wx*, + *Spm* tester. The intent was to ascertain if any of the plants producing ears with white pericarps and white seeds could produce mosaic pericarp plants if an active *Spm* were introduced. A poor seed set in the summer of 1988 necessitated bulking the seed from a number of ears for planting in Florida. Two ears of the 68 produced were mosaic pericarp. It should be noted that if there were two unlinked *Spm*'s present in mosaic pericarp plants sampled as some of the data seem to suggest, then only 1/5th of the white pericarp, white cob plants could be genotypically *P-mo/P-ww* minus *Spm*, and only 1/2 of their progeny would be mosaic pericarp. It's not possible, however, to compare the results derived from a bulked sample such as the one planted to any stated hypothesis. So while there was an indication of a *dSpm* inserted at *P* in *P-mo*, clearly more data are required to be certain that the seeds producing the mosaic pericarp plants did not come from a mosaic pericarp ear that had been misclassified even though this is unlikely.

Stocks that are *bz/bz*, *P-mo/P-wr* are now available and will make it possible to test directly the *Spm*(s) present using *bz-m13* as a tester allele. While it is clear that the *Spm* family of transposable elements is involved, the data are not compatible with the simplest hypotheses, which are that the *P-mo* allele is *P-rw+Spm* or *P-rw+dSpm* with either a linked or unlinked *Spm*.

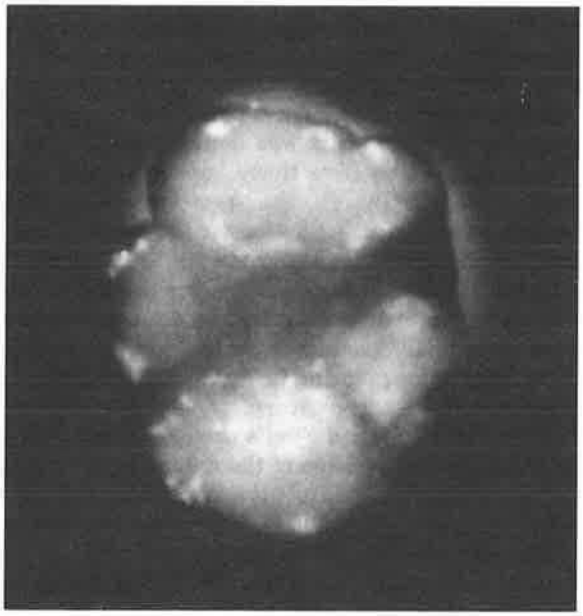
The fascicled ear (*Fas*) mutation

--Samuel Postlethwait and Oliver Nelson

We wish to call the attention of the maize genetics community to an interesting developmental variant that

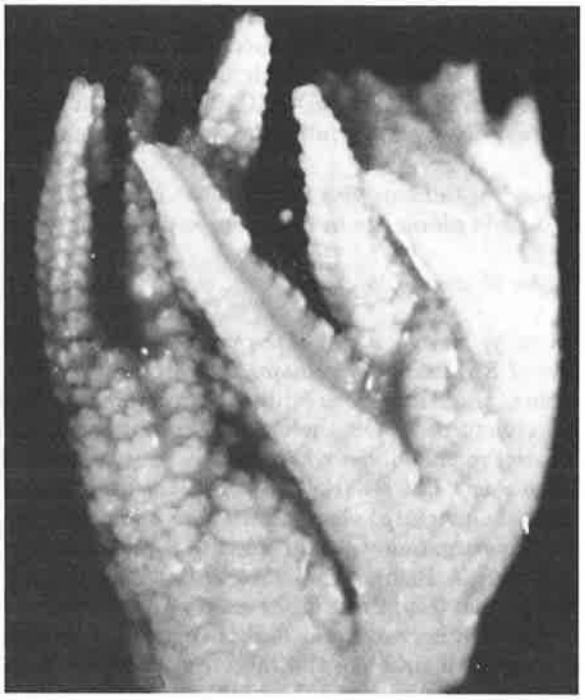
affects the floral structures of maize. We received this mutant stock over thirty years ago from Paul Weatherwax of Indiana University and have designated the mutation as fascicled ear (*Fas*). It is a dominant mutation with excellent penetrance and expressivity. Ms. Ruth Hessler, a student of Samuel Postlethwait, studied ear and tassel development in *Fas*/+ plants in comparison to their nonmutant sibs, but the results have not been published. Since we are unable to locate Ms. Hessler, there will not be a formal publication, but the increasing interest in studying maize development impels us to note the existence of this mutation and to describe the salient features of its expression.

The vegetative shoot apex of fascicled ear plants does not differ from that of nonmutant plants, but at the transition from a vegetative to a floral meristem, the meristem broadens until the width is double its height. The increase in width is followed by a division into two meristems that then broaden in width. The dichotomous branching is repeated (Fig. 1). Before the process ceases (when spikelet



and floret development is initiated), as many as 14 branches may be produced. It is possible that the mutation induces an upset in timing since the appearance of the meristems before division is reminiscent of a branch primordium before division to produce the two spikelet primordia giving rise to the double rows of florets on the cob axis. The product of this deviation from normal development is an ear in which a number of slender ears surround a depressed central area (Fig. 2). The apices of these branches may complete development in a normal fashion or may reinitiate cycles of rapid division producing a fasciated apex. The number of branches may differ from plant to plant within a progeny or even from ear to ear on the same plant.

Fewer kernels as a percent of ovules present mature on *Fas* ears than on their nonmutant sibs. The abortive kernels are usually on the inner surfaces of the branches.



Well-developed staminate structures that produce mature pollen typically terminate the branches.

As might be expected, the tassel of *Fas* plants is also affected. As tassel development commences, the apical meristem broadens and then divides. There may be several more divisions producing a highly branched central spike, and *Fas* plants can be first identified by the tassel morphology. The development of the *Fas* tassel further parallels ear development in that the branches on the central spike complete development normally.

Seed of this mutant will be sent to the Coop and can also be obtained from Oliver Nelson.

The identification of an intermediate allele of *D8*
--Oliver Nelson

The dominant mutation *D8* on chromosome 1 conditions the production of an extremely dwarfed plant that is nonresponsive to gibberellins. I have isolated from remnant seed of an EMS mutagenesis experiment set up some years ago by Prem Chourey and Hugo Dooner an intermediate allele of *D8* (*D8-81127*) that when heterozygous with a nonmutant allele (*D8-81127*/+) produces a plant that is 3 to 3.5 feet in height and sheds pollen freely. Homozygous plants (*D8-81127/D8-81127*) are extreme dwarfs, and the anthers are rarely exserted.

The evidence that *D8-81127* is allelic to *D8* comes from the following crosses. In 1986, *D8-81127*/+ plants were crossed by *D8*/+ plants. In 1988, the extreme dwarfs in a resultant progeny were crossed by N22 (+/+, *c*/*c*). In 1989, six different progenies from the N22 crosses were planted. Two progenies produced only dwarf plants. In both of these progenies, ca. one-half of the plants were extreme dwarfs (*D8*/+) while the remaining plants were typical *D8-81127*/+. In the other four progenies, there were tall (+/+) and extreme dwarf plants (*D8*/+) in approximately equal numbers.

Phosphoglucomutase activity in developing endosperms

--David Pan, Lisa Strelow and Oliver Nelson

The developing endosperms of most plants investigated have two phosphoglucomutase (PGM) isozymes. One is the amyloplast enzyme, the other is a cytosolic enzyme, and these isozymes are separable by starch gel electrophoresis. Assaying extracts of inbred maize endosperms 22 days postpollination by starch gel electrophoresis has shown that the majority of the inbreds have only one isozyme present. It has been demonstrated that the isozyme present in all inbreds is the amyloplast form. The inbreds B73, W22, SDP312, W552, A632, H49, B84, A540, B37, N22, and Oh43 have only amyloplast PGM. The inbreds W64A, Mo17, and W570 have both isozymes as does the sweet corn inbred, P39.

The separations by column chromatography (DEAE-cellulose) of the extracts from N22 and Oh43 reveal only a single peak of activity, indicating that the single zone of activity observed on SGE is unlikely to result from two isozymes that migrate to exactly the same zone.

The lack of the cytosolic isozyme has no discernible phenotypic consequence. It appears that this enzyme is completely dispensable for normal endosperm development. It is also possible that the absence of this enzyme is one of the cryptic deficiencies (along with many others) that contribute to the lessened vigor and lower productivity that characterize inbred lines and that is often redressed by crossing to an unrelated inbred line. This hypothesis is testable, and we are proceeding to do so.

Sequence of two stable *bz* derivatives from *bz-m13*

--Ron J. Okagaki, John W. Schiefelbein and Oliver E. Nelson

The *bz-m13* allele is an insertion of a 2.2 kb *dSpm* element 38 bp downstream from the intron in the *Bz1* gene. In the presence of an active *Spm* element approximately 40% of the kernels can be stable *bz1* derivatives, making this a rich source of stable derivative alleles. We have characterized 13 of these *bz1'* derivatives by Southern blot analysis, and determined the sequence from the region flanking the *dSpm* insertion site in two derivatives.

Southern blot analysis found one deletion in the sample, *bz'-10*; this derivative was cloned into the vector pUC119 after the sequence was amplified by the polymerase chain reaction. Three independent clones were sequenced using Sequenase (US Biochemicals). The *bz'-10* allele is a 49 bp deletion of *Bz1* sequences; the deletion begins in the 3' end of the intron, removes the *dSpm* and *Bz1* sequences between the breakpoints, and extends 8 to 11 bp downstream of the insertion site (Figure 1). Two unusual features of this deletion were immediately apparent. First, the deletion extends to both sides of the *dSpm* insertion site; other transposable element-associated deletions extend to one side of the insertion site. Second, the deletion breakpoints are within a 4 bp direct repeat. Short direct repeats are often found at deletion breakpoints in spontaneous deletion alleles in bacteria and other organisms, but they are not usually associated with transposable element deletions in maize. It is possible that *bz'-10* is a spontaneous deletion.

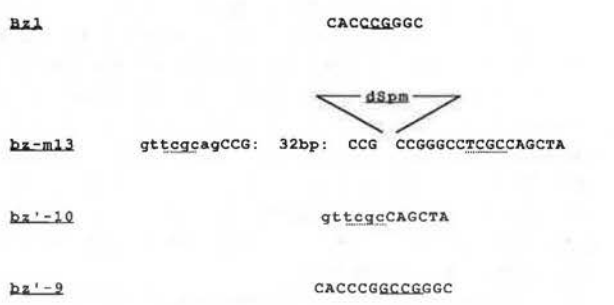


Figure 1. Sequence of *bz-m13* and two stable *bz1* derivatives. The top line shows the sequence from *Bz1* surrounding the *dSpm* insertion site; the three bases duplicated by the insertion are underlined. The second line gives the sequence from *bz-m13* flanking the insertion site and the sequences spanning the deletion breakpoints in *bz'-10*. Intron sequences are in lowercase letters, and the 4 basepair direct repeats are underlined with dotted lines. In *bz'-10*, the deletion begins in the 3' splice acceptor site and extends to 8-11 basepairs downstream of the *dSpm* insertion site. This deletion also maintains a 3' splice acceptor site, however the message is out of frame. In *bz'-9*, the 4 base pairs inserted are underlined.

The remaining 12 derivatives were indistinguishable from the progenitor allele by Southern blot analysis. One of these alleles, *bz'-9*, was cloned and sequenced. This derivative has a 4 bp insertion relative to *Bz1* sequences; the 3 bp host sequence duplication and one additional base are left behind. This sequence is similar to other stable derivative alleles examined by others.

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Combining ability for resistance to stalk rot, ear rot, common smut and head smut diseases

--M. Odiemah and I. Kovacs

Six inbred lines of maize were evaluated in a diallel set of 15 single crosses to study the inheritance of resistance to stalk rot, ear rot, common smut and head smut diseases by estimation of GCA and SCA effects. The entries were evaluated under artificial-natural infection at Martonvasar. Ratings for stalk rot and ear rot were recorded during harvest on individual plants per plot according to a scale from 1 to 9, while data of common and head smuts were based on the percentage of infected plants of fully grown plants for each plot. These entries were grown in an experiment of a randomized complete block design with four replications of two-row plots. Each row was 6m long and contained 10 hills (plants). The inter-row and intra-row distances were 70 and 30 cms, respectively. The analysis of variance was performed on plot means over two seasons. Partitioning of F1 hybrids into GCA and SCA effects was important for resistance to all diseases with the exception of common and head smuts where the SCA effects were not significant, GCA mean squares were substantially larger than SCA mean squares.

It means that additive gene action conditioning resistance to all diseases is of major importance in this set of diallel crosses. Generally, the present study indicated that both additive and nonadditive genetic effects are important in resistance of such diseases. Furthermore, the importance of additive genetic variation and the absence or relatively small magnitude of the nonadditive genetic effects for resistance to numerous diseases have been reported by several investigators. This indicates that most quantitative genetic resistance to maize diseases may have similar additive gene actions.

Estimates of general (GCA) and specific (SCA) combining ability effects are shown in Table 1. The GCA effects were significant for most inbred lines with all disease ratings. Resistance figure is of negative (-) direction because resistant reactions in entries were indicated by lower ratings or extremely resistant, whereas the higher ratings of positive (+) direction belonged to severe infections. Table 1 revealed that parental line 6 had the largest negative GCA effect for stalk rot followed in order by 5 and 4. By contrast, inbreds 1 and 2 had the largest positive GCA effects on susceptibility while inbred 3 had the least. Parental lines 3 and 4 appeared to have a negative GCA effect for ear rot, whereas parental lines 1, 4, 5 and 6 contributed positive effects. Parental lines 3 and 4 contributed negative GCA effects for common smut whereas the other parental lines contributed positive GCA effects. In head smut, parental lines 2, 3, 5 and 6 also contributed negative GCA effects, while lines 1 and 4 contributed positive effects.

Table 1. General GCA and specific SCA combining ability effects for the resistance to major diseases of six parental lines in maize averaged over two seasons.

Effect	Stalk rot	Ear rot	Common smut	Head smut
GCA:				
P_1	0.89	0.36	0.50	0.17
P_2	0.74	-0.43	0.09	-0.33
P_3	0.29	-0.51	-0.42	-0.47
P_4	-0.18	0.08	-0.10	0.69
P_5	-0.36	0.67	0.26	-0.67
P_6	-0.47	0.35	0.11	-0.55
SCA:				
Sc_{12}	0.35	0.26	0.28	0.22
Sc_{13}	0.55	-0.47	0.49	-0.51
Sc_{14}	-0.20	0.32	-0.31	0.17
Sc_{15}	0.11	-0.68	0.09	-0.56
Sc_{16}	-0.17	0.49	0.33	0.39
Sc_{23}	0.70	0.24	0.11	-0.54
Sc_{24}	0.05	0.33	0.34	-0.21
Sc_{25}	-0.61	0.36	-0.58	0.15
Sc_{26}	0.10	-0.52	-0.15	0.46
Sc_{34}	-0.63	-0.81	-0.13	0.27
Sc_{35}	-0.18	0.20	-0.50	0.56
Sc_{36}	-0.57	-0.43	-0.14	-0.60
Sc_{45}	0.05	-0.12	0.48	-0.22
Sc_{46}	0.40	0.35	-0.23	-0.12
Sc_{56}	0.12	0.66	-0.29	0.61
(SE) for:				
GCA:	0.16	0.13	0.20	0.27
SCA:	0.37	0.34	0.41	0.55

The majority of the significant SCA effects for stalk rot and ear rot were negative; the exceptions were Sc_{12}, Sc_{23}, Sc_{24}, SC_{46} and Sc_{56}. The SCA effects were nonsignificant in the analysis of variance for common and head smuts, although a negative effect was indicated in some single crosses.

From the standpoint of breeding, each parental line with negative GCA effect would be conducive to increasing resistance to disease directly. It may be possible to find recombinants of these genotypes with resistance to most diseases in a large population. Once such a composite population is established, it can then be improved for disease resistance by any suitable recurrent selection method.

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RFLP analysis of S4 lines selected for multiple borer resistance

--D. González de León, D. A. Hoisington, D. Jewell , J. Deutsch and J.A. Mihm

As part of the current development of applications of restriction fragment length polymorphisms (RFLPs) to the evaluation and breeding of tropical maize germplasm at the International Center for Wheat and Maize Improvement (CIMMYT, Mexico), we have been working on a collaborative project with the University of Missouri-Columbia on the utilization of RFLPs to monitor the transfer, and to determine the location, of chromosome segments responsible for resistance to corn borers.

The breeding objective at CIMMYT was to develop lines with a high level of resistance to Southwestern corn borer (SWCB) and improved agronomic traits. Earlier work at CIMMYT indicated that borer resistance may be under the control of several genes. Since there were no particular reasons for recovering the genotype of the chosen parental lines, we decided to develop a series of recombinant inbred lines, while selecting for resistance and then for agronomic performance at each successive cycle (S1 to S4).

The six S4 lines for which RFLP data are now available are part of the insect resistance breeding program at CIMMYT. These lines were derived from a single cross between Mp78:518 and an S3 line from CIMMYT's Population 47. Mp78:518 is a yellow endosperm line with very high multiple borer resistance (MBR) that was developed by the USDA-ARS in cooperation with the Mississippi Agricultural and Forestry Experiment Station; however, it is susceptible to ear rot when grown at CIMMYT stations in Mexico, develops iron chlorosis on calcareous soils and stalk lodges. The other parent , a white endosperm S3 line from CIMMYT's Pop.47, is intermediate to susceptible to corn borers. In contrast to

the first parent, this line is resistant to ear rots, tolerates the high pH of calcareous soils, and has a strong stalk. Each cycle was infested with Southwestern corn borer and only the most resistant fraction was carried forward to the next cycle. Additional selection pressure for improvement of other agronomic traits (particularly those contributed by the Pop.47 parent), was applied within the resistant fraction when appropriate.

Out of 101 loci analyzed for RFLPs, 91 were polymorphic between the two parents for at least one of the three enzymes used. All S4 lines were scored at each polymorphic locus and their genotypes determined (homozygous for one of the parents or heterozygous).

We are currently developing algorithms based on inbreeding theory (e.g., Haldane and Waddington, 1931, Genetics 16:357-374), for predicting the expected genomic composition of a given arm after n generations of self-fertilization based on the frequency of crossing over at each meiotic cycle. We shall then be able to calculate the probability of a given genomic composition, and thus estimate whether certain gene combinations significantly reflect the effects of selection for a specific trait. These calculations will take into account the multi-point maps now available for RFLP markers, as well as known cytogenetic features of maize such as chiasma frequencies along specific arms.

The overall expected heterozygosity of any particular S4 line (after five generations of selfing) is (1/2) = 3.125%. The observed values for heterozygosity were, on average, 4.5 times greater than expected.

The effects of selection on any particular area of the genome were estimated from the frequency of parental morphs along each chromosome. If no selection had taken place at a locus, one would expect a 1:1 contribution by each parent. For the six lines in the analysis, a total of 12 morphs (2 per line) is possible at any one locus. Ratios of 9:3, 10:2, 11:1 and 12:0 would be significant at the 90% confidence level (Fisher's Exact Test).

Since Mp78:518 is expected to contribute the majority of resistance factors, significant deviations toward that particular parent may indicate areas involved in borer resistance. Six regions showed such deviations (in 1L, 2, 3L, 5L, 9S and 10L). Chromosome 2 has several potential regions of importance as almost all of the loci were skewed towards the resistant parent.

Deviations toward the other parent may indicate regions of importance for agronomic traits found in Pop. 47; however, one cannot rule out the possibility that this parent might have contributed resistance factors in these regions. Eleven areas showed such deviations (in 1S, 1L, 3S, 3L, 4, 5S, 5L, 6, 7S, 8L and 10L). Chromosomes 4 and 6 may contain several regions of importance to the Pop. 47 plant type.

The results to date indicate possible areas of resistance and/or desirable agronomic traits in the S4 lines so far studied. We now plan to use this information in future studies of the inheritance of borer resistance. These studies will involve further analysis of additional S4 lines (both selected and unselected) and segregation analysis of the QTL loci responsible for borer resistance.

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Embryogenic cultures: release and development in liquid medium of proembryonic structures

--L.A. Manzocchi, G. Giovinazzo, S. Castelli

Considerable progress has been made recently in induction of embryogenic suspension cultures, from which it is now possible to obtain protoplasts capable of regenerating plants (reviewed in Shillito et al., Bio/Technology 7: 581, 1989). Maize does not seem therefore as reluctant to in vitro manipulation as previously believed, and it is reasonable to expect the possibility to develop for this plant in vitro cell systems able to carry on a developmental pattern in controlled conditions, analogous to the ones existing for better studied species such as carrot (Sung et al., Plant Mol. Biol. Rep. 2:216,1984).

With this aim, we have induced in summer 1989 highly embryogenic friable calli by plating immature embryos of A188 xW64A cross (from Ist. Sper. Cereal., Sez. Bergamo) on MS (Murashige and Skoog, Physiol. Plant. 15:473, 1962) agar medium as modified by Armstrong and Green (Planta 164:207, 1985); calli were subcultured every 15 days on N6 medium (Chu et al., Scientia Sinica 18:659, 1975) containing 2 mg/l 2,4-D. Incubation of calli in liquid media on a rotatory shaker resulted in a rapid release of cells, cell aggregates and proembryonic structures; although the study of their proliferation and differentiation is still in progress, we think it worthwhile to report some preliminary data.

Several media (B5, N6, MS) were tested to optimize cell release from embryogenic calli; the best results were obtained with a medium composed of MS salts (3/4 strength), 30 g/l sucrose, 1 g/l proline, 100 mg/l asparagine, 200 mg/l inositol, 1mg/l niacin, 0.5 mg/l thiamine, 0.2 mg/l pyridoxine, 0.2 mg/l Ca panthothenate, 1.5 mg/l 2,4-D (L medium). Medium with released cells can be taken from the flasks every five days and replaced with fresh medium, allowing proliferation and further release for long periods.

Three cell populations were separated by differential sieving through stainless steel filters, and cultivated in medium L (containing 1.5 mg/l 2,4-D), N6(+) with 2 mg/l 2,4-D, and N6(-) without auxin.

A first fraction, which passed through a 63 um sieve, was composed essentially of single elongated cells, and a few small clusters of dense cells; they divide slowly, only in media with 2,4-D, and no further differentiation is observed.

A second population was separated between 63 and 125 um: it still contains single elongated cells, but is predominantly composed of small round-shaped dense cells (as described by Shillito et al. for rapidly growing suspension cultures), originating compact clusters especially when cultivated in N6(+).

The fraction comprised between 125 and 500 um is represented by a heterogeneous population of compact cell clusters and larger proembryonic structures, either round-shaped or characteristically elongated or resembling the

torpedo stage of carrot embryonic development. Cultivation of this fraction in N6(-) does not stimulate either cellular division or increase in the number of proembryonic structures, a few of which develop not to complete embryos but to structures similar to roots. In N6(+) and L media there is apparently an evolution from cell clusters to large proembryonic structures: these, in turn, do not differentiate further, but, after some days, begin to proliferate cells from their surface evolving to small calli.

Although very preliminary, our data seem to indicate that compact cell clusters larger than 125 um released from embryogenic calli are possibly analogous to the proembryo bodies of better described embryogenic systems such as carrot; it is possibly feasible to separate them from other cell populations and to obtain their evolution in culture to embryonic structures; further studies on hormonal balances are necessary to allow in vitro expression of a complete embryonic developmental pattern.

Opaque-2 endosperm suspension cultures
--L.A. Manzocchi

Stable cell cultures derived from maize endosperms have been used to study endosperm physiology and biochemistry, taking advantage of the fact that cultures differentiate into storage cells, corresponding for most physiological features to the intact endosperm (reviewed in Felker and Goodwin, Plant Physiol. 88:1235-1239, 1988). More recently, attempts have been made to employ these cells as homologous systems for the study of promoters of zein genes (Manzocchi et al., Workshop on "Plant Biotechnology-Engineered Storage Products for the Agroindustry", EEC/Dechema, Bad Soden, 15-18 April 1989).

We have succeeded in establishing liquid suspension cultures of A69Y opaque-2 endosperm cells (seeds from the collection of the Ist. Sper. Cereal., Sez. Bergamo). Two-year-old calli, induced from immature endosperms as described (Manzocchi et al., 1989) and cultivated in agar medium containing Murashige and Skoog salts, 30 g/l sucrose, 2 g/l asparagine and 1 mg/l thiamine (MSE), were transplanted frequently (every 15 days) for 5 subcultures to make them more friable, and were transferred to liquid MSE medium.

Actively growing suspensions were obtained from the smaller cell clusters, and separated from larger aggregates at each subculture; the tendency of cells to aggregate in clusters larger than 500 um is higher than in control cultures from A69Y+ endosperms.

Endosperm suspension cultures accumulate zein in lower amount, with respect to fresh weight and total protein, compared to developing endosperms (Manzocchi et al., Plant Cell Rep. 7:639-643, 1989); nevertheless, as opposed to *o2* callus grown on solid media in which no zein protein could be detected, *o2* liquid cultures synthesize detectable amounts of zeins, approximately one half of the amount accumulated in wildtype cells.

SDS electrophoretic patterns of ethanol-soluble proteins show, for *o2* cells, the reduction of the heavier zein bands typical of the mutation.

Our data confirm, for the storage protein mutations, the phenotypic expression of endosperm mutations in cultures, as described for *wx* and *ae* by Saravitz and Boyer (Theor. Appl. Gen. 73:489, 1987).

Expression of engineered zein polypeptides in yeast
--B. Basso, L. Bernard, P. Ciceri and A. Viotti

It is commonly accepted that zein proteins consist of four domains: a head region of 30-40 amino acids, a central part of 8-9 repeats of about 15 amino acids each, separated by 2 or more glutamine residues, and a short tail of 8-10 amino acids.

In order to test the biological and functional importance of the various domains for the structure of the protein and for its aggregation into protein bodies, the yeast *S. cerevisiae* was employed as a heterologous system for the expression of the whole zein sequence coding for a 24 Kd protein (M6, Viotti et al., EMBO J. 4, 1985) or of truncated sequences representing only specific parts of this gene. The maize sequences were inserted in an *E. coli*/yeast shuttle vector (pEMBLyex2), under the yeast CYC1 gene promoter and under the control of the UAS-GAL enhancer. When a truncated gene had to be expressed, a modified form of pEMBLyex2 was employed, carrying an "ATG" starting codon in the vector insertion site.

The constructions to be tested (pyM6 = the whole gene; pyM6 T/H = the gene lacking the sequence coding for the signal peptide; pyM6P/H = the gene lacking the sequences coding for the signal peptide, the head region and the two first repeats) were used for yeast transformation. Yeast cells were grown in galactose medium and the cell content was then analyzed by SDS-PAGE following specific zein extraction; in each case the presence of zein was clearly detectable. Cells transformed with pyM6 and pyM6P/H were also analyzed by immuno-cytological detection experiments. Confirming previous results (I. Coraggio et al., EMBO J. 5, 1986 and Eur. J. Cell Biol. 47, 1988), in cells expressing the whole zein sequence, the protein was found localized mainly at the E.R. level, while, where a truncated sequence was expressed, the protein was localized in mitochondria, aggregated in protein body-like structures.

The microsequence analyses of the amino terminal regions of all the zein proteins extracted from the recombinant yeasts containing the entire genes reveal a regular processing in the maturation of the various signal peptides. Similar analyses of the polypeptides from the truncated genes showed that they were unprocessed. Other constructions, such as further shortened sequences or different lysine-inserted zeins, are under examination to complete and define the evidence here reported.

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Identification of new gibberellin sensitive dwarf mutants
--G. Todesco, E. Arreghini, D. Ceppi and G. Gavazzi

Even though extensive biochemical and genetic work particularly with maize, has contributed to elucidating the

sequence of events leading to GA1 (the only gibberellin active per se in the control of shoot elongation) many specific steps are still unresolved. Since the unraveling of the pathway was greatly facilitated by a series of GA mutants lacking either all or specific combinations of endogenous GA, we started a search for mutants with a dwarf phenotype. The aim is to identify new mutants with genetic lesions in steps of the gibberellin pathway different from those already described (Fig. 1).

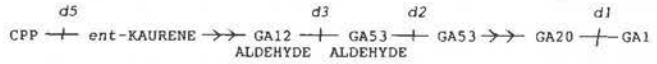


Figure 1. Genetic blocks in the pathway to GA1 identified in maize.

In maize five GA mutants have been identified, dwarf-1, dwarf-2, dwarf-3, dwarf-5 and anther ear-1, and for four of them the genetic block has been correlated to a specific metabolic step.

We induced seven mutants, all of them recessives, with a dwarf phenotype from the early seedling stage to maturity and resuming normal growth in response to exogenous GA (GA4 + GA7 10^{-5} M).

The segregation ratios obtained by selfing heterozygous plants together with the origin of each mutant and its provisional symbol are given in Table 1.

Table 1. Origin and segregation of recessive dwarf mutants.

Mutant symbol	Origin	Segregation +	Segregation dwarf	dwarf %
d*-2	EMS to seeds	188	55	22.6
d*-3	EMS to seeds	177	47	14.5
d*-4	Xrays to pollen	318	43	11.9
d*-6	EMS to pollen	305	54	15.0
d*-7	EMS to pollen	232	62	21.1
d*-8	Xrays to pollen	251	6	2.3
d*-9	spontaneous [A188xMu]	38	14	26.9

The seven mutants have been crossed with the known mutants *d1*, *d2*, *d3*, and *d5* to find out their allelic relationship to these four defined gene mutants.

The results of this allelism test, accomplished so far for five of the seven mutants isolated, are reported in Table 2. The test was performed by mating each homozygous *d** male mutant to at least three progeny plants of selfed *+/d* heterozygous parents or alternatively by pooling pollen of these progeny plants (at least three) to pollinate plants obtained by selfing *+/d* heterozygous parents. An average of 10 ears was tested for each specific *d/d** genetic combination by germinating a sample of 30 seeds and scoring for dwarf seedlings.

Table 2. Allelism test of five new GA mutants with *d1, d2, d3* and *d5*. The + and - symbols stand for lack of complementation and complementation respectively.

	d*-2	d*-3	d*-4	d*-6	d*-7
d1	-	+	+	+	+
d2	+	+	+	+	+
d3	+	+	+	+	-
d5	+	+	+	+	+

The test discloses allelism of *d*-2* with *d1* and *d*-7* with *d3* while *d*-3, d*-4* and *d*-6* show complementation with all four *d* mutants. These results suggest that *d*-3, d*-4* and *d*-6* represent mutational events affecting one or more genes not identifiable with the four *d* mutants. When crossed inter se, *d*-3* with *d*-6*, *d*-3* with *d*-4* and *d*-4* with *d*-6* all the pairwise combinations show complementation, a result expected if the three mutants belong to different genes (results not shown).

However, evidence of nonallelism based on complementation tests could be unreliable for at least two reasons:

(i) the reduced transmission of the *d** mutant vs. + as observed in selfing *+/d** heterozygotes (see Table 1) might account for the apparent complementation. However, this seems an unlikely explanation since an average test of 10 ears for each combination should be sufficient to recover the appropriate heterozygous combinations to test for allelism.

(ii) molecular evidence is available of situations where complementation takes place even among mutants belonging to the same gene.

More data will be obtained to confirm these preliminary results and to detect the role played by these new gene mutants in GA metabolism.

A cDNA clone of the maize α tubulin

--M. Mereghetti, G. Consonni and C. Tonelli

The α and β tubulins of most eukaryotes are encoded by small gene families (Cleveland and Sullivan Annu. Rev. Biochem. 54:331, 1985). A comparison of the amino acid sequences of the α and ß tubulins from numerous species suggests that the tubulins are extensively conserved in evolution.

In plants the α and β tubulin genes of *Arabidopsis* (Ludwig et al., Proc. Natl. Acad. Sci. 84:5833, 1987) and soybean (Guiltinan et al., Plant Mol. Biol. 10:171, 1987) have been cloned by cross-hybridization with *Chlamydomonas reinhardtii* tubulin probes (Silflow et al., Mol. Cell Biol. 5:2389, 1985) respectively.

In an attempt to clone the α tubulin of maize a cDNA library was established in lambda gt10 from poly(A)$^+$ RNA of scutellar nodes and mesocotyls of the W22 inbred line. This library was screened with α-1 tubulin gene of *Chlamydomonas* (Brunke et al., Nucl. Acid. Res. 10:1295, 1982; kindly provided by B. Burr) and a few positive recombinant phages were isolated. One of them containing a 1 Kb insert was further purified, subcloned in pBSKS (Stratagene), named pCTM5 and used in Southern and Northern analysis.

Genomic DNA of the W22 line was restricted with different enzymes, analyzed by Southern blot and hybridized to the 1 Kb cDNA probe using high stringency conditions. Between four and six bands of hybridization are observed depending on the type of restriction enzyme used. Further analysis is underway in order to estimate the number of α tubulin genes present in the maize genome.

To investigate the expression of the α tubulin genes different tissues have been analyzed. Total RNAs isolated from endosperms at 20 DAP, mesocotyls and scutellar

nodes, roots, coleoptiles, leaves, anthers, pollen and silks were analyzed by Northern blot and hybridized to the 1 Kb probe. A single band of approximately 1.5 Kb was observed in each sample. These results indicate that mRNAs homologous to our cDNA probe are present in all tissues analyzed and share the same size.

The genetic basis of *Sn* instability

--G. Gavazzi, M. Mereghetti, G. Consonni and C. Tonelli

We had previously shown that *Sn:bol3* differs from the other *Sn* accessions so far tested for its higher pigment potential in mesocotyl tissues and its instability. Instability relates to its frequent changes from an original condition, indicated as *Sn-s*, to *Sn-w*, where *-s* and *-w* stand for strong and weak and refer to the two levels of mesocotyl pigmentation. In heterozygous *Sn-bol3* genotypes (*R sn/r Sn*) weak derivatives are also recovered on the chromosome originally devoid of *Sn* as if the heterozygous association had promoted "contamination" of one chromosome (recipient) with *Sn* coming from the other (donor). Recombination between *R* and *Sn* can be ruled out since this event is not associated with outside marker recombination.

Of the *R* alleles tested in *R sn/r Sn* heterozygous genotypes, *R-r* appears the most efficient in eliciting "contamination" of the recipient chromosome. The effect of *R* constitution in trans on *Sn* instability is detected by germinating colored kernels derived from testcrossing the heterozygotes to a homozygous *r-g sn* line: the yield of nonparental seedlings with red mesocotyl in fact amounts to 50% if the *R* allele on the receiver chromosome is *R-r* and about 10% if it is *R-g*.

The levels of mesocotyl pigmentation ranging from a strong red to a very weak red are inherited and their transmission appears associated to *R* in its inheritance.

One way to demonstrate the trans effect induced by *R* in the heterozygotes and to prove its inheritance consists of planting colored kernels produced in the testcrosses of the heterozygotes, growing their plants, crossing them to *r-g sn* males and analyzing their progeny to see if those receiving the *R* marked chromosome acquired the potential for mesocotyl pigmentation. Results pertaining to this point using either *R-r* or *R-g* as a contrasting *R* allele in the recipient chromosome are given in Table 1.

Table 1. Mesocotyl pigment score of the progeny ears derived by growing colored kernels from the mating of *R sn /r Sn-s* females to *r-g sn* males. A sample of 50 seeds from each ear was germinated to ascertain the mesocotyl pigmentation conditioned by the *R* marked chromosome originally present in the parental *R sn/r Sn-s* genotype.

Parental genotype	n (1)	Mesocotyl pigmentation (2) S	I	W	NR(3)	% (4)
R-r sn / r Sn-s	51	16	4	9	22	56.8
R-g sn / r Sn-s	21	0	0	12	9	57.1

(1) number of progeny ears tested

(2) associated to the parental R-r chromosome originally present in the R sn/r Sn-s genotype

(3) S: strong red; I:intermediate red; W: weak red; NR: non red

(4) percent of *R* chromosomes leading to a more or less intense mesocotyl pigmentation, as inferred from germination tests of individual progeny ears

The results indicate that about 50% of the progeny plants from the heterozygotes receiving the *R* marked chromosome show the potential for mesocotyl pigmentation. However the level of pigmentation differs according to the *R* constitution of the recipient chromosome, being predominantly strong if the resident *R* allele is *R-r* and only weak if it is *R-g*.

To find out if the *Sn* derivatives isolated in the donor and recipient chromosome reflect identical or different genetic events we tested their response to *Pl*. Presence of *Pl* and *Sn* in the same genome in fact leads to constitutive pigment production in pericarp tissues while *Sn pl* genotypes are strictly light dependent for pigment accumulation in these tissues.

Starting material for this experiment was a sample of seeds obtained by pollinating a heterozygous *R-r sn/r Sn-s* female with a homozygous *r-g sn* male. Seeds were germinated and their seedlings classified for mesocotyl pigment as follows:

mesocotyl pigment score among: colored kernels				colorless kernels			
S	I	W	NR	S	I	W	NR
0	8	40	18	38	0	24	0

Seedling selections were grown in a greenhouse in winter 88 and the plants used as female parents in a cross with a homozygous *Pl* stock. At least 20 seeds from each resulting sib ear were grown in the field in summer 89 to ascertain their pericarp pigmentation. The results (Table 2) indicate that *Sn* derivatives can be grouped into two classes: (i) the parental *Sn-s* and its weak derivatives on the donor chromosome that maintain their capacity to interact with *Pl* unaltered and (ii) those recovered on the *R* marked homologue that lost their capacity to give a constitutive pericarp pigmentation in presence of *Pl*.

Table 2. Pericarp color in the progeny of different selections isolated from the mating *R-r sn/r Sn-s* x *r-g sn* and outcrossed to *Pl/Pl* stocks.

Selection Seed	Msc	Presumed Parental Chromos.	N° selections tested	n(1)	Pericarp red	nonred
Presumed parental chromosome constitution						
colored	interm.	R-r Sn-I	1	24	0	24
colored	weak	R-r Sn-w	1	20	0	20
colored	colorless	R-r sn	1	37	0	37
colorless	strong	r Sn-s	3	70	33	37
colorless	weak	r Sn-w	3	72	32	40

(1) number of sib plants obtained in the progeny of each selection outcrossed to *Pl* stock

This conclusion is in accord with another set of data obtained in the progeny from the cross *R sn/r Sn-s, Pl/Pl* X *r-g sn, pl*. The scoring of pericarp pigmentation in the progeny grown from cross (a) gave in fact the following results:

pericarp color in progeny ears from colored kernels		colorless kernels		n
red	nonred	red	nonred	
7	333	273	11	634

The results, consistent with the previous conclusion, allow one to map *Sn* in respect to *R* since the few nonparentals observed in the progeny of colored and colorless kernels are expected as a result of crossing over between *R* and *Sn*.

The recombination value so obtained, 2.88%, does not differ from the value obtained by the mesocotyl scoring in the progeny of testcrosses of *R-st sn/r Sn-s*, the only difference being that using *Pl* as a probe of the *Sn* constitution, recombination appears reciprocal while classification for *Sn* on the basis of mesocotyl score in testcrossed progeny leads to an apparently nonreciprocal recombination.

In conclusion it seems that the *Sn-w* derivatives arising in the progeny of the parental *Sn-s* allele represent changes of expression of the resident *Sn* allele while the *Sn* phenotype associated with *R* on the recipient chromosome as observed in the colored progeny of heterozygous *R sn/r Sn-s* plants is more likely interpretable as a change of expression of *R* rather than "contamination" or activation of a silent *Sn* residing on the recipient chromosome.

Thiocarbamate resistance and pollen selection

--M.Sari Gorla, E.Ottaviano and M.Villa

EPTC is a thiocarbamate herbicide (S-ethyl dipropylthiocarbamate) the main effect of which on plant metabolism is the inhibition of fatty acid biosynthesis. Since the chemical is not completely selective, it is used in association with antidotes serving to prevent injury to corn plants, while remaining active against weed species.

However, maize genotypes are characterized by different degrees of susceptibility; we analyzed about 40 inbred lines of different genetic origin with regard to their response to EPTC treatment. Seeds were soaked for 20 hours in EPTC solution (0.55 ml/l) and then planted in sand in pots covered with a plastic bag to prevent volatilization of the product. During seedling growth water was given without removing the bag, and after 12 days the effect of EPTC was evaluated: on the basis of plant injury, seedlings were scored from 1 (no damage) to 5 (completely deformed). A wide variability was observed among genotypes, ranging from complete susceptibility to resistance.

Two well-differentiated lines were chosen for the genetic analysis of the trait. By the procedure described above, P1, P2, F1, BC and F2 generation performances were evaluated; the data indicated that the character is controlled by few genes (2 or 3) and that resistance is due to recessive alleles. The observed segregations can be interpreted on the basis of the mechanism of EPTC metabolization in plants. The herbicidal compound is formed on metabolic sulphoxidation of EPTC by monoxygenase enzymes; the detoxification of EPTC-sulphoxide is mainly due to the formation of an EPTC-sulphoxide-glutathione conjugate, catalyzed by the glutathione S-transferase enzyme. The effect of corn-protective antidotes is in fact based on inducing high levels of glutathione and enhancing glutathione S-transferase activity; this process does not occur in weeds. Thus it is reasonable to think that EPTC resistance in maize can be the result of lack of sulphoxide production (inactivity of monoxygenase) or of a very efficient detoxification process (high levels of glutathione and of glutathione S-transferase). Work is in progress to characterize the lines with regard to the glutathione levels, and glutathione S-transferase and monoxygenase activity in the absence or presence of EPTC, antidote or both.

The effect of EPTC was also studied on pollen of the same lines: susceptibility or tolerance was evaluated on the basis of the germination percentage and pollen tube length on artificial medium supplemented with EPTC, in comparison with controls (germination and tube growth on standard medium). Since a high gametophytic-sporophytic correlation with regard to EPTC tolerance was observed, an experiment of gametophytic selection was carried out. The selection pressure was applied during the last phases of pollen maturation or during pollen function. Male inflorescences of F1 plants between susceptible and resistant genotypes were enclosed in a plexiglass chamber containing EPTC vapors for different times (from 3 to 24 h) during the pollen shedding period; the pollen produced was used to pollinate female plants of recessive genotype. Another group of female plants was treated in the same way after pollination, during pollen germination and the beginning of tube growth in the silks. The backcross progeny produced was evaluated for EPTC tolerance; seedlings of the BC progeny from treated pollen proved to be more tolerant than non-treated BC progeny. This response to selection, applied within plant, indicates that the observed gametophytic-sporophytic correlation for the trait is due to the expression of the same genes conferring herbicide resistance in both phases of the plant life cycle. It also indicates that the inclusion of pollen selection in breeding programs will serve to increase the effectiveness of selection methods.

ß-*glu1* null: a tissue specific mutation

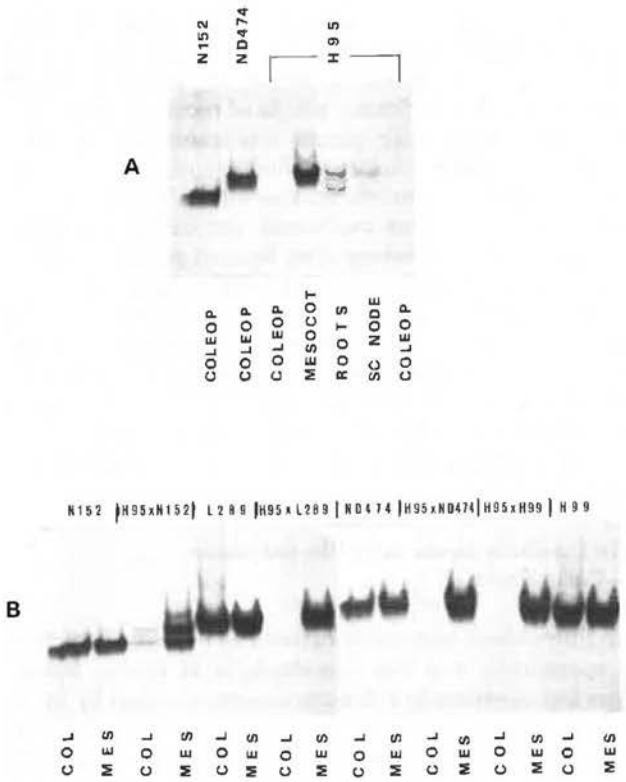
--Carla Frova

ß-glucosidase enzymatic activity is expressed in both the sporophyte and the gametophyte of maize. Sporophytic ß-glucosidase is a dimeric enzyme encoded by the ß-*glu1* gene, located on the long arm of chromosome 10 (Pryor, MNL 52:14, 1978). This locus is highly variable: 29 alleles, including a null, have been detected by electrophoretic analysis in a survey of several hundred genotypes (Stuber and Goodman, U.S. Agric. Res. Serv., 1982). On the contrary, in the male gametophyte, a single, invariant ß-GLU isozyme has been identified among numerous inbred lines, including *Glu1* nulls (Frova et al., MNL 56: 116, 1982; in Curr. Topics Biol. Med. Res., vol. 15, 1987; and unpublished), suggesting that two different ß-Glu genes are expressed in the haploid and diploid phases.

The genetical basis of the *Glu1* null phenotype is complex. GLU1 activity has been identified in hybrid *Glu1* null x *Glu1* plus seedlings. In some cases the appearance of two homodimers and the corresponding heterodimer has been reported (Goodman and Stuber, in Isozymes in Plant Genetics and Breeding, part B: 1, 1983; Rifaat and Esen, MNL 63:39, 1989). To explain these results Rifaat and Esen have proposed that the null phenotype is due to mutation of a

trans-acting regulatory element and that partial complementation occurs in the null x plus hybrids. The picture, however, seems to be more complex than that.

I have analyzed the ß-GLU zymograms produced by different seedling tissues (coleoptiles, mesocotyls, scutellar node and roots) of lines H95 (Null), ND474 (Slow), N152 (Fast), H99 and L289 (Intermediate) and their F1s. The accompanying Figure shows that: A) the null phenotype is present only in coleoptilar tissue of H95, while mesocotyls, roots and, to a less extent, scutellar node possess a clear ß-GLU activity, which identifies the line as ß-Glu SS; and: B) with the exception of H95 x N152, in all other hybrids the coleoptile presents a null phenotype. In all cases mesocotyls show the expected 1 (H95 x ß-Glu SS) or 3 (H95 x ß-Glu FF) band pattern.



The results suggest that the null phenotype in line H95 is: 1) tissue specific; and 2) that a trans acting regulatory element is indeed involved in its determination, but it appears to be a repressor rather than an activator, and behaves as dominant in null x plus F s. The reasons why in hybrid H95 x N152 coleoptiles ß-GLU activity, although weaker than normal, is expressed are at present unclear.

The data also show an additional, more anodal band in the roots of *Glu1* SS genotypes. Its absence in all *Glu1* FF inbreds analyzed (not shown) could be due to electrophoretic overlapping with the GLU1 FF homodimer, but further analyses are required to clarify this point.

Several questions remain unanswered, among which are: is the tissue specificity of the null phenotype peculiar to one inbred line (H95) or common to other *Glu1* nulls? Is the regulatory element linked to the *Glu1* locus and what is its precise mechanism of action? Is the additional ß-GLU found in roots the product of a different gene? All these points are currently under investigation.

ß-*glu1* expression during kernel development

--Carla Frova

In last year's MNL I reported on the expression of several enzymatic activities in different tissues of developing maize kernels, from 7 to 20 days after pollination (DAP). ß-GLU1 activity was detected in whole kernels and isolated scutella up to 14 DAP. Those results were obtained from the analysis of two inbred lines.

In order to identify the maternal and/or paternal origin of the ß-GLU activity, I have analyzed developing kernels (from 3 DAP to maturity) of several reciprocal F1s between inbred lines, characterized by the fast and slow variant of the enzyme. Had both paternally and maternally inherited genes been expressed, I would have expected to find the typical 3 band pattern, i.e. two homodimers and the corresponding heterodimer. In each case only the maternally determined isozyme was found instead, and the activity was strongest in the very early stages of development (3-7 DAP) and progressively declined thereafter. 3 weeks after pollination (21 DAP) and later, no ß-GLU activity was present either in whole kernels or in isolated tissues (scutellum, endosperm, aleurone and pericarp). These preliminary data suggest that ß-GLU activity in the developing kernel is not due to post zygotic expression of the *Glu1* genes, but rather inherited from the female parent.

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Glyphosate tolerance in cultured cells

--Milvia L. Racchi, Roberta Pelanda, G. Forlani[1], G. Musitelli[1] and E. Nielsen[1]

Glyphosate exerts its action blocking the shikimate pathway and EPSP synthase, the herbicide target enzyme, has been characterized and purified.

In monocots the amount of information on glyphosate effects and EPSP synthase properties is very limited. We have investigated the effects of glyphosate on growth and EPSP synthase activity in maize tissue culture. Cell cultures and calli of Black Mexican Sweet (BMS) do not show a significant reduction of growth in presence of increasing doses of glyphosate up to 10 mM (Fig.1).

On the contrary, seedling growth is severely inhibited by only 0.5 mM glyphosate. The tolerance observed in BMS cells is also showed by cells of other strains. In embryogenic cultures tolerance is confined to the cell proliferation stage while it is lost during the regeneration phase (Fig.2).

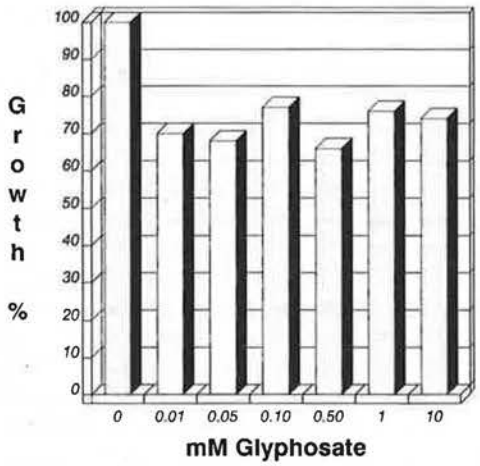

Figure 1. Glyphosate effect on BMS cell growth after 30 days of culture. The growth is expressed as % of the control.

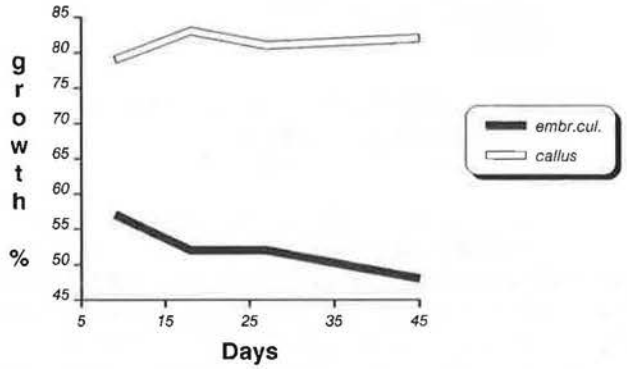

Figure 2. Glyphosate (0.2mM) effect on growth of callus and embryogenic cultures (A188xBMS) expressed as % of the control.

The glyphosate tolerance of maize cells is unusual since cells of other species are, in the same conditions, sensitive to the compound. The glyphosate inhibition kinetics of the target enzyme was investigated in plastid or cytoplasm-enriched extracts prepared from BMS cells. The results obtained reveal that the plastidial EPSP synthase activity is severely affected by micromolar concentrations of glyphosate, while the cytosolic enzyme maintains a remarkable activity at 10 mM glyphosate (Fig. 3).

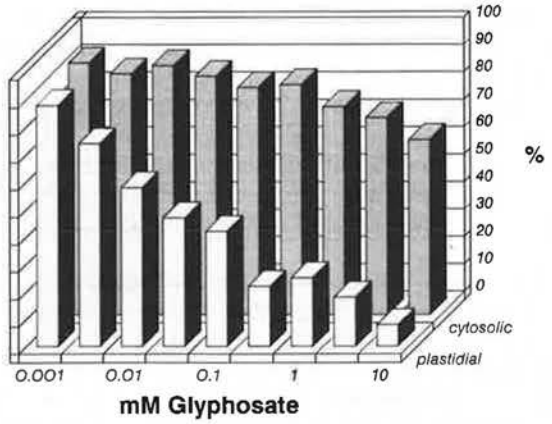

Figure 3. EPSP synthase activity in presence of different concentrations of glyphosate in plastid or cytoplasm enriched extracts prepared from BMS cells.

These results, confirmed in other maize cell lines, were not obtained in extracts prepared from cultured cells of carrot, where no tolerant EPSPs activity could be detected. The presence of two isoforms of EPSPs in monocots has been recently reported by Ream (1988) but no substantial differences in glyphosate sensitivity were described.

The existence in maize of a cytosolic glyphosate-resistant EPSPs form may allow cells to maintain a level of aromatic amino acids sufficient to sustain in vitro growth, thus explaining the tolerance to the herbicide.

Further evidence supporting the existence of two different EPSPs forms was recently obtained by means of the chromatographic separation of two EPSPs peaks from BMS cultured cell extracts. One of them is glyphosate-resistant and has a decreased affinity for the PEP substrate.

The presence and the expression level of the glyphosate insensitive EPSPs form need to be investigated at the plant level. In any case it could have significant implications relating to the study of the mechanism of glyphosate tolerance at cell level and during the differentiation processes and poses interesting questions concerning the physiological role of each EPSPs isoform.

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Isozyme patterns of inbred 346 somaclonal variants

--E. E. Khavkin, M. I. Orlova, Z. B. Shamina and V. G. Chernysheva

Regenerant plants (SC1) produced in callus culture from immature embryos of A654-derived inbred 346, as previously described (Chernysheva et al., Dokl. Akad. Nauk USSR 300:227, 1988), differed in several quantitative traits and manifested numerous morphological abnormalities eliminated in the SC2 generation. Two or three sibling SC2 plants were obtained from each of five SC1 plants with the following lineage: 1SC1 (3SC2 and 4SC2), 2SC1 (8SC2 and 10SC2), 3SC1 (1SC2 and 2SC2), 4SC1 (9SC2 and 11SC2) and 5SC1 (5SC2, 6SC2 and 7SC2). These SC2 varied from the initial plants in such traits as plant height, tassel length and the number of kernels per ear; particularly interesting was the significant diversity of sibs.

Several polyacrylamide and starch gel electrophoretic systems were employed to investigate acid phosphatase, alcohol dehydrogenase, esterase (E), glutamate-oxaloacetate-transaminase, isocitrate dehydrogenase (IDH), malate dehydrogenase, anodal and cathodal peroxidase (PRX) and 6-phosphogluconate dehydrogenase spectra in 3- to 5-day-old SC3 seedlings. While the seedlings of the control batch of inbred 346 exhibited uniform isozyme patterns, distinct alterations were found in two of ca. 20 analysed loci in at least seven of eleven investigated somaclonal variant seedlings

Two most characteristic additional bands were apparent in anodal esterase patterns of scutella (Fig. 1). Their mobility as related to E8 band was 0.99 and 0.89 in lithium

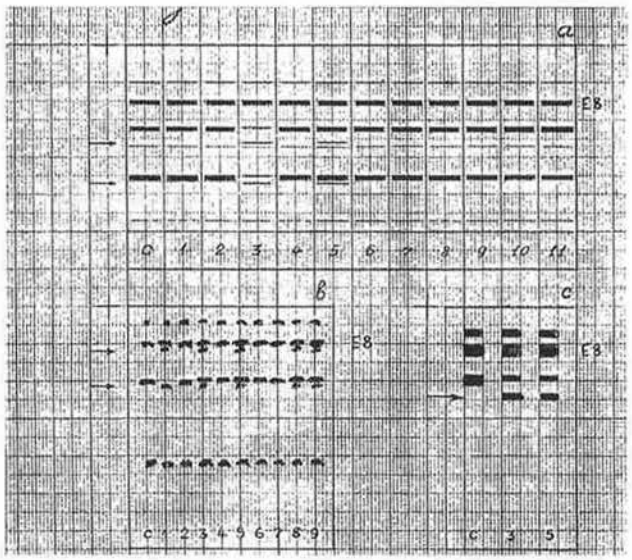

Figure 1. Esterase patterns of control (c) and somaclone (1-11) scutella as resolved by PAGE after Davis (a), in starch gel (b) and by neutral PAGE system (c).

borate//lithium borate - Tris-citrate starch gel system and 0.89 and 0.78 in PAGE after Davis. By the former data we may tentatively identify these bands with E5 electromorphs 190 and 340 as described by MacDonald and Brewbaker (Hawaii Agr. Exp. Sta. Tech. Bull., No. 89, 1975). It is noteworthy that the faster of these two bands (340?) was also present in the mesocotyl pattern both in the control and somaclones. In neutral PAGE system modified after Taber and Sherman (Ann. N. Y. Acad. Sci., 121:600, 1964) a single additional band with the relative mobility of 0.83 was found in scutella of somaclones. This band was also typical of mesocotyls.

The most prominent changes in anodal PRX pattern of SC3 mesocotyls were found in the zone with mobility of 0.2 relative to bromphenol blue marker (Fig. 2) which could be preliminarily assigned to PRX3 as described by Brewbaker and Johnson (MNL 46:29, 1972): in addition to a single band of the control pattern, one or two apparently new bands could be seen in this zone in three somaclones. Two more weakly stained electromorphs appeared: one in

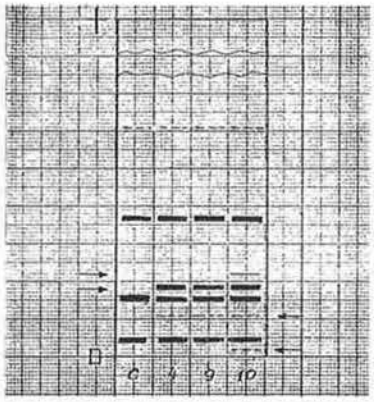

Figure 2. Peroxidase patterns of control (c) and somaclone (4, 9, 10) mesocotyls resolved by PAGE after Davis.

4, 9 and 10 SC3 mesocotyl spectra, while the second band with mobility of 0.02 (presumably PRX7) was found only in 10S C3.

Among several quantitative changes in isozyme patterns, variations of staining intensity of the ADH1 ADH2 band and the slowest IDH electromorph were particularly promising for further investigation.

Previous zymographic studies of somaclonal variation justified that single gene mutations of structural enzyme loci were exceptionally rare events (Brettell et al., Mol. Gen. Genet. 202:235, 1986). It seems more plausible to suggest that the changes in isozyme patterns reported here resulted rather from switching off some factors regulating organ-specific expression. The appearance of the mesocotyl-specific esterase band in the scutellar spectrum of several somaclonal variants as well as divergence of sibs both in isozyme patterns and morphological traits seem to support this suggestion.

NORMAL, ILLINOIS
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Recovery of B-A translocations from monosomic plants containing one B chromosome

--Gwen Shadley and David Weber

The *r-x1* deficiency is a submicroscopic deletion in maize that includes the *r* locus on chromosome ten. Because *r-x1* causes nondisjunction in the megagametophyte, many of the progeny of plants containing *r-x1* are monosomics and trisomics (Weber, 1973). *r-x1* also induces terminal deficiencies (Weber, 1983; Rhoades et al., 1986; Lin, 1987).

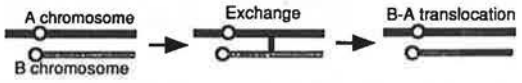
B chromosomes are accessory chromosomes of unknown origin which are present in certain maize lines. They have been shown to alter intergenic recombination, intragenic recombination, nondisjunction, and to interact with chromosome knobs (See Carlson, Annu. Rev. Genet. 16:5-23, 1978).

Because exchange has been found to take place between segments of nonhomologous A chromosomes which lack homologs in maize haploids (Weber and Alexander, 1973), we felt that it was possible that exchange might also occur between A chromosomes and B chromosomes in plants where both of these chromosome types were present as univalents. To determine if this could occur, we generated maize plants which were monosomic and contained one B chromosome; and we examined the progeny of these plants for the expected products of recombination between these nonhomologous chromosome types.

Inbred W22 plants with the *r-x1* deficiency were crossed by the inbred, Black Mexican, which contained B chromosomes. From this, we derived plants which contained one univalent A chromosome (a monosomic) and one univalent B chromosome as described below. If the nonhomologous univalent A chromosome paired with the nonhomologous univalent B chromosome in these plants and recombination occurred between the two nonhomolo-

gous univalent chromosomes as shown in Figure 1, a B-A translocation would be produced. If nondisjunction of the B-A chromosome then took place at the second microspore division, a mature pollen grain with one hypoploid sperm (which does not contain the B-A chromosome) and one hyperploid sperm (which contains two copies of the B-A chromosome) would be produced. If a pollen grain of this type fertilized a plant which was homozygous for a recessive endosperm marker which is located distal (between the breakpoint and the telomere on that chromosome arm) to the breakpoint on the A chromosome and the hypoploid sperm fertilized the polar nuclei, a kernel would be produced which would express the recessive phenotype of this marker in its endosperm. This kernel would also be hyperploid for this B-A translocation in its embryo (that is, it would contain the A-B chromosome and two copies of the B-A chromosome; therefore, it would contain the complete B-A translocation). Thus, several different events must each occur to produce B-A translocations by this mechanism.

Figure 1. Formation of a B-A translocation in a monosomic plant containing a B chromosome.



To determine if these events could occur, we generated *R/r-x1* plants which contained two B chromosomes. These plants were then crossed as female parents by Mangelsdorf's multiple chromosome tester (which is *bm2*; *lg*, *a*, *su*, *pr*, *y*, *gl*, *j*, *wx*, and *g* on chromosomes 1-10 respectively). Progeny of this cross expressing the *gl*, *j*, or *g* mutant phenotypes were identified as presumptive monosomics for chromosomes 7, 8, and 10 respectively, and plants expressing the distinctive phenotypes characteristic of plants monosomic for chromosomes 4 and 6 were also identified as presumptive monosomic-4 and -6 plants respectively. Nearly all of the progeny of this cross (including the presumptive monosomics) would also contain one B chromosome. Plants larger than typical of these monosomic types were discarded (which often contain deficiencies); thus, most of the presumptive monosomics would have been monosomics.

In order to determine if B-A translocations are produced by the mechanism indicated above, pollen from these monosomic plants containing one B chromosome was placed on silks of plants which contained a recessive marker mutant which is expressed in the endosperm as follows. Monosomic-4 plants were crossed to *c2* females, monosomic-6 to *su2* females, monosomic-7 to *o2* females, monosomic-8 to *pro* females, and monosomic-10 to *y9* and *o7* females. Ears produced from these crosses were screened for exceptional kernels which expressed the recessive mutant marker provided by the female tester parent. Each kernel which expressed the marker mutant phenotype was a potential isolate of a new B-A translocation with a breakpoint between the centromere and the mutant marker.

These exceptional kernels were then planted in a field nursery, and sporocyte samples were taken from the resultant plants for cytological analysis. Each of these plants was selfed and testcrossed onto plants which contained the original kernel marker to identify contaminants (which would be homozygous for the mutant marker).

They were also crossed as male parents onto plants carrying recessive seedling markers that mapped near or distal to the original kernel marker as follows: exceptional *c2* kernels produced by monosomic-4 plants were crossed to *gl3*, *gl4*, and *dp* females; exceptional *su2* kernels produced by monosomic-6 plants were crossed to *py* females; exceptional *o2* kernels produced by monosomic-7 plants were crossed to *v5* and *vp9* females; exceptional *pro* kernels produced by monosomic-8 plants were crossed to *v21* and *gl18* females, exceptional *y9* kernels produced by monosomic-10 plants were crossed to *oy*, and exceptional *o7* kernels produced by monosomic-10 plants were crossed to *l*, and *w2* females. Progeny of these crosses were planted in a sandbench and scored for the presence of the seedling markers. Expression of the seedling marker was taken as a positive indicator for the presence of a B-A translocation with a breakpoint in the A chromosome which is proximal (between the gene and the centromere) to the endosperm marker mutant.

Because the pachytene cytology was poor in these plants, the plants produced by the exceptional kernels were also crossed by KYS, an inbred with exceptionally favorable pachytene cytology. The isolates that showed unambiguous seedling segregation in the sandbench plantings were pursued more vigorously. The self of each isolate was crossed by KYS and sporocytes were collected from 5 to 15 of the resultant plants.

At this time, diakinesis in plants which germinated from the original exceptional kernels has been analyzed. Also all sporocytes from selfs of the isolate crossed by KYS from monosomic 2, 4, 6, 7, and 8 have been screened by observing cells in diakinesis for the presence of a ring or chain which is indicative of a translocation. Materials from monosomic 10 plants are about two thirds complete. Seven translocations have been identified at this time including B-A translocations from this material. Thus, B-A translocations are produced by the mechanism described above. It will be extremely interesting to determine if the breakpoints are randomly distributed or at specific positions on the chromosomes.

Much to our surprise, A-A translocations have also been identified in this material. Some of these A-A translocations possessed a univalent B chromosome and others did not. Weber (unpublished) has also recovered several A-A translocations in progeny of plants containing the *r-x1* deficiency. From these results, we can add the production of translocations to the list of effects known to be caused by the *r-x1* deficiency.

NORTHFIELD, MINNESOTA
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Centromeric breakage and the recovery of both telocentric fragments

--Katherine L. Rose and Rick W. Staub

We have recently isolated several spontaneously occurring mutants in a line of Black Mexican sweet corn containing high numbers of B chromosomes. One of these mutants (*r-BMSCB-3*) produced unexpected results in allele tests when crossed as a male onto *a1* and *c1* tester stocks. Many colorless, sectored, and mottled kernels were produced in highly variable numbers when different *r-BMSCB-3* plants were used as males. In some cases, the number of off-type kernels varied highly between crosses of a particular pollen parent to several tester females.

When root-tip chromosomes were examined to ascertain B chromosome number in test cross progeny, many plants were found with two telocentric fragments in addition to the B chromosomes which were present in most plants. One of these additional telocentric fragments is twice the length of the other and is hard to discern from a B chromosome in some mitotic spreads. However, in many cells the B chromosomes are more heterochromatic than either of the newly produced telocentric fragments, indicating that the new telocentrics are derived from an A chromosome (Figure 1).

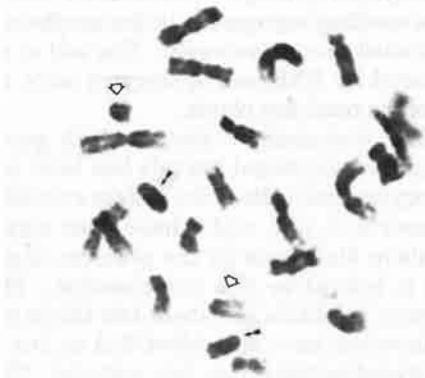

Figure 1. Mitotic chromosomes from plant containing two B chromosomes (small arrows) and both telocentric fragments (large arrows). The large telocentric fragment, though similar in length to a B chromosome, is clearly less heterochromatic and displays a separation of sister chromatids not seen in B chromosomes.

Karyotype analysis of plants with telocentric fragments indicated that the centromere breakage probably occurred in chromosome 3. Crosses to translocation stocks and marker gene stocks are being carried out to confirm this identification.

Pollen mother cells were examined from plants containing the two telocentric fragments and their normal homolog. Our observations indicate that each telocentric fragment pairs with one arm of the normal homolog and that subsequent prophase I centromere orientation generally yields one of two metaphase I trivalent configurations. A Type I configuration is produced when the centromeres of the two telocentric fragments align toward one pole and that of the normal homolog aligns toward the other pole. A V-shaped trivalent with three centromere spindle attachments is formed. This trivalent is consistently observed off the metaphase I plate (Figure 2). The two cen-

Figure 2. Type I trivalent configuration in metaphase I of meiosis in a plant containing both telocentric fragments and no B chromosomes. Both fragments are migrating to the same pole with opposite orientation of the normal homolog. The displacement of this trivalent from the metaphase I plate is common.

Figure 3. Type II trivalent configuration in metaphase I of meiosis from the same plant as in Figure 2. The two telocentric fragments are proceeding to opposite poles with the normal homolog stretched between them. Spindle attachment of the normal homolog is not apparent.

tromere spindle attachments of the telocentric fragments appear to provide for a stronger poleward force than does the single centromere spindle attachment of the normal homolog.

A Type II configuration is formed when the centromeres of the telocentric fragments align toward opposite poles. This alignment appears to preclude attachment of the normal homolog's centromere to the spindle and produces a rod-shaped trivalent in which the normal homolog is stretched between the telocentric fragments (Figure 3).

This breakage event could provide excellent material for the study of telocentric chromosomes and centromere structure and function as well as centromere orientation during meiosis. We recently created a stock that is homozygous for the broken chromosome with 2n = 22. It is still unclear what relationship B chromosomes may have had to the chromosome breakage event. Additionally, it is unclear how colorless, sectored, and mottled aleurones of progeny in test crosses are related to the telocentric fragments. Although it is easy to understand how these may be produced by instability of the large telocentric fragment in crosses to *a1* testers if the telocentric fragments are actually the arms of chromosome 3, it is more difficult to provide an explanation for these exceptional kernels in crosses to *c1* testers.

ORSAY, FRANCE
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Effect of gelling agent on callus initiation from immature embryos of inbred A188

--V. Tremellat, P. Vain and P. Flament

To obtain maize totipotent protoplasts, finely dispersed and embryogenic suspensions are to be used as starting material (Rhodes et al., Biotechnology 6:56-60, 1988). To establish such suspensions, friable and embryogenic calluses (referred to as type II calluses, as opposed to type I, which are embryogenic but compact) are needed. Unfortunately the ability to obtain type II callus is limited to a reduced number of genotypes (especially inbred A188) and remains difficult.

In order to enhance type II callus initiation, more favourable culture conditions have been researched. We have already shown that $AgNO_3$, when added to a modified Murashige and Skoog's medium, increases 4 to 6 fold the rate of type II callus initiation from inbred A188 (Vain et al., Plant Cell Tissue Organ Cult., in press). Here, the effect of three classical gelling agents on maize callus initiation rate is reported.

Fourteen-day-old embryos (1-2 mm long) were aseptically removed from self-pollinated kernels of inbred A188 and plated on Murashige and Skoog's modified medium complemented with 10 mg/l $AgNO_3$, with the embryo axis facing the medium. Cultivated embryos and/or calluses were subcultured every two weeks and callus production was scored at the fifth subculture. Results are given in the following table.

Gelling agent	Callus frequency	
	type I	type II
Gelrite (4g/l)	70/108	6/108
Agar (8g/l)	47/108	7/108
Agarose (6g/l)	70/108	27/108

Gelrite is from Siccap-Emmop (France), agar from Difco and agarose from Sigma (Type 1:low EEO); given concentrations are the most commonly used and give roughly the same compactness to the medium.

Both agarose and gelrite proved to be very well-suited for type I callus initiation (non-friable callus). For type II callus production, agarose gave the best results, showing a higher sensitivity of this type of callus to the impurities present in gelling agents. These results suggest that according to what is wanted and to the price of those components, using gelrite or agarose rather than agar may be worthwhile.

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The effect of C male sterile cytoplasm on morphological and agronomic traits of corn hybrids

--V. E. Miku and E. C. Partas

In order to determine the effect of C type of CMS on morphological and agronomic traits, 69 corn hybrids in C male sterile cytoplasm were compared with their normal counterparts.

Normal and C analogues of hybrids were examined during five years in one location in three-factor experiments including: density planting (2-3 densities each in 2-3

Table 1. The result of comparative study of corn hybrids in normal and male sterile cytoplasms.

Traits	Types of cytoplasm C	norm	LSD 005	Number of hybrids
Plant height,cm	221,3	224,7	1,8	36
Height to ear,cm	82,4	81,3	1,4	36
Length of: leaf,cm	77,7	77,7	0,6	36
tassel,cm	35,8	36,4	0,4	36
ear,cm	18,1	18,0	0,3	36
Width of leaf,cm	9,8	9,8	0,1	36
Number of: stalk knots	12,1	11,9	0,2	36
branches on tassel	12,2	12,0	0,3	36
kernel rows to cob	15,4	15,4	0,2	36
kernels per row	35,6	35,7	0,7	36
Grain moisture, %	26,7	26,9	0,4	48
Number of days from seedling to: silking	66,5	66,4	1,7	26
maturity	106,6	106,6	0,4	39
Grain yield, t/ha	7,3	7,2	0,05	69

replications, plot area 10 sq m); genotype (of 5-19 hybrids in each block); cytoplasm (C and normal).

Corn hybrids in C male sterile cytoplasm significantly differ from their normal counterparts only in plant height and tassel length (Table 1). There were not considerable differences between normal and C analogues, for all the rest of the morphological traits of the plant and ear. The C analogue hybrids have the same grain moisture when yielding, growing period, period from seedling to silking, but even more productive than normal one.

We may note that in drought years the yield of cytoplasmic male sterile hybrids was more (6.0%) than the yield of their normal counterparts, whereas in the favourable years differences were insignificant (1.5%). In addition it is necessary to observe, that grain yield of cytoplasmic male sterile hybrids depends upon their ability of pollen formation. In our investigation we concluded that in drought years, male sterile hybrids excel in grain yield (10.6%), while hybrids with restored fertility were only 3.8%. However, in favourable years differences in grain yield between male sterile and normal, as well as between restored and normal analogues were not so considerable.

Therefore we consider the utilization of the C male sterile cytoplasm acceptable in corn hybrid seed production.

PEORIA, ILLINOIS
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Identification of two C-zeins ("15" kDa or beta zeins) by RP-HPLC and SDS-PAGE.
--C. M. Wilson

C-zein is a high-methionine alcohol-soluble protein in corn endosperm protein bodies. Based on its mobility upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), it is also known as 15 kDa-zein, although its amino acid composition, derived from the sequence of a cDNA, indicates a molecular mass of 17.3 kDa (K. Pedersen et al, J. Biol. Chem. 261:6279, 1986). C-zeins from different inbreds differ slightly in relative SDS-PAGE mobilities (C. M. Wilson, Plant Physiol. 82:196, 1986). Paulis and Bietz (J. Cereal Sci. 4:205, 1986) found C-zein in the first protein peak eluted from a C_{18} reversed-phase high-performance liquid chromatography (RP-HPLC) column by an acetonitrile gradient.

When alcohol extracts were assayed by a modified RP-HPLC gradient, peak 1 from some inbreds eluted at 25.79 min, while that from other lines eluted an average of 0.37 min later. The early-eluting C-zein came from inbreds with the slow SDS-PAGE variant. C-zeins from two pairs of inbreds, B57-N28 and Oh43-W64A, and from their reciprocal F1 hybrid seeds were assayed by RP-HPLC. As expected, double peaks occurred in the F1 extracts, with the higher peak corresponding to that of the maternal parent. N28 and W64A have the slow SDS-PAGE C-zein band and the first HPLC peak, while B57 and Oh43 have the fast SDS-PAGE band and the second HPLC peak. Other inbreds with the slow SDS-PAGE variant include B37, BSSS-53, B84, L289, Oh45, Pa91, R802, Va35, and WF9; the fast variant also occurs in A619, B14A, B68, B73, M14, Mo17, R801, and W22.

HPLC is thus a convenient assay for identification of C-zein variants among inbreds and segregating populations. A manuscript is in preparation describing how the same HPLC system has identified additional variation among major A- and B-zeins, which were previously identified by IEF and SDS-PAGE.

A single nucleotide change in the DNA for C-zein could convert glutamine into a hydrophobic leucine residue. Such a substitution can increase SDS-PAGE mobility of a 20 kDa protein by 3% (W. W. deJong, Biochem. Biophys. Res. Commun. 82:532, 1978), probably because leucine binds more tightly to SDS. I suggest that such an amino acid replacement in C-zein might cause the modified molecule to move faster by SDS-PAGE, without changing molecular mass. Increased hydrophobicity of the modified C-zein would, however, cause it to bind more tightly to the RP-HPLC column and thus elute later.

PUSA, INDIA
Rajendra Agricultural University

Origin of Rabi (winter) maize in India
--V. K. Chaudhary and V. K. Shahi

Rabi (winter) cultivation of maize is a relatively new introduction to the state of Bihar in particular and the country as a whole. For the first time maize crop was grown in winter (starting from the 15th of October) on farmers' fields during 1961 following the release of high yielding hybrids. Its origin dates back to the period when high yielding hybrids bred in India were not becoming successful in the late 50's. Attempts were made in the state of Bihar during kharif (rainy) season (starting from June) in the year 1959 to grow hybrids like Texas-26, Texas-32 and Dixie-11 obtained from the United States of America (USA), knowing the fact that these varieties had given high yield in the past under Indian conditions in the farmer's field as well as on government farms. Seeds of double cross hybrids from USA were imported and also produced in the country from imported single cross hybrid seeds. This project was taken in collaboration with Dr. L. M. Humphrey, Agriculture adviser to the technical cooperation mission of USA. None of the hybrids from USA gave expected good yield and crops did not come up well during kharif season of 1959. On getting discouraging reports about poor performance of these hybrids from different parts of the state, the government of Bihar entrusted Dr. Rameshwar Singh, the then Maize and Millet Specialist, at Dholi-Pusa Centre, with the task of detail check-up of the crops grown during the season and directed him to submit a report indicating reasons for poor performance of the hybrids from USA. After investigation of field experiments grown at various places in the state it was found that besides other small managerial factors the crop had greatly suffered due to heavy rainfall which is of usual occurrence in this season. It was therefore decided by Dr.

Singh and his associates then to grow these hybrids, their single cross parents and the inbreds involved during Rabi season with the hope that they may do better in the milder winter condition of Bihar. Inbreds, single crosses and double crosses of the hybrids from the USA were grown in Rabi season at Dholi-Pusa campus which grew very well in winter condition and produced disease free vigorous crops. Crops grown from Texas-26 in a one acre area also gave a yield of 80 quintals per hectare. This was the turning point for revolutionizing maize cultivation in Bihar and other parts of the country. A mere glimpse of the possibility of getting higher yield from Rabi crop and also that the hybrids bred for Indian conditions were on the anvil, led to the start of a series of trials to find the best hybrid and agronomic practices for getting optimum yield from the crop. At the same time seed production and demonstrations on the farmers' fields were started. Multi-pronged attacks, without waiting for confirmative test of the possibility of growing maize in Rabi season, led to a maize revolution in this state (Singh, 1988). Total area under maize cultivation in Bihar during 1960-61 was nearly 8.5 lakh hectares grown only as kharif crop. At present out of a total area of nearly 8 lakh hectares, Rabi maize is being grown in an area of approximately 4 lakh hectares causing a shift of acreage from kharif to Rabi season. Rabi maize has the clear-cut comparative advantage of low incidence of diseases and insect pests, crops do not suffer on account of heavy rainfall, slow growth of weeds, etc. and hence, preferred by the farmers. These factors singly and in combination favoured the adoption of Rabi maize cultivation in Bihar. Later it caught the attention in other parts of the country like West Bengal, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Punjab, etc., where it is being grown successfully. Hence, Dholi-Pusa Centre, which is now the main campus of Rajendra Agricultural University, Bihar is the sheet of origin of Rabi maize cultivation which is fetching millions of rupees to the Indian farmers to boost their economy up. A concrete and significant effort indeed.

RALEIGH, NORTH CAROLINA
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Probes for the b-32 protein hybridize to loci on 7L and 8L

--Hank W. Bass, Paul H. Sisco, David L. Murray, and Rebecca S. Boston

The b-32 protein of maize endosperm is encoded by a small family of genes (Hartings et al., MNL 63:29, 1989). Expression of the b-32 genes has been reported to be affected by the zein regulatory mutations opaque-2 and opaque-6 (= *pro1*) (Di Fonzo et al., Mol. Gen. Genet. 212:481, 1988). We have used both cDNA and genomic clones encoding b-32 to probe genomic Southern blots of Ben Burr's recombinant inbred lines. Two loci hybridize to our b-32 probes, one on 8L between *BNLACT1* and *BNL2.369* , and the other on 7L between *BNL8.32* and *BNL7.61*. The cDNA probe, which corresponds to the major b-32 protein in kernels, shares greater identity with the locus on 8L than with the locus on 7L.

***Rf4* from A619 maps distal to *BNL13.05* on 8-S**

--Paul H. Sisco

Rf4 from the inbred A619 was shown to be a single dominant gene for restoration of cms-C by Kheyr-Pour et al. (Genetics 98:379-88, 1981) and was tentatively mapped to Chromosome 8 by A. Johnson using *wx* reciprocal translocation stocks (MNL 58:101-102, 1984). Using DNA extracted from backcross progeny from crosses using as male A619 (*Rf4/Rf4*) and as females cms-C conversions of W182BN, Oh51A, and NYD410 (*rf4/rf4*), we have found linkage between *Rf4* and two RFLP loci, *BNL13.05* and *BNL9.11* (Chi-Square = 47.8).

Rf4 - 13.5 cM - *BNL13.05* - 11.7cM - *BNL9.11*
(±3.6) (±5.6)

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Isolation and mapping of a cDNA probe for the b-70 protein

–Brian B. Shank, Hank W. Bass, Paul H. Sisco, Eleanora Wurtzel, and Rebecca S. Boston

An increase in the accumulation rate of the b-70 protein of maize endosperm has been correlated with the zein regulatory mutation floury-2 (Galante et al., Mol. Gen. Genet. 192:316-21, 1983). A cDNA clone encoding b-70 was isolated from a lambda gt11 cDNA library of endosperm mRNA (14DAP). This expression library yielded one clone encoding a protein that cross-reacted with b-70 antiserum and corresponded to an mRNA that increased in the *fl2* mutant.

We used this cDNA clone to probe genomic Southern blots from Ben Burr's recombinant inbred lines (Burr et al., Genetics 118:519-26, 1988). At low stringencies (Tm-35 C) several bands cross-hybridized with the probe, suggesting that the gene was a member of a multigene family. At higher stringencies (Tm-20 C) the probe mapped to a single locus near *BNL1.380* on Chromosome 5.

ST. PAUL, MINNESOTA
University of Minnesota

Partial concordance in inbred B37

--D.A. Muenchrath and R.L. Phillips

Concordance or nonconcordance of primordial germline cells in mature maize kernels is an especially important aspect of development that has implications in mutagenesis. With concordance, there is an equal probability

that both the male and female gametes produced by a plant derived from a mutagen-treated kernel would carry the same mutation; if gametes with the same mutation unite, the resulting zygote would be homozygous for the mutation. Concordance would permit the expression of recessive mutations in the M2 generation, the first selfed generation following mutagen treatment. Recessive mutations would not be apparent until the M3 generation if the germline cells are nonconcordant. Although maize is assumed to be nonconcordant, concordance may vary with the genotype, environment, or both.

Whole mature kernels of an inbred line, B37, were treated with 25 mM ethyl methanesulfonate (EMS) for 8 hours. The treated kernels were grown to maturity and self-pollinated. A sample of kernels from 200 of the resulting M2 ears was planted ear-to-row and scored for visible mutant phenotypes.

Approximately 11% of the 200 rows exhibited a mutant seedling or plant phenotype, with chlorophyll variants the most prevalent type. Twenty rows segregated 3:1, and one row segregated 1:1, normal to mutant phenotype, indicating primarily recessive inheritance.

Up to four ears were obtained upon self-pollinating plants in each of the 200 M2 rows. Among the resulting M3 ears, 10% segregated for mutant kernel phenotypes. The frequency of variant kernels on each of these ears was 25%, indicative of recessive inheritance.

Kernels were randomly selected from M3 ears produced in the M2 rows segregating for a mutant phenotype to obtain M3 plants. Ears from both normal and variant M2 plants were sampled and the kernels planted ear-to-row. Approximately half of the M3 rows did not show any mutant phenotype, suggesting the kernels sampled did not carry the mutation, or the variant phenotype was not heritable. Several variant phenotypes not observed in the M2 row were expressed in the M3 generation. Among the rows exhibiting a mutant phenotype, the segregation pattern was consistent with recessive inheritance.

Mutations expressed in M2 plants were expected to be dominant under the assumption of nonconcordance. Dominant mutations among EMS-treatment derived lines are known to be rare. The prevalent segregation ratio of 3 normal:1 mutant indicates these apparent mutants are likely to be recessives. The expression of recessive mutants in the M2 generation, together with the appearance of additional recessive mutants in the M3 generation, argue for partial concordance in the mature kernels of inbred B37.

Transient expression of foreign genes in endosperm tissue

--Roger E. Mitchell II and Irwin Rubenstein

We are developing a combination of techniques to allow high-quality expression studies on genes active during endosperm development.

The storage proteins that are contained in the protein bodies of endosperm tissue constitute approximately 60% of the protein in the mature kernel. Approximately 80% of these proteins are zeins encoded by 4 gene subfamilies; the remaining protein body proteins consist mainly of the zein associated proteins (ZAPs) encoded by a number of gene subfamilies (Rubenstein and Geraghty, Adv. in Cereal Sci. Tech. Vol VIII, Y. Pomeranz, ed., AACC, St. Paul, 1986). ZAP gene subfamilies contain few genes. This simplifies their molecular analysis and suggests that these genes contain strong promoter sequences. The ZAP3 subfamily of the maize inbred W22 consists of two genes (Z36A and Z36B) encoding proteins of about Mr 27,000. These genes have been isolated on a single cosmid clone and characterized in our lab (Geraghty, thesis, Univ. of MN, 1985). They constitute a tandem duplication of about 12 kilobases (kb) that is separated by 2.3 kb of unduplicated sequence. The genes are functional since cDNA clones have been isolated for both. The 5' sequences for these genes are virtually identical over a length of 3 kb.

Expression vectors have been constructed to study the biological activity of the untranslated 5' sequences of the ZAP3 genes. We have used site-directed mutagenesis to convert the base sequence of the ZAP3 gene Z36B initiation codon into the recognition sequence for the restriction endonuclease *Nco*I with no change being made in the gene's 5' untranslated region. The Z36B 5' region, with its presumed transcriptional control sequences, was then ligated to the protein coding regions of two reporter genes, chloramphenicol acetyl transferase (CAT) and beta glucuronidase (GUS). These reporter genes contain the transcriptional termination sequence from the nopaline-synthase (NOS) gene. The CAT gene was also modified to contain an initiator-codon-centered *Nco*I site; a GUS construct with an *Nco*I site was already available. The use of *Nco*I sites in this way allows for the construction of vectors retaining the exact relationship of the Z36B 5' sequences to their native initiation codon.

These vectors were delivered directly into developing endosperm by the use of DNA-coated gold particles accelerated by the high-voltage-induced explosion of a water droplet (McCabe et al.,Bio\Technology 6:923, 1988). Field or greenhouse grown plants of W22 were pollinated, and the entire cob harvested 12 to 20 days after pollination. The cobs were husked and surface sterilized, and endosperm target explants were prepared by excising cob sections containing 4 rows of 6 kernels each. The developing endosperm tissues were made accessible to particle bombardment by slicing off the top 1 mm of each kernel. The cob tissue was then trimmed to allow the explant to lie flat on the bottom of a small petri plate, and held in place with agar solidified nutrient medium.

Our work with the CAT reporter gene has utilized both the Z36B 5' sequence-CAT gene fusions described above and previously available constructs in which the CAT gene is fused to the strong, but non-tissue-specific, cauliflower mosaic virus (CaMV) promoter. Two days after bombardment the upper 2 mm of endosperm tissue was extracted and assayed with a standard CAT assay utilizing a radioactive substrate (^{14}C-chloramphenicol). CAT constructs containing the CaMV 35S promoter yielded detectable enzyme activity; those utilizing the Z36B 5' sequences gave a signal at least ten times as strong. We interpret these results as a good initial indication that these

fusions are being expressed in a manner related to their normal tissue specific expression, despite the alien mechanism by which they were introduced.

Preliminary experiments with the GUS reporter gene have utilized the vector pBI221, which contains the GUS gene fused to a CaMV 35S promoter. Endosperm tissue was bombarded with particles coated with this DNA and subsequently exposed to staining with a histological substrate (x-GUS). An insoluble blue product accumulated which allowed the detection of cells in which GUS gene expression occurred. The blue product was seen both in cells of the aleurone layer of the endosperm as well as in cells of the outer regions of the endosperm. The only non-endosperm tissue exposed in this particular experiment, the developing pericarp, did not show GUS expression, perhaps because the strength of its cell walls prevented efficient particle penetration. Expression was also not detected in the inner regions of the endosperm tissue. We do not yet know whether this was due to some unsuitability of this tissue for the CaMV 35S promoter, or whether the thin-walled cells in this area are too weak to withstand bombardment. Each tissue type shows maximal expression of foreign DNA over a narrow range of particle velocities (controlled in this case by adjusting the voltage used to explode the water drop). At too low a voltage, little or no expression is seen, presumably because the tissue's cell walls are not penetrated by the particles. Higher voltages fail to increase (and may even decrease) expression, perhaps because cell damage by the particles offsets any additional penetration.

The GUS activity of endosperm tissue bombarded with pBI221 DNA was also quantified by mixing an extract prepared in the same way as for the CAT assay with the substrate analog 4-methylumbelliferyl glucuronide, and measuring the product on a fluorometer. We expect to use our Z36B 5'-GUS gene constructs to increase the signal of the fluorometric assay, as well as to test for the tissue-specific expression of these vectors via our histological assay -- the x-GUS product should be seen in the cells of the storage endosperm, but not in the aleurone or other kernel tissue cells.

Simplified cloning techniques utilizing kanamycin resistant plasmids

--Roger E. Mitchell II, John Hunsperger, and Irwin Rubenstein

We have developed methods and vectors which utilize differential antibiotic resistance to manipulate DNA sequences. The beta-lactamase (ampicillin resistance gene, *Amp*^{r}) was removed from the pUC118 and pUC119 plasmids and replaced with the aminoglycoside 3'-phosphotransferase (kanamycin resistance, *Kan*^{r}) gene. Site-directed mutagenesis was used to eliminate the *Hin*dIII site present within the *Kan*^{r} gene, without changing its amino acid sequence. The resulting plasmids are termed pXC118 and pXC119, according to whether they were derived from pUC118 or pUC119. These pXC (eXChange) plasmids, pXC118 and pXC119, retain all of the standard features of their parent plasmids, including poly-cloning sequences containing several unique restriction sites, an M13 viral origin of replication allowing the synthesis of single-stranded DNA, a high-copy-number plasmid origin of replication which does not require chloramphenicol amplification to make double stranded DNA, and a unique *Eco* O109I restriction site in the vector backbone. As with the pUC vectors, the poly-cloning site is situated within the lacZ gene, with the orientation of the cloning site in the 118 form being opposite to that of the 119 form. Despite these similarities, however, the pXC plasmids are readily distinguishable from the pUC plasmids as a result of their different antibiotic resistance genes they encode and the different unique restriction sites which the resistance genes contain. In the case of the *Kan*^{r} gene of the pXC plasmids, the most useful distinguishing site remaining after mutagenesis is that of *Xho* I.

These plasmids have been used to develop a method to subclone DNA by sequence exchange. In the standard subcloning procedure, the recipient plasmid is cleaved with one or two restriction enzymes and the DNA fragments dephosphorylated to prevent direct ligation of the backbone or re-insertion of any fragments which may have been released. The donor plasmid is digested with the same enzymes, and the DNA fragment which is to be transferred is purified on an agarose gel, to prevent its re-insertion into the donor backbone upon ligation. This purification step is time consuming and can be difficult if the DNA fragment is small or if the donor plasmid is only available in small quantities. The pXC plasmids overcome these problems. By using a pXC plasmid as a recipient vector, the digested, dephosphorated recipient pXC DNA can be mixed directly with unpurified, digested donor pUC plasmid DNA and ligated. After the resultant constructs are transformed into *E. coli*, clones are selected on media containing kanamycin. Any construct not containing the recipient (pXC) backbone will fail to grow. It is, of course, possible for the donor (pUC) backbone to be inserted into the recipient rather than the intended fragment, but such clones occur infrequently and can be easily detected by virtue of their resistance to ampicillin. All of our sequence exchanges using these new plasmids have been successful. Exchanged fragment sizes have ranged from 51 up to 3000 base pairs in size. Furthermore, some fragments have been exchanged that were only available in such small quantities that they were barely visible on an agarose gel. Even in cases where the donor construct was available in abundance and the fragment to be transferred was large, this approach saves hours, and sometimes a full day. Of course, the roles of the pXC and pUC plasmids as recipient and donor can be reversed, and this has allowed us to make sequential transfers, simply by alternating their use.

The structure and expression of a maize ubiquitin fusion gene

--Keqin Chen, David A. Somers, and Irwin Rubenstein

Ubiquitin fusion genes encode a hybrid protein in which ubiquitin is fused at its carboxyl terminus to a tail sequence of either about 50 or 80 amino acid residues.

Similar ubiquitin fusion proteins have been found in yeast, humans, and plants. The amino acid sequence of the tail region is highly conserved among all eukaryotes investigated. The tail sequence is unrelated to the ubiquitin sequence and contains a putative metal-binding, nucleic acid-binding domain (Cys-X_{2-4}-Cys-X_{2-15}-Cys-X_{2-4}-Cys). Recent research indicates that these short tail polypeptides are located in ribosomes and may play a role in ribosome biogenesis. We have isolated a maize ubiquitin fusion gene and determined its structure. We have also studied the expression of this fusion gene in maize tissues.

An ubiquitin fusion gene clone was identified by screening a lambda maize W22 genomic library with a short oligonucleotide probe derived from the sequence for a yeast ubiquitin fusion gene. A 5 kb *Bam*HI fragment containing the ubiquitin fusion gene sequence was subcloned into the pUC119 vector. The sequence of the gene has been determined; it contains no introns. The fusion gene consists of a ubiquitin monomer sequence (228 bp long) and an extension sequence (234 bp long). The deduced amino acid sequence of the ubiquitin portion is identical to the sequence of the maize ubiquitins we have previously studied. The tail extension sequence consists of 78 residues and is highly homologous to the sequence found in yeast, human, and *Arabidopsis* (Fig. 1).

(76 maize ubiquitin amino acid residues)-A-K-K-R-K-K-K-E-Y-T-K-P-K-K-I-K-H-K-H-K-K-V-K-L-A-V-L-Q-F-Y-K-V-D-D-A-T-G-K-V-P-A-S-A-R-C-P-N-T-E-C-G-A-G-V-F-M-A-N-H-F-D-R-H-Y-C-G-K-C-G-L-T-Y-V-Y-N-Q-K-A

Figure 1. Deduced amino acid sequence of a maize ubiquitin fusion protein using the standard one-letter code to denote the amino acid residues: A = Alanine, R = Arginine, N = Asparagine, D = Aspartic Acid, C = Cysteine, Q = Glutamine, E = Glutamic Acid, G = Glycine, H = Histidine, I = Isoleucine, L = Leucine, K = Lysine, M = Methionine, F = Phenylalanine, P = Proline, S = Serine, T = Threonine, W = Tryptophane. Y = Tyrosine, and V = Valine.

The gene is transcribed as a 900-nucleotide transcript. Northern blots indicate that the gene is expressed at various levels in different maize tissues. For example, it is expressed at high levels in endosperm tissue (after pollination) and in the coleoptile tissue of young seedlings. The expression of the fusion gene appears to be regulated during endosperm development. Genomic Southern blots of maize inbred W22 DNA demonstrate that fusion gene sequences are present in more than one copy in the maize genome.

The promotor region of the fusion gene has been characterized by sequence analysis and S1 mapping of the transcription initiation site. An 800 nucleotide fragment of the promotor sequence has been fused to a GUS reporter gene to study the regulation of the fusion gene's expression. This DNA was delivered directly into Black Mexican suspension culture cells by means of DNA-coated gold particles accelerated by the high-voltage-induced explosion of a water droplet (McCabe et al., Bio\Technology 6:923, 1988). The GUS gene under the control of the fusion gene promotor has expressed.

Cloning of cDNA for dihydrodipicolinate synthase

--David A. Frisch, Andrew M. Tommey, Burle G. Gengenbach and David A. Somers

Dihydrodipicolinate synthase (DHPS) is the branch point enzyme leading to lysine biosynthesis in plants and is a key site for feedback regulation by lysine. Purified maize DHPS is inhibited 50% by 23 uM lysine (MNL 63:106, 1989) and in conjunction with aspartate kinase, imposes a strict limit on the cellular concentration of lysine. As one of the initial steps in establishing a basis for genetic manipulation of lysine biosynthesis in maize, we have isolated a maize cDNA clone for the DHPS enzyme.

We chose to try direct genetic complementation of the *Escherichia coli dapA*$^-$ auxotroph lacking DHPS enzyme activity. The procedure was similar to that used for direct genetic selection of maize glutamine synthetase cDNA (Snustad et al. Genetics 120:1111, 1988). *E. coli dapA*$^-$ cells require diaminopimelate, a lysine precursor, for growth. These auxotrophic cells were transformed by electroporation with size fractionated Black Mexican Sweet cDNA in pUC13. Selection for transformed, complemented cells on minimal medium containing ampicillin resulted in identification of five colonies. Plasmid DNA isolated from each colony yielded complementation frequencies comparable to the transformation frequency. DHPS activity in the five complemented cell lines (Table 1) was inhibited 86-90% by 100 uM lysine as expected of maize DHPS, but DHPS activity in nontransformed wildtype *dapA*$^+$ cells was inhibited less than 10%. No DHPS activity was detected in *dapA*$^-$ cells.

Table 1. DHPS activity in crude lysates of transformed *Escherichia coli* strains.

	Specific activity		Inhibition (%)
Cell line	Control	100 uM lysine	
Wildtype *dapA*$^+$	130	120	6
Auxotroph *dapA*$^-$	ND	ND	-
Transformant 1	230	24	89
Transformant 2	160	15	90
Transformant 3	190	22	88
Transformant 4	300	42	86
Transformant 5	260	27	89

ND = not detected. Specific activity values (0.001 OD/min/mg protein) have been multiplied by 10^6.

The cDNA insert was isolated as an *Eco*RI fragment from one of the cell lines and both strands were sequenced. The start of the coding region for maize DHPS was established by comparing the amino-terminus amino acid sequence of gel-purified DHPS monomer with the amino acid sequence derived from the nucleotide sequence. The apoprotein for DHPS is expected to have an amino-terminus transit sequence for transport into the plastid, but the purified mature protein is not expected to have the transit sequence. The amino-terminus sequence of Ala-Ile-Thr-Leu-Asp-Asp-Tyr-Leu of the purified protein was identified in the cDNA sequence as indicated in Figure 1. The nearest in-frame 5' ATG was located 162 bp upstream

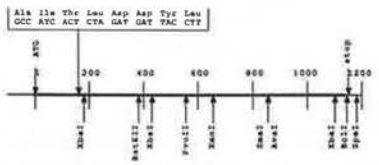

Figure 1. Representation of maize DHPS cDNA indicating the amino acid sequence of the mature protein and the corresponding cDNA sequence.

(designated 1 in Fig. 1) indicating that the transit peptide consisted of 54 amino acids. The complete DHPS coding sequence for the apoprotein was 1140 bp. The predicted molecular weight of the mature protein was 35,854, which was close to the 38,000 Mr estimate obtained from SDS-PAGE.

The cDNA clone hybridized to a single transcript of about 1400 nucleotides in northern blots of immature embryo and endosperm total RNA. A genomic clone containing at least part of the coding region has been identified in an EMBL-3 library of W22.

Endosperm amyloplast membrane proteins and their antibodies

--Jaakko Kangasjarvi, Dennis Mathews and Burle Gengenbach

Leaf chloroplasts and endosperm amyloplasts have distinctly different functions and are also expected to have differences in protein composition. We compared two methods for purification of amyloplast membrane proteins from early stages of developing endosperm. In one method (Gardner et al., Physiol. Plant. 69:541), the total microsomal fraction of an endosperm homogenate was isolated first. Then plastid membrane vesicles were concentrated at the 20/25% and 25/30% interfaces of a 20%, 25%, 30%, 36% and 44% sucrose step gradient. We also isolated intact amyloplasts according to the method of Echeverria et al. (Plant Physiol. 77:513, 1985; Plant Physiol. 86:786, 1988) and then lysed them and purified the membrane vesicles on a sucrose step gradient. Proteins extracted from membranes isolated by the two procedures were compared by SDS-PAGE and only a few differences in polypeptide patterns were observed (Figure 1).

Because we obtained low yields of amyloplasts and the amyloplast isolation procedure requires that amyloplasts contain starch grains (13 to 14 dap and older), we decided to use plastid membranes isolated from the microsomal fraction for the production of polyspecific polyclonal antibodies. Membranes for antibody production were isolated from 50 to 100 g of A188 and A619 x W64A endosperm 15 to 17 dap. Two chickens were injected with envelope membrane vesicles of A188 (800 ug of protein total/chicken) and two with vesicles of A619 x W64A amyloplast membranes (1 mg of protein total/chicken). Membrane vesicles were emulsified with complete Freunds adjuvant for the first injection and with incomplete Freunds for three boost injections. Antibodies were isolated from eggs after the fourth injection.

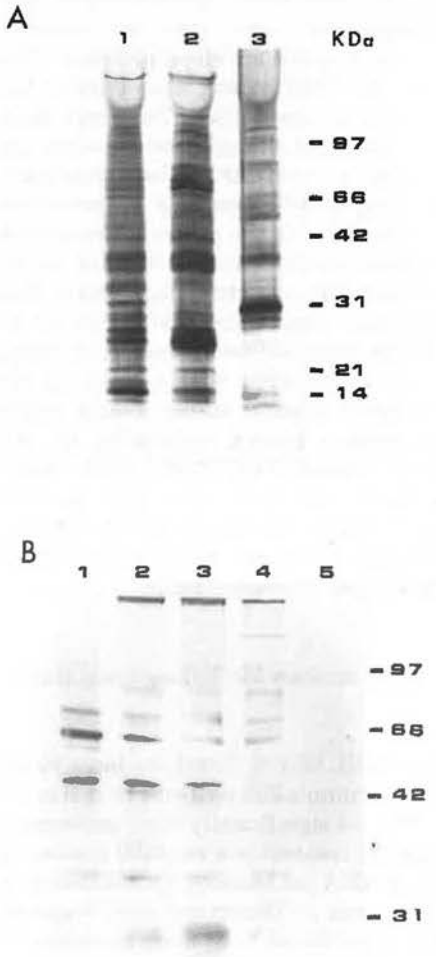

Figure 1. (A) Plastid envelope membrane proteins separated by 10-15% gradient SDS-PAGE. Envelope proteins from A188 12 dap endosperm microsomal fraction (Lane 1), A188 15 dap isolated amyloplasts (Lane 2), and leaf chloroplasts (Lane 3). Lane 1 has 67% of the total protein run in Lanes 2 and 3. (B) Endosperm plastid envelope membrane polyspecific antibodies reacted with envelope proteins separated by 10% SDS-PAGE. Envelope proteins from microsomal fraction of A188 8 dap (Lane 1) and 12 dap (Lane 2), and A619 X W64A 16 dap (Lane 4), from isolated amyloplasts of A188 15 dap (Lane 3), and from A188 leaf chloroplasts (Lane 5). An equal amount of protein was run in each lane.

Endosperms were collected and membranes isolated from 12 to 25 g of A188 (white endosperm) 8, 9, 10, 11, 12 and 16 dap and from an early season PAG hybrid (yellow endosperm) 8, 10, 12, 14 and 16 dap. For comparison, chloroplast envelope membrane proteins were isolated from 7 to 10-day-old maize seedlings using the method of Keegstra and Yousif (Meth. Enzymol. 118:316, 1986). Intact chloroplasts were isolated, purified on a Percoll gradient, lysed osmotically and the thylakoid membranes removed with centrifugation and the envelope vesicles purified on a sucrose step gradient. The protein fractions were analyzed on 10 to 15% and 8 to 25% acrylamide gradient SDS-PAGE.

Compared to chloroplast envelope membrane proteins, amyloplast envelope membrane protein profiles were quite different with only a few major bands with similar mobility detected (Fig. 1). The similarity of the patterns for envelope membranes isolated from the microsomal

fraction and from isolated intact amyloplasts indicates that the amyloplast envelope membranes were not contaminated with many non-amyloplast envelope proteins. The patterns for A188 and the PAG hybrid were similar but some major proteins were different sizes. The amyloplast membrane protein composition changed during early endosperm development but was constant in both genotypes after day 12. Three polyspecific antibody preparations were tested and found to differ in the proteins recognized. Compared to amyloplast proteins, the antibodies recognized only a few chloroplast proteins, suggesting that amyloplasts are a distinct plastid form with respect to envelope proteins. When immunofluorescence techniques were used with fresh endosperm tissue, most of the fluorescence was observed around starch grains which indicated that antibodies bound primarily to the amyloplast envelope membrane, but some weak fluorescence also was observed from the plasma membrane. These polyspecific antibodies are being used to isolate cDNA clones from an endosperm cDNA expression library for further characterization.

Plastid ORF170

--Jaakko Kangasjarvi, Andrew McCullough and Burle Gengenbach

In previous studies (MNL 63:105, 1989), we have found that plastid transcript accumulation patterns in maize endosperm total RNA differed significantly from patterns in leaf total RNA. Of specific interest is a ca. 2400 nucleotide transcript identified on RNA gel blots by hybridization to *Bam*HI restriction fragment 14 (Rodermel and Bogorad, MNL 63:155, 1989) that we found to be more abundant in endosperm than in leaf total RNA. Other transcripts homologous to *Bam*HI 14 were less abundant in endosperm. The 2400 nucleotide transcript accumulated progressively during endosperm development and became significantly more abundant between 8 and 12 DAP similar to the pattern for accumulation of spliced and unspliced transcripts for the *rps12* gene (MNL 63:105, 1989).

No genes are known to be located on the *Bam*HI 14 region in maize, but tobacco, *Marchantia*, rice and cauliflower have highly homologous open reading frames (167, 168, 170, 169 amino acids, respectively) in the corresponding region. Tobacco, rice and cauliflower ORFs have three exons (120 to 250 bp) separated by two type II introns (both about 750 bp). In *Marchantia* one of the introns is deleted. In tobacco and rice, the DNA homology extends into the introns and both introns contain an in-frame stop codon for exons I and II. Both exons I and II also begin with ATG. No function is known or has been proposed for the hypothetical protein.

To determine whether maize *Bam*HI 14 fragment also has an ORF with similar structure, two oligonucleotide primers were designed based on the rice sequences from regions that were most homologous with tobacco and *Marchantia* DNA sequences from the 5' (exon I) and 3' (exon III) regions of the coding sequence. The primers directed DNA synthesis towards each other on opposite strands. RNA was isolated from maize and rice leaves and roots and 16 DAP maize endosperm. cDNA was synthesized using the 3' oligonucleotide as the primer. Then the cDNA population was amplified by polymerase chain reaction (PCR) procedures using both primers. The products were separated on an agarose gel. Three products of about 1.9, 1.2 and 0.47 kb were obtained, which corresponded in size to that expected for the unspliced transcript, to transcripts with one of the two introns spliced, and to the mature message, respectively. The 0.47 kb product from the mature transcript was detected by PCR in all tissues tested and was the only transcript detected in leaves. The unspliced 1.9 kb product was the most abundant PCR product in endosperm. In the control experiment where leaf DNA was used for the PCR template, only the 1.9 kb fragment was amplified as expected for the intact unspliced sequence. The *Bam*HI 14 fragment hybridized to all three amplified products indicating their homology with maize plastid DNA. The amplified 0.47 kb product hybridized to a 2400 nucleotide transcript on gel blots of endosperm RNA.

Sequence data banks were searched for homology to the ORF170 sequence. No homology at the amino acid sequence level was identified when each exon from rice was used separately. When shorter 100% conserved amino acid sequences between tobacco, rice and *Marchantia* were used, however, we found homology to several proteins. The most common feature of these proteins was a function relating to some type of recognition or interaction with DNA. This possibility is being tested with the cloned sequence.

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Recombination event in cms-T regenerated plants during reversion to fertility

--Christiane M.-R. Fauron and Marie Havlik

The entire sequence complexity of the mitochondrial genome of the maize cytoplasmic male sterile cms-T is contained in a circular molecule also called master chromosome of 540 kb (Fauron et al., MGG 216:395, 1989). The master circle containing the entire sequence complexity is part of the multipartite structure arising via recombination at repeated sequences. In 1986 Dewey et al. (Cell 44:439) identified a rearranged sequence unique to the cms-T mitochondrial genome containing a 345 bp open reading frame (*T-urf13*) encoding a 13 kd polypeptide associated with the male sterile phenotype (Dewey et al., PNAS 84:5374, 1987). This sequence is located in cms-T on a 6.6 kb *Xho* I fragment that is absent in the revertant progenies obtained through tissue culture with the exception of two mutants, T4 (Umbeck and Gengenbach, Crop Sci. 23:584, 1983) and V18 (Brettell et al., MNL 56:13, 1982). This locus is located near a 4.6 kb repeat not found in the normal genome. It is either deleted (Fauron et al., Curr. Genet. 11:339, 1987; Rottman et al., EMBO J. 6:1541, 1987), or truncated (Wise et al., PNAS 84:2858, 1987) in the male fertile regenerated plants. We have shown that the dele-

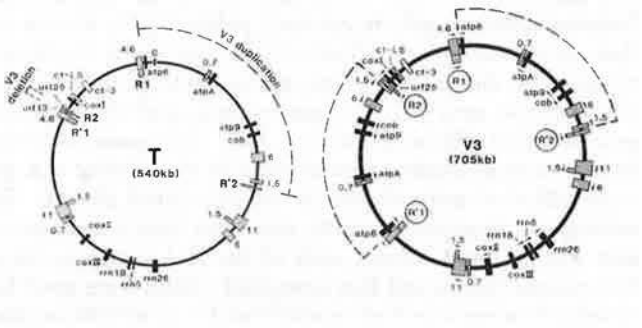

Figure 1. Comparison of the diagrams of the master chromosomes of cms-T and V3 mitochondrial genomes with the location of the repeated sequences and the rRNA and protein coding genes. Located on the cms-T circle are the 165 kb region found duplicated in the V3 genome and the 0.423 kb region deleted in the V3 genome. The duplicated region is also marked on the V3 circle.

tion of the *urf13* locus in the revertant V3 is the result of a recombination event involving two sets of repeats (Fauron et al., Genetics 123, 1990). As shown on Figure 1, associated with the 0.423 kb deletion encompassing the *T-urf13* gene is a duplication of 165 kb. The study of various independently isolated revertants has shown that different sets of repeated sequences might be involved in the recombinational events responsible for the mt genome reorganization (Fauron et al., TAG in press, 1990).

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University of Missouri and USDA/ARS

B-A translocations can be used as a tool to study quantitative genetic traits

--Ming-Tang Chang, E. H. Coe, Jr. and J. B. Beckett

In the past five years, sixteen B-A translocations (except 2S, 2L, 7S and 8S) were converted into four elite lines (A619, A632, B73 and Mo17). Morphologically, these elite background B-A translocations and their elite lines are identical. Genetic purity and degree of homozygosity of these translocations were tested by 10 isozyme loci (*Acp1*, *Acp4*, *Adh1*, *Mdh1*, *Mdh2*, *Pgd1*, *Pgd2*, *Phi*, *Pgm*, and *Idh2*), which represent five different chromosomes. Results showed that all sampled ears had 100% purity (no contaminants) and 100% homozygosity, except two ears showed allelic heterozygosity in one or two isozyme loci. Seeds from homozygous ears were used as materials for this study.

The 16 B-A translocations in A619, A632, B73 and Mo17 were planted in summer 1988. Reciprocal crosses of normal-A619 x TB-A632, normal-A632 x TB-A619, normal-B73 x TB-Mo17, and normal-Mo17 x TB-B73 were made. For each B-A translocation, 10 plants were used as male and crossed onto the normal elite line and also onto a TB tester. Results from the TB tester will separate these F1 hybrid ears into two classes: normal-elite x normal-elite and normal-elite x TB-elite.

The first quantitative genetic trait that we have studied is the kernel size/weight. Because of the non-disjunction of the B-A chromosomes, the endosperms of the F1 hybrid (normal-elite x TB-elite) will carry 2 doses, 3 doses, or 4 doses of the translocated chromosome arm. The change in genetic dose of a codominant gene specific for kernel size/weight will change the kernel size/weight distribution. The mean value should usually be greater in hyperploid endosperm than in hypoploid endosperm, if the gene action is greater with additive effect. The variance should always be greater than in normal F1 crosses. B. Y. Lin found that kernel size/weight reduction is caused by paternal imprinting, in which an endosperm factor (Ef) from the male is responsible for kernel size reduction. For this study the kernel size reduction of a specific translocation is determined by comparison of variance (F test) between F1-TB and F1-normal seed samples. Random 100-seed samples from each ear were used for this comparison. A significant F test implies that an endosperm-related factor is located on that chromosome arm segment. If the F test is insignificant, the chromosome arm segment is not carrying an endosperm-related factor.

Results from normal-ear vs. TB-ear kernel weight comparison can be categorized for the B-A translocation effect or gene action into four different types: 1) kernel weight distribution is skewed to lower values and the mean value is reduced as shown in Figure 1. An endosperm factor is located on the chromosome arm. 2) kernel weight distribution is skewed to higher values and the mean value is increased as shown in Figure 2. An endosperm factor is located on the chromosome arm. 3) kernel weight distribution is as shown in Figure 3. No endosperm factor on the chromosome arm. 4) kernel weight distribution is greater but mean value does not change as shown in Figure 4. An endosperm factor is located on the chromosome arm. A summary result of the endosperm

Figure 1. A 100 kernel weight distribution from normal and TB-1Sb ear.

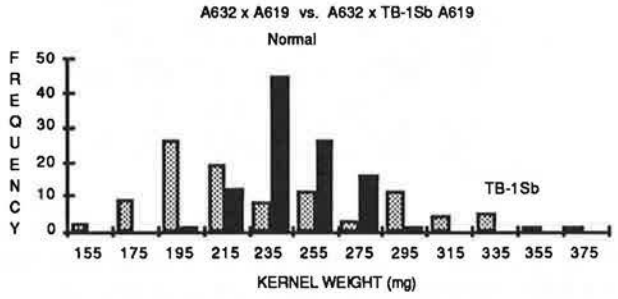


Figure 2. A 100 kernel weight distribution from normal and TB-1La ear.

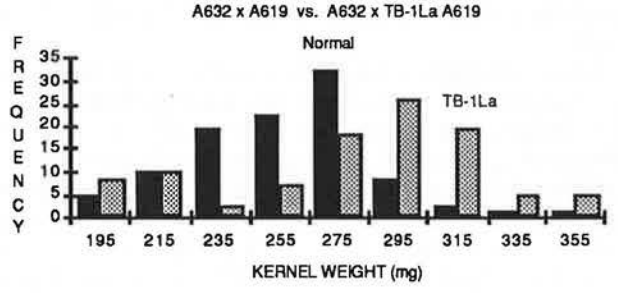


Figure 3. A 100 kernel weight distribution from normal and TB-3Sb ear.

A632 x A619 vs. A632 x TB-3Sb A619

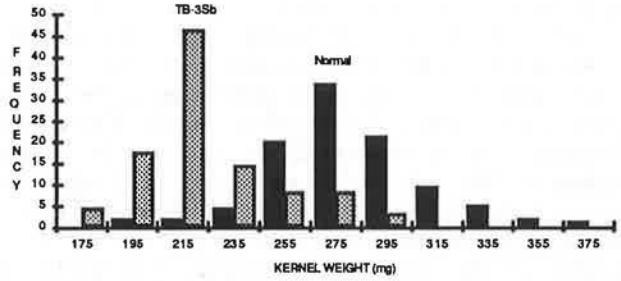


Figure 4. A 100 kernel weight distribution from normal and TB-10L19 ear.

A632 x A619 vs. A632 x TB-10L19 A619

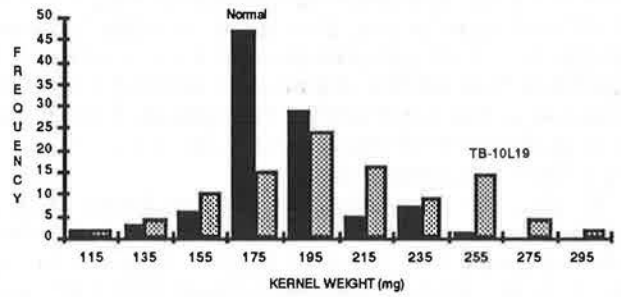


Table 1. Distribution analysis of endosperm factors (Efs) among four elite lines.

Chromosome Arm	A619	A632	B73	MO17
1Sb	▬			▬
1La	▬		▭	▭
3Sb	X	X		▬
3La	X			▬
4Sa	X	X		▬
4Lc	X			
5Sc		X	▬	▭
5La	X	▬		
6Sc	▭			X
6La	X	X		X
7Lb	▬			
8Lc	X	X		X
9Sd	X	X		▬
9Lc		X	▬	
10L19	▬	▬	▬	▬

Note: ▬ = kernel size significantly reduced.
▭ = results different from different ear samples.
X = no difference of kernel size.
blank = data not available.
A significant kernel size reduction implies that an endosperm factor (Efs) is located on that chromosome arm.

factor study is listed in Table 1. Results showed that the distribution of endosperm factors in the maize genome is quite different between elite lines. For example, Mo17 carries more endosperm factors than A619 and A632, which may imply that Mo17 will have larger effect than A619 and A632 in determining kernel size/weight in hybrid production.

The other quantitative genetic traits that were studied were tassel length, tassel branches, leaf length, leaf width, plant height, ear height, ear length and row numbers. Fifty-seed samples from selected B-A translocation F1 ears of A619 x A632-TB, A632 x A619-TB, B73 x Mo17-TB and Mo17 x B73-TB were planted in summer 1989. The hypoploid plants from each of the B-A translocations were identified by their smaller size and 50% pollen sterility. A few hypoploids were confirmed by RFLP (restriction fragment length polymorphism) polymorphic clones on that chromosome arm. Because the hypoploid plants are missing one chromosome arm, all genes in that particular chromosome arm will be hemizygous, and the additive genetic effect will be reduced to half. It is possible to determine a quantitative genetic trait by comparing the genetic effect of normal plants vs. hypoploid plants. By selfing the hypoploid plants, genes on that chromosome arm will be fixed. From each of the B-A translocations, five normal plants and five hypoploid plants were used for quantitative genetic trait measurments. A significant size reduction of a particular quantitative trait implies a quantative gene is located on that chromosome arm, otherwise the size reduction should be insignificant.

Results showed that the distribution of quantitative genetic factors in the maize genome is quite different between elite lines. The analysis of difference between elite lines is not finished. Therefore, we will be using results from A632 x A619-TB crosses as a model to describe the genetic effects of the B-A translocations on those quantitative genetic traits. The average tassel length of the normal F1 hybrid is about 15 inches and the hypoploid is about 6 inches. Tassel length ratio of normal/hypoploid is about 2.5 and not all the B-A translocations have dramatic effect as shown in Figure 5. The tassel branches of TB-1Sb hypoploids are dramatically reduced (13.33 in normal vs. 1.67 in hypoploids). In addition, TB-3La and TB-4Lc hypoploids had significant effect in reduction of tassel branches.

Figure 6 shows the analysis of normal/hypoploid ratio of leaf length, leaf width and plant height of A632 x A619-TB F1 hybrids. Results show that chromosome arm 1L had a significant effect on leaf length reduction, chromosome arm 1S, 5S and 5L had significant effect on leaf width reduction, and chromosome 1L had significant effect on plant height reduction. Figure 7 shows the analysis of normal/hypoploid ratio of ear height. The chromosome arm of 3L had clear effect in lowering the ear height of A632 x A619-TB F1 hybrids (36 inches in normal and 4.67

Figure 5. B-A translocation effects on tassel length and tassel branches of A632 x A619.

NORMAL / HYPOPLOID

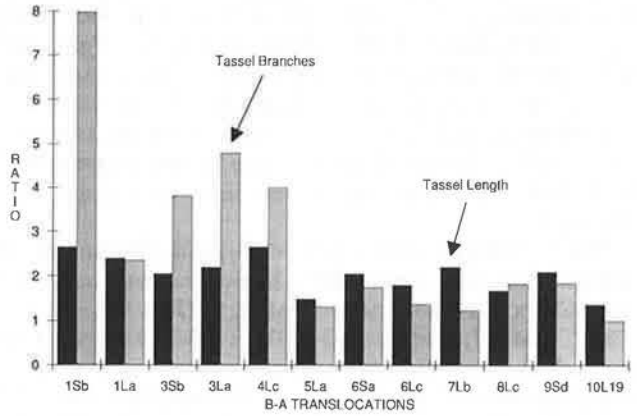


Figure 6. B-A translocation effects on leaf length, leaf width and plant height.

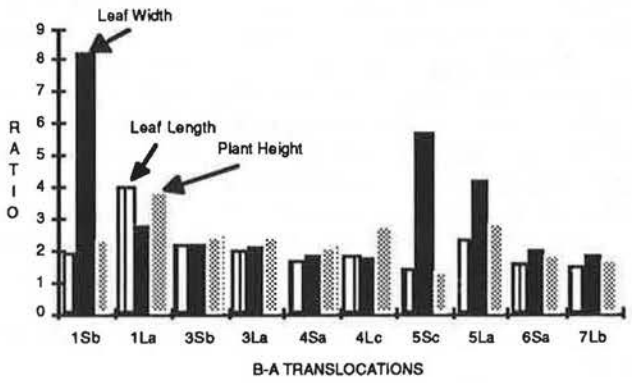


Figure 7. B-A translocation effect on hypoploid ear height of A632 x A619.

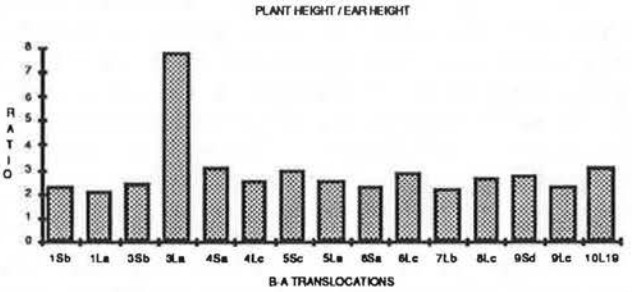
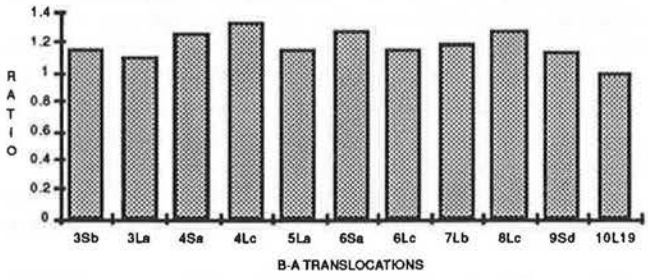


Figure 8. B-A translocation effect on row number of A632 x A619.



inches in hypoploids). Figure 8 shows the analysis of normal/hypoploid ratio of row numbers of A632 x A619-TB. Results show that row numbers were reduced from sixteen rows (normal) to twelve-fourteen rows (hypoploids) but the difference is not significant. The effect on ear length was the same as on row numbers. These results are very encouraging in showing that B-A translocations can be used as a tool to study quantitative genetic traits by dissecting each chromosome arm. The power of success of this study is highly dependent on stock purity and proper experimental design. Otherwise, results will not be repeatable.

STANFORD, CALIFORNIA
Stanford University

Reconstructing a *Bz2* allele using PCR

--Juli Nash and Virginia Walbot

Many maize genes have already been cloned by transposon tagging and many more are likely to be recovered in the future. The initial cloned fragment contains the gene of interest interrupted by a transposable element. In many cases it would be desirable to have the progenitor and/or the wild-type sequence; in the past, these have been recovered via cDNA cloning or by constructing a second genomic library. Using PCR (polymerase chain reaction) we demonstrate that progenitor alleles can be readily reconstructed by utilizing the existing transposon-containing clone and an amplified product from the progenitor that covers the site of insertion of the transposon.

The PCR technique requires only small amounts of DNA (<1ug) from the parental genotype. In this case 400ng of a single seedling mini-DNA preparation were used as the target DNA in the amplification step. A unique fragment of the *bz2-mu1* sequence including the entire *Mu* element was excised from the clone and replaced by the same amplified region of the *Bz2* locus from the progenitor genotype lacking the *Mu* insertion.

Figure 1

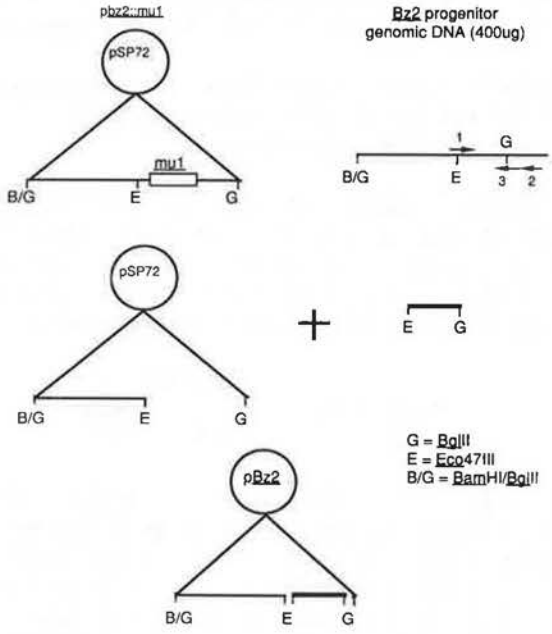


Table I

Primer #	Sequence (5'->3')
1	GGAGCCAGCGCTTCGCCGCGCACCCTGCAGCC
2	GGGCGATGACTTTTTAGACAA
3	GATAGTAGCTAGTGAGATCTTTTTTTC

Reaction	Primers	Product length
1	1 X 2	338bp
2	2 X 3	316bp

500ng of the unique *Eco*47111-*Bgl*II fragment of *Bz2* sequence were produced in two rounds of amplification. The first round primers (1 and 2 above) resulted in amplification of a 338 bp piece of DNA. The set of primers used in the second round of amplification (1 and 3 above) met two criteria: 1) they included the two unique restriction enzyme sites necessary for cloning and 2) at least one of the primers was internal to the fragment amplified in the first round yielding enrichment for the desired product. Buffers and reaction conditions were as suggested by the distributers of the *Taq* polymerase (Perkin-Elmer/Cetus). The amplification program used is 30X (1' 95 C, 1' 55 C, 2' 74 C) followed by 5X (1' 74 C).

Both the *bz2-mu1* clones and the PCR amplified product were digested with *Eco*47111 and *Bgl*II for compatible cloning. Digested products were separated in a 1% NuSieve low melting agarose gel. The 4kb *bz2-mu1* clone fragment and the 316 bp amplified genomic *Bz2* fragment were each cut out of the gel with a razor blade. The 1.5kb *Mu* containing fragment of the *bz2-mu1* clone was well separated and was left behind in the gel. Gel samples were melted at 68 C for 10 min. 5 ul samples of each DNA were mixed and cooled to 37 C. 10 ul of 2x ligase reaction buffer plus ligase (BRL) were added and the reaction was allowed to sit at room temperature for 4-20 hours. The sequence of one of the reconstructed *Bz2* clones indicates that the PCR amplified region of *Bz2* is identical to the corresponding *bz2-mu1* sequence except that the *Mu1* element and 9bp host sequence duplication were eliminated.

Effect of 5-azacytidine treatment on germination and Mutator transposable element activity

--Sally Otto and Virginia Walbot

Seeds were imbibed in water, 1 mM or 10 mM 5-azacytidine. In animals, this nucleoside analog inhibits DNA methylation, but its effects in plant cells are not known. We found that seed germination was reduced by approximately 50% in the 10 mM 5-azacytidine treatment:

	5-azacytidine	
Water Control	1 mM	10 mM
91.7	91.7	49.1

Because germination does not require DNA or RNA synthesis, this finding suggests that 5-azacytidine acts as a metabolic poison in a pathway outside of macromolecular nucleic acid synthesis. Interestingly, once germination was complete, plant viability, morphology and fertility were unaffected by the 5-azacytidine seed treatment. We had hypothesized that this analog might result in novel morphology by causing demethylation of the genome. Brown et al. (Theor. Appl. Genet. 78:321, 1989) recently found that 5-azacytidine treatment also had remarkably few effects in plant tissue cultures.

We also tested whether 5-azacytidine affected the level of activity of Mutator lines carrying the *bz2-mu1* reporter allele by scoring somatic instability in the progeny of the treated seed. Active lines were not stimulated to a significant extent, and inactive lines were not reactivated by analog treatment. Weakly active materials -- those with only a few spots per kernel and a lower than expected frequency of spotted kernels per ear--showed a dose-dependent rate of reactivation after analog treatment. In the water control 0.64% progeny kernels were spotted, while 1.77% of progeny kernels were spotted in the 1 mM 5-azacytidine treatment group, and 2.16% of progeny kernels were spotted from the 10 mM treatment group. This dependence of Mutator reactivation on 5-azacytidine treatment was significant at $p<0.05$, but not dramatic. Similarly, Martin et al. recently reported (EMBO J. 8:997, 1989) that *Tam*3 activity is only rarely modulated by 5-azacytidine treatment.

Mutator activity is maintained in exotic and inbred lines

--Virginia Walbot

A series of exotic lines was crossed with *bz2* tester in a W23 background, then selfed, to extract *bz2* with 50% exotic background. These lines were then crossed to and by *bz2* mutables (1.4 kb *Mu* insertions in the gene) in a mainly W23 background; somatic mutability was scored in the progeny with 25% exotic background. Alternatively, exotic lines were crossed with mutable materials, then selfed so that somatic mutability of the reporter allele could be scored in the progeny with 50% exotic background. In both types of test, mutability was maintained in Zapalote Chico, Tama Flint, Papago Flour, Maiz Chapalote, Tom Thumb, and Gourdseed. Mutable alleles of *Bz1* and *Bz2* have also been successfullly introgressed into the following inbred backgrounds: A188, K55, NI, W22, W23, and the Coop background in which the *an bz2* deletion is maintained. In both the standard and exotic lines, behavior of the mutable alleles is the same as we have previously reported: somatic mutability was maintained in most progeny but a few progeny became inactive. In our hands, all backgrounds are equivalent in maintaining Mutator activity. Our Mutator lines are all derived from the purple Mutator stock of D. S. Robertson.

Nature of insertion sequences in additional mutable alleles recovered from Mutator backgrounds

--Christine Warren, Anne Britt and Virginia Walbot

Since 1982 we have recovered a number of somatically unstable mutants in the anthocyanin pathway by crossing Robertson's purple Mutator stock with individual recessive testers. Using Southern hybridization in which the mutable is compared side-by-side to its progenitor we have succeeded in identifying the types of elements inserted at several alleles this past year. The *c2-mu1* allele we reported in 1985 (Walbot et al. in Genetics, Development & Evolution, edited by J. P. Gustafson, et al., p. 115) contains a 1.4 kb *Mu* element within the coding region. Two 1988 isolates, *bz1-mu3* and *bz2-mu3*, also contain 1.4 kb *Mu* elements within the transcription unit. Three *a2* mutables and *bz2-mu4* have been shown to contain insertions, but their nature is unknown; we have ruled out the presence of 1.4 kb and 1.7 kb *Mu* elements. Further tests are in progress to determine whether these contain examples of

the other types of *Mu* elements or whether these alleles contain insertions from a different transposable element family.

Evidence for somatic sectoring early in aleurone development with the *bz1-mu1* allele

--Virginia Walbot

Most excision events from *Mu*-induced mutants give rise to tiny spots of revertant tissue in the aleurone (1 - 100 cells) or short streaks in leaf tissue (1 - few hundred cells). Indeed, late timing is considered a feature of the Mutator system (D. S. Robertson, Science 213:1515, 1981). All of the *Mu*-induced mutables in our collection share this property. In 1987, however, a few progeny ears in a family carrying *bz1-mu1* had larger spots (250 - 2,000 cells). This allele contains a 1.4 kb *Mu1*-like element (Taylor et al., Maydica 31:31, 1986). The kernels with large spots (*bz1-mu1* or *bz1-mu1/bz1*) were selected, and selfed or crossed by *bz1* tester. Among the 77 progeny ears, 3 had both kernels with large sectors and fully purple kernels, putative germinal revertants. The remaining 74 ears contained many kernels with large sectors (250 - 2,000 cells), and most progeny ears had at least a few half- or quarter-kernel somatic reversion events. More careful analysis of the distribution of types is in progress. Even the preliminary results demonstrate, however, that selection for large spots can result in a stock with a reasonable frequency of early events, including likely germinal revertants. The nature of the original switch in timing is completely unknown. The only clue is that sister lines grown in adjacent field rows did not show this change in phenotype, suggesting that the switch was not caused by environmental conditions.

Mapping of loci affecting Mutator activity

--Avraham A. Levy and Virginia Walbot

The genetic basis of Mutator activity in typical Mutator lines is complex. Mutator activity cannot be explained by simple Mendelian genetic segregation of one or two autonomous elements. In this respect it is unique compared to other transposable elements in maize and most systems in other species. When an active Mutator line is crossed to a non-Mutator line most of the progeny are active. Moreover, the proportion of progeny turning off (losing their activity) varies in different crosses and Mutator lines; progeny of the same cross can show different levels of activity, as deduced from the variation in somatic reversion frequency. This type of genetic "behavior" is reminiscent of quantitative traits.

We tested the hypothesis that Mutator activity was regulated by several genes, as originally proposed by Robertson (Mutat. Res. 51:21-28, 1978). The difficulty in genetically identifying each of these units may have arisen from the possibilities that [1] more than one gene is required for activity and [2] the number of these genes varies as a result of segregation or of amplification (as was shown for the receptor elements). Variation in copy number of each unit may affect the degree of activity of a given progeny or family.

To test that hypothesis, and to partially overcome the difficulties described above, we looked for co-segregation of Mutator activity and known genetic markers.

a) An active Mutator line was selected (GG65) which contained a *Mu1.4* insertion in the Bronze-2 locus (*bz2-mu1* allele). In active progenies of this line, a characteristic fine spotting can be easily observed on the kernels; this reflects the excision of *Mu1.4* from the indicator allele. GG65 is derived from a Robertson purple Mutator line which was backcrossed four times to a *bz2* W23 background.

b) The Mangelsdorf tester (GG63) contains ten morphological traits differing from those of the Mutator line. Each trait is encoded by a single gene, corresponding to one of the ten maize chromosomes.

(GG65) active Mutator **x** (GG63) Mangelsdorf's tester

A/A bz2-mu1/bz2 | *a/a Bz2/Bz2*

winter 88: F1 kernels 100% purple

A/a bz2-mu1/Bz2 : *A/a bz2/Bz2*

spring 88:

71 F1 plants selfed

F2

33 ears with spotted K : 38 ears with no spots (*bz, Bz, pr*)

winter 89:

400 (K/ear) X 33 (ears) X 3/16 (*A/- bz2-mu1/bz2-mu1*)

~2500 F2 spotted K were scored.

In this material no progeny kernel had become completely inactive, therefore, the degree of spotting was scored visually for each kernel, as an indicator of Mutator activity. Note that all kernels have three doses of *bz2-mu1* in their aleurones, therefore, differences of intensity of spotting do not depend on the dosage of the reporter allele. Three classes were established:

2% = 47 K had very few (5-30) spots per kernel (LS = Low spotting)

20% = medium intensity of spotting (MS)

80% = high (HS) or very high (VHS) intensity of spotting.

The Mangelsdorf markers were scored as follows: sugary, red aleurone, and waxy were scored on the F2 kernels; glossy was scored on 2 week old seedlings; liguleless was scored on two month old plants; the other markers could not be scored. All 47 LS K were used from the population of 2500 spotted K. Kernels from the other groups (MS, HS, VHS) were sampled randomly.

If there is no association between a marker and Mutator activity, then the proportion of the recessive phenotype should be similar in all activity groups (LS->VHS). We used the G-test for heterogeneity to compare between proportions. No significant differences were found for sugary, waxy, and glossy, indicating that these markers are

Marker	individuals scored	Proportion of the recessive (Mangelsdorf) phenotype in each group				
		LS	MS	HS	VHS	total
Sugary	458	15/47	24/101	26/115	39/195	
		.32	.24	.22	.20	.23
Red	456	11/47	34/99	31/113	36/197	
Aleurone		.23	.34	.27	.18	.24
Waxy	349	8/32	18/73	15/87	38/157	
		.25	.25	.17	.24	.23
Glossy	357	6/39	28/77	23/94	32/147	
		.15	.36	.24	.22	.25
Liguleless	208	17/40	9/32	9/29	27/107	
		.425	.28	.29	.25	.30

probably not linked to a locus affecting Mutator activity. Significant differences were found for red aleurone and liguleless ($P(G_H)$=0.04 for both markers). For these markers, the frequency of the recessive allele was greater in the medium (0.34 for *pr*) and low spotted groups (0.425 for *lg*) than in the High + Very High spotting groups. Although the level of significance of *pr* association with spotting intensity is low, we found a similar trend of association in a small population of F_3 plants from the same cross, as well as in a small population from another cross (data not shown). These observations support the proposition that *Pr* is linked to a factor which affects Mutator activity. Interestingly, *Cy* has been mapped distal to *Pr* (Peterson, MNL 62:3, 1988). The locus we mapped from Robertson's stock may be homologous to *Cy*.

The five markers used in this study covered only a small portion of the genome (~10%), and did not explain all the variation in Mutator activity. Yet, two markers seem to be linked to loci affecting Mutator activity. This suggests that additional Mutator activity-related factors exist in the standard Robertson's stocks. We are currently mapping more precisely the factors which we identified, and we are looking for additional markers in the genome using RFLP analysis.

STANTON, MINNESOTA
The New Northrup King

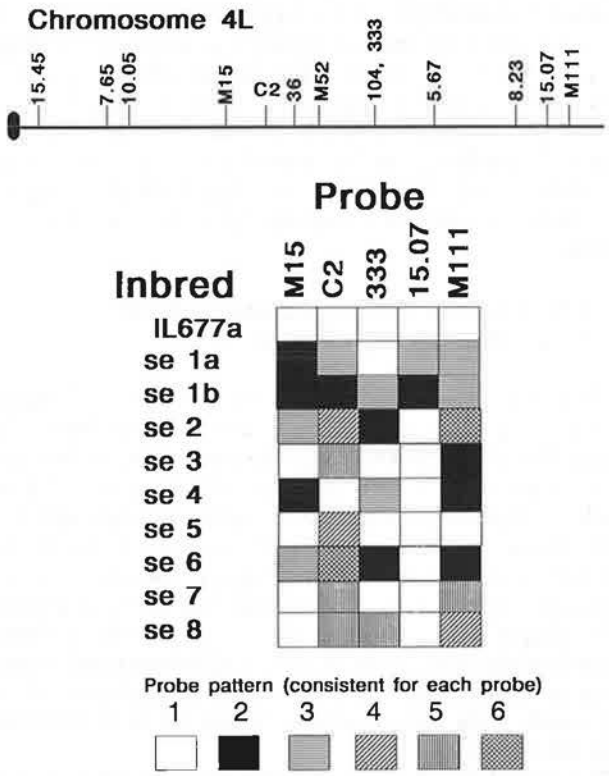
Linkage of sugary enhancer (*se*) to *15.07* on chromosome 4L

--Christine Bredenkamp, Diana Beckman, Michael Kiefer, Ann Majerus, Douglas Mead, Hope Sunderland, Carol Wangen and Edward Weck

The sugary enhancer (*se*) gene (Ferguson, Rhodes, and Dickinson, J. Hered. 69:377-80, 1978) is a recessive modifier of the *su1* locus, which is commonly used in sweet corn breeding. The *se* locus increases the sugar content of the kernel without the reduction of phytoglycogen commonly associated with the *sh2*, or "supersweet," varieties. LaBonte (Ph.D. thesis, University of Illinois, 1988) utilized the translocation stock TB-1LA-4L4692 and single kernel gas chromatographic sugar analyses to localize *se* to the long arm of chromosome 4, distal to *zb6*.

In order to map the sugary enhancer gene more precisely, we have analyzed a number of putative *se* inbreds with molecular markers (RFLPs) present on chromosome 4L. Figure 1 shows a schematic map of chromosome 4L along with hybridization patterns for 5 molecular markers. The hybridization banding patterns for each probe have been coded (pattern 1, none; pattern 2, black; pattern 3, horizontal lines). The codes for locus *M15* are independent of the codes for the other loci shown (*C2*, *333*, *15.07*, *M111*). Locus *15.07* shows the same banding pattern in IL677a, the original source of *se*, and 7 of the 8 other inbreds analyzed (*se* 1a and *se* 1b are independent isolates of the same inbred). This suggests that locus *15.07* is linked to *se*. The other probes tested in the region, *M15*, *C2*, *333*, and *M111*, are more polymorphic, having as many as 6 different patterns. This greater degree of polymorphism suggests that the chromosomal segment monitored by these probes was not transferred in construction of these *se* lines.

Figure 1.



The similarity of these inbred lines for locus *15.07* on chromosome 4L suggests that sugary enhancer is located between loci *333* and *15.07*. Loci *5.67* and *8.23* will be checked to determine if they also show the single hybridization pattern that suggests linkage. The differences observed between inbreds *se* 1a and *se* 1b and also with the other sugary enhancer inbreds, *se* 2-8, suggests that *se* 1a and b do not carry the sugary enhancer gene. In order to

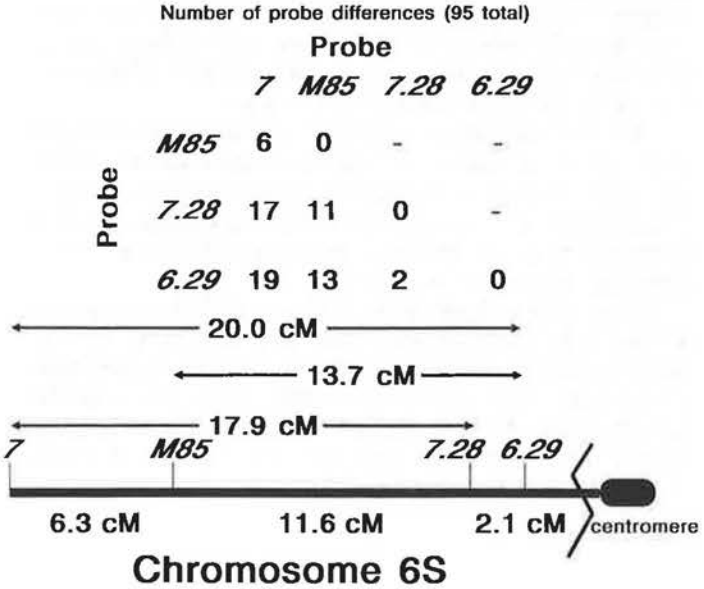
map the sugary enhancer gene more precisely, F2 or BC1 populations with IL677a as one parent will be constructed and analyzed for sugar content and chromosome 4L constitution.

RFLP mapping of chromosome 6S in a backcross 1 population

--Douglas Mead, Christine Bredenkamp, Michael Kiefer, Ann Majerus, Hope Sunderland, Carol Wangen, and Edward Weck

In order to establish the relative order of loci on chromosome 6S, a backcross 1 population of 95 plants from the cross (NK1 x NK2wx) x NK1 was utilized. The advantages of using a backcross population for mapping are: 1) the number of individuals displaying a recessive trait is greater than in an F2 population; 2) there are only two RFLP patterns to score; and 3) there is the possibility of gaining information about genomic constitution in a different set of genetic materials. The disadvantages of using a backcross population (compared with recombinant inbred lines) are: 1) each backcross 1 individual is different and only a limited amount of tissue can be obtained from each plant; 2) data can not be correlated across laboratories. The first disadvantage can be overcome by the utilization of wide crosses of heterotic elite materials to generate large amounts of tissue (we had great difficulty during the drought summer of 1988 in obtaining sufficient tissue samples from the recombinant inbred lines to allow the routine isolation of quality DNA). The second disadvantage is only significant if a universal mapping strategy is required.

In order to analyze the mapping data for loci on chromosome 6S in the cross (NK1 x NK2wx) x NK1, a SAS program was written which makes pairwise probe pattern comparisons. The number of differences between pairs of loci (including double crossovers) are shown in a "relatedness" triangle (Figure 1). The distance between loci was calculated (to the nearest 0.1 cM) using the number of probe differences. A map of chromosome 6S is shown in Figure 2. Comparison of this map with the 6S map from the 1988 MNL suggests that: 1) different mapping methods result in different relative amounts of recombination or 2) there is a parental line effect in the various mapping populations.



To examine these possibilities further, a number of probes on chromosome 3 and chromosome 9 were analyzed in the (NK1 x NK2wx) x NK1 BC 1 population and compared with the 1988 MNL maps (Figure 3a and b). The mapped distances differ significantly in the (NK1 x NK2wx) x NK1 population from those observed in the 1988 MNL maps. In this BC1 population the markers *M81* and *5.10* are not linked to *wx* or *8.17*. The presence of other numerous mapping differences makes it difficult to ascertain whether parental or test population differences are responsible for the discrepancies.

Figure 3a. Comparison of Chromosome 3 maps

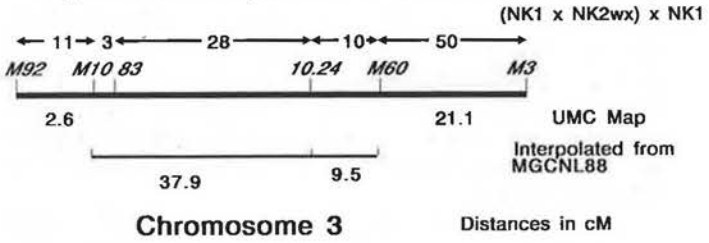
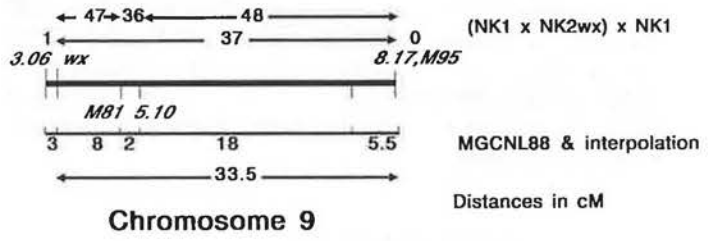


Figure 3b. Comparison of Chromosome 9 maps



The reason for examining the BC1 population was to map chromosome 6S markers as a prelude to incorporating the morphological markers into the RFLP map. A number of crosses will be made in an attempt to localize various morphological markers on the RFLP map.

TOCHIGI, JAPAN
National Grassland Research Institute

Heterofertilization exhibited by using highly haploid inducing line "Stock 6" and supplementary cross

--Akio Kato

Stock 6 has been known to be a highly haploid inducing line for about 30 years. However, the mechanism of haploid production is not solved, even though some presump-

tions were made. One of them is that "A part of the pollen of Stock 6 has only one sperm cell so the polar nucleus can be fertilized with it but the egg cell fails to be fertilized. Consequently the egg cell produces a haploid embryo apomictically."

I planned the experiment to prove the hypothesis in 1989, and an interesting result was observed. The method is the supplementary cross using the purple embryo marker *R-scm2* gene.

I crossed Stock 6 (*C-I/C-I, y/y*, white kernel, white scutellum) to Oh43 (*c1/c1, Y/Y, v1/v1*, yellow kernel, white scutellum) thinly. Twenty-four hours later I crossed X18G (*A1/A1, A2/A2, C1/C1, C2/C2, R-scm2/R-scm2, Y/Y*) to the same ears. The *R-scm2* gene makes the scutellum and aleurone deep purple. I obtained 609 seeds. One-hundred-ninety-eight seeds were yellow kernels with white scutella which means they are Oh43 x Stock 6 hybrids. Four-hundred-nine seeds were purple kernels with purple scutella which means they are Oh43 x X18G hybrids. Two seeds were purple kernels with white scutella. Seedlings from the two seeds did not exhibit virescence, and the fertility was normal. Genotypes of the two plants were both (*C-I/c1, Y/y*) so they are clearly Oh43 x Stock 6 hybrids. This means the egg cells were fertilized with Stock 6 and polar nuclei were fertilized with X18G. Such a phenomenon is known as heterofertilization.

I suppose it was the case that the pollen tube of Stock 6 released only one sperm cell and it fertilized the egg cell, and the polar nucleus failed to be fertilized. Twenty-four hours later the polar nucleus was fertilized with X18G sperm cell. If so this phenomenon supports the hypothesis described by several investigators.

However, in this case there is another possibility, that the pollen of Stock 6 germinated slowly and reached the embryo sac at the same time as the pollen of X18G, and they heterofertilized the polar nucleus and the egg cell. I think such a case must be very rare. But further experimentation is needed.

URBANA, ILLINOIS
University of Illinois

Shrunken-5

--G. F. Sprague

Data presented earlier (MNL 61:96) indicated that *Sh5* was on chromosome 5, linked to *Pr* with approximately 22% crossing over. Data from the cross + *v3 pr/sh5* + + permit the establishment of the linear order. The F2 data are given below:

+++	++ *pr*	+ *v3* +	+ *v3 pr*
199	38	28	77
sh5 ++	*sh5* + *pr*	*sh5 v3* +	*sh5 v3 pr*
96	7	2	8

Sh5-V3 17 *V3-Pr* 32 *Sh5-Pr* 34

It should be possible to obtain backcross data next season.

Glossies

--G. F. Sprague

Tests have been completed on *gl*-PI262490* and it is not allelic to any of the standard glossies. It has, tentatively, been assigned the symbol *gl23*.

gl14 has been located to chromosome 2 on the basis of linkage with *wt*. In an attempt to establish linear sequence, crosses were made to *v4*. Unexpectedly, the F2 of this cross segregated 15+:1*gl*. This suggests that what has been designated *gl14* is really a double recessive, *gl14 gl14, gl24 gl24*. If this is true *gl24* is commonly present in recessive form, as in all previous crosses *gl14* has behaved as a single locus recessive. Apparently *wt* is not expressed in *v4* seedlings as no *wt v4* plants were observed in a population of over 400 seedlings.

Non-allelism of two threonine accumulating mutants

--David R. Duncan and Jack M. Widholm

The addition of both lysine and threonine to maize tissue culture medium inhibits callus growth through the feedback inhibition of the aspartate biosynthetic pathway. Ultimately, the tissue is starved for methionine, and the addition of methionine to the culture medium can reverse the growth inhibition. Through their inhibition of growth, the combination of lysine and threonine has been used as a selection agent in regenerable maize callus cultures for the recovery of mutants that accumulate threonine (K. A. Hibberd and C. E. Green, Proc. Natl. Acad. Sci. 79:559-563, 1982; and S. Miao, D. R. Duncan and J. M. Widholm, Plant Cell Tissue Organ Cult. 14:3-14, 1988).

It was the goal of this work to determine if the mutant gene selected by Hibberd and Green (LT19) was allelic to that of the mutant selected in our lab. Homozygous LT19 seed, supplied by Burle Gengenbach, was crossed with our homozygous LT-R3. Randomly selected F1 plants were selfed and their progeny were screened for the mutant phenotype by assaying the growth of excised, 1 cm long, root tips on H medium (D. R. Duncan, M. E. Williams, B. E. Zehr and J. M. Widholm, Planta 165:322-331, 1985) containing 3 mM lysine and 3 mM threonine.

Of 198 F2 segregants tested, 17 expressed the wildtype phenotype of no root growth on the assay medium. A chi square analysis of these data showed a close fit ($p > .10$) to the 15:1 ratio of two independent, dominant genes. No difference in response was seen for reciprocal crosses of the homozygous parental material.

These data indicate that LT19 and LT-R3 are non-allelic genes for the same phenotype. At this point we do not know if these genes code for different enzymes, isozymes of the same enzyme or perhaps different subunits of the same enzyme. We have also not studied the allelism of LT-R3 and LT20, another threonine accumulating mutant (D. A. Frisch and B. G. Gengenbach).

Characterization of globulin-1 alleles

--Faith C. Belanger and Alan L. Kriz

A major storage protein of maize embryos is an Mr 63,000 polypeptide designated GLB1. This protein is encoded by the single gene *Glb1*, for which several size alleles and a null allele have been described (Schwartz, MGG 174:233, 1979; Kriz and Schwartz, Plant Physiol. 82:1069, 1986). We recently reported the nucleotide sequence of a cDNA clone corresponding to the Small (*S*) allele of the gene (Belanger and Kriz, Plant Physiol. 91:636, 1989).

To further characterize the *Glb1* gene, we have isolated genomic clones corresponding to the Large (*L*) and null alleles. The *Glb1-L* clone was obtained from a partial lambda library of sequences from the inbred line W64A, and the clone for the null allele was obtained by digesting DNA from *Glb1-0/0* plants with *Eco*RI, fractionating the reaction on an agarose gel, and eluting fragments of about 3.4 kb which were subsequently cloned into LambdaZAP. We have determined the entire nucleotide sequence of the 3.4 kb *Eco*RI clone corresponding to the *L* allele. Both strands were completely sequenced. This sequence includes 380 nucleotides 5', and 767 nucleotides 3', to the sequence of the cDNA clone pcGlb1S. Primer extension analysis indicates that the 5' end of the *Glb1-L* transcript maps to a position 45 nucleotides upstream of the initiator methionine codon. A TATA sequence element is found 33 nucleotides upstream of the transcription start site. Sequences with homology to the ABA response elements from the *Em* gene of wheat (Marcotte et al., Plant Cell 1:969, 1989) are present 116 and 75 nucleotides 5' to the start of transcription, consistent with the observation that *Glb1* expression is positively regulated by ABA (Kriz et al., Plant Physiol., in press). *Glb1-L* contains four introns ranging in size from 82 to 113 nucleotides. The size difference observed in the proteins encoded by the *L* and *S* alleles appears to be largely attributable to a 36-nucleotide duplication in the 3' portion of the coding region.

Nucleotide sequence analysis of the null allele is almost complete. From sequence comparisons and Southern blot analysis of maize DNA it appears the null allele is more closely related to the *L* allele than to the *S* allele. The null allele possesses the small 3' duplication present in the *L* allele, and there are no nucleotide differences between the two alleles in the 410 nucleotides upstream of the initiator methionine codon. The null allele appears to possess the four introns, and the nucleotide sequences immediately surrounding the splice sites are no different from those in the *L* allele. There are, however, differences from the *L* sequence within the introns. There are also several differences in the 3' regions of the two alleles, including a small deletion and insertion. Experiments are in progress to determine the nature of the defect in the null allele, which may very well be due to post-transcriptional processing errors.

Molecular characterization of the *Glb2* gene

--Nancy H. Wallace and Alan L. Kriz

Normal maize embryos contain high levels of saline-soluble, water-insoluble globulin storage proteins. The most abundant globulin component is the *Mr* 63,000 product of the *Glb1* gene, which has recently been characterized at the molecular level (above report). The second most abundant globulin is designated GLB2, which is encoded by the *Glb2* gene (Kriz, Biochem. Genet. 27:239, 1989). We have isolated and characterized at the nucleotide sequence level cDNA clones corresponding to *Glb2*. Clones were isolated from an embryo-specific cDNA library by using antiserum raised against whole embryo globulin. Immunoreactive plaques were picked and purified by a secondary screen. A tertiary screening was performed using as probe the radiolabelled insert from a *Glb1*-specific cDNA clone. Those plaques reacting with whole globulin antiserum but not with the *Glb1* probe were considered to be potential *Glb2* cDNA clones. The clone with the largest insert (1600 bp) was radioactively labelled and used as a probe in a Southern blot analysis of five other potential *Glb2* clones. All five showed hybridization with the radiolabelled probe. The largest clone was subjected to nucleotide sequence analysis. To obtain the 5' untranslated region for analysis, a *Pst*I fragment of ~180 bp from the 5' end of the 1600 bp clone obtained above was used to screen the cDNA library for longer clones. Such a clone was isolated and subjected to nucleotide sequence analysis. This 1638 bp clone, designated pcGlb2, contains a 1350 bp open reading frame corresponding to a polypeptide of 450 amino acids. The translated region contains a putative signal peptide of 23 amino acids, followed by 15 amino acids which correspond to the amino terminus of GLB2 as determined by direct protein sequencing of the mature polypeptide. The predicted molecular weight of the mature polypeptide (47,380 daltons) is in good agreement with the value of 45,000 daltons empirically determined from SDS gel analysis. We are currently characterizing genomic clones corresponding to the *Glb2* gene.

Tissue specificity of *Glb2* expression has been examined by Northern blot analysis of total RNA from various plant tissues. *Glb2* transcripts are found only in the developing embryo and not in endosperm, seedling or unfertilized ear. Western blot analysis comparing the globulin protein profiles of embryo and endosperm of the maize inbred line Va26 shows the GLB2 protein to be present only in the embryos. This is in contrast to the situation observed for *Glb1* transcripts and proteins, which are present at low levels in endosperm tissues. It has also been determined by Northern blot analysis that *Glb2* transcript is present between 21 DAP to 36 DAP and absent in mature dry embryos. This expression pattern also differs from that of *Glb1* in that *Glb1* transcripts, first detectable at 15 DAP, persist in mature embryos. Lastly, embryos homozygous for a *Glb2* null allele lack the *Glb2* transcript as shown by Northern blot analysis. Isolation of genomic clones corresponding to the null allele will eventually be performed.

Globulin gene expression in embryos of viviparous mutants

--Renato Paiva and Alan L. Kriz

Vivipary is the phenomenon in which seed maturation fails to go to completion and, as a consequence, germina-

tion initiates while the kernel is still attached to the ear. To better understand the mechanism involved in genetically controlled vivipary, we have examined the expression of genes encoding embryo storage proteins in sib normal and viviparous (*vp*) kernels. In normal maize embryos, specific globulin storage proteins which accumulate to high levels during seed development and maturation are rapidly degraded during the early stages of germination. The most abundant embryo globulin components are encoded by the *Glb1* and *Glb2* genes, which have been characterized at the molecular level in our laboratory (see above notes). We previously used expression of these genes as markers of embryo maturation (proteins and transcripts present) and germination (proteins and transcripts absent) to determine if viviparous embryos undergoing precocious germination had switched from a "maturation program" to a "germination program" of gene expression. These experiments demonstrated that precociously germinating embryos of the Class II ABA-deficient mutants (*vp2*, *vp5*, *vp7*, and *vp9*), as well as *vp8*, contained significant amounts of *Glb1* proteins and transcripts (Kriz et al., Plant Physiol., in press; MNL 63:116, 1989), indicating that these embryos exhibit characteristics of both maturation (*Glb1* expression) and germination (i.e., radicle protrusion) processes.

During this past summer, embryos were collected from ears of various ages segregating for the different *vp* mutants. These include normal and mutant sib embryos and endosperm from developmentally staged ears segregating for each of the viviparous mutants *vp1, vp2, vp7, vp8,* and *vp9*. With the exception of *vp8*, which is difficult to classify prior to the onset of precocious germination, samples were obtained at various days after pollination (DAP) prior to, and during, the period of precocious germination. Embryos were pooled from three selfed ears of the same family pollinated on the same day. Since proteins encoded by the *Glb* genes are rapidly degraded during the early stages of seed germination, we anticipated that these proteins might be present in *vp/vp* embryos prior to the onset of precocious germination, and that they would be degraded in germinating embryos older than those previously examined (as reported in last year's newsletter). This does not turn out to be the case. Proteins were extracted from sibling normal and mutant (*vp*) embryos and subjected to immunoblot analysis to visualize specific proteins encoded by the maize *Glb1* and *Glb2* genes. None of these proteins are detected in *vp1/vp1* (Class I; ABA-insensitive) embryos at any of the embryo ages examined, regardless of whether precocious germination is apparent. In embryos homozygous for either *vp2*, *vp7*, or *vp9* (Class II; ABA-deficient), globulins are absent in younger embryos, but are apparent in older embryos, even though these older embryos are actively germinating.

Results consistent with those obtained from the immunoblot analyses were observed when levels of *Glb1* transcripts were examined in embryos by RNA blot analysis. *Glb1* transcripts, usually first detectable at 15 DAP, are normally present throughout most of embryo development. These transcripts are not detectable in *vp1/vp1* embryos at any age, providing additional evidence that *Glb1* expression is absent in homozygous *vp1* embryos; since *Glb1* expression is apparently regulated by ABA (Kriz et al., 1990), this is probably due to the ABA-insensitive nature of this mutant. Although *Glb1* is expressed in Class II mutant embryos after 18 DAP, the rate of accumulation of *Glb1* transcripts in these mutant embryos is delayed relative to that observed in normal sib embryos.

Although the results obtained upon analysis of *Glb1* expression in the Class II *vp* mutants were somewhat unexpected, they clearly demonstrate that the pattern of gene expression during precocious germination of the *vp* mutants, at least with respect to the maize *Glb* genes, is different from that of normal germination. The increased levels of *Glb1* expression in older embryos of the Class II *vp* mutants may be due to accumulation of ABA as a function of embryo age. Although ABA levels in the Class II mutants are much lower than those of normal embryos (Neill et al., Planta 169:87, 1986), these low levels may be sufficient to promote expression of *Glb1*. We are in the process of determining ABA levels in the embryo samples collected this past summer to ascertain if there is any correlation between ABA content and level of *Glb1* expression in these materials.

Use of A-B translocations to identify chromosomal locations of dominant genes

--Earl B. Patterson and John R. Laughnan

The use of A-B translocation stocks to identify chromosome arm locations of recessive genes is well known; it is not generally recognized that these stocks can also be used to locate dominant genes. In the first step of such a procedure the dominant marker stock is crossed as female parent with specific balanced or hyperploid A-B translocation heterozygotes. Appropriate analysis of either or both hyperploid and hypoploid progeny from this cross will yield linkage information. If, for example, the initial cross involves the dominant allele *Ts5* (tassel seed-5; chromosome 4S) and TB-4Sa, the F1 hyperploid progeny from this cross will be $4(Ts5)/4^{B}B^{4(+)}B^{4(+)}$ and F1 hypoploid offspring will be $4(Ts5)/4^{B}$.

The F1 hyperploid offspring are crossed as male parents onto a tester stock, *su1* in this case, to verify their A-B interchange status and onto a non-*Ts5* tester strain for linkage analysis. The $4(Ts5)/4^{B}B^{4(+)}B^{4(+)}$ F1 hyperploid plant is expected to produce both $4(Ts5)B^{4(+)}$ and $4^{B}B^{4(+)}$ microspores. Pollen grains whose tube nuclei are $4^{B}B^{4(+)}$, having a balanced nuclear genotype, under pollen competition are expected to function to the exclusion of pollen with the unbalanced $4(Ts5)B^{4(+)}$ genotype and, except for crossovers derived from exchanges between chromosomes 4 and B^4 proximal to *Ts5*, should transmit only the wild-type allele (*Ts5*$^+$) carried in B^4. Testcross progeny should include balanced A-B interchange heterozygotes, $4(+)/4^{B}B^{4(+)}$, and both products of microspore nondisjunction, the $4(+)/4^{B}$ hypoploid and the $4(+)/4^{B}B^{4(+)}B^{4(+)}$ hyperploid. All hypoploid offspring should exhibit normal tassels (*Ts5*$^+$) since they do not have a B^4 chromosome that could, by crossing over, carry *Ts5*. Balanced and hyperploid offspring are expected to be mainly *Ts5*$^+$, with

occasional crossover *Ts5* plants whose frequency is a function of the recombination distance between *Ts5* and the B^4 breakpoint, and of the effective pairing frequency between chromosomes 4 and B^4 in that region.

Needless to say, if the initial cross were to involve the *Ts5* strain and a nonchromosome-4 A-B translocation stock, e.g., TB-7Lb, the F1 hyperploid would carry two normal chromosomes 4 and have the genotype *Ts5*/+; its cross onto the *Ts5*$^+$ *Ts5*$^+$ tester strain would be expected to produce a 1:1 ratio of *Ts5*:+ among the TB-7Lb balanced, hyperploid and hypoploid progeny.

Since the A-B hyperploid testcross procedure described above is based on exclusion of the A chromosome (chromosome 4 in our example) from pollen transmission, it can identify the chromosome, but not the specific arm, that carries the dominant gene in question. For example, it is expected that TB-4S or TB-4L strains would indicate that *Ts5* is in chromosome 4 but that neither would necessarily clearly place it in 4S.

The F1 4(*Ts5*)4^B hypoploid from the initial cross produces an unequivocal basis for linkage assignment. Since the 4^B chromosome is lethal to both male and female gametophytes, self-pollination of this hypoploid will yield only tassel seed (*Ts5*/*Ts5*) offspring, and crosses with nontasselseed testers, involving the F1 hypoploid as both male and female parent, are expected to produce only tassel seed (*Ts5*/*Ts5*$^+$) progeny. Again, if the initial cross were to involve the *Ts5* strain and a nonchromosome-4 A-B translocation stock, F1 hypoploids would carry two normal chromosomes 4 and have the genotype *Ts5*/*Ts5*$^+$; self-pollination of this hypoploid should produce a progeny ratio of 3 *Ts5*:1 +, and both kinds of testcrosses are expected to produce 1:1 ratios for *Ts5*:+ among the progeny.

For locating dominant genes to chromosome, compound A-B translocations have a use similar to that of simple A-B translocations. They differ in that a simple A-B translocation is derived from a single chromosome of the "A" complement, whereas a compound A-B translocation includes chromosome segments from two chromosomes of the "A" complement.

The origin of the compound TB-1Sb-2L4464 may serve as an example (Rakha, F. A. and D. S. Robertson, Genetics 65:223, 1970). As a preliminary to extraction of the compound, a female parent carrying TB-1Sb(S.05) may be crossed by a male parent homozygous for T1-2(4464) (1S.53; 2L.28) to produce the F1 combination $1^B B^{1}2/1^2 2^1$; this combination is completely balanced and homologous pairing can occur in all regions of the "A" chromosomes. Part of this pairing involves the region between the interchange point in TB-1Sb(S.05) and the 1S interchange point in T1-2(4464)(S.53). A crossover in this region leads to formation of a compound interchange chromosome, namely $B^{1,2}$, which by nondisjunction can lead simultaneously to hyperploidy in progeny for the region between 1S.05 and 1S.53 and for the terminal 2L segment distal to 2L.28, or alternatively may result in hypoploidy for the same segments. Together, a 1^B chromosome and a $B^{1,2}$ chromosome contain the same "A" complement chromatin as the original 1^2 chromosome. Since the combination $1^2 2^1$ is balanced, the combination $1^B B^{1,2} 2^1$ is also balanced.

Hyperploid stocks of TB-1Sb-2L4464 may be perpetuated by crossing female parents from standard chromosome stocks with hyperploid male plants and again selecting and testing for hyperploid plants in the progeny: $12/2^1 1^B B^{1,2} B^{1,2}$. The nondisjunctional capability of the male parent may be confirmed by the occurrence of virescent progeny plants from crosses onto homozygous (or heterozygous) *v4* female testers. If testcrosses are made onto homozygous *v4* plants and yield some virescent plants, nonvirescent progeny plants have a good chance of being hyperploid. Hyperploid compound A-B stocks free of the recessive *v4* allele may also be perpetuated by using the *v4* stocks only to test chromosomal constitution and using nonmutant standard female parent stocks for perpetuation. In the latter instance, the frequency of hyperploid plants from kernels selected for planting may be enhanced by choosing smaller size kernels that have an increased probability of having hypoploid endosperms and hyperploid embryos.

TB-1Sb-2L4464 may be used to test for the chromosome location of the dominant allele *Ch* (chocolate pericarp; 2L). In the initial cross, a homozygous *Ch* female parent, when pollinated by hyperploid TB-1Sb-2L4464 plants, is expected to produce hyperploid (1 2(*Ch*)/$2^1 1^B B^{1,2} B^{1,2}$), hypoploid (1 2(*Ch*)/$2^1 1^B$) and balanced (1 2(*Ch*)/$2^1 1^B B^{1,2}$) progeny plants. From hyperploid plants, those microspore tube nuclei which carry a balanced chromosome complement will virtually always be represented by the chromosome combination $2^1 1^B B^{1,2}$; combinations carrying a standard chromosome 1, a standard chromosome 2, or both, and accompanied by a $B^{1,2}$ chromosome are unbalanced, and presumably noncompetitive. If the *Ch* allele is carried on either the standard chromosome 1 or the standard chromosome 2, it is expected to be transmitted to progeny only if it is transferred by crossing over to that one of the three interchange chromosome (2^1, 1^B or $B^{1,2}$) which carries the locus. The frequency of that transfer will be a function of the recombination distance between *Ch* and the adjacent interchange point.

The F1 hyperploid offspring produced from the initial cross onto the *Ch* female stock are then crossed onto the tester stock, *v4*, to verify the A-B nondisjunctional capability and onto a nonchocolate tester strain for linkage analysis. As explained in the previous paragraph, if the *Ch* allele has been introduced into the F1 hyperploid on a standard chromosome 1 or a standard chromosome 2, linkage analysis of the testcross progeny is expected to reveal transmission of the *Ch* allele to progeny to be significantly less than 50%.

From an initial cross of a *Ch* female stock by TB-1Sb-2L4464, hypoploid progeny would be of the constitution 1 2(*Ch*)/$2^1 1^B$. From such hypoploids the only chromosome combination transmissible through either female or male gametophytes is 1 2. If the locus of *Ch* is represented on a $B^{1,2}$ chromosome, inasmuch as the hypoploid plant lacks this chromosome, there is no possibility of the transfer of a *Ch*$^+$ allele to a standard chromosome 1 or a standard chromosome 2 by crossing over. As a result, self-pollinating an hypoploid plant will yield all *Ch*/*Ch* progeny and

testcrosses to nonchocolate in either direction will yield all *Ch/Ch*$^{+}$ progeny. If the *Ch*$^{+}$ allele is present in the hypoploid plant on either the 2^{1} or the 1^{B} chromosome, it may by crossing over be transferred to a standard 1 or standard 2 chromosome and be transmitted to progeny with less than 50% frequency.

In brief, when a compound A-B translocation is used to test for location of a dominant gene, the immediate positive evidence of linkage does not distinguish which of the two possible chromosome assignments is correct. However, the question may be resolved in other ways by further evidence from A-B translocation crosses. If there is linkage of *Ch* to TB-1Sb-2L4464, but not to TB-1Sb itself, then *Ch* is assigned to chromosome 2. Alternatively, if *Ch* is linked to TB-1Sb-2L4464 and to a second compound involving chromosome 2 and a chromosome other than chromosome 1, then *Ch* is assigned to chromosome 2. Finally, *Ch* is assigned to chromosome 2 if a simple A-B translocation involving chromosome 2 shows linkage.

TB-1La-4L4692 may be used to test for the chromosome location of the dominant allele *Tu* (tunicate; 4L). In the initial cross, a standard chromosome female parent carrying the *Tu* allele is pollinated by hyperploid TB-1La-4L4692 to produce hyperploid progeny plants carrying the *Tu* allele: $1\ 4/4^{1}1^{B}B^{1,4}B^{1,4}$. The nondisjunctional capability of hyperploids may be demonstrated when crosses onto female *c2 R-scm2* tester yield some progeny kernels simultaneously displaying colorless aleurone and colored scutellum. The same tested hyperploid F1 plants may be crossed onto a nontunicate tester strain for linkage analysis. Linkage and transmission characteristics follow the same pattern as detailed for TB-1Sb-2L4464, in that F1 tunicate hyperploid plants produce balanced microspore nuclei whose chromosome complement ($4^{1}1^{B}B^{1,4}$) consists entirely of interchange chromosomes. In testcrosses of tunicate hyperploid plants onto nontunicate female testers, linkage of the *Tu* locus to the compound TB-1La-4L4692 is shown by transmission of the *Tu* allele to be significantly less than 50% of progeny plants.

We have preliminary data involving the hyperploid method that confirm, or are consistent with, the location of three dominant genes; *Ch* (chocolate; chromosome 2L), *Tu* (tunicate; chromosome 4L) and *Ts5* (tassel seed-5; chromosome 4S). The respective A-B translocation stocks used were TB-1Sb-2L4464, TB-1La-4L4692 and TB-4Sa. In each case small kernels were taken from ears of the initial cross to select for hyperploid plants that were in turn crossed as male parents onto vigorous nonmutant tester plants.

From the testcross of a single F1 *Ch* hyperploid plant a total of 43 plants were scored; four had chocolate pericarp and 39 were normal. Included were five hypoploids, all normal.

There were also 43 plants in the testcross progeny of a single F1 *Tu* hyperploid. Six were tunicate and 37 were normal. Included were six hypoploids, all with normal phenotype.

There were 56 plants in a testcross progeny of a single F1 *Ts5* hyperploid. Eight were tassel seed and 48 were normal. There were only two hypoploid plants, both normal.

In all three cases the significant departure from a 1:1 ratio for mutant:normal is consistent with the established location of *Ch* in chromosome 2 and of *Tu* and *Ts5* in chromosome 4.

We are currently testing the F1 hypoploid procedure for location of dominant genes. Both this and the F1 hyperploid method described above are being used to locate *Rf* genes that restore male fertility in cms-S and cms-C strains.

Correlation of tassel and ear reversion events in cms-S

--Susan Gabay-Laughnan and J. R. Laughnan

In maize the first vertical division of the embryo initial determines the right and left halves of the plant, a plant that is bilaterally symmetrical through the midribs of the leaves. This pattern of development was deduced by studying gamma- and X-ray induced losses of chromosome segments in dry seeds whose embryos were heterozygous for various marker genes (Steffensen, Amer. J. Bot. 55:354, 1968; Coe and Neuffer, in The Clonal Basis of Development, pp. 113-129, 1978).

Plants in the M825 inbred background have an average of over 35 tassel branches and ten percent of cms-S plants exhibit spontaneous cytoplasmic reversion to fertility, expressed as fertile-sterile tassel sectors or totally fertile tassels in families of plants expected to be all male sterile. Only plants with large tassel sectors, in which the main rachis is included in the reversion event, or those with totally-fertile tassels have been observed to have correlated reversion events in their ears. We have carried out a study of plants with these two types of events in stocks of cms-VG, cms-I, cms-ML and cms-RD WF9, cms-S 38-11, cms-VG N6, cms-VG K55 and cms-ML M14, all of which have been converted to the M825 nuclear background by ten recurrent backcrosses using M825 inbred line as the male parent. Ears on such plants were pollinated to determine whether they were included in the reversion events; the progeny were scored as male-fertile, male-sterile or mixed (both fertile and sterile). Tassels of plants with large sectors were mapped to determine the positional relationship of the sector with the ears borne on these plants. Ears subtending the fertile tassel sectors were considered to be "in", those subtending the sterile portion of a sectored tassel were considered to be "out", and in a third class ears were judged near enough to the predicted fertile-sterile tassel interface to be considered "on the border".

The studies by Steffensen and by Coe and Neuffer referred to above predict that fertile tassel sectors larger than one-half of the tassel are not likely to occur. Such fertile sectors were observed, however, in about 25% (12 cases) of the 52 plants with large tassel sectors that were studied intensively. Tassels of these 52 plants with large fertile sectors were mapped and ears on these plants were pollinated by M825 maintainer (nonrestoring) plants:

Position of ear	Male sterile	Male fertile	Mixed
Out	21	0	0
Border	13	1	1
In	5	8	3

The result from the 16 "in" cases was unexpected but, as it turned out, not inconsistent with the results obtained from ears borne on plants with totally fertile tassels. These were also expected to produce entirely fertile progeny. Of 36 such plants analyzed, only 22 produced entirely male-fertile progeny. Four produced mixed progeny and ten produced male-sterile progeny. A total of 31% of the ears considered to be "in" large fertile sectors, and 28% of the ears borne on plants with totally fertile tassels gave male-sterile progeny.

The above observations indicate a correspondence between male-fertile "in" tassel sectors or entirely fertile tassels and male-fertile progeny from ears on such plants crossed with nonrestoring pollen; in other words, in these cases the male-fertile products of the cytoplasmic reversion event are found in both tassel and ear of the same plant. Even so, in about 30% of plants with this type of tassel fertility the progeny from their crossed ears were male-sterile. Why is there not perfect correspondence between tassel and ear fertility in these cases? We believe the answer lies in the timing of the primary event that leads to fertility. If it occurs in the mother cell that divides to produce the two daughter cells that define right and left halves of the mature plant, or in one of those two daughter cells, both tassel and ear initials should carry the reversion, and correspondence of male-fertile elements in tassel and subtending ear is expected. On the other hand, if the reversion event occurs much later, after cells ancestral to tassel primordia and those ancestral to ear initials are defined, noncorrespondence between male-fertile elements in tassel and ear is expected. This model is consistent with numbers of instances in which ears on male-sterile plants crossed by maintainer plants have produced male-fertile progeny.

The theory of midrib symmetry for the corn plant is not consistent with the position of all fertile tassel sectors, numbers of which, including large ones, have been found to overlap the midrib borderline. It might be argued that "sorting out" of mitochondria can explain these observations since the theory was developed as a result of studies of sectors that occurred following the loss of nuclear genes. We think this is unlikely since such "sorting out" of organelles should still exhibit correspondence with cell lineage patterns; it is more likely that these "nonconforming" fertile tassel sectors result from discrepant or noncoincidental patterns of cell division during very early stages of embryo development.

Are *Mu*-homologous sequences present in mitochondria?

--Carol Leja, Susan Gabay-Laughnan and J. R. Laughnan

Several inverted and direct repeats are known to be present in the mitochondrial genome of maize. Homologous repeats may recombine with each other and, consequently, the DNA is reorganized. Cytoplasmic reversion in cms-S lines of maize is associated with loss of specific repeat sequences and/or reorganization of contiguous DNA sequences. Two experiments were performed to determine the following: (1) whether or not sequences homologous to the terminus of *Mu*, which may serve as additional sites for recombination, are present in the mitochondrial genome; (2) if present, do the termini indicate the presence of complete elements that could be associated with apparent loss and reappearance of mitochondrial DNA sequences, and (3) if present, do these elements account for the highly unstable nature of a WF9-RD cytoplasmic revertant.

Mitochondrial DNA from WF9-RD sterile and a corresponding cytoplasmic revertant (cyto-rev) was digested with *Hin*dIII and *Eco*RI. The *Hin*dIII and *Eco*RI digests were electrophoresed in a 1% agarose gel and blotted onto a nylon membrane. The Southern blot was probed with a clone obtained from Vicki Chandler. The clone, pDTE1, contains approximately 220 bp of the *Mu* terminus. No homology to the *Mu* terminus was detected in the mitochondrial DNA.

Mitochondrial RNA from WF9-N x M825[10], WF9-RD sterile, WF9-RD cyto-rev, WF9-RD cyto-rev x M825[7] (fertile) and WF9-RD cyto-rev x M825[8] (sterile) was electrophoresed in a 1.2% agarose gel and blotted onto a nylon membrane. When the Northern blot was probed with pDTE1, no hybridization signal was obtained.

The reappearance of the R repeat

--Carol Leja, Susan Gabay-Laughnan and J. R. Laughnan

Mitochondrial DNA (mtDNA) from cytoplasmic male-sterile maize of the S-type (cms-S) is characterized by the presence of two plasmid-like, double-stranded, linear DNA molecules of low molecular weight, S1 and S2. The relative amounts of S1 and S2 in cms-S strains are determined by the nuclear background. In most inbred lines with cms-S, S1 and S2 are present in equimolar amounts. However, M825 maintains S1 in greater amount relative to S2, and 38-11 maintains the reverse relationship (Stadler Symp. 13:93, 1981). The S1 and S2 molecules have terminal inverted repeats (IR) of 208 bp.

Cytoplasmic reversion in M825 and 38-11 is characterized by the loss of the S1 and S2 molecules and rearrangements of S2 or S1 homologous sequences in the main mitochondrial genome, respectively. The S1 and S2 targets for recombination, specifically the IR-homologous sequences present in the *sigma*-R and *psi*-R regions of the main mitochondrial genome, are not affected (Theor. Appl. Genet. 76:609, 1988). However, linear chromosome termini which have homology to the R repeat, the 2kb sequence common to regions of the main mitochondrial genome termed *sigma'* and *psi'* and that contains the IR-homologous sequence, are lost (Theor. Appl. Genet. 76:609, 1988; Phil. Trans. R. Soc. Lond. Ser. B 319:149, 1988; Nature 310:292, 1984).

In contrast, cytoplasmic reversion in the inbred line WF9 is characterized by the maintenance of S1 and S2 in equimolar amounts and the lack of rearrangements involving S1 and S2 homologous sequences in the main mitochondrial genome (Theor. Appl. Genet. 75:659, 1988). Interestingly, it is the S1 and S2 targets for recombination that are lost (Theor. Appl. Genet. 76:609, 1988).

To further characterize the molecular aspects of cytoplasmic reversion involving the R repeat, two sibling, WF9-RD cytoplasmic revertants (cyto-revs) were converted to the M825 nuclear background by backcrossing with M825 as the recurrent male parent. After the first backcross to M825, some exceptional sterile plants were obtained from one of these cyto-rev sources. Both the fertile and sterile plants were propagated by continued backcrossing to M825.

MtDNA from WF9-N x $M825^{10}$, WF9-RD (sterile), WF9-RD cyto-rev 81-47-13 (fertile), WF9-RD cyto-rev 81-47-15 (fertile), 81-47-13 x $M825^{6}$ (fertile), 81-47-15 x $M825^{6}$ (fertile), 81-47-15 x $M825^{6}$ (sterile), 81-47-15 x $M825^{7}$ (sterile), and 81-47-15 x $M825^{6}$ (sterile; independent case) was digested with *Bam*HI and electrophoresed on a 1% agarose gel and blotted onto a nylon membrane. The Southern blot was probed with IS1E5, a clone specific for the R repeat. Consistent with the findings of Small et al. (Theor. Appl. Genet. 76:609-618, 1988), the sibling WF9-RD cyto-revs lost the S1 and S2 target sites for recombination (See Figure 1, lanes 3 and 4.). However, in contrast to the WF9-S cyto-rev described by Small et al., the chromosome termini (R*) were also lost.

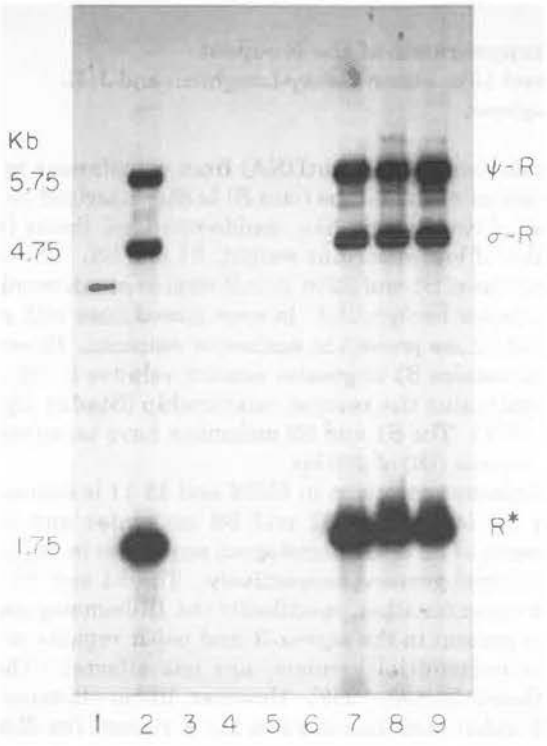


Figure 1. Hybridization of IS1E5 (specific for the R repeat) to a Southern blot of *Bam*HI-digested mtDNA. Lanes: (1) WF9-N x $M825^{10}$ (2) WF9-RD (sterile) (3) WF9-RD cyto-rev 81-47-13 (fertile) (4) WF9-RD cyto-rev 81-47-15 (fertile) (5) 81-47-13 x $M825^{6}$ (fertile) (6) 81-47-15 x $M825^{6}$ (fertile) (7) 81-47-15 x $M825^{6}$ (sterile; independent case from that in lanes 8 and 9) (8) 81-47-15 x $M825^{7}$ (sterile) (9)81-47-15 x $M825^{6}$(sterile).

Even after conversion of the sibling WF9-RD cyto-revs to the M825 nuclear background, the male-fertile plants neither lost the S1 and S2 episomes nor regained the target sites for recombination normally retained by M825 cyto-revs (lanes 5 and 6). Reversion to fertility in WF9, however, did not affect the ability of S1 and S2 to change their relative copy number in response to the M825 nuclear background. The relative abundance of S1 and S2 was that characteristic of M825 (MNL 62:109, 1988).

The exceptional sterile plants obtained after the first backcross of the sibling WF9-RD cyto-revs to M825 that were continually backcrossed also had S1 and S2 in the relative abundance characteristic of M825. However, these sterile plants apparently regained the S1 and S2 target sites for recombination and the R* linear chromosome termini (lanes 7-9). This observation is similar to the findings of Wang and Gengenbach (Maydica 34:217, 1989), who noted the appearance of the *S-atpA-3* gene from progenitors in which it was not detected. A plausible explanation for the loss and reappearance of the R repeat is the reduction of the sequence to undetectable levels followed by selective amplification.

The effect of the nucleus on mitochondrial transcripts

--Carol Leja, Susan Gabay-Laughnan and J. R. Laughnan

The nucleus has been demonstrated to have an effect on mitochondrial DNA (mtDNA). The relative amounts of S1 and S2 -- two self-replicating, linear DNA molecules present in the mitochondria of cytoplasmic male-sterile maize of the S-type (cms-S) -- are determined by the nuclear background. The nucleus also affects what changes occur in the mtDNA when male-sterile plants undergo cytoplasmic reversion to male fertility. Even in the absence of cytoplasmic reversion, however, the mtDNA can be altered by changing the nuclear background.

The effect of nuclear genotype on mitochondrial gene expression in maize is less well characterized. Studies have focused on the region of the mitochondrial genome implicated in cytoplasmic male sterility and toxin sensitivity in T cytoplasm. *Urf13-T* transcripts have been found to be altered in cms-T (T-type cytoplasmic male-sterile) plants into which the nuclear restorer gene, *Rf1*, had been introduced (Cell 44:439, 1986; PNAS 84:5374, 1987). Nuclear background, apart from the presence of fertility restoration genes, has been shown to affect the abundance of *urf13-T* transcripts and even to direct the synthesis of an ORF25-specific transcript (Mol. Gen. Genet. 210:399, 1987). The mRNA processing event that generates a 1.1kb transcript from ORF25 may involve more than one nuclear locus (Genome 30 (Suppl. 1):316, 1988).

We report here on the effect of nuclear background on mitochondrial transcripts of a gene that has not been implicated in cytoplasmic male sterility in S cytoplasm. A Northern blot of mitochondrial RNA from WF9-N x $M825^{10}$, WF9-RD (sterile; subtype of S), WF9-RD cyto-

plasmic revertant (cyto-rev; fertile) 81-47-15, 81-47-15 x M825[7] (fertile) and 81-47-15 x M825[8] (sterile; phenotype identified after first backcross to M825) was hybridized with clone TA22, a 4.2kb *Hin*dIII DNA fragment containing the *atp-alpha* gene and its flanking sequences as isolated from cms-T (Plt. Physiol. 79: 571, 1985).

Figure 1(a) shows the hybridization signal obtained after 19.5 hours of exposure of the hybridized blot to film. Two main transcripts are detected in the M825 nuclear background whereas the larger transcript predominates in the WF9 nuclear background. The sizes of the two transcripts are approximately the same as those described by Braun and Levings for cms-T (Plt. Physiol. 79:571, 1985). Also consistent with their data are the relative intensities of the two bands, the smaller transcript producing a more intense hybridization signal. Comparison of the TA22 hybridization signals with those obtained for a clone specific for the coding region of *atp-alpha* led Braun and Levings to suggest that the larger band (5,000 nts) may represent the primary transcript and the smaller band (2,600 nts) may represent the mature transcript. A more complex transcriptional pattern was noted in B37-N (Curr. Genet. 10:321-328, 1985). Three major transcripts, two of which approximate the two described by Braun and Levings, and several minor transcripts were detected. However, identical transcript sizes identical to those of B37-N were found in all male-sterile lines examined. The data for the M825

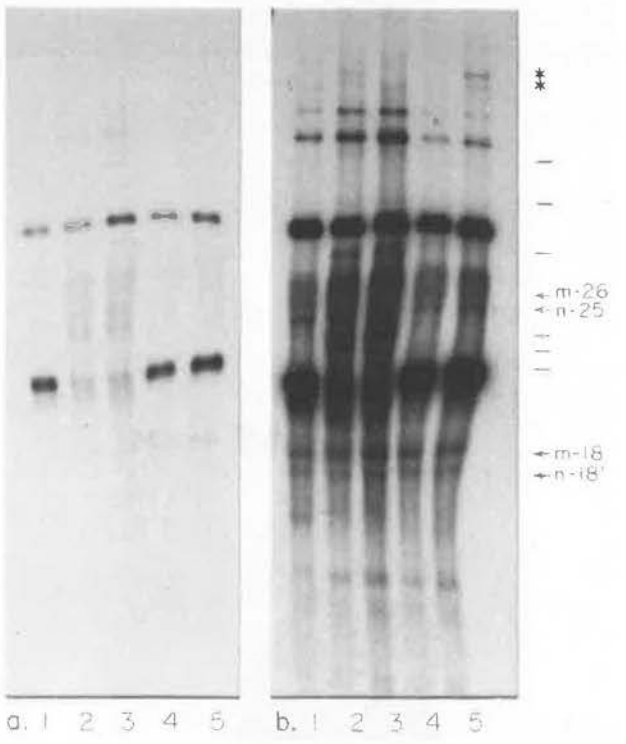


Figure 1. Hybridization of TA22 to Northern blot. (a) 19.5 hr. exposure. (b) 11 day exposure (same blot). Lanes: (1)WF9-N x M825[10] (2)WF9-RD (sterile) (3)WF9-RD cyto-rev 81-47-15 (fertile) (4)81-47-15 x M825[7] (fertile) (5)81-47-15 x M825[8] (sterile). (*) indicates transcripts which appear to be present in sterile plants only. (-) indicates transcripts which appear to be present in WF9 only (not all are indicated). Positions of rRNAs on gel are indicated with arrows. The approximate sizes of the nuclear (n) and mitochondrial (m) rRNAs are given in Svedberg units.

nuclear background shown in Figure 1a (lanes 1, 4 and 5) are consistent with the above findings. The two major transcripts detected in normal cytoplasm (lane 1) are also detected in S cytoplasm (lanes 4 and 5).

The fact that normal and sterile cytoplasms exhibit identical transcript sizes is more significant when one considers the existence of different genomic arrangements and varying copy number of the *atp-alpha* gene. Small et al. have characterized four arrangements based on variability in the 3' flanking sequence (EMBO J. 6:865, 1987). T cytoplasm lines contain predominantly one gene having the type 4 organization. C cytoplasm lines also contain predominantly one gene but it has the type 1 organization. On the other hand, most normal (N) and S cytoplasms contain two *atp-alpha* arrangements in equal abundance. Both WF9-N and M825-N mtDNA contains type 1 and 2 arrangements, whereas WF9-RD contains type 2 and 3 arrangements. Variability in the region 3' to the *atp-alpha* gene does not result in different RNA transcripts. Therefore, it appears that this 3' region is not transcribed.

The fact that the two major transcripts are much larger than the coding region of the gene (1,524 nts) suggests that these transcripts contain extensive 5' and 3' untranslated regions. Although the nuclear background does not appear to affect which of the *atp-alpha* genomic arrangements are predominant in any one cytoplasm, the nucleus does seem to exert an influence on *atp-alpha* transcripts. "Mature" *atp-alpha* transcripts are abundantly present in the M825 nuclear background. However, in the WF9 nuclear background (Figure 1a, lanes 2 and 3), very few, if any, "mature" transcripts are present. Interestingly, this lack of "mature" transcripts is not associated with an increased level of "primary" transcript. Instead, additional transcripts of various sizes are present (Figure 1b, lanes 2 and 3). Although an 11-day exposure of the Northern hybridized with *atp-alpha* reveals the presence of several minor transcripts in both the M825 and WF9 nuclear backgrounds, the pattern of transcripts is inherently more complex in the WF9 nuclear background. Also, some of the minor, high molecular weight transcripts present in both nuclear backgrounds appear to be more abundant in WF9. These transcripts may result from multiple initiation/termination events, post-transcriptional processing or recombination between transcribed regions of different mitochondrial genes. The two minor transcripts of high molecular weight, which appear to be present only in sterile plants, may represent substoichiometric, sterile-specific, genomic arrangements of the *atp-alpha* gene. Although some of the detected transcripts may represent other genes which have flanking sequences in common with the *atp-alpha* gene, the almost complete lack of "mature" *atp-alpha* transcripts in the WF9 nuclear background (Figure 1a, lanes 2 and 3) as compared with the M825 nuclear background (Figure 1a, lanes 1, 4 and 5) provides additional evidence for nuclear-cytoplasmic interaction. The WF9 nucleus appears to affect the expression of the *atp-alpha* gene in RD cytoplasm primarily at the level of post-transcriptional processing.

Methylation in maize mitochondrial DNA

--Gracia Zabala, Carmen Oliver, Susan Gabay-Laughnan and John R. Laughnan

Several researchers have reported the absence of methylation at the internal cytosine residue in 5'-C·C·G·G-3' sequences in mitochondrial DNA (mtDNA), based on the identical restriction patterns obtained with the pair of isoschizomers *Msp*I (cuts 5'-C·C·G·G-3'and 5'-C·m^{5}C·G·G-3') and *Hpa*II (cuts 5'-C·C·G·G-3' but not 5'-C·m^{5}C·G·G-3') (Groot and Kroon, Biochim. Biophys. Acta 564:355, 1979; Bonen et al., FEBS Let. 111:340, 1980; Borck and Walbot, Genetics 102:109, 1982). These results prompted the prevalent belief that mtDNA is, in general, unmethylated.

We have analyzed DNA methylation of mtDNA from a cytoplasmic male-sterile strain (cms-S) and a cytoplasmic revertant (CR) obtained from the sterile strain, using two pairs of m^{5}C-sensitive and -insensitive restriction enzyme isoschizomers, *Hpa*II and *Msp*I, as well as *Eco*RII and *Bst*NI. *Eco*RII cannot cleave the sequence 5'-C·m^{5}C·N·G·G-3' (N = A or T), whereas *Bst*NI can . Both enzymes digest the unmethylated sequence 5'-C·C·N·G·G-3'.

As in previous reports (Groot and Kroon, Biochim. Biophys. Acta 564:355, 1979; Bonen et al., FEBS Let. 111:340, 1980; Borck and Walbot, Genetics 102:109, 1982), we found no differences in the restriction patterns obtained with *Msp*I and *Hpa*II, indicating the absence of methylation of the CpG type. In contrast, the restriction patterns obtained with the pair of isoschizomers *Eco*RII and *Bst*NI revealed several differences. Figure 1 shows three DNA fragments of 8.3, 6.2 and 5.3 kb present in the *Eco*RII (E) digests of both sterile (S) and cytoplasmic revertant (CR) mtDNA which are absent from *Bst*NI digests of identical samples. A 3.0 kb fragment is also present in the *Bst*NI digests, but is missing from the *Eco*RII digests. Since reactions were carried out in the presence of 1mM spermidine to facilitate DNA digestion, differences in restriction patterns between samples digested with these two enzymes are not the result of an artifactual partial digestion by *Eco*RII. Changes in DNA and enzyme concentration as well as incubation time did not alter the digestion patterns shown in Figure 1. We have repeated this experiment with two other sets of mtDNA samples and the results have been consistent.

In addition to differences in restriction patterns observed in ethidium bromide-stained agarose gels, Southern blot analyses with mtDNA probes have revealed further

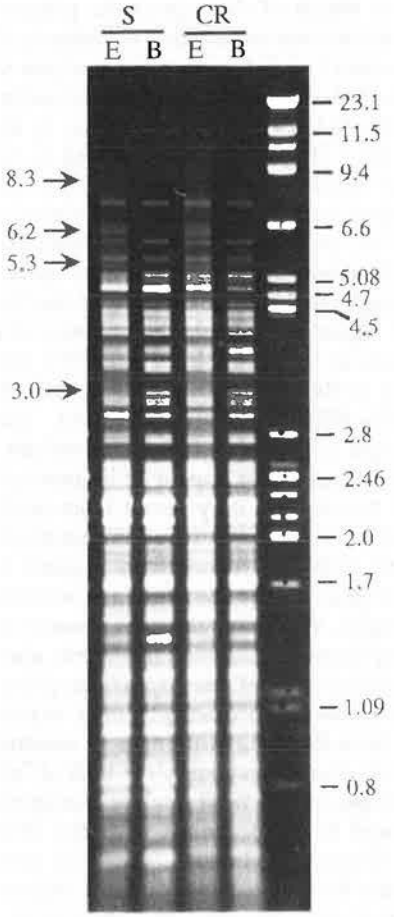


Figure 1. Ethidium bromide-stained agarose gel of *Eco*RII- (E) and *Bst*NI-digested (B) mtDNA of sterile (S) and cytoplasmic revertant (CR) samples. The arrows to the left of the figure indicate major differences in the digestion patterns of the methylation sensitive enzyme (*Eco*RII) and its isoschizomer *Bst*NI. Molecular weights are given in kb.

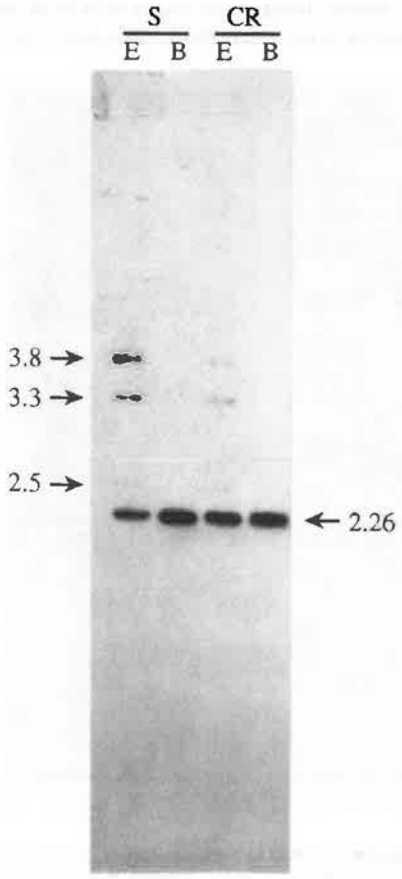


Figure 2. Hybridization of M2C1 (specific probe for the mitochondrial *cox1* gene) to a Southern blot of *Eco*RII- (E) and *Bst*NI-digested (B) mtDNA from sterile (S) and cytoplasmic revertant (CR) samples. The arrows to the left of the figure indicate DNA fragments that the methylation sensitive enzyme *Eco*RII does not digest but that its isoschizomer *Bst*NI does. Molecular weights are given in kb.

restriction fragment polymorphisms between the *Eco*RII and *Bst*NI digests. Figure 2 shows three bands (3.8, 3.3 and 2.5 kb) hybridizing to the probe M2C1 (specific for the *cox1* mitochondrial gene) in the *Eco*RII digests which are absent in the *Bst*NI digests. The low abundance of these additional bands hybridizing to the M2C1 probe in the *Eco*RII digests compared to that of the 2.26 kb unique hybridization signal in the *Bst*NI digests suggests that only a portion of *cox1* or its flanking sequences is methylated.

Southern hybridization analysis with the IS1E5 probe (a mtDNA sequence neighboring the *cox1* sequence) also revealed incomplete digestion with *Eco*RII (data not shown). In contrast, no significant difference was observed when Southern blots were hybridized to a mtDNA probe containing the *atp*A gene (data not shown).

Our results suggest that the mtDNA of six different maize samples has methylated sequences of the CpNpG type. We have found no differences in methylation between mtDNA from sterile and revertant samples. It will be of interest to determine whether methylation plays a role in transcriptional regulation during mitochondrial development or stress, as has been shown in the chloroplast of sycamore and tomato (Ngernprasirtsiri et al., PNAS 85:4750, 1988; Ngernprasirtsiri et al., Plant. Physiol. 88:16, 1988).

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Malonated flavonoids in maize

--O. Ceska and E. D. Styles

Two acylated anthocyanins in maize have been characterized recently by Harborne and Self (Phytochemistry 26:2417, 1987); they are: 1) cyanidin 3-(6"-malonylglucoside) and 2) cyanidin 3-dimalonylglucoside. Compounds acylated with aliphatic dicarboxylic acids are relatively unstable in solutions containing HCl. Standard extraction procedures using methanolic HCl result in intermediate methyl ester formation and the eventual loss of the acyl group. Substitution of a weaker acid such as acetic or formic acid allows for the extraction of the acylated compounds without change. In the past we have always extracted anthocyanin-containing tissues with methanolic HCl, and we have always obtained several anthocyanin spots on TLC chromatograms in addition to the simple cyanidin 3-glucoside. These extra spots were almost certainly the result of an interaction between HCl and the malonated anthocyanins. When we use acetic acid in aqueous methanol for the extraction we get only two acylated anthocyanins in addition to the spot of cyanidin 3-glucoside. In tissues that have predominantly pelargonidin compounds (*pr pr* genotypes) we find, as with the cyanidin compounds, the simple 3-glucoside of pelargonidin plus two acylated derivatives, and in tissues that have peonidin (cherry pericarp), we find the simple 3-glucoside peonidin, and again, two acylated derivatives.

Malonated anthocyanins can be easily separated by electrophoresis on Whatman No. 3 paper in acetate buffer of pH 4.4 at 40V/cm and 0.5mA/cm. The usual time quoted in the literature is 2 hours, but we have found that a much longer time is required for good separation (up to 18 hours).

When we analyze the methanolic extracts of tissues that normally have strong anthocyanin concentration but are brown because of *a1*, *a2*, or *bz2* blocks, we consistently find two flavonol compounds in addition to the quercetin and isorhamnetin 3-glucosides. They behave similarly on TLC but their Rf values in 15% acetic acid are slightly higher than those of the other flavonols. Only the flavonol based on quercetin was isolated in sufficient quantity for partial characterization. Total acidic hydrolysis as well as enzymic hydrolysis gave quercetin and glucose. Diagnostic UV spectra were identical with quercetin 3-glucoside. On chromatography in water the Rf value of quercetin 3-glucoside was 0.8 whereas the Rf value of unknown flavonol was 80, suggesting the presence of acyl group. Paper electrophoresis under the same conditions as described for malonated anthocyanins was used to find out if malonic acid was present. As a control, crude methanol-acetic acid-water (8:1:1) extract of cherry *Pl* pericarp containing malonated anthocyanins was used. Anionic mobilities were 2.5 cm for flavonol, 2.5 cm for cyanidin 3-malonylglucoside and 5 cm for cyanidin 3-dimalonylglucoside. This confirmed the presence of malonic acid in quercetin 3-glucoside. However, on saponification no malonic acid could be detected, probably due to the small amount of compound available. FAB mass spectrum gave clearly [M]+ = 551 which is molecular weight of quercetin 3-monomalonylglucoside but fragmentation indicating loss of malonic acid and mass of the aglycone did not show well. Again, the amount of compound was probably inadequate. Work is now in progress to isolate more of this flavonol so saponification and FAB MS can be repeated.

Malonylation is considered an important step because it appears to stabilize anthocyanins in the acidic environment of the vacuole (Harborne, Phytochemistry 25:1887, 1986) and also it may facilitate the transport of flavonoid glucosides through the tonoplast into the vacuole (Mattern et al., Planta 167:183, 1986).

Malonated anthocyanins are fairly widespread (Harborne, Phytochemistry 25:1887, 1986), but only a few malonated flavonols have been reported. Quercetin 3-malonylglucoside, together with other malonated flavonoids, were produced by parsley cell cultures after irradiation with UV (Kreuzaler and Hahlbrock, Phytochemistry 12:1149, 1973). Quercetin 3-malonylglucoside was also reported by Woeldecke and Hermann (Z. Naturforsch. 29C:335, 1974) and more recently by Geslin and Verbist (J. Natur. Prod. 48:111, 1985) from *Salicornia europaea*.

It is not clear why malonated quercetin 3-glucoside should be produced in maize only when there is a block in anthocyanin synthesis. Perhaps malonyltransferase can accept other substrates, in this case quercetin 3-glucoside, if no anthocyanins are available. Or perhaps malonated quercetin 3-glucoside is formed as a reaction of the plant to obvious stress. In the case of the above-mentioned genotypes, brown pigments form in the place of anthocyanins, and frequently their accumulation is accompanied by tissue necrosis, the ultimate stress response.

Modifying the tissue specific expression of some *R* alleles

--E. Derek Styles

In last year's News Letter (p. 124) I described how the expressions of *R* alleles may be limited, enhanced or modified by factors other than the so-called complementary genes. This is a follow-up to that article, reporting on the expression of some *R* alleles in the presence of *a3* and/or *Pl*.

R-mb: a) with *A3* and *pl* (normal for W22 lines) and *b*: marbled aleurone, slight pigment in coleoptile tip, green plant otherwise, including anthers; b) with *b*, *a3* or *Pl*: similar to above except that the anthers have scattered pigment, distributed more or less longitudinally.

R-scm ('self-color' mutations from *R-mb*): a) with *A3*, *pl* and *b*: full colored aleurone, but otherwise similar to *R-mb* in plant color; b) with *b*, *a3* or *Pl*: scattered pigment in anthers with either *a3* or *Pl*. Red plant tissues with *a3*.

R-nj: a) with *A3*, *pl* and *b*: aleurone pigment mostly in crown, red seedlings, anthers and silks, some glume and brace root pigment, otherwise green plant; b) with *a3* and *Pl*: aleurone, seedlings, anthers and silks as above, sheath edges and leaf midribs pigmented, cherry pericarp.

R-nj-6 (a compound allele derived by R. A. Brink from *R-st:nj* (stippled crown)): a) with *A3*, *pl*, and *b*: uneven aleurone pigment, mostly in the crown, green plant and anthers; b) with *Pl*: aleurone pigment as above, green plant, but red anthers. *a3 Pl* combination to be tested next year.

The above information on the Navajo alleles supplements our report on these alleles in the 1973 News Letter (Kyle and Styles, MNL 47:184) where we noted that the pericarp of developing *R-nj:Cudu* and *R-nj-6* seeds developed pigment when the seeds were removed from the cob and placed under light.

The point in reporting on the expressions of these *R* alleles is to re-emphasize the point I tried to make last year, i.e., if *R* is a 'regulatory locus', it is important to recognize those factors that can regulate the regulator. Beyond differences that can be demonstrated when different alleles are compared against a common genetic background are differences that can be demonstrated only when different alleles are compared against different genetic backgrounds. Thus some alleles seem to increase the limits of their tissue specific activities with *a3* and/or *Pl*, whereas others do not. What part of the locus is responding? What is the difference between those alleles that respond and those that do not? And is the response simply that of boosting a subliminal capacity, or is it creating or releasing a different tissue specific potential?

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Anther size vs. glume size, a delicate balance

--Walton C. Galinat

The cooperative action of genes toward achieving success in a common objective is no better illustrated than the association between independent genes for anther and glume size in the male flowers. An increase or decrease in one component must be followed by a corresponding change in the other or functional male sterility will result. When the glumes are too big for their anthers, the anthers may not emerge to disperse their pollen. If the glumes are too short, the anthers are prematurely exposed with lethal sun-burning the consequence.

Both the anthers and glumes of the annual teosintes of Mexico are smaller than those in corn except for vestigial glume (*Vg*) corn. One of the viability problems of *Vg* corn is that the immature anthers are exposed to sun-burning and blasting. Why, then, not transfer the small anthers of teosinte into *Vg* corn? We have been attempting to do this for about five years, generally with unsuccessful results except for one lineage. In most cases the small anthers of teosinte appear linked to small glumes that have an additive effect with the *Vg* gene, producing an inviable combination. Apparently with the exceptional case, the small anthers and small glumes of teosinte have been separated by crossing over. Some promising isolates have been made that should lead to improved fully male-fertile lines that are homozygous *Vg*.

Increased transmission of full vegetative multiranking through pollen from the central spike of the tassel in comparison with pollen from the lateral branches

--Walton C. Galinat

Pollen from different parts of the same tassel was used to self-pollinate different ears on the same plant with full expression of vegetative multiranking (full *mrv*). Of 144 plants derived from pollination by the central spike with floral multiranking, 4.4% had full expression of vegetative multiranking. Of 126 plants derived from pollination by the lateral, two-ranked branches from the same tassel onto the other ear borne on the same plant, only 1.8% showed full expression of vegetative multiranking. The difference in transmission rate is attributed to an epigenic difference in ranking between the central spike of the tassel and its lateral branches. In the presence of the *mrv* gene, this difference in ranking tends to be carried over into the next generation as if pollen from the central spike was not fully set back to two-ranking for the next cycle. In teosinte there is no need for a reversion system for phyllotaxy because both the floral and vegetative phases are two-ranked. The *ub* (unbranched tassel) gene is being transferred to the *mrv* stocks in an attempt to stabilize the *mrv* expression in the vegetative phase.

Coroico as a resource for maize improvement

--Walton C. Galinat

Long separated and isolated from the main pathways of corn's evolution, the race Coroico has retained at least two primitive genes, *is* (cupulate interspace) and *tpe* (thin pericarp) that are now unknown in Mexican corn, although they appear to occur in the oldest Tehuacan maize (7,200 years) and do occur in the teosintes. The *is*

gene segregates out in the F2 of corn-teosinte hybrids as a single recessive gene. Allelism tests between *is* from teosinte and Coroico have not yet been made. Apparently the Guarany Indians from the interior lowlands east of the Andes in Bolivia, Peru and Brazil received and cultivated the primitive ancestor of Coroico several thousand years ago soon after its spread southward from Mexico. In their hands, Coroico evolved at least two other associated traits that are unique and of potential economic importance.

The interspace (*is*) factor exposes a bare face of rachis between the apex (upper lip) of the cupule and the glume cushion of the diverging spikelet(s) above. Botanically, the interspace represents the backside of the internode from the alternate rachis segment (phytomer). When condensation is sufficiently low for the length of the interspace to become equal to the length of the cupule below, then there is enough extra space to interlock the pedicellate member from an adjacent pair while the sessile member stays in line with its cupule row. The result is a 50% reduction in the kernel-row number. The most common kernel row number in Coroico is nine, on long slender ears with enlarged butts that are 18-rowed.

The exposure of the kernel from the teosinte fruitcase required that it adapt by evolving two new systems of kernel protection. An inner system of either a thickened pericarp or a thickened aleurone that would guard against "self-popping" of mature kernels due to weather and, secondly, a new outside system of husk leaves for varmint protection. The Coroico kernels have the primitive thin pericarp caused by the *tpe* gene of only three to four cell thickness. When expressed in a teosinte background, where apparently the *tpe* of Coroico originated, the pericarp is only two to three cells thick and resistant to self-popping. Viability of teosinte seed is, thereby, insured by the physical support derived from containment within the fruitcase. Protection of the exposed kernel from self-popping in Coroico under the direction of the Guarany Indians in South America has taken the aleurone pathway while in Central America the pathway was by the pericarp. Sweet corn improvement based on a tender or thin pericarp may be best achieved by use of the Coroico complex (see my item here on multi-layered expression). The *tpe* gene is incompletely dominant and in some backgrounds it may be inviable due to pericarp splitting and pathogen infection. The multiple aleurone factor may be inherited as a single dominant gene (Wolf et al., Crop Sci. 12:440, 1972).

Multi-layered expression of aleurone-specific genes
--Walton C. Galinat

In the process of recombining the two-celled pericarp from teosinte with the multiple layered aleurone from Coroico in a sweet corn background, a faint blue color was detected. In addition to the prime components of selection, the blue color was held onto in the hope that it would be an easy tool to identify the multiple-layered segregants. In fact, this has proven to be the case. As the number of aleurone layers increased, there was a dosage effect on increases in the intensity of blueness. Freehand sections through the kernels showed the blue was present in all layers. It may be expected that other aleurone-specific genes will have dosage effects in the multi-layered aleurone and, thereby, increase the total output of their special phenotype. This dosage effect with increased layering appears also to be manifest with genes for the bronze or orange colored aleurone that is common in Coroico. The most primitive kernels from Tehuacan had this same bronze-orange aleurone; identified as the race Nal Tel, it may have been the distant ancestor of Coroico. The Tehuacan kernels were not sectioned and Nal Tel is not known at present to have multiple aleurone. It seems probable that the multi-layered condition evolved under the eye and mind of the Guarany Indians. The aesthetic beauty of these intensely orange ears with multi-layered aleurone was probably the direct reason for their selection by the Guarany Indians. But the multiple aleurone may also have other benefits not initially intended such as increased protein in an opaque-2 background (Nelson and Chang, Crop Sci. 14:374, 1974) and a potential to cope with increased kernel size.

It is hoped that this stock with thin pericarp and thick aleurone will not only result in new high quality sweet corn but that it will also be useful in studies of gene action in the aleurone and of the chemical improvement of the nutritive value of this digestible layer or layers.

Seed of my blue multi-aleurone, thin pericarp stock has already been supplied for research purposes to Dr. Prem Chourey (Univ. of Fla., Gainesville) for studies of gene action and to Dr. Victor Raboy (Montana State Univ., Bozeman) for studies of phytic acid.

The sweet corn inbred Illinois 677a is reported by Dusty Rhodes to contain some Coroico germplasm. The inbred has been important as a source of the *se* (sugary enhancer) gene. It has only a single aleurone layer, unlike Coroico.

The combination of *Ma* and *Tpe* such as occurs in Coroico with *sh2* (shrunken) endosperm is a better means to cope with the *sh2* germination problems than the currently used thick pericarp which gives poor eating quality.

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Utility of *Tpi3* and *Tpi4* variants in quality control of hybrid popcorn seed production
--P. K. Bretting and J. F. Wendel

Popcorn grown commercially in the U. S. is relatively depauperate in isozymatic variation as compared to dent corn, and is also isozymatically somewhat distinct from the latter (Stuber and Goodman, USDA/ARS Agric. Res. Results ARR-S-16, 1983). Isozymatic monitoring of quality in hybrid popcorn seed production therefore might require slight modifications of the methodology (Smith and Weissinger, MNL 58:103-105, 1984; Stuber et al., NCARS

Techn. Bull. 286, 1988) commonly employed with dent corn.

Electrophoretic analysis of maize TPI (triosephosphate isomerase) isozymes (Wendel et al., J. Hered. 80:218-228, 1989) suggested that "their usefulness as genetic markers is somewhat hindered by relatively low levels of polymorphism, particularly in domestic germplasm" (op. cit., p. 227). Nevertheless, a preliminary survey of popcorn inbred lines and hybrids revealed allelic variation at *Tpi3* and *Tpi4* that is useful for quality control.

For example, P608--a widely grown Purdue public hybrid--is the single cross between SG1533 and HP72-11, two lines fixed for different alleles (2 and 4) at *Tpi3* (Table 1). Plants in a seed lot of P608 with genotypes other than *Tpi3* -2/4 would thus be offtypes. Genotypes at other isozymatic loci would help classify the offtypes as selfs, outcrosses, or contaminants. Allelic variation at *Tpi4* may be similarly employed. Preliminary studies suggest that allelic variants at *Tpi3* and *Tpi4* also may be valuable for quality control of hybrid sweet corn production.

Table 1. *Tpi* genotypes[a] of selected U. S. popcorn inbred lines.

Inbred line	Tpi1	Tpi2	Tpi3	Tpi4	Tpi5
HP62-02	4/4	4/4	4/4	4/4	8/8
HP72-11	4/4	4/4	4/4	4/4	8/8
HP301	4/4	4/4	2/2	4/4	8/8
IDS53	4/4	4/4	2/2	4/4	8/8
SG1533	4/4	4/4	2/2	4/4	8/8
4722Ht	4/4	4/4	4/4	4/4	8/8
Proprietary 1	4/4	4/4	4/4	1/1	8/8

[a]Allelic and locus designations follow Wendel et al. and Stuber et al. (op. cit.)

We thank R. B. Ashman, A. Galbreth, and J. Sandage for their comments, and C. Shaner for technical assistance.

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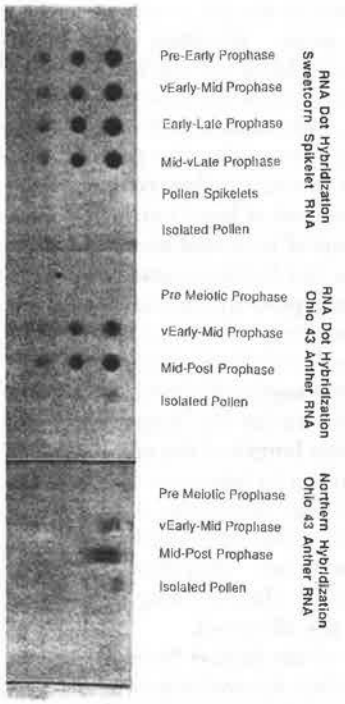
Stage-specific expression of small *hsp* gene RNA in anthers

--R. A. Bouchard and D. B. Walden

Two previous reports (MNL 60:71-72; MNL 62:114) described the recovery of a maize genomic clone showing homology to small *hsp* (heat shock protein) genes, based on its homology to cDNA clones for a major class of transcripts developmentally expressed during prophase of meiosis in lily. Evidence of sequence homology (P. S. Dietrich, R. A. Bouchard, E. M. Silva and R. M. Sinibaldi, J. Cell. Biol. 105:245a, 1987) and transient expression of reporter genes (Silva et al., J. Cell. Biol. 105:245a, 1987) both indicate that the isolate actually represents a maize small *hsp* gene, as does heat-induced accumulation of transcripts in seedling tissues (MNL 62:88). The question therefore arises as to whether this gene, or close relatives, might be developmentally expressed during the meiotic interval in maize in the absence of heat stress, just as corresponding



genes are in lily, as well as yeast and fruitflies (Lindquist and Craig, Annu. Rev. Genet., 1988).

We therefore isolated RNAs from staged premeiotic tassels, from anthers containing meiotic and haploid microsporocytes and microspores (see the accompanying report for procedures used to obtain this material), and from anthers containing mature pollen as well as from shed pollen. RNA was also prepared from spikelets, representing somewhat coarser staging. We have performed preliminary RNA-Dot and Northern hybridization studies on these RNAs to detect transcripts of the 18kd clone gene or closely related sequences. While further work will be required for precise kinetics, it is evident that transcripts homologous to the small *hsp* clone are absent in premeiotic tassels, accumulate substantially during the period of meiotic prophase with peak abundance during late prophase, and are virtually undetectable by the time mature pollen is present. These patterns are evident in the RNA-Dot analyses of Seneca 43 spikelet RNAs and Ohio 43 anther RNAs, and the accompanying Northern analyses of Ohio 43 RNAs only, as shown in the accompanying figure. While developmental expression of small *hsp* gene in the absence of stress certainly occurs in anthers containing PMCs of the appropriate stages, it cannot yet be associated with the prophase meiocytes unequivocally. Further work is planned to refine our picture of the timing of developmental expression, to identify whether developmental expression is a property of all or only some of the maize small *hsp* genes, and ultimately to determine whether developmental expression is in fact localized in the meiocyte.

Preparation of staged anther material for molecular analysis

--R. A. Bouchard and D. B. Walden

The development of the microsporocyte and the anther

containing it are central in the life cycle of all higher plants, including maize. The events occurring in the microsporocyte itself, from meiosis through differentiation of mature pollen grains, are crucial to the development of male fertility, as are vital changes that occur in coordinate fashion in the surrounding anther tissues. The system where the most progress has been made in the study of these events (particularly meiotic development) is the developing anther of lily. This is due to the unique advantages the system provides, a key aspect being the highly synchronized microspore development in lily, which is tightly correlated to external bud length (Erickson, Amer. J. Bot. 35:729, 1948), making it possible to sort large numbers of specific stages quickly. Another advantage is the relatively large quantity of staged material which can be obtained, making biochemical-scale preparations feasible. Previous efforts to extend these advantages to maize have focused on using probes derived from lily to recover cognate sequences from maize (MNL 60:71-72; MNL 62:114). We now report our first efforts at a more direct approach: the sorting of biochemical quantities of staged maize material in order to study microsporocyte and anther development at the molecular level more directly.

The chief difficulty in preparing staged material from maize is the lack of synchrony among developing anthers. In a single tassel, one may find anthers in spikelets of the lowest lateral branches containing microsporocytes in early meiotic phase, while microsporocytes of spikelets midway up the main spike will have already reached the uninucleate microspore stage. Spikelets of a particular portion of the tassel, such as the central spike, are in closer synchrony with one another. However, even at the level of the individual spikelet, the existence of two florets at different points of growth means that two different stages of anther and microsporocyte development are represented. We find, in agreement with Chang and Neuffer (Genome 32:232, 1989), that gross morphological characteristics, such as tassel length or distance from base of plant to base of tassel, are not particularly useful even for gross tagging. Moreover, in contrast to lily, length of the "bud" (the glume of a floret) is not a reliable index of anther stage in our hands. We find that only anther length itself, determined for a sample of anthers from the tassel actually being processed, is a sufficiently sensitive guide for reasonably fine-scale sorting of material.

To prepare staged anther material, we begin by harvesting whole plants early in the day. These are quickly returned to the laboratory, and the tassels are dissected out and divided into central spike and lateral branch portions. These are placed in large petri dishes containing Kimwipes moistened with distilled water and held on ice until processed. Initial spikelet samples for anther staging are removed from the upper, middle, and lower portions of the central spike, and from the upper and lower regions of the main lateral branches. Lengths of the spikelets, and of the three anthers from each floret of a spikelet, are determined using the ocular micrometer of a dissecting microscope. The stage of anther development is determined from an aceto-orcein squash: "stage" being defined as the stage of meiosis or microspore development of the microsporocytes of the anther. The stages used by Chang and Neuffer were generally employed, except that meiosis could sometimes be partitioned into early-middle (leptotene-zygotene) and middle-late (pachytene-divisions) substages. A usable anther length/developmental stage profile of the tassel can be produced in this way in a couple of hours. Once this has been obtained, the information can be used to sort anthers from the tassel of origin for approximate stage by their external lengths alone.

Since a number of hours are required for this bulk sorting, it is essential that the material be kept chilled and humid at all times. We place the base of a dissecting microscope used for this in a bed of crushed ice and process groups of spikelets on microscope slides sitting directly on the chilled stage. Each spikelet is opened under magnification using the beaded ends of sealed glass needles made from drawn-out Pasteur pipettes, and the triad of anthers in each floret is freed by crushing the undeveloped filaments, which leaves the anthers themselves undamaged. The length of each anther (or the whole triad, if as often happens all three are the same length) is checked with the ocular micrometer and the anthers are pushed into the appropriate pile at the base of the slide. If spikelets from a given portion of the tassel are processed together, all anthers will fall into two or occasionally three adjacent stage classes. At intervals, we collect the piles of anthers of each stage in marked, pre-weighed Eppendorf tubes, determine and record the weight of anthers in each tube, and snap-freeze in liquid nitrogen. The material can then be safely stored at -70 C until needed. With experience, several hundred milligrams of anthers of a given stage can be collected from a single tassel in a few day's work, an amount sufficient for examining stage-specific differences at the levels of whole cell RNA preparations or major cellular proteins.

STAGE OF PMC DEVELOPMENT VS ANTHER LENGTH (MM) FOR 3 STRAINS

Stage of Development	Seneca 60	KYS	Ohio 43
Premeiotic PMCs. For all strains, the last wave of synchronous mitosis in the PMCs occurs as the anthers reach 0.75mm.	<1.25	<1.00	<1.00
Early Prophase (Leptotene through Zygotene)	1.25-2.00	1.00-1.75	1.00-1.75
Later Prophase (Pachytene through Quartet)	2.00-3.25	1.75-2.75	1.75-3.25
Uninucleate Microspore (To haploid mitosis I)	>3.25	2.75-3.75	3.25-4.25
Binucleate Microspore (To haploid mitosis II)	ND	3.75-5.00+	4.25-5.25+
Trinucleate Microspore (To mature Pollen)	ND	>5.00-9.00-	>5.25-10.0-

For greatest accuracy, as noted, we found it best to stage a sample of anthers from every tassel processed before sorting its anthers. Nevertheless, there was a fair degree of consistency in the relationship of anther length to microsporocyte stage from plant to plant of a given strain over the course of the work. The table above gives the approximate relationships as observed in tassels of field grown plants, which represented several successive plantings of each strain, harvested from the last week in July through the third week in August at London, Ontario. We must stress, however, that the boundaries are approxi-

mate, and may well vary even for the same strains in other environments.

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Character and inheritance of a new Y-type cytoplasmic male-sterile line

--Tai-chen Qin and De-xiang Dun

It is a useful approach to create new types of male-sterile lines by means of combining sterile cytoplasms of restorer lines with maintainer nuclear backgrounds. In this way, we have bred YII types of male-sterile lines. Research on their resistant reactions against HmT, fertility restoration patterns, hereditary traits, light microscopy, electron microscopic ultrastructure and biochemical character has been conducted rather systematically for several years. According to the results, the Y-type sterile line was roughly considered as a new one in maize and different from C, T and S groups. However, a further testing is needed before it can be accurately assigned to a group. The Y-type sterile line has shown some favorable characters, such as the stable inheritance of pollen abortion, readily being applied in hybrid production, possessing many restorer lines in multitudinous inbred lines and strong resistance against HmT and so on. It is regarded as a valuable germplasm source and has important application value in seed production.

Cytoplasmic male-sterility: identification of the number of the restorer genes

--Tai-chen Qin, Miang-liang Xu and De-xiang Dun

The number of restorer genes in two male-sterile cytoplasms, C-group and Y-type, were identified on the basis of differing restoration responses in F1 hybrids, as well as self and backcross progenies of 14 tester lines in C Huangze 4 and Y Mo17. The results indicated that three restorer gene loci are involved in the restoration of C-group and Y-type. Among them two gene loci, *Rf4* and *Rf5*, which showed full restoring capacity, are required both in C-group and Y-type for fertility restoration, and act in a duplicate dominant fashion. The third one, however, is not the same between C-group and Y-type (*Rf6* in C-group and *Rf7* in Y-type). Either *Rf6* or *Rf7* can partially restore pollen fertility and this is further modified by environment. Finally, the *Rf7* gene probably inhibits the expression of fertility restoration of the *Rf5* gene.

III. ZEALAND 1990

This is a summary of selected genetic research information (e.g., new factors; mapping; cloning) reported in this News Letter and in recent literature ("r" refers to numbered references in the Recent Maize Publications section). The Symbol Index refers by number to all current published research involving genetic materials; comments on this new aid would be welcome.
BS = Base Sequence; BSH = Broad Sense Heritability; gca, sca = general and specific combining ability; QTL = Quantitative Trait Loci; RM = Restriction Map; unc. = uncovered
* in symbols identifies loci needing allelism tests, documentation, or standardization of the symbol.

CHROMOSOME 1
Sod4, two cDNAs; BS; mapped near *P1* --Cannon & r67; Weber & r674
Kn1-2F11 contains *Ds2* insertion, genomic, RM; *Kn1-O* deletion --Hake & r226
D8, Mpl1 both 3-4 m.u. distal to *Adh1* and *lw1*, between T1-3(5267)(1L.72,3L.73) & T1-3(5242)(1L.90,3L.65) --Harberd & r234
Glb1-0, *-L*, *-I*, *-S*, *-V*; cDNA, BS --Kriz r326
P1-VV P1-RR-2 P1-RR*-1 P1-WW-1112*: genomic, RM --Lechelt & r349
Adh1-Cm, RM, BS --Osterman & r448
bz2 cDNA, RM --Theres & r627
TB positions, 1S through 1L: *NPI109 NPI234 P1* TB-1Sb *NPI304* TB-1La *NPI40 NPI236 NPI54 NPIPhy1 NPI82 NPI238* --Weber & r674
Mono-1, nulli-1 pollen development --Zhao & r712
PIO200044 - 5 - *hm1* - 5 - *PIO200644* --Johal & 64:36,37
idd-2286A*, indeterminate dwarf, unc. by TB-1La --Neuffer 64:52

CHROMOSOME 2
Ht1 near *UMC122* --Hoisington & r253
hcf106, Mu1 insert, genomic: RM; on 2 (B. Burr) --Martienssen & r384
B1 probed with *r1-nj:1* clone --Robbins & r515
TB positions, 2S through 2L: *NPI320 NPI57 B1* TB-3La-2S6270 *NPI11 NPISSu2* TB-1Sb-2L4464 *NPI123 NPI337 NPI210* --Weber & r674
Tpi2 unc. by TB-1Sb2L4464; *fl1* - 15 - *v4* - 17 - *Tpi2* --Wendel & r677
Mono-2, nulli-2 pollen development --Zhao & r712

CHROMOSOME 3
a1 & *a1-m1Cache rDt* element, *A1'* revertant: RM, BS --Brown & r49
vp1-mum1, *-mum2*, *-mum3*, *-w1*, *-w2*, *-w3*; clone, RM --McCarty & r398
TB positions, 3S through 3L: *NPIMe1 NPI249 NPI89 NPI219* TB-3Sb TB-3La *NPI83 NPI70 NPI90 NPI78 NPI212* TB-3La-2S6270 *NPI91* --Weber & r674
Tpi4 not unc. by TB-3Sb, TB-3La, TB-3Lf, TB3-Lg; *d1* - 12 - *Tpi4* - 11 - *Lg3*; *Pgd2* arm location uncertain --Wendel & r677
Mono-3, nulli-3 pollen development --Zhao & r712
D-1991*, semidwarf, linked with *wx1* T3-9c --Neuffer 64:51

CHROMOSOME 4
Cat3 on 4S --Smith r578
TB positions, 4S through 4L: (*NPI77 NPI27 NPI95*) TB-4Sa TB-1La-4L4692 *NPI250* TB-9Sb-4L6504 *NPI208 NPI36* TB-7Lb-4L4698 *NPI333* --Weber & r674
Zeins linked with *su1*, including zein-A1/30.5, -A1/37, -A2/28, -B8/32, -B8/54.5, -B6/36, -B7/17.5, -A2/60, -A3/14, -A3/19, -A3/33, -A3/33.5, -B9/49, -B9/35 --Wilson & r693
orp1 on 4S per TB-4Sa and TB-4Lf ratios in hypos and hypers; *orp1* - 1 - *su1*; *BNL15.45* - *orp1* - *BNL7.20L* --Wright & r700, 64:50
Mono-4, nulli-4 pollen development --Zhao & r712
Ms44 (was *Ms*-7255*), male sterile, *Ms44* - 14 - *Ms41* Albertsen & 64:52
se1 unc. by TB-1La-4L4692 (Labonte thesis); *se1* near *BNL15.07* and *NPI333* --Bredenkamp & 64:108
c2-m1 Spm, by inverse polymerase chain reaction & RFLPs, maps on 4L --Earp & 64:2
Ts5 - 1 - *dek7* - 5 - *su1* --Neuffer 64:52
su1 - *gl4* - 14 - *dek31* --Sheridan 64:64

CHROMOSOME 5
TB positions, 5S through 5L: *NPI409 NPI75 NPI282 NPI233* TB-5Sc TB-5La *NPI60 NPI115 NPI237 NPI74* --Weber & r674
NPI346-TPI (was "*Tpi5*") redesignated *Tpi6* (by ed.) --Wendel & r677
Mrh-37962 linked with *wx1* T5-9c --Dempsey & 64:31
pr1 - 29 - *Dap1* - 15 - *v2*; *pr1* - 6 - *v12* - 24 - *Dap1* --Stinard & 64:12
D-2319*, semidwarf, linked with *wx1* T5-9c; *Nl2* (was *Rgd2, Rgd*-1445*) renamed; *Nl2* - 10 - *a2* - 3 - *bt1* --Neuffer 64:52
b70 protein probe maps near *BNL1.380* --Shank & 64:97
sh5 - 17 - *v3* - 32 - *pr1* --Sprague 64:110

CHROMOSOME 6
Dehydration-induced protein (17 kDa, dehydrin) (*Dhn1*, *UMC170-DHN*, location on 6L), cDNA, BS --Close & r91
Mdm1, Maize dwarf mosaic virus R; *UMC85* - 0.4 - *Mdm1* - 0.8 - *BNL6.29* - 4.9 - *Y1* - 2.1 - *UMC59* - 36.6 - *UMC21*; MDMV R linked with *Y1* in several lines --McMullen & r400; Scott r558
TB positions, 6S through 6L: (*NPI235 NPI245*) TB-6Sa TB-6Lc *Y1 NPI223 NPI265* TB-6Lb *NPI252 NPI280* --Weber & r674
Mono-6, nulli-6 pollen development --Zhao & r712
sbd1 (was *wxl*-2292*), sunburned, unc. by TB-6L --Neuffer 64:52

CHROMOSOME 7
Rcm1, rectifier of teosinte-cytoplasm miniature seed/retarded growth; miniature pollen, gametophytic, closely associated; *Rcm1-w*, weak allele; *Rcm1* - 3.3 - *vp9* --Allen & r6
o2, BS --DiFonzo & r133; Hartings & r238; Maddaloni & r373

TB positions, 7S through 7L: *NPI28 NPI111* TB-7Lb *NPI216 NPI112* TB-7Lb-4L4698 *NPI283 NPI431* --Weber & r674
o2 - 20 - *gl1* - 23 - *Tpi1* --Wendel & r677
Zeins linked with T7-9(4363) & *y8*, including zein-D/55, -B9/22, -B9/10, -B8/38 --Wilson & r693
Mono-7, nulli-7 pollen development --Zhao & r712
BNL8.32 - (b32 probe) - *BNL7.61* --Bass & 64:97
Rs4 (was *Rs*-1606*), rough sheath, 1% recomb. with *wx1* T7-9(4363), 14% with *wx1* T7-9g --Neuffer 64:51

CHROMOSOME 8
TB positions, 8S through 8L: *NPI114 NPI110 NPI64 NPI37* TB-8La *NPIPdk2* --Weber & r674
Tpi3, *Mdh1* not unc. by TB-8Lc, *Idh1* unc. by TB-8Lc; *Tpi3* - 17 - *Mdh1* - 18 - *Idh1* - 29 - *Tpi5*; *NPI346* locus "*Tpi5*" on chromosome 5 redesignated *Tpi6* by ed. --Wendel & r677
Mono-8, nulli-8 pollen development --Zhao & r712
BNLAct1 - (b32 probe = *pro1*?) -*BNL2.369* --Bass & 64:97
Rf4 - 14 - *BNL13.05* - 12 - *BNL9.11* --Sisco 64:97

CHROMOSOME 9
c1 clone homologous sequences: cDNAs Zm1 & Zm38, BS --Marocco & r382
TB positions, 9S through 9L: *NPI253* (*NPI343 NPI211*) *sh1 NPI266* (TB-9Sb TB-9Sb-4L6504) (*NPI300 BNL3.06*) *wx1* TB-9Sd *NPIPep1* TB-9Lc (*BNL5.10 NPI454 NPI416 NPI222 UMC20*) *BNL7.13 NPI80 NPI293 NPICss2* TB-9La *NPI209 NPI97* --Weber & r674
Mono-9, nulli-9 pollen development --Zhao & r712

CHROMOSOME 10
r1-ch:Hopi recombination analysis negative --Racchi & r503
r1-nj:1 probe --Robbins & r515
TB positions, 10S through 10L: *NPI285 NPI105* TB-10Sc TB-10L19 *NPI85* TB-10L36 *NPI92 NPI350* --Weber & r674
orp2 - 13 - *g1*; *orp2* - 18 - *R1*; *PIO06003* (or *PIO06005*) and *orp2* closely linked --Wright & r700, 64:50
Mono-10, nulli-10 pollen development --Zhao & r712
Seed (S) component of *R1*, BS --Perrot & r469
Gs4 (was *Gs*-1439*), green striped, linked with *wx1* T9-10b; *Gs4* - 23 - *R1* --Neuffer 64:51

UNPLACED
Rcm2, rectifier (weak) of teosinte-cytoplasm miniature seed/retarded growth; *Rcm3*, rectifier from *Z. diploperennis*; *Rcm*-X*, from *Z. luxurians* --Allen & r6
*mr1**, *mr2**, *mr3**, etc. (*mrv1*, etc.) new designations for multi-ranking vs. two-ranking (*tr1*) in ear; *tpe1*, thin pericarp; *Ma1* (*Ma1**), multiple aleurone --Galinat r193; 64:120
Glb2-0 --Kriz r326
dsy3, *dsy4*, desynaptic --Golubovskaya r212
atn1, anaerobic tolerant null --Lemke-Keyes & r354
MYG-1, maternally inherited yellow-green --Mourad & r424
ba3 (was *ba*-861059b*) barren stalk --Pan & 64:8
Betaine-deficient (stress response), recessive --Rhodes & r512
LT19* and LT-R3*, lysine-threonine R, not allelic --Duncan & 64:110
cif1, cross-incompatible female; *cim1* & *cim2*, cross-incompatible male --Rashid & 64:8
Wi3 (was *Wi*-1614*), wilted; *Rld*-1441* and *Rld*-1990*, rolled leaf, closely linked or allelic --Neuffer 64:51
Fas1, fascicled ear --Nelson & 64:81
Rf7, partial restorer of cms-Y --Qin & 64:124
gl23 (was *gl*-PI262490*); *gl24* duplicate factor with *gl14* --Sprague 64:110

B CHROMOSOME

TRANSPOSABLE ELEMENTS
rDt element RM, BS --Brown & r49
tnpA protein of *En-1*, binding motifs --Gierl & r206
Bs1 RM, BS --Jin & r275; Johns & r278
Mrh, *Uq* in BSSS population --Peterson r472
Cy & *Mu* relationship --Schnable & r552
rcy:Mu7 RM, BS --Schnable & r553
Mu4, *Mu5* RM, BS --Talbert & r614

NUCLEAR cDNA, GENOMIC CLONES, AND PROBES
Ant-G1, Ant-G2 (*Ant1*, *Ant2*), adenine nucleotide translocator (ADP/ATP carrier, mitochondrial), genomic, RM, BS --Bathgate & r21
Signal recognition protein (SRP), 7SL RNA, BS --Campos & r64
Ubiquitin cDNA, BS --Christensen & r87
Autonomously replicating sequences (ARS), genomic, pMARS26 --Eckdahl & r155
Small nuclear RNA (snRNA) U1, U2, U4, U5, U6 forms --Egeland & r158
Zein-pml1, -A1, -20kDaA20, -27kDaA, -4, -10kDa, -15kDa, -19, -7, glutelin, actin: BS --Elliston & r163
Embryo ABA- and water stress-induced cDNA clone pMA12 (Gomez &, Nature 334:262, 1988) --Gomez & r479
Nitrate reductase (NRase, NADH:NRase) & glyceraldehyde-3-phosphate dehydrogenase (GAPDHase), BS --Gowri & r215
axr1, auxin-binding protein (ABP) cDNA, BS --Hesse & r248; Inohara & r266; Tillmann & r630
Pyruvate decarboxylase (PDC) cDNA, BS --Kelley r297
Zein-10kDa genomic: RM, BS --Kirihara & r307
S14 ribosomal protein, 40S subunit (*Cry1*, S14-40S, *Mch1*, *Mch2*), cDNA: RM, BS --Larkin & r338

Pollen-specific cDNA clones: pZmc13, RM, BS, genomic Zmg13; pZmc58 & Zmg58; Zmc26; pZmc46; pZmc26; pZmc30 --Hanson & r232, Mascarenhas r387 r388 r389 r390
Gpc1, Gpc2, Gpc3, glyceraldehyde-3-phosphate dehydrogenase (GAPDHase), cytosolic, genomic, BS --Martinez & r385; Russell & r530
PEPcase, genomic: RM, BS --Hudspeth & r262; Matsuoka & r395
NADP-malate dehydrogenase (NADP-MDH), chloroplastic, cDNA & genomic, BS --Metzler & r404
Zein-19kDa, genomic, pMS1 & pMS2, BS --Quayle & r499
NADP-dependent malic enzyme (NADP-ME; ME), chloroplastic, cDNA, RM, BS --Rothermel & r525
Chlorophyll binding protein (CAB), *Cab*-1*, RM, BS --Sullivan & r603
Zein-19kDA: BS --Wandelt & r668
Sod3 cDNA, RM, BS; transit peptide deletion analysis --White & r681
Bacteriophage M13 protein III probe hybridizes (hypervariable minisatellites) to 0.56% of genome --Zimmerman & r713

CHLOROPLAST
atpF, petB, petD, rpl16, psbB, psbH, atpH, atpI, atpA, atpB, atpE, rbcL gene clusters, splicing --Barkan r17
Z. perennis, luxurians, diploperennis, mays subsp., *T. pilosum, T. dactyloides*: restriction analysis, introgression --Doebley r136
tRNAval, rRNA16S, tRNAile, tRNAala, rRNA23S restriction analysis --Gauly & r199
petE BS --Haley & r229
atpB, rbcL transcription with insertions & deletions --Hanley-Bowdoin & r231
psbA BS --Zaitlin & r707

MITOCHONDRIA
T-urf13 deletion mutations, mapping toxin sensitivity & binding of DCCD protectant --Braun & r42 r43
Z. luxurians 22kDa protein, nucleus-regulated --Cooper & r97
cms-T RM, gene sequence, RM; *T-urf13* map location --Fauron & r166 r167
tRNAser, tRNApseudo, nad3, rps12, RM, BS --Gualberto & r221
NCS3, RM and BS alterations --Hunt & r263
atp6, T-urf13 initiation and processing sites, BS --Kennell & r298
2.3kb plasmid BS; chloroplastic *tRNApro(CAA) & tRNAtrp(UGG)* --Leon & r356
rRNA26S, BS --Maloney & r378
NCS5, NCS6 expressions similar; *coxII* altered in both; expressions of NCS4 --Newton & r435, 64:52
R2-integrated, BS --O'Brien & r443
hsp60, homolog to groEL (*E. coli*) & *hsp60* (*S. cerevisiae, T. thermophila*) --Prasad & r489
tRNAglu(UUC), tRNAser(UGA), tRNAtyr(GUA), tRNAlys(UUU), tRNAmet(CAU), BS --Sangare & r539, r540, r541
RU, BS --Small & r577
S-atpA1, S-atpA2, S-atpA3, RM --Wang & r673

RESISTANCE/TOLERANCE/QUANTITATIVE INHERITANCE/QTLs/GERMPLASM
Salt R --Ashraf & r11; Hajibagheri & r225
Maize Rough Dwarf Virus (MRDV) R --Bar-Tsur & r15
Intra- vs. interpopulation synthetics --Becker & r23
Hybrid improvement by interpopulation crosses, F2xF2 vs. BC1xBC1 --Bernardo & r28
Leaf surface wax variations among lines --Blaker & r32
Flowering, *pg11 pg12* --Bosch & r38
Flint-dent introgression; N response --Brun & r52 r53
Betaine levels in lines (stress response) --Brunk & r54
Earworm (*Heliothis zea* Boddie) R vs. silk pH & silk browning --Byrne & r60
Southern leaf blight (*Bipolaris maydis* (Nisik.) Shoemaker) race O vs. yield --Byrnes & r61
Variety-cross evaluation toward synthetics --Camussi & r65
S2 vs. S2 random-mated counterparts vs. S8 --Carlone & r68
Tropical germplasm accessions: short- vs. long-day evaluations --Castillo-Gonzalez & r70
R to *Bipolaris maydis* race T (*Bmt**), one or two genes --Ceballos & r71
Inhibitor of *Ht2* (*Iht**) from B14 --Ceballos & r72
Somatic embryogenesis inheritance --Close & r90; Willman & r692
Prolificacy selection vs. fertility & density --Coors & r98
Vegetative phase vs. filling period selection --Corke & r104
Grain-fill in opaque-2 --Cross & r109
Dry-down rate, diallel --Cross & r111
Selection for yield with international testing; gxe --Crossa & r114
Ear and stalk rot (*Fusarium moniliforme* Sheldon) R selection --DeLeon & r127
Embryo size heterosis --Djisbar & r135
Chlorosis R selection --Dolstra & r138
Nitrate reductase selection vs. N use efficiency --Eichelberger & r159 r160
Drought R selection --Fischer & r182
European Corn Borer (*Ostrinia nubilalis* Hubner) R selection, DIMBOA correlated --Grombacher & r220
Selection vs. genetic drift & variance --Helms & r246 r247
Southern leaf blight race O, sources of R --Holley & r255
Fusarium moniliforme R sources --Holley & r256
S2 vs. testcross selection --Horner & r257
Heritability vs. selection effectiveness --Johnson r279
Water stress selection --Johnson & r282
Inbreeding in open-pollinated populations --Kahler & r287
Two-spotted spider mite (*Tetranychus urticae* K.) R in line 41:2504B --Kamali & r289
European Corn Borer R diallel, gca, sca, reciprocal effects --Khalifa & r301

Maize Streak Virus (MSV) R --Kim & r303
Mg in ear leaf --Kovacevic & r323
Methomyl R & male fertility from tissue culture selection, cms-T --Kuehnle & r328
Pollen competitive ability selection --Landi & r335
Drought tolerance, ABA levels; maternal inheritance --Larque-Saavedra & r340
Somaclonal variations, height, maturity, dry matter --Lazanyi r345
RFLPs, diallel, gca, sca; heterotic groups: yield QTLs on 1, 3, 5, 7, 8 --Lee & r351
Organ-specific proteins, 2D: qualitative co-dominant (62 mesocotyl, 54 sheath, 40 blade), qualitative dominant (2 meso, 7 sh), quantitative co-dominant (42 meso, 50 sh, 16 bl), quantitative dominant (5 meso, 6 sh) --Leonardi & r357
Aluminum tolerance selection --Magnavaca & r374
Quality-protein-maize (QPM), normal-appearing *o2* kernels; selection --Magnavaca & r375 r376
Favorable-allele donor identification --Misevic r409 r410
Oil selection, diallel --Misevic & r411 r413
Gene flow among maturity groups in populations --Nevado & r434
European Corn Borer R selection --Nyhus & r440
Index selection response; genetic drift --Odhiambo & r445
Conicalness of ear, BSH --Ordas & r447
Stalk rot (*Colletotrichum graminicola* (Ces.) Wils R diallel --Pereira & r468
Phytic acid in Illinois High Oil & Illinois High Protein selections --Raboy & r501
Double cross prediction --Reddy & r508
Dwarf vs. normal subpopulations --Rocheford & r517
Goss's bacterial wilt (*Clavibacter michiganense* ssp. *nebraskense*) R linkage to *wx* translocations, chromosome 4 --Rocheford & r518
Growth rate correlation with sucrose-P-synthase level --Rocher & r520
Cold-tolerance selection, chlorosis, chlorophyll fluorescence --Schapendonk & r548
Maize dwarf mosaic virus R linked to *Y1* --Scott r558
Slow-rusting (*Puccinia polysora* Underw.) R --Scott r559
Oil percentage selection --Silvela & r571
Autotetraploid single vs. double crosses; vs. isogenic diploids --Sockness & r582 r583
Barrenness under stress, diallel --Sotchenko r585
Multiplicative selection index, full-sib --Stromberg & r597
Nitrate uptake selection --Teyker & r623
Weevil (*Sitophilus zeamais* Motschulsky) R diallel, gca, sca --Tipping & r632
Pericarp thickness vs. field emergence selection, *sh2* --Tracy & r636
Genetic drift vs. selection --Tragesser & r637
Kernel quality, stalk & root lodging R selection --Verderio & r652
Banded leaf and sheath blight (*Rhizoctonia solani*) R, gca, sca --Vimla & r654
Fall armyworm (*Spodoptera frugiperda* J.E. Smith) & Southwestern corn borer (*Diatraea grandiosella* Dyar) R selection, gca, sca --Williams & r690
Earworm (*Heliothis zea* Boddie) R in *PI* sources --Wilson & r694
Favorable-allele estimation by diallel, F2, testcrosses --Zanoni & r708 r709 r710
Low-P tolerance, RFLPs: QTLs on 3, 4, 6mid, 6L Reiter & 64:80

--Assembled unrestricted by Prof. Ligate

COORDINATORS' REPORTS

CHROMOSOME 1L

Mapping breakpoints of Reciprocal Translocation Stocks:

*wx*T1-9(8389) [1L.74,9L.13] : The 1L breakpoint for this commonly used translocation stock maps between *bz2* and *gs1*. Data from 350 testcross progeny produced the following approximate recombination distances in centiMorgans:

bz2 - 4 - T - 8 - *gs1* - 30 - *bm2*

T1-9(8389): The 1L breakpoint for the non-waxy version of this stock mapped to the same position.

T1-4g [1L.95,4L.35]: the 1L breakpoint maps distal to *bm2*. Data from 142 testcross progeny produced the following approximate distances:

bz2 - 22 - *gs1* - 3 - *bm2* - 7 - T

Note that recombination is reduced around the translocation breakpoint so that map distances are much less than in the standard map.

*wx*T1-9(035-10) [1L.89,9S.67]: The version of this stock which we got from the Maize Coop showed no linkage to *bz2*, *gs1*, or *bm2*. It may be misidentified as to breakpoint (Earl Patterson, personal communication).

Paul H. Sisco

CHROMOSOME 6S

McMullen and Louie (Mol. Plant Microbe Interactions 2:309-14, 1989) have mapped a major gene for resistance to maize dwarf mosaic virus strain A, *Mdm1*, to the interval between RFLP markers *UMC85* and *BNL6.29*. Mead et al. in this volume of the newsletter have determined distances between the following probes: *NPI7*-6.3cM-*UMC85*-11.6cM-*BNL7.28*-2.1cM-*BNL6.29*.

Ed Weck

IV. MAIZE GENETICS COOPERATION STOCK CENTER

It has been a matter of interest whether the increased levels of seed requests the past few years will be sustained. During calendar 1987, 2270 seed samples were supplied in response to 184 requests (letters or phone calls). The corresponding figures for 1988 were 3349 samples and 215 requests. In 1989 about 3000 samples were supplied in response to 222 requests. It appears that the transition to a higher level of requests has a strong probability of being continued.

The summer, 1989 season was the best of the past four years, though some irrigation was necessary to counteract periods of drouth. The improved field conditions were reflected in a larger than usual harvest.

The following categories of stocks were important components of the field and greenhouse plantings of the past year:

(1) There was a special increase of B-A translocations because of the continuing substantial level of requests.

(2) Stocks of defective kernel (*dek*) mutants submitted by Dr. Neuffer and Dr. Sheridan were propagated for the first time.

(3) Increases were obtained of many dominant or recessive new symbolized, mostly located genes submitted by Dr. Neuffer.

(4) There were increases of additional miscellaneous located, symbolized genes newly received from various sources.

(5) There were increases of stocks in low supply, all chromosomes. Extensive plantings were made of selected stocks of chromosomes 5, 6, 7, 8, 9 and 10.

(6) There were plantings of new accessions of unidentified, untested genes. There were substantial selfing blocks of mutants expressed as seedling traits.

(7) Numerous field plantings were made to test or confirm genetic constitutions with regard to mature plant traits.

(8) Greenhouse sandbench plantings were made to determine or verify genotypes relative to seedling traits.

During the fall of 1989 source samples of several dozen new accessions of *wx*-marked reciprocal translocations were received that were developed by Dr. D. S. Robertson. When combined as a supplement to our previous stocks, this enlarged collection will provide substantially improved coverage of the chromosome complement as a technique for locating genes to chromosome. From this overall collection, a smaller, selected listing will probably be recommended as a basis for generalized, routine initial screening to determine chromosome locations of gene loci by those who wish to use the *wx*-marked translocation technique.

In general, the sequential listing of chromosome stocks in the annual catalog has followed the pattern of beginning with markers at the left, or short arm, end of each linkage map and proceeding to the right. The rationale for this ordering has been to simplify the search for tester combinations marking particular chromosome regions. Once storage drawer space becomes limiting, however, there is reduced flexibility in intercalating stocks of new marker genes that may be mapped to approximate positions. Some of the additions to this year's catalog consist of single-trait stocks. Most of these newly-available mutant alleles which have been assigned to chromosome have been grouped together in the catalog at the end of the listing for each chromosome, partly as a matter of storage convenience.

Details of listings of catalog stock items change from year to year. Requests should be based on the most recent listing. Occasionally, it is necessary to discontinue distribution of certain items because of inadequate supplies or because of errors detected in pedigrees. When these items are requested before catalog corrections can be made, an effort is made to substitute with similar marker combinations and to explain the reason for the substitution.

We are making a concerted effort to work through the stocks of untested, unidentified mutant genes to rejuvenate seed supplies when appropriate and to assemble stocks into common categories. In the process, results of any allelism testing and mapping studies will be summarized to determine the extent of current information. When this sorting has been completed, it should be much easier to organize plantings of particular categories of mutant genes for the purpose of efficient and comprehensive allelism testing both on this project and by cooperators.

There is a continuing need to submit basic stocks of the collection to the National Seed Storage Laboratory in order to provide an alternative site to guard against loss. Under current staffing, there has been no opportunity to fulfill this important objective, but it remains an item of unmet high priority.

Additional personnel were added to the Stock Center program in the spring of 1989. Dr. Susan Gabay-Laughnan, who has extensive experience in a broad range of maize genetics, is now assisting part-time with all aspects of the Stock Center program. In addition, Janet Day is serving part-time as a laboratory specialist, assisting in both laboratory and field nursery operations.

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CATALOG OF STOCKS

CHROMOSOME 1

101A *sr zb4 P-WW*
101B *sr P-WR*
101C *sr P-WW*
101D *sr P-RR*
101F *sr ts2 P-RR*
102B *sr P-WR an bm2*
102C *sr P-RW ad bm2*
103C *sr P-WR bm2*
103D *vp5*
103E *zb4 ms17 P-WW*
103G *sr P-RR bm2*
104B *zb4 ts2 P-WW bm2*
105A *zb4 P-WW*
105C *zb4 P-WW br*
105E *ms17*
105F *ms17 P-WW*
106A *zb4 P-WW bm2*
106B *ts2 P-RR*
106C *ts2 P-WW bm2*
107A *P-CR*
107B *P-RR*
107C *P-RW*
107D *P-CW*
107E *P-MO*
107F *P-VV*
107G *P-OR*
108C *P-RR br f an gs bm2*
109A *P-RR an ad bm2*
109B *P-RR an gs bm2*
109D *P-RR ad bm2*
109E *P-WR br f*
110A *P-WR an Kn bm2*
110B *P-WR an Kn*
110C *P-WR an ad bm2*
110D *P-WR an bm2*
110E *P-WR ad bm2*
110F *P-WR br Vg*
110G *P-WR br f gs bm2*
110H *P-WR br f bm2*
110K *P-WR br*
111A *P-WW rs2*
111B *P-WW rs2 br*
111D *P-WW hm br f*
112B *P-WW br f bm2*
112E *as*

112H *P-WW br*
113A *as br2*
113B *rd*
113C *br f*
113E *br f Kn*
113K *hm; hm2*
113L *Hm; hm2*
114B *br f Kn bm2*
114D *Vg*
114E *br Vg f*
114F *hm br2*
115A *Vg an bm2*
115B *Vg br2 bm2*
115D *bz2-m; m A A2 C Pr*
115E *br2 Vg*
116A *bz2-m; M A A2 C R Pr*
116C *an bm2*
116D *an-bz2-6923* (Df)
116I *bz2 gs Ts6 bm2*
117A *br2*
117B *br2 bm2*
117D *tb*-8963*
117E *Kn*
118A *Kn Ts6*
118B *Kn bm2*
118C *lw*
119B *up8*
119C *gs*
119D *gs bm2*
119E *Ts6*
119F *bm2*
120A *id*
120B *nec2*
120C *ms9*
120D *ms12*
120F *Mpl1*
121A *ms14*
121B *mi*
121C *D8*
121D *Lls*
121F *fg*
121J *ms14 br2*
122A TB-1La
122B TB-1Sb
123 Primary trisomic 1
124 *v*-5688*
124 *j*-5828*
124 *w*-8345*
124 *v*-5588*
124 *w*-018-3*
124 *w*-4791*
124 *w*-6577*
124 *w*-8054*
124 *v*-032-3*
124 *v*-8943*
124 *yg*-8574*
125A *Les2*
127 *dek1*
127 *dek2*
127 *dek22*
127 *Les7*
127 *Msc1*
127 *Tlr1*
127 *mi*-8043*
127 *gt*

CHROMOSOME 2

201A *ws3 lg gl2 B*
201F *ws3 lg gl2 b*
202C *ws3 lg gl2 fl v4*
202E *ws3 lg gl2 b v4*
203B *al=y3*
203D *al lg*
205B *lg*
205C *lg gl2*
206A *lg gl2 B*
206B *lg gl2 B gs2*
207A *lg gl2 B gl11*
208B *lg gl2 B sk*
208D *lg gl2 B v4*
208E *lg gl2 b*
208F *lg gl2 b gs2*
208G *lg gl2 b gs2 Ch*
208H *gl2*
209E *lg gl2 b sk*
209F *lg gl2 b sk fl*
210A *lg gl2 b sk v4*
211A *lg gl2 b fl*
211D *gl2 b wt*
212A *lg gl2 b wt v4*
212B *lg gl2 b fl v4*
212C *lg gl2 b fl v4 Ch*
212D *lg gl2 b v4*
212E *lg gl2 b v4 Ch*
213A *lg gl2 mn v4*
213B *lg gl2 wt*
213C *lg gl2 w3*
213D *lg gl2 w3 Ch*
213E *lg gl2 b Ch*
213F *lg B-V Ch*
214C *d5*
214D *B gl11*
214E *B ts*
214F *gl2 v4 Ch*
214G *lg gs2 v4*
215B *gl11*
215C *ut*
215E *fl*
215G *fl v4*
216A *fl v4 Ch*
216D *fl w3*
216F *fl w3 Ch*
217A *ts*
217B *v4*
217E *w3 Ht Ch*
218A *w3*
218B *w3 Ht1*
218C *w3 Ch*
218D *Ht* (source A and B)
218E *ba2*
218F *B ba2*
219A *B-Peru ("R2"); rAC*
219B *b("r2"); r-g AC*
219C *Ch*
219F *B-Peru; bz2 r-g; AC*
220A *Les*
220B 2 2T T2/ *ws3 lg gl2* (T=*Tripsacum*)
221A *gs2*
221B *B gs2*
222A TB-1Sb-2L 4464
222B TB-3La-2S 6270
223A Primary trisomic 2
224A *w*-4670*
224B *v*-5537*
224F *w*-062-3*
224G *yel*-8630*
224H *whp*
224J *ij-mos*-7335*
224K *gl-nec*-8495*
227 *dek3*
227 *dek4*
227 *dek16*
227 *dek23*
227 *Les4*

CHROMOSOME 3

301A *cr*
301B *cr d*
301C *cr d Lg3*
301E *cr ts4 na* lg2
302A *d=d-6016* (rosette)
302B *d rt*
302E *d* (tall)
303A *d rt Lg3*
303B *d Rf lg2*
303F *g2 =v19 =pg14 =g5*
304A *d ys3*
304B *d ys3 Rg*
304G *Lg3 Rg*
305A *d Lg3*
305D *d Rg*
307C *pm*
308B *d ts4*
308C *d lg2 a-m;* A2 C R Dt
308E *ra2*
308G *d ts4 a-m;* A2 C R Dt
309D *ra2 Rg lg2*
310A *ra2 ts4*
310C *ra2 lg2*
310D *Cg*
311A *cl*
311C *cl; Clm3*
311D *cl-p; Clm4*
311E *rt*
311F *ys3*
311G *ys3 Lg3*
312C *ys3 ts4 lg2*
312D *Lg3*
313A *gl6*
313C *gl6 Lg3 Rg*
313E *gl6 Lg3*
314F *gl6 Rg lg2*
314G *gl6 lg2*
315B *Rg gl6*
316A *ts4*
316B *ts4 na*
318A *ig*
318B *ba*
318C *w*-7748 = y10*
319A *lg2 A-b et; A2 C R Dt*
319B *lg2 a-m sh2 et; A2 C R Dt*
319C *lg2 a-m et; A2 C R dt*
319D *lg2 a-m et; A2 C R Dt*
319F *lg2 a-st et; A2 C R Dt*
320A *lg2*
320D *A sh2; A2 C R B Pl dt*
320F *A sh2; A2 C R b pl*
320I *A sh2; A2 C R*
321A *A-d31; A2 C R*
322A *A-d31 sh2; A2 C R dt*
322B *A-d31 sh2; A2 C R Dt*
322E *a-m; A2 C R B Pl dt*
322F *a-m; A2 C R b pl dt*
322G *a; A2 C C2 R*
323A *a-m; A2 C R Dt*
323C *a-m sh2; A2 C R B Pl dt*
324A *a-st; A2 C R Dt*
324B *a-st sh2; A2 C R Dt*
324E *a-st et; A2 C R Dt*
324G *a-st; A2 C R dt*
325A *a-p et; A2 C R dt*
325B *a-p et; A2 C R B Pl Dt*
325C *a-x1*
325D *a-x3*
325G *a3*
325K *a-m3 sh2-m1; Ac*
326A *sh2*
326B *vp*

326C *Rp3*
327A TB-3La
327B TB-3Sb
327C TB-3Lc
328A Primary trisomic 3
329 *v*-9003*
329 *v*-8623*
329 *w*-022-15*
329 *yd2*
329 *w*-062-3*
329 *w*-8336*
330A *h*
331E TB-3Lf
331F TB-3Lg
331I TB-3Lj
331K TB-3Ll
332 *dek5*
332 *dek24*
332 *W r k*
332 *gl19*
332 *dek6*
332 *dek17*
332 *Spc*
332 *Lxm*
332 *ms23*

CHROMOSOME 4

401A *Rp4*
401B *Ga*
401D *Ga-S*
402A *st*
402C *st fl2*
402D *Ts5*
403A *Ts5 fl2*
405B *la*
405D *la su gl3*
405G *la su gl4*
406C *fl2*
407B *fl2 su bm3*
407D *su*
407E *su-am*
408B *su bm3*
408E *bm3*
409A *su zb6 Tu*
410D *su zb6 gl3*
412C *su gl3*
412E *su j2 gl3*
413B *su gl4*
414A *bt2*
414B *gl4*
414C *gl4 o*
415A *j2*
415C *j2 C2; A A2 C R*
416A *Tu*
416B *Tu-l* 1st
416C *Tu-l* 2nd
416D *Tu-d*
416E *Tu-md*
417A *j2 gl3*
417B *v8*
417C *gl3*
417D *gl3 o*
418A *gl3 dp*
418B *c2; A A2 C R*
418C *C2; A A2 C R*
418D *C2-Idf* (Active-1) *; A A2 C R*
418E *dp*
418F *o*
418G *v17*
419B *su gl3 ra3*
419F *Dt6 gl3; a-m A2 C R*
420A *Dt4 su; a-m A2 C R*
420B TB-9Sb-4L6504
420C *nec-rd*
420D *yel*-8457*
420E *fra2*
420I TB-9Sb-4L6222
421A TB-4Sa
421B TB-lLa-4L 4692
421C TB-7Lb-4L4698
422A Primary trisomic 4
423A TB-4Lb
423B TB-4Lc
423C TB-4Ld
423D TB-4Le
423E TB-4Lf
427 *dek7*
427 *dek25*
427 *Ysk1*
427 *orp1*
427 *dek8*
427 *dek10*
427 *Ms41*

CHROMOSOME 5

501A *am a2; A C R*
501B *lu*
501C *lu sh4*
501D *ms13*
501E *gl17*
501H *gl17 a2 bt; A C R*
502A *gl17 a2 bt v2; A C R*
502B *A2 vp7 =ps pr; A C R*
502D *A2 bm pr; A C R*
503A *A2 bm pr ys; A C R*
503D *A2 bt v3 pr; A C R*
504A *A2 bt pr; A C R*
504B *A2 bm pr ys v2; A C R*
505C *A2 bt ga2 pr; A C R*
506A *A2 v3 pr; A C R*
506B *A2 pr; A C R*
506C *A2 pr v2; A C R*
506D *A2 pr na2; A C R*
505B *A2 pr ys; A C R*
506E *A2 pr zb3; A C R*
506F *A2 pr v12; A C R*
506L *A2 pr br3; A C R*
507A *a2; A C R*
508B *a2 bm bt pr ys; A C R*
508F *a2 bm pr ys; A C R*
510A *a2 bm pr v2; A C R*
511A *a2 bt v3 pr; A C R*
511B *a2 bt v3 Pr; A C R*
512B *a2 v3 pr; A C R*
512C *a2 bt ga2 pr; A C R*
513A *a2 pr; A C R*
513G *a2; A C R*
515A *vp2*
515C *vp7 =ps*
515D *bm*
516A *bm yg; Ch*
516B *bt*
516C *ms5*
516D *td ae*
517A *v3*
517B *ae*
518A *sh4*
518B *gl8*
518C *na2*
518D *lw2*
518F *sh4 v2*
519A *ys*
519B *eg*
519C *v2*
519D *yg*
519E *pr yg; A C R*
520B *v12*
520C *br3*
522A TB-5La
522B TB-5Lb
522C TB-5Sc
523A Primary trisomic 5
527 *dek18*
527 *dek9*
527 *dek26*
527 *dek27*
527 *grt1*

CHROMOSOME 6

601C *rgd y*
601D *rgd Y*
601E *po = ms6*
601F *po y pl*
602J *y = pb = w-m*
603A *y l10*
603B *y l11*
603C *y l12*
603D *y w15*
604A *y pb4 pl*
604B *y pb4 Pl*
604F *y ms-si*
604I *Y ms*
605A *y wi Pl*
605C *y pg11 ; Wx pg12*
606A *Y pg11; Wx pg12*
606B *y pg11 ; wx pg12*
606C *Y pg11 ; wx pg12*
606E *y pl*
606F *y Pl*
607A *y Pl Bh ; c sh wx A A2 R*
607B *y pl Bh ; c sh wx A A2 R*
607C *y su2*
609A *Y pb4*
609B *Y wi pl*
609C *Y wi Pl*
609D *Y su2*
609E *ms-si*
610B *Pl Dt2 ; a-m A2 C R*
610C *pl sm ; P-RR*
611A *Pl sm ; P-RR*
611D *Pt*
611E *w*
611F *Pl sm Pt;P-RR*
611H *py*
612A *w14*
612B *ms6*
612D *oro*
613A 2NOR; *a2 bm pr v2*
614A TB-6Lb
614B TB-6Sa
614C TB-6Lc
615A Primary trisomic 6
627 *dek28*
627 *dek19*

CHROMOSOME 7

701A *Hs o2 v5 ra gl*
701B *In-D*
701D *o2*
702A *o2 v5*
702B *o2 v5 ra gl*
702E *o2 v5 ra gl ij*
703A *o2 v5 gl*
704B *o2 ra gl sl*
704C *o2 v5 gl sl*
705A *o2 gl*
705B *o2 gl sl*
705C *o2 ij*

705D *a2 bd*
706E *gl Tp*
707A *y8 v5 gl*
707B *in ; A2 pr A C R*
707D *v5*
707E *vp9*
707F *y8 gl*
708A *ra*
708G *y8*
709A *gl*
710A *gl Tp*
710E *gl o5 = pg**
710H *ms7 gl Tp*
711A *Tp*
711B *ij*
711G *ts-br*
712A *ms7*
713A *Bn*
713B *bd*
714B *o5*
714C *o5 mn2 gl*
714D *va*
715A *Dt3; a-m A2 C R*
715C *gl Dt3; a-m A C R*
716A *v*-8647*
716B *yel*-7748*
716F *Les9*
716G *y8*
717A TB-7Lb
718A Primary trisomic 7
727 dek11

CHROMOSOME 8

801A *gl18*
801B *v16*
801D *v16 ms8 j*
801F *v16 j gl18*
801G *v16 gl18*
803A *ms8*
803D *ms8 gl18*
804A *v21*
805A *fl3*
805E *el*
806A TB-8La
806B TB-8Lb
809A TB-8Lc
827 dek20
827 dek29
827 Bif
827 Sdw1
827 Clt1

CHROMOSOME 9

901B *yg2 C sh bz; A A2 R*
901C *yg2 C sh bz wx; A A2 R*
901D *yg2 C-I sh bz wx; A A2 R*
901E *yg2 C bz wx; A A2 R*
902A *yg2 c sh bz wx; A A2 R*
902B *yg2 c sh wx; A A2 R*
902C *yg2 c sh wx gl15; A A2 R*
902D *yg2 c sh wx gl15 K-S9; A A2* R
902E *yg2 c bz wx; A A2 R*
924A *wd-Ring C-I; A A2 R*
903A *C sh bz; A A2 R*
903B *C sh bz wx; A A2 R*
903D *C-I sh bz wx; A A2 R*
904B *C sh; A A2 R*
904C *C sh wx; A A2 R*
904D *C wx ar; A A2 R*
905A *C sh wx K-L9; A A2 R*
905B *C sh ms2; A A2 R*
905C *C bz Wx; A A2 R*
905D *K-L9 C sh wx; K10 A A2 R*
905E *C sh wx v; A A2 R*
906A *C Ds wx; A A2 R Pr y*
906C *C-I Ds Wx; A A2 R*
906D *C-I; A A2 R*
906G *C-I Ds wx; A A2 R*
907A *C wx; A A2 R*
907E *C-I wx; A A2 R y*
908B *C wx v; A A2 R*
908D *C wx gl15; A A2 R*
908F *C wx da;A R*
909A *C wx Bf; A A2 R*
909B *c bz wx; A A2 R*
909C *c sh bz wx; A A2 R y*
909D *c sh wx; A A2 R*
909E *c sh wx v ; A A2 R*
909F *c sh wx gl15; A A2 R*
910B *c sh wx gl15 Bf; A A2 R*
910C *c sh wx bk2; A A2 R*
910D *c; A A2 R*
910G *sh-bz-x2 Wx; A C R*
911A *c wx; A A2 R y*
911B *c wx v; A A2 R*
911C *c wx gl15; A A2 R*
911D *c wx Bf; A A2 R*
912A *sh*
912B *sh wx v*
912E *lo2*
913A *sh wx*
914A *wx d3*
915A *wx* (Other alleles from O. Nelson avail.)
915B *wx-a*
915C *w11*
916A *wx v*
916C *wx bk2*
917A *wx Bf*
917C *v*
917D *ms2*
917E *gl15*
917F *d3*
918A *gl15 Bf*
918D *Wc*
918F *Wx Bf*
918H *Wc bm4*
919A *bm4*
919B *Bf bm4*
919C *l6*
919D *l7*
920A *yel*-034-16*
920B *w*-4889*
920C *w*-8889*
920E *w*-8950*
920F *w*-9000*
920G Tp9 N9 N3 Df3
921A TB-9La
921B TB-9Sb
921C TB-9Lc
921D TB-9Sd
922A Primary trisomic 9
927 dek12
927 dek13
927 dek30
927 Les8
927 Zb8
927 Dt7; a-r; A2 CR

CHROMOSOME 10

X01A *oy*
X01B *oy R; A A2 C*
X01C *oy bf2*
X01E *oy bf2 R; A A2 C*
X02A *oy ms11*
X02G *oy zn*
X02I *oy bf2 ms10*
X03B *Og*
X04A *Og du R; A C R*
X04B *ms11*
X04D *bf2*
X05A *bf2 zn*
X05E *bf2 sr2*
X06C *nl g R; A A2 C*
X07C *y9*
X07D *nl*
X09B *li g R; A A2 C*
X09F *ms10*
X10A *du*
X10D *du g r; A C R*
X10F *zn*
X11A *zn g*
X11F *g r; A A2 C*
X12A *g r sr2*
X12E *g R;A A2 C*
X13D *g r-r sr2; A A2 C*
X14A *Isr r-r; A A2 C*
X15C *R-g; A A2 C*
X15D *r-ch; Pl A C*
X16B *r K10; A A2 C*
X16C *R-ch ;A A2 C B pl*
X16D *r sr2; A A2 C*
X17A *r-g; A A2 C*
X17B *r-r; A A2 C*
X17C *R-mb; A A2 C*
X17D *R-nj; A A2 C*
X17E *R-r; A A2 C*
X17F *R-nj* purple embryo Chase; *A A2 C*
X18C *R-st; A A2 C*
X18D *R-sk; A A2 C*
X18E *R-st Mst*
X18G *R-scm2; bz2 A A2 C C2*
X19A *Lc*
X25A *R-scm2; a-st A2 C C2*
X25B *R-scm2; c2 A A2 C*
X25C *R-scm122; pr A A2 C C2*
X25D *R-scm2; a2 A C C2*
X25E *R-scm2; c A A2 C2*
X19B *w2*
X19C *w2 l*
X19D *o7*
X20B *l*
X20C *v18*
X20F *yel*-8721*
X21A TB-10La
X22A TB-10Sc
X21B TB-10L19
X21C TB-10Lb
X23A Primary trisomic 10
X26A *r-x1; AC*
X27 *dek14*
X27 *dek15*
X27 *dek21*
X27 *Les6*
X27 *gl21*
X27 *Vsr1*
X27 *Oy*-700*
X27 *orp2*

UNPLACED GENES

U140 *dek31*
U140 *dv*
U140 *dy*
U140 *l3*
U140 *l4*
U141 *ms22*
U141 *ms24*
U141 *o9*

U141 *o10*
U141 *o11*
U142 *o12*
U142 *o13*
U142 *rd3*
U142 *ub*

MULTIPLE GENE STOCKS

M141A *A A2 C C2 R-g Pr B Pl*
M141B *A A2 C C2 R-g Pr B pl*
M141C *A A2 C C2 R-g b Pl*
M141D *A A2 C C2 R-g b pl*
M241A *A A2 C C2 r-g Pr B Pl*
MX17A *A A2 C C2 r-g Pr b pl*
M241B *A A2 C C2 r-g Pr B pl*
M340A *A A2 c C2 R-g Pr B pl*
M241C *A A2 C C2 R-r Pr B Pl*
M341B *A A2 C C2 R-r Pr B pl*
M341C *A A2 C C2 R-r Pr b Pl*
M341F *A A2 C C2 R-r Pr b pl*
M441A *A A2 C C2 R-r Pr B Pl wx*
M441B *A A2 C C2 R-r Pr B pl wx*
M441F *A A2 C C2 R-g Pr b pl wx*
M541 *A A2 C C2 R Pr*
M641A *A2 C C2 R Pr wx*
M641D *A A2 C C2 r Pr y wx*
MX41A *A A2 C C2 R pr y wx gl*
M941A *A A2 c C2 R Pr y wx*
M341D *A A2 c C2 R-r Pr B Pl*
M341E *A A2 c C2 R-g Pr b pl*
M441D *A A2 C C2 r-r Pr B Pl*
M441E *A A2 c C2 r-r Pr B Pl*
MX41B *su pr y gl wx ; A A2 C C2 R*
M841A *A su pr ; A2 C C2 R*
MX41C *bz2 a c2 a2 pr Y/y c bz wx r*
M841B *a su A2 C C2 R*
MX40A *bm2 lg a su pr y gl j wx g*
M841C colored scutellum; *A A2 C C2 R Pr*
M841E " " ; *A A2 C C2 R pr*
MX41D *a su pr y gl wx A A2 C C2 R*
M741C Stock 6 : Hi-haploid *R-r B Pl*
M741 " " *A C R-g* col scutellum
M741 " " *y C-I wx A R-g*

POPCORNS

P142A Amber Pearl
P142B Argentine
P142C Black Beauty
P242a Hulless
P242B Ladyfinger
P242C Ohio Yellow
P342A Red
P342B Strawberry
P342C Supergold
P342D South American
P442A Tom Thumb
P442B White Rice

EXOTICS AND VARIETIES

E542A Black Mexican Sweet Corn (with B-chromosomes)
E542B Black Mexican Sweet Corn (without B-chromosomes)
E642A Knobless Tama Flint
E642C Knobless Wilbur's Flint
E442A Gaspe Flint
E642B Gourdseed
E742A Maiz Chapalote
E942B Missouri Cob Corn
E742B Papago Flour Corn
E742C Parker's Flint
E842A Tama Flint
E942A Winnebago Flint
E842B Zapalote Chico

TETRAPLOID STOCKS
N103A *P-RR*
N103D *P-WR*
N104B *pr ; A A2 C R*
N107C Synthetic B
N107B W23 conversion
N104C *su wx*
N106D *sh Wx ; Y*
N106E *sh wx ; y*

CYTOPLASMIC TRAITS

C337 NCS2
C337 NCS3

CYTOPLASMIC STERILES AND RESTORERS

C836A WF9-(T) *rf rf2*
C836B WF9 *rf rf2*
C736A R213 *Rf rf2*
C736C B37 *Rf rf2*
C736D N6 *Rf rf2*
C736B Ky21 *RfRf2*

WAXY RECIPROCAL TRANSLOCATIONS*

WX01A *wx* 1-9c (1S.48 ; 9L.22)
WX01B *wx* 1-9 5622 (1L.10; 9L.12)
WX03A *wx* 1-9 8389 (1L.74 ; 9L.13)
WX04A *wx* 2-9c (2S.49 ; 9S.33)
WX05A *wx* 2-9b (2S.18 ; 9L.22)
WX06A *wx* 2-9d (2L.83 ; 9L.27)
WX07A *wx* 3-9 8447 (3S.44 ; 9L.14)
WX08A *wx* 3-9c (3L.09 ; 9L.12)
WX10A *wx* 4-9e (4S.53 ; 9L.26)
WX11A *wx* 4-9g (4S.27 ; 9L.27)
WX12A *wx* 4-9 5657 (4L.33 ; 9S.25)
WX13A *wx* 4-9b (4L.90 ; 9L.29)
WX14A *wx* 5-9c (5S.07 ; 9L.10)
WX15A *wx* 5-9 4817 (5L.06 ; 9S.07)
WX16A *wx* 5-9d (5L.14 ; 9L.10)
WX17A *wx* 5-9a (5L.69 ; 9S.17)
WX18A *wx* 6-9 4778 (6S.80 ; 9L.30)
WX20A *wx* y 6-9b (6L.10 ; 9S.37)
WX21A *wx* 6-9 4505 (6L.13 ; 9ctr.)
WX22A *wx* 7-9 4363 (7ctr. ; 9ctr.)
WX23A *wx* 7-9a (7L.63 ; 9S.07)
WX24A *wx* 8-9d (8L.09 ; 9L.16)
WX25A *wx* 8-9 6673 (8L.35 ; 9S.31)
WX26A *wx* 9-10 8630 (9S.28 ; 10L.37)
WX27A *wx* 9-10b (9S.13 ; 10S.40)
WX28A *wx* 5-9 8386 (5L.87 ; 9S.13)

NON-WAXY RECIPROCAL TRANSLOCATIONS*

Wx30A *Wx* 1-9c (1S.48 ; 9L.22)
Wx30B *Wx* 1-9 4995 (1L.19 ; 9S.20)
Wx30C *Wx* 1-9 8389 (1L.74 ; 9L.13)
Wx31A *Wx* 2-9c (2L.49 ; 9S.33)
Wx31B *Wx* 2-9b (2S.18 ; 9L.22)
Wx32A *Wx* 3-9 8447 (3S.44 ; 9L.14)
Wx32B *Wx* 3-9 8562 (3L.65 ; 9L.22)
Wx32C *Wx* 3-9c (3L.09 ; 9L.12)
Wx33A *Wx* 4-9e (4S.53 ; 9L.26)
Wx33B *Wx* 4-9 5657 (4L.33 ; 9S.25)
Wx33C *Wx* 4-9g (4S.27 ; 9L.27)
Wx34A *Wx* 5-9c (5S.07 ; 9L.10)
Wx34B *Wx* 5-9 4817 (5L.06 ; 9S.07)
Wx34C *Wx* 4-9b (4L.90 ; 9L.29)
Wx35A *Wx* 5-9 8386 (5L.87 ; 9S.13)
Wx35B *Wx* 5-9a (5L.69 ; 9S.17)
Wx35C *Wx* 5-9d (5L.14 ; 9L.10)
Wx36A *Wx* 6-9 4778 (6S.80 ; 9L.30)
Wx37A *Wx* 6-9 8768 (6L.89 ; 9S.61)
Wx37B *Wx* 7-9 4363 (7ctr. ; 9ctr.)
Wx37C *Wx* 6-9 4505 (6L.13 ; 9ctr.)
Wx38A *Wx* 7-9a (7L.63 ; 9S.07)
Wx38B *Wx* 8-9d (8L.09 ; 9L.16)
Wx38C *Wx* 8-9 6673 (8L.35 ; 9S.31)
Wx39A *Wx* 9-10 8630 (9S.28 ; 10L.37)
Wx39B *Wx* 9-10b (9S.13 ; 10S.40)

* = Single cross of homozygotes between M14 and W23 versions will be supplied if available

INVERSIONS

I143A Inv 1a (1S.86-L.50)
I143B Inv 1c (1S.35-L.01)
I143C Inv 1d (1L.55-L.92)
I143D Inv 1 5131-10 (1L.46-L.82)
I444A Inv 2a (2S.70-L.80)
I243A Inv 2 8865 (2S.06-L.05)
I243B Inv 2 5392-4 (2L.13-L.51)
I343A Inv 3a (3L.38-L.95)
I343B Inv 3L (3L.19-L.72)
I343C Inv 3 3716 (3L.09-L.81)
I443A Inv 4b (4L.40-L.96)
I443B Inv 4c (4S.86-L.62)
I543A Inv 4e (4L.16-L.81)
I743A Inv 5 8623 (5S.67-L.69)
I743B Inv 6 8452 (6S.77-L.33)
I843A Inv 6 8604 (6S.85-L.32)
I743C Inv 6 3712 (6S.76-L.63)
I943A Inv 7 5803 (7L.17-L.61)
I943B Inv 7 8540 (7L.12-L.92)
I943C Inv 7 3717 (7S.32-L.30)
IX43A Inv 8a (8S.38-S.15)
I344A Inv 9a (9S.70-L.90)
IX43B Inv 9b (9S.05-L.87)
IX43C Inv 9c (9S.10-L.67)

Cooperators (that means you) need the Stock Center.

The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors that you have reported in this News Letter 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 of the stocks for research when you publish, and advice or help you have received in development of your research project.

V. GENE LIST AND WORKING MAPS

Following is the current genelist, arranged by gene symbol, identifying the unit factors for which stocks are available in the Maize Genetics Stock Center (Department of Agronomy, University of Illinois, Urbana, Illinois 61801), those for which variants exist in generally available strains (e.g. isozyme variants), and those upon which current or recent research studies have been published or have been reported in the Maize Genetics Cooperation Newsletter. The information tabulated includes the chromosome (L=long arm, S=short arm) and map position or approximate position, the name and phenotype, availability from the Stock Center (S), a photograph (P) in the Mutants of Maize (Neuffer, M.G., et al. 1968, Crop Sci. Soc. Amer., Madison, WI), and references to the original descriptions.

Following the gene list are the current working maps for each chromosome. The traditional linkage map based on conventional factors and isozymes is presented in the center. Each linkage map represents the order and recombinational distances, in centimorgans (1% recombination = 1 cM), for those genes for which sufficient information is available to make a reasonable judgment of their location. Each chromosome is arranged beginning with the most distal gene in the short arm. Locations of the centromeres are indicated according to the best available data from cytogenetic studies. The physical map of each chromosome, immediately to the left of each linkage map, is drawn with the length of each arm in proportion to the ratio of the length of that arm to the length on chromosome 1. Locations of the B-A translocations, which generate hemizygous segments, are shown as TB-...; placement on the physical map is in accordance with observed breakpoints; placement on the linkage map is in relation to genes uncovered or not uncovered. The vertical line associated with simple B-A translocations represents the segment within which the breakpoint is located (genes distal to the line on that arm should be uncovered). In the case of compound translocations, the associated vertical line on the linkage map for the first arm involved (e.g., 1L of TB-1La-5S8041) defines the segment within which the second breakpoint is located (genes distal to the line are not uncovered). On the map of the second arm involved (5S, in the example), genes distal to the associated line are uncovered (as they are with simple B-A translocations). TB's shown spanning one or more genes may or may not uncover the indicated gene or genes. Immediately to the right of the linkage map are those genes that have some information leading to a "rough" placement on the map, either near a gene already on the map or to a region of the map. Further to the right are those genes which have been only placed to chromosome (represented by the vertical line with arrows at both ends) or to one arm (represented by a vertical line running from near the centromere to the end of the arm).

To left of each chromosome's physical map are the current versions of the public restriction fragment length polymorphism (RFLP) maps developed by B. Burr at the Brookhaven National Labs (on the left) and D. Hoisington at the University of Missouri (on the right). On the right of the Missouri map are preliminary localizations of conventional markers based on work at Missouri and that provided by other researchers. The horizontal ticks indicate the RFLP loci used in mapping the gene or genes.

The Integrated Mapping Project, developed under the encouragement, advice and efforts of the maize community, is in the second of 3 years of funding from NSF. The focus of the prioritized work under this support is (1) to set a universally usable framework of RFLP markers; (2) to define physical locations with B-A and A-A translocations; and (3) to map a selected group of conventional markers (see notes in this Newsletter). It is important to stress that this effort in no way decreases the need for others to map (either traditionally or with RFLPs). What this project will do is provide a means by which these data can be assembled and distributed to all interested research workers.

The importance of placing loci defined by probes of known function cannot be overstressed. In a number of cases these provide very accurate ties to the conventional map and, in the very least, provide functional significance to a particular region of the genome that will be important as further additional studies (particularly in the area of quantitative genetics) progress. Therefore, if you have a clone for a known function and know or believe that it hybridizes to a maize genomic sequence, please attempt to map the locus (or loci). This can be accomplished in a couple of ways (and we recommend doing both). First, the set of recombinant inbreds should be probed and the data sent to Ben Burr for analysis. Secondly, it would be appreciated if the probe could be sent to Missouri for mapping in F2 and testcross populations. We would also be able to use the probe in our correlation to physical and conventional markers. We have included in this Newsletter a sample form of the desired information for each clone you provide. If you have any questions regarding mapping of RFLP loci (both old and new), please call or write.

As usual, any comments and/or changes to the maps are greatly appreciated.

Ed Coe, Dave Hoisington and Shiaoman Chao

SYMBOL	LOCATION	NAME/PHENOTYPE	S	P	REF
a1	3L-149.0	anthocyaninless: colorless aleurone, green or brown plant, brown pericarp with *P1-RR* ; for alleles and interactions, see Coe et al., 1988; RFLP loci(probe): *BNL-A1* (pAmu2), *NPI51-A1* (), *NPI467-A1* ()	S	P	81
A1-2	1L	anthocyanin candidate: RFLP locus(probe): *NPI482* ()			373
A1-3	2L	anthocyanin candidate: RFLP locus(probe): *NPI468* ()			373
A1-4	5L	anthocyanin candidate: RFLP locus(probe): *NPI469* ()			373
A1-5	7S	anthocyanin candidate: RFLP locus(probe): *NPI470* ()			373
a2	5S-35	anthocyaninless: like *a1*, but red pericarp with *P1-RR*	S	P	151
a3	3L-132	anthocyanin: recessive intensifier of expression of *R1* and *B1* in plant tissues	S	P	192
Ac		activator: designator for autonomous transposable elements; regulates *Ds* transposition and dissociation; ex. *Ac9* designates element isolated from *wx1-m9*		P	212
Ac2		activator: similar to *Ac*			66
Aco1	4S	aconitase: electrophoretic mobility; monomeric			364
Aco2	?	aconitase: electrophoretic mobility			364
Aco3	?	aconitase: electrophoretic mobility			364
Aco4	?	aconitase: electrophoretic mobility; monomeric			364
Acp1	9L	acid phosphatase (was *Ap1, Acph1, Phos*): electrophoretic mobility; cytosolic; dimeric			76a 124
Acp2	?	acid phosphatase (was *Ap2*): electrophoretic mobility; dimeric			76a 162
Acp4	1L-176	acid phosphatase: electrophoretic mobility; monomeric			162
Act1	8	actin candidate: RFLP loci(probe): *BNL-ACT1* (pMAcI), *NPI368-ACT1* ()			372 37
ad1	1L-108	adherent: seedling leaves, tassel branches, and occasionally top leaves adhere	S	P	165
Adh1	1L-128	alcohol dehydrogenase: electrophoretic mobility; null allele is known; dimeric; intra/interlocus hybrid bands occur; RFLP locus(probe): *NPI21-ADH1* ()	S		317

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Adh2	4S-46	alcohol dehydrogenase: electrophoretic mobility; null allele is known; dimeric; intra/interlocus hybrid bands occur; RFLP loci(probe): *BNL-ADH2* (pZML841), *NPI228-ADH2* ()			315
Adk1	6S-0	adenylate kinase: electrophoretic mobility; plastidial			365
Adp1	1L	ADP glucose pyrophosphorylase candidate: RFLP locus(probe): *NPI309-ADP1* ()			373
Adp2	2L-near *Ht1*	ADP glucose pyrophosphorylase candidate: RFLP locus(probe): *NPI310-ADP2* ()			373
Adp3	4	ADP glucose pyrophosphorylase candidate: RFLP locus(probe): *NPI314-ADP3* ()			373
Adp4	8	ADP glucose pyrophosphorylase candidate: RFLP locus(probe): *NPI318-ADP4* ()			373
Adp5	10S	ADP glucose pyrophosphorylase candidate: RFLP locus(probe): *NPI319-ADP5* ()			373
Adr1	?	alcohol dehydrogenase regulator			177
ae1	5L-57	amylose extender: glassy, tarnished endosperm; high amylose content; starch branching enzyme IIb	S	P	363
afd1	?	absence of first division: male and female sterility; anaphase I equatorial			120
AGR		Agrigenetics: designator for loci defined by restriction fragment polymorphisms			
agt1	?	ageotropic: primary root unresponsive to gravity			73
al1	2S-4	albescent plant: variably cross-banded to white leaves; pale yellow endosperm, some alleles viviparous (see Coe et al., 1988)	S	P	273
alh1	1L-near *bm2*	histone Ia (was *H1a*): electrophoretic mobility			348
Alpha		*A1* locus component (see *Beta*): determines reduced aleurone and plant color, brown pericarp			181
Alr1	2L	aleurain analog candidate: RFLP locus(probe): *NPI348-ALR1* ()			372
Alr2	7L	aleurain analog candidate: RFLP locus(probe): *NPI349-ALR2* ()			372
am1	5S-20	ameiotic: male and female sterility; anaphase I equatorial	S	P	264 287
Amp1	1L-near *f1*	aminopeptidase: electrophoretic mobility; cytosolic; monomeric			262
Amp2	1-near *hm1*	aminopeptidase: electrophoretic mobility; monomeric			262
Amp3	5S-near *a2*	aminopeptidase: electrophoretic mobility; monomeric			262
Amp4	?	aminopeptidase: electrophoretic mobility; monomeric			262
Amy1	?	alpha amylase: electrophoretic mobility; monomeric			41
Amy2	5S-near *Mdh5*	beta amylase: electrophoretic mobility; monomeric			40
an1	1L-104	anther ear: andromonoecious dwarf, intermediate stature; few tassel branches; responds to gibberellins; *an1-6923* includes deletion of *Bz2*	S	P	78 88
anl1	5S-near *lu1*	anthocyaninless lethal: colorless aleurone; small kernels; embryo lethal			47
Ant1	5L	adenine nucleotide translocator candidate: RFLP locus(probe): *UMC142-ANT* (pZmc-ATP-01)			9
Ant2	?	adenine nucleotide translocator candidate (cDNA probe)			9a
aph1	?	aphid resistance			39
ar1	9L-62	argentia: virescent seedling, greens rapidly; husk leaf tips striped	S	P	92
ARS		designator for autonomously replicating sequences			
as1	1-56	asynaptic: synaptic failure in male and female	S	P	16
Asr1	4S-19	absence of seminal roots			225
Atc1		(see *Zb8*)			
atn1	?	anaerobic tolerant null: enhances survival of ADH-null under anoxia			
ats1	8	atrazine susceptible: lacks glutathione S-transferase			128
axr1	?	auxin receptor candidate (cDNA probe)			136a
Bl	2S-49	colored plant: anthocyanin in major plant tissues; some alleles affect aleurone and embryo color (for alleles, see Coe et al., 1988)	S	P	84
B chr		B chromosome: supernumerary chromosome		P	281
ba1	3L-102	barren stalk: ear shoots and most tassel branches and spikelets absent	S	P	137
ba2	2-near *ts1*	barren stalk: like *ba1*, but tassel more normal	S		137
ba3	?	barren stalk			264a
baf1	9S-near *w11*	barren stalk fastigiate (was *ba*-s*): ear shoots often absent; tassel branches erect			48
bd1	7L-109	branched silkless: ear silkless, branched at base; tassel proliferated, bushy	S	P	168
beta		*A1* locus component (see alpha): determines aleurone and plant color, red pericarp			181
Bf1	9L-137	blue fluorescent: homozygous seedlings (homozygous or heterozygous anthers) fluoresce blue under ultraviolet; anthranilic acid present	S	P	354
bf2	10L-30	blue fluorescent: similar to *Bf1* in expression; shows earlier, stronger seedling fluorescence than *Bf1*	S		2
Bg		Bergamo: regulatory element mediating *o2-mr*			301
Bh1	6L-50	blotched: colored patches on colorless (*c1*) aleurone	S	P	83
Bif1	8	barren inflorescence (was *Bif*-1440*): ear and tassel have many fewer spikelets, bare rachis appendages	S		253
bk2	9L-82	brittle stalk: brittle plant parts after 4-leaf stage	S	P	180
Blh1	1S	bleached (was *Bleached-1593*): pale green midveins and base in upper leaves			248
bm1	5S-41	brown midrib: brown pigment over vascular bundles of leaf sheath, midrib, and blade	S	P	91
bm2	1L-161	brown midrib: like *bm1*	S		36
bm3	4-near *bt2*	brown midrib: like *bm1* (C.R. Burnham, 1935, unpublished data)	S		89 175
bm4	9L-141	brown midrib: like *bm1*	S		33
Bn1	7L-71	brown aleurone: yellowish brown aleurone color	S		173
BNL		Brookhaven National Laboratory: designator for loci defined by restriction fragment polymorphisms			
br1	1L-81	brachytic: short internodes, short plant; no response to gibberellins	S	P	164 167

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
br2	1L-near *hm1*	brachytic: like *br1*	S		183
br3	5	brachytic: like *br1*	S		329
brn1	3S-19	brown aleurone: brown kernel, brown embryo; seedling lethal			299
BS1	?	barley stripe: transposable element, retrovirus-like; 1-5 copies in genome			156
bs1	?	barren sterile			217
bt1	5L-42	brittle endosperm: mature kernel collapsed, angular, often translucent and brittle; affects starch-granule-bound phospho-oligosaccharide synthase	S	P	203 367
bt2	4S-67	brittle endosperm: like *bt1* ; ADP glucose pyrophosphorylase electrophoretic mobility; (compare *sh2*) (G.F. Sprague, 1935, unpublished data)	S		89 355
btn1	?	brittle node			163
bu1	?	leaf burn: leaves show burning, sometimes horizontal bands, accentuated by high temperature			107
bv1	5L-47	brevis plant: short internodes, short plant	S		185
bv2	?	brevis plant: plant height 30-50% of normal			274
bx1	4S	benzoxazinless: absence of cyclic hydroxamates (blue color in crushed root tip with FeCl3), which inhibit *Ostrinia nubilalis* and *Helminthosporium turcicum*			58
bz1	9S-31	bronze: modifies purple aleurone and plant color to pale or reddish brown; anthers yellow-fluorescent; UDPG-flavonol 3-O-glucosyl transferase; allele *bz1-m4* = *sh1-bz1-m4* ; RFLP loci(probe): *BNL-BZ1* (pMBzPA), *NPI8-BZ1*	S	P	286
bz2	1L-106	bronze: like *bz1* ; anthers not fluorescent; *an1-6923* mutation includes deletion for *Bz2*	S	P	260
C1	9S-26	colored aleurone: *c1* colorless; *C1-I* dominant colorless; *c1-p* pigment inducible by light (see Coe et al., 1988); RFLP locus(probe): *BNL-C1* (pEco1.0)	S	P	75
c2	4L-117	colorless: colorless aleurone, reduced plant color; chalcone synthase; *C2-Idf* dominant inhibitor (see Coe et al., 1988); RFLP locus(probe): *BNL-C2* (pC2-c46)	S	P	45 127
Cab1	3L-near *Mdh3*	chlorophyll a/b binding protein candidate: RFLP locus(probe): *NPI477-CAB1* ()			373
Cab2	7L	chlorophyll a/b binding protein candidate: RFLP locus(probe): *NPI478-CAB2* ()			373
Cab3	7L	chlorophyll a/b binding protein candidate: RFLP locus(probe): *NPI478-CAB3* ()			373
Cab4	8L	chlorophyll a/b binding protein candidate: RFLP locus(probe): *NPI479-CAB4* ()			373
Car1	1S	catalase regulator: enzyme activity level increased			307
Cat1	5S-near *Mdh5*	catalase: electrophoretic mobility; cytosolic/glyoxysomal; tetrameric; intra/interlocus hybrid bands occur			19
Cat2	1S	catalase: electrophoretic mobility; null allele is known; cytosolic/glyoxysomal; tetrameric; intra/interlocus hybrid bands occur			304
Cat3	4S	catalase: electrophoretic mobility; null allele is known; mitochondrial; tetrameric; no hybrid bands			306
Cdh1	?	cinnamyl alcohol dehydrogenase: electrophoretic mobility			101
Ce1	?	curled entangled: rolled leaves tend to be entangled			42 265
cfl2	?	complementary to *fl2*			263
cfr1	1S	coupling factor reduction: chloroplast ATP synthase affected			76
Cg1	3S-35	corngrass: narrow leaves, extreme tillering	S	P	328
Cg2	?	corngrass: like *Cg1* ; mutable			197
Cgl1	?	*Colletotrichum graminicola* resistance			8
Ch1	2L-155	chocolate pericarp: dark brown pericarp	S	P	4
Cin		Cinteotl corn insert: repetitive sequences dispersed in the genome			320
cl1	3S-60	chlorophyll: white to green seedlings, depending upon *Clm1* ; pale yellow endosperm	S		90
clh1	?	histone Ic: electrophoretic mobility			348
Clm1	8	modifier of *cl1* : greens *cl1* seedlings; does not restore endosperm carotenoids	S		90
Clt1	8	clumped tassel (was *Clt*-985*): variable dwarfing, developmental anomalies	S		110 252
cm1	10L-near *R1*	chloroplast mutator: like *ij1*	S		350
cms-C		cytoplasmic male sterility: female-transmitted male sterility, C type; restored by *Rf4*			17
cms-S		cytoplasmic male sterility: female-transmitted male sterility, S type; restored by *Rf3*			159 161
cms-T		cytoplasmic male sterility: female-transmitted male sterility, Texas type; restored by *Rf1* *Rf2*			159 161
cp1	7S-near *vp9*	collapsed: endosperm collapsed and partially defective			195
cp2	7S-near *vp9*	collapsed: endosperm rough, collapsed, partially defective; seedling very light green with darker streaks; lethal			256
cr1	3S-26	crinkly leaves: plant short; leaves broad, crinkled, foreshortened	S	P	85
Css1	9L-near *gl15*	sucrose synthase (= *Sus2-ne*): sucrose synthase-2 of embryo and other tissues; (compare *sh1*); RFLP loci(probe): *BNL-CSS1* (pshD13), *NPI121-CSS1* ()			209
ct1	8	compact plant: semi-dwarf plant, ear furcated			241
ct2	1S	compact plant: semi-dwarf plant with club tassel			113
ctDNA		chloroplast DNA: sequences or loci in chloroplast genome			
cto1	?	cob turned out: ear inverted to a sheet or tube, kernels internally placed; variable expression			361
Cx1	10L-near *bf2*	catechol oxidase: electrophoretic mobility; null allele is known; monomeric; no hybrid bands			280
Cy	5L-near *pr1*	: regulatory element mediating *bz1-rcy*			309
d1	3S-44	dwarf plant: plant andromonoecious, short, compact; responds to gibberellins; *d1-t* intermediate in height	S	P	78
d2	3	dwarf plant: like *d1*			352
d3	9S-59	dwarf plant: like *d1*	S		63

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
d5	2S-34	dwarf plant: like *d1*	S		352
D8	1L-133	dwarf plant: dominant, resembles *d1* ; not responsive to gibberellins; (compare *Mpl1*)	S	P	271
da1	9	dilute aleurone: aleurone color diluted	S		96
Dap1	5L-near *Got2*	dappled aleurone: patches of normal and abnormal aleurone cells			347
db1	?	dichotomously branching plants (= *dib*): variable location of dichotomy, usually at 4-8th node (possible association with aneuploidy)	S		216 217
dek1	1S-27	defective kernel (was *clf1, gay1, clf*-792*): germless; floury endosperm; anthocyanins and carotenoids absent; cultured embryos not obtained	S		254 255
dek2	1L	defective kernel (was *dsc*-1315A*): discolored, scarred endosperm; lethal; cultured embryos green	S		254 255
dek3	2S	defective kernel (was *gm*-1289*): germless; cultured embryos white with green stripe	S		254 255
dek4	2L	defective kernel (was *clf*-1024A*): germless; floury endosperm; cultured embryos green, narrow leaved	S		254 255
dek5	3S	defective kernel (was *sh*-874A*): shrunken endosperm; white seedling with green stripes	S		254 255
dek6	3L	defective kernel (was *sh*-627D*): shrunken endosperm; lethal; cultured embryos normal	S		254 255
dek7	4S-near *Ts5*	defective kernel (was *su*-211C*): shrunken sugary endosperm; white seedling with green stripes	S		254 255
dek8	4L	defective kernel (was *sh*-1156A*): shrunken endosperm; lethal; cultured embryos green, small	S		254 255
dek9	5L	defective kernel (was *crp*-1365*): crumpled endosperm; lethal; anthocyanins and carotenoids reduced; cultured embryos not obtained	S		254 255
dek10	4L	defective kernel (was *cp*-1176A*): collapsed endosperm; lethal; cultured embryos green, curled, stubby	S		254 255
dek11	7L	defective kernel (was *et*-788*): etched endosperm; lethal; cultured embryos white with green stripes	S		254 255
dek12	9S	defective kernel (was *cp*-873*): collapsed endosperm; lethal; cultured embryos green, narrow-leaved, curled	S		254 255
dek13	9L	defective kernel (was *o*-744*): defective opaque endosperm; lethal; cultured embryos pale green with green stripes	S		254 255
dek14	10S	defective kernel (was *cp*-1435*): collapsed endosperm; lethal; cultured embryos yellow-green	S		254 255
dek15	10L	defective kernel (was *cp*-1427A*): collapsed floury endosperm; lethal; cultured embryos green	S		254 255
dek16	2L	defective kernel (was *fl*-1414*): floury endosperm; lethal; cultured embryos normal	S		321
dek17	3L	defective kernel (was *cp*-330D*): collapsed endosperm; lethal; cultured embryos not obtained	S		321
dek18	5S	defective kernel (was *cp*-931A*): collapsed endosperm; lethal; cultured embryos green, narrow-leaved	S		321
dek19	6L	defective kernel (was *o*-1296A*): collapsed opaque endosperm; lethal; cultured embryos green	S		321
dek20	8L	defective kernel (was *cp*-1392A*): collapsed endosperm; lethal; cultured embryos green	S		321
dek21	10L	defective kernel (was *msc*-1330*): aleurone mosaic of reduced anthocyanins; reduced carotenoids; lethal; cultured embryos white; (compare *w2*)	S		321
dek22	1L	defective kernel (was *cp*-1113A*): collapsed endosperm; lethal; cultured embryos not obtained	S		44 322
dek23	2L	defective kernel (was *dcr*-1428*): defective crown; lethal; cultured embryos not obtained	S		44 322
dek24	3S	defective kernel (was *cp*-1283*): collapsed endosperm; lethal; cultured embryos normal	S		322
dek25	4S	defective kernel (was *sh*-1167A*): shrunken endosperm; lethal; cultured embryos normal	S		322
dek26	5L	defective kernel (was *cp*-1331*): collapsed endosperm; lethal; cultured embryos normal	S		322
dek27	5L	defective kernel (was *cp*-1380A*): collapsed endosperm; lethal; cultured embryos green	S		322
dek28	6S	defective kernel (was *o*-1307A*): opaque endosperm	S		322
dek29	8L	defective kernel (was *cp*-1387A*): collapsed endosperm; viable; cultured embryos green, narrow-leaved	S		322
dek30	9L	defective kernel (was *fl*-1391*): floury endosperm; lethal; cultured embryos green, narrow-leaved	S		322
dek31	4L-near *Tu1*	defective kernel (was *ptd*-1130*): pitted endosperm; lethal	S		323
dep1	6	defective pistils			218
Df		deficiency: general symbol for loss of segments of chromosome			
Dhn1	6L	dehydrin candidate: dehydration-induced protein;RFLP locus (probe): *UMC170-DHN*			44a
Dia1	2-near *v4*	diaphorase: electrophoretic mobility; cytosolic; monomeric			364
Dia2	1L-near *bm2*	diaphorase: electrophoretic mobility; cytosolic; dimeric			364
dp1	4L-137	distal pale: seedling leaf tip virescent (E.G. Anderson, unpublished)	S		
Ds		dissociation: designator for transposable factors regulated by *Ac* ; modifies gene function and/or chromosome breakage (termed " *Ds-2* "); ex. *Ds2* designates element isolated from *Adh1-2F11*	S	P	212
dSpm		defective suppressor-mutator: designator for transposable factors regulated by *Spm*			308
dsy1	?	desynaptic: male and female sterility; synaptic failure			118
dsy2	?	desynaptic: like *dsy1*			117
dsy3	?	desynaptic: like *dsy1*			115a
dsy4	?	desynaptic: like *dsy1*			115a

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Dt1	9S-0	dotted: regulated controlling element at *A1* ; responding *a1-m* alleles express colored dots on colorless kernels and purple sectors on brown plants	S	P	282
Dt2	6L-44	dotted: like *Dt1*	S		261
Dt3	7L	dotted: like *Dt1*, but expression variable	S		261
Dt4	4	dotted: like *Dt1*, but dots chiefly on crown of kernel	S		67
Dt5	9S-near *yg2*	dotted: like *Dt1*			67
Dt6	4-near *su1*	dotted: like *Dt1*	S		339
du1	10L-28	dull endosperm: glassy, tarnished endosperm; affects soluble starch synthase and branching enzyme IIa (P.C. Mangelsdorf, 1935, unpublished data)	S		89 204
dv1	?	divergent spindle: chromosomes unoriented at metaphase I; partial male and female sterility	S		43
dy1	?	desynaptic: chromosomes unpaired in microsporocytes; partial male and female sterility	S		240
E1	7L	esterase: electrophoretic mobility; null allele is known; dimeric; intralocus hybrid bands occur			312
E2	?	esterase: presence-absence			314
E3	3S	esterase: electrophoretic mobility; dimeric; intralocus hybrid bands occur			313
E4	3S-near *cl1*	esterase (was *Est4*): electrophoretic mobility; null allele is known; monomeric			132
E5-I	?	esterase (duplicate factor with *E5-II*): electrophoretic mobility			199
E5-II	?	esterase (duplicate factor with *E5-I*): electrophoretic mobility			199
E6	?	esterase: presence-absence			199
E7	?	esterase: presence-absence			199
E8	3S-14	esterase: electrophoretic mobility; null allele is known; dimeric; intralocus hybrid bands occur			199
E9	?	esterase: electrophoretic mobility; null allele is known			199
E10	?	esterase: electrophoretic mobility			199
eg1	5L	expanded glumes: glumes open at right angle	S		34
Ej1		(= *Isr1*)			
el1	8L	elongate: chromosomes uncoiled during meiotic metaphase and anaphase in male and female; frequent unreduced gametes	S	P	287
Emu1	2	endogenous *Mu* : RFLP locus(probe): *NPI347-EMU1* ()			372
En		enhancer: transposable element (equivalent to *Spm*); autonomous, regulates I transposition (e.g. at *g2-m* = *pg-m* = *pg14-m*)		P	269
Enp1	6L-near *y1*	endopeptidase: electrophoretic mobility; null allele is known; monomeric			215
Est		(see *E*)			
et1	3L-161	etched: pitted, scarred endosperm; virescent seedling	S	P	343
f1	1L-86	fine stripe: virescent seedling, fine white stripes on base and margin of older leaves	S	P	189
fae1	?	fasciated ear: small, rounded ears branched at their tips			323
Fas1	?	fasicled ear: ears and tassels branch dichotomously, may fasciate			204a
Fbr1	?	few-branched (was *Fbr*-1602*): tassel reduced to 0-3 branches; bract replaces next-to-bottom branch			114
Fcu		factor Cuna: controlling element of *r1-cu*			122
fl1	2S-68	floury endosperm (= *o4*): endosperm opaque, soft; dosage effect	S	P	136
fl2	4S-58	floury: endosperm opaque, soft; dosage effect (W.J. Mumm, 1935, unpublished data)	S		89 244
fl3	8L-0	floury: endosperm opaque, soft; dosage effect	S		237
Flt		flint: designator for factors determining flint endosperm type			229
g1	10L-47	golden plant: seedling and plant with distinct yellow cast	S	P	78 80
g2 / *g5*	3S-0	golden plant (= *g5* = *pg14* = *v19*): like *g1*, but more extreme; sheaths whitish yellow-green (= *g2*)	S		148
G6	9S-near *l7*	golden plant (was *G*-1585*): like *g1* ; lighter yellowish sheaths			257
Ga1	4S-32	gametophyte factor (= *ga9*): *Ga1* pollen grains competitively superior to *ga1* on *Ga1* silks; *Ga1-S* super-gametophyte	S		160
ga2	5L-55	gametophyte factor: *Ga2* pollen grains competitively superior to *ga2*	S		32
ga7	3L-167	gametophyte factor: *ga7* pollen from heterozygotes 10-15% functional regardless of silk genotype			284
ga8	9S-near *lo2*	gametophyte factor: *Ga8* pollen grains competitively superior to *ga8* on *Ga8* silks			311
ga9		(= *ga1*)			
ga10	5	gametophyte factor			121
Gdh1	1L-near *vp8*	glutamic dehydrogenase: electrophoretic mobility; null allele is known; intra/interlocus hybrid bands occur			278
Gdh2	10	glutamic dehydrogenase: electrophoretic mobility; intralocus hybrid bands occur			123
Ger		glucoside earworm resistance: designator for earworm resistance factors from Cateto Palha Roxa			229
gl1	7L-36	glossy: cuticle wax altered; leaf surface bright, water adheres	S	P	173
gl2	2S-30	glossy: like *gl1*	S	P	134
gl3	4L-112	glossy: like *gl1*	S		134
gl4	4L-81	glossy (= *gl16*): like *gl1* (G.F. Sprague, unpublished)	S		
gl5	?	glossy (was *gl5-1*, duplicate factor with *gl20*): like *gl1* (G.F. Sprague, 1935, unpublished data)			89 340
gl6	3L-69	glossy: like *gl1* (G.F. Sprague, 1935, unpublished data)	S		89
gl7	3L	glossy (= *gl12*): like *gl1* (G.F. Sprague, 1935, unpublished data)			89
gl8	5L-68	glossy (= *gl10*): like *gl1* (G.F. Sprague, 1935, unpublished data)	S		89
gl9	?	glossy: expression poor (G.F. Sprague, 1935, unpublished data)			89
gl10		(= *gl8*)			
gl11	2S-near *B1*	glossy: like *gl1* ; abnormal seedling morphology	S		337

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
gl12		(= *gl7*)			
gl14	2	glossy (duplicate factor with *gl24*): like *gl1*			3
gl15	9L-66	glossy: like *gl1* ; expressed after 3rd leaf	S	P	3
gl16		(= *gl4*)			
gl17	5S-34	glossy: like *gl1*, but semi-dwarf with necrotic crossbands on leaves	S		288
gl18	8L-near *fl3*	glossy: like *gl1* ; expression poor	S		
gl19	3S	glossy (was *gl*-169*): like *gl1* ; lethal	S		249
gl20	?	glossy (was *gl5-2*, duplicate factor with *gl5*): like *gl1*			340
gl21	10S	glossy (was *gl*-478B*, duplicate factor with *gl22*): like *gl1*	S		249
gl22	?	glossy (was *gl*-478C*, duplicate factor with *gl21*): like *gl1*			247
gl23	?	glossy (was *gl*-PI262490*): like *gl1*			341a
gl24	?	glossy (duplicate factor with *gl14*): like *gl1*			341a
Glb1	1L-121	globulin (was *Prot1*): Mr 63,000, electrophoretic mobility; null allele is known; embryo protein			316 172
Glb2	?	globulin: Mr45,000, presence-absence			172
Glu1	10L-near *bf2*	beta glucosidase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intralocus hybrid bands occur			279
Got1	3L-near *Me1*	glutamate-oxaloacetate transaminase (possibly = *Ta1*): electrophoretic mobility; null allele is known; glyoxysomal; dimeric; intralocus hybrid bands occur			305
Got2	5L-96	glutamate-oxaloacetate transaminase: electrophoretic mobility; null allele is known; plastidial; dimeric; intralocus hybrid bands occur			125
Got3	5S-near *a2*	glutamate-oxaloacetic transaminase: electrophoretic mobility; null allele is known; mitochondrial; dimeric; intralocus hybrid bands occur			125
Gpa1	?	glyceraldehyde-3-phosphate dehydrogenase, chloroplstic, A subunit (cDNA probe pZm57)			28a
Gpc1	?	glyceraldehyde-3-phosphate dehydrogenase. cytosolic, C subunit (cDNA probe pZm9)			28a 299a
Gpc2	?	glyceraldehyde-3-phosphate dehydrogenase, cytosolic, C subunit (cDNA probe pGAPC2)			299a
Gpc3	?	glyceraldehyde-3-phosphate dehydrogenase, cytosolic, C subunit (cDNA probe pGAPC3)			299a
grt1	5L	green tip (was *grt*-1308B*): pale yellow seedling with green first leaf tip; lethal	S		249
gs1	1L-135	green stripe: grayish green stripes between vascular bundles on leaves; tissue wilts	S	P	83 223
gs2	2S-54	green stripe: like *gs1*, but pale green stripes; no wilting (G.F. Sprague, 1935, unpublished data)	S	P	89
gs3	6L	green stripe (was *gs*-268*): like *gs2*			249
Gs4	10	green stripe (was *Gs*-1439*.): like gs1			248a
gt1	1	grassy tillers: numerous basal branches; vegetatively totipotent in combination with *id1* and *pe1*	S		318
h1	3	soft starch: endosperm soft, opaque	S		235
hcf1	2L	high chlorophyll fluorescence: affects NADP+ oxidoreductase; green seedling			220
hcf2	1L	high chlorophyll fluorescence: missing cytochrome f/b6 complex; yellow-green seedling			220
hcf3	1S	high chlorophyll fluorescence (= *hcf9*): missing PSII thylakoid membrane core complex; green seedling			220
hcf4	1L	high chlorophyll fluorescence: affects CO2 fixation; green seedling			221
hcf5	6S	high chlorophyll fluorescence: affects PSII reaction; green seedling			222
hcf6	1S	high chlorophyll fluorescence: missing cytochrome f/b6 complex; green seedling			184
hcf9		(= *hcf3*)			
hcf12	1L	high chlorophyll fluorescence			184
hcf13	1L	high chlorophyll fluorescence: affects CO2 fixation; green seedling			184 221
hcf15	2L	high chlorophyll fluorescence: affects photophosphorylation; yellow-green seedling			184
hcf18	5L-near *pr1*	high chlorophyll fluorescence (= *hcf43*): major loss of PSI; other thylakoid complexes reduced; yellow-green seedling			221
hcf19	3L	high chlorophyll fluorescence: affects PSII thylakoid membrane core complex; green/yellow-green seedling			184 221
hcf21	5L	high chlorophyll fluorescence: affects CO2 fixation, Rubisco; green seedling			221
hcf23	4S	high chlorophyll fluorescence: affects photophosphorylation; near-white seedling			184 221
hcf26	6S	high chlorophyll fluorescence: affects electron transport; yellow-green, viable seedling			184 221
hcf28	10L	high chlorophyll fluorescence: affects CO2 fixation; green seedling			222
hcf31	1S	high chlorophyll fluorescence: missing chlorophyll a/b binding protein; yellow-green seedling			222
hcf34	6L	high chlorophyll fluorescence: affects photophosphorylation; yellow-green seedling			184 221
hcf36	6L	high chlorophyll fluorescence: affects electron transport; green seedling			222
hcf38	5L	high chlorophyll fluorescence: affects cytochrome f/b6 complex, alpha and beta components of CF1; green seedling			184
hcf41	1L	high chlorophyll fluorescence: affects PSII thylakoid membrane core complex; green seedling			184 221
hcf42	9L	high chlorophyll fluorescence: affects Rubisco; green/yellow-green seedling			221
hcf43		(= *hcf18*)			
hcf44	1L	high chlorophyll fluorescence: affects PSI membrane core complex; pale-green seedling			221
hcf46	3L	high chlorophyll fluorescence			184
hcf47	10S	high chlorophyll fluorescence: affects cytochromes; yellow-green seedling			222
hcf48	6L	high chlorophyll fluorescence: affects electron transport; yellow-green seedling			222
hcf50	1L	high chlorophyll fluorescence: missing PSI thylakoid membrane core complex; green seedling			221
hcf101	7L	high chlorophyll fluorescence (was *Mu-5**): affects PSI thylakoid membrane core complex			222
hcf102	8L	high chlorophyll fluorescence: affects cytochrome f/b6 complex (D. Miles, unpublished)			

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
hcf103	7L	high chlorophyll fluorescence: affects PSII			56
hcf104	7L	high chlorophyll fluorescence: photosystem I-deficient			56
hcf106	2-near *ts1*	high chlorophyll fluorescence: affects PSI, PSII, cytochrome f/b6			204a
hcf108	5	high chlorophyll fluorescence: ATPase-deficient			56
hcf111	7L	high chlorophyll fluorescence: cytochrome b/f-deficient			56
hcf113	9S	high chlorophyll fluorescence: multiple effects; yellow-green seedlings			55
hcf114		(= *hcf103*)			
hcf316	10S	high chlorophyll fluorescence: affects chlorophyll a/b binding protein; yellow-green seedling			222
hcf323	6S	high chlorophyll fluorescence: affects photophosphorylation, coupling factor; green seedling			222
hcf408	6L	high chlorophyll fluorescence: affects chlorophyll a/b binding protein; yellow-green seedling			222
Hex1	3S-near *Cg1*	hexokinase: electrophoretic mobility; null allele is known; cytosolic; monomeric			366
Hex2	6L-near *Pt1*	hexokinase: electrophoretic mobility; null allele is known; cytosolic; monomeric			366
hm1	1L-64	*Helminthosporium carbonum* susceptibility: disease lesions vs. yellowish flecks (resistant) on leaves with race 1	S	P	359
hm2	9L-near *bk2*	*Helminthosporium carbonum* susceptibility: like *hm1* ; masked by *Hm1*			243
Hs1	7S-0	hairy sheath: abundant hairs on leaf sheath	S	P	353
Hsf1	5	hairy sheath frayed (was *Hsf*-1595*): pubescent sheaths and leaf margins; liguled enations at leaf margins			21
Hsp1	8L	heat shock protein (70kD) candidate: RFLP locus(probe): *NPI119-HSP1* ()			372
Ht1	2L-121	*Helminthosporium turcicum* resistance	S		141
Ht2	?	*Helminthosporium turcicum* resistance			142
Ht3	?	*Helminthosporium turcicum* resistance: (from *Tripsacum floridanum*)			143
I		inhibitor (= *C1-I*, inhibitor allele at *C1* locus): also commonly used as a general symbol for inhibition and for the controlling elements responding to *En*			71
id1	1L-near *an1*	indeterminate growth: requires extended growth and short days for flowering; vegetatively totipotent with *gt1* and *pe1*	S		327
Idh1	8L	isocitrate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			125
Idh2	6L-near *w14*	isocitrate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			125
ig1	3L-90	indeterminate gametophyte: low male fertility, polyembryony, heterofertilization, polyploidy, androgenesis (male and female affected)	S		169
ij1	7L-52	iojap striping: many variable white stripes on leaves; conditions chloroplast defects that are cytoplasmically inherited	S	P	147
ij2	1L	iojap striping: like *ij1* ; chloroplast inheritance unknown			249
in1	7S-20	intensifier: intensifies aleurone anthocyanin pigments; *In1-D* dominant dilute	S	P	98
Inv		Inversion: general symbol for inversion of a segment of chromosome	S	P	
is1	?	cupulate interspace			104
Isr1	10L-near *R1*	inhibitor of striate (was *Ej1*): reduces expression of *sr2* and other leaf-striping factors	S		170
j1	8L-42	japonica striping: white stripes on leaf and sheath; not expressed in seedling	S	P	80
j2	4L-106	japonica striping: extreme white striping of leaves, etc. (R.A. Emerson, 1935, unpublished data)	S	P	89
K		knob: general symbol for constitutive heterochromatic elements			
K3L	3L-115	knob: constitutive heterochromatic element			65
K10	10L-near *sr2*	abnormal-10: heterochromatic appendage on long arm of chromosome 10; neocentric activity distorts segregation of linked genes	S	P	194
Kn1	1L-near *Adh1*	knotted: localized proliferation of tissue at vascular bundles on leaf	S	P	30
Kn2	?	knotted: finger-like projections of leaf at the ligule			100
Krn		kernel row number: designator for factors determining kernel row number			229
l1	10L-near *R1*	luteus: yellow pigment in white tissue of chlorophyll mutants *w1*, *w2*, *j1*, *ij1*, etc.	S	P	187 188
l4	?	luteus: lethal yellow seedling	S	P	153
l6	9S-near *bz1*	luteus: like *l4* (W.H. Eyster, 1935, unpublished data)	S		89
l7	9S-42	luteus: yellow seedling and plant; lethal	S		96
l10	6L-19	luteus: like *l4* ; fails to convert protochlorophyllide to chlorophyllide	S		294
l11	6S	luteus (was *l*-4120*): yellow seedling with green leaf tips; lethal	S		7
l12	6L-16	luteus (was *l*-4920*): like *l11*	S		59
l13	10L-91	luteus (was *l*-59A*, *l*-Neuffer2*): dark yellow, lethal seedling; fails to convert protoporphyrin IX to Mg-protoporphyrin			205 249
l15	6L-30	luteus (was *l*-Blandy3*, *l*-Brawn*): like *l4*			298
l16	1S	luteus (was *l*-515*): like *l4* ; leaves bleach to paler yellow in patches			249
l17	1L	luteus (was *l*-544*): like *l4* ; leaves with lighter yellow crossbands			249
l18	2L	luteus (was *l*-1940*): like *l4*			249
l19	10S	luteus (was *l*-425*): like *l4*			249
la1	4S-55	lazy plant: prostrate growth habit	S	P	154
lbl1	?	leaf bladeless: leaf blade reduced to absent; low temperature enhances expression			219
Lc1	10L-65	red leaf color: anthocyanin in coleoptile, nodes, auricle, leaf blade, etc.; (compare *Sn1*)	S		72
Lcs1	?	thylakoid membrane polypeptide: electrophoretic mobility			232
Lct1	?	thylakoid membrane polypeptide: electrophoretic mobility			232

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
lct2	?	thylakoid membrane polypeptide: presence-absence			232
Les1	2S-58	lesion (was *Les*-843*): large necrotic lesions resembling disease lesions formed by fungal infections on susceptible lines	S		250
Les2	1S-near *sr1*	lesion (was *Les*-845A*): small white lesions resembling disease lesions formed by fungal infections on resistant lines	S		250
Les3	10	lesion: like *Les1*			6
Les4	2L	lesion (was *Les*-1375*): late expression of large necrotic lesions	S		139
Les5	1S	lesion (was *Les*-1449*): like *Les2*			139
Les6	10S	lesion (was *Les*-1451*): like *Les4*	S		139
Les7	?	lesion (was *Les*-1461*): late expression of small chlorotic lesions	S		139
Les8	9S-near *lo2*	lesion (was *Les*-2005*): late expression of small, pale green lesions	S		139
Les9	7L-near *ra1*	lesion (was *Les*-2008*): late expression of small necrotic lesions	S		139
Les10	2-near *v4*	lesion (was *Les*-A607*): like *Les1*			140
Lfy1	?	leafy: increased number of leaves			319
lg1	2S-11	liguleless: ligule and auricle missing; leaves upright, enveloping	S	P	7879
lg2	3L-101	liguleless: like *lg1*, less extreme	S	P	25
Lg3	3-65	liguleless: dominant, no ligule; leaves upright, broad, often concave and pleated	S	P	266
li1	10L-near *bf2*	lineate leaves: fine, white striations on basal half of mature leaves	S	P	54
lls1	1S	lethal leaf spot: chlorotic-necrotic lesions resembling *Helminthosporium carbonum* infection	S		360
ln1	6	linoleic acid: lower ratio of oleate to linoleate in kernel			60
lo2	9S-50	lethal ovule: ovules containing *lo2* gametophyte abort	S		240
loc1	?	low oil content in kernel: associated with albino seedlings			275
lp1	4	lethal pollen: *lp1* pollen fails in competition with *Lp1*			239
lte1	2	latente: drought, heat, aluminum tolerance; frost resistance; from Michoacan 21; dominance varies			226
Lte2	10L-near *g1*	latente: drought, heat, aluminum tolerance; from Cateto; epistatic to *lte1*			227
lty1	?	light yellow endosperm			69
lty2	?	light yellow endosperm			69
lu1	5S-29	lutescent: pale yellow green leaves	S		325
lw1	1L-near *Adh1*	lemon white: white seedling, pale yellow endosperm	S		357
lw2	5L-near *pr1*	lemon white: like *lw1*	S	P	357
lw3	5L-near *v2*	lemon white (duplicate factor with *lw4*): like *lw1*			357
lw4	4-near *zb6*	lemon white (duplicate factor with *lw3*): like *lw1*			357
Lxm1	3	lax midrib (was *Lxm*-1600*): leaves with wide, flat, flexible midrib	S		246
lyc1		: (= *ps1-lyc*)			
mal1	9	multiple aleurone layering: recessive interacts with two complementary dominants *Mal2* and an unnamed factor, giving multiple cell layers			224
Mal2	4	multiple aleurone layering: (see *mal1*)			224
Mc1	?	mucronate: opaque endosperm			302
Mch1	?	maize CRY1 homolog: ribosomal protein gene family (cDNA probe)			180a
Mch2	?	maize CRY1 homolog: ribosomal protein gene family (cDNA probe)			180a
Mdh1	8	malate dehydrogenase: electrophoretic mobility; null allele is known; mitochondrial; dimeric; intra/interlocus hybrid bands occur			258
Mdh2	6L-near *w14*	malate dehydrogenase: electrophoretic mobility; null allele is known; mitochondrial; dimeric; intra/interlocus hybrid bands occur			258
Mdh3	3L-146	malate dehydrogenase: electrophoretic mobility; null allele is known; mitochondrial; dimeric; intra/interlocus hybrid bands occur			258
Mdh4	1L-near *an1*	malate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			258
Mdh5	5S-17	malate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			258
Mdm1	6-near *w15*	maize dwarf mosaic virus resistance			214a
Me1	3L-125	NADP malic enzyme: electrophoretic mobility; null allele is known; tetrameric; RFLP locus(probe): *NPI231-ME1* () [may be ambiguous]			125
Me2	6L	NADP malic enzyme candidate: RFLP locus(probe): *NPI330-ME2* ()			372
Mei1	?	meiosis: chromosomes sticky in metaphase I; male sterile			115 116
mep1	5L	modifier of endosperm protein: affects quantities of *Prot1* protein forms			316
Mer		Maya earworm resistance: designator for earworm resistance factors from IAC Maya			228
mg1	?	miniature germ (replaces *mg* of Wentz): germ 1/4 to 1/3 of normal; viable			178
mi1	1	midget plant: small plant (H.S. Perry, 1935, unpublished data)	S		89
mmm1	1L-near *an1*	modifier of mitochondrial malate dehydrogenases: mobilities			258
mn1	2-near *fl1*	miniature seed: small, somewhat defective kernel; fully viable	S	P	196
mn2	7	miniature seed: small kernel, loose pericarp; extremely defective but will germinate (R.J. Lambert, unpublished)	S		
Mod		modifier: inactive *Spm* element, enhances excisions elicited by active *Spm*			214
Mp		modulator of pericarp: transposable factor affecting *P1* locus; parallel to *Ac-Ds*			27
Mpi1		transposable element: 10-15 copies in the genome			369
Mpl1	1L-near *Adh1*	miniplant: dominant, andromonoecious, intermediate dwarf; compare *D8*; not responsive to gibberellins	S		130
Mr	9S-near *l7*	mutator of *R-m* : transposable factor, regulates *R1-m* mutation		P	38

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Mrh	5	mutator: controlling element of *a1-m-rh*			289
ms1	6L-near *si1*	male sterile: anthers shriveled, not usually exserted; affected at microspore vacuolation	S		330
ms2	9L-64	male sterile: like *ms1* ; affected between vacuolation and pore formation	S		94 96
ms3	3	male sterile: anthers shrivelled; not usually exserted			94 96
ms4		(= *po1*)			
ms5	5-near *v3*	male sterile: anthers not exserted; affected at microspore mitosis	S		14
ms7	7L-near *ra1*	male sterile: like *ms2*	S		14
ms8	8L-28	male sterile: like *ms5* ; affected in meiosis	S	P	14
ms9	1S-near *P1*	male sterile: like *ms5* ; affected in meiosis	S		14
ms10	10L-near *bf2*	male sterile: like *ms5* ; affected at microspore vacuolation	S		14
ms11	10	male sterile: like *ms5* ; affected at microspore mitosis	S		14
ms12	1	male sterile: like *ms1* ; affected at microspore vacuolation	S		14
ms13	5S	male sterile: like *ms5* ; affected at microspore vacuolation	S		14
ms14	1-near *as1*	male sterile: like *ms5* ; affected at microspore mitosis	S		14
ms17	1S-23	male sterile: like *ms1* ; affected variably in meiosis	S		86
ms20	?	male sterile			96
Ms21	6	male sterile: pollen grains developing in presence of *Ms21* are defective and nonfunctional if *sks1*, normal if *Sks1*			182 310
ms22	?	male sterile: affected in meiosis	S		368
ms23	3L	male sterile (allelic to *ms*-Bear7*): affected in meiosis	S		368
ms24	?	male sterile: like *ms1* ; affected in microspore mitosis	S		368
ms28	?	male sterile: anaphase I disturbed, spindle persists			116
Ms41	4L	male sterile (was *Ms*-1995*)	S		257
ms43	8L	male sterile: anaphase I impaired			115 116
Ms44	4L	male sterile (was *Ms*-7255*)			00
Msc1	1L	mosaic (was *Msc*-791A*): aleurone mosaic for anthocyanin color	S		257
Msc2	5S	mosaic (was *Msc*-1124B*): aleurone mosaic for anthocyanin color			257
Mst1	10L-67	modifier of *R-st* : affects expression of *R1-st*	S		5
mtDNA		mitochondrial DNA: sequences or loci in the mitochondrial genome			
Mu		mutator: freely transposable element; *Mu1* designates element isolated from *Adh1-S3034*			297
Mut	2S-near *gl2*	mutator: controlling element for *bz1-m-rh*			289
Mv1	?	resistance to maize mosaic virus I ("corn stripe")			23
na1	3L-113	nana plant: short, erect dwarf; no response to gibberellins	S	P	146 186
na2	5S-near *bt1*	nana plant: like *na1* (H.S. Perry, unpublished)	S		
NCS1		nonchromosomal stripe: maternally inherited light green leaf striping			326
NCS2		nonchromosomal stripe: maternally inherited pale green and depressed striping; mitochondrial	S		46
NCS3		nonchromosomal stripe: maternally inherited striations, distorted plants; mitochondrial	S		46
nec1	8L-near *fl3*	necrotic (was *nec*-6697, sienna*-7748*): chlorotic seedling that stays rolled, wilts and dies			206
nec2	1S-34	necrotic (was *nec*-8147, olive-necrotic-8147, ON-8147*): green seedling develops necrotic lesions at 2-3 leaf stage; lethal (E.G. Anderson, 1952, unpublished data)	S		
nec3	5-near *bt1*	necrotic (was *nec*-409*): seedling emerge with tightly rolled leaves that turn brown and die without unrolling; manually unrolled leaves tan with dark brown crossbands			245
nec4	2S-near *d5*	necrotic (was *nec*-516B*): seedling yellow, leaf tips necrotic; lethal			138
nec5	4L	necrotic (was *nec*-642A*): pale green seedling becoming necrotic; dark brown exudate; lethal			249
nec6	5S-near *a2*	necrotic (was *nec*-493*): like *nec3*			249
nec7	5L	necrotic (was *nec*-756B*): seedling becoming necrotic in crossbands; lethal			249
NIU		Northern Illinois University: designator for loci defined by restriction fragment polymorphisms			
nl1	10L-near *bf2*	narrow leaf: leaf blade narrow, some white streaks (R.A. Emerson, 1935, unpublished data)	S	P	89
Nl2	5-near *lu1*	narrow leaf (was *Rgd2, Rgd*-1445*): leaves narrow and distorted; tillering			253
NOR	6S	nucleolus organizer: codes for ribosomal RNA	S		210
NPI		Native Plants, Inc.: designator for loci defined by restriction fragment polymorphisms			
o1	4L-near *gl3*	opaque endosperm: endosperm starch soft, opaque (W.R. Singleton and D.F. Jones, 1935, unpublished data)	S		89 244
o2	7S-16	opaque endosperm: like *o1* (W.R. Singleton and D.F. Jones, 1935, unpublished data); high lysine content; regulates b32 protein (see *pro1*) reduced lysine degradation (lysine-ketoglutaric reductase); RFLP loci(probe): *BNL-O2* (pXho0.9), *NPI480-O2* ()	S	P	89 244
o4		(= *fl1*)			
o5	7L-near *ra1*	opaque endosperm: like *o1* ; virescent to yellow or white seedlings	S		293
o6		(= *pro1*)			
o7	10L-87	opaque: like *o1* ; high lysine content	S		230
o9	?	opaque endosperm: crown opaque and light in color, frequently with a cavity; base or abgerminal side of kernel often corneous	S		238
o10	?	opaque endosperm: like *o1*	S		238
o11	?	opaque endosperm: thin, opaque, somewhat shrunken kernels with greyish cast	S		238
o12	?	opaque endosperm: thin, etched or scarred kernels, variable in size; plants chlorophyll deficient and small, with pollen but few ears	S		238
o13	?	opaque endosperm: opaque, etched kernels with rim of corneous starch on abgerminal side	S		238
Oec1	4	oxygen-evolving complex protein candidate: RFLP locus(probe): *NPI472-OEC* ()			373

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Oec2	2S-near *B1*	oxygen-evolving complex protein candidate: RFLP locus(probe): *NPI473-OEC* ()			373
Oec3	5S-near *Pgm2*	oxygen-evolving complex protein candidate: RFLP locus(probe): *NPI474-OEC* ()			373
Oec4	7L	oxygen-evolving complex protein candidate: RFLP locus(probe): *NPI475-OEC* ()			373
Oec5	8	oxygen-evolving complex protein candidate: RFLP locus(probe): *NPI476-OEC* ()			373
Og1	10S-16	old gold stripe: variable bright yellow stripes on leaf blade	S	P	192
ora2	?	orange endosperm			68
ora3	?	orange endosperm			69
oro1	6S	orobanche: yellow to tan necrotic with cross-banding when grown under light-dark cycle; some chlorophyll with *Orom1* ; fails to convert Mg-protoporphyrin monomethyl ester to protochlorophyllide	S		205
oro2	?	orobanche: like *oro1*			205
Orom1	?	orobanche modifier: partially corrects chlorophyll loss in *oro1*			205
orp1	4S-near *su1*	orange pericarp (duplicate factor with *orp2*): pericarp orange over *orp1 orp2* kernels; lethal	S		251
orp2	10L	orange pericarp (duplicate factor with *orp1*)	S		251
oy1	10S-12	oil yellow: seedling oily greenish-yellow; viable; fails to convert protoporphyrin IX to Mg-protoporphyrin; *oy1-t* tinged green; *oy1-1039, oy1-1040* lethal; *Oy1-700* dominant yellow-green (see Coe et al., 1988)	S	P	95
P		plant color component at *R1* : anthocyanin pigmentation in seedling leaf tip, coleoptile, anthers			344 345
P1	1S-26	pericarp color: red pigment in cob and pericarp (for alleles, see Coe et al., 1988); RFLP locus(probe): *NPI370-P1* ()	S	P	77 193
pam1	?	plural abnormalities of meiosis: desynchronized meiotic divisions and premeiotic mitosis; male sterile, incompletely female sterile			119
pam2	?	plural abnormalities of meiosis: like *pam1*			117
pb1	6L-near *y1*	piebald leaves: very light, irregular green bands on leaf	S	P	64
pb4	6L-near *y1*	piebald leaves: like *pb1*	S		64
pd1	?	paired rows: single vs. paired pistillate spikelets; *pd1* is found in teosinte also			179
Pdf1	?	thylakoid membrane polypeptide: dominant increase in electrophoretic mobility			233
Pdk1	6L	pyruvate, Pi dikinase candidate: RFLP loci(probe): *UMC173-PDK* (p1-9), *NPI229-PDK1* ()			372
Pdk2	8L	pyruvate, Pi dikinase candidate: RFLP loci(probe): *NPI230-PDK2* ()			372
pe1	?	perennialism: vegetatively totipotent in combinations with *gt1* and *id1*			318
Pep1	9-near *pg12*	phosphoenol pyruvate carboxylase candidate: RFLP locus(probe): *NPI332-PEP1* ()			372
pg11	6L-38	pale green (duplicate factor with *pg12*): seedling light yellowish green; mature plant pale and vigorous	S	P	285
pg12	9-61	pale green (duplicate factor with *pg11*)	S		285
pg13	?	pale green: seedling light yellowish green; stunted growth			324
pg14		(= *g2*)	S	P	269
pg15	1S	pale green (was *ppg*-340B*): seedling light yellowish green; bleaches to near white in patches; lethal			249
pg16	1L	pale green (was *pg*-219*): seedling light yellowish green			249
Pgd1	6-near *rgd1*	6-phosphogluconate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			125
Pgd2	3L-near *Rg1*	6-phosphogluconate dehydrogenase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intra/interlocus hybrid bands occur			125
PGE		Plant Gene Expression Center: designator for loci defined by restriction fragment polymorphisms			
Pgm1	1L-near *Glb1*	phosphoglucomutase: electrophoretic mobility; null allele is known; cytosolic; monomeric			125
Pgm2	5S-0	phosphoglucomutase: electrophoretic mobility; null allele is known; cytosolic; monomeric			125
Ph1	4S-0	pith abscission: cob disarticulation			105
Phi1	1L-149	phosphohexose isomerase: electrophoretic mobility; null allele is known; cytosolic; dimeric; intralocus hybrid bands occur			125
Phy1	1L-near *Adh1*	phytochrome candidate: RFLP loci(probe): *BNL-PHY1* (pcPhy101), *NPI251-PHY1* ()			372
Phy2	5S-near *Pgm2*	phytochrome candidate: RFLP loci(probe): *BNL-PHY2* (pcPhy101), *NPI369-PHY2* ()			372
pi1	?	pistillate florets (duplicate factor with *pi2*): secondary florets develop ("Country Gentlemen" or "Shoe Peg" expression) in *pi1 pi2* ears			144
pi2	?	pistillate florets (duplicate factor with *pi1*)			144
PIO		Pioneer Hi-Bred, International: designator for loci defined by restriction fragment polymorphisms			
Pl1	6L-49	purple plant: sunlight-independent purple pigment in plant; RFLP locus(probe): *BNL-PL1* (pH3-Sal0.6)	S	P	84
pm1	3L-near *ts4*	pale midrib: midrib and adjacent tissue lighter green; reduced plant vigor	S	P	26
Pn1	7L-112	papyrescent glumes: long, thin papery glumes on ear and tassel		P	106
po1	6S-4	polymitotic (= *ms4*): repeats 2nd meiotic division in male and female	S	P	12
ppg1	5L	pale pale green (was *cb*-199A*): white seedling with faint green; white necrotic crossbands; lethal			249
pr1	5L-67	red aleurone: changes purple aleurone to red; flavonoid 3'-hydroxylase	S	P	75
pro1	8L-near *fl3*	proline requiring (= *o6*): crumpled opaque kernel; b32 protein isoforms and null; green-striped lethal seedling			108

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Prot1		(= *Glb1*)			
ps1	5S-39	pink scutellum (= *vp7*): viviparous; endosperm and scutellum pink, seedling white with pink flush; *ps1-lyc* not viviparous	S	P	336
Pt1	6L-60	polytypic ear: proliferation produces irregular growth on ear and tassel	S	P	242
Px1	2L	peroxidase: electrophoretic mobility; null allele is known; monomeric			129
Px2	?	peroxidase: electrophoretic mobility; monomeric			198
Px3	7L-near *Pn1*	peroxidase: electrophoretic mobility; monomeric			198
Px4	?	peroxidase: electrophoretic mobility; null allele is known; monomeric			198
Px5	?	peroxidase: presence-absence			198
Px6	?	peroxidase: presence-absence			198
Px7	?	peroxidase: electrophoretic mobility; null allele is known; monomeric			198
Px8	?	peroxidase: electrophoretic mobility; monomeric			24
Px9	?	peroxidase: electrophoretic mobility; null allele is known; monomeric			24
py1	6L-69	pigmy plant: leaves short, pointed; fine white streaks	S	P	352
py2	1L	pigmy: like *py1*			249
pyd1	9S-near *yg2*	pale yellow deficiency: pale yellow seedling; deficiency for short terminal segment of chromosome arm; lethal; (for alleles, see Coe et al., 1988)			211
R1	10L-61	colored: red or purple color in aleurone and/or anthers, leaf tip, brace roots, etc.; (for alleles, see Coe et al., 1988); RFLP locus(probe): *NPI308-R1* ()	S	P	75
ra1	7L-32	ramosa: ear and tassel many-branched; tassel branches taper to tip	S	P	14 111
ra2	3S-49	ramosa: irregular kernel placement; tassel many-branched, upright (R.A. Brink, 1935, unpublished data)	S	P	89 259
ra3	4	ramosa: (H.S. Perry, 1954, unpublished data)	S		
rbcS		(= *Ssu*)			
rBg		: receptor of *Bg*			301
Rcm1	7-near *vp9*	rectifier: restores miniature seed of teosinte cytoplasm to normal			0
Rcm2	?	rectifier: weakly restores miniature seed of teosinte cytoplasm to normal			0
Rcm3	?	rectifier: restores miniature seed of teosinte cytoplasm to normal; from *Z. diploperennis*			0
rcu		: receptor of *Fcu*			122
rcy		: receptor of *Cy*			309
rd1	1L-near *Adh1*	reduced plant: semi-dwarf plant	S		241
rd2	6L	reduced plant: like *rd1*, but not as extreme			114
rDNA		ribosomal DNA: rDNA5.8S, rDNA18S and rDNA25S located in NOR on 6S; rDNA5S on 2L near *Ht1*			
rDt		receptor of Dotted			334
Rf1	3S-near *Wrk1*	fertility restorer: restores fertility to cms-T; complementary to *Rf2*	S		158
Rf2	9-near *wx1*	fertility restorer: see *Rf1*	S		74
Rf3	2L	fertility restorer: restores fertility to cms-S			31
Rf4	8	fertility restorer (complementary with *Rf5* and *Rf6*): restores fertility to cms-C			126
Rf5	?	fertility restorer (complementary with *Rf4* and *Rf6*): restores fertility to cms-C			362
Rf6	?	fertility restorer (complementary with *Rf4* and *Rf5*): restores fertility to cms-C			362
Rf7	?	fertility restorer: partially restores fertility to cms-Y			280a
Rg1	3-67	ragged leaves: defective tissue between veins of older leaves, causing holes and tearing	S	P	28
rgd1	6-8	ragged seedling: seedling leaves narrow, thread-like, have difficulty in emerging	S	P	174
Rgd2		(= *Nl2*)			253
rgo1	?	reversed germ orientation: embryo faces base of ear; variable frequency, maternal trait			300
rhm1	6-near *rgd1*	resistance to *Helminthosporium maydis* : chlorotic-lesion reaction with race O			331
Ri1	4S-27	rind abscission: cob disarticulation			105
rMrh		: receptor of *Mrh*			289
rMut		: receptor of *Mut*			289
RNY		Rockefeller University: designator for loci defined by restriction fragment polymorphisms			
Rp1	10S-0	resistance to *Puccinia sorghi*		P	200 201
Rp3	3-near *Rg1*	resistance to *Puccinia sorghi*	S		370
Rp4	4S-24	resistance to *Puccinia sorghi*	S		370
Rp5	10S-near *Rp1*	resistance to *Puccinia sorghi*			303
Rp6	10S-near *Rp1*	resistance to *Puccinia sorghi*			370
RPA		Rhone-Poulenc Agrochimie: designator for loci defined by restriction fragment polymorphisms			
Rpp9	10S-near *Rp1*	resistance to *Puccinia polysora* and *P. sorghi*			358
Rs1	?	rough sheath: extreme ligule disorganization			171
rs2	1-near *as1*	rough sheath	S		171
Rs4	7	rough sheath (was *Rs*-1606*): leaf sheaths rough, vascular bundles enlarged			248a
rt1	3S-near *cl1*	rootless: secondary roots few or absent	S	P	150
ruq		: receptor of *Uq*			102
S		seed color component at *R1* : anthocyanin pigmentation in aleurone			344
Sad1	10L-near *bf2*	shikimate dehydrogenase: electrophoretic mobility; plastidial; monomeric			364

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
sbd1	6L	sunburned (was wxl*-2292): sun-exposed leaves greyish-waxy			248a
Sdw1	8	semi-dwarf plant (was *Sdw*-1592*): shortened internodes, erect leaves	S		20
se1	4L-near *dp1*	sugary-enhancer: high sugar content with su1; light yellow endosperm; freely wrinkled in Ill677a			97
sen1	3	soft endosperm (duplicate factor with *sen2*): endosperm soft, opaque			346
sen2	7	soft endosperm (duplicate factor with *sen1*)			346
sen3	1	soft endosperm (duplicate factor with *sen1*): like *sen1*			346
sen4	?	soft endosperm (duplicate factor with *sen3*)			346
sen5	2	soft endosperm (duplicate factor with *sen6*): like *sen1*			346
sen6	5	soft endosperm (duplicate factor with *sen5*)			346
sft1	?	small flint type: ears on *sft1* plants produce only small flint endosperms; +/*sft1* ears are normal			70
Sg1	?	string cob: reduced pedicels	S	P	103
sh1	9S-29	shrunken: inflated endosperm collapses on drying, forming smoothly indented kernels; sucrose synthase-1 of endosperm (compare *Css1*); homotetramer; RFLP loci(probe): *BNL-SH1* (Pst38), *NPI15-SH1* ()	S	P	145
sh2	3L-149.2	shrunken: inflated, transparent, sweet kernels collapse on drying, becoming angular and brittle; ADPG pyrophosphorylase (compare *bt2*)	S	P	202
sh4	5L	shrunken: collapsed, chalky endosperm	S		356
sh5	5-near *lu1*	shrunken: sides of kernel collapsed			341a
si1	6L-20	silky (= *ms-si*): multiple silks in ear; sterile tassel with silks	S		99
sk1	2S-56	silkless ears: pistils abort, no silks	S		157
Sks1	2L-near *v4*	suppressor of sterility: pollen grains developing in presence of *Ms21* are defective and nonfunctional if *sks1*, normal if *Sks1*			182 310
sl1	7L-50	slashed leaves: leaves slit longitudinally by necrotic streaks	S		134
sm1	6L-59	salmon silks: silks salmon color with *P1-RR*, brown in *P1-WW*	S	P	1
Sn1	10L-near *R1*	scutellar node color: anthocyanin in coleoptile, nodes, auricle, leaf blade, etc. (compare *Lc1*)			109
Sod1	?	superoxide dismutase: electrophoretic mobility; plastidial; dimeric; intralocus hybrid bands occur			10
Sod2	7L	superoxide dismutase: RFLP locus(probe): *NPI419-SOD2* ()			372
Sod2-2	9	superoxide dismutase: RFLP locus(probe): *NPI463-SOD* ()			373
Sod3	?	superoxide dismutase: electrophoretic mobility; mitochondrial; tetrameric; intralocus hybrid bands occur			10
Sod4	1S-near *P1*	superoxide dismutase: electrophoretic mobility; cytosolic; dimeric; intralocus hybrid bands occur; RFLP locus(probe): *NPI412-SOD4* ()			10
Spc1	3L-near *ig1*	speckled (was *Spc*-1376*, *Les*-1376*): brown speckling on leaves and sheath at flowering; supporting tissues weak	S		253
spc2	1L	speckled (was *spc*-262A*): green seedling with light green speckles			249
spc3	3L	speckled (was *pg*-553C*): green seedling with dark and light green speckles			249
Spm		suppressor-mutator: autonomous transposable element (equivalent to *En*); regulates *dSpm* transposition and function at *a1-m1*, *a1-m2*, *bz1-m13*, etc.			213
spt1	2L	spotted (was *spt*-464*): pale green, weak seedlings with dark green spots			249
spt2	4S	spotted (was *pg spt*-1269A*): like *spt1*			249
sr1	1S-0	striate leaves: many white striations or stripes on leaves (A.M. Brunson, 1935, unpublished data)	S		89
sr2	10L-95	striate leaves: white stripes on blade and sheath of upper leaves	S	P	155
sr3	10S	striate leaves: virescent and striate to striped	S	P	113
sr4	6L	striate leaves (was *stp*-65A*): seedlings pale luteus, later leaves white-striped			247
Ss2		(see *Css1*)			
Ssu1	4L	ribulose bisphosphate carboxylase small subunit candidate: RFLP loci(probe): *BNL17.05-SSU1(rbcS)* (pC1), *RPA9B-SSU* (pZmcRPA:SSU), *NPI331-SSU* ()			372
Ssu2	2-near *ts1*	ribulose bisphosphate carboxylase small subunit candidate: RFLP locus(probe): *RPA9A-SSU* (pZmcRPA:SSU)			372
st1	4S-62	sticky chromosome: small plant, striate leaves, pitted kernels resulting from sticky chromosomes; *st1-e* heightened by high temperature	S	P	15
su1	4S-66	sugary: endosperm wrinkled and translucent when dry; sweet at milk stage; starch debranching enzyme I; *su1-am* sugary-amylaceous; *su1-st* recessive starchy (see Coe et al., 1988)	S	P	57
su2	6L-58	sugary: endosperm glassy, translucent, sometimes wrinkled	S		96
Sup1	?	suppressor: modifies *o2* kernels to semi-transparent			207
Sus1		(= *Css1*)			
sy1	?	yellow scutellum			335
T		reciprocal translocation: general symbol for exchange of parts between two nonhomologous chromosomes	S	P	
Ta1	?	transaminase (possibly = *Got1*): electrophoretic mobility; dimeric; intralocus hybrid bands occur			198
tb1	1L-near *Adh1*	teosinte branched: many tillers; ear branches tassel-like	S		35
td1	5-near *bt1*	thick tassel dwarf: (E.G. Anderson, unpublished)	S		
te1	3	terminal ear: stalked ear appendages at tip; varying to infolded ears			208
Thc1	?	thiocarbamate sensitive: sensitive to Eradicane			270
tl1	?	tasselless			217
Tlr1	1L	tillered (was *Tlr*-1590*): extreme tillering	S		257

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Tp1	7L-46	teopod: many tillers, narrow leaves, many small partially podded ears, tassel simple	S	P	191
Tp2	10L-45	teopod: like *Tp1*		P	268
tpe1	?	thin pericarp: reduced cell number in pericarp (from Coroica)			105a
Tpi1	7L-59	triose phosphate isomerase: electrophoretic mobility; plastidial; dimeric; intra/interlocus hybrids occur with *Tpi2*			366a
Tpi2	2L-100	triose phosphate isomerase: electrophoretic mobility; plastidial; dimeric; intra/interlocus hybrids occur with *Tpi1*			366a
Tpi3	8	triose phosphate isomerase: electrophoretic mobility; cytosolic; dimeric; intra/interlocus hybrids occur with *Tpi4* & *Tpi5*; RFLP locus(probe): *NPI344-TPI* ()			366a
Tpi4	3L-near *Wrk1*	triose phosphate isomerase: electrophoretic mobility; cytosolic; dimeric; intra/interlocus hybrids occur with *Tpi3* & *Tpi5*;RFLP locus(probe): *NPI345-TPI* ()			366a
Tpi5	8L	triose phosphate isomerase: electrophoretic mobility; cytosolic; dimeric intra/interlocus hybrids occur with *Tpi3* & *Tpi4*			366a
Tpi6	5L	triose phosphate isomerase candidate: RFLP locus(probe): *NPI346-TPI* ()			366a 372
tpm1	?	thylakoid peptide modifier: dominant decrease in electrophoretic mobility			231
tr1	?	two-ranked ear: distichous vs. decussate phyllotaxy in ear axis			179
tru1	?	tassels replace upper ears: upper ear branches tassel-like, tillers bear ears			323
ts1	2S-74	tassel seed: tassel pistillate and pendant; if removed, small ear with irregular kernel placement develops	S		82
ts2	1S-24	tassel seed: like *ts1*, but branches variably pistillate and staminate	S	P	82
ts4	3L-73	tassel seed: tassel compact silky mass, upright, with pistillate and staminate florets; ear silky and proliferated	S	P	272
Ts5	4S-53	tassel seed: tassel upright with scattered, short silks; branches mostly pistillate toward the base	S		87
Ts6	1L-158	tassel seed: tassel pistillate to mixed, compact; ear with irregular kernel placement	S	P	259
Tu1	4L-101	tunicate: kernels enclosed in long glumes; tassel glumes large, coarse	S	P	5253
Tub1	1L-near *Adh1*	alpha tubulin candidate: RFLP locus(probe): *BNL17.04-TUB* (pUC9alpha-1)			37
ub1	?	unbranched: tassel with one spike	S	P	256
ubi1	4L	ubiquitin candidate: RFLP locus(probe): *AGR1002* ()			236
ubi2	5	ubiquitin candidate: RFLP locus(probe): *AGR1002* ()			236
Ufo1	?	unstable factor for orange: anthers, silks, and most other plant parts orange with *P1-WR* or *P1-RR*; growth retarded			351
UMC		University of Missouri, Columbia: designator for loci defined by restriction fragment polymorphisms			
Uq		ubiquitous: controlling element mediating *a1-ruq*			102
v1	9L-63	virescent: yellowish white seedling, greens rapidly; low temperature exaggerates	S	P	62
v2	5L-107	virescent: like *v1*, but greens slowly; low temperature exaggerates	S	P	80
v3	5L-45	virescent: light yellow seedling, greens rapidly; low temperature exaggerates	S	P	62
v4	2L-83	virescent: like *v2*	S	P	62
v5	7S-24	virescent: like *v1*, but older leaves have white stripes	S	P	62
v8	4L-near *Tu1*	virescent: like *v2*; lethal	S		63
v12	5L-near *ys1*	virescent: like *v3*	S		273
v13	?	virescent: first leaf with green tip; greens slowly	S		273
v16	8L-14	virescent: like *v2*	S		273
v17	4	virescent: like *v1*, but greening from base to tip	S		273
v18	10	virescent: like *v1*	S		273
v19		(= *g2*)			
v21	8L	virescent (was *v*-25*, *v*-A552*): grainy virescent, greening from tips and margins inward	S		18
v22	1L-near *an1*	virescent (was *v*-8983*): like *v1* (E.G. Anderson, unpublished)	S		
v23	4-near *su1*	virescent (was *v*-8914*): like *v1* (E.G. Anderson, unpublished)			
v24	2L	virescent (was *v*-424*): like *v1*			249
v25	1S	virescent (was *v*-17*): greenish white seedling; greens from base upward			249
v26	2S	virescent (was *v*-453*): yellowish white seedling with green leaf tip and midrib			249
v27	7L	virescent (was *v*-590A*): like *v1*			249
v28	9S	virescent (was *v*-27*): like *v1*			249
v29	10L	virescent (was *v*-418*): grainy virescent			249
v30	9L-87	virescent (was *v*-8587*): like *v1*			47
va1	7L-near *ij1*	variable sterile: variable male and female fertility; cytokinesis fails in anaphase I	S		13
Vg1	1L-85	vestigial glume: glumes very small, cob and anthers exposed	S	P	338
vp1	3L-near *ts4*	viviparous: embryo fails to become dormant, viable if transplanted; some alleles dormant; chlorophyll and carotenoids unaffected; anthocyanins in aleurone suppressed; RFLP locus(probe): *BNL-VP1* (pVPM1B)	S		93
vp2	5S-38	viviparous: embryo fails to become dormant; white endosperm, white seedling; anthocyanins unaffected	S	P	93
vp5	1S-1	viviparous: like *vp2*	S	P	290
vp7		(= *ps1*)			
vp8	1L-154	viviparous: embryo fails to become dormant; chlorophyll and carotenoids unaffected; small, pointed-leaf seedlings	S		291

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
vp9	7S-25	viviparous (also known as *y7*): like *vp2* ; *vp9-4889* dormant, pale aleurone, pale green seedling	S	P	291
Vsr1	10L	virescent striped (was *Vsr*-1446*): virescent seedling; greens to white and yellow striped plant	S		257
w1	6L-near *w14*	white: white seedling	S		7879 188
w2	10L-77	white: white seedling; endosperm pitted and spotted (compare *dek21*)	S		190
w3	2L-111	white: like *vp2* ; *w3-8686* pale endosperm, pale green seedling in dim light	S	P	190
w11	9S-54	white: like *w1*	S		63
w14	6L-78	white (was *w*-8657*): like *w1*	S		59
w15	6L-13	white (was *w*-8896*): like *w1* ; fails to convert protochlorophyllide to chlorophyllide	S		59
w16	7S-near *vp9*	white: like *w1*			234
w17	7S-near *Hs1*	white: like *w1*			234
w18	1L	white seedling (was *w*-495A*, allelic to *w*-571C*): like *w1*			247
Wc1	9L-107	white cap: kernel with white crown and pale yellow endosperm	S		176
wd1	9S-near *yg2*	white deficiency: white seedling: deficiency for distal half of first chromomere of short arm (for alleles, see Coe et al., 1988)	S	P	211
wgs1	5L	white green sectors (was *sct*-206B*): white seedling with green sectors			249
whp1	2L	white pollen: duplicate factor with *c2* for yellow pollen and for anthocyanins; RFLP locus(probe): *BNL17.03-WHP* (PC2-c46)	S		49
wi1	6L-near *y1*	wilted: chronic wilting, leaves not as cool as normal; delayed differentiation of metaxylem vessels	S		277
Wi2	3	wilted: top leaves wilt under moisture/temperature stress			248
Wi3	?	wilted: like *Wi2*			248a
wlu1	3L	white luteus (was *wl*-28*): pale yellow seedling; lethal			249
wlu2	7L	white luteus (was *wl*-543A*): like *wlu1*			249
wlu3	8L	white luteus (was *wl*-203A*): like *wlu1*			249
wlu4	9L	white luteus (was *wl*-41A*): like *wlu1*			249
wlu5	1L-near *br1*	white luteus (was *wl*-266A*): like *wlu1*			247
Wrk1	3S-62	wrinkled kernel (was *Wr*-1020*): kernels small and wrinkled	S		257
Wrp1	?	wrinkled plant: dominant dwarf, leaves and culm longitudinally corrugated; dosage effect			22
ws1	?	white sheath: light yellow leaf sheaths; duplicate factor with *ws2*			166
ws2	?	white sheath: see *ws1*			166
ws3	2S-0	white sheath: white leaf sheath, culm, husks	S	P	283
Ws4	1S	white sheath (was *Pale green-1589*): seedlings and plants lighter green in sheaths			
wsp		weak striped plant: maternally inherited pale striping			29
wt1	2S-60	white tip: tip of first leaf white and blunt	S		342
wt2	4S	white tip (was *cb*-10*): seedling with white leaf tip and crossbands on first 2 leaves			249
wx1	9S-56	waxy: amylopectin (stained red by iodine) replaces amylose (blue staining) in endosperm and pollen; starch-granule-bound NDP-starch glucosyl transferase; (for alleles, see Coe et al., 1988); RFLP loci(probe): *BNL-WX1* (pBF225), *UMC25-WX1* (pBF225), *NPI16-WX1* ()	S	P	51
wyg1	7L-near *ra1*	white yellow green			234
y1	6L-17	white endosperm: reduced carotenoid pigments in endosperm; some alleles affect chlorophyll in seedlings (e.g. *y1-8549* ; see Coe et al., 1988)	S	P	57
y3	2S-near *al1*	white endosperm (compare *al1*): like *y1*	S		267
y7		(= *vp9-y7*)			
y8	7S-18	white endosperm: pale yellow endosperm	S		152
y9	10S-24	white endosperm: pale yellow endosperm, slightly viviparous; green to pale green seedlings and plants	S		296
y10	3L	white endosperm (was *w*-7748*): pale yellow endosperm; white seedling; lethal	S		292
y11	?	white endosperm: pale yellow endosperm; green seedling			341
y12	?	white endosperm: like *y11*			341
yd2	3L-near *lg2*	yellow dwarf	S		295
yg1	5L-near *v2*	yellow-green: yellow-green seedling and plant	S		91
yg2	9S-7	yellow-green: like *yg1* (for alleles, see Coe et al., 1988)	S	P	149
YNH		Yale University: designator for loci defined by restriction fragment polymorphisms			
ys1	5L-75	yellow stripe: yellow tissue between leaf veins, reflects iron deficiency symptoms	S	P	11
ys2	1S	yellow stripe: yellow tissue between leaf veins			276
ys3	3L-near *gl6*	yellow stripe: like *ys1*	S		371
Ysk1	4-near *su1*	yellow streaked (was *Ysk*-844*): longitudinal yellow streaks top 3rd of mature leaves	S		253
z1		(= *vp9-z* = *y7-z*)			
zb1	?	zebra crossbands: yellowish crossbands on older leaves	S		61
zb2	?	zebra crossbands: crossbands on seedling leaves	S		349
zb3	5L-near *v2*	zebra crossbands: yellowish crossbands on older leaves (M. Demerec, 1935, unpublished data)	S		89
zb4	1S-19	zebra crossbands: regularly spaced crossbands on earlier leaves; enhanced by cool temperatures	S	P	133
zb6	4-79	zebra crossbands: regularly spaced crossbands on earlier leaves; enhanced by cool temperatures	S		135
zb7	1L-near *Adh1*	zebra crossbands (was *zb*-101*): lighter green crossbands on seedlings; glossy			249

SYMBOL	LOCATION	NAME,PHENOTYPE	S	P	REF
Zb8	9-near *wx1*	zebra crossbands (was *Atc1*, *Cb*-1443*): yellow-green crossbands on older leaves; strong anthocyanin expression in leaf tip and blade	S		253 257
Zer		Zapalote Chico earworm resistance: designator for earworm resistance factors from Zapalote Chico			228
zn1	10L-26	zebra necrotic: necrotic tissue appears between veins in transverse leaf bands on half-grown or older plants	S	P	131
zn2	?	zebra necrotic: like *zn1*	S		112
Zp		zein polypeptide: designator for loci determining zein polypeptides			332 333
zpg1	?	zebra-stripe pale green			69

REFERENCES TO ORIGINAL DESCRIPTIONS AND DESIGNATIONS
(gene symbols referenced are included within brackets)
(MNL = Maize Genetics Cooperation Newsletter)

00. Albertsen, M.C. and M. G. Neuffer. 1990. MNL 64:52. (*Ms44*)
0. Allen, J. O., et al. 1989. Maydica 34:277-290. (*Rcm1 Rcm2 Rcm3*)
1. Anderson, E.G. 1921. Cornell Univ. Agric. Exp. Stn. Memoir 48:533-554. (*sm1*)
2. Anderson, E.G. 1953. MNL 27:5. (*bf2*)
3. Anderson, E.G. 1955. MNL 29:5-6. (*gl14 gl15 gl18*)
4. Anderson, E.G. and R.A. Emerson. 1931. Am. Nat. 65:253-257. (*Ch1*)
5. Ashman, R.B. 1960. Genetics 45:19-34. (*Mst1*)
6. Ashman, R.B. and A.J. Ullstrup. 1976. J. Hered. 67:220-222. (*Les3*)
7. Bachmann, M.D., et al. 1973. J. Ultrastruct. Res. 45:384-406. (*l11*)
8. Badu-Apraku, B., et al. 1987. Plant Breed. 98:194-199. (*Cgl1*)
9. Baker, A. and C. J. Leaver. 1985. Nucl. Ac. Res. 13:5357-5867. (*Ant1*)
9a. Bathgate, B., et al. 1989. Eur. J. Biochem. 183:303-310. (*Ant2*)
10. Baum, J.A. and J.G. Scandalios. 1982. J. Hered. 73:95-100. (*Sod1 Sod3 Sod4*)
11. Beadle, G.W. 1929. Am. Nat. 63:189-192. (*ys1*)
12. Beadle, G.W. 1931. Cornell Univ. Agric. Exp. Stn. Memoir 135:1-12. (*po1*)
13. Beadle, G.W. 1932. Cytologia 3:142-155. (*va1*)
14. Beadle, G.W. 1932. Genetics 17:413-431. (*ms5 ms7 ms8 ms9 ms10 ms11 ms12 ms13 ms14 ra1*)
15. Beadle, G.W. 1932. Ztschr. ind. Abst. Vererbungsl. 63:195-217. (*st1*)
16. Beadle, G.W. and B. McClintock. 1928. Science 68:433. (*as1*)
17. Beckett, J.B. 1971. Crop Sci. 11:724-727. (cms-C)
18. Beckett, J.B. and M.G. Neuffer. 1973. MNL 47:147. (*v21*)
19. Beckman, L., et al. 1964. Science 146:1174-1175. (*Cat1*)
20. Bird, R.M. and M.G. Neuffer. 1985. MNL 59:42. (*Sdw1*)
21. Bird, R.M. and M.G. Neuffer. 1985. p. 818-822. *In* M. Freeling (ed.) Plant genetics. Alan R. Liss, New York. (*Hsf1*)
22. Bockholt, A.J. and J.D. Smith. 1989. MNL 63:56. (*Wrp1*)
23. Brewbaker, J.L. 1974. Proc. 29th Ann. Corn and Sorghum Res. Conf. p. 118-133. (*Mv1*)
24. Brewbaker, J.L. and Y. Hasegawa. 1974. MNL 48:35-37. (*Px8 Px9*)
25. Brink, R.A. 1933. J. Hered. 24:325-326. (*lg2*)
26. Brink, R.A. 1935. J. Hered. 26:249-251. (*pm1*)
27. Brink, R.A. and R.A. Nilan. 1952. Genetics 37:519-544. (*Mp*)
28. Brink, R.A. and P.H. Senn. 1931. J. Hered. 22:155-161. (*Rg1*)
28a. Brinkmann, H., et al. 1987. J. Mol. Evol. 26:320-328. (*Gpa1 Gpc1*)
29. Brown, W.L. and D.N. Duvick. 1958. MNL 32:120-121. (*wsp*)
30. Bryan, A.A. and J.E. Sass. 1941. J. Hered. 32:342-346. (*Kn1*)
31. Buchert, J.G. 1961. Proc. Natl. Acad. Sci. 47:1436-1440. (*Rf3*)
32. Burnham, C.R. 1936. J. Am. Soc. Agron. 28:968-975. (*ga2*)
33. Burnham, C.R. 1947. MNL 21:36. (*bm4*)
34. Burnham, C.R. 1958. MNL 32:93. (*eg1*)
35. Burnham, C.R. 1961. MNL 35:87. (*tb1*)
36. Burnham, C.R. and R.A. Brink. 1932. J. Am. Soc. Agron. 24:960-963. (*bm2*)
37. Burr, B., et al. 1988. Genetics 118:519-526. (*Act1 Ssu1 Tub1*)
38. Chang, M.T. and M.G. Neuffer. 1987. J. Hered. 78:163-170. (*Mr*)
39. Chang, S-H. and J.L. Brewbaker. 1976. MNL 50:31-32. (*aph1*)
40. Chao, S.E. and J.G. Scandalios. 1969. Biochem. Genet. 3:537-547. (*Amy2*)
41. Chao, S.E. and J.G. Scandalios. 1971. Genetics 69:47-61. (*Amy1*)
42. Chourey, P.S. and C. Mouli. 1975. Genetics 77:s11. (*Ce1*)
43. Clark, F.J. 1940. Am. J. Bot. 27:547-559. (*dv1*)
44. Clark, J.K. and W.F. Sheridan. 1986. J. Hered. 77:83-92. (*dek22 dek23*)
44a. Close, T.J., et al. 1989. Plant Mol. Biol. 13:95-108. (*Dhn1*)
46. Coe, E.H. 1983. Maydica 28:151-168. (NCS2 NCS3)
47. Coe, E.H. 1987. MNL 61:47. (*anl1 v30*)
48. Coe, E.H. and J.B. Beckett. 1987. MNL 61:46-47. (*baf1*)
49. Coe, E.H., et al. 1981. J. Hered. 72:318-320. (*whp1*)
50. Coe, E. H., et al. 1988. p 81-258.*In* G.F. Sprague and J.W. Dudley (ed.) Corn and corn improvement Amer. Soc. Agron., Madison
51. Collins, G.N. 1909. USDA, Plant Indus. Bur. Bull. 161:1-30. (*wx1*)
52. Collins, G.N. 1917. J. Agric. Res. 9:383-395. (*Tu1*)
53. Collins, G.N. 1917. Proc. Natl. Acad. Sci. 3:345-349. (*Tu1*)

54. Collins, G.N. and J.H. Kempton. 1920. J. Hered. 11:3-6. (*li1*)
55. Cook, W.B. and C.D. Miles. 1988. MNL 62:50. (*hcf113*)
56. Cook, W.B. and C.D. Miles. 1989. MNL63:65-66. (*hcf103 hcf104 hcf108 hcf111 hcf114*)
57. Correns, C. 1901. Bibliotheca Bot. 53:1-161. (*su1 yl*)
58. Couture, R.M., et al. 1971. Phys. Plant Pathol. 1:515-521. (*Bx1*)
59. Cox, E.L. and D.B. Dickinson. 1971. Biochem. Genet. 5:15-25. (*l12 w14 w15*)
60. de la Roche, I.A., et al. 1971. Crop Sci. 11:856-859. (*ln1*)
61. Demerec, M. 1921. J. Hered. 12:406-407. (*zb1*)
62. Demerec, M. 1924. Cornell Univ. Agric. Exp. Stn. Memoir 84. (*v1 v3 v4 v5*)
63. Demerec, M. 1926. Am. Nat. 60:172-176. (*d3 v8 w11*)
64. Demerec, M. 1926. J. Hered. 17:301-306. (*pb1 pb4*)
65. Dempsey, E.D. 1971. MNL 45:58. (K3L)
66. Dempsey, E.D. 1985. p. 311-316. *In* M. Freeling (ed.) Plant genetics. Alan R. Liss, New York. (*Ac2*)
67. Doerschug, E.B. 1973. Theor. Appl. Genet. 43:182-189. (*Dt4 Dt5*)
68. Dollinger, E.J. 1984. MNL 58:209-210. (*ora2*)
69. Dollinger, E.J. 1985. Crop Sci. 25:819-821. (*lty1 lty2 ora3 zpg1*)
70. Dollinger, E.J. 1987. MNL 61:103. (*sft1*)
71. Dooner, H.K. and J.L. Kermicle. 1971. Genetics 67:427-436. (*I*)
72. Dooner, H.K. and J.L. Kermicle. 1976. Genetics 82:309-322. (*Lc1*)
73. Doyle, G.G. 1978. MNL 52:77. (*agt1*)
74. Duvick, D.N. 1965. Adv. Genet. 13:1-56. (*Rf2*)
75. East, E.M. and H.K. Hayes. 1911. Conn. Agric. Exp. Stn. Bull. 167. (*C1 pr1 R1*)
76. Echt, C., et al. 1987. Molec. Gen. Genet. 208:230-234. (*cfr1*)
76a. El-Metainy, A.Y., and A.A. Omar. 1981. Biochem. Genet. 19:635-640. (*Acp1 Acp2*)
77. Emerson, R.A. 1911. Nebr. Agric. Exp. Stn. Ann. Rep. 24:59-90. (*P1*)
78. Emerson, R.A. 1912. Am. Breeders Assoc. Ann. Rep. 8:385-399. (*an1 d1 g1 lg1 w1*)
79. Emerson, R.A. 1912. Nebr. Agric. Exp. Stn. Ann. Rep. 25:81-88. (*lg1 w1*)
80. Emerson, R.A. 1912. Nebr. Agric. Exp. Stn. Ann. Rep. 25:89-105. (*g1 j1 v2*)
81. Emerson, R.A. 1918. Cornell Univ. Agric. Exp. Stn. Memoir 16. (*a1*)
82. Emerson, R.A. 1920. J. Hered. 11:65-76. (*ts1 ts2*)
83. Emerson, R.A. 1921. Am. J. Bot. 8:411-424. (*Bh1 gs1*)
84. Emerson, R.A. 1921. Cornell Univ. Agric. Exp. Stn. Memoir 39. (*B1 Pl1*)
85. Emerson, R.A. 1921. J. Hered. 12:267-270. (*cr1*)
86. Emerson, R.A. 1932. Science 75:566. (*ms17*)
87. Emerson, R.A. 1932. Sixth Int. Congress Genet. Proc. 1:141-152. (*Ts5*)
88. Emerson, R.A. and S.H. Emerson. 1922. Genetics 7:203-236. (*an1*)
89. Emerson, R.A., et al. 1935. Cornell Univ. Agric. Exp. Stn. Memoir 180. (*bm3 bt2 du1 fl2 gl5 gl6 gl7 gl8 gl9 gs2 j2 l6 mi1 nl1 o1 o2 ra2 sr1 zb3*)
90. Everett, H.L. 1949. Proc. Natl. Acad. Sci. 35:628-634. (*cl1 Clm1*)
91. Eyster, W.H. 1926. Science 64:22. (*bm1 yg1*)
92. Eyster, W.H. 1929. Ztschr. ind. Abst. Vererbungsl. 49:105-130. (*ar1*)
93. Eyster, W.H. 1931. Genetics 16:574-590. (*vp1 vp2*)
94. Eyster, W.H. 1931. J. Hered. 22:99-102. (*ms2 ms3*)
95. Eyster, W.H. 1933. Am. Nat. 67:75. (*oy1*)
96. Eyster, W.H. 1934. Bibliographia Genetica. 11:187-392. (*da1 l7 ms2 ms3 ms20 su2*)
97. Ferguson, J.E., et al. 1978. J. Hered. 69:377-380. (*se1*)
98. Fraser, A.C. 1924. J. Hered. 15:119-123. (*in1*)
99. Fraser, A.C. 1933. J. Hered. 24:41-46. (*si1*)
100. Freeling, M. and S. Hake. 1985. Genetics 111:617-634. (*Kn2*)
101. Freeling, M. and J.C. Woodman. 1978. MNL 52:9-10. (*Cdh1*)
102. Friedemann, P. and P.A. Peterson. 1982. Mol. Gen. Genet. 187:19-29. (*ruq Uq*)
103. Galinat, W.C. 1969. Mass. Agric. Exp. Stn. Bull. 577:1-19. (*Sg1*)
104. Galinat, W.C. 1971. MNL 45:98-99. (*is1*)
105. Galinat, W.C. 1975. MNL 49:100-102. (*Ph1 Ri1*)
105a. Galinat, W. C. 1990. MNL 64:120-121. (*tpe1*)
106. Galinat, W.C. and P.C. Mangelsdorf. 1957. MNL 31:67. (*Pn1*)
107. Galinat, W.C., et al. 1978. MNL 52:58. (*bu1*)
108. Gavazzi, G., et al. 1975. Theor. Appl. Genet. 46:339-346. (*pro1*)
109. Gavazzi, G., et al. 1986. p. 91-103 *In* G.M. Reddy and E.H. Coe (ed.) Gene structure and function in higher plants Oxford-IBH, New Delhi. (*Sn1*)
110. Gelinas, D.A., et al. 1966. Am. J. Bot. 53:615. (*Clt1*)
111. Gernert, W.B. 1912. Am. Nat. 46:616-622. (*ra1*)
112. Giesbrecht, J. 1965. J. Hered. 56:118, 130. (*zn2*)
113. Glover, D.V. 1968. MNL 42:151. (*ct2 sr3*)
114. Glover, D.V. 1970. Crop Sci. 10:611-612. (*rd2*)
115. Golubovskaya, I.N. 1979. Int. Rev. Cytol. 58:247-290. (*Mei1 ms43*)
115a. Golubovskaya, I. N. 1990. Advan. Genet. 26:149-192. (*dsy3 dsy4*)
116. Golubovskaya, I.N. and D.V. Sitnikova. 1980. Genetika 16:656-666. (*Mei1 ms28 ms43*)
117. Golubovskaya, I.N. and N.B. Khristolyubova. 1985. p. 723-738 *In* M. Freeling (ed.) Plant genetics Alan R. Liss, New York. (*dsy2 pam2*)
118. Golubovskaya, I.N. and A.S. Mashnenkov. 1976. Genetika. 12:7-14. (*dsy1*)
119. Golubovskaya, I.N. and A.S. Mashnenkov. 1977. Genetika. 13:1910-1921. (*pam1*)
120. Golubovskaya, I.N., et al. 1975. Genetika. 11:11-17. (*afd1*)
121. Gonella, J.A. and P.A. Peterson. 1975. MNL 49:71-73. (*ga10*)

122. Gonella, J.A. and P.A. Peterson. 1977. Genetics 85:629-645. (*Fcu rcu*)
123. Goodman, M.M. and C.W. Stuber. 1982. MNL 56:125. (*Gdh2*)
124. Goodman, M.M. and C.W. Stuber. 1983. Maydica 28:169-188. (*Acp1 Acp2*)
125. Goodman, M.M., et al. 1980. Genetics 96:697-710. (*Got2 Got3 Idh1 Idh2 Me1 Pgd1 Pgd2 Pgm1 Pgm2 Phi1*)
126. Gracen, V.E., et al. 1979. Proc. 34th Ann. Corn and Sorghum Res. Conf. p. 76-91. (*Rf4*)
127. Greenblatt, I.M. and R. A. Brink. 1954. J. Hered. 45:47-50. (*c2*)
128. Grogan, C.O., et al. 1963. Crop Sci. 3:451. (*ats1*)
129. Hamill, D.E., and J.L. Brewbaker. 1969. Physiol. Plant. 22:945-958. (*Px1*)
130. Harberd, N., and M. Freeling. 1989. Genetics 121:827-838. (*Mpl1*)
131. Horovitz, S. 1948. MNL 22:42. (*zn1*)
132. Harris, J.W. 1968. Genetics 60:186-187. (*E4*)
133. Hayes, H.K. 1932. J. Hered. 23:415-419. (*zb4*)
134. Hayes, H.K. and H.E. Brewbaker. 1928. Am. Nat. 62:228-235. (*gl2 gl3 sl1*)
135. Hayes, H.K. and M.S. Chang. 1938. MNL 12:2. (*zb6*)
136. Hayes, H.K. and E.M. East. 1915. Conn. Agric. Exp. Stn. Bull. 188:1-31. (*fl1*)
137. Hofmeyr, J.D.J. 1930. Unpub. thesis, Cornell. (*ba1 ba2*)
138. Hoisington, D.A. and M. G. Neuffer. 1983. MNL 57:159-160. (*nec4*)
139. Hoisington, D.A. 1985. MNL 60:50-51. (*Les4 Les5 Les6 Les7 Les8 Les9*)
140. Hoisington, D.A. 1987. MNL 61:48-49. (*Les10*)
141. Hooker, A.L. 1963. Crop Sci. 3:381-383. (*Ht1*)
142. Hooker, A.L. 1977. Crop Sci. 17:132-135. (*Ht2*)
143. Hooker, A.L. 1981. MNL 55:87-88. (*Ht3*)
144. Huelsen, W.A. and M.C. Gillis. 1929. Ill. Agric. Exp. Stn. Bull. 320. (*pi1 pi2*)
145. Hutchison, C.B. 1921. J. Hered. 12:76-83. (*sh1*)
146. Hutchison, C.B. 1922. Cornell Univ. Agric. Exp. Stn. Memoir 60:1419-1473. (*na1*)
147. Jenkins, M.T. 1924. J. Hered. 15:467-472. (*ij1*)
148. Jenkins, M.T. 1926. Am. Nat. 60:484-488. (*g2*)
149. Jenkins, M.T. 1927. Genetics 12:492-518. (*yg2*)
150. Jenkins, M.T. 1930. J. Hered. 21:79-80. (*rt1*)
151. Jenkins, M.T. 1932. J. Agric. Res. 44:495-502. (*a2*)
152. Jenkins, M.T. 1947. MNL 21:33. (*y8*)
153. Jenkins, M.T. and M.A. Bell. 1930. Genetics 15:253-282. (*l4*)
154. Jenkins, M.T. and F. Gerhardt. 1931. Iowa Agric. Exp. Stn. Res. Bull. 138:121-151. (*la1*)
155. Joachim, G. and C.R. Burnham. 1953. MNL 27:66. (*sr2*)
156. Johns, M.A., et al. 1985. EMBO J. 4:1093-1102. (BS1)
157. Jones, D.F. 1925. J. Hered. 16:339-341. (*sk1*)
158. Jones, D.F. 1951. Proc. Natl. Acad. Sci. 37:408-410. (*Rf1*)
159. Jones, D.F. 1954. Proc. IX Int. Genet. Cong. 1225-1237. (cms-S cms-T)
160. Jones, D.F. and P.C. Mangelsdorf. 1925. Anat. Rec. 31:351. (*Ga1*)
161. Josephson, L.M. 1955. Empire J. Exp. Agric. 23(89):1-10 (cms-S cms-T)
162. Kahler, A.L. 1983. J. Hered. 74:239-246. (*Acp2 Acp4*)
163. Kang, M.S. 1981. MNL 55:26. (*btn1*)
164. Kempton, J.H. 1920. J. Hered. 11:111-115. (*br1*)
165. Kempton, J.H. 1920. J. Hered. 11:317-322. (*ad1*)
166. Kempton, J.H. 1921. Am. Nat. 56:461-464. (*ws1 ws2*)
167. Kempton, J.H. 1921. USDA Bull. 925:1-28. (*br1*)
168. Kempton, J.H. 1934. J. Hered. 25:29-32. (*bd1*)
169. Kermicle, J.L. 1969. Science 166:1422-1424. (*ig1*)
170. Kermicle, J.L. and J.D. Axtell. 1981. Maydica 26:185-197. (*Isr1*)
171. Khadzhinov, M.I. 1937. Bull. Appl. Bot. Gen. Plant Breed. Ser. II. 7:247-258. (*Rs1 rs2*)
172. Kriz, A.L. 1989. Biochem. Genet. 27:239-251. (*Glb1 Glb2*)
173. Kvakan, P. 1924. Cornell Univ. Agric. Exp. Stn. Memoir 83:1-22. (*Bn1 gl1*)
174. Kramer, H.H. 1957. MNL 31:120. (*rgd1*)
175. Kuc, J., et al. 1968. Phytochemistry 7:1435-1436. (*bm3*)
176. Kulkarni, C.G. 1927. Mich. Acad. Sci. Arts and Letters Papers. 6:253-273. (*Wc1*)
177. Lai, Y-K. and J.G. Scandalios. 1980. Devel. Genet. 1:311-324. (*Adr1*)
178. Lambert, R.J. and G.F. Sprague. 1987. MNL61:96. (*mg1*)
179. Langham, D.G. 1940. Genetics 25:88-107. (*pd1 tr1*)
180. Langham, D.G. 1940. MNL 14:21. (*bk2*)
180a. Larkin, J. C., et al. 1989. Genes Devel. 3:500-509. (*Mch1 Mch2*)
181. Laughnan, J. R. 1949. Proc. Natl. Acad. Sci. 35:167-178. (*Alpha Beta*)
181a. Lemke-Keyes, C. A. and M. M. Sachs. 1989. J. Hered. 80:316-319. (*atn1*)
182. Leng, E.R. and L.F. Bauman. 1955. Agron. J. 47:189-191. (*Ms21 Sks1*)
183. Leng, E.R. and M.L. Vineyard. 1951. MNL 25:31-32. (*br2*)
184. Leto, K.J. 1982. p. 317-325. *In* W.F. Sheridan (ed.) Maize for biological research. Plant Mol. Biol. Assoc., Charlottesville, VA. (*hcf6 hcf12 hcf13 hcf15 hcf19 hcf23 hcf26 hcf34 hcf38* ITOhcf41 *hcf46*)
185. Li, H.W. 1931. J. Hered. 22:14-16. (*bv1*)
186. Li, H.W. 1937. J. Hered. 24:279-281. (*na1*)
187. Lindstrom, E.W. 1917. Am. Nat. 51:225-237. (*l1*)
188. Lindstrom, E.W. 1918. Cornell Univ. Agric. Exp. Stn. Memoir 13:1-68. (*l1 w1*)
189. Lindstrom, E.W. 1921. Genetics 6:91-110. (*f1*)
190. Lindstrom, E.W. 1924. Genetics 9:305-326. (*w2 w3*)
191. Lindstrom, E.W. 1925. J. Hered. 16:135-140. (*Tp1*)
192. Lindstrom, E.W. 1935. Iowa St. Coll. J. Sci. 9:451-459. (*a3 Og1*)

193. Lock, R.H. 1906. Roy. Bot. Gard. Annals 3:95-184. (*P1*)
194. Longley, A.E. 1932. J. Agric. Res. 54:835-862. (K10)
195. Lorenzoni, C., et al. 1974. MNL 48:19-20. (*cp1*)
196. Lowe, J. and O.E. Nelson, Jr. 1946. Genetics 31:525-533. (*mn1*)
197. Lysikov, V.N., et al. 1984. Sov. Genet. 20:72-80. (*Cg2*)
198. Macdonald, T. and J.L. Brewbaker. 1972. J. Hered. 63:11-14. (*Px2 Px3 Px4 Px5 Px6 Px7 Ta1*)
199. Macdonald, T. and J.L. Brewbaker. 1974. J. Hered. 65:37-42. (*E5-I E5-II E6 E7 E8 E9 E10*)
200. Mains, E.B. 1926. J Hered. 17:313-325. (*Rp1*)
201. Mains, E.B. 1931. J. Agric. Res. 43:419-430. (*Rp1*)
202. Mains, E.B. 1949. J. Hered. 40:21-24. (*sh2*)
203. Mangelsdorf, P.C. 1926. Conn. Agric. Exp. Stn. Bull. 279:509-614. (*bt1*)
204. Mangelsdorf, P.C. 1947. Genetics 32:448-458. (*du1*)
204a. Martienssen, R.A., et al. 1989. EMBO J. 8:1633-1639. (*hcf106*)
204b. Martinez, P., et al. 1989. J. Mol. Biol. 208:551-565. (*Gpc1*)
206. Mascia, P.N. and D.S. Robertson. 1980. J. Hered. 71:19-24. (*nec1*)
207. Mashnenkov, A.S. and M.I. Khadjinov. 1979. Proc. IX Eucarpia Corn and Sorghum Sect. p. 447-450. (*Sup1*)
208. Matthews, D.L., et al. 1974. J. Agric. Sci. 82:433-435. (*te1*)
209. McCarty, D.P., et al. 1986. Proc. Natl. Acad. Sci. 83:9099-9103. (*Css1*)
210. McClintock, B. 1934. Z. Zellforsch. Mikrosk. Anat. 21:294-328. (NOR)
211. McClintock, B. 1944. Genetics 29:478-502. (*pyd1 wd1*)
212. McClintock, B. 1950. Proc. Natl. Acad. Sci. 36:344-355. (*Ac Ds*)
213. McClintock, B. 1956. Brookhaven Symp. Biol. 8:58-74. (*Spm*)
214. McClintock, B. 1957. Carnegie Inst. Wash. Yrbk. 56:393-401. (*Mod*)
214a. McMullen, M.D., and R. Louie. 1989. Mol. Plant-Microbe Interact. 2:309-314. (*Mdm1*)
215. Melville, J.C. and J.G. Scandalios, J.G. 1972. Biochem. Genet. 7:15-31. (*Enp1*)
216. Micu, V. 1980. MNL 54:63-64. (*db1*)
217. Micu, V. 1981. Genetical Studies of Maize. Shtiintsa, Kishinev, Mold. SSR (*bs1 db1 tl1*)
218. Micu, V. and S.I. Mustyatsa. 1978. Genetika. 14:365-368. (*dep1*)
219. Miles, C.D. 1989. MNL 63:66-67. (*lbl1*)
220. Miles, C.D. and D.J. Daniel. 1974. Plant Phys. 53:589-595. (*hcf1 hcf2 hcf3*)
221. Miles, D. 1982. p. 75-107. *In* M. Edelman, et al. (ed.) Methods in chloroplast molecular biology. Elsevier, Amsterdam. (*hcf4 hcf13 hcf18 hcf19 hcf21 hcf23 hcf26 hcf34 hcf41 hcf42 hcf44 hcf50*)
222. Miles, D., et al. 1986. p. 361-365 *In* K.E. Steinbeck, et al. (ed.) Molecular biology of the photosynthetic apparatus Cold Spring Harbor Laboratory. (*hcf5 hcf28 hcf31 hcf36 hcf47 hcf48 hcf101 hcf316 hcf323 hcf408*)
223. Miles, F.C. 1915. J. Genetics 4:193-214. (*gs1*)
224. Miranda, L.T. de. 1980. MNL 54:15-18. (*mal1 Mal2*)
225. Miranda, L.T. de. 1980. MNL 54:19. (*Asr1*)
226. Miranda, L.T. de. 1981. MNL 55:18-19. (*lte1*)
227. Miranda, L.T. de, et al. 1982. MNL 56:28-30. (*Lte2*)
228. Miranda, L.T. de, et al. 1982. MNL 56:30-32. (Mer Zer)
229. Miranda, L.T. de, et al. 1984. MNL 58:38-46. (Ger Flt Krn)
230. Misra, P.S., et al. 1972. Science 176:1425-1427. (*o7*)
231. Modena, S.A. 1983. MNL 57:38. (*tpm1*)
232. Modena, S.A. 1984. MNL 58:79-82. (*Lcs1 Lct1 lct2*)
233. Modena, S.A. 1984. MNL 58:211-212. (*Pdf1*)
234. Motto, M., et al. 1983. Maydica 28:25-39. (*w16 w17 wyg1*)
235. Mumm, W.J. 1929. Anat. Rec. 44:279. (*hl*)
236. Murphy, M., et al. 1988. MNL 62:89-91. (*ubi1 ubi2*)
237. Nelson, O.E. 1976. MNL 50:114. (*fl3*)
238. Nelson, O.E. 1981. MNL 55:68. (*o9 o10 o11 o12 o13*)
239. Nelson, O.E. 1981. MNL 55:68-73. (*lp1*)
240. Nelson, O.E. and G.B. Clary. 1952. J. Hered. 43:205-210. (*dy1 lo2*)
241. Nelson, O.E. and A.J. Ohlrogge. 1957. Science 125:1200. (*ct1 rd1*)
242. Nelson, O.E. and S.N. 277dethwait. 1954. Am. J. Bot. 41:739-748. (*Pt1*)
243. Nelson, O.E. and A.J. Ullstrup. 1964. J. Hered. 55:194-199. (*hm2*)
244. Nelson, O.E., et al. 1965. Science 150:1469-1470. (*fl2 o1 o2*)
245. Neuffer, M.G. 1973. MNL 47:150. (*nec3*)
246. Neuffer, M.G. 1988. MNL 62:53. (*Lxm1*)
247. Neuffer, M.G. 1988. MNL 63:62. (*gl22 sr4 w18 wlu5*)
248. Neuffer, M.G. 1989. MNL 63:62-63. (*Blh1 Fbr1 Wi2 Wrp1 Ws4*)
248a. Neuffer, M.G. 1990. MNL 64:51-52. (*Gs4 Rs4 sbd1 Wi3*)
249. Neuffer, M.G. and J.B. Beckett. 1987. MNL 61:50. (*gl19 gl21 grt1 gs3 ij2 l13 l16 l17 l18 l19 nec5 nec6 nec7 pg15 pg16 ppg1 py2 spc2 spc3 spt1 spt2 v24 v25 v26 v27 v28 v29 wgs1 wlu1 wlu2 wlu3 wlu4 wt2 zb7*)
250. Neuffer, M.G. and O.H. Calvert. 1975. J. Hered. 66:265-270. (*Les1 Les2*)
251. Neuffer, M.G. and M.T. Chang. 1986. MNL 60:55. (*orp1 orp2*)
252. Neuffer, M.G. and D. England. 1984. MNL 58:77-78. (*Clt1*)
253. Neuffer, M.G. and K.A. Sheridan. 1977. MNL 51:60. (*Bif1 Nl2 Spc1 Ysk1 Zb8*)
254. Neuffer, M.G. and W.F. Sheridan. 1980. Genetics 95:929-944. (*dek1 dek2 dek3 dek4 dek5 dek6 dek7 dek8 dek9 dek10 dek11 dek12 dek13 dek14 dek15*)
255. Neuffer, M.G. and W.F. Sheridan. 1980. MNL 55:29-30. (*dek1 dek2 dek3 dek4 dek5 dek6 dek7 dek8 dek9 dek10 dek11 dek12 dek13 dek14 dek15*)
256. Neuffer, M.G., et al. 1968. The mutants of maize. Crop Sci. Soc. Amer., Madison, Wisc. (*cp2 ubl*)
257. Neuffer, M.G., et al. 1987. MNL 61:50-51. (*G6 Ms41 Msc1 Msc2 Tlr1 Vsr1 Wrk1 Zb8*)
258. Newton, K.J. and D. Schwartz. 1980. Genetics 95:425-442. (*Mdh1 Mdh2 Mdh3 Mdh4 Mdh5 mmm1*)

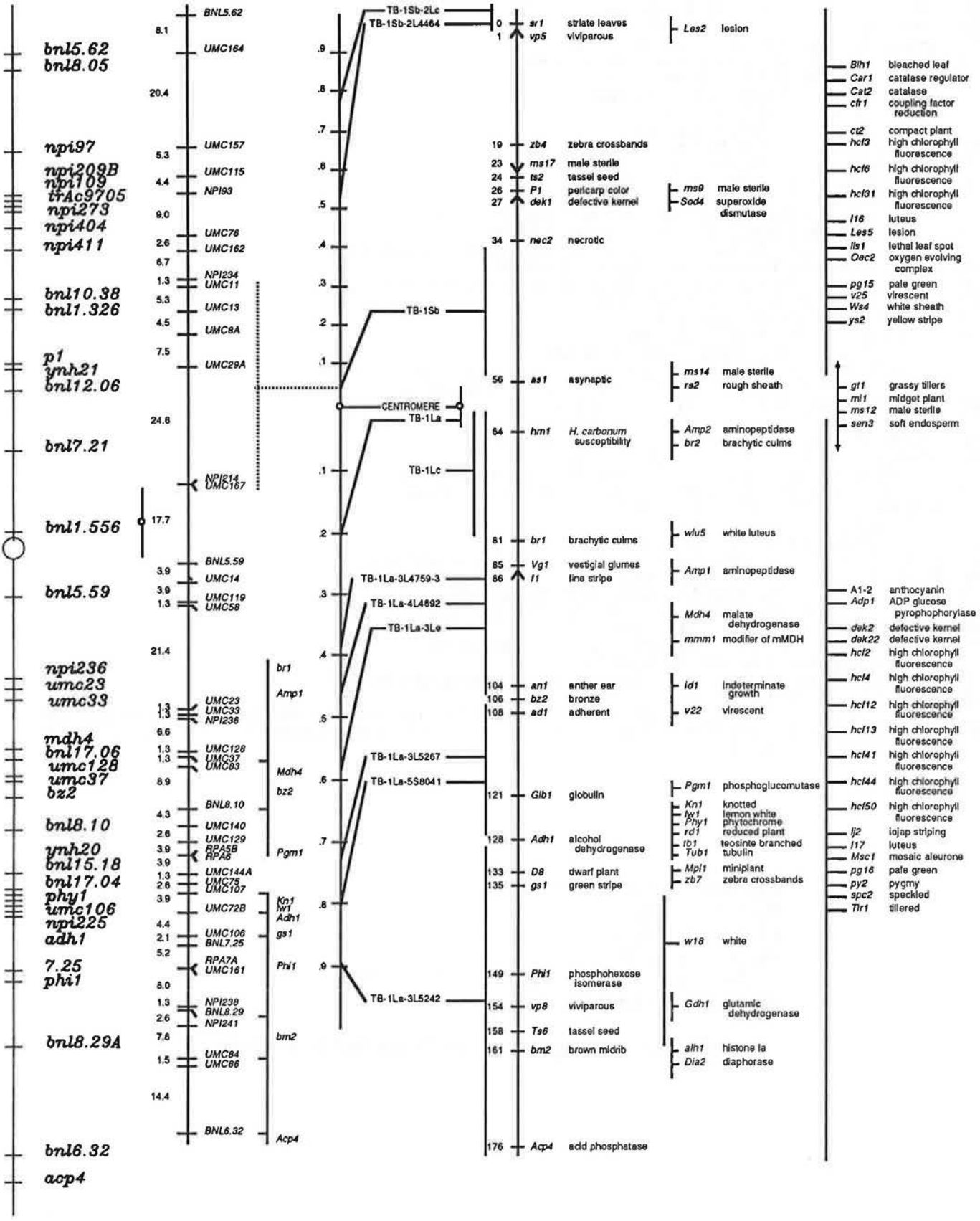
259. Nickerson, N.H. and E.E. Dale. 1955. Ann. Mo. Bot. Gard. 42:195-212. (*ra2 Ts6*)
260. Nuffer, M.G. 1954. MNL 28:63-64. (*bz2*)
261. Nuffer, M.G. 1955. Science 121:399-400. (*Dt2 Dt3*)
262. Ott, L. and J.G. Scandalios. 1978. Genetics 89:137-146. (*Amp1 Amp2 Amp3 Amp4*)
263. Paliy, A.F. and A.I. Rotar. 1979. Genetika 15:478-481. (*cfl2*)
264. Palmer, R.G. 1971. Chromosoma 35:233-246. (*am1*)
264a. Pan, Y. B., and P. A. Peterson. 1990. MNL 64:8-9. (*ba3*)
266. Perry, H.S. 1939. MNL 13:7. (*Lg3*)
267. Perry, H.S. and G.F. Sprague. 1936. J. Am. Soc. Agron. 28:990-996. (*y3*)
268. Peterson, H. 1959. MNL 33:41. (*Tp2*)
269. Peterson, P.A. 1960. Genetics 45:115-133. (*En pgl4*)
270. Pfund, J.H. and C.W. Crum. 1977. Agron. Abstr. p. 66. (*Thc1*)
271. Phinney, B.O. 1956. Proc. Natl. Acad. Sci. 42:185-189. (*D8*)
272. Phipps, I.F. 1928. J. Hered. 19:399-404. (*ts4*)
273. Phipps, I.F. 1929. Cornell Univ. Agric. Exp. Stn. Memoir 125:1-63. (*al1 v12 v13 v16 v17 v18*)
274. Piovarci, A. 1982. MNL 56:157. (*bv2*)
275. Plewa, M. 1979. MNL 53:93-96. (*loc1*)
276. Pogna, N.E., et al. 1982. MNL 56:153. (*ys2*)
277. Postlethwait, S.N. and O.E. Nelson, Jr. 1957. Am. J. Bot. 44:628-633. (*wi1*)
277a. Postlethwait, S. N. and O. E. Nelson, Jr. 1990. MNL 64:81-82. (*Fas1*)
278. Pryor, A.J. 1974. Heredity 32:397-401. (*Gdh1*)
279. Pryor, A.J. 1976. MNL 50:15-16. (*Glu1*)
280. Pryor, T. and D. Schwartz. 1973. Genetics 75:75-92. (*Cx1*)
280a. Qin, T-c. et al. 1990. MNL 64:124. (*Rf7*)
281. Randolph, L.F. 1928. Anat. Rec. 41:102. (B chr)
282. Rhoades, M.M. 1935. Am. Nat. 69:74-75. (*Dt1*)
283. Rhoades, M.M. 1939. Genetics 24:62. (*ws3*)
284. Rhoades, M.M. 1948. MNL 22:9. (*ga7*)
285. Rhoades, M.M. 1951. Am. Nat. 85:105-110. (*pg11 pg12*)
286. Rhoades, M.M. 1952. Am. Nat. 86:105-106. (*bz1*)
287. Rhoades, M.M. 1956. MNL 30:38-42. (*am1 el1*)
288. Rhoades, M.M. and E. Dempsey. 1954. MNL 28:58. (*gl17*)
289. Rhoades, M.M. and E. Dempsey. 1982. MNL 56:21-22. (*Mrh Mut rMrh rMut*)
290. Robertson, D.S. 1952. Proc. Natl. Acad. Sci. 38:580-583. (*vp5*)
291. Robertson, D.S. 1955. Genetics 40:745-760. (*vp8 vp9*)
292. Robertson, D.S. 1961. Genetics 46:649-662. (*y10*)
293. Robertson, D.S. 1967. MNL 41:94. (*o5*)
294. Robertson, D.S. 1973. MNL 47:82. (*l10*)
295. Robertson, D.S. 1974. MNL 48:70. (*yd2*)
296. Robertson, D.S. 1975. J. Hered. 66:127-130. (*y9*)
297. Robertson, D.S. 1978. Mutat. Res. 51:21-28. (*Mu*)
298. Robertson, D.S. 1981. MNL 55:115. (*l15*)
299. Robertson, D.S. 1984. MNL 58:18. (*brn1*)
299a. Russell, D. A., and M. M. Sachs. 1989. Plant Cell 1:793-803. (*Gpc1 Gpc2 Gpc3*)
300. Sachan, J.K. and K.R. Sarkar. 1978. MNL 52:119-120. (*rgo1*)
301. Salamini, F. 1980. Cold Spr. Harb. Symp. 45:467-476. (*Bg rBg*)
302. Salamini, F., et al. 1983. Theor. Appl. Genet. 65:123-128. (*Mc1*)
303. Saxena, K.M.S. and A.L. Hooker. 1968. Proc. Natl. Acad. Sci. 61:1300-1305. (*Rp5*)
304. Scandalios, J.G., et al. 1972. Arch. Biochem. Biophys. 153:695-705. (*Cat2*)
305. Scandalios, J.G., et al. 1975. Biochem. Genet. 13:759-769. (*Got1*)
306. Scandalios, J.G., et al. 1980. Mol. Gen. Genet. 179:33-41. (*Cat3*)
307. Scandalios, J.G., et al. 1980. Proc. Natl. Acad. Sci. 77:5360-5364. (*Car1*)
308. Schiefelbein, J.W., et al. 1985. Proc. Natl. Acad. Sci. 82:4783-4787. (*dSpm*)
309. Schnable, P.S. and P.A. Peterson. 1986. Maydica 31:59-82. (*Cy rcy*)
310. Schwartz, D. 1951. Genetics 36:676-696. (*Ms21 Sks1*)
311. Schwartz, D. 1951. MNL 25:30. (*ga8*)
312. Schwartz, D. 1960. Proc. Natl. Acad. Sci. 46:1210-1215. (*E1*)
313. Schwartz, D. 1964. Proc. Natl. Acad. Sci. 51:602-605. (*E3*)
314. Schwartz, D. 1965. Proc. XI Int. Genet. Cong. 2:131-135. (*E2*)
315. Schwartz, D. 1966. Proc. Natl. Acad. Sci. 56:1431-1436. (*Adh2*)
316. Schwartz, D. 1979. Mol. Gen. Genet. 174:233-240. (*mep1 Prot1*)
317. Schwartz, D. and T. Endo. 1966. Genetics 53:709-715. (*Adh1*)
318. Shaver, D.L. 1967. J. Hered. 58:270-273. (*gt1 pe1*)
319. Shaver, D. 1983. Proc. Annu. Corn Sorghum Res. Conf. 38:161-180. (*Lfy1*)
320. Shepherd, N.S., et al. 1982. Mol. Gen. Genet. 188:266-271. (*Cin*)
321. Sheridan, W.F., et al. 1984. MNL 58:98-99. (*dek16 dek17 dek18 dek19 dek20 dek21*)
322. Sheridan, W.F., et al. 1986. MNL 60:64. (*dek22 dek23 dek24 dek25 dek26 dek27 dek28 dek29 dek30*)
323. Sheridan, W. F. 1988. Ann. Rev. Genet. 22:353-385. (*dek31 fae1 tru1*)
324. Shortess, D.K. and R.P. Amby. 1979. Maydica 24:215-221. (*pg13*)
325. Shortess, D.K., et al. 1968. Genetics 58:227-235. (*lu1*)
326. Shumway, L.K. and L.F. Bauman. 1967. Genetics 55:33-38. (NCS1)
327. Singleton, W.R. 1946. J. Hered. 37:61-64. (*id1*)
328. Singleton, W.R. 1951. Am. Nat. 85:81-96. (*Cg1*)
329. Singleton, W.R. 1959. MNL 33:3. (*br3*)

330. Singleton, W.R. and D.F. Jones. 1930. J. Hered. 21:266-268. (*ms1*)
331. Smith, D.R. and A.L. Hooker. 1973. Crop Sci. 13:330-331. (*rhm1*)
332. Soave, C., et al. 1978. Theor. Appl. Genet. 52:263-267. (Zp)
333. Soave, C., et al. 1981. Genetics 97:363-377. (Zp)
334. Sorrentino, J.J., et al. 1987. MNL 61:103. (*rDt*)
335. Sprague, G.F. 1932. U.S. Tech. Bull. 292:1-43. (*sy1*)
336. Sprague, G.F. 1936. J. Am. Soc. Agron. 28:472-478. (*ps1*)
337. Sprague, G.F. 1938. MNL 12:2. (*gl11*)
338. Sprague, G.F. 1939. J. Hered. 30:143-145. (*Vg1*)
339. Sprague, G.F. 1984. MNL 58:197. (*Dt6*)
340. Sprague, G.F. 1987. MNL 61:96. (*gl5 gl20*)
341. Sprague, G.F. 1987. MNL 61:96. (*y11, y12*)
341a. Sprague, G.F. 1990. MNL 64:110. (*gl23 gl24 sh5*)
342. Sprague, G.F., et al. 1965. MNL 39:164. (*wt1*)
343. Stadler, L.J. 1940. MNL 14:26. (*et1*)
344. Stadler, L.J. and M.H. Emmerling. 1956. Genetics 41:124-137. (*P S*)
345. Stadler, L.J. and M.G. Nuffer. 1953. Science 117:471-472. (*P*)
346. Stierwalt, T.R. and P.L. Crane. 1974. MNL 48:139. (*sen1 sen2 sen3 sen4 sen5 sen6*)
347. Stinard, P.S. and D.S. Robertson. 1987. MNL 61:7-9. (*Dap1*)
348. Stout, J.T. and R.L. Phillips. 1973. Proc. Natl. Acad. Sci. 70:3043-3047. (*alh1 clh1*)
349. Stroman, G.N. 1924. Genetics 9:493-512. (*zb2*)
350. Stroup, D. 1970. J. Hered. 61:139-141. (*cm1*)
351. Styles, E.D., et al. 1987. MNL 61:100. (*Ufo1*)
352. Suttle, A.D.. 1924. Unpub. thesis, Cornell. (*d2 d5 py1*)
353. Tavcar, A. 1932. Jugoslav. Akad. Znanosti i Umjetnosti Prestampo. 244:74-93. (*Hs1*)
354. Teas, H.J. and E.G. Anderson. 1951. Proc. Natl. Acad. Sci. 37:645-649. (*Bf1*)
355. Teas, H.J. and A.N. Teas. 1953. J.Hered. 44:156-158. (*bt2*)
356. Tsai, C. and O.E. Nelson. 1969. Genetics 61:813-821. (*sh4*)
357. Tulpule, S.H. 1954. Am. J. Bot. 41:294-301. (*lw1 lw2 lw3 lw4*)
358. Ullstrup, A.J. 1965. Phytopathology 55:425-428. (*Rpp9*)
359. Ullstrup, A.J. and A.M. Brunson. 1947. J. Am. Soc. Agron. 39:606-609. (*hm1*)
360. Ullstrup, A.J. and A.F. Troyer. 1968. Phytopathology 57:1282-1283. (*lls1*)
361. Vahrusheva, E.I. 1975. MNL 49:95-96. (*cto1*)
362. Vidakovic, M. 1988. Maydica 33:51-64. (*Rf5 Rf6*)
363. Vineyard, M.L. and R.P. Bear. 1952. MNL 26:5. (*ae1*)
364. Wendel, J.F., et al. 1985. MNL 59:87-88. (*Aco1 Aco2 Aco3 Aco4 Dia1 Dia2 Sad1*)
365. Wendel, J.F., et al. 1986. MNL 60:109. (*Adk1*)
366. Wendel, J.F., et al. 1986. Theor. Appl. Genet. 72:178-185. (*Hex1 Hex2*)
366a. Wendel, J.F., et al. 1989. J. Hered. 80:218-228. (*Tpi1 Tpi2 Tpi3 Tpi4 Tpi5 Tpi6*)
367. Wentz, J.B. 1926. J. Hered. 17:327-329. (*bt1*)
368. West, D.P. and M.C. Albertsen. 1985. MNL 59:87. (*ms22 ms23 ms24*)
369. Weydemann, U., et al. 1988. MNL 62:48. (*Mpi1*)
370. Wilkinson, D.R. and A.L. Hooker. 1968. Phytopathology 58:605-608. (*Rp3 Rp4 Rp6*)
371. Wright, J.E. 1961. MNL 35:111. (*ys3*)
372. Wright, S., et al. 1987. MNL 61:89-90. (*Act1 Alr1 Alr2 Emu1 Hsp1 Me2 Pdk1 Pdk2 Pep1 Phy1 Phy2 Sod2 Sod4 Ssu1 Ssu2 Tpi6*)
373. Wright, S. and T. Helentjaris. 1988. MNL 62:104. (A1-2 A1-3 A1-4 A1-5 *Adp1 Adp2 Adp3 Adp4 Adp5 Cab1 Cab2 Cab3 Cab4 Oec1 Oec2 Oec3 Oec4 Oec5* Sod2-2)

Map maker, Map maker
Make me a map
Probe me a blot
Find me a gene
Night after night in the lab all aclone
Make me the perfect map!

(Thanks, and apology, to Susan Melia-Hancock)

CHROMOSOME 1



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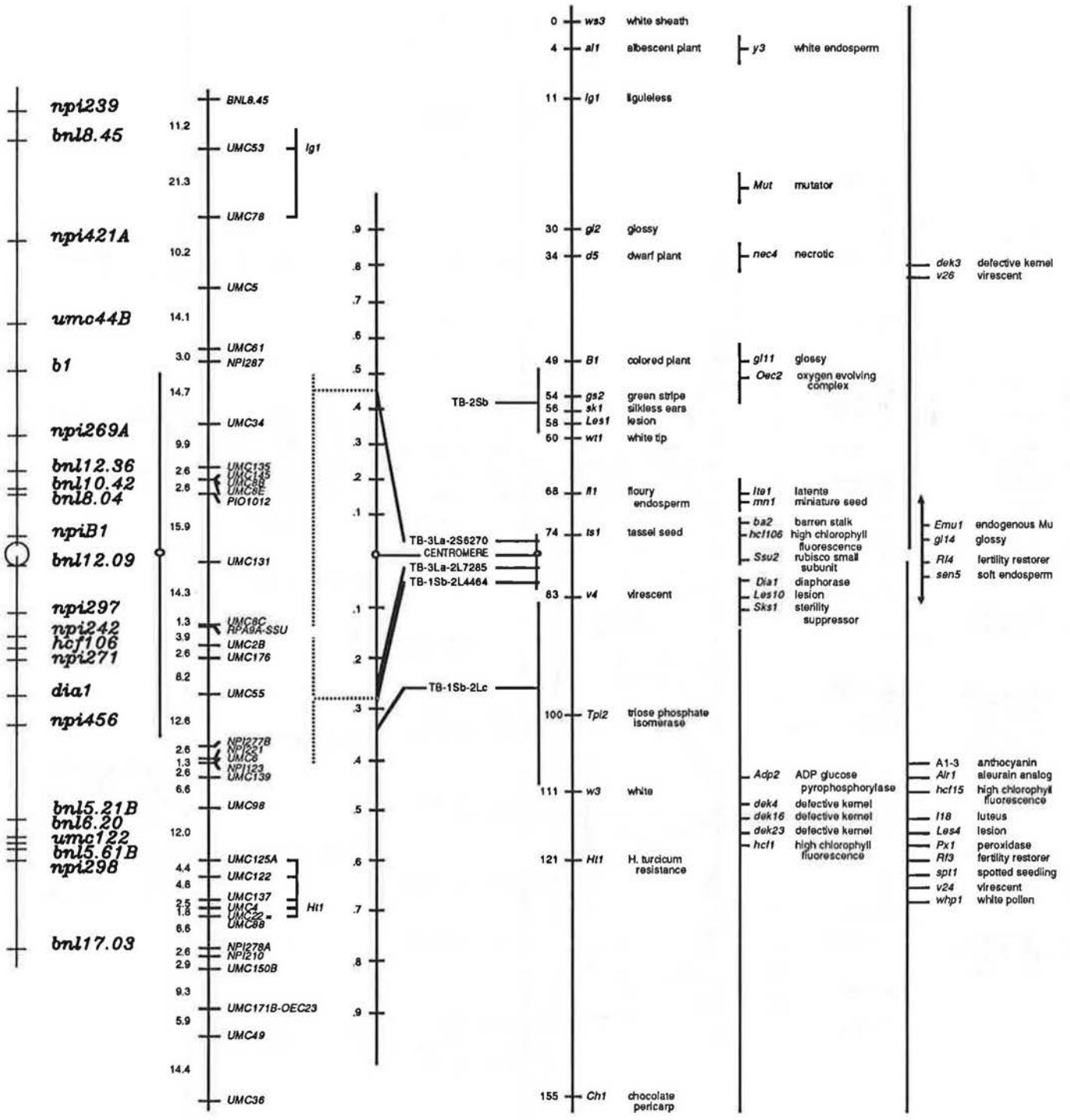

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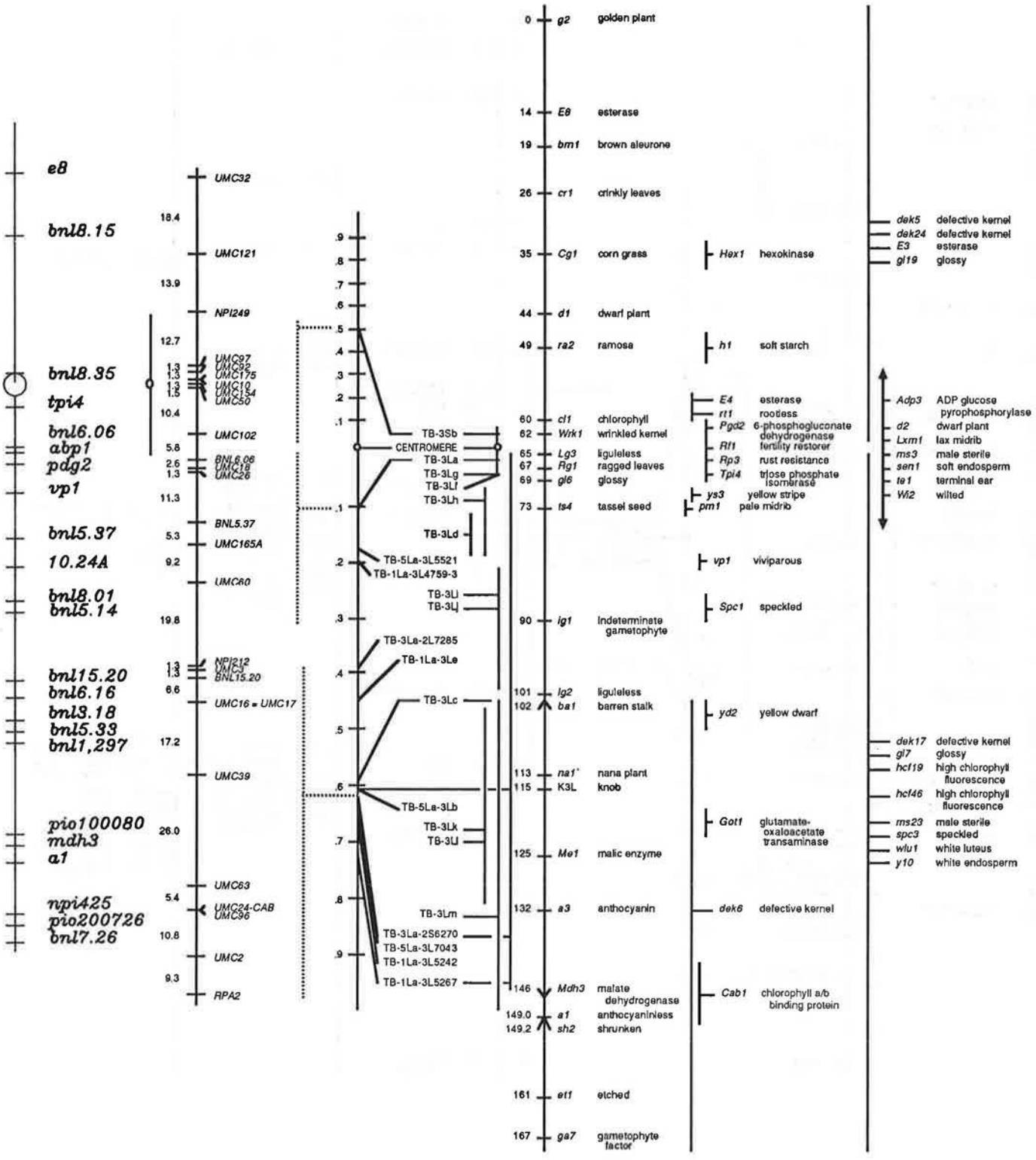

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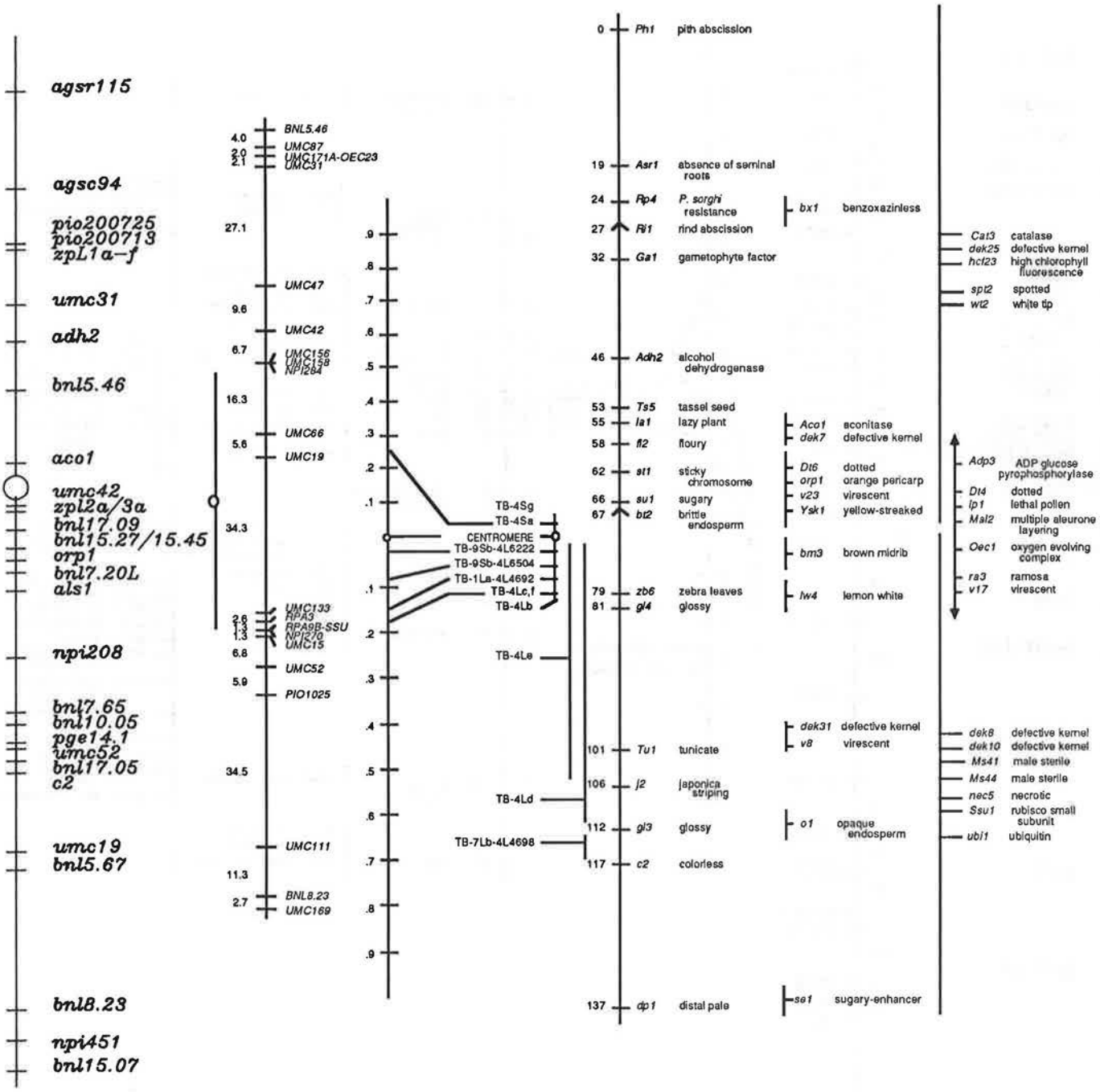

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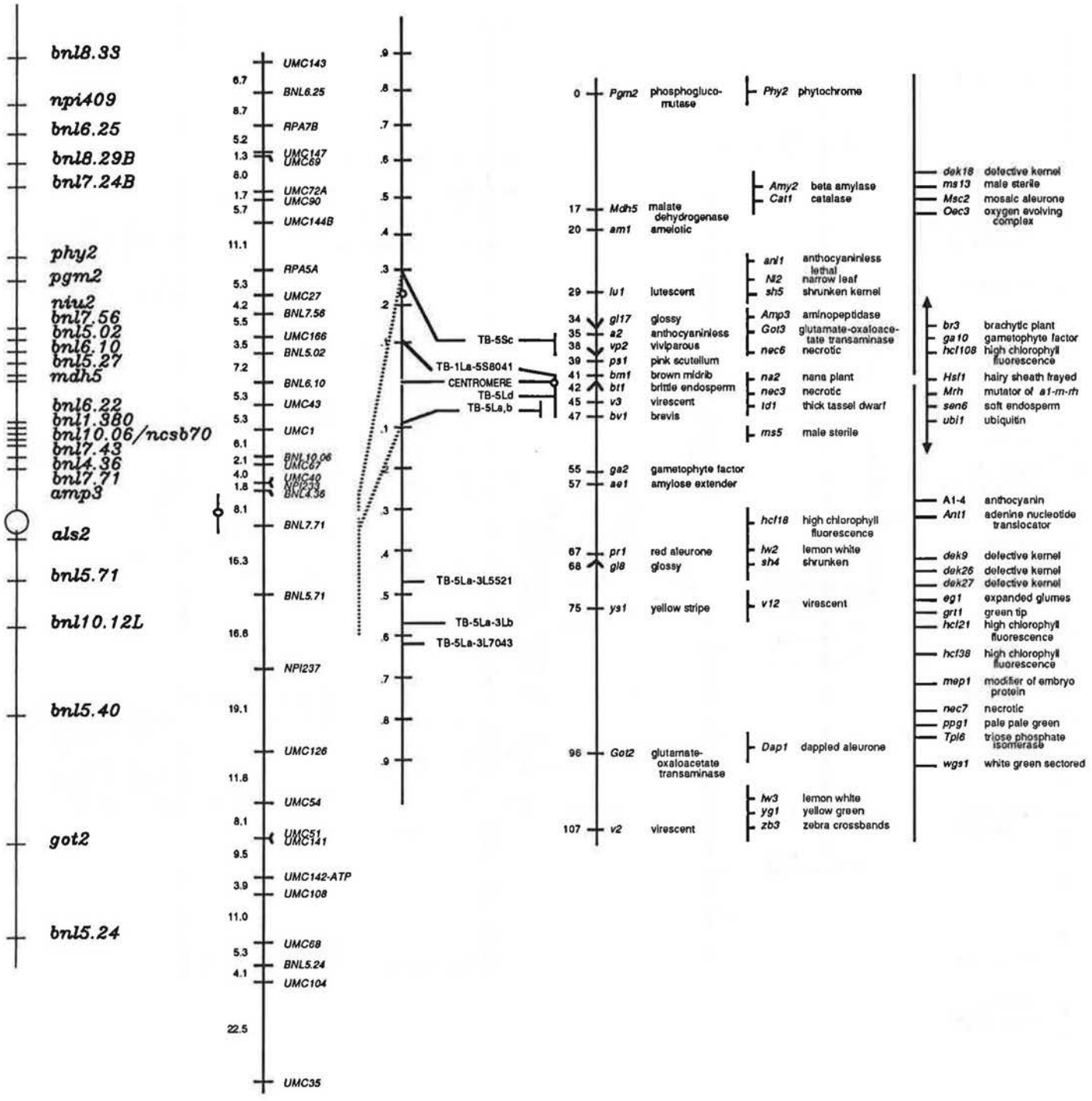

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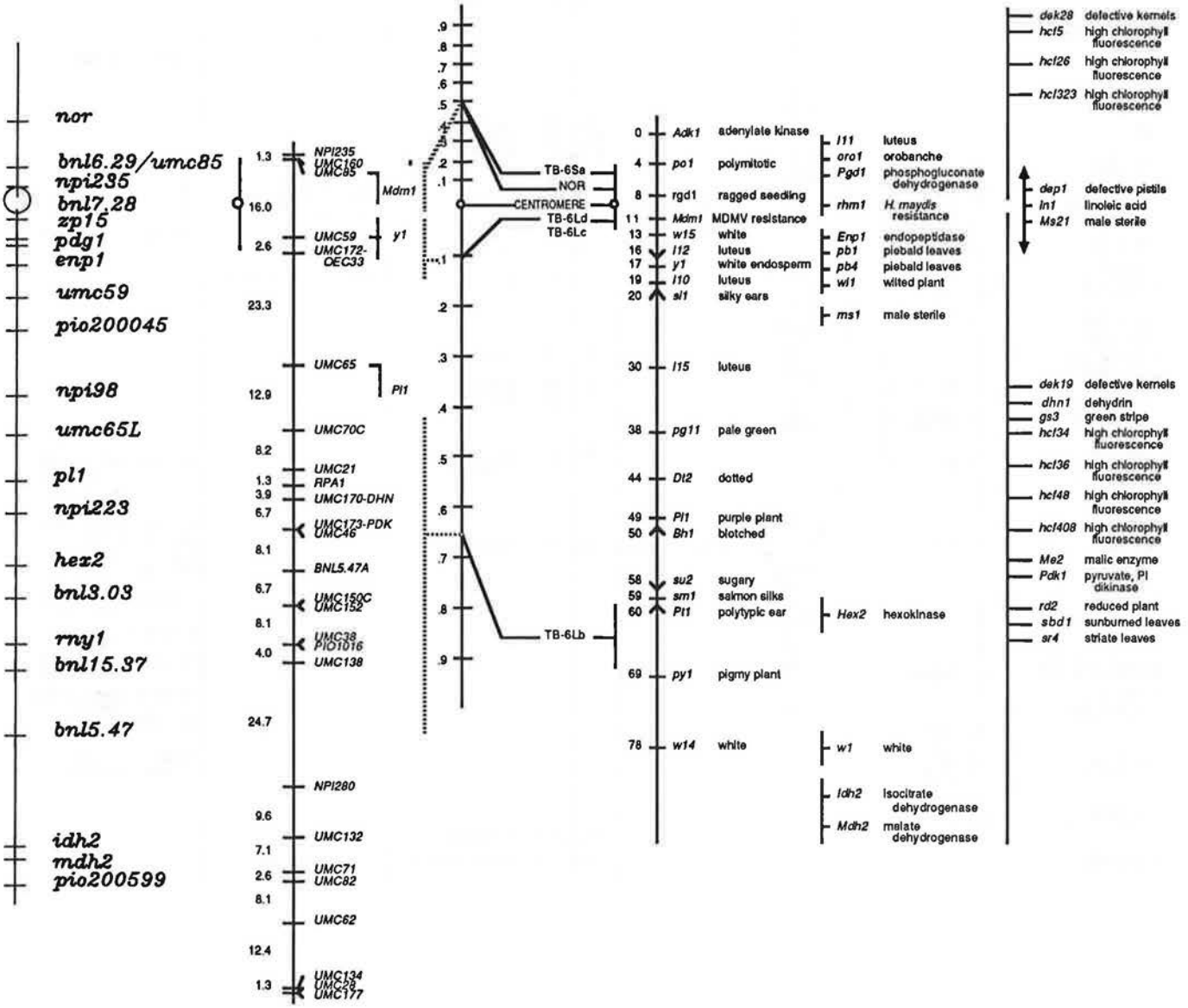

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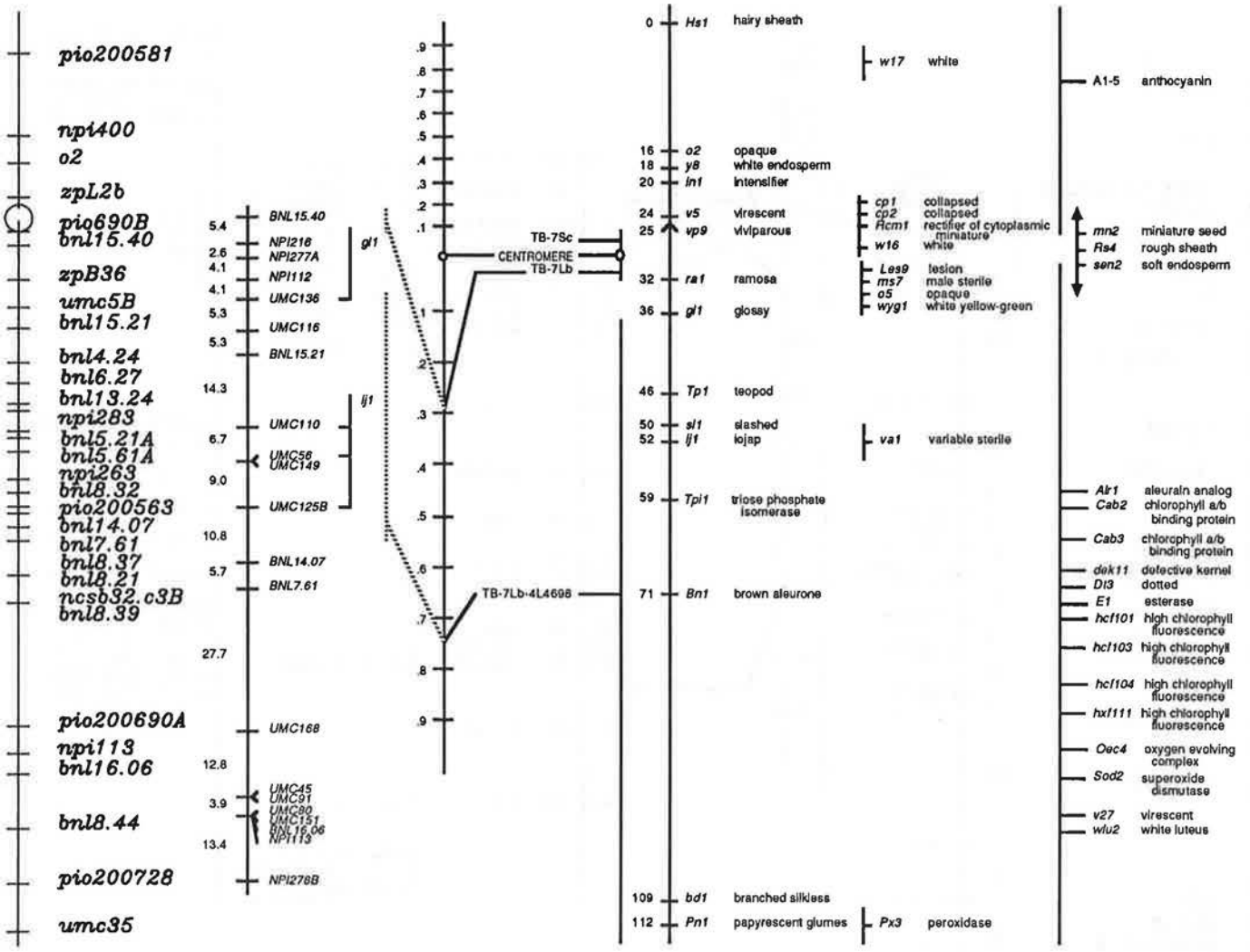
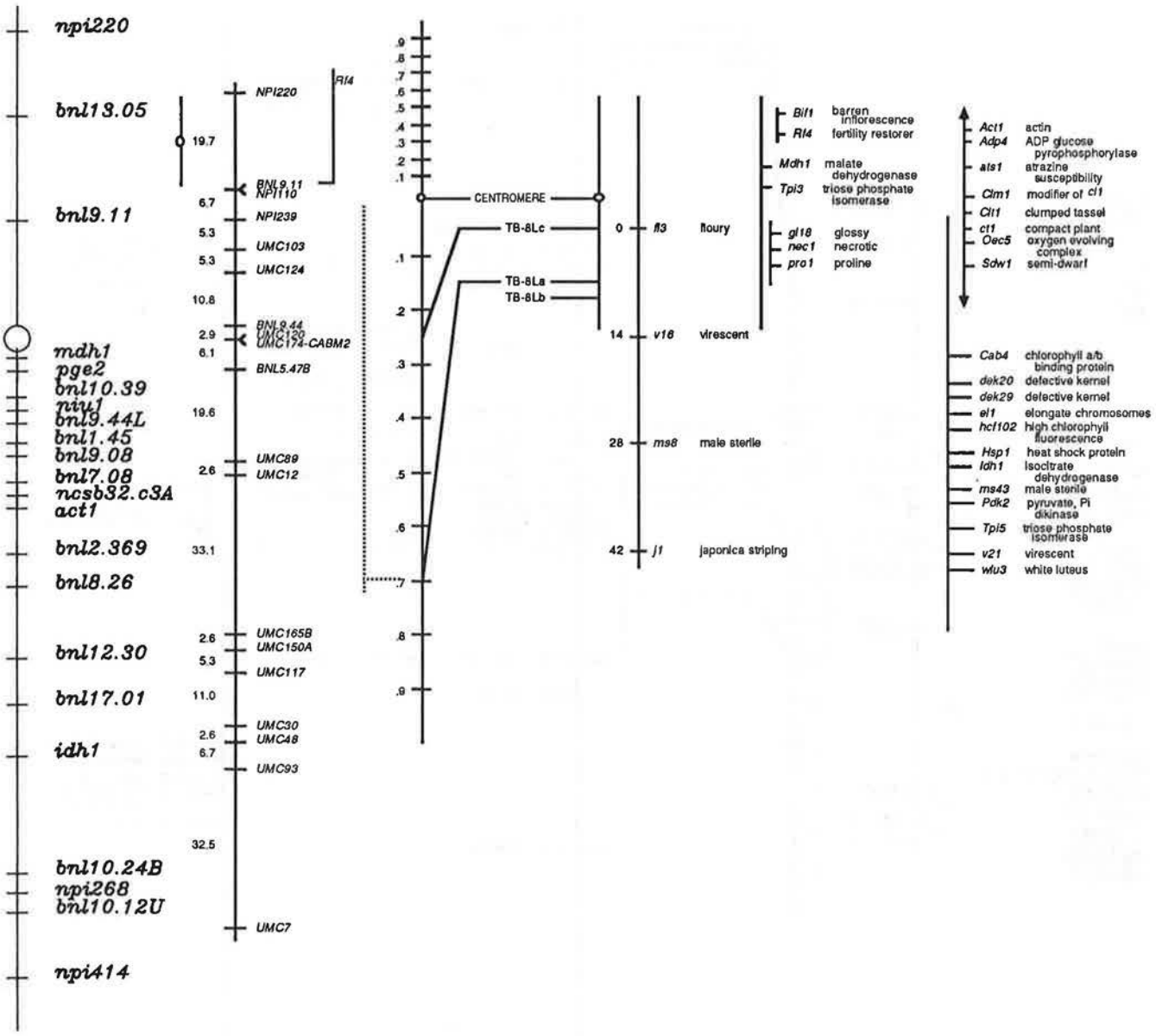


CHROMOSOME 8



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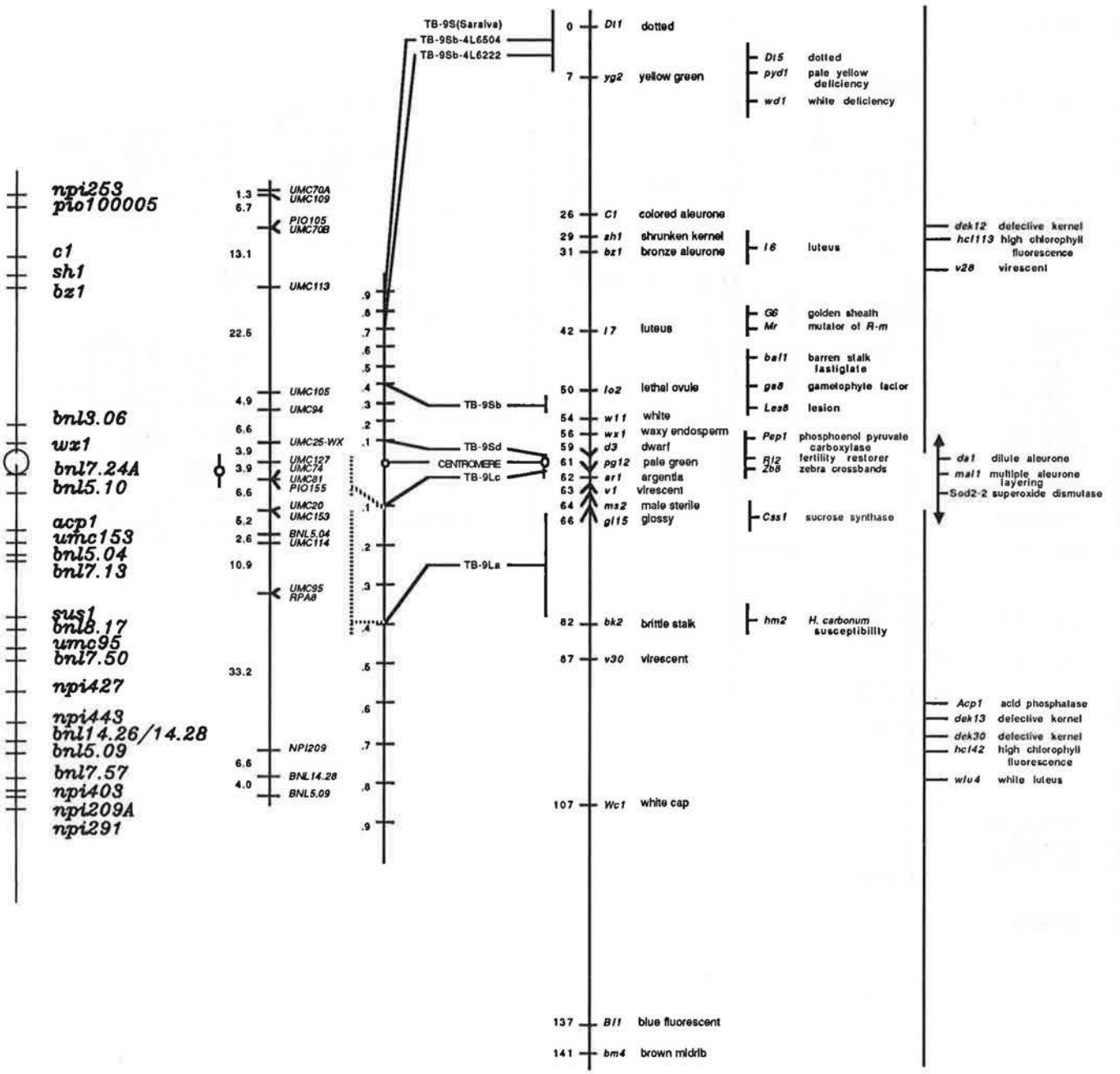

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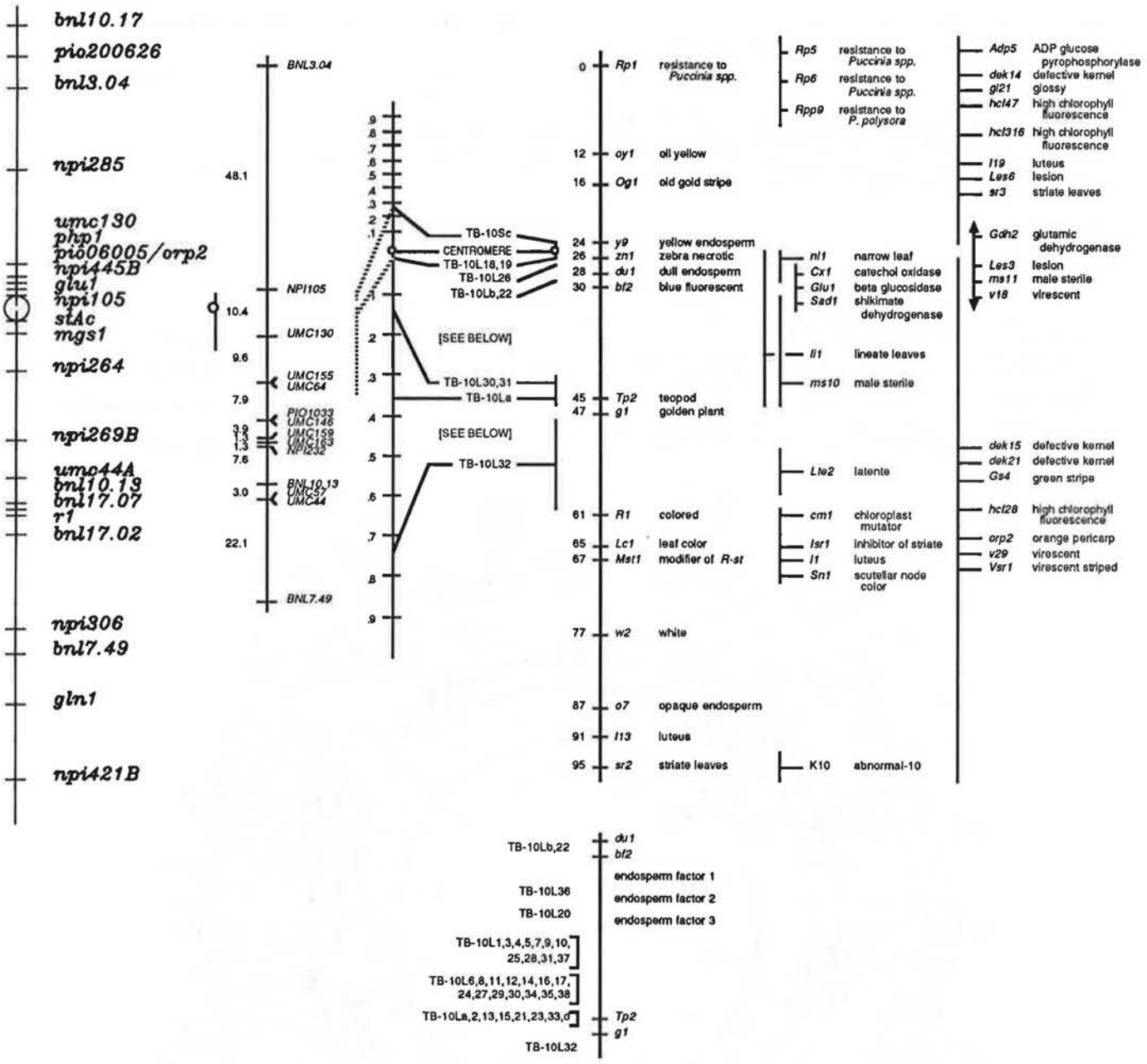

GENETIC MAP OF THE ZEA MAYS PLASTID CHROMOSOME

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The complete sequences of 6 maize chloroplast genes have been reported since last year's News Letter. The locations of these genes are shown on the circular map of the chromosome (below), and their products are briefly described in the following table. See the 1987 and 1988 News Letters (MNL 62: 148; MNL 63: 155) for discussions of maize plastid chromosome organization and plastid gene nomenclature, as well as for descriptions of the other sequenced genes on the map.

REFERENCES

Haley, J. and L. Bogorad. 1988. A 4-kDa maize chloroplast polypeptide associated with the cytochrome b_6-f complex: subunit 5, encoded by the chloroplast petE gene. Proc. Natl. Acad. Sci. USA 86: 1534-1538.

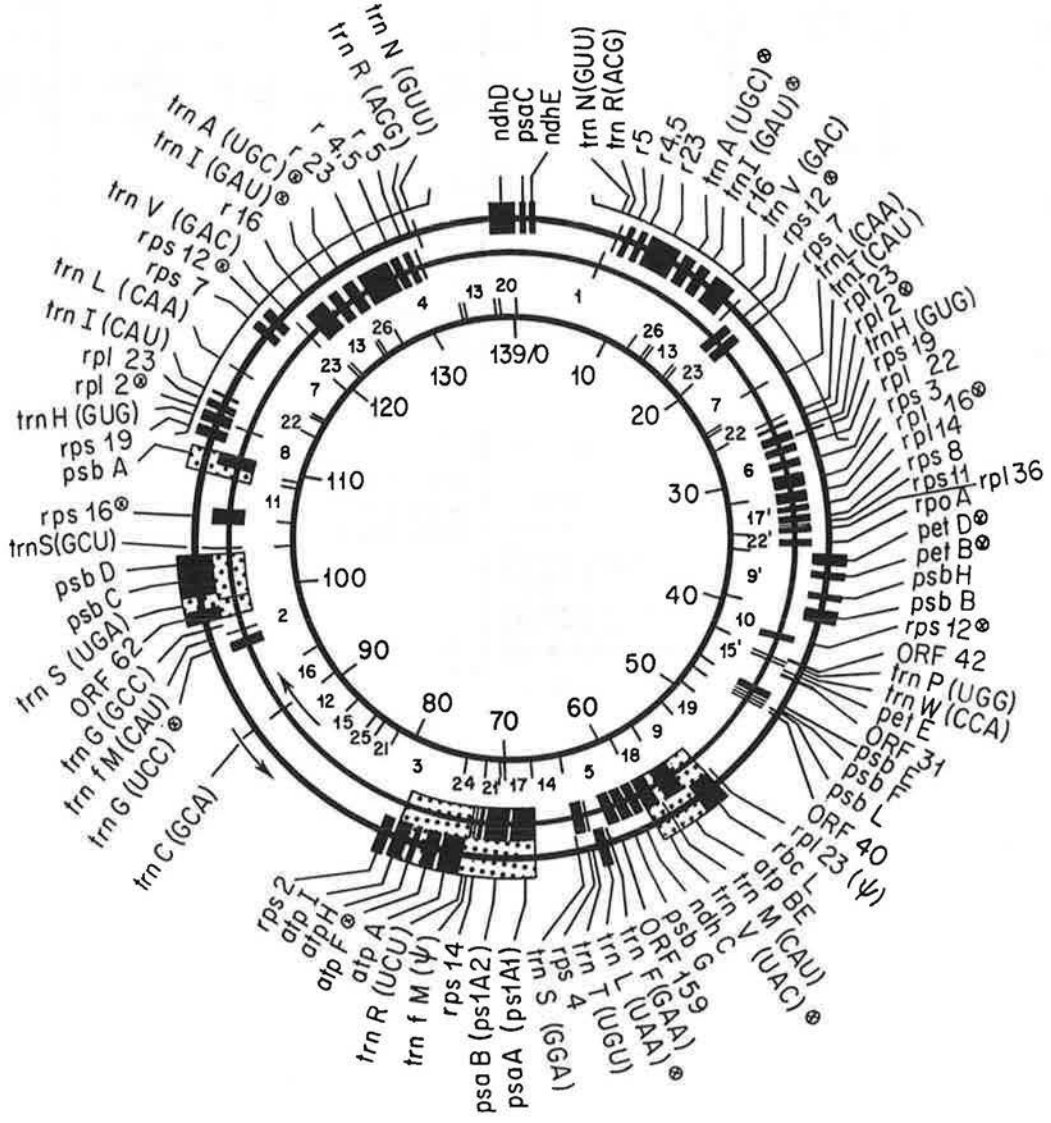
McLaughlin, W.E. and I.M. Larrinua. 1988. The sequence of the maize plastid encoded rpl23 locus. Nucleic Acids Res. 16: 8183.

Markmann-Mulisch, U., K. von Knoblauch, A. Lehmann and A.R. Subramanian. 1987. Nucleotide sequence and linkage map position of the secX gene in maize chloroplast and evidence that it encodes a protein belonging to the 50S ribosomal subunit. Biochem. Int. 15: 1057-1067.

Markmann-Mulisch, U. and A.R. Subramanian. 1988. Nucleotide sequence of maize chloroplast rps11 with conserved amino acid sequence between eukaryotes, bacteria and plastids. Biochem. Int. 17: 655-664.

RECENTLY REPORTED ZEA MAYS PLASTID GENES AND GENE PRODUCTS

Gene Product	Gene	Reference
Photosystem II Components:		
5 Kd O_2-evolving core component	psbL	J. Haley and L. Bogorad, submitted
Cytochrome b_6/f Complex:		
Subunit 5 (4 Kd)	petE	Haley and Bogorad, 1989
70S Ribosomal Proteins:		
S2	rps2	Stahl et al., in preparation
S11	rps11	Markmann-Mulisch and Subramanian, 1988
L23	rpl23	McLaughlin and Larrinua, 1988
L36	rpl36	Markmann-Mulisch et al., 1988



Genetic map of the Zea Mays mitochondrial genome from the maize fertile cytoplasm N and the male sterile cytoplasm cmsT (genotype B37)

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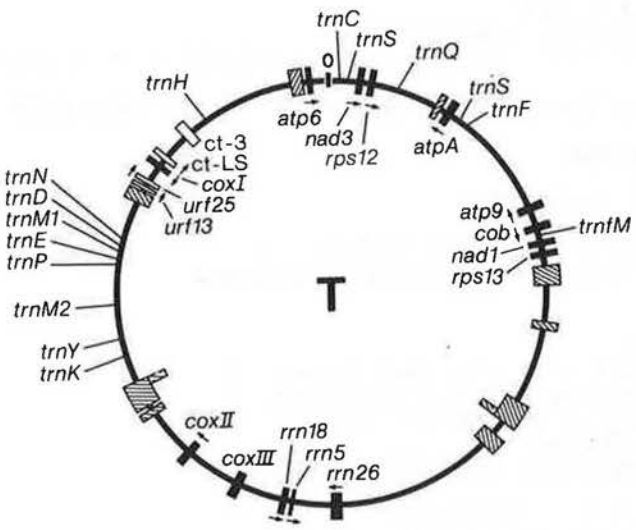
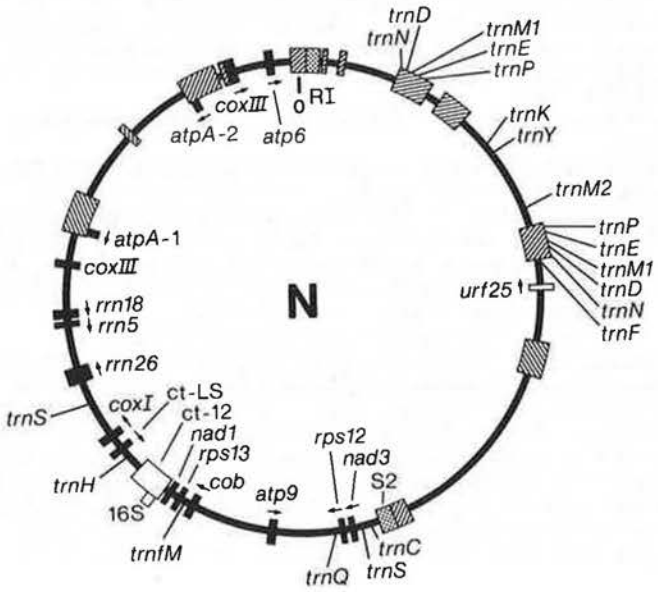
Since last year's news letter, the totality of the maize mitochondrial tRNA genes have been localized on the mt master-chromosome of the fertile line (B37) and the sterile line cmsT (B37). The two genomes contain only 16 tRNA genes with 14 different anticodons which correspond to 13 amino acids (Sangare et al. 1990). They are found scattered all around the genome. Six tRNA genes have been shown to originate from chloroplast DNA insertions and to be expressed in the mitochondria. They have been located on the circular map (figure 1) and a description of the genes is given in table 1. More details about the organization of the rRNA genes and proteins coding genes can be found on last year's Maize News Letter (MNL 63: 152). The restriction enzyme mapping data with BamHI, XhoI, SmaI can also be found on the last year's news letter (MNL63: 153) or the following publications: Lonsdale et al. 1984, Fauron et al. 1988, 1989. More information about those maps is available upon request to C. M.-R. Fauron.

Table 1. Maize mitochondrial genome tRNA genes.

transfer RNA	genes	anticodon	references
Asp	trnD	GUC	Parks, et al. 1985
Asn	trnN	GUU	Sangare, et al. 1990
Cys	trnC	GCU	Wintz, et al. 1988
Gln	trnQ	UUG	Sangare, et al. 1990
Glu	trnE	UUC	Sangare, et al. 1989
His	trnH	GUC	Iams, et al. 1985
Lys	trnK	UUU	Sangare, et al. 1989
fMet	trnfM	CAU	Parks, et al. 1984
m-Met-1	trnM-1	CAU	Parks, et al. 1984
m-Met-2	trnM-2	CAU	Sangare, et al. 1989
Phe	trnF	AAA	Marechal, et al. 1985 Sangare, et al. 1990
Pro	trnP	UGC	Sangare, et al. 1990
Ser	trnS	GCU	Wintz, et al. 1988
Ser	trnS	UGA	Sangare, et al. 1989
Trp	trnW (linear 2-3kb plasmid)	CCA	Marechal, et al. 1987 Leon, et al. 1989
Tyr	trnY	GUA	Sangare, et al. 1989





References

Fauron, C.M.-R., M. Havlik, D. Lonsdale, and L. Nichols. 1989. Mitochondrial genome organization of the maize cytoplasmic male sterile type T. Mol. Gen. Genetics 216: 395-401.

Fauron, C.M.-R. and M. Havlik. 1989. The maize mitochondrial genomes of the normal type and the cytoplasmic male sterile type T have very different organization. Current Genetics 15: 149-154.

Fauron, C.M.-R. and M. Havlik. 1988. The BamHI, XhoI, SmaI, restriction enzyme maps of the normal maize mitochondrial genome genotype B37. Nuc. Acid. Res. 16:10395.

Iams, K.P. and J.H. Sinclair. 1982. Mapping the mitochondrial DNA of Zea mays: ribosomal gene localization. Proc. Natl. Acad. Sci. USA 79:5926-5929.

Leon, P., V. Walbot, and P. Bedinger. 1989. Molecular analysis of the linear 2.3 kb plasmid of maize mitochondria: apparent capture of tRNA genes. Nuc. Acids Res. 17:4089-4099.

Lonsdale, D.M., T.P. Hodge, and C.M.-R. Fauron. 1984. The physical map and organization of the mitochondrial genome from the fertile cytoplasm of maize. Nuc. Acids Res. 12:9249-9261.

Marechal, L., P. Guillemaut, J.M. Grienenberger, G. Jeannin, and J.H. Weil. 1985. Structure of bean mitochondrial $tRNA^{Phe}$ gene on the mitochondrial genomes of maize and wheat. FEBS Lett. 184:289-293.

Marechal, L., P. Runeberg-Roos, J.M. Grienenberger, J. Colin, J.H. Weil, B. Lejeune, F. Quetier, and D.M. Lonsdale. 1987. Homology in the region containing a $tRNA^{Trp}$ gene and a (complete or partial) $tRNA^{Pro}$ gene in wheat mitochondrial and chloroplast genomes. Curr. Genet. 12:91-98.

Parks, T.D., W.G. Dougherty, CSIII Levings, and D.H. Timothy. 1984. Identification of two methionine transfer RNA genes in the maize mitochondrial genome. Plant Physiol. 76:1079-1082.

Parks, T.D., W.G. Dougherty, CSIII Levings, and D.H. Timothy. 1985. Identification of an aspartate transfer RNA gene in maize mitochondrial DNA. Curr. Genet. 9:517-519.

Sangaré, A., D. Lonsdale, J.H. Weil, and J.M. Grienenberger. 1989. Sequence analysis of the $tRNA^{Tyr}$ and $tRNA^{Lys}$ genes and evidence for the transcription of a chloroplast-like $tRNA^{Met}$ in maize mitochondria. Curr. Genet. 16:195-201.

Sangaré, A., J.H. Weil, and J.M. Grienenberger. 1989. Nucleotide sequence of a maize mitochondrial $tRNA^{Ser}$(UGA) gene. Nuc. Acids Res. 17:7979-7979.

Sangaré, A., J.H. Weil, and J.M. Grienenberger. 1989. Nucleotide sequence of a maize mitochondrial $tRNA^{Glu}$(UUC) gene. Nuc. Acids Res. 17:5837-5837.

Sangaré, A., J.H. Weil, J.M. Grienenberger, C. Fauron, and D. Lonsdale. 1990. Localization and organization of tRNA genes on the mitochondrial genomes of fertile and male sterile lines of maize. Mol. Gen. Genetics (submitted).

Wintz, H., J.M. Grienenberger, J.H. Weil, and D.M. Lonsdale. 1988. Location and nucleotide sequence of two tRNA genes and a tRNA pseudo-gene in the maize mitochondrial genome: evidence for the transcription of a chloroplast gene in mitochondria. Curr. Genet. 13:247-254.

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VII. RECENT MAIZE PUBLICATIONS

1. Abadi, R; Levy, Y; Bar-Tsur, A, 1989. Physiological races of *Exserohilum turcicum* in Israel. Phytoparasitica 17:23-30.
2. Ajayi, OA; Clarke, B, 1989. High velocity shearing of maize kernels. J. Agr. Eng. Res. 42:15-26.
3. Akhtar, M; Garraway, MO, 1989. High temperature stress-induced severity of disease in maize may involve solubilization of proteins. Curr. Topics Plant Biochem. Physiol. 8:280.
4. Alcantara, EN; Wyse, DL, 1988. Glyphosate as harvest aid for corn (*Zea mays*). Weed Technol. 2:410-413.
5. Allard, RW, 1990. Future directions in plant population genetics, evolution, and breeding. Pp. 1-19 in Plant Population Genetics, Breeding, and Genetic Resources. A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, ed., Sunderland, MA: Sinauer Assoc.
6. Allen, JO; Emenhiser, GK; Kermicle, JL, 1989. Miniature kernel and plant - interaction between teosinte cytoplasmic genomes and maize nuclear genomes. Maydica 34:277-290.
7. Anonymous, 1989. Herschel L. Roman. Ann. Rev. Genet. 23:vii.
8. Anthony, CG, 1988. Mechanization and maize; agriculture and the politics of technology transfer in East Africa. New York: Columbia Univ. Press.
9. Antonelli, NM; Stadler, J, 1989. Chemical methods for efficient direct gene transfer to maize cells: Treatment with polyethylene glycol or polybrene. J. Genet. Breed. 43:113-122.
10. Ashman, RB, 1989. Registration of HPXD-1 and HPXD-2 popcorn (maize) germplasms. Crop Sci. 29:1331-1332.
11. Ashraf, M; McNeilly, T, 1989. Effect of salinity on some cultivars of maize. Maydica 34:179-189.
12. Ayuk-Takem, JA, 1988. Stability analyses of early and late maturing highland maize (*Zea mays* L.) varieties in Cameroon. Maydica 33:295-317.
13. Bailey, BA; Larson, RL, 1989. The distinction between hydroxamic acid and flavonoid glycosylation in maize. Curr. Topics Plant Biochem. Physiol. 8:295.
14. Bailey, BA; Larson, RL, 1989. Hydroxamic acid glucosyltransferases from maize seedlings. Plant Physiol. 90:1071-1076.
15. Bar-Tsur, A; Saadi, H; Antignus, Y, 1988. Resistance of corn genotypes to maize rough dwarf virus. Maydica 33:189-200.
16. Bargman, TJ; Hanners, GD; Becker, R; Saunders, RM; Rupnow, JH, 1989. Compositional and nutritional evaluation of Eastern Gamagrass (*Tripsacum dactyloides* (L.) L.), a perennial relative of maize (*Zea mays* L.). Lebensm.-Wiss. Technol. 22:208-212.
17. Barkan, A, 1989. Tissue-dependent plastid RNA splicing in maize: transcripts from four plastid genes are predominantly unspliced in leaf meristems and roots. Plant Cell 1:437-445.
18. Barlow, PW, 1989. Experimental modification of cell division patterns in the root meristem of *Zea mays*. Ann. Bot. 64:13-20.
19. Barnabas, B; Kovacs, G; Abranyi, A; Pfahler, P, 1988. Effect of pollen storage by drying and deep-freezing on the expression of different agronomic traits in maize (*Zea mays* L.). Euphytica 39:221-226.
20. Basra, AS; Dhillon, R; Malik, CP, 1989. Influence of seed pre-treatments with plant growth regulators on metabolic alterations of germinating maize embryos under stressing temperature regimes. Ann. Bot. 64:37-42.
21. Bathgate, B; Baker, A; Leaver, CJ, 1989. Two genes encode the adenine nucleotide translocator of maize mitochondria--isolation, characterization and expression of the structural genes. Eur. J. Biochem. 183:303-310.
22. Bazhok, HU; Razumovich, AN, 1988. Effectiveness of primary photosynthetic reactions in maize lines and hybrids. Vyestsi Akad. Navuk BSSR Syer. Biyal. Navuk:19-24.
23. Becker, HC; Schnell, FW, 1988. Performance of advanced generations of eight maize synthetics differing in number and genetic diversity of constituent lines. Maydica 33:151-162.
24. Belanger, FC; Kriz, AL, 1989. Molecular characterization of the major maize embryo globulin encoded by the *Glb1* gene. Plant Physiol. 91:636-643.
25. Belousov, AA; Ignatova, SA; Lukyanyuk, SF, 1989. Utilization of isolated embryo culture on lysin-threonine medium in maize breeding for grain quality. Genetika 25:1802-1810.
26. Bennet, RJ; Breen, CM, 1989. Towards understanding root growth responses to environmental signals - the effect of aluminium on maize. S. Afr. J. Sci. 85:9-11.
27. Bennett, MA; Gorski, SF, 1989. Response of sweet corn (*Zea mays*) endosperm mutants to chloracetamide and thiocarbamate herbicides. Weed Technol. 3:475-478.
28. Bernardo, R; Johnson, GR; Dudley, JW; Meghji, MR, 1989. Evaluation of F2 X F2 and BC1 X BC1 maize interpopulation crosses. Crop Sci. 29:1377-1381.
29. Berthiaume, L; Beaudry, D; Lazure, C; Tolan, DR; Sygusch, J, 1989. Recombinant anaerobic maize aldolase: overexpression, characterization, and metabolic implications. Arch. Biochem. Biophys. 272:281-300.
30. Bianchi, G; Avato, P; Scarpa, O; Murelli, C; Audisio, G; Rossini, A, 1989. Composition and structure of maize epicuticular wax esters. Phytochemistry 28:165-172.
31. Bird, RM, 1989. Maize, man and vegetation in north-central Peru. Pp. 447-468 in Evolutionary Ecology of Plants. J. H. Bock and Y. B. Linhart, ed., Boulder, CO: Westview Press.
32. Blaker, TW; Greyson, RI; Walden, DB, 1989. Variation among inbred lines of maize for leaf surface wax composition. Crop Sci. 29:28-32.
33. Bochnia, T; Hominska, A, 1989. Use of an x-ray microprobe to study plant cell physiology. Wiad. Bot. 33:19-32.
34. Bocsi, J, 1989. Cold tolerance of maize (*Zea mays* L.). Novenytermeles 38:267-273.
35. Bocsi, J; Kovacs, G, 1989. Effect of gibberellic acid on low-temperature germination and on heredity of low-temperature germination in maize (*Zea mays* L.). Novenytermeles 38:97-104.
36. Boka, K; Fridvalszky, I; Herdi, F, 1989. Histological and cytological effects of EPTC treatment in maize. Novenytermeles 38:209-220.
37. Boothe, JG; Walden, DB, 1989. Multivariate analyses of polypeptide synthesis in developing maize embryos. Theor. Appl. Genet. 77:495-500.
38. Bosch, LL; Casanas, F; Nuez, F, 1989. Synchronization of flowering in maize (*Zea mays* L.) by the genetic system *pg11 pg12*. Euphytica 41:129-134.
39. Boulton, MI; Buchholz, WG; Marks, MS; Markham, PG; Davies, JW, 1989. Specificity of *Agrobacterium*-mediated delivery of maize streak virus DNA to members of the Gramineae. Plant Mol. Biol. 12:31-40.
40. Bowen, KL; Pedersen, WL, 1989. A model of corn growth and disease development for instructional purposes. Plant Dis. 73:83-95.
41. Bowman, DT, 1989. Plot configuration in corn yield trials. Crop Sci. 29:1202-1206.

42. Braun, CJ; Siedow, JN; Levings, CS, 1989. The *T-Urf13* gene is responsible for toxin sensitivity in maize and E. coli. Pp. 79-85 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss Inc.
43. Braun, CJ; Siedow, JN; Williams, ME; Levings, CS, III, 1989. Mutations in the maize mitochondrial *T-urf13* gene eliminate sensitivity to a fungal pathotoxin. Proc. Nat. Acad. Sci. USA 86:4435-4439.
44. Bretting, PK; Goodman, MM, 1989. Karyotypic variation in Mesoamerican races of maize and its systematic significance. Econ. Bot. 43:107-124.
45. Brink, DE; Price, SC; Martinez, C, 1989. Monoclonal antibodies against zeins. Seed Sci. Technol. 17:91-98.
46. Brink, DE; Price, SC; Nguyen, H; Fuerts, G; Martinez, C, 1989. Genetic purity assessment of commercial single cross maize hybrids: isoelectric focusing of zeins. Seed Sci. Technol. 17:1-6.
47. Brinkmann, H; Cerff, R; Salomon, M; Soll, J, 1989. Cloning and sequence analysis of complementary DNAs encoding the cytosolic precursors of subunits GAPA and GAPB of chloroplast glyceraldehyde-3-phosphate dehydrogenase from pea and spinach. Plant Mol. Biol. 13:81-94.
48. Brown, AHD; Clegg, MT; Kahler, AL; Weir, BS, ed., 1990. Plant Population Genetics, Breeding, and Genetic Resources. Sunderland, MA: Sinauer Assoc.
49. Brown, JJ; Mattes, MG; O'Reilly, C; Shepherd, NS, 1989. Molecular characterization of *rDt*, a maize transposon of the "Dotted" controlling element system. Mol. Gen. Genet. 215:239-244.
50. Brown, WE; Robertson, DS; Bennetzen, JL, 1989. Molecular analysis of multiple Mutator-derived alleles of the bronze locus of maize. Genetics 122:439-446.
51. Bruder, K; Mohr, I, 1989. Comparison of infection methods for testing the resistance to stalk rot in maize. Archiv Phytopathol. Pflanzenschutz 25:365-373.
52. Brun, EL; Dudley, JW, 1989. Breeding potential in the USA and Argentina of corn populations containing different proportions of flint and dent germplasm. Crop Sci. 29:570-577.
53. Brun, EL; Dudley, JW, 1989. Nitrogen response in the USA and Argentina of corn populations with different proportions of flint and dent germplasm. Crop Sci. 29:565-569.
54. Brunk, DG; Rich, PJ; Rhodes, D, 1989. Genotypic variation for glycinebetaine among public inbreds of maize. Plant Physiol. 91:1122-1125.
55. Brush, SB; Corrales, MB; Schmidt, E, 1988. Agricultural development and maize diversity in Mexico. Hum. Ecol. 16:307-328.
56. Bryant, JA, 1988. At last transgenic cereal plants from genetically engineered protoplasts. Trends Biotechnol. 6:291-292.
57. Burch, LR; Horgan, R, 1989. The purification of cytokinin oxidase from *Zea mays* kernels. Phytochemistry 28:1313-1320.
58. Burton, JD; Gronwald, JW; Somers, DA; Gengenbach, BG; Wyse, DL, 1989. Inhibition of corn acetyl coenzyme A carboxylase by cyclohexanedione and aryloxyphenoxypropionate herbicides. Pestic. Biochem. Physiol. 34:76-85.
59. Bush, MB; Piperno, DR; Colinvaux, PA, 1989. A 6,000 year history of an Amazonian maize cultivation. Nature 340:303-304.
60. Byrne, PF; Darrah, LL; Simpson, KB; Keaster, AJ; Barry, BD; Zuber, MS, 1989. Maize silk pH as an indicator of resistance to the corn earworm (Lepidoptera: Noctuidae). Environ. Entomol. 18:356-360.
61. Byrnes, KJ; Pataky, JK; White, DG, 1989. Relationships between yield of 3 maize hybrids and severity of southern leaf blight caused by race O of *Bipolaris maydis*. Plant Disease 73:834-840.
62. Caldwell, EEO; Peterson, PA, 1989. Diversity of transposable-element interactions: The *Uq* transposable-element system in maize controls four *c-m* mutants exhibiting unique responses to *Uq-13*. Maydica 34:89-105.
63. Callis, J; Fromm, M; Walbot, V, 1988. Heat inducible expression of a chimeric maize hsp70CAT gene in maize protoplasts. Plant Physiol. 88:965-968.
64. Campos, N; Palau, J; Zwieb, C, 1989. Diversity of 7 SL RNA from the signal recognition particle of maize endosperm. Nucl. Acid. Res. 17:1573-1588.
65. Camussi, A; Landi, P; Bertolini, M, 1988. Analysis of variety crosses to develop early base population for reciprocal recurrent selection in maize. Maydica 33:269-281.
66. Camussi, A; Ottaviano, E; Basso, B; Pirillo, E, 1989. Photosynthetic rate as a yield component of maize in environmental restrictive conditions. J. Genet. Breed. 43:37-44.
67. Cannon, RE; Scandalios, JG, 1989. Two cDNAs encode two nearly identical Cu/Zn superoxide dismutase proteins in maize. Mol. Gen. Genet. 219:1-8.
68. Carlone, MR; Russell, WA, 1989. Evaluation of S2 maize lines reproduced for several generations by random mating within lines. 2. Comparisons for testcross performance of original and advanced S2 and S8 lines. Crop Sci. 29:899-904.
69. Carswell, GK; Johnson, CM; Shillito, RD; Harms, CT, 1989. O-acetyl-salicylic acid promotes colony formation from protoplasts of an elite maize inbred. Plant Cell Rep. 8:282-284.
70. Castillo-Gonzalez, F; Goodman, MM, 1989. Agronomic evaluation of Latin American maize accessions. Crop Sci. 29:853-861.
71. Ceballos, H; Gracen, VE, 1988. A new source of resistance to *Bipolaris maydis* race T in maize. Maydica 33:233-246.
72. Ceballos, H; Gracen, VE, 1989. A dominant inhibitor gene inhibits the expression of *Ht2* against *Exserohilum turcicum* race 2 in corn inbred lines related to B14. Z. Pflanzenzucht. 102:35-44.
73. Ceska, O, 1988. Flavonoid analysis of corn tissues. Pp. 139-142 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
74. Chang, MT; Neuffer, MG, 1989. Maize microsporogenesis. Genome 32:232-244.
75. Chaves, L; Vencovsky, R; Geraldi, IO, 1989. A nonlinear model applied to genotype X environment interaction analysis in maize. Pesqui. Agropecu. Bras. 24:259-268.
76. Checheneva, TN; Morgun, VV; Ruban, TA, 1988. Regeneration of plants from different types of callus tissues of inbred lines and maize hybrids. Dokl. Akad. Nauk Ukr. SSR Ser. B Geol. Khim. Biol. Nauki:80-83.
77. Chemeris, AV; Vakhitov, VA, 1989. Primary structure of 5.8S ribosomal RNA gene and ribosomal DNA internal transcribed spacer in diploid wheat (*Triticum urartu* Thum. ex Gandil). Mol. Biol. 23:320-326.
78. Chemeris, AV; Vakhitov, VA; Akhemtzyanov, AA, 1989. Organization of ribosomal RNA genes in plants of different tribes of the cereal family. Mol. Biol. 23:327-335.
79. Chen, C; Lien, T-J, 1989. Studies on genotype-environment interactions in maize. Acad. Sin. Inst. Bot. Monogr. Ser.:9-28.
80. Chen, W; Duan, S, 1988. The cytological observation of anther development in C-cytoplasmic male sterile corn. Acta Agron. Sin. 14:177-181.
81. Chen, Y; Wang, M, 1988. Studies on recurrent selection for specific combining ability in two maize populations. Acta Agron. Sin. 14:221-226.
82. Chen, YC; Chourey, PS, 1989. Spatial and temporal expression of the 2 sucrose synthase genes in maize - immunohistological evidence. Theor. Appl. Genet. 78:553-559.

83. Cheng, DSK; Wang, AS, 1989. Process for regenerating corn U.S. patent-4843005. Off. Gaz. U. S. Pat. Trademark Off. Pat. 1103:2540.
84. Cheng, PC; Wright, DC, 1989. Effects of 3-(p-chlorophenyl)-6-methoxy-s-triazine-2,4 (1H, 3H) dione triethanolamine (DPX-3778) treatment on the floral development of maize. Can. J. Bot. 67:327-331.
85. Chernyshev, AI; Golovkin, MV; Mil'shina, NV; Gazumyan, AK; Ananyev, EV, 1988. Molecular-genetic organisation of mobile elements of the *Ac-Ds* family in cereal genomes: identification of barley DNA sequences homologous to the maize *Ac* element. Genetika 24:1918-1927.
86. Chirikova, GB; Vershinin, AV, 1989. Cytoplasmic genomes of cereals. Genetika 25:773-783.
87. Christensen, AH; Quail, PH, 1989. Sequence analysis and transcriptional regulation by heat shock of polyubiquitin transcripts from maize. Plant Mol. Biol. 12:619-632.
88. Clegg, MT, 1990. Molecular diversity in plant populations. Pp. 98-115 in Plant Population Genetics, Breeding, and Genetic Resources. A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, ed., Sunderland, MA: Sinauer Assoc.
89. Cliquet, JB; Morotgaudry, JF, 1989. Comparative study of maize leaf and stalk RuBPcase. Comp. Rend. Acad. Sci. Ser. III-Sci. Vie 309:583-586.
90. Close, KR; Gallagher-Ludeman, LA, 1989. Structure-activity relationships of auxin-like plant growth regulators and genetic influences on the culture induction response in maize (*Zea mays* L.). Plant Sci. 61:245-252.
91. Close, TJ; Kortt, AA; Chandler, PM, 1989. A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol. Biol. 13:95-108.
92. Cobb, BG; Hannah, LC, 1988. Shrunken-1 encoded sucrose synthase is not required for sucrose synthesis in the maize endosperm. Plant Physiol. 88:1219-1221.
93. Coe, E; Gabay-Laughnan, S; Patterson, EB, 1989. Laughnan, John R. - Genetics from *A* to *Zea* in 3 Score and 10. Maydica 34:191-195.
94. Coelho, AM; Morais, ARD; eGama, EEG; Silva, BGD.; Cornelio, WMDO, 1988. Stability estimates for yield in maize cultivars for the state of Minas Gerais, Brazil. Pesqui. Agropecu. Bras. 23:1015-1024.
95. Cone, KC; Burr, B, 1988. Molecular and genetic analyses of the light requirement for anthocyanin synthesis in maize. Pp. 143-145 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
96. Consonni, G; Gavazzi, G; Tonelli, C, 1988. Interaction of light with *Sn*, a tissue specific gene. Analysis of in vivo protein synthesis. Pp. 115-122 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
97. Cooper, P; Newton, KJ, 1989. Maize nuclear background regulates the synthesis of a 22-kDa polypeptide in *Zea luxurians* mitochondria. Proc. Nat. Acad. Sci. USA 86:7423-7426.
98. Coors, JG; Mardones, MC, 1989. 12 cycles of mass selection for prolificacy in maize. 1. Direct and correlated responses. Crop Sci. 29:262-266.
99. Coors, JG; Mardones, MC, 1989. Registration of W3L Comp-Hs C4 and W3L Comp-S1 C4 maize germplasm. Crop Sci. 29:1579-1580.
100. Coors, JG; Mardones, MC, 1989. Registration of W552 parental line, W570 parental line, W572 parental line and W576 parental line of maize. Crop Sci. 29:247-248.
101. Coors, JG; Mardones, MC, 1989. Registration of Wgg(MP) C15 maize germplasm. Crop Sci. 29:1579.
102. Coors, JG; Staub, JE; Senalik, D; Rinehart, CA, 1989. Microcomputer simulation of expected gains from eight methods of intrapopulation recurrent selection. Agron. J. 81:317-319.
103. Coraggio, I; Martegani, E; Compagno, C; Porro, D; Alberghina, L; Bernard, L; Faoro, F; Viotti, A, 1988. Differential targeting and accumulation of normal and modified zein polypeptides in transformed yeast. Eur. J. Cell Biol. 47:165-172.
104. Corke, H; Kannenberg, LW, 1989. Selection for vegetative phase and actual filling period duration in short season maize. Crop Sci. 29:607-612.
105. Coumans, MP; Sohota, S; Swanson, EB, 1989. Plant development from isolated microspores of *Zea mays* L. Plant Cell Rep. 7:618-621.
106. Craker, LE; Waldron, PF, 1989. Acid rain and seed yield reductions in corn. J. Environ. Qual. 18:127-129.
107. Cross, HZ, 1989. Registration of ND262, ND263, and ND264 parental lines of maize. Crop Sci. 29:839.
108. Cross, HZ, 1989. Registration of NDSB(MS)C8 and NDSG(MS)C8 maize germplasms. Crop Sci. 29:494.
109. Cross, HZ; Dosso, H, 1989. *R-nj* aleurone color selection and grain-filling responses in opaque-2 maize. Euphytica 43:269-274.
110. Cross, HZ; Dosso, H, 1989. *R-nj* aleurone color selection and nitrogen response in opaque-2 maize. Can. J. Plant Sci. 69:741-748.
111. Cross, HZ; Kabir, KM, 1989. Evaluation of field dry-down rates in early maize. Crop Sci. 29:54-58.
112. Crossa, J, 1989. Methodologies for estimating the sample size required for genetic conservation of outbreeding crops. Theor. Appl. Genet. 77:153-161.
113. Crossa, J, 1989. Theoretical considerations for the introgression of exotic germplasm into adapted maize populations. Maydica 34:53-62.
114. Crossa, J; Gardner, CO, 1989. Predicted and realized grain yield responses to full-sib family selection in CIMMYT maize (*Zea mays* L.) populations. Theor. Appl. Genet. 77:33-38.
115. Crossa, J; Westcott, B; Gonzalez, C, 1989. The yield stability of CIMMYT's maize germplasm. Euphytica 40:245-252.
116. Cutler, AJ; Saleem, M; Coffey, A; Loewen, MK, 1989. Role of oxidative stress in cereal protoplast recalcitrance. Plant Cell Tissue Organ Cult. 18:113-128.
117. Dai, J; Xie, Y, 1988. Study of Y type male sterile cytoplasm in maize. Acta Agron. Sin. 14:110-116.
118. Darrah, LL; West, DR; Wassom, CE; Poneleit, CG; Bockholt, AJ; Crane, PL; Barry, BD; Elmore, R; York, JO; Lundquist, R; Colbert, TR; Martin, P; Feyerabend, W, 1988. White food corn: 1988 national crop performance. Univ. Mo. Agr. Exp. Stn., Columbia, MO.
119. Das, R; Sharma, AK; Sopory, SK, 1989. Regulation of NADH-glutamate dehydrogenase activity by phytochrome, calcium and calmodulin in *Zea mays*. Plant Cell Physiol. 30:317-324.
120. Dash, S; Peterson, PA, 1989. Chromosome constructs for transposon tagging of desirable genes in different parts of the maize genome. Maydica 34:247-261.
121. daSilva, TRG; Furlani, AMC; Felippe, GM, 1988. Correlation between nitrogen content and dry matter in 11 maize germplasms. Rev. Bras. Biol. 48:639-644.
122. Datta, JK; Katsumi, M, 1989. Changes in the sugar content of the leaf sheath of dwarf maize seedlings associated with gibberellin-induced growth. Plant Cell Physiol. 30:143-145.

123. Davis, FM; Ng, SS; Williams, WP, 1989. Mechanisms of resistance in corn to leaf feeding by Southwestern corn borer and European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 82:919-922.
124. Debnath, SC; Sarkar, KR, 1989. Quantitative genetic analysis of grain yield and some other agronomic traits in maize. Pak. J. Sci. Ind. Res. 32:253-256.
125. Deinum, B, 1988. Genetic and environmental variation in quality of forage maize in Europe. Neth. J. Agr. Sci. 36:400-.
126. Deinum, B; Struik, PC, 1989. Genetic variation in digestibility of forage maize (*Zea mays* L.) and its estimation by near infrared reflectance spectroscopy (NIRS). An analysis. Euphytica 42:89-98.
127. DeLeon, C; Pandey, S, 1989. Improvement of resistance to ear and stalk rots and agronomic traits in tropical maize gene pools. Crop Sci. 29:12-17.
128. DeRose, RT; Ma, D-P; Kwon, I-S; Hasnain, SE; Klassy, RC; Hall, TC, 1989. Characterization of the kafirin gene family from sorghum reveals extensive homology with zein from maize. Plant Mol. Biol. 12:245-256.
129. deSouza, IRP; Saraiva, LS, 1989. Genetic analysis of partial fertility restoration of male-sterile C-cytoplasm in maize (*Zea mays* L.). Rev. Ceres 36:94-105.
130. deSouza, IRP; Saraiva, LS, 1989. Genetics of fertility restoration in maize (*Zea mays* L.) with male-sterile C-type cytoplasm. Rev. Brasil. Genetica 12:303-312.
131. DeVienne, D; Leonardi, A; Damerval, C, 1988. Genetic aspects of variation of protein amounts in maize and pea. Electrophoresis 9:742-750.
132. Dhillon, BS; Khehra, AS, 1989. Modified S1 recurrent selection in maize improvement. Crop Sci. 29:226-228.
133. DiFonzo, N; Hartings, H; Lazzaroni, N; Salamini, F; Thompson, R; Motto, M, 1989. The sequence of the zein regulatory gene opaque-2 (*O2*) of *Zea mays*. Nucl. Acid. Res. 17:7532.
134. Dimitrov, G; Boev, A; Ivanova, T, 1988. Use of genetic cms designations in maize seed production. Rasteniev'd. Nauki 25:5-9.
135. Djisbar, A; Gardner, FP, 1989. Heterosis for embryo size and source and sink components of maize. Crop Sci. 29:985-992.
136. Doebley, J, 1989. Molecular evidence for a missing wild relative of maize and the introgression of its chloroplast genome into *Zea perennis*. Evolution 43:1555-1559.
137. Doehlert, DC, 1989. Separation and characterization of 4 hexose kinases from developing maize kernels. Plant Physiol. 89:1042-1048.
138. Dolstra, O; Jongmans, MA; deJong, K, 1988. Improvement and significance of resistance to low-temperature damage in maize (*Zea mays* L.). I. Chlorosis resistance. Euphytica Suppl.:117-124.
139. Dooner, HK; Belachew, A, 1989. Transposition pattern of the maize element *Ac* from the *bz-m2(Ac)* allele. Genetics 122:447-458.
140. Doring, H-P, 1989. Tagging genes with maize transposable elements: An overview. Maydica 34:73-88.
141. Dotson, SB; Somers, DA, 1989. Differential metabolism of sodium azide in maize callus and germinating embryos. Mutat. Res. 213:157-164.
142. Dourleijn, CJ; Den Nijs, APM; Dolstra, O, 1988. Description and evaluation of a device for measuring vertical pulling resistance in maize (*Zea mays* L.). Euphytica Suppl.:69-76.
143. DuBay, DT, 1989. Direct effects of simulated acid rain on sexual reproduction in corn. J. Environ. Qual. 18:217-221.
144. Dudley, JW, 1988. Theory for identification of lines or populations useful for improvement of elite single crosses. Pp. 451-461 in Proc. 2nd Int. Conf. Quant. Genet., Univ. North Carolina, Raleigh, NC; B. S. Weir, E. J. Eisen, M. M. Goodman and G. Namkoong, ed., Sunderland, MA: Sinauer Assoc., Inc.
145. Dugas, CM; Li, QN; Khan, IA; Nothnagel, EA, 1989. Lateral diffusion in the plasma membrane of maize protoplasts with implications for cell culture. Planta 179:387-396.
146. Duncan, DR; Singletary, GW; Below, FE; Widholm, JM, 1989. Increased induction of regenerable callus cultures from cultured kernels of the maize inbred FR27rhm. Plant Cell Rep. 8:350-353.
147. Duncan, DR; Widholm, JM, 1989. Differential response to potassium permanganate of regenerable and of non-regenerable tissue cell walls from maize callus cultures. Plant Sci. 61:91-104.
148. Dupuis, I; Zhao, ZX; Dumas, C, 1988. Normal and abnormal embryo development after in vitro fertilization in maize. Pp. 309-313 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
149. Duvick, DN, 1989. Possible genetic causes of increased variability in U. S. maize yields. Pp. 147-156 in Variability in Grain Yields: Implications for Agricultural Research and Policy in Developing Countries. J. R. Anderson and P. B. R. Hazell, ed., Baltimore: Johns Hopkins Univ. Press.
150. Dwyer, LM; Stewart, DW; Balchin, D; Houwing, L; Marur, CJ; Hamilton, RI, 1989. Photosynthetic rates of six maize cultivars during development. Agron. J. 81:597-602.
151. Dwyer, LM; Tollenaar, M, 1989. Genetic improvement in photosynthetic response of hybrid maize cultivars: 1959 to 1988. Can. J. Plant Sci. 69:81-92.
152. Eagles, HA; Hardacre, AK, 1989. Synthetic populations of maize containing highland Mexican or highland Peruvian germplasm. Crop Sci. 29:660-665.
153. Eagles, HA; Hardacre, AK; Bansal, RK, 1989. Testcross performance of maize lines from backcross populations containing highland Mexican or highland Peruvian germplasm. Euphytica 41:263-272.
154. Eberhart, SA; Briggs, RW; Raycraft, J; Linder, JO, 1989. Registration of 9 maize germplasm populations. Crop Sci. 29:243-244.
155. Eckdahl, TT; Bennetzen, JL; Anderson, JN, 1989. DNA structures associated with autonomously replicating sequences from plants. Plant Mol. Biol. 12:507-516.
156. Efimov, VA; Buryakova, AA; Pashkova, IN; Chakhmakhcheva, OG, 1988. Synthesis and cloning of artificial zein genes. Bioorg. Khim. 14:1538-1544.
157. Efron, Y; Kim, SK; Fajemisin, JM; Mareck, JH; Tang, CY; Dabrowski, ZT; Rossel, HW; Thottappilly, G; Buddenhagen, IW, 1989. Breeding for resistance to maize streak virus - a multidisciplinary team approach. Plant Breeding 103:1-36.
158. Egeland, DB; Sturtevant, AP; Schuler, MA, 1989. Molecular analysis of dicot and monocot small nuclear RNA populations. Plant Cell 1:633-643.
159. Eichelberger, KD; Lambert, RJ; Below, FE; Hageman, RH, 1989. Divergent phenotypic recurrent selection for nitrate reductase activity in maize.1. Selection and correlated responses. Crop Sci. 29:1393-1397.
160. Eichelberger, KD; Lambert, RJ; Below, FE; Hageman, RH, 1989. Divergent phenotypic recurrent selection for nitrate reductase activity in maize.2. Efficient use of fertilizer nitrogen. Crop Sci. 29:1398-1402.
161. El-Hosary, AA, 1985. Study of combining ability in some top crosses in maize. Egypt. J. Agron. 10:39-48.
162. El-Hosary, AA, 1986. Estimation of genetic variance in open pollinated variety with reference to expected gain from selection. Egypt. J. Agron. 11:119-128.

163. Elliston, K; Messing, J, 1989. The molecular architecture of plant genes and their regulation. Pp. 115-139 in Plant Biotechnology. S. D. Kung and C. J. Arntzen, ed., Stoneham: Butterworths.
164. Esen, A; Hilu, KW, 1989. Immunological affinities among subfamilies of the Poaceae. Am. J. Bot. 76:196-203.
165. Esen, A; Mohammad, K; Schurig, GG; Aycock, HS, 1989. Monoclonal antibodies to zein discriminate certain maize inbreds and genotypes. J. Hered. 80:17-23.
166. Fauron, C; Havlik, M, 1989. The maize mitochondrial genome of the normal type and the cytoplasmic male sterile type T have very different organization. Curr. Genet. 15:149-154.
167. Fauron, C; Havlik, M; Lonsdale, D; Nichols, L, 1989. Mitochondrial genome organization of the maize cytoplasmic male sterile type T. Mol. Gen. Genet. 216:395-401.
168. Fedoroff, N, 1989. About maize transposable elements and development. Cell 56:181-191.
169. Fedoroff, N, 1989. The heritable activation of cryptic Suppressor-mutator elements by an active element. Genetics 121:591-608.
170. Fedoroff, N; Masson, P; Banks, J, 1987. Regulation of the maize Suppressor-mutator element. Pp. 63-70 in Eukaryotic Transposable Elements as Mutagenic Agents. M. E. Lambert, J. F. McDonald and I. B. Weinstein, ed., Cold Spring Harbor, New York: Cold Spring Harbor Press.
171. Fedoroff, N; Masson, P; Banks, JA, 1988. A new understanding of how maize transposable elements are controlled in development. Carnegie Inst. Wash. Year Book 87:45-52.
172. Fedoroff, N; Masson, P; Banks, JA, 1989. Mutations, epimutations, and the developmental programming of the maize suppressor-mutator transposable element. BioEssays 10:139-144.
173. Fedoroff, NV, 1987. Mobile genetic elements in maize. Pp. 97-125 in Developmental Genetics of Higher Organisms: A Primer in Developmental Biology. G. M. Malacinski, ed., New York: Macmillan Publ. Co.
174. Fedoroff, NV, 1989. Maize transposable elements. Pp. 375-411 in Mobile DNA. D. E. Berg and M. M. Howe, ed., Washington: Amer. Soc. Microbiol.
175. Fedoroff, NV, 1989. Maize transposable elements in development and evolution. Amer. Zool. 29:549-556.
176. Fedoroff, NV; Baker, B, 1989. Plant gene vectors and genetic transformation - the structure, function, and uses of maize transposable elements. Pp. 101-132 in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes. J. Schell and I. K. Vasil, ed., San Diego: Academic Press.
177. Fedoroff, NV; Banks, JA; Masson, P, 1989. Developmental determination of *Spm* expression. Pp. 51-64 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss Inc.
178. Felker, FC; Goodwin, JC, 1988. Sugar uptake by maize endosperm suspension cultures. Plant Physiol. 88:1235-1239.
179. Felker, FC; Miernyk, JA; Crawford, CG, 1989. Characterization of maize endosperm-derived suspension cells throughout the culture cycle and enhancement of tissue friability. Plant Cell Tissue Organ Cult. 18:153-166.
180. Filippov, GL; Vishnevsky, NV; Gubenko, VA; Zhurba, GM; Zhakote, AG; Kruglitsky, LV, 1988. Physiological peculiarities of maize lines with improved cold resistance. Fiziol. Biokhim. Kult. Rast. 20:581-585.
181. Finnegan, EJ; Taylor, BH; Craig, S; Dennis, ES, 1989. Transposable elements can be used to study cell lineages in transgenic plants. Plant Cell 1:757-764.
182. Fischer, KS; Edmeades, GO; Johnson, EC, 1989. Selection for the improvement of maize yield under moisture-deficits. Field Crops Res. 22:227-243.
183. Fragoso, LL; Nichols, SE; Levings, CS, 1989. Rearrangements in maize mitochondrial genes. Genome 31:160-168.
184. Fransz, PF; deRuijter, NCA; Schel, JHN, 1989. Isozymes as biochemical and cytochemical markers in embryogenic callus cultures of maize (*Zea mays* L.). Plant Cell Rep. 8:67-70.
185. Frey, M; Tavantzis, SM; Saedler, H, 1989. The maize *En-1/Spm* element transposes in potato. Mol. Gen. Genet. 217:172-177.
186. Frey, NM; McConnell, RL; Duvick, DN, 1988. Maize breeding and seed product development. Pp. 69-72 in Genetic Improvements of Agriculturally Important Crops. R. T. Fraley, N. M. Frey and J. M. Schell, ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
187. Frova, C, 1988. Pre-anthesis and post-anthesis gene expression in maize pollen. Pp. 15-20 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
188. Frova, C; Taramino, G; Binelli, G, 1989. Heat-shock proteins during pollen development in maize. Develop. Genet. 10:324-332.
189. Fujioka, S; Yamane, H; Spray, CR; Gaskin, P; Macmillan, J; Phinney, BO; Takahashi, N, 1988. Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, dwarf-1, dwarf-2, dwarf-3 and dwarf-5 seedlings of *Zea mays* L. Plant Physiol. 88:1367-1372.
190. Gage, DA; Fong, F; Zeevaart, JAD, 1989. Abscisic acid biosynthesis in isolated embryos of *Zea mays* L. Plant Physiol. 89:1039-1041.
191. Galcheva-Gargova, ZI; Marinova, EI; Koleva, ST, 1988. Isolation of nuclear shells from plant cells. Plant Cell Environ. 11:819-826.
192. Galinat, WC, 1989. The Singleton Sweet Corn Bibliography. Amherst: Univ. Mass. Agric. Exp. Stn.
193. Galinat, WC, 1989. The teosinte progenitors of corn as tools for its improvement. Proc. Annu. Corn Sorghum Res. Conf. 43:180-193.
194. Gallais, A, 1988. Heterosis: Its genetic basis and its utilization in plant breeding. Euphytica 39:95-104.
195. Gallie, DR; Lucas, WJ; Walbot, V, 1989. Visualizing mRNA expression in plant protoplasts: factors influencing efficient mRNA uptake and translation. Plant Cell 1:301-311.
196. Garraway, MO; Akhtar, M; Wokoma, ECW, 1989. Effect of high temperature stress on peroxidase activity and electrolyte leakage in maize in relation to sporulation of *Bipolaris maydis* race T. Phytopathology 79:800-805.
197. Gasser, CS; Fraley, RT, 1989. Genetically engineering plants for crop improvement. Science 244:1293-1299.
198. Gatenby, AA; Rothstein, SJ; Nomura, M, 1989. Translational coupling of the maize chloroplast *atp*B and *atp*E genes. Proc. Nat. Acad. Sci. USA 86:4066-4070.
199. Gauly, A; Kossel, H, 1989. Evidence for tissue-specific cytosine-methylation of plastid DNA from *Zea mays*. Curr. Genet. 15:371-376.
200. Gemel, J; Ciesla, E; Kaniuga, Z, 1989. Different response of two *Zea mays* inbreds to chilling stress measured by chloroplast galactolipase activity and free fatty acid levels. Acta Physiol. Plant. 11:3-12.
201. Gemel, J; Saczynska, V; Kaniuga, Z, 1989. Composition of non-esterified fatty acids in chloroplasts of closely related chill-sensitive plants. Phytochemistry 28:1813-1816.
202. Genova, I, 1988. Combining ability of local and introduced maize populations. Genet. Sel. 21:494-498.

203. Genova, I, 1989. Genetic and breeding evaluation of introduced and Bulgarian maize lines for protein content in the grain. Genet. Sel. 22:120-125.
204. Gentinetta, E; Manusardi, C; Bertolini, M; Snidaro, M; Bressan, M; Lorenzoni, C; Maggiore, T, 1988. Environmental stability and adaptability for agronomical and qualitative characters of opaque-2 maize hybrids. Riv. Agron. 22:75-80.
205. Gibson, DM; Christen, AA, 1989. Slurry-producing agents provide buoyancy and allow growth of plant suspension cells in stationary culture. Biotechnol. Tech. 3:305-308.
206. Gierl, A; Lutticke, S; Saedler, H, 1988. *TnpA* product encoded by the transposable element *En-1* of *Zea mays* is a DNA binding protein. EMBO J. 7:4045-4053.
207. Gierl, A; Saedler, H, 1989. The *En/Spm* transposable element of *Zea mays*. Plant Mol. Biol.13:261-266.
208. Gierl, A; Saedler, H; Peterson, PA, 1989. Maize transposable elements. Ann. Rev. Genet. 23:71-85.
209. Gikoshvili, TI; Beletskii, IP; Vilenchik, MM; Kuzin, AM, 1988. Mechanisms of induction of cross thermoresistance and radioresistance of *Zea mays* seedlings. Radiobiologiya 28:714-716.
210. Gobel, E; Lorz, H, 1988. Genetic manipulation of cereals. Oxford Surv. Plant Mol. Cell Biol. 5:1-22.
211. Goday, A; Torrent, M; Ludevid, MD; Puigdomenech, P, 1988. The use of two-dimensional gel electrophoresis in the analysis of organ-specific maize proteins. Electrophoresis 9:738-741.
212. Golubovskaya, IN, 1989. Meiosis in maize - *mei* genes and conception of genetic control of meiosis. Adv. Genet. 26:149-192.
213. Goodman, MM, 1988. The history and evolution of maize. CRC Crit. Rev. Plant Sci. 7:197-220.
214. Goodman, MM; Thompson, DL; Hill, WH; Payne, GA, 1989. Registration of NC258 and NC262 parental lines of maize. Crop Sci. 29:1334.
215. Gowri, G; Campbell, WH, 1989. cDNA clones for corn leaf NADH:nitrate reductase and chloroplast NAD(P)+:glyceraldehyde-3-phosphate dehydrogenase--characterization of the clones and analysis of the expression of the genes in leaves as influenced by nitrate in the light and dark. Plant Physiol. 90:792-798.
216. Graham, GG; Lembcke, J; Lancho, E; Morales, E, 1989. Quality protein maize - digestibility and utilization by recovering malnourished infants. Pediatrics 83:416-421.
217. Grasser, KD; Maier, U-G; Feix, G, 1989. A nuclear casein type II kinase from maize endosperm phosphorylating HMG proteins. Biochem. Biophys. Res. Commun. 162:456-463.
218. Greer, DH; Hardacre, AK, 1989. Photoinhibition of photosynthesis and its recovery in two maize hybrids varying in low temperature tolerance. Aust. J. Plant Physiol. 16:189-198.
219. Grimsley, N; Hohn, B; Ramos, C; Kado, C; Rogowsky, P, 1989. DNA transfer from *Agrobacterium* to *Zea mays* or *Brassica* by agroinfection is dependent on bacterial virulence functions. Mol. Gen. Genet. 217:309-316.
220. Grombacher, AW; Russell, WA; Guthrie, WD, 1989. Resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. J. Kansas Entomol. Soc. 62:103-107.
221. Gualberto, JM; Wintz, H; Weil, JH; Grienenberger, J-M, 1988. The genes coding for subunit 3 of NADH dehydrogenase and for ribosomal protein S12 are present in the wheat and maize mitochondrial genomes and are co-transcribed. Mol. Gen. Genet. 215:118-127.
222. Gudz, YV, 1989. The principles for selection of parent lines while developing corn hybrids with a wide adaptive potential. Tsitol. Genet. 23:52-54.
223. Gutierrez, C; Castanera, P; Torres, V, 1988. Wound-induced changes in DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) concentration in maize plants caused by *Sesamia nonagroides* (Lepidoptera: Noctuidae). Ann. Appl. Biol. 113:447-454.
224. Ha, G; Hallauer, AR; Bailey, TB, Jr., 1989. Nonlinear relationship between single-cross hybrids and their parental lines. Rev. Bras. Genet. 12:287-302.
225. Hajibagheri, MA; Yeo, AR; Flowers, TJ; Collins, JC, 1989. Salinity resistance in *Zea mays* - fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. Plant Cell Env. 12:753-757.
226. Hake, S; Vollbrecht, EV; Freeling, M, 1989. Cloning knotted, the dominant morphological mutant in maize using *Ds2* as a transposon tag. EMBO J. 8:15-22.
227. Hake, S; Walko, R; Lane, B; Lowe, B, 1989. Transposon mutagenesis in developmental biology: methodology and results. Curr. Topics Plant Biochem. Physiol. 8:237-250.
228. Halda, L; Denic, M; Todorovic, M; Neyra, AC, 1988. Isolation and characterization of rhizoplane bacteria associated with maize. Mikrobiologija 25:29-44.
229. Haley, J; Bogorad, L, 1989. A 4-kDa maize chloroplast polypeptide associated with the cytochrome b6-f complex: subunit 5, encoded by the chloroplast *pet*E gene. Proc. Nat. Acad. Sci. USA 86:1534-1538.
230. Hallauer, AR, 1988. Genotype-environment interaction. Pp. 488-491 in Proc. 2nd Int. Conf. Quant. Genet., Univ. North Carolina, Raleigh, NC; B. S. Weir, E. J. Eisen, M. M. Goodman and G. Namkoong, ed., Sunderland, MA: Sinauer Assoc., Inc.
231. Hanley-Bowdoin, L; Chua, N-H, 1989. Transcriptional interaction between the promoters of the maize chloroplast genes which encode the beta subunit of ATP synthase and the large subunit of ribulose 1,5-bisphosphate carboxylase. Mol. Gen. Genet. 215:217-224.
232. Hanson, DD; Hamilton, DA; Travis, JL; Bashe, DM; Mascarenhas, JP, 1989. Characterization of a pollen-specific cDNA clone from *Zea mays* and its expression. Plant Cell 1:173-179.
233. Hanson, MR, 1989. Tracking down plant genes: Paths, patterns, and footprints. Plant Cell 1:169-172.
234. Harberd, NP; Freeling, M, 1989. Genetics of dominant gibberellin-insensitive dwarfism in maize. Genetics 121:827-838.
235. Hardacre, AK; Eagles, HA, 1989. The temperature response of young hybrid maize plants adapted to different climates. New Zealand J. Crop Hort. Sci. 17:9-18.
236. Hardacre, AK; Greer, DH, 1989. Differences in growth in response to temperature of maize hybrids varying in low temperature tolerance. Aust. J. Plant Physiol. 16:181-188.
237. Harris, MJ; DeMason, DA, 1989. Comparative kernel structure of three endosperm mutants of *Zea mays* L. relating to seed viability and seedling vigor. Bot. Gaz. 150:50-62.
238. Hartings, H; Maddaloni, M; Lazzaroni, N; DiFonzo, N; Motto, M; Salamini, F; Thompson, R, 1989. The *O2* gene which regulates zein deposition in maize endosperm encodes a protein with structural homologies to transcriptional activators. EMBO J. 8:2795-2801.
239. Hartung, RC; Poneleit, CG; Cornelius, PL, 1989. Direct and correlated responses to selection for rate and duration of grain fill in maize. Crop Sci. 29:740-745.
240. Hauptli, H; Williams, S, 1988. Maize in vitro pollination with single pollen grains. Plant Sci. 58:231-237.

241. Hauptmann, RM; Ashraf, M; Vasil, V; Hannah, LC; Vasil, IK; Ferl, R, 1988. Promoter strength comparisons of maize shrunken-1 and alcohol dehydrogenase-1 and dehydrogenase-2 promoters in monocotyledonous and dicotyledonous species. Plant Physiol. 88:1063-1066.
242. Headrick, JM; Pataky, JK, 1989. Resistance to kernel infection by *Fusarium moniliforme* in inbred lines of sweet corn and the effect of infection on emergence. Plant Dis. 73:887-892.
243. Hehl, R; Baker, B, 1989. Induced transposition of *Ds* by a stable *Ac* in crosses of transgenic tobacco plants. Mol. Gen. Genet. 217:53-59.
244. Heinlein, M; Starlinger, P, 1989. Tissue- and cell-specific expression of the two sucrose synthase isoenzymes in developing maize kernels. Mol. Gen. Genet. 215:441-446.
245. Helentjaris, T, 1988. Use of RFLP analysis to identify genes involved in complex traits of agronomic importance. Pp. 27-30 in Genetic Improvements of Agriculturally Important Crops. R. T. Fraley, N. M. Frey and J. M. Schell, ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
246. Helms, TC; Hallauer, AR; Smith, OS, 1989. Genetic drift and selection evaluated from recurrent selection programs in maize. Crop Sci. 29:602-607.
247. Helms, TC; Hallauer, AR; Smith, OS, 1989. Genetic variability estimates in improved and nonimproved 'Iowa Stiff Stalk Synthetic' maize populations. Crop Sci. 29:959-962.
248. Hesse, T; Feldwisch, J; Balshusemann, D; Bauw, G; Puype, M; Vandekerckhove, J; Lobler, M; Klambt, D; Schell, J; Palme, K, 1989. Molecular cloning and structural analysis of a gene from *Zea mays* (L.) coding for a putative receptor for the plant hormone auxin. EMBO J. 8:2453-2461.
249. Hiatt, A, 1989. Polyamine synthesis in maize cell lines. Plant Physiol. 90:1378-1381.
250. Hibbard, BE; Bjostad, LB, 1989. Corn semiochemicals and their effects on insecticide efficacy and insecticide repellency toward Western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82:773-781.
251. Ho, THD; Sachs, MM, 1989. Stress-induced proteins - characterization and the regulation of their synthesis. Biochemistry of Plants 15:347-378.
252. Hoekstra, FA; Crowe, LM; Crowe, JH, 1989. Differential desiccation sensitivity of corn and *Pennisetum* pollen linked to their sucrose contents. Plant Cell Environ. 12:83-91.
253. Hoisington, DA; Coe, EH, Jr., 1989. Methods for correlating RFLP maps with conventional genetic and physical maps in maize. Pp. 19-24 in Current Communications in Molecular Biology - Development and Application of Molecular Markers to Problems in Plant Genetics. T. Helentjaris and B. Burr, ed., Cold Spring Harbor: Cold Spring Harbor Lab.
254. Hole, DJ; Smith, JD; Cobb, BG, 1989. Regulation of embryo dormancy by manipulation of abscisic acid in kernels and associated cob tissue of *Zea mays* L. cultured in vitro. Plant Physiol. 91:101-105.
255. Holley, RN; Goodman, MM, 1989. New sources of resistance to Southern corn leaf blight from tropical hybrid maize derivatives. Plant Dis. 73:562-564.
256. Holley, RN; Hamilton, PB; Goodman, MM, 1989. Evaluation of tropical maize germ plasm for resistance to kernel colonization by *Fusarium moniliforme*. Plant Dis. 73:578-580.
257. Horner, ES; Magloire, E; Morera, JA, 1989. Comparison of selection for S2 progeny vs. testcross performance for population improvement in maize. Crop Sci. 29:868-874.
258. Horowitz, NH, 1990. George Wells Beadle (1903-1989). Genetics 124:1-6.
259. Hsu, C-F, 1988. The development and utilization of maize germplasm in China. Pp. 87-90 in Crop Genetic Resources of East Asia. S. Suzuki, ed., Rome: Int. Board Plant Genet. Resources.
260. Huang, Y-C; Tseng, M-T; Hsieh, J-H, 1989. Preliminary study on the relation between unselected inbred and hybrid traits in maize. Acad. Sin. Inst. Bot. Monogr. Ser.:1-8.
261. Huang, Y-W; Dennis, ES, 1989. Factors influencing stable transformation of maize protoplasts by electroporation. Plant Cell Tissue Organ Cult. 18:281-296.
262. Hudspeth, RL; Grula, JW, 1989. Structure and expression of the maize gene encoding the phosphoenolpyruvate carboxylase isozyme involved in C4 photosynthesis. Plant Mol. Biol. 12:579-592.
263. Hunt, M; Newton, K, 1989. A molecular analysis of the NCS3 mitochondrial mutant. Curr. Topics Plant Biochem. Physiol. 8:269.
264. Iglesias, CA; Hallauer, AR, 1989. S2 recurrent selection in maize populations with exotic germplasm. Maydica 34:133-140.
265. Ikeda, M; Tulloch, AP; Hoffman, LL, 1989. A plant growth inhibitor from immature seeds of corn. Agr. Biol. Chem. 53:569-584.
266. Inohara, N; Shimomura, S; Fukui, T; Futai, M, 1989. Auxin-binding protein located in the endoplasmic reticulum of maize shoots: molecular cloning and complete primary structure. Proc. Nat. Acad. Sci. USA 86:3564-3568.
267. Iremiren, GO, 1989. Response of maize to trash burning and nitrogen fertilizer in a newly opened secondary forest. J. Agric. Sci. 113:207-210.
268. Irish, EE; Nelson, T, 1989. Sex determination in monoecious and dioecious plants. Plant Cell 1:737-744.
269. Isenhour, DJ; Wiseman, BR; Layton, RC, 1989. Enhanced predation by *Orius insidiosus* (Hemiptera: Anthocoridae) on larvae of *Heliothis zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) caused by prey feeding on resistant corn genotypes. Environ. Entomol. 18:418-422.
270. Ivanchenko, MG; Georgieva, EI, 1988. Electrophoretic heterogeneity of maize histones. Electrophoresis 9:812-815.
271. Ivanov, P; Zlatanova, J, 1989. Histone content in the cytoplasm of dry maize embryo. Fiziol. Rast. 15:22-27.
272. James, MG; Stadler, J, 1989. Molecular characterization of Mutator systems in maize embryogenic callus cultures indicates *Mu* element activity in vitro. Theor. Appl. Genet. 77:383-393.
273. Jenkins, CLD; Edwards, GE; Andrews, J, 1989. Reduction in chlorophyll content without a corresponding reduction in photosynthesis and carbon assimilation enzymes in yellow-green oil yellow mutants of maize. Photosynth. Res. 20:191-.
274. Jensen, SD; Williams, NE, 1989. Inbred corn line PHK29 (U. S. patent 4812600). Off. Gaz. U. S. Pat. Trademark Off. Pat. 1100:1095-1096.
275. Jin, Y-K; Bennetzen, JL, 1989. Structure and coding properties of *Bs1*, a maize retrovirus-like transposon. Proc. Nat. Acad. Sci. USA 86:6235-6239.
276. Johannessen, CL; Parker, AZ, 1989. Maize ears sculptured in 12th and 13th century A.D. India as indicators of pre-Columbian diffusion. Econ. Bot. 43:164-180.
277. Johannessen, S; Hastorf, CA, 1989. Corn and culture in Central Andean prehistory. Science 244:690-691.
278. Johns, MA; Babcock, MS; Fuerstenberg, SM; Fuerstenberg, SI; Freeling, M; Simpson, RB, 1989. An unusually compact retrotransposon in maize. Plant Mol. Biol. 12:633-642.

279. Johnson, B, 1989. The probability of selecting genetically superior S2 lines from a maize population. Maydica 34:5-14.
280. Johnson, J; Cobb, BG; Drew, MC, 1989. Hypoxic induction of anoxia tolerance in root tips of *Zea mays*. Plant Physiol. 91:837-841.
281. Johnson, MW, 1989. Registration of PA356, PA376 and PA891 parental lines of maize. Crop Sci. 29:1333-1334.
282. Johnson, SS; Geadelmann, JL, 1989. Influence of water stress on grain yield response to recurrent selection in maize. Crop Sci. 29:558-564.
283. Jondle, DJ; Coors, JG; Duke, SH, 1989. Maize leaf beta-1,3-glucanase activity in relation to resistance to *Exserohilum turcicum*. Can. J. Bot. 67:263-266.
284. Jones, AM; Lamerson, P; Venis, MA, 1989. Comparison of site-I auxin binding and a 22-kilodalton auxin-binding protein in maize. Planta 179:409-413.
285. Jones, JDG; Carland, FM; Maliga, P; Dooner, HK, 1989. Visual detection of transposition of the maize element activator (*Ac*) in tobacco seedlings. Science 244:204-207.
286. Juliano, BO; Gonzales, LA, 1986. Rice and corn technology and policy in the Philippines. Trans. Natl. Acad. Sci. Technol., Repub. Philippines 8:5-35.
287. Kahler, AL; Shaw, DV; Allard, RW, 1989. Nonrandom mating on tasseled and detasseled plants in an open pollinated population of maize. Maydica 34:15-21.
288. Kaiser, SAKM, 1987. Evaluation of maize genotypes to brown spot disease. Indian J. Plant Pathol. 5:198-202.
289. Kamali, K; Dicke, FF; Guthrie, WD, 1989. Resistance-susceptibility of maize genotypes to artificial infestations by twospotted spider mites. Crop Sci. 29:936-938.
290. Kaminskaya, LM; Kudzyelka, LI; Khatylyuva, LU, 1988. Stability parameters of interlinear maize hybrids as a function of their heterogeneity. Vyestsi Akad. Navuk BSSR Syer. Biyal. Navuk:14-19.
291. Kang, MS; Gorman, DP, 1989. Genotype X environment interaction in maize. Agron. J. 81:662-664.
292. Kang, MS; Lillehoj, EB; Marshall, JG; Hall, W, 1988. Preharvest aflatoxin levels in corn hybrid kernels in Louisiana. Cereal Res. Commun. 16:237-244.
293. Kang, MS; Pham, HN, 1989. Reply to interrelationships among and repeatability of several stability statistics estimated from international maize trials. Crop Sci. 29:1335.
294. Kang, MS; Zuber, MS, 1989. Combining ability for grain moisture, husk moisture, and maturity in maize with yellow and white endosperms. Crop Sci. 29:689-692.
295. Kasamo, K; Kagita, F; Arai, Y, 1989. Isolation of subunits of coupling factor 1 from maize and spinach chloroplasts and properties of combinations of subunits with ATPase activity. Plant Cell Physiol. 30:729-738.
296. Keifer, DW, 1989. Tolerance of corn (*Zea mays*) lines to clomazone. Weed Sci. 37:622-628.
297. Kelley, PM, 1989. Maize pyruvate decarboxylase messenger RNA is induced anaerobically. Plant Mol. Biol. 13:213-222.
298. Kennell, JC; Pring, DR, 1989. Initiation and processing of *atp6*, *T-urf13* and ORF221 transcripts from mitochondria of T cytoplasm maize. Mol. Gen. Genet. 216:16-24.
299. Kerhoas, C; Dumas, C, 1988. Pollen quality in *Zea mays* as a prerequisite for sperm cell isolation and pollen transformation. Pp. 97-104 in Plant Sperm Cells as Tools for Biotechnology. H. J. Wilms and C. J. Keljzer, ed., Wageningen: Pudoc.
300. Kevresan, S; Kandrac, J; Grujic, S, 1988. Auxin-induced changes in the population of translation products of maize mesocotyl polysomes in the cell-free system. Iugosl. Physiol. Pharm. Acta 24:343-349.
301. Khalifa, I; Drolsom, N, 1988. Combining ability for European corn borer resistance and three agronomic traits in maize. Maydica 33:247-259.
302. Kidd, AD; Francis, D; Bennett, MD, 1989. Replicon size and rate of DNA replication fork movement are correlated in grasses. Exp. Cell Res. 184:262-267.
303. Kim, S-K; Efron, Y; Fajemisin, JM; Buddenhagen, IW, 1989. Mode of gene action for resistance in maize to maize streak virus. Crop Sci. 29:890-894.
304. Kim, S-K; Guthrie, WD; Hallauer, AR; Russell, WA; Brewbaker, JL; Hong, CS, 1989. Evaluation of tropical and subtropical corn lines for resistance to second-generation European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 82:1245-1250.
305. Kim, S-K; Hallauer, AR; Guthrie, WD; Barry, D; Lamkey, KR; Hong, CS, 1989. Genetic resistance of tropical corn inbreds to second-generation European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 82:1207-1211.
306. Kim, SH; Terry, ME; Hoops, P; Dauwalder, M; Roux, SJ, 1988. Production and characterization of monoclonal antibodies to wall-localized peroxidases from corn seedlings. Plant Physiol. 88:1446-1453.
307. Kirihara, JA; Petri, JB; Messing, J, 1988. Isolation and sequence of a gene encoding a methionine-rich 10-kDa zein protein from maize. Gene 71:359-370.
308. Klein, TM; Kornstein, L; Sanford, JC; Fromm, ME, 1989. Genetic transformation of maize cells by particle bombardment. Plant Physiol. 91:440-444.
309. Klein, TM; Roth, BA; Fromm, ME, 1989. Advances in direct gene transfer into cereals. Genet. Eng. 11:13-31.
310. Klein, TM; Roth, BA; Fromm, ME, 1989. Regulation of anthocyanin biosynthetic genes introduced into intact maize tissues by microprojectiles. Proc. Nat. Acad. Sci. USA 86:6681-6685.
311. Klosgen, RB; Saedler, H; Weil, J-H, 1989. The amyloplast-targeting transit peptide of the waxy protein of maize also mediates protein transport in vitro into chloroplasts. Mol. Gen. Genet. 217:155-161.
312. Kniep, KR; Mason, SC, 1989. Kernel breakage and density of normal and opaque-2 maize grain as influenced by irrigation and nitrogen. Crop Sci. 29:158-163.
313. Kodrzycki, R; Boston, RS; Larkins, BA, 1989. The opaque-2 mutation of maize differentially reduces zein gene transcription. Plant Cell 1:105-114.
314. Kohler, R; Leuoth, A; Jeroch, H; Flachowsky, G; Gebhardt, G; Hielscher, H; Kappel, W, 1989. Studies of the content and the digestibility of cell wall substances in remaining plants of various maize hybrids. Arch. Tierernahr. 39:187-192.
315. Koinuma, K; Mochizuki, N, 1989. Field inoculation methods for evaluating the resistance to banded leaf and sheath blight (*Rhizoctonia solani*)in maize. Bull. Natl. Grassl. Res. Inst. :13-18.
316. Konstantinov, YM; Lutsenko, GN; Podsosonny, VA; Zykova, VV, 1989. Possible mechanism of DNA transcription disturbance in maize mitochondria under membrane lipid peroxidation. Fiziol. Biokhim. Kul't. Rast. 21:483-487.
317. Konstantinov, YM; Podsosonny, VA; Lutsenko, GN; Rivkin, MI, 1989. DNA synthesis in intact maize mitochondria treated by PBR bacterial vector plasmids. Biopolim. Kletka 5:98-102.
318. Kopyl'chuk, GP; Kostyshin, SS; Marchenko, MM, 1989. Phosphorylation of nuclear proteins in hybridization. Dokl. Akad. Nauk Ukr. SSR Ser. B Geol. Khim. Biol. Nauki:69-71.

319. Koranyi, P, 1989. Characterisation of maize (*Zea mays* L.) seed samples by the electrophoretic patterns of their protein monomers. Seed Sci. Technol. 17:153-160.
320. Koranyi, P, 1989. Simple purity checking of maize (*Zea mays* L.) lines and hybrids by protein monomer analysis. Seed Sci. Technol. 17:161-168.
321. Kostandi, SF; Geisler, G, 1989. Maize smut induced by *Ustilago maydis* (D.C.) Corda - reaction of maize hybrids and lines to smut disease. Z. Acker-Pflanzenbau 162:149-156.
322. Kotting, K; Hofner, W, 1988. The ontogenesis of *Zea mays* L. and their modification by plant growth regulators. Z. Acker-Pflanzenbau 161:221-233.
323. Kovacevic, V; Vujevic, S, 1989. Inheritance of ear-leaf magnesium uptake in maize plants. Magnesium Bull. 11:22-24.
324. Kovacs, G, 1989. Selection for cold hardiness in pollen populations of maize. Novenytermeles 38:9-14.
325. Kramer, V; Lahners, K; Back, E; Privalle, LS; Rothstein, S, 1989. Transient accumulation of nitrite reductase messenger RNA in maize following the addition of nitrate. Plant Physiol. 90:1214-1220.
326. Kriz, AL, 1989. Characterization of embryo globulins encoded by the maize *Glb* genes. Biochem. Genet. 27:239-252.
327. Kubica, S; Baluska, F; Gasparikova, O, 1989. Pattern of nucleic acids synthesis in the root apex of *Zea mays* L. Biologia 44:201-208.
328. Kuehnle, AR; Earle, ED, 1989. In vitro selection for methomyl resistance in cms-T maize. Theor. Appl. Genet. 78:672-682.
329. Kumar, H, 1988. Oviposition and larval behaviour of stalk-borer (*Chilo partellus*) on susceptible and resistant varieties of maize (*Zea mays*). Indian J. Agr. Sci. 58:918-921.
330. Kunze, R; Starlinger, P, 1989. The putative transposase of transposable element *Ac* from *Zea mays* L. interacts with subterminal sequences of *Ac*. EMBO J. 8:3177-3185.
331. Lambert, RJ, 1989. Registration of 3 yellow dent maize lines R225, R226, and R227. Crop Sci. 29:1586-1587.
332. Lambert, RJ, 1989. Registration of Rbs10 (S1 - Hs) C3 corn germplasm. Crop Sci. 29:1580.
333. Lander, ES; Botstein, D, 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.
334. Landi, P; Frascaroli, E, 1988. Pollen-style interaction in *Zea mays* L. Pp. 315-320 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
335. Landi, P; Frascaroli, E; Tuberosa, R; Conti, S, 1989. Comparison between responses to gametophytic and sporophytic recurrent selection in maize (*Zea mays* L.). Theor. Appl. Genet. 77:761-767.
336. Landi, P; Vicari, A; Catizone, P, 1989. Response of maize (*Zea mays* L.) inbred lines and hybrids to chlorsulfuron. Weed Res. 29:265-272.
337. Langdale, JA; Lane, B; Freeling, M; Nelson, T, 1989. Cell lineage analysis of maize bundle sheath and mesophyll cells. Develop. Biol. 133:128-139.
338. Larkin, JC; Hunsperger, JP; Culley, D; Rubenstein, I; Silflow, CD, 1989. The organization and expression of a maize ribosomal protein gene family. Gene. Develop. 3:500-509.
339. Larkins, BA; Lending, CR; Wallace, JC; Galili, G; Kawata, EE; Geetha, KB; Kriz, AL; Martin, DN; Bracker, CE, 1989. Zein gene expression during maize endosperm development. Pp. 109-120 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss.
340. Larque-Saavedra, A; Rodriguez, MT, 1989. Evidences for maternal inheritance of abscisic acid in relation to drought tolerance in *Zea mays* L. Phyton 49:145-150.
341. Larson, RL, 1988. Genetics, precursors and enzymes in flavonoid biosynthesis in maize. Pp. 71-76 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
342. Larson, RL, 1989. Flavonoid 3'-0-methylation by a *Zea mays* L. preparation. Biochem. Physiol. Pflanz. 184:453-460.
343. Laurie, DA, 1989. Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv. Highbury X *Zea mays* cv. Seneca-60. Z. Pflanzenzucht. 103:133-140.
344. Law, MD; Moyer, JW; Payne, GA, 1989. Effect of host resistance on pathogenesis of maize dwarf mosaic virus. Phytopathology 79:757-761.
345. Lazanyi, J, 1988. Variation in the R3 generation of CHI-31 maize inbred line after in vitro regeneration of immature embryos. Cereal Res. Commun. 16:251-257.
346. Lazarowitz, SG; Pinder, AJ; Damsteegt, VD; Rogers, SG, 1989. Maize streak virus genes essential for systemic spread and symptom development. EMBO J. 8:1023-1032.
347. Lazarowitz, SG; Pinder, AJ, 1989. Molecular genetics of maize streak virus. Pp. 167-184 in Molecular Biology of Plant-Pathogen Interactions. B. Staskawicz, P. Ahlquist and O. Yoder, ed., New York: Alan R. Liss.
348. Leblova, S; Malik, M; Fojta, M, 1989. Isolation and characterization of maize pyruvate decarboxylase. Biologia 4:329-338.
349. Lechelt, C; Peterson, T; Laird, A; Chen, JC; Dellaporta, SL; Dennis, E; Peacock, WJ; Starlinger, P, 1989. Isolation and molecular analysis of the maize *P*-locus. Mol. Gen. Genet. 219:225-234.
350. Lecommandeur, D; Daussant, J, 1989. Polymorphism in maize, oats and sorghum alpha-amylases. Phytochemistry 28:2921-2925.
351. Lee, M; Godshalk, EB; Lamkey, KR; Woodman, WW, 1989. Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. Crop Sci. 29:1067-1071.
352. Lee, T-C, Wu, H-P.; Lu, H-S; Liu, K-S; Ho, C-L; Shieh, G-J, 1989. Quantitative inheritance of vegetative growth and kernel growth characteristics in maize. Acad. Sin. Inst. Bot. Monogr. Ser.:29-46.
353. Leisner, SM; Gelvin, SB, 1989. Multiple domains exist within the upstream activator sequence of the octopine synthase gene. Plant Cell 1:925-936.
354. Lemke-Keyes, CA; Sachs, MM, 1989. Anaerobic tolerant null - a mutant that allows *Adh1* nulls to survive anaerobic treatment. J. Hered. 80:316-319.
355. Lending, CR; Larkins, BA, 1989. Changes in the zein composition of protein bodies during maize endosperm development. Plant Cell 1:1011-1023.
356. Leon, P; Walbot, V; Bedinger, P, 1989. Molecular analysis of the linear 2.3 kb plasmid of maize mitochondria: Apparent capture of tRNA genes. Nucl. Acid. Res. 17:4089-4100.
357. Leonardi, A; Damerval, C; DeVienne, D, 1988. Organ-specific variability and inheritance of maize proteins revealed by two-dimensional electrophoresis. Genet. Res. 52:97-103.
358. Levings, CS, III; Brown, GG, 1989. Molecular biology of plant mitochondria. Cell 56:171-180.
359. Leznicki, AJ; Bandurski, RS, 1988. Enzymic synthesis of indole-3-acetyl-1-0-beta-d-glucose. 1. Partial purification and characterization of the enzyme from *Zea mays*. Plant Physiol. 88:1474-1480.

360. Lin, CS, 1989. Interrelationships among and repeatability of several stability statistics estimated from international maize trials. Crop Sci. 29:1334.
361. Lin, K, 1989. Studies on the resistance of corn inbred lines and hybrids to maize dwarf mosaic virus strain B. Sci. Agric. Sin. 22:57-61.
362. Liu, J, 1988. Diallel analysis of peroxidase isozyme in maize. Acta Genet. Sin. 15:265-269.
363. Lonsdale, DM, 1989. The plant mitochondrial genome. Biochemistry of Plants 15:229-295.
364. Ludwig, SR; Habera, LF; Dellaporta, SL; Wessler, SR, 1989. *Lc*, a member of the maize *R* gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region. Proc. Nat. Acad. Sci. USA 86:7092-7096.
365. Lupotto, E; Lusardi, MC, 1988. Secondary somatic embryogenesis from regenerating plantlets of the inbred line B79 of maize (*Zea mays* L.). Switch from type 1 to type 2 callus and effect on the regenerative potential. Maydica 33:163-177.
366. Lynch, J; Polito, VS; Lauchli, A, 1989. Salinity stress increases cytoplasmic Ca activity in maize root protoplasts. Plant Physiol. 90:1271-1274.
367. Lyznik, LA; Kamo, KK; Grimes, HD; Ryan, R; Chang, K-L; Hodges, TK, 1989. Stable transformation of maize: the impact of feeder cells on protoplast growth and transformation efficiency. Plant Cell Rep. 8:292-295.
368. Lyznik, LA; Ryan, RD; Ritchie, SW; Hodges, TK, 1989. Stable co-transformation of maize protoplasts with *gusA* and *neo* genes. Plant Mol. Biol. 13:151-161.
369. Lyznik, LA; Tsai, CY, 1989. Protein synthesis in endosperm cell cultures of maize (*Zea mays* L.). Plant Sci. 63:105-114.
370. Maas, C; Werr, W, 1989. Mechanism and optimized conditions for PEG mediated DNA transfection into plant protoplasts. Plant Cell Rep. 8:148-151.
371. Maas, SJ; Dunlap, JR, 1989. Reflectance, transmittance, and absorptance of light by normal, etiolated, and albino corn leaves. Agron. J. 81:105-110.
372. Macmillan, J; Phinney, BO, 1987. Biochemical genetics and the regulation of stem elongation by gibberellins. Pp. 156-171 in Physiology of Cell Expansion During Plant Growth, D. J. Gosgrove and D. P. Knievel, ed., Rockville: Amer. Soc. Plant Physiol.
373. Maddaloni, M; DiFonzo, N; Hartings, H; Lazzaroni, N; Salamini, F; Thompson, R; Motto, M, 1989. The sequence of the zein regulatory gene opaque-2 (*O2*) of *Zea mays*. Nucl. Acid. Res. 17:7532.
374. Magnavaca, R; eGama, EEG; Bahia Filho, AFC; Fernandes, FT, 1988. Double-cross hybrid selection for aluminum tolerance. Pesqui. Agropecu. Bras. 23:970-978.
375. Magnavaca, R; Oliveira, AC; Morais, AR; eGama, EEG; dosSantos, MX, 1989. Family hybrid selection of quality protein maize (*Zea mays* L.). Maydica 34:63-71.
376. Magnavaca, R; Paiva, E; Winkler, EI; Carvalho, HWLD; Silva Filho, MDC; Peixoto, MJVVD, 1988. Evaluation of high quality protein maize populations. Pesqui. Agropecu. Bras. 23:1263-1268.
377. Maguire, MP, 1989. Nuclear compression at premeiotic mitosis: implications for homologue pairing. Cytologia 54:37-44.
378. Maloney, AP; Traynor, PL; Levings, CS, III; Walbot, V, 1989. Identification in maize mitochondrial 26S rRNA of a short 5'-end sequence possibly involved in transcription initiation and processing. Curr. Genet. 15:207-212.
379. Manzocchi, LA; Bianchi, MW; Viotti, A, 1989. Expression of zein in long term cultures of wildtype and opaque-2 maize endosperms. Plant Cell Rep. 7:639-643.
380. Marchenko, MM; Oplachko, LT; Kostyshin, SS; Yazlovitskaya, LS, 1989. Characteristics of protein synthesis on endogenous messenger RNAs in cell-free systems from germs of different maize forms. Biopolim. Kletka 5:103-106.
381. Marlow, GC; Wurst, DE; Loschke, DC, 1988. The use of ultrathin-layer polyacrylamide gel isoelectric focusing in two-dimensional analysis of plant and fungal proteins. Electrophoresis 9:693-704.
382. Marocco, A; Wissenbach, M; Becker, D; Paz-Ares, J; Saedler, H; Salamini, F; Rohde, W, 1989. Multiple genes are transcribed in *Hordeum vulgare* and *Zea mays* that carry the DNA binding domain of the myb oncoproteins. Mol. Gen. Genet. 216:183-187.
383. Marquez-Sanchez, F; Gomez-Montiel, N, 1988. Seed increase of half-sib families in the modified ear-to-row method of selection in maize with additional genetic gain. Maydica 33:223-226.
384. Martienssen, RA; Barkan, A; Freeling, M; Taylor, WC, 1989. Molecular cloning of a maize gene involved in photosynthetic membrane organization that is regulated by Robertson's Mutator. EMBO J. 8:1633-1639.
385. Martinez, P; Martin, W; Cerff, R, 1989. Structure, evolution and anaerobic regulation of a nuclear gene encoding cytosolic glyceraldehyde-3-phosphate dehydrogenase from maize. J. Mol. Biol. 208:551-566.
386. Mascarenhas, JP, 1988. Anther- and pollen-expressed genes. Pp. 97-115 in Temporal and Spatial Regulation of Plant Genes. D. P. S. Verma and R. B. Goldberg, ed., New York: Springer-Verlag Wien.
387. Mascarenhas, JP, 1989. Characterization of genes that are expressed in pollen. Pp. 99-105 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss Inc.
388. Mascarenhas, JP, 1989. The isolation and expression of pollen-expressed genes. Curr. Sci. 58:1008-1015.
389. Mascarenhas, JP, 1989. The male gametophyte of flowering plants. Plant Cell 1:657-664.
390. Mascarenhas, JP; Hamilton, DA; Bashe, DM, 1989. Genes expressed during pollen development. Pp. 108-113 in Plant Reproduction: From Floral Induction to Pollination. E. Lord and G. Bernier, ed., Amer. Soc. Plant Physiol.
391. Masson, P; Banks, J; Surosky, R; Kingsbury, J; Fedoroff, N, 1987. Structure and regulation of the maize Suppressor-mutator transposable element. Pp. 589-597 in Plant Molecular Biology. D. v. Wettstein and N.-H. Chua, ed., New York: Plenum Press.
392. Masson, P; Fedoroff, NV, 1989. Mobility of the maize Suppressor-mutator element in transgenic tobacco cells. Proc. Nat. Acad. Sci. USA 86:2219-2223.
393. Masson, P; Rutherford, G; Banks, JA; Fedoroff, N, 1989. Essential large transcripts of the maize *Spm* transposable element are generated by alternative splicing. Cell 58:755-765.
394. Mather, DE; Kannenberg, LW, 1988. Expected responses to ratio-based selection for maize grain yield and maturity. Maydica 33:227-232.
395. Matsuoka, M; Minami, E, 1989. Complete structure of the gene for phosphoenolpyruvate carboxylase from maize. Eur. J. Biochem. 18:593-598.
396. Matthys-Rochon, E; Dumas, C, 1988. The male germ unit - retrospect and prospects. Pp. 51-60 in Plant Sperm Cells as Tools for Biotechnology. H. J. Wilms and C. J. Keljzer, ed., Wageningen: Pudoc.
397. McCabe, DE; Platt, SG; Paau, AS, 1989. Biological inoculant for corn (U. S. patent 4828600). Off. Gaz. U. S. Pat. Trademark Off. Pat. 1102:1082.

398. McCarty, DR; Carson, CB; Stinard, PS; Robertson, DS, 1989. Molecular analysis of viviparous-1: an abscisic acid-insensitive mutant of maize. Plant Cell 1:523-532.
399. McDaniel, CN; Poethig, RS, 1989. From here to there in maize - a fate map of the shoot apical meristem of the germinating corn embryo. Pp. 3-11 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss.
400. McMullen, MD; Louie, R, 1989. The linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. Mol. Plant-Microbe Interact. 2:309-314.
401. Meagher, RB; Berry-Lowe, S; Rice, K, 1989. Molecular evolution of the small subunit of ribulose bisphosphate carboxylase: nucleotide substitution and gene conversion. Genetics 123:845-863.
402. Meints, RH; Hu, W; Schuster, A; Timothy, DH; Levings, CS, 1989. Electron microscopy study of the plasmid-like DNAs of maize mitochondria. Maydica 34:197-205.
403. Melis, M; Rijkenberg, FHJ, 1988. Moisture stress in the screening of maize cultivars for stalk rot resistance and yield. Plant Dis. 72:1061-1063.
404. Metzler, MC; Rothermel, BA; Nelson, T, 1989. Maize NADP-malate dehydrogenase: cDNA cloning, sequence, and mRNA characterization. Plant Mol. Biol. 12:713-722.
405. Miernyk, JA, 1989. Extracellular secretion of an endoprotease by maize endosperm cultures. Curr. Topics Plant Biochem. Physiol. 8:308.
406. Milic, V; Saric, MR, 1988. Efficacy of *Azotobacter* in dependence of maize genotype and nitrogen content in nutritive solution. Mikrobiologija 25:45-56.
407. Mino, M; Inoue, M, 1988. Hybrid vigor in relation to lipid and protein metabolism in germinating maize kernels. Jpn. J. Breed. 38:428-436.
408. Mino, M; Inoue, M, 1989. DNA synthesis and nuclease activity during germination of a heterotic F1 hybrid of maize. Can. J. Bot. 67:73-75.
409. Misevic, D, 1989. Evaluation of three test statistics used to identify maize inbred lines with new favorable alleles not present in elite single cross. Theor. Appl. Genet. 77:402-408.
410. Misevic, D, 1989. Identification of inbred lines as a source of new alleles for improvement of elite maize single crosses. Crop Sci. 29:1120-1125.
411. Misevic, D; Alexander, DE, 1989. 24 cycles of phenotypic recurrent selection for percent oil in maize. 1. Per se and testcross performance. Crop Sci. 29:320-324.
412. Misevic, D; Dumanovic, J, 1989. Examination of methods for choosing locations for preliminary maize yield testing. Euphytica 44:173-179.
413. Misevic, D; Maric, A; Alexander, DE; Dumanovic, J; Ratkovic, S, 1989. Population cross diallel among high oil populations of maize. Crop Sci. 29:613-617.
414. Mitchell, JC; Petolino, JF, 1988. Heat stress effects on isolated reproductive organs of maize. J. Plant Physiol. 133:625-628.
415. Moentono, MD, 1988. Evaluation of corn inbred lines for high combining abilities for grain yield and root lodging resistance. Indones. J. Crop Sci. 3:23-34.
416. Moore, R; McClelen, CE, 1989. Characterizing pathways by which gravitropic effectors could move from the root cap to the root of primary roots of *Zea mays*. Ann. Bot. 64:415-423.
417. Morel, JL; Bitton, G; Chaudhry, GR; Awong, J, 1989. Fate of genetically modified microorganisms in the corn rhizosphere. Curr. Microbiol. 18:355-360.
418. Morera, JA, 1989. Comparison of 2 breeding methods in corn. 1. Effect of breeding method on combining ability of 3rd-cycle lines. Turrialba 39:63-68.
419. Morera, JA, 1989. Comparison of 2 breeding methods in corn. 2. Determination of inbreeding depression. Turrialba 39:68-71.
420. Mortenson, E; Dreyfuss, G, 1989. RNP in maize protein. Nature 337:312.
421. Morton, CM; Lawson, DL; Bedinger, P, 1989. Morphological study of the maize male sterile mutant *ms7*. Maydica 34:239-245.
422. Moskaleva, OV; Polevoy, VV, 1989. Effect of plant hormones on the growth of isolated organs of maize seedlings as dependent on their age. Fiziol. Biokhim. Kult. Rast. 21:278-285.
423. Motto, M; Thompson, R; DiFonzo, N; Ponziani, G; Soave, C; Maddaloni, M; Hartings, H; Salamini, F, 1989. Transpositional ability of the *Bg-rbg* system of maize transposable elements. Maydica 34:107-122.
424. Mourad, G; Polacco, M; Skogen-Hagenson, MJ; Morris, D; Robertson, D, 1989. A maternally inherited mutant of *Zea mays* L. lacks the cytochrome b/f complex. Curr. Genet. 16:109-116.
425. Moustafa, RAK; Duncan, DR; Widholm, JM, 1989. The effect of gamma radiation and N-ethyl-N-nitrosourea on cultured maize callus growth and plant regeneration. Plant Cell Tissue Organ Cult. 17:121-132.
426. Mulligan, RM; Leon, P; Calvin, N; Walbot, V, 1989. Introduction of DNA into maize and rice mitochondria by electroporation. Maydica 34:207-216.
427. Nandal, DPS; Agarwal, SK, 1989. Response of winter maize (Zea-mays) to sowing date, irrigation and nitrogen levels in North-West India. Indian Journal of Agricultural Sciences 59:629-633.
428. Napier, RM; Venis, MA; Bolton, MA; Richardson, LI; Butcher, GW, 1988. Preparation and characterisation of monoclonal and polyclonal antibodies to maize membrane auxin-binding protein. Planta 176:519-526.
429. Naskidashvili, PP; Dzhindzhikhadze, ZP; Koridze, NR, 1988. Inheritance of the vegetation period duration character in F1 F2 and backcrossing BC1 BC3 generations during hybridization on early and late-maturing forms of maize. Soobshch. Akad. Nauk Gruz. SSR 132:349-352.
430. Nelson, OE, 1990. Quantitative changes in maize loci induced by transposable elements. Pp. 116-127 in Plant Population Genetics, Breeding, and Genetic Resources. A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, ed., Sunderland, MA: Sinauer Assoc.
431. Nelson, T; Langdale, J, 1989. Differentiation of bundle sheath and mesophyll cells in C4 leaves depends on light and vein placement. Curr. Topics Plant Biochem. Physiol. 8:251-260.
432. Nelson, T; Langdale, JA, 1989. Patterns of leaf development in C4 plants. Plant Cell 1:3-13.
433. Nemchinov, LG; Buzhoryanu, VV; Tertyak, DD, 1988. Effect of dwarf mosaic virus on the ultrastructure of maize anthers. Izv. Akad. Nauk Mold. SSR Ser. Biol. Khim. Nauk:19-24.
434. Nevado, ME; Cross, HZ; Johnson, KM, 1989. Combining ability related to reduced gene flow among maturity groups within maize populations. Crop Sci. 29:928-932.
435. Newton, KJ; Coe, EH; Gabay-Laughnan, S; Laughnan, JR, 1989. Abnormal growth phenotypes and mitochondrial mutations in maize. Maydica 34:291-296.

436. Ngernprasirtsiri, J; Chollet, R; Kobayashi, H; Sugiyama, T; Akazawa, T, 1989. DNA methylation and the differential expression of C4 photosynthesis genes in mesophyll and bundle sheath cells of greening maize leaves. J. Biol. Chem. 264:8241-8248.
437. Ninomya, Y; Okuno, K; Glover, DV; Fuwa, H, 1989. Some properties of starches of sugary-1; brittle-1 maize (*Zea mays* L.). Starke 41:165-166.
438. Norton, DC, 1989. Host efficiencies of *Zea diploperennis* and *Z. perennis* for *Pratylenchus* spp. J. Nematol. 21:547-548.
439. Nyhus, KA; Russell, WA; Guthrie, WD, 1988. Response of two maize synthetics to recurrent selection for resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and *Diplodia* stalk rot. J. Econ. Entomol. 81:1792-1798.
440. Nyhus, KA; Russell, WA; Guthrie, WD, 1989. Changes in agronomic traits associated with recurrent selection in 2 maize synthetics. Crop Sci. 29:269-275.
441. Nyhus, KA; Russell, WA; Guthrie, WD, 1989. Distributions among S1 lines for European corn borer (Lepidoptera: Pyralidae) and stalk rot resistance ratings in two maize synthetics improved by recurrent selection. J. Econ. Entomol. 82:239-245.
442. Nyhus, KA; Russell, WA; Guthrie, WD; Martinson, CA, 1989. Reaction of two maize synthetics to anthracnose stalk rot and Northern corn leaf blight following recurrent selection for resistance to *Diplodia* stalk rot and European corn borer. Phytopathology 79:166-169.
443. O'Brien, C; Zabala, G; Walbot, V, 1989. Integrated R2 sequence in mitochondria of fertile B37N maize encodes and expresses a 130 kD polypeptide similar to that encoded by the S2 episome of S-type male sterile plants. Nucl. Acid. Res. 17:405-422.
444. Oard, JH; Paige, D; Dvorak, J, 1989. Chimeric gene expression using maize intron in cultured cells of breadwheat. Plant Cell Rep. 8:156-160.
445. Odhiambo, MO; Compton, WA, 1989. 5 cycles of replicated S1 vs. reciprocal full-sib index selection in maize. Crop Sci. 29:314-319.
446. Onenanyoli, AHA; Fasoulas, AC, 1989. Yield response to honeycomb selection in maize. Euphytica 40:43-48.
447. Ordas, A; DeRon, AM, 1988. A method to measure conicalness in maize. Maydica 33:261-267.
448. Osterman, JC; Dennis, ES, 1989. Molecular analysis of the *Adh1-Cm* allele of maize. Plant Mol. Biol. 13:203-212.
449. Ottaviano, E; Gorla, MS; Frova, C; Pe, E, 1988. Male gametophytic selection in higher plants. Pp. 35-42 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
450. Ottaviano, E; Mulcahy, DL, 1989. Genetics of angiosperm pollen. Adv. Genet. 26:1-64.
451. Ozias-Akins, P; Dujardin, M; Hanna, WW; Vasil, IK, 1989. Quantitative variation recovered from tissue cultures of an apomictic, interspecific *Pennisetum* hybrid. Maydica 34:123-132.
452. Padgette, SR; Dellacioppa, G; Shah, DM; Fraley, RT; Kishore, GM, 1989. Selective herbicide tolerance through protein engineering. Pp. 441-476 in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes. J. Schell and I. K. Vasil, ed., San Diego: Academic Press.
453. Palmer, JD; Jansen, RK; Michaels, HJ; Chase, MW; Manhart, JR, 1988. Chloroplast DNA variation and plant phylogeny. Ann. Missouri Bot. Gard. 75:1180-1206.
454. Palmer, PG; Gerbeth-Jones, S, 1988. A scanning electron microscope survey of the epidermis of East African grasses V. and West African supplement. Smithson. Contrib. Bot.:1-157.
455. Pan, YB; Peterson, PA, 1989. Tagging of a maize gene involved in kernel development by an activated *Uq* transposable element. Mol. Gen. Genet. 219:324-327.
456. Pareddy, DR; Greyson, RI, 1989. Effects of amino acids on the development of spikelets in cultured tassels of *Zea mays*. Can. J. Bot. 67:1331-1335.
457. Pareddy, DR; Greyson, RI, 1989. Studies on sucrose requirements of cultured maize tassels. Can. J. Bot. 67:225-229.
458. Pareddy, DR; Greyson, RI; Walden, DB, 1989. Production of normal, germinable and viable pollen from in vitro-cultured maize tassels. Theor. Appl. Genet. 77:521-526.
459. Pareddy, DR; Petolino, JF, 1989. Tassel culture of elite inbreds of maize. Crop Sci. 29:1564-1566.
460. Pataky, JK; Headrick, JM, 1989. Management of common rust on sweet corn with resistance and fungicides. J. Prod. Agric. 2:362-369.
461. Paul, AL; Ferl, RJ, 1989. Chromatin structure and gene expression. Pp. 355-370 in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes. J. Schell and I. K. Vasil, ed., San Diego: Academic Press.
462. Paulsen, MR; Wigger, WD; Litchfield, JB; Sinclair, JB, 1989. Computer image analyses for detection of maize and soybean kernel quality factors. J. Agric. Eng. Res. 43:93-101.
463. Payne, GA; Thompson, DL; Lillehoj, EB; Zuber, MS; Adkins, CR, 1988. Effect of temperature on the preharvest infection of maize kernels by *Aspergillus flavus*. Phytopathology 78:1376-1380.
464. Peplinski, AJ; Paulsen, MR; Anderson, RA; Kwolek, WF, 1989. Physical, chemical, and dry-milling characteristics of corn hybrids from various genotypes. Cereal Chem. 66:117-120.
465. Pepo, P; Pasztor, K; Palij, AF; Pepo, P, 1989. Genetical analysis of maize mutant lines. Novenytermeles 38:193-200.
466. Pereira, A; Saedler, H, 1989. Transpositional behavior of the maize *En/Spm* element in transgenic tobacco. EMBO J. 8:1315-1321.
467. Pereira, MB; Vencovsky, R, 1989. Limits in recurrent selection: I Factors affecting the increment in allelic frequencies. Pesqui. Agropecu. Bras. 23:769-780.
468. Pereira, OAP; Balmer, E; Miranda Filho, JB, 1989. Inheritance of resistance to stalk rot, caused by *Colletotrichum graminicola* (CES.) Wils, in maize (*Zea mays* L.). Rev. Brasil. Genet. 12:53-65.
469. Perrot, GH; Cone, KC, 1989. Nucleotide sequence of the maize *R-S* gene. Nucl. Acid. Res. 17:8003.
470. Perrot-Rechenmann, C; Joannes, M; Squalli, D; Lebacq, P, 1989. Detection of phosphoenolpyruvate and ribulose 1,5-bisphosphate carboxylase transcripts in maize leaves by in situ hybridization with sulfonated cDNA probes. J. Histochem. Cytochem. 37:423-428.
471. Pescitelli, SM; Mitchell, JC; Jones, AM; Pareddy, DR; Petolino, JF, 1989. High frequency androgenesis from isolated microspores of maize. Plant Cell Rep. 7:673-676.
472. Peterson, PA, 1988. The evolutionary role of mobile element transpositions in plants. Pp. 31-47 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
473. Peterson, SS; Scriber, JM; Coors, JG, 1988. Silica, cellulose and their interactive effects on the feeding performance of the Southern armyworm *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). J. Kansas Entomol. Soc. 6:169-177.
474. Petrovic, R; Kerecki, B; Sataric, I, 1988. Variation of root length plant height and content of microelements under stress temperature in corn (*Zea mays* L.). Acta Biol. Med. Exp. 13:97-100.

475. Pfahler, PL; Barnett, RD, 1988. Forced pollen shedding effects on pollen diameter and early seedling growth in maize. Pp. 271-276 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
476. Pietraface, WJ; Elliott, WM, 1989. Culture of albino corn and sorghum. Phyton 49:31-34.
477. Pinter, L; Schmidt, J; Kelemen, G; Szabo, J; Henics, Z, 1988. Complex evaluation of different corn genotypes for CCM (Corn-Cob-Mix) use. Maydica 33:283-294.
478. Pinto, LRM; daSilva, JC; Silva, MdA; Sediyama, CS, 1989. Estimation of inbreeding depression and grain yield of lines by the analyses of means of a diallel among eight corn varieties (*Zea mays* L.). Rev. Brasil. Genet. 12:67-80.
479. Pla, M; Goday, A; Vilardell, J; Gomez, J; Pages, M, 1989. Differential regulation of ABA-induced 23-25 kDa proteins in embryo and vegetative tissues of the viviparous mutants of maize. Plant Mol. Biol. 13:385-394.
480. Planckaert, F; Walbot, V, 1989. Molecular and genetic characterization of *Mu* transposable elements in *Zea mays* - behavior in callus culture and regenerated plants. Genetics 123:567-578.
481. Planckaert, F; Walbot, V, 1989. Transient gene expression after electroporation of protoplasts derived from embryogenic maize callus. Plant Cell Rep. 8:144-147.
482. Poethig, S, 1989. Clonal analysis of the cell lineage of the maize leaf. Curr. Topics Plant Biochem. Physiol. 8:261-264.
483. Poethig, S, 1989. Genetic modifiers of heterochronic mutations in maize. Pp. 25-36 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss.
484. Pollak, LM; Pham, HN, 1989. Classification of maize testing locations in Sub-Saharan Africa by using agroclimatic data. Maydica 34:43-51.
485. Pollak, LM; Thomas-Compton, MA, 1989. Charles O. Gardner: An appreciation. Maydica 34:1-3.
486. Poneleit, CG; Evans, KO; Green, RC, 1989. Registration of KyWS1, KyWS2, KyWS3, KyWS4, KyWS5, and KyWS6 maize germplasms. Crop Sci. 29:1580-1581.
487. Power, AG, 1988. Leafhopper response to genetically diverse maize stands. Entomol. Exp. Appl. 49:213-220.
488. Prasad, R; Singh, S; Paroda, RS, 1988. Combining ability analysis in a maize diallel. Indian J. Genet. Plant Breed. 48:19-24.
489. Prasad, TK; Hallberg, RL, 1989. Identification and metabolic characterization of the *Zea mays* mitochondrial homolog of the *Escherichia coli* gro-el protein. Plant Mol. Biol. 12:609-618.
490. Pressey, R; Reger, BJ, 1988. Polygalacturonase in pollen from corn and other grasses. Plant Sci. 59:57-62.
491. Price, CA, 1989. George Wells Beadle, 1903-1989. Plant Mol. Biol. Rep. 7:180-181.
492. Price, CA, 1989. Nomenclature for cloned plant genes. Plant Mol. Biol. Rep. 7:99-103.
493. Pring, DR; Lonsdale, DM, 1989. Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize. Annu. Rev. Phytopathol. 27:483-504.
494. Prioli, LM; daSilva, WJ, 1989. Somatic embryogenesis and plant regeneration capacity in tropical maize inbreds. Rev. Bras. Genet. 12:553-566.
495. Prioli, LM; Sondahl, MR, 1989. Plant regeneration and recovery of fertile plants from protoplasts of maize (*Zea mays* L.). Bio/Technology 7:589-594.
496. Privalle, LS; Lahners, KN; Mullins, MA; Rothstein, S, 1989. Nitrate effects on nitrate reductase activity and nitrite reductase messenger RNA levels in maize suspension cultures. Plant Physiol. 90:962-967.
497. Pylneva, PN, 1989. Physiological and biochemical characteristics of high-lysine corn seed. Fiziol. Biokhim. Kult. Rast. 21:211-217.
498. Quang, PD; Szundy, T, 1989. Some features of cold hardiness in S2 families of maize. Novenytermeles 38:15-20.
499. Quayle, TJA; Brown, JWS; Feix, G, 1989. Analysis of distal flanking regions of maize 19-kDa zein genes. Gene 80:249-257.
500. Quigley, F; Brinkmann, H; Martin, WF; Cerff, R, 1989. Strong functional GC pressure in a light-regulated maize gene encoding subunit GAPA of chloroplast glyceraldehyde-3-phosphate dehydrogenase: Implications for the evolution of GAPA pseudogenes. J. Mol. Evol. 29:412-421.
501. Raboy, V; Below, FE; Dickinson, DB, 1989. Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. J. Hered. 80:311-315.
502. Raboy, V; Kim, H-Y; Schiefelbein, JW; Nelson, OE, Jr., 1989. Deletions in a *dSpm* insert in a maize bronze-1 allele alter RNA processing and gene expression. Genetics 122:695-703.
503. Racchi, ML; Gavazzi, GA, 1988. Light and germination as promoters of pigment accumulation in maize seed tissues: role of *r-ch:Hopi*. Pp. 123-132 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
504. Ramos, JMV; Lopez, S; Vazquez, E; Murillo, E, 1988. DNA integrity and DNA polymerase activity in deteriorated maize embryo axes. J. Plant Physiol. 133:600-604.
505. Rao, KV; Suprasanna, P; Reddy, GM, 1988. Biochemical characterization of color-inhibitor in maize anthocyanin biosynthesis. Indian J. Exp. Biol. 26:929-931.
506. Rao, KV; Suprasanna, P; Reddy, GM, 1989. Enzyme and isozyme patterns in embryogenic glume derived calli of *Zea mays* L. Proc. Indian Nat. Sci. Acad. (Part B) 55:177-180.
507. Reddy, GM, 1988. Gene action studies in biosynthesis of flavonoids and related pigments in maize. Pp. 133-137 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
508. Reddy, PRR; Kumar, RS; Satyanarayana, E; Sarma, MY, 1988. A simple and rapid method of predicting the best double cross hybrids in maize (*Zea mays* L.). Maydica 33:213-222.
509. Rees, CAB; Gullons, AM; Walden, DB, 1989. Heat shock protein synthesis induced by methomyl in maize (*Zea mays* L.) seedlings. Plant Physiol. 90:1256-1261.
510. Reid, CD; Lampman, RL, 1989. Olfactory responses of *Orius insidiosus* (Hemiptera: Anthocoridae) to volatiles of corn silks. J. Chem. Ecol. 15:1109-1116.
511. Rhodes, C, 1989. Corn - from protoplasts to fertile plants. Bio/Technology 7:548.
512. Rhodes, D; Rich, PJ; Brunk, DG; Ju, GC; Rhodes, JC; Pauly, MH; Hansen, LA, 1989. Development of 2 isogenic sweet corn hybrids differing for glycinebetaine content. Plant Physiol. 91:1112-1121.
513. Riedell, WE, 1989. Western corn rootworm damage in maize - greenhouse technique and plant response. Crop Sci. 29:412-415.
514. Ritchings, BW; Tracy, WF, 1989. Day length, temperature, and the expression of the corngrass gene in maize (*Zea mays* L.). J. Hered. 80:324-326.
515. Robbins, TP; Chen, J; Norell, MA; Dellaporta, SL, 1988. Molecular and genetic analysis of the *R* and *B* loci in maize. Pp. 105-113 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
516. Roberts, JKM; Chang, K; Webster, C; Callis, J; Walbot, V, 1989. Dependence of ethanolic fermentation, cytoplasmic pH regulation, and viability on the activity of alcohol dehydrogenase in hypoxic maize root tips. Plant Physiol. 89:1275-1278.

517. Rocheford, TR; Bridges, WC, Jr.; Johnson, BE; Gardner, CO, 1989. Evaluation of normal and dwarf subpopulations extracted from an exotic x adapted cross of maize. Maydica 34:33-41.
518. Rocheford, TR; Gardner, CO; Vidaver, AK, 1989. Genetic studies of resistance in maize (*Zea mays* L.) to Goss's bacterial wilt and blight (*Clavibacter michiganense* ssp. *nebraskense*). J. Hered. 80:351-356.
519. Rocher, JP, 1988. Comparison of carbohydrate compartmentation in relation to photosynthesis, assimilate export and growth in a range of maize genotypes. Aust. J. Plant Physiol. 15:677-686.
520. Rocher, JP; Prioul, JL; Lecharny, A; Reyss, A; Joussaume, M, 1989. Genetic variability in carbon fixation, sucrose-P-synthase and ADP glucose pyrophosphorylase in maize plants of differing growth rate. Plant Physiol. 89:416-420.
521. Roeckel, P; Dupuis, I; Detchepare, S; Matthys-Rochon, E; Dumas, C, 1989. Isolation and viability of sperm cells from corn (*Zea mays*) and kale (*Brassica oleracea*) pollen grains. Pp. 105-110 in Plant Sperm Cells as Tools for Biotechnology. H. J. Wilms and C. J. Keljzer, ed., Wageningen: Pudoc.
522. Rogachenko, AD; Gulyaev, BI; Pryadkina, GA; Ferents, AF, 1988. Specific surface density of maize hybrid leaves in sowings. Fiziol. Biokhim. Kul't. Rast. 20:327-330.
523. Rollo, F; Amici, A; Salvi, R; Garbuglia, AR, 1989. Characterization of a mitochondrial and a nuclear maize DNA sequence by polymerase chain reaction: potential for use as molecular probes. J. Genet. Breed. 43:91-98.
524. Romanova, IM; Lysikov, VN, 1989. Effect of superhigh doses of gamma-irradiation of the pollen on maize genetic variability. Izv. Akad. Nauk Mold. SSR Ser. Biol. Khim. Nauk:32-35.
525. Rothermel, BA; Nelson, T, 1989. Primary structure of the maize NADP-dependent malic enzyme. J. Biol. Chem. 264:19587-19592.
526. Rowland, LJ; Chen, Y-C; Chourey, PS, 1989. Anaerobic treatment alters the cell specific expression of *Adh-1*, *Sh*, and *Sus* genes in roots of maize seedlings. Mol. Gen. Genet. 218:33-40.
527. Rowland, LJ; Robertson, DS; Strommer, J, 1989. Chromosome breakage undetectable in active *Mu* lines of maize. Genetics 122:205-210.
528. Rusche, ML, 1988. The male germ unit of *Zea mays* in the mature pollen grain. Pp. 61-68 in Plant Sperm Cells as Tools for Biotechnology. H. J. Wilms and C. J. Keljzer, ed., Wageningen: Pudoc.
529. Rusche, ML; Mogensen, HL, 1988. The male germ unit of *Zea mays* - quantitative ultrastructure and 3-dimensional analysis. Pp. 221-225 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
530. Russell, DA; Sachs, MM, 1989. Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. Plant Cell 1:793-803.
531. Russell, WA, 1989. Registration of B90 and B91 parental inbred lines of maize. Crop Sci. 29:1101-1102.
532. Saab, IN; Pritchard, J; Voetberg, GS; Sharp, RE, 1989. Increased endogenous abscisic acid is required for root growth at low water potentials. Curr. Topics Plant Biochem. Physiol. 8:281.
533. Saccomani, M; Ferrari, G, 1989. Sulfate influx and efflux in seedlings of maize genotypes of different productivity. Maydica 34:171-177.
534. Sadykov, AS; Mukhamedov, RS; Tuneev, VM; Gizatullin, RZ; Abdukarimov, AA, 1988. Identification of sequences homologous to the mobile element of maize in the nuclear and chloroplast genomes of cotton. Biopolim. Kletka 4:218-220.
535. Sampoux, JP; Gallais, A; Lefort-Buson, M, 1989. Combining forage and grain tests for both forage and grain maize breeding. Agronomie 9:667-676.
536. Sampoux, JP; Gallais, A; Lefort-Buson, M, 1989. S1 value combined with topcross value for forage maize selection. Agronomie 9:511-520.
537. Sanchez de Jimenez, E; Vargas, M; Aguilar, R; Jimenez, E, 1988. Age-dependent responsiveness to cell differentiation stimulus in maize callus culture. Plant Physiol. Biochem. 26:723-732.
538. Sander, KW; Barrett, M, 1989. Differential imazaquin tolerance and behavior in selected corn (*Zea mays*) hybrids. Weed Sci. 37:290-295.
539. Sangare, A; Lonsdale, D; Weil, J-H; Grienenberger, J-M, 1989. Sequence analysis of the *tRNATyr* and *tRNALys* genes and evidence for the transcription of a chloroplast-like *tRNAMet* in maize mitochondria. Curr. Genet. 16:195-202.
540. Sangare, A; Weil, J-H; Grienenberger, J-M, 1989. Nucleotide sequence of a maize mitochondrial *tRNAGlu* (UUC) gene. Nucl. Acid. Res. 17:5837.
541. Sangare, A; Weil, J-H; Grienenberger, J-M, 1989. Nucleotide sequence of a maize mitochondrial $tRNA^{Ser}$ (UGA) gene. Nucl. Acid. Res. 17:7979.
542. Sano, H; Kamada, I; Youssefian, S; Wabiko, H, 1989. Correlation between DNA undermethylation and dwarfism in maize. Biochim. Biophys. Acta 1009:35-38.
543. Sanou, J; Sauvaire, D, 1988. Selection of maize varieties adapted to a semi-intensive level of crop management in Burkina Faso. Agron. Trop. 43:99-105.
544. Saric, Z; Saric, M; Govedarica, M; Stankovic, Z., 1987. Efficiency of *Azotobacter* strains depending on maize genotype and nitrogen level. Mikrobiologija 24:95-106.
545. Sasakawa, H; Sugiyama, T, 1989. Contents of carbon-assimilating enzymes in leaves of maize hybrids released between 1936 and 1976. Soil Sci. Plant Nutr. 35:161-169.
546. Saugy, M; Mayor, G; Pilet, PE, 1989. Endogenous ABA in growing maize roots - light effects. Plant Physiol. 89:622-627.
547. Savich, IM, 1988. Ambiguity of the effect of urea on isoperoxidase activity in various maize strains. Izv. Akad. Nauk Kaz. SSR Ser. Biol.:29-34.
548. Schapendonk, AHCM; Dolstra, O; Van Kooten, O, 1989. The use of chlorophyll fluorescence as a screening method for cold tolerance in maize. Photosyn. Res. 20:235-247.
549. Schmidt, DH; Tracy, WF, 1989. Duration of imbibition affects seed leachate conductivity in sweet corn. Hortscience 24:346-347.
550. Schmitz, ML; Maier, U-G; Brown, JWS; Feix, G, 1989. Specific binding of nuclear proteins to the promoter region of a maize nuclear rRNA gene unit. J. Biol. Chem. 264:1467-1472.
551. Schnabl, H; Weissenbock, G; Sachs, G; Scharf, H, 1989. Cellular distribution of UV-absorbing compounds in guard and subsidiary cells of *Zea mays* L. J. Plant Physiol. 135:249-252.
552. Schnable, PS; Peterson, PA, 1989. Genetic evidence of a relationship between two maize transposable element systems: *Cy* and Mutator. Mol. Gen. Genet. 215:317-321.
553. Schnable, PS; Peterson, PA; Saedler, H, 1989. The *bz-rcy* allele of the *Cy* transposable element system of *Zea mays* contains a *Mu*-like element insertion. Mol. Gen. Genet. 217:459-463.

554. Schnell, FW, 1988. Quantitative genetics in crop improvement. Pp. 462-464 in Proc. 2nd Int. Conf. Quant. Genet., Univ. North Carolina, Raleigh, NC; B. S. Weir, E. J. Eisen, M. M. Goodman and G. Namkoong, ed., Sunderland, MA: Sinauer Assoc., Inc.
555. Schwartz, D, 1989. Gene-controlled cytosine demethylation in the promoter region of the *Ac* transposable element in maize. Proc. Nat. Acad. Sci. USA 86:2789-2793.
556. Schwartz, D, 1989. Pattern of *Ac* transposition in maize. Genetics 121:125-128.
557. Schy, WE; Plewa, MJ, 1989. Molecular dosimetry studies of forward mutation induced at the *yg2* locus in maize by ethyl methanesulfonate. Mutat. Res. 211:231-242.
558. Scott, GE, 1989. Linkage between maize dwarf mosaic virus resistance and endosperm color in maize. Crop Sci. 29:1478-1480.
559. Scott, GE; Zummo, N, 1989. Effect of genes with slow-rusting characteristics on Southern Corn Rust in maize. Plant Dis. 73:114-116.
560. Segebart, RL, 1989. Inbred corn line PHV78 (U. S. patent 4812599). Off. Gaz. U. S. Pat. Trademark Off. Pat. 1100:1095
561. Setter, TL; Flannigan, BA, 1989. Relationship between photosynthate supply and endosperm development in maize. Ann. Bot. 64:481-487.
562. Shah, AA; Stegemann, H, 1989. Electrophoretic evaluation of inbred lines of maize. Biochem. Physiol. Pflanz. 184:293-302.
563. Shamina, NV, 1988. A study of genetic control of spindle formation and operation using mutation affecting chromosome segregation in male meiosis of maize. Tsitologiya 30:1301-1306.
564. Sharma, AK; Sarma, JSP, 1988. Chromosome structure rearrangements and genome relationship in Maydeae. Feddes Repert 99:291-337.
565. Sharma, ML; Mishra, DP, 1988. Effect of carbofuran on galactolipid and phospholipid contents of maize chloroplasts. Int. J. Trop. Agric. 6:117-124.
566. Shatters, RG, Jr.; Hekman, WE; Miernyk, JA, 1989. Construction of a plasmid encoding a maize "neosecretory" protein for use in molecular analysis of targeting signals. Curr. Topics Plant Biochem. Physiol. 8:309.
567. Shen, DL; Wu, M, 1989. Transmission electron microscopic study of maize pachytene chromosome-6. Stain Technol. 64:65-74.
568. Shevardnadze, GA; Goginashvili, KA, 1988. Correlation between some phenotypic characteristic of multiple ear maize. Soobshch. Akad. Nauk Gruz. SSR 131:597-600.
569. Shillito, RD; Carswell, GK; Johnson, CM; Dimaio, JJ; Harms, CT, 1989. Regeneration of fertile plants from protoplasts of elite inbred maize. Bio/technology 7:581-588.
570. Shotwell, MA; Larkins, BA, 1989. The biochemistry and molecular biology of seed storage proteins. Biochemistry of Plants 15:297-345.
571. Silvela, L; Rodgers, R; Barrera, A; Alexander, DE, 1989. Effect of selection intensity and population size on percent oil in maize, *Zea mays* L. Theor. Appl. Genet. 78:298-304.
572. Simeonov, N, 1989. Productivity of inter-subspecies hybrids of grain maize. Rasteniev'd. Nauki 26:3-7.
573. Sinclair, TR; Horie, T, 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency - a review. Crop Sci. 29:90-98.
574. Singletary, GW; Below, FE, 1989. Growth and composition of maize kernels cultured in vitro with varying supplies of carbon and nitrogen. Plant Physiol. 89:341-346.
575. Sisco, PH; Goodman, MM; Thompson, DL, 1989. Registration of NC264 parental line of maize. Crop Sci. 29:248.
576. Skadsen, RW; Scandalios, JG, 1989. Pretranslational control of the levels of glyoxysomal protein gene expression by the embryonic axis in maize. Develop. Genetics 10:1-10.
577. Small, I; Suffolk, R; Leaver, CJ, 1989. Evolution of plant mitochondrial genomes via substoichiometric intermediates. Cell 58:69-76.
578. Smith, JSC, 1988. Identification of pedigrees of hybrid maize (*Zea mays* L.) cultivars by isozyme electrophoresis and reversed-phase high-performance liquid chromatography. Euphytica 39:199-205.
579. Smith, JSC, 1989. The characterization and assessment of genetic diversity among maize (*Zea mays* L.) hybrids that are widely grown in France - chromatographic data and isozymic data. Euphytica 43:73-85.
580. Smith, JSC; Smith, OS, 1989. The description and assessment of distance between inbred lines of maize: I. The use of morphological traits as descriptors. Maydica 34:141-150.
581. Smith, JSC; Smith, OS, 1989. The description and assessment of distances between inbred lines of maize: II. The utility of morphological, biochemical, and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34:151-161.
582. Sockness, BA; Dudley, JW, 1989. Morphology and yield of isogenic diploid and tetraploid maize inbreds and hybrids. Crop Sci. 29:1029-1032.
583. Sockness, BA; Dudley, JW, 1989. Performance of single and double cross autotetraploid maize hybrids with different levels of inbreeding. Crop Sci. 29:875-879.
584. Solis, C; Sanchez de Jimenez, E; Loyola-Vargas, VM; Carabez, A; Lotina-Hennsen, B, 1989. The biogenesis of chloroplasts in tissue cultures of a C-3 and a C-4 plant. Plant Cell Physiol. 3:609-616.
585. Sotchenko, VS; Zaganmandzhiev, NL; Frolov, AN, 1989. A diallel analysis of corn barrenness as an index of resistance to stress conditions. Genetika 25:941-945.
586. Southworth, D; Platt-Aloia, KA; Thomson, WW, 1988. Freeze fracture of sperm and vegetative cells in *Zea mays* pollen. J. Ultrastruct. Mol. Struct. Res. 101:165-172.
587. Souza, CL, Jr.; Miranda, JBF, 1989. Genetic variability in two maize (*Zea mays* L.) populations and its relationship with intra- and interpopulation recurrent selection. Rev. Brasil. Genetica 12:271-286.
588. Spike, BP; Tollefson, JJ, 1988. Western corn rootworm (Coleoptera: Chrysomelidae) larval survival and damage potential to corn subjected to nitrogen and plant density treatments. J. Econ. Entomol. 81:1450-1455.
589. Spike, BP; Tollefson, JJ, 1989. Relationship of plant phenology to corn yield loss resulting from Western corn rootworm (Coleoptera: Chrysomelidae) larval injury, nitrogen deficiency, and high plant density. J. Econ. Entomol. 82:226-231.
590. Springer, PS; Zimmer, EA; Bennetzen, JL, 1989. Genomic organization of the ribosomal DNA of sorghum and its close relatives. Theor. Appl. Genet. 77:844-850.
591. Stadler, J; Phillips, R; Leonard, M, 1989. Mitotic blocking agents for suspension cultures of maize 'Black Mexican Sweet' cell lines. Genome 32:475-478.
592. Starlinger, P; Coupland, G; Fusswinkel, H; Heinlein, M; Kunze, R; Laufs, J; Li, M; Stochaj, U; Baker, B; Schell, J; Both, C; Lee, J; Oellig, C; Doerfler, W; Schwartz, D, 1989. Studies on transposable element *Ac* in *Zea mays*. Pp. 65-78 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss.

593. Steinmuller, K; Ley, AC; Steinmetz, AA; Sayre, RT; Bogorad, L, 1989. Characterization of the *ndh*C-*psb*G-ORF157/159 operon of maize plastid DNA and of the cyanobacterium *Synechocystis* sp. PCC6803. Mol. Gen. Genet. 216:60-69.
594. Stevanovic, M, 1988. Agrotechnical measures significant for utilization of maize genetic potential. Agrohemija:333-342
595. Stoilov, L; Mirkova, V; Zlatanova, J, 1989. Transcriptional activity and DNA supercoiling during early germination in maize. Plant Sci. 63:59-66.
596. Stoilov, M; Kisova, B, 1989. Some results and problems of applying experimental mutagenesis in maize breeding. Genet. Sel. 22:85-90.
597. Stromberg, LD; Compton, WA, 1989. 10 cycles of full-sib selection in maize. Crop Sci. 29:1170-1172.
598. Stuber, CW, 1990. Molecular markers in the manipulation of quantitative characters. Pp. 334-350 in Plant Population Genetics, Breeding, and Genetic Resources. A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, ed., Sunderland, MA: Sinauer Assoc.
599. Styles, DE; Gavazzi, GA; Racchi, ML, 1988. The Genetics of Flavonoids. Milan: Edizioni Unicopli.
600. Styles, ED; Ceska, O, 1989. Pericarp flavonoids in genetic strains of *Zea mays*. Maydica 34:227-238.
601. Sudjana, A, 1988. Six cycles of selection for early flowering in maize (*Zea mays* L.). Indones. J. Crop Sci. 3:35-42.
602. Suenaga, K; Nakajima, K, 1989. Efficient production of haploid wheat (*Triticum aestivum*) through crosses between Japanese wheat and maize (*Zea mays*). Plant Cell Rep. 8:263-266.
603. Sullivan, TD; Christensen, AH; Quail, PH, 1989. Isolation and characterization of a maize chlorophyll a/b binding protein gene that produces high levels of mRNA in the dark. Mol. Gen. Genet. 215:431-440.
604. Sun, CS; Prioli, LM; Sondahl, MR, 1989. Regeneration of haploid and dihaploid plants from protoplasts of supersweet (*sh2 sh2*) corn. Plant Cell Rep. 8:313-316.
605. Suparyono; Pataky, JK, 1989. Influence of host resistance and growth stage at the time of inoculation on Stewart's Wilt and Goss's Wilt development and sweet corn hybrid yield. Plant Dis. 73:339-344.
606. Suparyono; Pataky, JK, 1989. Relationships between incidence and severity of Stewart's and Goss's bacterial wilts and yield of sweet corn hybrids. Crop Protection 8:363-368.
607. Suprasanna, P; Rao, KV; Reddy, GM, 1989. Effect of 2,4-D analogues on callus cultures in maize. Curr. Sci. 58:35-37.
608. Suprasanna, P; Rao, KV; Reddy, GM, 1989. Effect of growth regulators on anthocyanin synthesis in maize endosperms cultured in vitro. Biol. Plant. 31:177-181.
609. Suprasanna, P; Rao, KV; Reddy, GM, 1989. Genetically controlled anthocyanin synthesis in callus cultures of *Zea mays* L. Proc. Indian Acad. Sci.-Plant Sci. 99:293-295.
610. Suprasanna, P; Rao, KV; Reddy, GM, 1989. Hydroxylation pattern of certain intermediates in anthocyanin biosynthesis in maize. Indian J. Exp. Biol. 27:917-918.
611. Sylvester, AW; Cande, WZ; Freeling, M, 1989. Ligule formation during maize leaf development: changes in cell shape and microtubule orientation in wild type and developmental mutants. Curr. Topics Plant Biochem. Physiol. 8:298.
612. Szundy, T, 1989. Phenotypic variability of some traits of maize hybrids obtained from heterozygote strains. Novenytermeles 38:201-208.
613. Takeda, C; Takeda, Y; Hizukuri, S, 1989. Structure of amylomaize amylose. Cereal Chem. 66:22-25.
614. Talbert, LE; Patterson, GI; Chandler, VL, 1989. *Mu* transposable elements are structurally diverse and distributed throughout the genus *Zea*. J. Mol. Evol. 29:28-39.
615. Taliercio, EW; Chourey, PS, 1989. Post-transcriptional control of sucrose synthase expression in anaerobic seedlings of maize. Plant Physiol. 90:1359-1364.
616. Tarasyuk, AN, 1989. Reduction of homologous pairing of telomere chromosome arm segments in maize meiosis as affected by high temperature. Tsitol. Genet. 23:8-14.
617. Tauer, LW; Love, J, 1989. The potential economic impact of herbicide-resistant corn in the USA. J. Prod. Agric. 2:202-207.
618. Taylor, BH; Finnegan, EJ; Dennis, ES; Peacock, WJ, 1989. The maize transposable element *Ac* excises in progeny of transformed tobacco. Plant Mol. Biol. 13:109-120.
619. Taylor, LP; Briggs, WR, 1988. Genetic regulation and photocontrol of anthocyanin synthesis during maize seedling development. Pp. 147-148 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
620. Taylor, PA; Hopkins, WG, 1989. Characterization of chloroplasts in a tinged (*tn*) mutant of maize (*Zea mays* L.). J. Plant Physiol. 134:81-84.
621. Taylor, WC, 1989. Regulatory interactions between nuclear and plastid genomes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:211-233.
622. Taylor, WC, 1989. Transcriptional regulation by a circadian rhythm. Plant Cell 1:259-264.
623. Teyker, RH; Moll, RH; Jackson, WA, 1989. Divergent selection among maize seedlings for nitrate uptake. Crop Sci. 29:879-884.
624. Thakur, RP; Leonard, KJ; Jones, RK, 1989. Characterization of a new race of *Exserohilum turcicum* virulent on corn with resistance gene *HtN*. Plant Dis. 73:151-155.
625. Thakur, RP; Leonard, KJ; Leath, S, 1989. Effects of temperature and light on virulence of *Exserohilum turcicum* on corn. Phytopathology 79:631-635.
626. Thakur, RP; Leonard, KJ; Pataky, JK, 1989. Smut gall development in adult corn plants inoculated with *Ustilago maydis*. Plant Dis. 73:921-925.
627. Theres, N; Schmitz, G; Scheele, T; Starlinger, P, 1988. The *Bz2* locus in maize: cloning and transcription studies. Pp. 97-104 in The Genetics of Flavonoids. D. E. Styles, G. A. Gavazzi and M. L. Racchi, ed., Milan: Edizioni Unicopli.
628. Thompson, GA; Larkins, BA, 1989. Structural elements regulating zein gene expression. BioEssays 10:108-113.
629. Tiba, SD; Frean, ML, 1989. A comparative study of the structure-function relationship of cross veins in leaves of *Digitaria eriantha* and *Zea mays*. Ann. Bot. 63:433-439.
630. Tillmann, U; Viola, G; Kayser, B; Siemeister, G; Hesse, T; Palme, K; Lobler, M; Klambt, D, 1989. cDNA clones of the auxin-binding protein from corn coleoptiles (*Zea mays* L.): Isolation and characterization by immunological methods. EMBO J. 8:2463-2467.
631. Timothy, DH; Harvey, PH; Dowswell, CR, 1988. Development and spread of improved maize varieties and hybrids in developing countries. Washington: Agency Int. Devel.
632. Tipping, PW; Cornelius, PL; Legg, DE; Poneleit, CG; Rodriguez, JG, 1989. Inheritance of resistance in whole kernel maize to oviposition by the maize weevil (Coleoptera, Curculionidae). J. Econ. Entomol. 82:1466-1469.

633. Titok, VV; Razumovich, AN; Khotylyova, LV, 1989. Features of integral parameters' inheritance of energy metabolism in seedlings of hybrid maize forms. Genetika 25:1223-1229.
634. Tollenaar, M, 1989. Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. Crop Sci. 29:1365-1371.
635. Tonelli, C; Salvi, C; Gavazzi, G; Longo, GPM; Rossi, G; Longo, C, 1989. Benzyladenine partially restores the normal phenotype in a chlorophyll mutant of maize. J. Plant Physiol. 133:713-718.
636. Tracy, WF; Juvik, JA, 1989. Pericarp thickness of a shrunken-2 population of maize selected for improved field emergence. Crop Sci. 29:72-74.
637. Tragesser, SL; Youngquist, WC; Smith, OS; Compton, WA, 1989. Drift vs. selection effects from five recurrent selection programs in maize. Maydica 34:23-32.
638. Traore, M; Sullivan, CY; Rosowski, JR; Lee, KW, 1989. Comparative leaf surface morphology and the glossy characteristic of sorghum, maize, and pearl millet. Ann. Bot. 64:447-453.
639. Tsay, H-S, 1989. Factors affecting haploid plant regeneration from maize anther culture. Acad. Sin. Inst. Bot. Monogr. Ser.:157-166.
640. Tyson, RH; Rees, TA, 1988. Failure to detect UDP-glucose phosphorylase in the developing endosperm of maize and wheat. Plant Sci. 59:71-76.
641. Ulger, AC; Becker, HC, 1989. Influence of year and nitrogen treatment on the degree of heterosis in maize. Maydica 34:163-170.
642. Urrelo, R; Wright, VF, 1989. Development and behavior of immature stages of the maize weevil (Coleoptera, Curculionidae) within kernels of resistant and susceptible maize. Ann. Entomol. Soc. Amer. 82:712-716.
643. Urrelo, R; Wright, VF, 1989. Influence of susceptible and resistant maize accessions on the development of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) with initial feeding in specific kernel areas. J. Kansas Entomol. Soc. 62:32-43.
644. Urrelo, R; Wright, VF, 1989. Oviposition performance of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) on resistant and susceptible maize accessions. J. Kansas Entomol. Soc. 62:23-31.
645. Urrelo, R; Wright, VV; Mills, RB; Wassom, CE, 1989. Screening for resistance against the maize weevil *Sitophilus zeamais* (Motsch) (Coleoptera, Curculionidae) in Peruvian maize accessions. Turrialba 39:9-17.
646. Vain, P; Yean, H; Flament, P, 1989. Enhancement of production and regeneration of embryogenic type II callus in *Zea mays* L. by AgNO3. Plant Cell Tissue Organ Cult. 18:143-152.
647. Vakili, NG; Bailey, TB, 1989. Yield response of corn hybrids and inbred lines to phylloplane treatment with mycopathogenic fungi. Crop Sci. 29:183-190.
648. VanSluys, MA; Tempe, J, 1989. Behavior of the maize transposable element Activator in *Daucus carota*. Mol. Gen. Genet. 219:313-319.
649. Vaughn, KC; Campbell, WH, 1988. Immunogold localization of nitrate reductase in maize leaves. Plant Physiol. 88:1354-1357.
650. Vencill, WK; Hatzios, KK; Wilson, HP, 1989. Growth and physiological responses of normal, dwarf, and albino corn (*Zea mays*) to clomazone treatments. Pesticide Bioch. Physiol. 35:81-88.
651. Vencovsky, R; Miranda Filho, JB; Souza, CL, Jr., 1988. Quantitative genetics and corn breeding in Brazil. Pp. 465-477 in Proc. 2nd Int. Conf. Quant. Genet., Univ. North Carolina, Raleigh, NC; B. S. Weir, E. J. Eisen, M. M. Goodman and G. Namkoong, ed., Sunderland, MA: Sinauer Assoc., Inc.
652. Verderio, A; Bertolini, M; Gentinetta, E; Lorenzoni, C; Maggiore, T; Motto, M, 1988. Recurrent selection for seed quality and agronomic traits in the DOo2 maize variety. Maydica 33:201-211.
653. Vereshchagina, AB; Gandrabur, SI, 1988. Behavioral reactions of cereal aphids fed on corn of varying resistance. Sov. J. Ecol. 19:98-101.
654. Vimla, B; Mukherjee, BK; Ahuja, VP, 1988. Combining ability analysis for resistance to banded leaf and sheath blight of maize. Indian J. Genet. Plant Breed. 48:75-80.
655. Vinh, DN, 1989. Factors affecting the formation and differentiation of embryogenic callus cultured in vitro of immature maize embryoids. Genet. Sel. 22:45-51.
656. Vinnichenko, AN; Fedenko, VS; Shupranova, LV; Shtemenko, NI; Sysoeva, MV, 1989. Isolation and biochemical characterization of opaque-2 maize zein. Fiziol. Biokhim. Kult. Rast. 21:388-392.
657. Vinnichenko, AN; Shtemenko, NI; Fedenko, VS; Tumanov, GN; Glushko, LP; Kashenkova, RV, 1988. Study of the lipid composition in the opaque-2 corn grain. Khim. Prir. Soedin:360-363.
658. Vishnyakov, AA; Kometiani, DG, 1988. Karyotypic characterization of some plants from the southern USSR. Dokl. Vses. Ordena Lenina Ordena Trud. Krasnogo Znameni Akad. S-Kh. Nauk Im V. I. Lenina:8-10.
659. Vodkin, MH; Vodkin, LO, 1989. A conserved zinc finger domain in higher plants. Plant Mol. Biol. 12:593-594.
660. Vozda, J; Kubecova, B, 1989. Performance assessment in maize hybrids in microenvironments and macroenvironments. Rostl. Vyroba 35:101-108.
661. Wadsworth, GJ; Scandalios, JG, 1989. Differential expression of the maize catalase genes during kernel development: The role of steady-state mRNA levels. Develop. Genet. 10:304-310.
662. Wagner, VT; Dumas, C; Mogensen, HL, 1989. Morphometric analysis of isolated *Zea mays* sperm. J. Cell Sci. 93:179-184.
663. Wagner, VT; Song, Y; Matthys-Rochon, E; Dumas, C, 1988. The isolated embryo sac of *Zea mays* - structural and ultrastructural observations. Pp. 125-130 in Sexual Reproduction in Higher Plants, E. Pacini, P. Gori and M. Cresti, ed., Berlin: Springer-Verlag.
664. Wagner, VT; Song, YC; Matthys-Rochon, E; Dumas, C, 1988. Observations on the isolated embryo sac of *Zea mays* L. Plant Sci. 59:127-132.
665. Walbot, V, 1989. Is anatomy destiny in plants. Pp. 37-50 in Molecular Basis of Plant Development. R. Goldberg, ed., New York: Alan R. Liss.
666. Walden, DB; Greyson, RI; Bommineni, VR; Pareddy, DR; Sanchez, JP; Banasikowska, E; Kudirka, DT, 1989. Maize meristem culture and recovery of mature plants. Maydica 34:263-275.
667. Wan, Y; Petolino, JF; Widholm, JM, 1989. Efficient production of doubled haploid plants through colchicine treatment of anther-derived maize callus. Theor. Appl. Genet. 77:889-892.
668. Wandelt, C; Feix, G, 1989. Sequence of a 21 kd zein gene from maize containing an in-frame stop codon. Nucl. Acid. Res. 17:2354.
669. Wang, H; Cutler, AJ; Saleem, M; Fowke, LC, 1989. DNA replication in maize leaf protoplasts. Plant Cell Tissue Organ Cult. 18:33-46.

670. Wang, H; Cutler, AJ; Saleem, M; Fowke, LC, 1989. Microtubules in maize leaf protoplasts in relation to donor tissue and in vitro culture. Protoplasma 150:48-53.
671. Wang, H; Cutler, AJ; Saleem, M; Fowke, LC, 1989. Microtubules in maize protoplasts derived from cell suspension cultures - effect of calcium and magnesium ions. Eur. J. Cell Biol. 49:80-86.
672. Wang, L, 1987. The rise of corn. Pp. 174-175 in Feeding a Billion; Frontiers of Chinese Agriculture. S. Wittwer, Y. Yu, H. Sun and L. Wang, ed., East Lansing: Michigan State Univ. Press.
673. Wang, Y; Gengenbach, B, 1989. Heterogeneity and changes in mitochondrial *atp*A genes among related cms-S cytoplasm lines of maize. Maydica 34:217-225.
674. Weber, D; Helentjaris, T, 1989. Mapping RFLP loci in maize using B-A translocations. Genetics 121:583-590.
675. Weir, BS; Eisen, EJ; Goodman, MM; Namkoong, G, 1988. Proceedings of the Second International Conference on Quantitative Genetics. Sunderland: Sinauer Associates.
676. Weissinger, A; Tomes, D; Maddock, S; Fromm, M; Sanford, J, 1988. Maize transformation via microprojectile bombardment. Pp. 21-26 in Genetic Improvements of Agriculturally Important Crops, R. T. Fraley, N. M. Frey, J. Schell, ed., Cold Spring Harbor: Cold Spring Harbor Lab.
677. Wendel, JF; Stuber, CW; Goodman, MM; Beckett, JB, 1989. Duplicated plastid and triplicated cytosolic isozymes of triosephosphate isomerase in maize (*Zea mays* L.). J. Hered. 80:218-228.
678. Wessler, SR, 1989. The splicing of maize transposable elements from pre-mRNA - a minireview. Gene 82:127-133.
679. Westgate, ME; Grant, DLT, 1989. Water deficits and reproduction in maize - response of the reproductive tissue to water deficits at anthesis and mid-grain fill. Plant Physiol. 91:862-867.
680. Westhoff, P; Grune, H; Schrubar, H; Oswald, A; Streubel, M; Ljungberg, U; Herrmann, RG, 1988. Mechanisms of plastid and nuclear gene expression during thylakoid membrane biogenesis in higher plants. Pp. 261-276 in Photosynthetic Light-Harvesting Systems: Organization and Function, H. Scheer and S. Schneider, ed., Berlin: Walter De Gruyter.
681. White, JA; Scandalios, JG, 1989. Deletion analysis of the maize mitochondrial superoxide dismutase transit peptide. Proc. Nat. Acad. Sci. USA 86:3534-3538.
682. White, RP; McRae, KB, 1989. Recent advances in maturity and yield of grain corn hybrids tested in the maritimes. Can. J. Plant Sci. 69:1259-1263.
683. Wicks, ZW, III; Carson, ML; Scholten, GG, 1989. Registration of SD44 parental line of maize. Crop Sci. 29:839-840.
684. Wicks, ZW, III; Carson, ML; Scholten, GG, 1989. Registration of SD46 parental line of maize. Crop Sci. 29:840.
685. Widstrom, NW; Bagby, MO; Carr, ME, 1989. Registration of GT-SSRS-SX and GT-SSRS-PX maize germplasms. Crop Sci. 29:243.
686. Wilkes, G, 1988. Intellectual property rights--plant patenting--potential impact on the seed industry. Proc. Annu. Farm Seed Conf. 34:10-24.
687. Wilkes, G, 1989. Germplasm preservation: Objectives and needs. Pp. 13-41 in Biotic Diversity and Germplasm Preservation, Global Imperatives, L. Knutson and A. K. Stoner, ed., Netherlands: Kluwer Academic Publ.
688. Wilkes, G, 1989. Maize: Domestication, racial evolution, and spread. Pp. 440-455 in Foraging and Farming: The Evolution of Plant Exploitation, D. R. Harris and G. C. Hillman, ed., London: Unwin Hyman.
689. Williams, RJ; Leopold, AC, 1989. The glassy state in corn embryos. Plant Physiol. 89:977-981.
690. Williams, WP; Buckley, PM; Davis, FM, 1989. Combining ability for resistance in corn to fall armyworm and Southwestern corn borer. Crop Sci. 29:913-915.
691. Williamson, JD; Galili, G; Larkins, BA; Gelvin, SB, 1988. The synthesis of a 19-kilodalton zein protein in transgenic petunia plants. Plant Physiol. 88:1002-1007.
692. Willman, MR; Schroll, SM; Hodges, TK, 1989. Inheritance of somatic embryogenesis and plantlet regeneration from primary (Type 1) callus in maize. In Vitro Cell Develop. Biol. 25:95-100.
693. Wilson, CM; Sprague, GF; Nelsen, TC, 1989. Linkages among zein genes determined by isoelectric focusing. Theor. Appl. Genet. 77:217-226.
694. Wilson, RL; Wiseman, BR, 1988. Field and laboratory evaluation of selected maize plant introductions for corn earworm responses at two locations. Maydica 33:179-187.
695. Windham, GL; Rhoades, WP, 1988. Reproduction of *Meloidogyne javanica* on corn hybrids and inbreds. Ann. Appl. Nematol. 2:25-28.
696. Wiseman, BR; Isenhour, DJ, 1989. Effects of temperature on development of corn earworm (Lepidoptera: Noctuidae) on meridic diets of resistant and susceptible corn silks. Environ. Entomol. 18:683-686.
697. Wolfe, KH; Gouy, M; Yang, Y-W; Sharp, PM; Li, W-H, 1989. Date of the monocot-dicot divergence estimated from chloroplast DNA sequence data. Proc. Nat. Acad. Sci. USA 86:6201-6205.
698. Wolswinkel, P; Ammerlaan, A, 1989. Effect of the osmotic environment on assimilate transport in isolated developing embryos of maize (*Zea mays*). Ann. Bot. 63:705-.
699. Wosnick, MA; Barnett, RW; Carlson, JE, 1989. Total chemical synthesis and expression in *Escherichia coli* of a maize glutathione transferase (GST) gene. Gene 76:153-160.
700. Wright, AD; Neuffer, MG, 1989. Orange pericarp in maize - filial expression in a maternal tissue. J. Hered. 80:229-233.
701. Wrischer, M, 1989. Ultrastructural localization of photosynthetic activity in thylakoids during chloroplast development in maize. Planta 177:18-23.
702. Wu, M, 1989. Applications of nucleic acid electron microscopy and in situ hybridization techniques in the study of plant genomes. Pp. 355-372 in Plant Biotechnology. S. D. Kung and C. J. Arntzen, ed., Stoneham: Butterworths.
703. Wu, Z, 1988. Study of eight agronomic characters in corn under two environments. Acta Agron. Sin. 14:39-45.
704. Xia, R; Liu, J, 1989. The cytological study of cytoplasmic male sterility in maize. Acta Agron. Sin. 15:97-103.
705. Xia, T; Liu, J, 1988. Study on the relation between cytoplasmic male sterility in maize and cyanide resistant respiration in tissues. Sci. Agric. Sin. 21:39-43.
706. Xie, Y; Dai, J, 1988. Study on identification of male sterile cytoplasms using analysis of mitochondrial DNA in maize. Acta Genet. Sin. 15:335-339.
707. Zaitlin, D; Hu, J; Bogorad, L, 1989. Binding and transcription of relaxed DNA templates by fractions of maize and chloroplast extracts. Proc. Nat. Acad. Sci. USA 86:876-880.
708. Zanoni, U; Dudley, JW, 1989. Comparison of different methods of identifying inbreds useful for improving elite maize hybrids. Crop Sci. 29:577-582.
709. Zanoni, U; Dudley, JW, 1989. Testcross evaluation of F2 populations from maize inbreds selected for unique favorable alleles. Crop Sci. 29:589-595.

710. Zanoni, U; Dudley, JW, 1989. Use of F2 generations to identify inbreds with favorable alleles not present in an elite hybrid. Crop Sci. 29:583-589.
711. Zhao, Z; Gu, M, 1988. Cytogenetic studies of chemically-induced parthenogenic maize plants. Acta Genet. Sin. 15:89-94.
712. Zhao, Z-Y; Weber, DF, 1989. Male gametophyte development in monosomics of maize. Genome 32:155-164.
713. Zimmerman, PA; Lang-Unnasch, N; Cullis, CA, 1989. Polymorphic regions in plant genomes detected by an M13 probe. Genome 32:824-828.
714. Zuber, MS; Lillehoj, EB, 1988. Aflatoxin contamination in maize and its biocontrol. Pp. 85-92 in Biocontrol of Plant Diseases. K. G. Mukerji and K. L. Garg, ed., Boca Raton: CRC Press.
715. Zummo, N; Scott, GE, 1989. Evaluation of field inoculation techniques for screening maize genotypes against kernel infection by *Aspergillus flavus* in Mississippi. Plant Dis. 73:313-316.

Quote Without Comment

The naming of the few

Until a few years ago, genes were quite properly the province of geneticists. The identification of a gene required the identification of a heritable character; a genetic locus required the segregation of that character from other known characters. Among higher plants, maize has been the best studied. By 1987 about 650 loci had been mapped in the nuclear genome of maize; this represents perhaps 5 percent of the total number of genes. Almost a fifth of the mapped loci affect the electrophoretic mobility of an enzyme (isozyme characters) and could, therefore, be structural genes for those proteins. A smaller number affect other biochemical characters, such as anthocyanin accumulation. Most of the remainder are morphological characters.

In the early days of maize genetics it was quite acceptable to identify a gene by one or two letters: *B* designated a character in which anthocyanin accumulated in major tissues; *bm*, was brown midrib; *d* was dwarf; *E* affected esterase; *o*, opaque endosperm; *su*, sugary. Lower case referred to a recessive gene, upper case to a dominant gene. When additional loci that produced the same phenotype were discovered, a number was added: d3, E8, *o2*, *su1*, etc. But one or two letters per gene limits one to 26 or 676 genes, respectively, so that a system in which genes are represented by one or two letters will run out of symbols. More recently maize geneticists agreed to use three letters (= 17,576 symbols), but the earlier designations were allowed to stand. Only a handful of the genetic loci in maize has been isolated from genomic libraries and sequenced.

--C. A. Price, 1989

Speaking of Terms:

Are the new orange factors (pericarp; plant) related to nehi corn?

VIII. SYMBOL INDEX

("r" refers to numbered references in the Recent Maize Publications section)

IX. AUTHOR AND NAME INDEX

("r" refers to numbered references in the Recent Maize Publications section)
(* identifies articles authored in this Newsletter)

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March, 1990

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LINE ANALYZED	ENZYME(S) TRIED	# BANDS SEEN	APPROX. MW

FOR THE MAIZE RFLP LABORATORY IT IS OPTIMAL TO RECEIVE 20-30 UG OF PLASMID AS A DRIED PELLET. IF POSSIBLE, WE WOULD ALSO LIKE TO RECEIVE STABS.

AMOUNT OF PURIFIED PLASMID SUPPLIED (10-100 ug): _____

VECTOR: _____ ENZYME(S) TO CUT OUT INSERT: _____

HOST OF SUPPLIED STAB CULTURE: _____ MEDIA: _____

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