MAIZE GENETICS COOPERATION

NEWSLETTER

76

May 15, 2002

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

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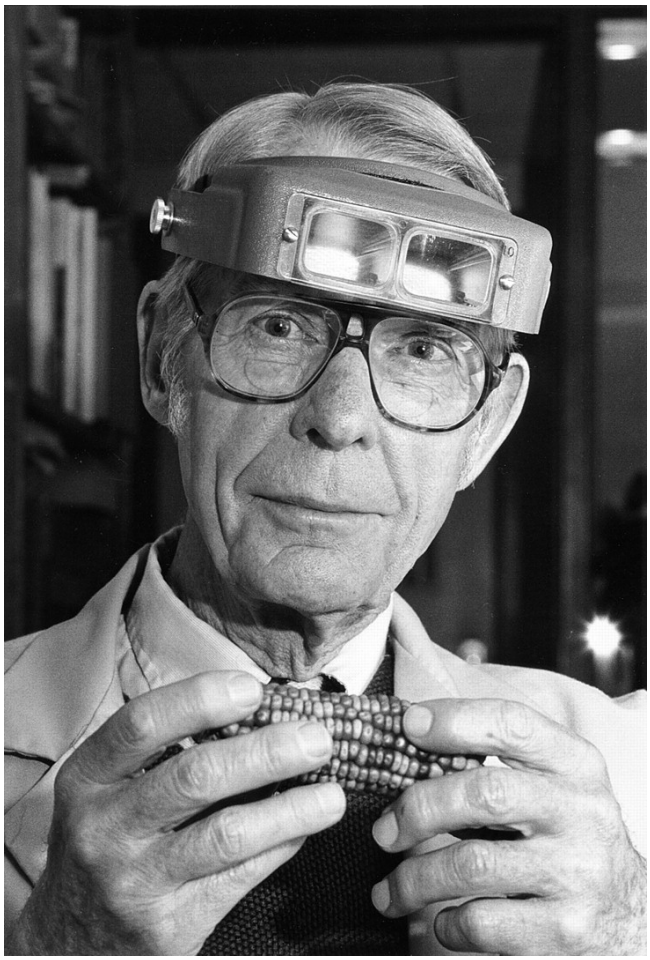
Oliver Evans Nelson, Jr. (August 16, 1920 – November 6, 2001)

--Hannah, C, Burr, B, Dooner, H
University of Florida, Brookhaven National Laboratory and Rutgers University



Noted maize geneticist Oliver Nelson of the University of Wisconsin died in a Madison hospital November 6, 2001 after a long battle with both osteoporosis and Parkinson's disease. Having become acutely ill that afternoon, Oliver refused transport to the hospital in order to finish the communication of a paper concerning enhanced starch synthesis to the Proceedings of the National Academy of Sciences. He died soon after reaching the hospital.

Born in Seattle, Washington, Oliver was raised in the vicinity of New Haven, Connecticut and first experienced maize genetics as a summer high school assistant in the Department of Genetics, Connecticut Agricultural Experiment Station. Oliver graduated from Colgate *magna cum laude* in Botany at the age of 21. He subsequently received his M.S. and Ph.D. degrees from Yale under the direction of D. F. Jones.

Oliver joined the faculty at Purdue University as an Assistant Professor in 1947 and was promoted to Professor in 1954. To the great delight of R. A. Brink, Oliver left Purdue in 1969 for the University of Wisconsin. He spent the rest of his career there, having "retired" to emeritus status in 1991.

Throughout his long career, Oliver pursued many aspects of maize genetics and biochemistry. Initially hired at Purdue as a popcorn breeder (at which he was quite successful -- some of his better lines were used in the popcorn industry long after he ended his breeding efforts), he found that most popcorn lines carried the *Ga1-s* allele and as such were cross-incompatible with dents, carrying the *ga1* allele, when used as females. This discovery led to a systematic study of the *ga1* locus. With L.R. House he showed that non-reciprocal cross-sterility was the result of poor *ga1* pollen tube growth on *Ga1-s* silks.

Oliver soon focused on the *waxy1* locus to address questions of fundamental importance to biologists of the time, as well as to test predictions about transposable elements, then in vogue. Exploiting the finding of R.A. Brink and M. Demerec that the *wx1* gene functions in the gametophytic genotype, Oliver realized that pollen populations of sufficient size to detect rare recombination within the gene could be quickly examined. If independent mutations of the *waxy* gene occurred at different sites, he could place these relative to each other by constructing a fine structure recombination map through the occurrence of rare wild-type recombinant grains. Looking at 50,000 pollen grains at a time he first showed that no wild-type pollen grains could be observed in plants that had two copies of the same *waxy* allele, but that rare, blue-black staining grains could be observed at frequencies between 0 to 80 per 100,000 in plants heterozygous for two independent *waxy* alleles. Characteristically, he also showed for one of these combinations that he could observe the same frequency of recombination in the seed. As he used stocks with flanking markers in this important control, this also allowed him to orient the mutations he was mapping relative to the other genes on the chromosome. Even though the frequency of recombination was not additive across the locus, he was able to construct his map using overlapping deletions. All told, the sites of 31 *waxy* mutants were mapped in this way. Among them were a number of stable transposable element-induced alleles. Oliver showed that these were distributed throughout the locus rather than being concentrated in a control region at one end of the gene. The construction of the first fine structure map of a plant gene, plus that done with the *rosy* locus of *Drosophila*, provided us with the most detailed glimpse of the structure of a gene before DNA sequencing. When the *wx1* gene was cloned, Wessler and Varagona remapped 13 of the mutants at the DNA level. An excellent correlation between the genetic and physical maps was observed.

Oliver considered himself a biochemical geneticist. He began his career at a time when there was great excitement to learn how genes worked and what they could tell us about biochemical pathways. Oliver saw no reason that such studies could not also be pursued in higher plants. Since starch was the major component of corn seed, it stood to reason that mutations of this process would have a visible mutant phenotype. He and his colleagues conducted a systematic investigation of the deficiencies of a number of kernel mutations and over the course of three decades identified the biochemical defects associated with eight starch mutants including those responsible for *wx1*, *sh1, sh2, sh4, bt1, bt2, du1* and *su1*. Placing these mutants in the biochemical pathways allowed us to have a better understanding of how this process operates in the corn kernel. This accomplishment is made even more impressive when one realizes that virtually all of this was done before recombinant DNA methodology was commonplace.

The starch work produced a number of "firsts". Identification

of the lesion with *wx1*, a starch-bound ADP-glucose glucosyl transferase, to our knowledge, represented the first case in a higher plant in which the biochemical lesion of a gene with a visible mutant phenotype was elucidated. At the pathway level, the work with *wx1* provided the first and unexpected demonstration that amylose, the straight-chained glucose polymer is **not** a precursor for the branched polymer of glucose, amylopectin, a fact still not presented correctly in many plant physiology textbooks.

The elucidation of the enzyme associated with *su1*, in association with subsequent cloning and characterization of the gene in the Myers-James lab at Iowa State University, showed that **debranching** of alpha 1,6 bonds in starch is essential for the synthesis of wild-type levels of starch. This, too, was unexpected based on the then current view of starch synthesis. The cloning and identification of the protein associated with *bt1,* coupled with subsequent physiological/biochemical studies at Penn State University, provided the first definitive evidence that ADP-glucose, the precursor for starch, is synthesized in the cytosol (and not in the plastid) in the cereal endosperm.

As mentioned, Oliver's pursuit of *wx1* was aimed initially at understanding the nature of the relationship between the gene and its associated transposable elements. This interest persisted throughout his career at Wisconsin. This line of investigation, to some extent with the *sh2* locus, but in much more detail with the *bz1* gene, also led to a number of "first" observations. These included the first reports that inserted transposable elements led to the production of a structurally altered protein. This was as expected if the elements were inserted throughout the gene — as shown in his *wx1* recombination investigations – but was contrary to the idea that transposable elements act as normal regulatory elements of gene expression. Another first was the report that excision events led to a heterogeneous group of revertant alleles that displayed different protein properties, thus anticipating the alterations in coding regions created by transposon excision. These insights came before the application of recombinant DNA methodology to the study of transposable elements.

In the 80s, when the tools of molecular biology made it possible to address questions at the DNA level, Oliver's interest shifted to the transposable elements themselves. Another “first”, in what immediately became classical work, his lab --in collaboration with Nina Fedoroff's-- isolated for the first time a plant gene by a then novel procedure of transposon tagging. The maize elements have been subsequently exported to other species and have revolutionized gene isolation procedures not only in maize but also in other plants. The second main contribution from Oliver's lab during his last decade of activity, and one that involved perhaps the largest number of students and postdocs that he had in his career at any one time, was the elucidation of many of the changes that these highly unstable elements are capable of undergoing.

Perhaps the greatest acclaim given to Oliver came for the work done during the period 1962 to 1969. These studies, done primarily in collaboration with Ed Mertz of Purdue University, showed that levels of the essential amino acids, lysine and tryptophan, could be enhanced by mutation. The discovery that certain amino acids were enhanced in *opaque2* and *floury2,* was of tremendous importance to maize breeding programs; however, problems of kernel softness and yield drag precluded early use in the corn industry. Only now, through development of “modified opaque” maize, is the *o2* mutation being incorporated into corn lines of commerce.

A little known fact about the early *opaque2* and *floury2* work exemplifies Oliver’s uncanny ability at understanding biology through mutant analysis. Before the understanding of *o2* and *fl2,* biochemists at Purdue were massively screening, with little success, maize lines for amino acid content. Oliver knew that lines selected at the University of Illinois for enhanced protein content exhibited an enhanced level of zeins. Because zeins contain little to no lysine, these lines were of little value for feeding monogastric animals. These lines also exhibited a translucent phenotype. Oliver reasoned that mutants with the opposite phenotype (opaque) might have reduced zein content. If non-zein proteins increased, lysine content would increase. Accordingly, four opaque mutants were analyzed: *o1, o2, fl1* and *fl2.* Lysine was doubled in two of the four mutants.

In addition to his many research accomplishments, Oliver was a tireless worker for the maize genetics community. Oliver played an instrumental role in hosting the maize meetings following its move from Allerton House in central Illinois to the various sites in Wisconsin and the Chicago area. He chaired and wrote the substantially unchanged “A Standard For Maize Genetics Nomenclature”. Oliver also served as Chairman of the Genetics Department at Wisconsin, an Associate Editor for Plant Physiology and devoted more than his fair share of time to grant panels in Washington.

Oliver won many prestigious awards during his career. These included an honorary Doctorate of Agriculture from Purdue University in 1973, The Thomas Hunt Morgan Medal from the Genetics Society of America in 1997, the Stephen Hales Prize from the Ameican Society of Plant Physiologists (1998), the Herbert Newby McCoy Award (1967), the John Scott Medal (1967), the Holblitzele National Award in Agricultural Sciences (1968) and the Commemorative Medal of the Federal Land Bank System (1968). He was elected to the National Academy of Sciences in 1972.

Oliver was reserved, unfailingly courteous, rigorously honest, and forthright. He was an insightful and observant scientist. His strength was the use of genetics - mutant phenotypes and segregational analysis - to answer biologically and agronomically important questions. This careful and perceptive analytical bent of his was the hallmark of his career. Oliver played an instrumental role in maize genetics, plant biology, and the maize genetics community. He taught by example. He provided our introduction to a life-long career with the corn plant, and he gave us an everlasting appreciation of the values of Mendelism and appropriate controls. Not only was he a mentor to the three of us in the early stages of our careers, he continued to provide guidance, advice, wisdom and an unconditional friendship to us throughout the rest of his life. He will be missed.

I. Foreword

The Maize Genetics Cooperation Newsletter exists for the benefit of the maize community as an informal vehicle for communication. Its inception and continuation has been to foster cooperation among those interested in investigating maize. Your submissions are encouraged to disseminate knowledge about our field that might otherwise go unrecorded.

For this issue, we solicited reports on genome projects that include maize in some form. We received an enthusiastic response to these requests and for this we thank those investigators who contributed. The objectives of including these notes were severalfold. First, they provide the maize community with information about the studies being conducted and the resources being developed. Further, by providing a compendium for what is currently being performed, the reports might serve as an inspiration for future projects that will enrich the ability to use maize as a model system.

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes can be submitted at any time and are entered into MaizeDB. The deadline for the next print copy, volume 77, is January 1, 2003. Electronic submission is encouraged by sending your contributions as attachments, or as text of an email, to Newsletter@chaco.agron.missouri.edu.

We encourage the community to carry studies of general scientific interest to the formal literature. However, there is a great need to share technical tips, protocols, mutant descriptions, map information, ideas and other isolated information useful in the lab and field.

As in the past, Shirley Kowalewski has been responsible for assembly and correcting of the copy. She has performed this task with speed, precision and a great sense of humor.

Mary Polacco
James A. Birchler
Co-editors

AMES, IOWA
USDA-ARS

Phenotypic characterization of inbred lines and their *o2* conversions

--Scott, MP

Peter Loesh was a plant breeder at Iowa State University from 1971 to 1980. During this time, he worked extensively with the *o2* mutation in an effort to improve the nutritional quality of maize. Unfortunately, he passed away suddenly, and this work was not brought to a conclusion. Some of his effort was devoted to developing *o2* back-cross conversions of public domain inbred lines. Because Dr. Loesh's field books have been lost, the extent of recovery of the recurrent parent phenotype is not known.

The objective of this study was to characterize Dr. Loesh's *o2* converted lines relative to their recurrent parents and to verify that they have the *o2* genotype. To accomplish this, I planted one row of each line adjacent to one row of the corresponding recurrent parent at the Iowa State University Agronomy Farm in the summer of 2001. The resulting plants were characterized phenotypically on the basis of their plant height, ear height and pollination date (see Table). With the exceptions noted in the table, the inbreds were phenotypically similar to their recurrent parents.

In order to verify that the Loesh inbreds carried the *o2* mutation, each of these lines was crossed to an *o2* mutant tester from the Maize Co-op Stock Center (701D). In all cases, the *o2* genotype was confirmed.

These lines will be valuable for comparing the effect of different genetic backgrounds on the *o2* mutation, and may also be useful to breeding programs aimed at improving nutritional quality with the *o2* mutation. Seed of these lines has been sent to the Maize Co-op Stock Center for maintenance and distribution.

Table. Phenotypic characterization of inbred lines and their *o2* conversions.

Pedigree	plt ht[a]	ear ht	pol date[b]	comments
A257	NS	NS	-1	
A619	* *	NS	-3	
B14A	* *	NS	-2	*o2* small kernels
B45	NS	*	0	
B46	*	NS	2	*O2* red cob, *o2* white cob
B57	NS	NS	-6	
B66	NS	NS	0	
M14	NS	*	-9	

[a]Plant and ear height measurements are the average of the first five plants in the row. NS indicates the difference between the inbred line and its *o2* conversion is not significant, ** significant to P=0.05, * significant to P=0.1

[b]Pollen date = (number of days after planting when half the plants in the *O2* row first shed pollen) – (number of days after planting when half the plants in the *o2* row first shed pollen)

BEIJING, CHINA
Academia Sinica

Common and different band for isozyme of the multiplasmic lines in maize (Zea mays L.)

--Zeng, M, Yang, T

The term "multiplasm" was originally introduced into the research of CMS (cytoplasmic male sterility) by Grogan (1971). At the present time, "multiplasm" in the broad sense generally indicates a set of genetic materials: a homonucleo-hetero cytoplasmic line, a homocytoplasm-hetero nucleus line and different lines for single, double, multiple genes, including isogenic and near-isogenic lines. Multiplasm in a narrow sense indicates only the genetic material (line) of homeonucleo-hetero cytoplasm. Breeding for a multiplasmic line, has not only provided a new model of investigation into the genetics of cytoplasmic and nuclear genes and nucleoplasmic genetic interactions, but also has provided a fresh channel for avoiding a single cytoplasmic resource in germplasm and has increased the diversity of germplasm resource in crop production. This study surveys the isozymic band by electrophoresis.

Eleven multiplasmic lines of maize were used as experimental materials, i.e. (Fli) Mo17 [original Mo17 line, (Fli) was shown as flint cytoplasm], (su1)Mo17, (sh2)Mo17, (bt1)Mo17, (Pop)Mo17, (wx)Mo17, (Teo)Mo17, (cms-T)Mo17, (cms-S)Mo17, (cms-C)Mo17, (cms-21A)Mo17. (Fli)Mo17 was used as a control (CK) to carry out the analyses. The common degree of nuclear genes was 99.95% in 10 of the multiplasmic lines.

Isozymes were determined as described by Zeng (Determinations of the biochemistry for CMS and genetics for their restoration in maize. Science in China, 25(3):283~296, 1987). The electrophoreses were conducted analyzing the POD and EST of kernel at the 9th day after pollination, embryo at the 15th, 25th, and 34th day after pollination, endosperm at the 15th, 25th, and 34th day after pollination, and young shoot of mature seed, unfolded leaf at the 6-leaf, 8-leaf, and 14-leaf stage, emergent tassel stage, pollination stage, 15th day after pollination, and tassel at emergent tassel, and pollen at pollination stage, respectively.

Experimental Results. The results obtained indicate there were 20 POD bands, and 18 EST bands in total. Comparing the 11 multiplasmic lines, the zymograms for POD electrophoresis of the

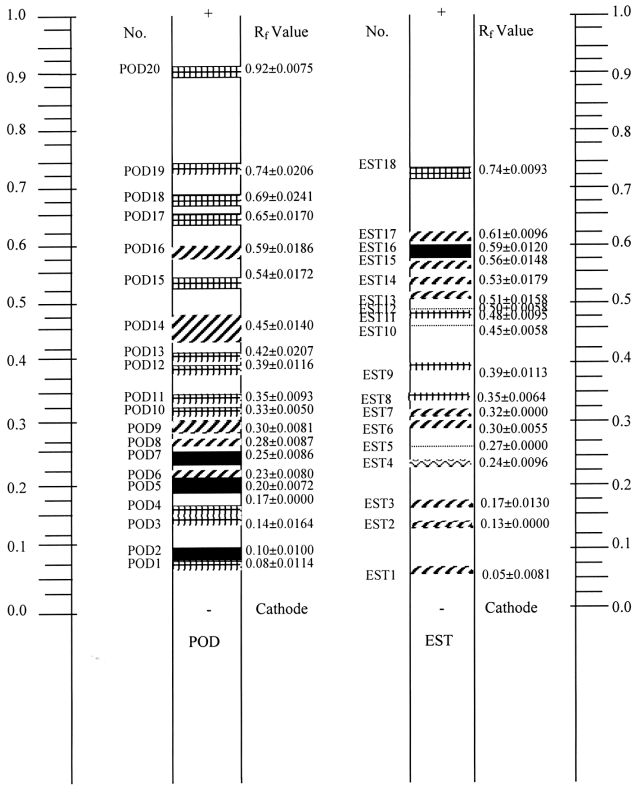


Figure 1 Zymograms of peroxidaes and esterase isozyme in multiplasms
The following order is one of the increasing activities of isozymic bands.

different tissues at different stages varied clearly, formed the same bands and specific bands, that of embryo at the 34th day after pollination and pollen at pollination was uniform. The difference of zymogram for EST electrophoresis of the 11 multiplasmic lines mainly appeared in the tissues of the kernel at the 9th day, the embryo at the 25th day and 34th day, the endosperm at the 34th day after pollination, and the tassel at the emergent tassel and the pollen at the pollination stage, formed the same bands and specific bands also. But EST of the other tissues (that of the embryo at the 15th day, the endosperm at the 25th day and the endosperm at the 25th day after pollination, and the young shoot, the leaf at 6th leaf, at 8th leaf, at 14th leaf, at emergent tassel, at pollination, at the 15th day after pollination stage) was all uniform.

BELGRADE-ZEMUN, YUGOSLAVIA
Maize Research Institute

The antagonistic effect of endophytic bacteria on phytopathogenic fungi

--Dondo Gosic, S, Drinic Mladenovic, S, Konstantinov, K

Diseases caused by phytopathogenic fungi are a serious problem in maize production, as they can result in premature ripeness of maize. Infected plants produce ears of poorer quality, and lodged plants are not suitable for mechanised harvest, etc. Control by the application of chemicals is limited due to resistance of fungi and destruction of antagonistic soil mycoflora.

The biological control of disease affecting plants has gained much attention in the past two decades as a way of reducing the use of chemical products. The existence and active metabolism of endophytic bacteria within plant tissue make them potential candidates for biological control of plant resistance to phytopathogenic fungi. The effects of endophytic bacteria isolated from maize and sunflower on growth of phytopathogenic fungi *B. zeicola, E. turcicum, F. oxysporum, Ph. zeae, M. bolleyi, N. oryzae* were observed in the present study under in vitro and in vivo conditions.

Two bacterial strains were selected for these investigations: *Bacillus licheniformis*, isolated from maize embryo, and *Seratia liquefaciens*, isolated from sunflower. The bacterial strains were determined by the application of API and Microlog 1 system. The slightly modified method of Gutterson et al. (Journal of Bacteriology, vol.165, No.3, 696-703, 1986) was applied to monitor fungi/bacterial interaction. The effects of bacteria on fungal development and growth were evaluated in dual cultures on media optimal for growth of bacteria (LB) and fungi (PDA) in the course of 3 and 7 days.

According to the results obtained, it can be concluded that effects of both bacteria on all fungi were antagonistic. Inhibition of fungal growth occurred without physical contact between the bacterium and the fungus. The typical appearance of dual culture growth on both mediums is shown in Figure 1.

The growth rate of fungi is expressed as the colony diameter of dual or separated cultures through seven days. The greatest antagonistic effect was obtained in dual cultures with *B. zeicola* and *Ph. zeae*. A greater effect on fungal growth was achieved on the LB medium. Results of fungal growth in dual cultures in relation to the control are presented in Figure 2.

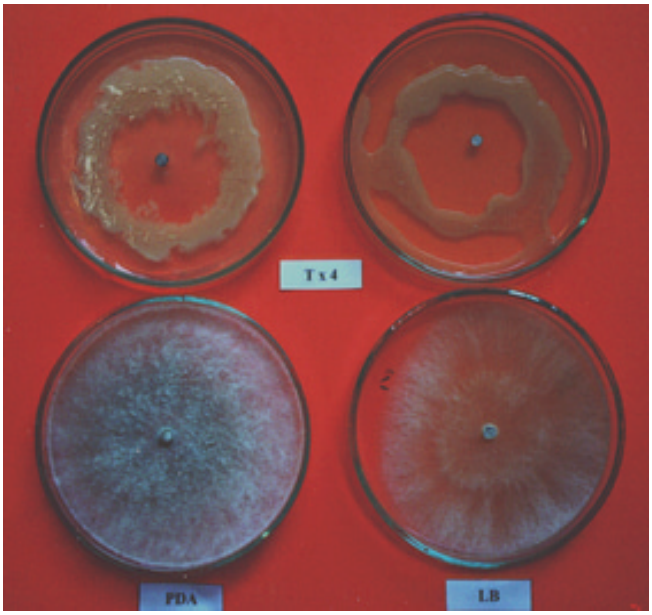


Fig. 1. Separate and dual cultures of *Bacillus licheniformis* and *Seratia liquefaciens* on PDA and LB medium.

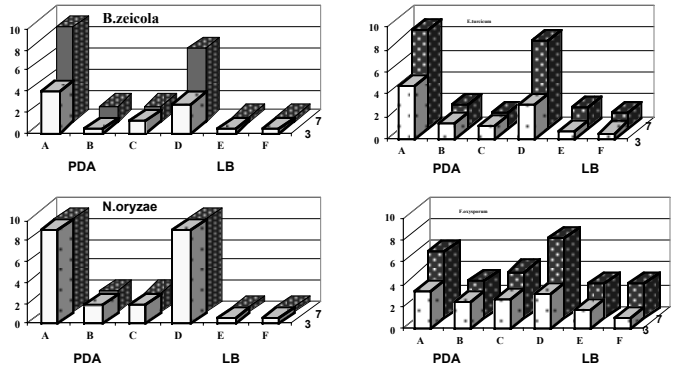


Fig 2. Colony diameter (cm)of fungi in separate (A, D) and dual cultures with B.licheniformis (B,E) and S.liquefaciens (C, F) during 3 and 7 days of incubation on PDA and LB media

Endophytic bacteria release components in the medium that reduce fungal growth. Proteins were isolated from bacteria grown as single cultures, as well as from bacteria interacting with fungi, and were separated on polyacrylamide gels. A greater number of new polypeptide fractions in relation to the control were determined in the interactions with observed fungi. The studies on the molecular basis of this antagonism are in progress.

Two fungi *B. zeicola* and *F. oxysporum*, i.e. *M. bolleyi* and *N. oryzae*, were selected for investigation of antagonistic effects of *B. licheniformis* and *S. liquefaciens*, respectively, under in vivo conditions. Untreated seed and seed treated only with fungi were used as the control. The length of the shoots and roots of treated and control maize plants were measured and results are presented in Figure 3.

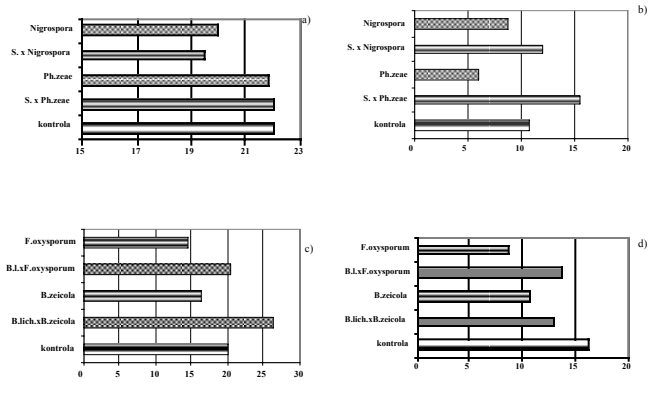


Fig. 3. The length of shoots (a, c) and roots (b, d) of plants treated with bacteria and fungi as well as plants treated only with fungi and control plants.

Greater lengths of roots and shoots were detected in plants treated with both bacteria and fungi than in plants treated only with fungi.

The results obtained point out that endophytic bacteria isolated from maize and sunflower can be used in biological control of observed phytopathogenic fungi of maize.

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Maize Rpd3-type histone deacetylase and retinoblastoma-related proteins cooperate in repressing gene transcription in a plant system

--Rossi, V, Locatelli, S, Lanzanova, C, Motto, M

In eukaryotic cells transcriptional activation is a dynamic and complex process, involving direct recruitment of co-activator complexes and assembly of the functional transcriptional machinery at the target gene promoter. Recent findings, indicating that several transcriptional co-activators and co-repressors possess histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities, have revealed a direct mechanistic link between chromatin modification and transcriptional regulation (Strahl, BD and Allis, CD, Nature 403:41- 45, 2000).

The mammalian pRb/E2F complex has been reported to recruit chromatin remodeling enzymes, including Rpd3-type HDACs, to actively repress transcription of genes involved in G1/S transition during cell cycle progression (Harbour, JW and Dean, DC, Genes Dev 14:2393-2409, 2000). To investigate whether the maize Rpd3-type histone deacetylase, ZmRpd3, (Rossi, V, et al., Mol Gen Genet 258:288-296, 1998) and the maize retinoblastoma-related protein, ZmRBR, can repress transcription, we prepared a set of effector plasmids, in which either the complete ZmRpd3I coding sequence or the ZmRBR1 large pocket region were fused to the DNA-binding domain of the tetracycline repressor (TetR). The expression of these chimeric genes was driven by the 35S constitutive promoter of the cauliflower mosaic virus (CaMV). The TET system is based on the targeting of TetR-fused proteins to a synthetic promoter containing four tet operators upstream of a minimal promoter (TAX promoter, Bohner, S, et al., Plant J 14:87-95, 1999). Our experimental strategy was based on the following considerations. First, the reporter plasmid, containing the β-glucuronidase gene (GUS) under the control of the TAX promoter was stably integrated into the tobacco genome (tobacco TAX transgenic lines) allowing formation of a natural chromatin structure. Second, analysis of the repression activity was carried out by means of competition assays, in which tobacco protoplasts from TAX lines were co-transformed with a TetR-fusion of the strong viral activator VP16 together with one of the TetR-fusion plasmids expected to repress transcription. To avoid heterodimerization of the TetR domains, which might interfere with VP16 transactivation efficiency, we employed different TetR DNA-binding domains unable to heterodimerize (TetR-B for VP16 fusions and TetR-B/E for the other TetR-fusions, respectively (Forster, K, et al., Nucl Acids Res 27:708-710, 1999). Third, the effect on transcriptional activity of the GUS gene, due solely to competition for binding the TET operators, was assessed by co-transforming TetR-B-VP16 (TET-VP16) and a TetR-B/E-fused maize zein (TET-zein), a structural protein unable to control transcriptional activity (Spena, A, et al., J Mol Biol 169:799-811, 1983). Therefore, evaluation of transcription repression activities of ZmRpd3I/ZmRBR1 TetR-fusion proteins was achieved by comparison with results obtained from TetR-B/E-zein transformed protoplasts.

The results of these experiments showed that VP16 strongly activated expression of the GUS gene, which was only slightly reduced by the presence of competing TetR-zein. Conversely, a plasmid carrying the ZmRpd3I TetR-fusion strongly reduced GUS activity. Furthermore, both a mutation in histidine 153, believed to be critical for HDAC activity, as well as treatment with the HDAC inhibitor TSA, were able to relieve the repression activity of ZmRpd3I. These results indicate that ZmRpd3I is likely to use its HDAC activity to repress transcription when artificially targeted to a promoter within a chromatin context. A negative effect on gene transcription was also observed for the ZmRBR1 TetR-fusion, although it was lower with respect to ZmRpd3I and almost insensitive to TSA treatment. Significantly, GUS activity was reduced to background levels by the simultaneous presence of TET-RBR and CaMV-Rpd3, a plasmid expressing ZmRpd3I not fused to the TetR DNA-binding domain. In this case, TSA relieved the effect of ZmRBR1 on GUS activity repression to a level observed with ZmRBR1 alone. The introduction of the C653F mutation, which affects A/B pocket integrity, resulted in a complete loss of transcription repression activity regardless of whether CaMV-Rpd3 was present or not. These findings suggest that ZmRBR1 possesses an HDAC-independent capacity to repress gene transcription, which relies on the integrity of the A/B pocket. However, our results also indicate that the ability of ZmRBR1 to recruit a ZmRpd3I-related HDAC enhances its effect in repressing gene transcription. The integrity of the A/B pocket domain is also a prerequisite for this repression mechanism. Finally, we observed that similar results were obtained when the reporter plasmid (pUC-TAX-GUS/INT) was co-transformed into protoplasts prepared from tobacco wild type plants together with the above described effector plasmids. In this last experiment the reporter plasmid may not adopt a complete chromatin structure suggesting that non-histone proteins may also be ZmRBR1/ZmRpd3I substrates.

Genetic relatedness and variability among maize inbred lines selected in Italy

--Hartings, H, Chittò, A, Bertolini, M, Verderio, A, Motto, M

In breeding programs, information on genetic relationships within and between species is used for organizing germplasm collections, identifying heterotic groups within crops, and selecting parents for purposes of crossing. In this respect, DNA fingerprinting offers the possibility of studying genetic variation and relationships at the molecular level.

To address the issue of genetic relatedness and variability between the inbred lines developed in Italy by the Bergamo Maize Station, an AFLP analysis (Vos, P et al., Ac. Res. 23:4407-4414, 1995) was performed on a series of 71 inbred lines, considered representative of optimized breeding material. Twenty reference lines, encompassing the major heterotic groups available in the U.S. Corn Belt were included in the analyses in order to maximize genetic variability across the data set. Hence, the reference lines supplied a basis of genetic diversity to which the Italian inbred lines were related in the evaluation of their relative genetic relationships.

AFLP analysis of the Italian and reference inbred lines produced stable and repeatable profiles, which allowed us to unequivocally fingerprint each inbred line analyzed. A total of 682 polymorphic bands were revealed by the use of 5 PstI/MseI and 9 EcoRI/MseI primer combinations (PCs). E/M PCs displayed between 29 and 65 polymorphic bands resulting in an average of 47.56 12.68 markers. P/M PCs appeared less variable, disclosing between 41 and 54 polymorphic bands with an average of 50.8 5.63 markers. E/M PCs displayed an average Polymorphism Information Content (PICav) value of 0.33 0.027, while an average PICav of 0.35 0.014 was obtained with P/M PCs. In general, PICav values ranged from 0.28 to 0.36, demonstrating the good discriminatory power of the markers identified.

Scoring of the markers allowed the construction of a 682x91 binary array, which was consequently utilized to compute genetic distance (GD) values (Nei, M and Li, WH, Proc. Natl. Acad. Sci. USA 76:5269-5273, 1979) for all pairs of inbred lines considered. GD values ranged from 0.124 for inbred lines Lo876 and Lo1064, both derived from Lo876o2, to 0.62 for inbreds Lo3 and Lo903. An average GD of 0.437 0.012 was calculated for the entire data set.

Distance measures were subsequently used to construct a hierarchical tree using the UPGMA method using an NTSYS-PC program. Table 1 summarizes the grouping of inbred lines, presenting the major groups identified as well as their disclosed subgroups. Cluster analysis largely agreed with pedigree information as can be established by comparing the pedigree with clustering information.

For data with a hierarchical structure, analysis of molecular variance (AMOVA) allows the study of patterns of genetic variation within and between groups through the examination of variance. This assay can be extended to evaluate molecular marker data even in the absence of replicated values for samples (Law, JR et al., Euphytica 102:335-342, 1998). An AMOVA of the AFLP data based on the grouping obtained in cluster analysis of the inbred lines considered is presented in Table 2. Clusters were used to recompose, in broad terms, BSSS, LSC, and unrelated heterotic groups. The amalgamation into heterotic groups was performed using both a small number of larger clusters, as well as a larger number of clusters of reduced size. In both cases, the within-population (clusters) components of variance dominated the AMOVA, accounting for 73% to 79% of the variation, with less than five percent representing variation between heterotic groups. Changes in the grouping pattern applied had no significant effect on the distribution of variation. Furthermore, the genetic distance between clusters (F_{st} values) exceeded both the degree of inbreeding within clusters (F_{sc} values) and the degree of relatedness between genes within inbred lines (F_{ct} values) in all cases.

Table 1. Principal groups of inbred lines as identified by cluster analysis.

		BSSS1 (22) [a]		BSSS2 (9)	References (12)
	Lo950	Lo903	Lo999	Lo876	Lo986
	Lo951	Lo904	Lo1055	Lo1016	A71
	Lo960	Lo1086	Lo1137	Lo1064	A69Y
	Lo964	Lo1101	Lo1141	Lo1066	B57
	Lo1054	Lo1106	B37	Lo1067	CI187-2
	Lo1053	Lo1127		Lo1123	FR5
	Lo1087	Lo1167		Lo1169	H55
	Lo1094	B73		Lo1170	N6
	Lo1173			A632	Oh07
					Os420
					W64A
					Wf9
Ave [b]		0.32		0.37	0.47
Min		0.13		0.12	0.27
Max		0.48		0.49	0.54

	LSC1 (21)		LSC2 (18)		Lo932 (4)
	Lo1059	Lo863	Lo881	Lo902	Lo932
	Lo1061	Lo1077	Lo1038	Lo924	Lo937
	Lo1063	Lo1095	Lo1056	Lo976	Lo944
	Lo1076	Lo1096	Lo1035	Lo1124	W153
	Lo1156	Lo1125	Lo1090	Lo1126	
	Lo1157	Lo1128	Lo1140	Lo1142	
	Lo1158	Lo1172	C103	Lo1166	
	Lo1159	Lo1176	Va59	Mo17	
	Lo1160	Lo1182	T8	Oh43	
	Lo1162				
	Lo1168				
	Lo1171				
Ave	0.37		0.40		0.33
Min	0.13		0.15		0.19
Max	0.51		0.54		0.47

[a] numbers in parentheses represent the number of inbreds in each group;
[b] Ave: average GD, Min: lowest GD, Max: highest GD within groups.
BSSS: Iowa Stiff Stalk Synthetic; LSC: Lancaster Sure Crop

Table 2. Summary of AMOVA for AFLP data from Italian and U.S. Corn Belt Inbred Lines.

Nested AMOVA Variance Components						
	Between Groups	Between Populations Within Groups	Within Populations			
Populations	V(A)%	V(B)%	V(C)%	F_{st}	F_{sc}	F_{ct}
Large	2.61	18.31	79.1	0.209	0.188	0.026
Small	4.27	22.38	73.4	0.266	0.234	0.043

Heterogeneity within breeding groups was further analyzed by computing a series of diversity statistical indices from the AFLP data (Table 3). Estimates of θ, which is the product of population gene number and mutation rate, were computed based on the number of polymorphic sites ($\hat{\theta}_S$) and on the mean number of pairwise differences ($\hat{\theta}_\pi$). Both estimates hold under the assumption of random mating, population equilibrium and neutral mutations. If these assumptions are valid, $\hat{\theta}_S$ has smaller stochastic variance than $\hat{\theta}_\pi$. However, since $\hat{\theta}_\pi$ is independent of sample size while $\hat{\theta}_S$ is not, $\hat{\theta}_\pi$ may be a more reliable estimate of gene diversity within heterotic groups. None of the breeding groups analyzed

showed significant variation in $\hat{\theta}_S$ and $\hat{\theta}_\pi$ values. Subsequently, the average gene diversity per site was computed. This parameter of diversity, as determined on the entire population of inbreds analyzed, equaled 0.33 ± 0.16 and varied within a narrow range (0.24 – 0.33) when determined for the heterotic groups identified. Expansion or contraction of the heterotic groups was assayed by statistics developed by Tajima (Tajima, F, Genetics 123:585-595, 1989). Tajima's D statistics, computed on the heterotic groups listed in Table 3, did not reveal any expansion or contraction of groups, since none of the values obtained reached statistical significance using coalescent simulation (Hudson, R.R., Oxford Surways in Evalutionary Biology, Oxford Univ. Press, 1-44, 1990), or parametric approximation assuming a beta-distribution (Tajima, 1989).

Table 3. Summary of diversity statistics for Italian and U.S. Corn Belt Inbred Lines.

		Groups				
	All Inbreds	Reference[a]	BSSS1	BSS2	LSC1	LSC2
No. inbreds	91	20	22	9	21	18
No. polymorphic sites	682	475	442	412	235	329
Theta(S)	0.16	0.33	0.27	0.36	0.28	0.29
S.D.[b] Theta(S)	0.04	0.13	0.09	0.15	0.09	0.10
Theta(π)	0.25	0.38	0.36	0.40	0.36	0.35
S.D. Theta(π)	0.12	0.20	0.17	0.22	0.18	0.18
Average gene diversity	0.33	0.33	0.24	0.26	0.27	0.30
+/- average diversity	0.16	0.17	0.12	0.14	0.14	0.15
Tajima's D	2.24	0.68	0.92	0.45	1.15	0.91

[a] All Reference lines; [b] S.D. Standard Deviation

In this study, AMOVA, performed on genetic structures obtained by means of two different amalgamation schemes showed that the within cluster component of variance largely exceeds the variance between heterotic groups, regardless of the amalgamation scheme selected. Moreover, additional statistical indices of diversity show that no significant differences occur between the variability encountered within identified heterotic groups and the overall level of genetic diversity among the inbred lines taken into consideration. It can therefore be concluded that breeding activity has by no means caused a decline of genetic variability within heterotic groups. On the contrary, levels of genetic diversity have remained substantially unchanged over time and hence, plant breeding has resulted in a qualitative rather than a quantitative shift in diversity. In conclusion, this study has shown that a large genetic variability occurs among maize germplasm available in Italy. This variability can be exploited in hybrid and line development for further yield improvement.

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Mu Killer (*MuK*) causes epigenetic silencing of *Mutator* elements

--Slotkin, RK, Freeling, M, Lisch, D

The *Mutator* family of transposable elements is under the control of the autonomous *Mu* element *MuDR*, which encodes transcripts essential for both its own and nonautonomous *Mu* element transposition. The loss of *Mutator* activity can occur in at least three previously described ways: the autonomous *MuDR* element can segregate away, transpose to a transpositionally weak position, or internally delete creating a non-functional element (Lisch and Freeling, Maydica 39:289-300, 1994). Otherwise functional *MuDR* elements have also been observed to become epigenetically silenced. This silencing is correlated with methylation of both *MuDR* and nonautonomous *Mu* elements. Here we report a dominant locus, *Mu Killer* (*MuK*), which results in epigenetic silencing of a functional *MuDR* element at a known active position.

Normally in our minimal line, which carries a single *MuDR* element at a known position (*MuDR(p1)*) and a single *Mu1* element, methylation only occurs in progeny that lack a functional *MuDR* element. In families segregating *Mu Killer*, however, half the progeny that carry *MuDR(p1)* have methylated *Mu1* TIRs. This methylation is associated with a reduction or elimination of *Mu1* excision activity.

Mu Killer activity was originally identified when a high color background was crossed to a minimal *Mutator* line carrying a *MuDR* element at an unknown position. It was observed that this *MuDR* element became silenced in the progeny of this cross (Lisch and Freeling, 1994). In subsequent generations, the silencing effect was genetically separated from any full-length *MuDR* elements.

In the winter of 2000, a male carrying an active *MuDR(p1)* element was crossed to both a *Mu* permissive tester and *MuK* ears. The progeny of these crosses were analyzed by Southern blot. As expected, all (12 of 12 examined) of the progeny of the *Mu* permissive tester that carried *MuDR(p1)* had hypomethylated *Mu1* elements. In contrast, of the progeny of the *MuK* plant that inherited *MuDR(p1)*, 40 out of 78 (51.3%) had methylated *Mu1* elements, as expected for the independent segregation of *MuDR(p1)* and *MuK*. All of these *Mu1* methylated plants had been grown from weakly spotted or pale kernels carrying a full-length *MuDR(p1)*. Pale kernels from this *MuK* ear were planted in the summer of 2001 and the resulting progeny were screened by DNA gel blot for the presence of *MuDR*. Although only half the progeny in this family were expected to carry *MuK*, none of those that carried *MuDR(p1)* exhibited signs of reactivation, demonstrating that even in the absence of *MuK* (due to segregation), silenced *MuDR* elements remain silent. Silenced *MuDR(p1)* elements also remained inactive when crossed to *Mu* permissive plants that did not contain *MuDR* (0/~1200 spotted kernels). Thus, *MuK* causes a heritable alteration of *MuDR* elements.

To test the heritability of *MuK* itself, several progeny plants from the above cross that lacked *MuDR* and that segregated for *MuK* were again crossed as females to males carrying *MuDR(p1)*, and their progeny was analyzed by Southern blot. In one family that showed evidence of *MuK* activity (reduced excisions), 47.6% of the progeny that carried *MuDR(p1)* were methylated and inactive showing that *MuK* segregation was heritable (Table 1). In contrast, families derived from crosses between the same *MuDR(p1)* active plant and permissive testers resulted in all progeny with *MuDR(p1)* having hypomethylated *Mu1* TIRs, demonstrating that *MuDR* was not inactivating prior to contact with the *MuK* ear.

Mu Killer's effect is not only on *Mu1* TIRs. *MuDR* TIRs also become methylated in the presence of *MuK*. Thus, the inactivation of *Mu1* is most likely a byproduct of *MuDR* silencing. *Mu Killer*'s effect on *MuDR* is also not confined to the *MuDR* element at p1. *MuK* has been observed to silence single *MuDR* elements at other

Table 1

Female plant x single copy full-length *MuDR(p1)*

Female genotype and generation	*Mu* permissive generation 1	*Mu Killer* generation 1	*Mu* permissive generation 2	*Mu Killer* generation 2[1]
MuDR(p1) with hypomethylated Mu1	12[2]	38	18[2]	11
MuDR(p1) with hypermethylated Mu1	0	40	0	10

[1] *Mu Killer* generation 2 individuals are the pale kernel progeny of *Mu Killer* generation 1
[2] The weakest spotted kernels of the *Mu* permissive progeny were tested for *Mu1* TIR methylation

positions as well (Lisch and Freeling, 1994). Experiments are underway to determine if *Mu Killer* has the ability to silence complex *Mu* active lines currently used for transposon tagging. To date, *MuK* has been found not to cosegregate with any background *MuDR* deletion derivative.

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Development and preliminary evaluation of near-isogenic maize lines differing for a QTL which affects leaf ABA concentration

--Landi, P, Salvi, S, Sanguineti, MC, Stefanelli, S, Tuberosa, R

In plants grown under stress conditions, particularly drought, the concentration of abscisic acid in the leaf (L-ABA) increases rapidly (Hartung, W and Davies, WJ, in Davies, WJ and Jones, HG (eds.), 1991); this suggests that L-ABA can be involved in attenuating the stress-induced injuries. However, with respect to the effects associated with genetic variation in L-ABA, controversial results have been reported in cereals (Quarrie, SA, in Davies, WJ and Jones, HG (eds.), 1991). As an example in maize, positive relationships have been found between L-ABA and drought tolerance by Pekic, S and Quarrie, SA (J Plant Phys 127:203-217, 1987 and Ann Bot 61:669-678, 1988) while negative relationships have been found by Landi, P et al. (Maydica 40:179-186, 1995) and by Sanguineti, MC et al. (J Exp Bot 50:1289-1297, 1999). These controversial findings can be likely related to the complex genetic control of the investigated traits and their interaction with different genetic backgrounds and environmental factors (e.g., timing and intensity of drought stresses).

To gain information on the genetic control of L-ABA in maize, we analyzed the segregating populations derived from the cross Os420 x IABO78; parental inbred Os420 was chosen because it shows high L-ABA, especially under drought, while inbred IABO78 shows low L-ABA (Conti, S et al., Euphytica 78:81-89, 1994). The studies on such populations allowed us to identify an important QTL affecting L-ABA located on chromosome 2 between the RFLP loci *umc34* and *csu4a* (Salvi, S et al., MNL 71:15-16, 1997; Tuberosa, R et al., Theor Appl Genet 97:744-755, 1998). To investigate the effects of this QTL on L-ABA and on related physiological traits, we undertook a backcross breeding program aimed at developing near-isogenic lines for this QTL. As emphasized by Prioul, JL et al. (J Exp Bot 48:1151-1163, 1997), near-isogenic lines are a useful "tool" to study the physiological basis of plant responses across environments.

In this note, we summarize the development of the near-isogenic lines and provide results of their preliminary evaluation.

As source material, we used the cross between Os420, providing the allele with increasing effect (+) on L-ABA at the target QTL, and IABO78, providing the allele with decreasing effect (-) on L-ABA. To develop the near-isogenic lines for both parents two backcross procedures were followed. In the first backcross procedure, Os420 was used as recurrent parent and IABO78 as the donor, while in the second one the role of the parents was reversed. The backcross procedure was aided by using the two closely linked RFLP loci *umc34* and *csu4a* (ca. 8 cM apart) flanking the target QTL. If for simplicity we indicate *umc34* as *A* and *csu4a* as *B*, then the genotype of Os420 can be indicated as *A1B1/A1B1* and the genotype of IABO78 can be indicated as *A2B2/A2B2*. Plants heterozygous *A1B1/A2B2* were also considered to be heterozygous for the two parental chromosome segments flanked by the two RFLP loci, assuming that no double crossover occurred between the two segments. These heterozygous plants were backcrossed for five generations and then selfed for one generation. Four BC5F2 families were developed throughout the breeding process because, starting from the BC2 generation, two different families were grown for each recurrent parent (the two families were identified as .1 and .2).

In 2000, the four BC5F2 families were grown near Bologna, under mild drought stress conditions in a randomized block design with three replications. All plants were assayed for their genotype at the two RFLP loci *umc34* and *csu4a*. Plants that proved to be homozygous for the marker alleles provided by Os420 (i.e., *A1B1/A1B1*) were assumed to be homozygous (+/+) at the target QTL controlling L-ABA; analogously, plants homozygous for the two marker alleles provided by IABO78 (i.e., *A2B2/A2B2*) were assumed to be homozygous (-/-). Such BC5F2 homozygous plants were selfed to finally produce the BC5F3 near-isogenic lines. These were analyzed for L-ABA in order to obtain preliminary information on the effectiveness of the backcross-assisted procedure and on the additive effect of the QTL. In each plot, leaf samples for L-ABA analysis were collected at tassel appearance from four plants of the (+/+) near-isogenic line and from four plants of the (-/-) near-isogenic line.

The mean values for L-ABA of the four near-isogenic lines derived from Os420 as recurrent parent were higher than the mean values of the four near-isogenic lines derived from IABO78 (Table 1). Within each family, the (+/+) line showed a higher mean value than the corresponding (-/-) line; on a family mean basis, the additive effect was 14.8 and 14.0% for the two Os420 families and 8.2 and 10.2% for the two IABO78 families. Even if the additive effect of the target QTL was significant ($P \leq 0.05$) only for the two Os420 families, the interaction "family x additive effect" was not significant. Across the four families, the additive effect was

Table 1. Mean values for L-ABA (ng ABA g^{-1} DW) at tassel appearance of the near-isogenic lines (+/+) and (-/-) of the BC5F2 families derived from Os420 and IABO78 as recurrent parents.

Family	Near-isogenic lines		Additive effect		
	(+/+)	(-/-)	[a]	(%)[b]	[c]
Os420.1	315	234	41	14.8	*
Os420.2	358	270	44	14.0	*
IABO78.1	211	179	16	8.2	ns
IABO78.2	232	189	22	10.2	ns
Mean	279	218	31	12.3	**

[a] Calculated as [(+/+) - (-/-)]/2.
[b] Referred to the family mean.
[c] * and ** indicate significance levels of $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = not significant.

highly significant ($P \leq 0.01$) and equal to 31 ng ABA g^{-1} DW, corresponding to 12.3% of the overall mean. It is worth mentioning that in our previous investigation conducted on F4 families derived from the same single cross (Tuberosa, R et al., Theor Appl Genet 97:744-755, 1998), the additive effect at this QTL was 49 ng ABA g^{-1} DW, corresponding to 12.1% of the overall mean.

The results of this preliminary investigation suggest that the target QTL was successfully transferred by the marker-assisted backcross and tend to confirm the relative importance of its additive effect in controlling L-ABA. As the next step, the near-isogenic BC5F3 lines (+/+) and (-/-) will be tested in field trials conducted at different levels of water stress. In addition, crosses between the lines derived from Os420 and from IABO78 will be produced (according to the factorial scheme), in order to investigate, in the absence of inbreeding depression, the QTL effects on physiological and agronomic traits related to drought tolerance.

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Bio-photonic crystal: SHG imaging

--Cheng, PC, Sun, CK, Lin, BL, Chu, SW

The intrinsic optical sectioning properties in non-linear optical microscopy has drawn much interest in biological sciences (Denk et al., Science 248:73, 1990; Cheng et al., J. Microscopy 189:199-212, 1997); in conjunction with numerous fluorescence bio-probes, multi-photon fluorescence microscopy has become a powerful tool in deep tissue imaging. The technology has also been applied to micro-spectroscopy at high spatial resolution (Lin et al., SPIE Proceedings 4082:100-104, 2000), In addition to the fluorescence signals generated by the multi-photon excitation process, non-linear phenomena such as harmonic generation also provide useful information for the structure and optical properties of a specimen. Simultaneous recording of the spectral response in an image (x-y-λ) provides insight for the nature of the signal. Due to low attenuation of biological sample in the infrared (Lin et al., MGCNL 75:61-62, 2001), one not only achieves deep penetration in the sample, but also opens the possibility of imaging modalities in second-harmonic generation (SHG) and third harmonic generation (THG). In addition, both visible and near infrared regions are open to efficient detection while the pump wavelength is far away from the sensitivity range of silicon-based detectors. The use of longer illumination wavelength dramatically reduces the background auto-fluorescence and photo-damage.

Two-photon fluorescence, SHG and THG imaging was performed using a Spectra-Physics Millennia IR (1064 nm) pumped Cr-forsterite laser operating at 1230mn (110MHz, 130fs) or a Coherent Verdi pumped Spectra-Physics Tsunami mode-locked Ti-sapphire laser operated at 780mn with 100fs pulse at 82MHz.. A modified Olympus BX microscope equipped with a confocal scanning unit (Olympus Fluoview FVX) was used. A TE-cooled CCD equipped SpectraPro-150 spectrometer was used. Spectro-microscopy was performed in transmission mode by using a computer-controlled scanning stage.

Similar to the two-photon fluorescence, second-harmonic generation (SHG), which is a X(2) second-order non-linear process, provides strong nonlinear signals but occurs only in noncentrosymmetric media. SHG process can thus be used to image bio-interface and orderly arranged structure, where centro-symmetry is broken. In SHG, only virtual state transition is involved, thus no photo-damage and bleaching from the process results. With a square dependence on the incident illumination intensity, the second-harmonic generation process also provides optical sectioning resolution as the two-photon fluorescence process if the same excitation wavelengths are used. There are a number of maize structures capable of generating strong SHG signals, these include cell walls (Fig. 1), starch granules, silica cells and possibly stacked membrane structures such as grana in the chloroplast. Since they behave similarly to photonic crystals, we suggest the use of "bio-photonic crystals" to describe these types of structures. In fact, a piece of potato tuber is capable of producing a strong frequency-doubled beam similar to those non-linear crystals used in the photonic industry.

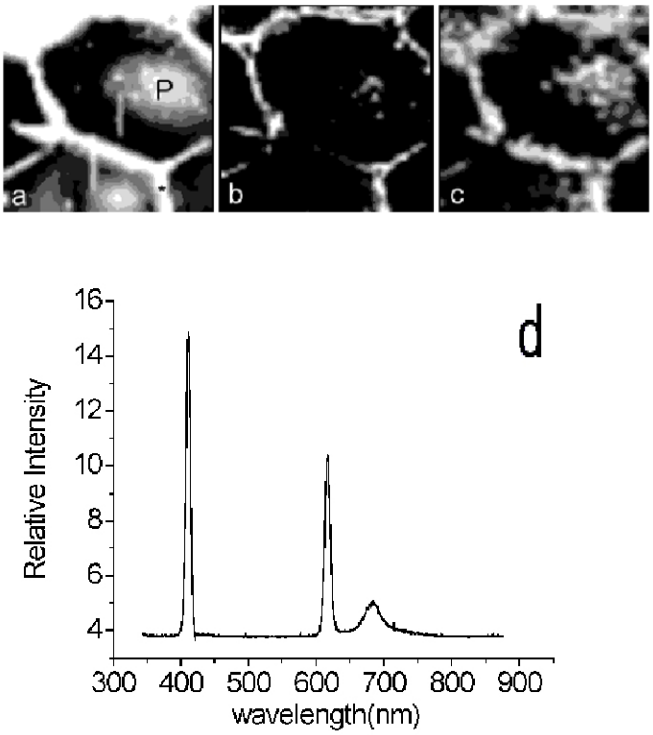

Figure 1. Multi-modality of maize parenchyma cell showing (a) THG, (b) SHG and (c) two-photon excited fluorescence. The bright patch (P) in (a) is the transverse wall of parenchyma cell. (d) Selected spectrum taken from parenchyma cell; the excitation wavelength was 1230nm, the peak at 680nm is two-photon excited autofluorescent, the 615nm and 410nm peaks corresponding the SHG and THG signals.

Third-harmonic (THG) is an X(3) third-order non-linear process. Like SHG, it involves only a virtual state, makes no energy deposition during the conversion process, and thus no photo-damage is expected. Due to the large refractive index difference between the fundamental and THG light and the positive dispersion in biological specimens, effective THG generation occurs only in thin layer or on the interface. This coherent length effect provides the THG process an excellent axial resolution in detecting the surface of cellular organelles, membranes and cell walls.

Simultaneously detecting the multi-photo fluorescence, SHG and THG provide a new way of studying biological specimens. This

type of multi-modality nonlinear microscopy allows co-localization of biologically significant fluorescence signals with structural information corresponding to SHG and THG signals. Figure 1 shows a set of images (THG, SHG and red fluorescence) and a selected spectrum obtained from the parenchyma cells of the maize stem. Recording the spectrum of each pixel in an image ensures positive identification of the origin of signals.

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Genetic diversity among tropical maize inbred lines as revealed by SSR markers

--Laborda, PR, Oliveira, KM, Garcia, AAF, Paterniani, MEAGZ, Souza, AP

Diversity of maize inbreds has a major importance to the process of maize improvement. In Brazil, little is known about the variability of this crop and an analysis of its potential is extremely necessary. The use of codominant molecular markers such as the microsatellites, or Simple Sequence Repeats - SSRs, is an appropriate and useful way of acquiring data about diversity when the genome is being surveyed. This work has the objective of (1) analyzing the diversity among 95 maize inbred lines chosen from the IAC (Instituto Agronômico de Campinas) germplasm bank and (2) dividing them into heterotic groups. This germplasm bank is composed of old lines as well as recent lines, introduced from CIMMYT.

Amplifications were carried out using 50 ng of DNA and a "touchdown" PCR with annealing temperatures varying from 65 C to 55 C. The PCR products were visualized in 4% agarose/metaphor gels stained with ethidium bromide. In order to facilitate the genotyping of each microsatellite, an "allele pattern" was established among 28 inbreds. This pattern could represent the allele diversity and was organized as a molecular weight marker, which was used later for comparison with the other 67 inbreds.

To select the best primer pairs, three sets of tests were performed: the first among three lines, the second among eight lines and the third among 28 lines. The second was the most efficient of all, being able to reveal the polymorphism between the inbreds and not wasting much effort and time. A total of 193 microsatellite primer pairs were tested, and classifications according to amplification quality and genotyping difficulty were done. Most primer pairs had a good amplification quality; nevertheless, many of them were also classified as "difficult" for genotyping, with little difference in the number of base pairs among the amplification products obtained from the 95 inbreds. From the 193 microsatellites tested, 14 were chosen for the diversity analysis.

Results in Table 1 show the number of alleles for each locus studied and its PIC (or genic diversity) values. Among the 14 SSRs, a mean of 5.7 alleles/locus was observed and the mean for the genic diversity was 0.6. These results show the great discriminatory abilities of the loci analyzed and suggest large diversity among the germplasms being studied. The dendrogram presented in Figure 1 shows the genetic distances as provided by the modified Rogers coefficient (TFPGA and NTSYS/UPGMA 2.02 used). This index was chosen due to its Euclidian behavior and due to the fact that it may be compared to other measures of this kind, since it is calculated using means among the loci studied. Further, it considers allelic frequencies, which are extremely important when working with codominant markers. The cophenetic value was 0.57, which indicates that the schematic representation of the dendrogram was not able to show with confidence the distance matrix provided by the index. Moreover, it was not possible to visualize defined groups within the dendrogram, which may be caused by the insufficient number of loci analyzed until now. In order to provide more confident schematic representation, the genetic distances in the distance matrix were grouped and represented in Figure 2. Notably, the majority of the distances are between 0.6 and 0.9, which indicates great divergence among the inbreds.

Table 1. Number of alleles obtained for each SSR locus and the respective PIC values.

Locus	**Number of Alleles**	PIC
bnlg1621b	10	0.8614
bnlg1724	6	0.4676
phi022	3	0.5199
umc1069	8	0.7076
umc1122	4	0.5058
umc1221	9	0.7981
umc1230	6	0.7898
umc1252	2	0.3006
umc1357	4	0.6409
umc1395	3	0.5203
umc1416	2	0.4943
umc1639	5	0.7521
umc1804	15	0.8754
umc1943	4	0.3166
Mean	**5.7**	**0.6107**

The results presented in this work suggest that the tropical germplasm under analysis is an excellent material for hybrid breeding programs. A greater characterization of this germplasm will provide a larger understanding of the potential tropical maize has and will provide quality data for the maize improvement programs in Brazil.

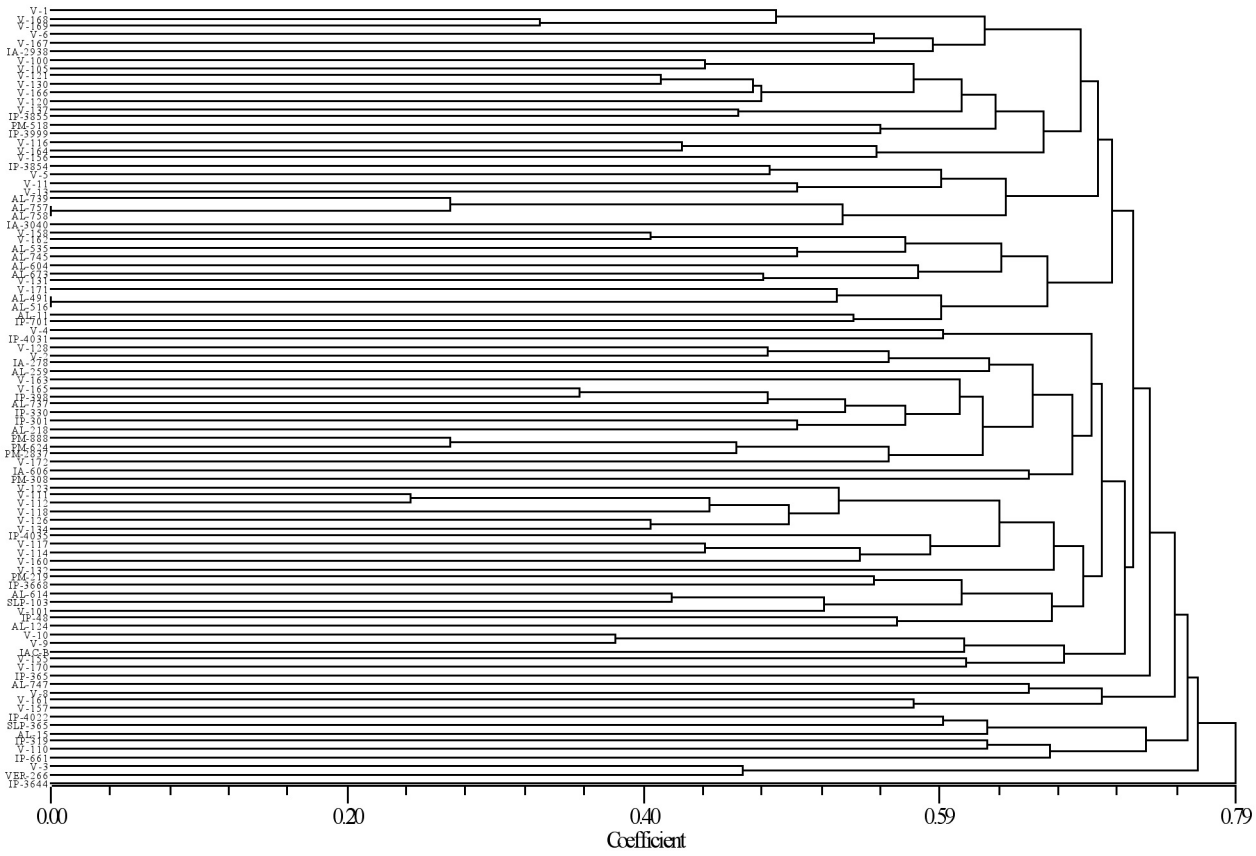

Figure 1. Dendrogram showing the genetic distances according to the modified Rogers coefficient and to the UPGMA clustering method. Cophenetic value = 0.5767.

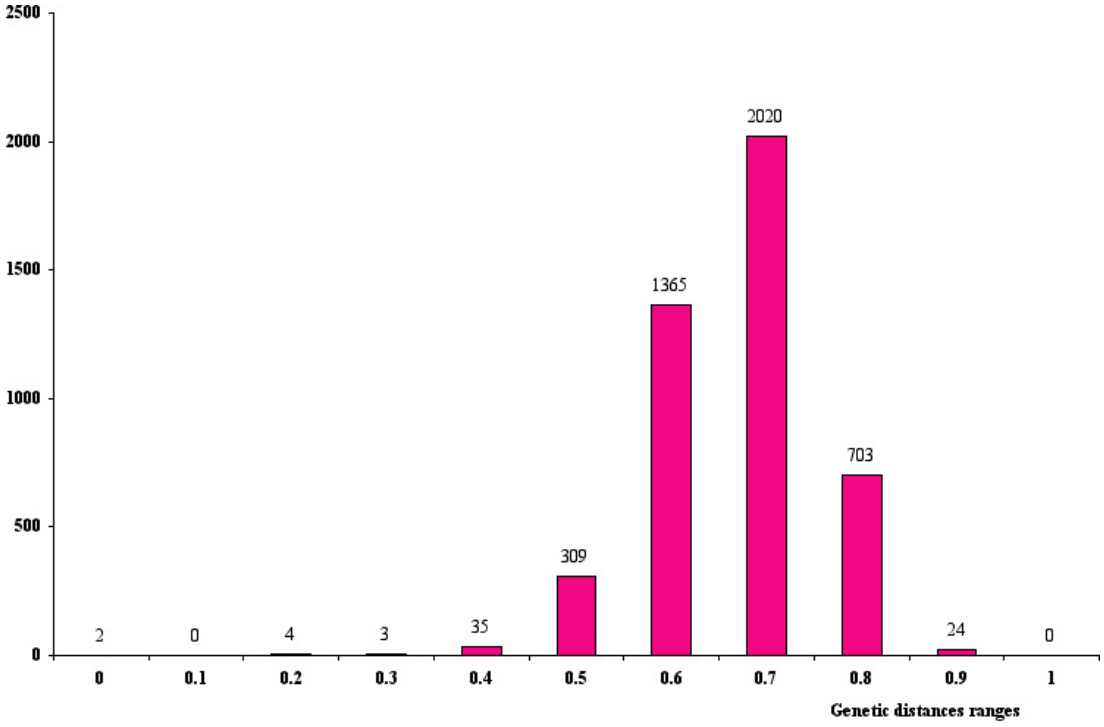

Figure 2. Rate of occurrence of the genetic distances in the distance matrix obtained by using the modified Rogers coefficient.

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More studies of day length effect on cloning gene in tetraploid maize

--Ting, YC, Tran, L

In order to find out how short-day illumination (12 hrs or less) affects the expression of cloning gene in tetraploid maize (*Zea mays* L.), several tests were conducted in the last few years. Descriptions of these tests follow:--In July of 1999, a short-day treatment was applied to 11 adult plants heterozygous for the cloning gene (*Clg clg clg clg*), by covering them with black plastic barrels from 6:00 PM to 10:00 AM the next day. At that time, those plants were a little over three months old and their male inflorescences just began to initiate. The treatment continued for three months. One month after the treatment was discontinued, contrary to expectation, none of the treated plants responded positively by regenerating tassel plantlets. However, three of the control-plants having the same genetic background manifested a weak expression of plantlet regeneration by growing out three to five plantlets per tassel. It was intriguing! When those control-plants showing regeneration were sib-crossed, many well developed kernels were obtained. Therefore, it was deemed necessary to make another test on the transmission of the cloning gene. Subsequently, in early November of the year 2000, 12 selected kernels of one of the above parental plants were sown in the greenhouse. In the first week of February of this year, the same number of kernels of another parental plant were sown next to the first planting. Last May, it was observed that six adult plants of the first planting were healthy and well developed, and four of the second planting did as well. About three weeks later, it was surprising to find that only one of six of the first group and two of the second responded respectively by regenerating vigorous tassel plantlets. Figure 1 depicts one of the tassels bearing many plantlets. This plant was one of the pedigree plants of the fifth generation of cloning gene transmission. In the middle of last June, a further test on the behavior of the cloning gene was carried out. Of each of the above positive respondents, 10 plantlets were detached and planted in the summer experimental plot. The plantlets were at the three- to four-leaf stage in development. In the first week of July, it was found that one of the plantlets grew to about two feet tall and regenerated plantlets. However, in contrast, all of the other plantlets continued to develop into normal adult plants and, on average, they reached about six feet in height two months later. They all failed to regenerate plantlets. The above plantlet transmission experiment was conducted under long-day (more than 12 hrs) condition, from the time of their initiation to the stage of their seed maturation. Up to the present, it appears that a tentative conclusion can be drawn on the inheritance of the cloning gene in the tetraploid, starchy and semiperennial maize. It is dominant. The expression of its phenotype was more likely to be affected by day length; short day was more effective in the induction of plantlet regeneration than long day. Nevertheless, under a short day regime, the stage of plant growth may play a certain role in the sensitivity of the cloning gene to day length.

Figure 1.

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The use of haploid plants for evaluation of population genetic structure

--Rotarenco, VA, Mihailov ME

The utilization of matroclinal haploids in breeding programs and for genetic analysis has become possible based upon studies on the segregation of the haploid population using marker genes (Lashermes et al., 1988; Chalyk, ST and Chebotar, OD, 2000), as well as studies on the correlation between the traits of diploid inbred lines and those of the haploids derived from them (Chase, 1964, Chalyk, ST and Ostrovskiy, VV, 1993). The studies have shown that directed elimination of the genotypes and/or ovule selectivity does not occur when matroclinal haploids are induced, and that the correlation coefficients for most traits between haploids and diploids are quite high. Thus, haploids represent a randomly segregating population and might be employed for genetic analysis and selection of the genotypes possessing economic traits.

Our preliminary findings have demonstrated that recurrent selection involving haploid plants seems to be quite efficient in the improvement of synthetic populations (Chalyk, ST and Rotarenco, VA, MNL 73, 1999). Selection efficiency in this case is associated

with the fact that only additive and epistatic gene effects are expressed in haploids; increasing the frequency of favorable alleles is the basis of the improvement of heterogeneous populations for the traits selected. In our opinion, this specific feature of haploids might be utilized to evaluate breeding and genetic values of populations as well as to assess selection efficiency.

The comparison of haploids demonstrated the importance of additive and epistatic gene effects in the development of the trait. This information might be especially useful in breeding programs and in the prediction of selection efficiency.

The study was aimed at the comparison of the haploid plants derived from different cycles of haploid recurrent selection for some quantitative traits. Simultaneously, we conducted an experiment to assess the genetic results of selection in two diploid populations (SP and SA) for five traits. The goal of the experiment was to obtain estimates of additive variance for each cycle of selection (C0,C1,C2) in both populations. The values of variance among half-sib families in each selection cycle was multiplied by four to estimate values of additive variance.

Table 1 shows the values of five traits of haploids resulting from three cycles in the SP population. Haploids of the later selection cycles (C1 and C2) significantly exceeded those derived from the initial population (C0) for most traits. The findings demonstrated the changes in the population due to selection, i.e. the changes in the frequency of the favorable alleles possessing additive and epistatic effects.

Table 1. Trait means and coefficients of variation in haploids derived from three selection cycles in the SP population.

Traits	Selection Cycles	Traits Means, cm	Coefficient of Variation (%)
Ear Length	SPC0	7.03±0.3	29.2
	SPC1	7.85±0.4***	23.4
	SPC2	7.66±0.3***	25.3
Plant Height	SPC0	47.12±1.8	29.2
	SPC1	50.13±2.1***	26.5
	SPC2	56.47±1.9***	23.2
Height to the ear	SPC0	6.64±0.5	60
	SPC1	7.56±0.8***	70.6
	SPC2	6.76±0.6	64.3
Leaf Length	SPC0	27.19±0.7	20.5
	SPC1	28.67±1.8***	23
	SPC2	29.19±0.8***	20.5
Leaf Width	SPC0	4.07±0.09	16.8
	SPC1	4.15±0.1**	18.6
	SPC2	3.89±0.1	20.3

,* The differences from the haploids of the initial population (C0) are significant at 1% and 0.1% significance level, respectively.

The material was evaluated under extremely adverse environmental conditions for maize production, occurring in Moldova in the year of 2000. There was no rainfall during two months (May and June). This significantly reduced the total yield level of the SP and SA diploid populations (Tables 2 and 3). The unfavorable growing conditions are likely to have caused a considerable reduction in the variation between the initial population and subsequent selection cycles, especially in the SP population.

The values of the additive variance and corresponding coefficients of variation were higher in the initial SPC0 population than in the SAC0 population (Tables 2 and 3). Theoretically, selection would be expected to be more effective in the SP population, but the three years' trials show that selection was considerably more efficient in the SA population compared to the SP population.

Table 2. Trait means, coefficients of variation and estimates of additive variance of three selection cycles of the SP diploid population.

Traits	Selection Cycles	Trait Means	Coefficient of Variation	Additive Variance
Productivity	SPC0	64.4±2.7	25.2	1033.9
	SPC1	64.1±1.7	17.4	365.8
	SPC2	66.9±2.7	19.6	732.3
Ear Length	SPC0	13.3±0.3	12	9.9
	SPC1	14.2±0.15**	6.8	2.64
	SPC2	13.8±0.2	7.8	4.95
Ear Diameter	SPC0	3.8±0.03	5.5	0.17
	SPC1	3.8±0.01	3	0.03
	SPC2	3.9±0.04*	5	0.16
Number of Seed Rows	SPC0	12.7±0.13	6.2	2.2
	SPC1	13±0.1	5.2	1.1
	SPC2	13.6±0.2***	6.7	3.5
Plant Height	SPC0	115.8±2.5	13	1161.9
	SPC1	125.3±1.6	8.5	458.4
	SPC2	138.6±1.7*	6.8	471.4

*,**,*** The differences from the initial population (C0) are significant at 5%, 1% and 0.1% significance level, respectively.

Table 3. Trait means, coefficients of variation and estimates of additive variance of three selection cycles of the SA diploid population.

Traits	Selection Cycles	Trait Means	Coefficient of Variation	Additive Variance
Productivity	SAC0	54.02±1.5	17	264.5
	SAC1	70.5±1.8***	16.2	420.4
	SAC2	65.9±2.3***	23.2	865.7
Ear Length	SAC0	13.8±0.14	6.5	2.6
	SAC1	14.06±0.2	7.7	3.95
	SAC2	14.2±0.15***	7.2	3.7
Number of Seed Rows	SAC0	3.5±0.02	3.7	0.05
	SAC1	3.7±0.02***	3.6	0.05
	SAC2	3.62±0.04**	7.3	0.26
Number of Seed Rows	SAC0	12.2±0.09	4.8	1.04
	SAC1	12.5±0.09*	4.6	0.25
	SAC2	12.5±0.1*	5.5	1.64
Plant Height	SAC0	120.3±1.4	7.5	319.5
	SAC1	123.7±1.2	6.5	249
	SAC2	135.3±1.8***	9	624.3

*,**,*** The differences from the initial population (C0) are significant at 5%, 1% and 0.1% significance level, respectively.

Judging by the additive variance in subsequent selection cycles (C1, C2) of both populations, a supposition might be made that the SP population was losing its potential for further significant improvement, while the variation among the half-sib families was increasing in the SA population, which indicates that further selection might be promising.

The fact that two synthetic populations differ in their responsiveness to the same selection method is a common phenomenon, observed by many researchers. We would like once more to underline some discrepancy between the prediction of the selection results in populations based on the assessment of half-sib families and observed results. Our method of estimation, as well as the extreme environmental conditions of the year could be responsible, but the literature suggests that such results have been quite usual.

Coefficients of variation for plant height and ear length of the haploid population were greater than those of the half-sib families in the same selection cycles. Since genetic variation in the haploid population was expressed mostly by the non-allelic gene effects. This significant difference between the haploid and diploid popu-

lation is likely to be the main reason for the discrepancy mentioned above. Based on the result of this comparison we may suggest that the variation among the half-sib families in each selection cycle in this case, did not quite reflect the variation among the genes possessing non-allelic effects. Hence, the additive variances obtained due to this variation could not reflect the differences for these gene effects between both populations and selection cycles in these populations.

In our opinion, a comparative analysis of populations at the haploid level to determine their genetic structure and to assess changes occurring in them might be promising. The necessity of special crossing for progeny testing is not necessary. A simple comparison of the values of haploid traits might provide the information on the efficiency of the selection, while the variations of the traits might prompt whether further selection is promising.

Additionally, haploid plants may be used to make it easy to obtain estimates of the heritability in the narrow sense. Haploids, as has already been mentioned, do not possess intra-allelic gene interactions and effects are based only on the additive and epistatic contributions of alleles. In haploid populations, narrow and broad sense heritabilities are equivalent in the absence of epistasis.

In our viewpoint, use of haploid plants might make the studies in population genetics significantly easier.

The supposed pleiotropic effect of the *p1* locus

--Mihailov, ME, Chernov, AA

In F2 plants of two maize hybrids (Chernovitskaya 21 x 2-9m and Ku123 x 2-9m) the color of dry tassels was recorded a month after flowering. The 124 F2 plants of Chernovitskaya 21 x 2-9m were classified: 85 - dark tassel, 35 - light tassel, 4 - doubtful. The 88 F2 plants of Ku123 x 2-9m: 58 - dark tassel, 26 - light tassel, 4 - doubtful.

It was revealed after harvest that the dry tassel color correlates with the color of cob. Dark tassel corresponds, as a rule, to red cob, and light tassel to white cob (Table 1-2).

Table 1. Segregation for color of cob and dry tassel in F2 (Chernovitskaya 21 x 2-9m).

Colour of dry tassel	Red cob	White cob
dark	82	3
doubtful	3	1
light	1	34

Table 2. Segregation for colour of cob and dry tassel in F2 (Ku123 x 2-9m).

Colour of dry tassel	Red cob	White cob
dark	57	1
doubtful	2	2
light	2	24

As is seen in the tables, there are recombinant plants. Two versions are possible:

1) The dry tassel color is caused by a locus closely linked (rf=3-4%) with the *p1* locus controlling cob coloration;

2) There is a pleiotropic effect of the *p1* locus and recombinants appear because of modification or classification error.

Maize embryo culture as a test for productivity

--Chernov, AA, Mihailov, ME

The 6 inbred lines of the maize were tested in embryo culture with hormonal treatment. The embryos with scutella have been excised from the grains to remove the influence of endosperm, and then were grown in agar medium with 4-amino,3,5,6-trichlorpicoline acid, 1 mg/l. The seedlings were analysed for coleoptile length, coleoptile weight and weight/length ratio. For each trait these lines were grouped into 3 classes: high (3), middle (2) and low (1). The seedling phenotype was compared with productivity (Table 1).

Table 1. Productivity and embryo culture phenotype of maize lines

	Phenotypic classes				
Line	coleoptile length	coleoptile weight	weight/length	Marginality	Productivity, gm/plant
A619	2	2	2	0	75+11
MK109	1	1	3		113+2
MK133	2	3	3	0.67	80+6
MK196	2	2	2	0	34+4
W32	2	1	2	0.33	69+4
W64	3	3	1	1	95+6

It is proved that the lines of highest productivity are marginal for seedling traits, and the line of lowest productivity belongs to the middle classes. The marginality parameter was deduced from the three seedling traits. The Spearman rank correlation between marginality and productivity is 0.89 ($P<0.05$). This dependence is explicable since biosynthesis and organogenesis are based on hormonal reactions.

New scheme of haploid recurrent selection in maize

--Chalyk, ST, Rotarenco, VA, Bylich, VG

In a previous publication, we suggested utilization of haploid plants in recurrent selection (Chalyk and Rotarenco, MNL 1999). Subsequent testing of synthetic populations has shown high efficiency of haploid sib recurrent selection (HSRS) (Chalyk and Rotarenco, Rus J Genet 2001).

In the scheme we used, selection was only carried out for haploid plants. We presume this allowed an increased frequency of genes with nonallelic interactions.

In order to create high-yield maize hybrids, it is necessary to select genotypes with all types of gene interactions contributing to the yield formation. Genes with allelic interactions should be combined with nonallelic ones. To improve synthetic populations more efficiently and to increase frequency of genes with both allelic and nonallelic interactions, a new scheme of haploid recurrent selection, haploid & diploid recurrent selection (H&DRS), is used in the Institute of Genetics, Moldova. In this scheme, selection of haploid plants is combined with selection of diploid plants. Diploid plants are selected based on their combining ability. The new scheme, H&DRS, is a two-step scheme, the same as HSRS.

For comparison purposes, below we present both schemes of recurrent selection – HSRS and H&DRS.

HSRS scheme:

Step 1: Haploids are obtained from a synthetic population.

Step 2: The haploids are pollinated with pollen taken from diploid plants of the improving synthetic population. Selection is carried out only for haploid plants.

The new scheme, H&DRS, differs from HSRS in that in Step 2 diploid plants are used twice. First, diploid plants serve as a source of pollen for haploids. Second, combining ability of diploid plants is tested. In order to test the combining ability, diploid plants from the improving synthetic population are self-pollinated and at the same time crossed with a tester. Hybrids obtained as a result of crossing are tested in Step 1 of the following cycle of

selection.

H&DRS scheme: First Cycle of Selection

Step 1: Haploids are obtained from a synthetic population.

Step 2: a) The haploids are pollinated with pollen of diploid plants of the same synthetic population. The best haploids are selected.

b) Diploid plants, which serve as a source of pollen for the haploids, are self-pollinated and crossed with a tester. The tester is the maternal component of the cross.

Second Cycle of Selection

Step 1: a) New haploids are obtained from the population grown from seeds of the haploid plants selected in the previous cycle of selection.

b) Hybrids are tested, which were obtained in the previous cycle of selection.

Step 2: a) Haploids, obtained in Step 1, are pollinated with a mixture of pollen taken from progeny of the diploid plants, which displayed high combining ability. The best haploids are selected.

b) Diploid plants, which are a source of pollen for haploids, are self- pollinated and crossed with a tester in order to assess their combining ability.

Subsequent selection is performed the same as the second cycle of selection. The advantage of the H&DRS scheme over conventional recurrent selection schemes is that it allows the intensive selection of genotypes with additive and epistatic effects at the haploid plants level. Genotypes with high effects of overdominance and dominance are selected at the diploid level based on testing of hybrids. When haploids are pollinated with pollen of diploid plants, genes with high effects of overdominance and dominance are added to those with high additive and epistatic effects. The combination of these types of gene interactions must ensure high efficiency of the synthetic population improvement.

Determination of the combining ability of diploid plants in the H&DRS scheme is quite common for a recurrent selection. Lines, synthetic populations or hybrids can be used as a tester. In our work, a reciprocal version of the H&DRS scheme is used. The work is conducted with two synthetic populations – SP and SA. When combining ability is tested, they serve as each other's tester.

Diploid plants, which are selected for their combining ability and are used as a source of pollen for haploid plants, are assessed and selected based on many other traits. Before their pollen is used to pollinate haploids, they are selected based on their maturity group, resistance to lodging, resistance to diseases, etc.

When haploid plants are used, it is possible to obtain homozygous lines rapidly. During each cycle of selection, we treat a portion of haploid plants with colchicine in order to double their chromosome number. Thus, each cycle of the selection improves synthetic populations and at the same time produces homozygous lines by an accelerated method.

The authors believe that both HSRS and H&DRS schemes will be useful for maize breeders.

The influence of the temperature, sufficient humidity and illumination duration on the (*Cg2*/+) hybrid expression

--Rusanovskaya, EG, Krivov, NV

The expression of heterochronous mutations depends on growing conditions. The reaction of the plants carrying the *Cg* gene to the differences in the light period duration has been first observed by W.R. Singleton (MNL 23, 1949). We evaluated the *Cg2* gene expression by the male inflorescence which has had no branches in mutant plants and was only represented by a central axle. Besides, we measured plant height, panicle length, shoot, node and producing ear corn numbers, leaf length and width, and ear corn diameter in the maize hybrid plants.

As we expected, 100% penetration of the *Cg2* gene was observed in the Cg2-220 x *sr1 bm2*, Cg2-220 x *lg1 gl2 v4*, *lg1 gl2 v4* x Cg2-220, and *y1 pb4* x Cg2-143-41 combinations grown in both the field and greenhouse. Ordinary branched panicles were only observed in a significant part of the plants in the *+o2 gl*/Cg2-220 + + hybrid, which did not differ from the common maize phenotypically (Table 1), i.e. growing of hybrid offspring in the greenhouse contributes to the increase in percentage of plants with the heterochronous Cg2 phenotype.

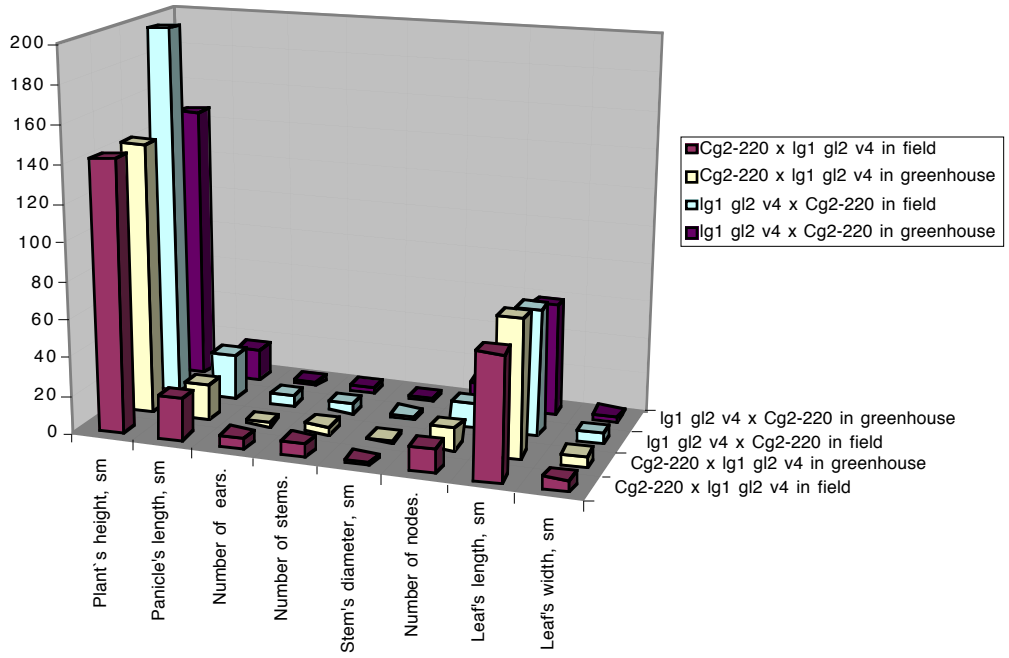
Table 1. The influence of growing conditions on the *Cg2* expression combination.

	Combination	wt	Cg2	% wt plant
Field	Cg2-220 x o2 gl	131	14	86.2
Greenhouse		6	26	17.8
Field	o2 gl x Cg2-220	39	23	62.9
Greenhouse		10	11	47.6

The hybrids grown in the greenhouse had few tillers with shorter and narrower leaves and shortened panicles and reduced plant heights (Graph 1). Graph 1 shows that the cytoplasm of the maternal line changes the distribution pattern of these traits in hybrid plants, i.e. if a marker line is used as a maternal plant, the hybrid offspring are taller and less tillered.

Three major factors, including temperature, efficient water supply and light period duration, influenced the formation of the maize hybrid plants. A four hour illumination in the greenhouse significantly increased the winter daylight. Therefore, the impact of this factor on the reduction of the hybrid plants in the greenhouse was insufficient. A low temperature in the greenhouse making 20 C2 at a sufficient water supply can contribute to the modification of quantitative traits in maize hybrids, which are largely heterozygous for *Cg2*.

Cytoplasm's role in the hybrids reactions on the cultivating conditions (field and greenhouse)



Postradiation effect of the electric field

--Ikhim, YG, Bliandur, OV, Lysikov, VN

Sufficiently many works have been carried out to assess the influence of electric fields on the plant organism. But the specific action of this factor during the postradiation period has not attracted close attention. While studying the impact of the electric field in the radiation mutagenesis we have come across the expressions of the effects both stimulating repair systems and enhancing the radiation damage.

We have set an experiment involving the radiation of 7-day-old seedlings of the 19-3-3 maize line to study these phenomena. A dose of 6 Gy, which is sublethal for seedlings, was applied for a control effect. The radiation was accomplished through a hard γ-irradiation using a PXM-γ-20 unit. Following a 10 min radiation, the Petri dishes containing the seedlings were exposed 1, 5, and 10 min to a 2 kW electric field. Rootlets were fixed to estimate chromosome aberrations and the findings were compared with control, in which the seedlings were not exposed to postradiation treatment. The rootlets were fixed at different periods, immediately after the treatment (0 min), after 30 min and 90 min to assess the dynamic action of the repair systems.

The number of chromosome aberrations, %

Treatment	Fixation time		
	0 min	30 min	90 min
Control - 6Gy	48.36	48.09	48.62
1) 6Gy + 1 min EF	51.14	49.02	46.43
2) 6Gy + 5 min EF	62.70	63.97	65.29
3) 6Gy+10 min EF	71.08	71.54	71.85

The findings are summarized in the table. All the treatments increased the chromosome aberration with respect to the control.

Effect of high and low temperature on male gametophyte viability of waxy maize

--Kravchenko, OA, Kravchenko, AN

The aim of this study was to reveal the effect of high and low temperature on male gametophyte viability at the pollen germination stage and pollen tube growth stage. Inbred lines 346 and 502 and their waxy counterparts, as well as their MR5 progeny (obtained from irradiated immature embryos in vitro), were taken as experimental material. Fresh collected pollen from each genotype was planted on the nutrient medium (developed by Cook F.S., Walden D.B., Can.J.Bot. 43:779-786) and subjected to low (+12 C, for 6 hours) and high (+35 C, for 20 minutes) temperature treatment. At the same time, pollen of control variants was cultivated in normal (+24 C) temperature conditions. On average, 500-700 pollen grains from each genotype were analyzed to determined pollen viability.

A significant variability of male gametophyte viability among plants of the MR5 generation was found. The reliable effect of genotype and temperature was revealed by two factor analysis of variance (Table 1). Results indicate that pollen viability of MR5 progeny of inbred lines 346 and 502, as well as that of their waxy counterparts, was genotype dependent. It should be noted that

Table 1. Genotype and temperature effect (%) on male gametophyte viability.

	Factors:		
MR5 progeny of inbred lines:	genotype	temperature	their interaction
346+/+	50.12***	7.41***	41.62***
346wx1wx1	55.34***	15.8***	23.57***
502+/+	47.02***	24.6***	26.43***
502wx1wx1	52.31***	17.7***	22.12***

***- P<0.001

pollen viability of MR5 progeny of inbred line 502 was more temperature dependent in comparison with MR5 progeny of other inbred lines. Generally, low and high temperature treatment resulted in a decrease of maize pollen viability. However, among the genotypes under analysis there were some which demonstrated high male gametophyte viability in both low and high temperature conditions.

Production of matroclinous maize haploids following natural and artificial pollination with a haploid inducer

--Rotarenco, VA

Studies carried out recently have significantly contributed to the solution of the main problem of haploidy in maize, which means the possibility for wide production of haploid plants. Mass production of haploids has become possible, mostly due to the development of efficient genetic inducers, which make it possible to obtain matroclinous haploids from practically any genotype (Tyrnov and Zavalishna, 1984; Zabirova, 1996; Chalyk, 1999).

We observed earlier that the percentage of the haploid yield varied somewhat following natural and artificial pollination with inducer pollen. Therefore we set a task for the current year (2001) of comparing the percentage of the seeds with a haploid embryo following natural and artificial pollination with an inducer of matroclinous haploids.

Two homozygous lines (092 and Rf-7) and a synthetic population (SA) were pollinated with the pollen of MHI line (Moldavian Haploid Inducer) (Chalyk, MNL 73, 1999). The material was grown also in an isolation plot, where it was pollinated with pollen of the MHI line.

The results presented in the Table show that, following artificial pollination, the percentage of the seeds possessing a haploid embryo in the inbred lines is more than twice as high in comparison with natural pollination at the isolation plot. A significant difference between the natural and artificial pollination by this criteria is also observed in the heterogeneous population.

Table 1. Percentage of haploids after natural and artificial pollination.

Genotypes	Number of seeds with haploid embryos, %	
	Natural pollination	Artificial pollination
092	3.8	8.8
Rf-7	2.3	6.0
SA (population)	2.5	4.3

The reason for the difference in the haploid yields was, most probably, a delayed pollination in this case. The delayed pollination is known to be one of the techniques of producing matroclinous haploids in various crops, including maize. Pollination with the inducer pollen was carried out when a mass appearance of stigmata occurred, as is usually done. In our opinion, this delay caused an increase in the haploid yield in comparison with the material grown in an isolation plot. It should be noted that the inducer line was planted three times with a 10 day interval in the isolation plot to increase the period of pollen availability. Thus, the ears of the material under study were able to be pollinated during the whole period of their flowering.

Accordingly it has been established that the delayed pollination might contribute to a significant growth of the matroclinous haploid yield in maize when haploid inducers are used.

Study of the phenomenon of antidromy with diploid and haploid maize

--Rotarenco, VA, Maslobrod, SN

The phenomenon of antidromy, which is noticed with many species of plants and particularly well marked in grasses, was described by G. Maclosky (1895; 1896). The antidromy phenomenon consists of the periodic change of spirally - rolled up leaf tubes counterclockwise (leftness) and clockwise (rightness) with every new stalk's node. According to the data by Sulima (1970) left (L) and right (D) leaves of most gramineous plants alternate on the stalk with exceptional consistency. The exception to the antidromy rule is maize.

Together with the right antidromy plants (symmetrical) there are some plants of maize with an infringed antidromy (asymmetrical). The latter have an infringed alternation of L and D leaves on the stalk's nodes, which determines leftness and rightness of plants: L-plants appear to have an abundance of L-leaves, frequently repeating on several nodes after the other; D-plants on the contrary were noted to have a superfluous forming of D-leaves also repeating on several successive nodes. Zone of antidromy infringement is situated as a rule on the nodes from an ear to the panicle of a maize plant (Sulima,1970).

We wished to determine the amount of infringed antidromy (asymmetrical plants) on the diploid and haploid levels. We studied the character of alternation of L and D leaves on the nodes from the ear to the panicle. Diploids and their haploid analogues of two homozygous lines (092 and Rf-7) and also the synthetic population (SP), no less than 200 plants of every variant, were used. The results are presented in Table 1.

Table 1. Part of asymmetrical plants (with an infringed antidromy) on diploid (2n) and haploid (n) levels.

Genotype	Number of asymmetrical plants, (%)	
	2n	n
092	6.8	37.0
Rf-7	6.6	27.7
SP(population)	25.4	41.5

Sulima supposed that the infringements of antidromy with maize were not only harmless or indifferent for the species, but were even useful. According to his hypothesis, antidromy infringements on a maize stalk are a sort of filter regulating the flow of metabolites between the panicle and the ear. This filter should be considered as an evolutionarily expedient mechanism of regulation of the direction of the main flow of metabolites with its reorientation to the middle of the stalk, where the main consumer of substances, the ear is situated. Sulima and colleagues have analyzed 12 male (without ears) and 9 female (without panicles) forms of maize. Male forms had 30% and female had 92% with infringed antidromy. Such a high density of infringements is characteristic only for female plants (Sulima, 1970).

Based on Sulima's hypothesis it can be supposed that an increase of the number of dissymmetrical plants among haploids is caused by the fact that their panicles are practically completely sterile, but the ears are partially fertile. Through this the flow of metabolites in haploids blockades by means of antidromy infringements and reorientates mainly to the ears. In our work we received a confirmation of Sulima's hypothesis comparing numbers of asymmetrical plants in cycles of selection in the SP population. For the improvement of this population we use haploid recurrent selection (Chalyk and Rotarenco, MNL 1999). The results ob-

tained on the diploid and haploid level are given in Table 2. An increase of the number of plants with an infringed antidromy in the cycle of selection takes place in both cases. Thus together with an increase of the ear's parameters (productivity) in our case, an increase in the number of asymmetrical plants takes place in the population.

Table 2. Part of asymmetrical - left (L) and right (D) plants in the cycles of selection on diploid (2n) and haploid (n) levels in SP population.

Cycles of selection	(2n)			(n)		
	Asymmetrical plants,%	Of them		Asymmetrical plants,%	Of them	
		L, %	D, %		L, %	D, %
C0	16.2	43	57	36.2	53	47
C1	22.2	45	55	44.1	43	57
C2	32.9	30	70	36.0	37	63
C3	30.1	36	64	49.1	44	56

In the work by Maslobrod and others (2000, 2001) on tomatoes, it was shown that L-plants had a 15-20% higher yield than D-plants. In the work by Sulima (1970) D-plants of maize hybrids were more productive. In our investigation all the plants with an infringed antidromy were also divided into L and D-plants. It was determined that the number of D-plants with reference to L-plants, both on the diploid and haploid levels, increases in cycles of selection (Table 2). Thus the selection for yield leads to an increase in the number of D-plants with reference to L-plants. This indirectly confirms the fact that D-plants have a greater yield. Also a comparative analysis of quantitative traits (Table 3) has shown that D-plants on the diploid level essentially exceed L-plants for plant traits. On the haploid level, the differences in the traits studied are not essential. It is connected with the fact that haploids practically don't have correlations between traits of a plant and the ear (Rotarenco, MNL 2000).

Table 3. Parameters of quantitative plant's traits of L and D-plants in SP population on diploid (2n) and haploid (n) levels.

Traits	(2n)		(n)	
	L-plants	D-plants	L-plants	D-plants
Plant height, cm	181.7	197.6***	85.3	87.9
Height to the ear, cm	55	59.4**	18	17.7
Leaf length, cm	63.6	69***	34.4	35.6
Number of nodes	11.1	11.3*	8.1	8.5

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

Some additional investigations should be carried out. However, the first experiments have already shown the certain connection between the antidromy infringement and maize productivity.

The influence of preplant γ–ray treatment of maize hybrids on bioisomery of the germs, grown from pairs of adjoining seeds. Electromagnetic mechanism

--Maslobrod, SN, Ganea, AI, Corlateanu, LB, Romanova, IM, Lysikov, VN

It has been shown that from a pair of maize seeds with adjoining germs, reflection symmetric germs predominate (RSG): a left germ (*l*) grows from one seed of the pair, and a right germ (*d*) grows from the other seed. The phenomenon depends on the genotype, physiologic state of the components (seeds and germs) of the pair, environmental temperature, and electromagnetic fields. In the previous experiments, a different physiologic state of the components of the pair was achieved by differential swelling (Maslobrod, SN, Shabala, SN, Tretiakov, NN, 1994; Maslobrod, SN, Shabala, SN et al., 1994; Maslobrod, SN, 2001).

The results of experiments where the differences in the state of the seeds of the pair were achieved by γ-ray treatment of the seeds are given in this report. Initially, hybrid maize Moldavian 450 seeds were treated with a mutagenic dose (500Gy), which results in a drastic decrease of seed germination capacity and germ growth rate. The seeds were planted in pots filled with loamy black earth. The seeds were kept at 24 C during the daytime and at 20 C during the night. At the 15th day the height of the germs was measured, and their bioisomery was determined. The *l* germs have the first leaf rolled counter-clockwise, and the *d* germs have the first leaf rolled clockwise (Maslobrod SN, Shabala SN, Tretiakov NN, 1994). The number of RSG pairs (*ld+dl*), and the number of pairs with the same sign of bioisomery (*ll* and *dd*) were counted. The number of repeats for each variant is no less than 100. The variants of the experiment are: 1) normal single seeds (N), 2) irradiated single seeds (γ), 3) a pair of N seeds, with adjoining germs and situated horizontally one after another (NN), 4) similar pair of γ seeds (γγ), and 5) mixed pair (N γ). As appears from Table 1, the height of the germs of the NN pair is significantly above the control (N), which is a result of the interaction of germinating seeds (group effect - Shabala, SN, Maslobrod, SN, 1978; Titov, YV, 1978). In the γ γ pair stimulation of the germ growth is absent in comparison with the γ variant. It is also absent in the N γ pair. Thus, N seeds do not affect γ seeds in respect to germ growth, probably, because of the high radiation dose. The total number of the germs remains practically unchanged in comparison with the control N, except for the NN variant. Another criterion of pair seed interaction – the number of RSG (*ld+dl*) has the following pattern. The number of RSG in NN pairs is significantly above the control (i.e. RSG with no interaction of pair seeds, which is 442.5% for the given variant - Maslobrod SN, Shabala SN et al., 1994). The number of RSG in the pure pair γ γ is slightly decreased, and in the mixed pair Nγ it exceeds the level of RSG of the NN pair. Thus, some interaction between N and γ seeds in the mixed pairs is observed; it is manifested by the change of germ geometric parameters, and not growth parameters. We had observed the intensification of the reflection symmetry effect earlier, in the case of different physiological activity of the components of the pair (Maslobrod, SN, Shabala, SN, Tretiakov, NN, 1994; Maslobrod, SN, Shabala, SN et al., 1994; Maslobrod, SN, 2001). It is worth noting that in pure and mixed pairs with participation of γ irradiated seeds the number of *ll* pairs increases. Is this a coincidence or a regularity? Is there a new type of interaction of germinating seeds involved? A test experiment on estimating the pattern of *ld+dl, ll* and *dd* pair formation from normal seeds, with the germs adjoined at different spatial orientation, horizontal (one after the other) and vertical (one above the other), was carried out. Additional variants with filters were used: plates of organic glass up to 1 mm thickness were placed between the seeds. Thus water-ionic (chemical) communication channel between seeds and communication channel by electric current were excluded, and the communication channel by seed electromagnetic fields was kept (Shabala SN, Maslobrod SN, 1978). The corresponding horizontal variants with no filter (NN), with filter (N|N) and vertical without filter (N) and with filter (N̲) are given in Table 2.

N N

Table 1. Growth and bioisomeric characteristics of maize hybrid Moldavian 450 germs when the seeds were treated with γ radiation dose 500 Gy

Variant of the experiment	Component of the germ pair	Germ height, mm	Number of l germs, %	Number of bioisomeric germ pairs, %		
				ld+dl	ll	dd
N	N	84.21.5	64.7			
		46.62.3*	61.1			
N N	N	95.62.5*	42.5	55.0	15.0	30.0
		50.63.4*	64.7	52.2	31.8	13.0
N	N	79.24.0	66.7	61.0	31.7	7.3
		43.32.7*	61.0			

* Differences from the control at 5% significance level

Table 2. Number of bioisomeric germ pairs of maize hybrid Moldavian 450 when seed pairs where arranged in horizontal and vertical directions with filter and with no filter, %

Variant of the experiment	ld+dl	ll	dd
N N	63.6	19.5	16.9
N|N	60.8	26.6	12.6
N N	55.4	23.5	21.1
N N	61.4	19.6	19.0

A new phenomenon was found: the RSG effect does not depend on the pattern of contacting seeds in horizontal or vertical directions. As can be seen from Table 2, the number of RSG in all variants is significantly above control, i.e. more evidence that the RSG effect appears to be due to the interaction of electromagnetic fields of germinating seeds was obtained. It expands our concept about the types of interactions between seeds and germs with the above-mentioned spatial orientation (Maslobrod SN, 2000). The number of *ll* and *dd* pairs is approximately equal, except for the N/N variant. Thus, in the pure pairs of N seeds only one electromagnetic mechanism of RSG formation due to the combination of left-hand helical and right-hand helical fields of the seeds functions. The above-mentioned variants were used for checking up an additional mechanism of *ll* pair formation in mixed pairs. An object in which the mechanism of RSG formation is absent was chosen, in order to increase the purity of the experiment. Such an object turned out to be maize hybrid Moldavian 291 (in other cases the RSG effect can be observed in this hybrid - Maslobrod, SN, Shabala, SN, Tretiakov, NN, 1994; Maslobrod, SN, Shabala, SN et al., 1994). In our case the number *of ld+dl, ll* and *dd* pairs in Moldavian 291 was, correspondingly, in %: 49.2; 25.6 and 25.2. In addition, another seed radiation treatment dose was chosen, the rate of germ growth does not decrease at this dose, and their elimination does not occur (100 Gy). According to Table 3, the number of RSG in all variants of seed pattern was at the control level (442.5%). Thus, the above mentioned mechanism of RSG

Table 3. Number of bioisomeric germ pairs of maize hybrid M291 when normal and irradiated with 100 Gy seeds were arranged in horizontal and vertical directions with filter and with no filter, %

Variant of the experiment	ld+dl	ll	dd
N	45.4	36.4	18.2
N	45.6	31.1	22.8
N	42.2	35.1	22.7
N	43.9	38.5	17.6
N	45.8	34.4	19.8
N	38.1	36.1	25.8
Mm	43.51.2	35.31.0	21.21.3

formation in Moldavian 291 seeds was not detected. At the same time the number of *ll* pairs is significantly larger than that of *dd* pairs in case of both direct contact of seeds through the germs and indirect contact through the filter. We tend to think that here an additional mechanism of interaction of germinating seeds, also of an electromagnetic nature, is clearly manifested. This mechanism is absent in the pairs of seeds, the physiological activity of which was achieved by adequate factors, such as natural aging of seeds and different term of their swelling, and is manifested under the action of specific factors, such as radiation. In conclusion, we can note that the formation of the symmetry of two germinating seeds and germs, apparently is carried out by electromagnetic fields, not only of different spiralization signs (left and right), but also of the same signs (left and left). Probably, this is a demonstration of the principle of asymmetry of living and non-living nature (Hehstrom, RA, Condeputy, DK, 1990).

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Maize pollen expresses a truncated version of the transcriptional coactivator Ada2

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Transcriptional adaptors and coactivators play important roles in the process of transcription by RNA polymerase II. A genetic screen in yeast led to the identification of the putative adaptors ADA2 (Berger et al., Cell 70(2): 251-65, 1992) which had properties consistent with an adaptor role in transcriptional activation. The Ada2 proteins of different species have been shown to potentiate transcription in vivo and may function as adaptors to bridge physical interactions between DNA-bound activators and the basal transcriptional machinery (Candau et al., J Biol Chem 271(9): 5237-45, 1996). All Ada2 sequences isolated so far share an N-terminal zinc finger motif and a sequence related to the myb DNA-binding domain, as well as a C-terminal Ada3 interaction domain. It has been shown for different species that the N-terminal region of the Ada2 protein is sufficient for an in vitro interaction with Gcn5. Arabidopsis was the first organism for which two Ada2 genes were identified (Stockinger et al., Nucleic Acids Res 29(7): 1524-33, 2001). It was speculated that the two genes may be expressed in different cell types within leaves, roots or other tissues.

We have previously reported on the isolation of central components of the yeast GCN5/ADA2 complex from maize (Becker, MNL 73:22, 1999).

Further analysis of ZmAda2 showed it is present as a small gene family of very closely related sequences (unpublished sequencing results) in the maize genome. Using a probe consisting of the entire cDNA derived from endosperm mRNA on Northern blots, we detected a 2.0 kb mRNA species in all tissues examined except for pollen, which expresses a 1.7 kb version.

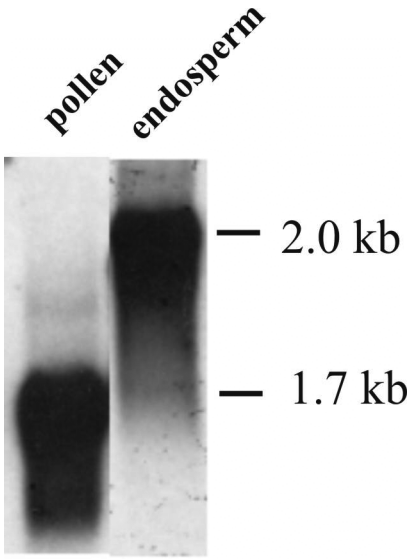

Figure 1. Northern blot analysis of ZmAda2. A 1.7 kb mRNA is detected in pollen, whereas in endosperm (and other tissues examined) a 2.0 kb mRNA is detected.

We isolated clones corresponding to the smaller ZmAda2 transcript from a maize pollen cDNA library, and found the pollen transcript (ZmAda2-Pollen) lacks the N-terminal region of the longer, somatic ZmAda2 transcript. The full length ZmAda2 encodes a 565 amino acid long polypeptide, whereas ZmAda2-Pollen, encodes a 387 amino acid long polypeptide, which, apart from being truncated, is identical to the ZmAda2 protein sequence. With an anti-ZmAda2 antibody we are able to detect both predicted proteins in the corresponding maize tissues. ZmAda2-Pollen lacks both the N-terminal zinc finger motif and the myb DNA-binding-like domain. Both full-length and truncated Ada2 proteins were expressed as GST-fusions in *E. coli*. While full-length ZmAda2 is able to interact with ZmGCN5 in yeast two-hybrid assays, as well as when expressed in *E. coli* and used in a pull-down assay, ZmAda2-Pollen is not able to interact with ZmGCN5, presumably because of the missing N-terminal domain. Conceivably, ZmAda2-pollen might act as a dominant-negative factor to inhibit targeted histone acetylation by GCN5 or other coactivator activities, in the pollen cells. This might also be related to the production of histone variants in pollen. Further experiments are directed at the possibility that the truncated ZmAda2 protein might influence chromatin organization during pollen maturation.

COLOGNE, GERMANY
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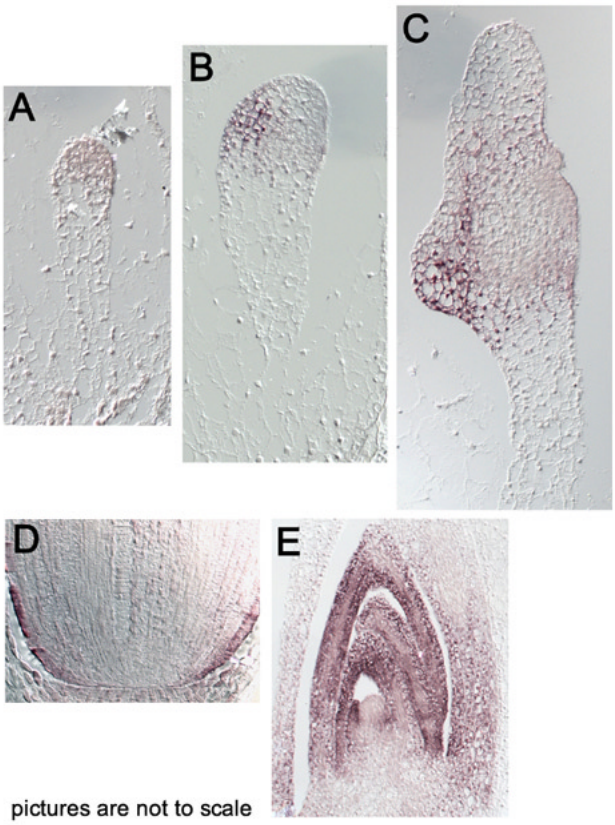
Dynamics of *Shrunken1* (*Sh1*) transcriptional activity during maize embryogenesis

--Bommert, P, Werr, W

We have investigated the expression pattern of the *Sh1* gene at different stages of embryogenesis to test its suitability as a molecular marker for pattern formation. As a probe DIG-labeled antisense RNA was generated using the unique 3'UTR of the *Sh1* mRNA as a template. We have never observed any *Sh1* activity in the suspensor of the developing maize embryo.

Sh1 transcripts are absent, or below the detection level in the embryo proper during the proembryo-stage (Fig. A). In the



Figures A-E.

transition-stage embryo, *Sh1* transcripts accumulate in the abaxial hemisphere of the embryo-proper. These *Sh1* expressing cells contribute to the development of the scutellum and rapidly enlarge at the end of the transition stage. This cellular enlargement is the main mechanism responsible for the outgrowth of the scutellum (Elster et al., Dev. Genes Evol. 210:300-310, 2000). In contrast, cells located adaxially in the region of the SAM-anlage stay compact and remain free of *Sh1* activity (Fig. B). A series of longitudinal sections through coleoptilar-stage embryos show that the *Sh1* transcriptional activity is not equally distributed throughout the scutellum, but a patchy pattern appears to be associated with domains of rapid cellular enlargement (Fig. C).

At later embryonic stages, *Sh1* is downregulated in the elaborated scutellum, whereas expression at high levels is now detected in the root/shoot axis. Strong expression is found in the rhizodermis, but lower *Sh1* transcript levels are observed in the central cylinder of the root. No *Sh1* transcripts are observed in the root meristem (Fig. D), which is similar to the embryonic shoot apical meristem. Both primary meristems are therefore essentially free of *Sh1* activity although cells in the meristems strictly depend on the import of carbohydrates.

In the shoot, high *Sh1* transcript levels are found in the epidermal layer of developing leaves. This *Sh1* activity in embryonic leaves is not restricted to the epidermis but extends into the parenchymal cell layer, although at a lower level relative to that in the epidermal layer, additionally weak expression can be detected in expanding coleoptile (Fig. E). Activation of the *Sh1* promoter in maize is thus linked to the initiation of leaf primordia as has been

described for the tomato SAM (Pien et al., Plant J. 25:663-674, 2001).

In conclusion, transcriptional activity of the *Sh1* gene is highly dynamic during embryogenesis. The absence of *Sh1* transcripts in the primary root and shoot meristems with high rates of cell proliferation argues against a simple sink-based control. In contrast, the obvious link between cellular enlargement and *Sh1* transcription during scutellum development, supports a direct contribution to cell wall synthesis (Amor et al., Proc. Natl. Acad. Sci. USA 92:9353-9357, 1995), an assumption compatible with the repression of *Sh1* promoter activity by the cellulose biosynthesis inhibitor dichlorobenzonitril (Maas et al., EMBO J. 11:3447-345, 1990). One interesting new aspect of the *Sh1* pattern is the preferentially high activity in the epidermal cells of the primary root and young leaves, which may be related to functional specificities of both outer cell layers.

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The development of a Plant Ontology™ Consortium and Plant Ontologies

–Vincent, PLD, Coe, EH, Polacco, M

Abstract: The goal of the Plant Ontology™ Consortium is to produce structured controlled vocabularies, arranged in ontologies, that can be applied to plant-based database information even as knowledge of the biology of the relevant plant taxa (e.g. development, anatomy, morphology, genomics, proteomics...) is accumulating and changing.

Background: Plant databases are rapidly expanding in number, size and complexity. These information-rich databases face the challenge of accurately and consistently documenting features such as gene structures, products and functions, phenotypes, traits, developmental stages and anatomical parts besides other information. It will be increasingly desirable for inter-database queries to be performed between these plant-based databases to exploit comparative genomic strategies to elucidate functional aspects of plant biology. However, terms used to describe comparable objects in each database are sometimes quite variable and limit the ability to accurately and successfully query information in and across different databases. One solution to this problem involves the development and application of ontologies of structured controlled vocabularies.

What is an ontology? An ontology is a classification methodology for formalizing a subject's knowledge in a structured way (typically for consumption by an electronic database). Dictionaries and encyclopedias are examples of ontologies, as are many web-based entities, such as Yahoo and Excite, and so is the schema for a database. A more formal definition of ontology is available from: http://www-ksl.stanford.edu/onto-std/mailarchive/0136.html. In the world of structured information, ontologies, comprising controlled vocabularies, play a very important role in facilitating information retrieval. Furthermore, the definitions that accompany the controlled vocabulary terms facilitate the consistent use of the controlled vocabulary terms in database curation.

In biology-based ontologies the controlled vocabulary terms are arranged in an ontology in such a way that their placement reflects the known or putative biological associations between the objects represented by the controlled vocabulary terms. Consequently, considerable effort must be invested into the compilation of the controlled vocabulary terms and the definitions of these terms and the correct design of the ontologies which comprise these controlled vocabularies. If the ontology of the relationships between the terms is incorrect, the information retrieved via a database search is likely to be incorrect or useless. The converse is also likely to be true. The same applies to the controlled vocabulary terms used, although the use of synonyms can overcome difficulties which occur through the use of local terms which lack wider/international recognition.

While it is relatively easy to design an ontology based on concrete facts such as names, birthdates etc., it is considerably more difficult to design an ontology based on knowledge that as yet does not have unanimous support or which is not yet well understood. However, the Plant Ontology™ Consortium is attempting to develop various plant ontologies that will represent our current and future understanding of relationships amongst various plant-based knowledge domains. These ontologies would provide shared, common vocabularies of defined terms to describe various knowledge domains in plant-based databases. The relationships between elements (represented by controlled vocabulary terms) within and between ontologies would be represented by the use of Directed Acyclic Graphs (DAGs). A DAG is similar to a hierarchical structure but with the ability to have more than one 'parent' for an element in the hierarchy. DAGs are able to represent biological relationships more readily than typical hierarchical structures.

Some of the structured controlled vocabularies being developed would be generic enough to facilitate inter-database queries for related organisms (e.g. monocots and dicots).

Other ontologies would be taxon-specific but would still be able to be interrogated for inter-taxon comparisons. For example, an inter-database query for phenotypes involving the inflorescence should produce 'tassel' and 'ear' phenotypes in maize (*Zea mays*) and comparable inflorescence phenotypes in *Arabidopsis*. The relevant associated genomic information can then be obtained from each database for further analysis.

There is little doubt that the plant sciences community, worldwide, could benefit from having controlled vocabularies of terms arranged in ontologies. Furthermore, that the use of these controlled vocabularies would contribute towards consistent data curation and so contribute to the information management needs of the plant sciences.

The Plant Ontology™ (PO) Consortium is extending a paradigm developed by the Gene Ontology™ Consortium. The Gene Ontology™ (GO) Consortium (http://www.geneontology.org) has been developing ontologies and associated controlled vocabularies for several years. The objective of the GO consortium has been the development of ontologies and controlled vocabularies for three knowledge domains: the molecular function, biological process and cellular component of gene products. These ontologies are being developed for a generic eukaryotic cell. Several research groups have been annotating their databases according to the controlled vocabularies contained in the ontologies produced by the GO consortium.

The Plant Ontology™ consortium is a collaboration between representatives of model organism databases and currently comprises the following participants: Gramene (A Comparative

Mapping Resource for Grains - http://www.gramene.org/; the International Rice Research Institute (IRRI - http://www.irri.org/Index.htm) associated with The International Crop Information System (ICIS) database (http://www.cgiar.org/icis/); MaizeDB (http://www.agron.missouri.edu/) and the Maize Mapping Project (http://www.cafnr.missouri.edu/mmp/); The Arabidopsis Information Resource (TAIR - http://www.arabidopsis.org/). These collaborations are focusing on using and extending the GO paradigm to the very pressing need for ontology and controlled vocabulary development for plant-based databases. The GO paradigm is effectively described in the *General Documentation* at http://www.geneontology.org/GO.doc.html and in Gene Ontology: tool for the unification of biology, The Gene Ontology Consortium (2000) Nature Genet. 25:25-29 (http://www.geneontology.org/GO_nature_genetics_2000.pdf). Database representatives of other plant-based databases (e.g. *Medicago truncatula*, soybean (*Glycine max*), *Phaseolus vulgaris*, cassava (*Manihot esculenta*) will be invited to become involved in the collaborative efforts of the Plant Ontology™ Consortium.

A website for the Plant Ontology™ Consortium is to be developed. The URL will be: www.plantontology.org.

Relevant entities in MaizeDB (http://www.agron.missouri.edu/) will be annotated with Plant Ontology™ I.D. numbers (PO:id) as these become available and annotation is implemented.

Further information on the Plant Ontology™ Consortium can be obtained from Leszek Vincent (Leszek@missouri.edu).

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The role of weather conditions in donor plant cultivation in corn anther culture

--Satarova, TN, Chernousova, NM

The process of embryoid induction in anther culture is determined not only by the period of anther cultivation in vitro, but also by the period of donor plant growing. The environment factors change physiological features of initial plants and can promote the increase or reduction of androgenic response. During the work on anther cultivation of corn at the Institute of Grain Farming, UAAS some genotypes were studied over a few years under identical soil conditions of donor plant growing and uniform technique of cultivation in vitro. This allows characterization of the variability of embryoid induction depending on the weather conditions, which changed from year to year.

Donor plants were grown in a field in 1993-1999 in the southeast of Ukraine (Dniepropetrovsk). Annually complete mineral fertilizer N60P60K30 was applied by plowing. In the spring the field was treated with soil herbicide harness 3 l/hec. Sowing was carried out at the end of April - the beginning of May. The cutting of tassels for anther culture was made in July. Anther planting in vitro was on nutrient medium YP according to Genovesi, AD and Collins, GB (Crop Sci 22:1137-1144, 1982) after cold pretreatment at a temperature of 8 C for 14 days. Anthers from 5-7 plants were taken for one variant of the experiment. Anthers were used at the stage of young bicellular pollen grain. Anthers at the appropriate stage of development were isolated from sites on the central core of each tassel. The percentage of responsive anthers (anther response) and the number of embryoids per 100 anthers cultivated were registered after 6 weeks of cultivation in vitro. The experimental results were compared with meteorological data of the corresponding years. The coefficients of pair and multiple correlations between the change of weather characteristics and two parameters of androgenic induction were counted.

The frequencies of anther response and numbers of embryoids per 100 anthers for various genotypes in different years are submitted in Tables 1 and 2.

Table 1. Anther response (%) for corn genotypes in different years of investigations.

Genotype	Years							F/F0.01**
	1993	1994	1995	1996	1997	1998	1999	
And44	0.64a*	-	12.14b	0a	1.08a	1.19a	0a	85.24/3.02
H99xWf9	0a	0.30a	1.32b	0.37a	0a	-	-	12.85/3.32
Wf9xH99	0.34a	0.56a	3.78b	-	0a	-	0a	26.99/3.32
B14xWf9	17.70a	3.43b	6.92c	0.29e	3.93b	-	1.71be	169.12/3.02
Wf9xB14	-	2.94a	10.99b	0.26c	0c	-	1.94ac	190.50/3.32
Wf9xLH148	-	5.68a	-	0b	-	15.86c	-	56.45/4.61
And44xLH148	1.07a	0.13ac	2.78b	2.20b	0.24ad	-	0.07cd	11.30/3.02
Wf9xAnd44	0.21a	-	4.41b	-	0a	-	2.38c	45.75/3.78
Average	2.75a	0.94b	3.06c	0.65be	0.71bf	1.48d	0.46def	126.8/2.80

* - Data for one genotype with the same letter are not significant at the level 0.01. ** F - the criterion of Fisher for the given genotype, F0.01 - standard criterion of Fisher at the level 0.01.

Table 2. Number of embryoids per 100 anthers for corn genotypes in different years of investigations.

Genotypes	Years							F/F0.01**
	1993	1994	1995	1996	1997	1998	1999	
And44	0.64a*	-	29.57b	0a	1.16a	1.63a	0a	325.03/3.02
H99xWf9	0a	0.30a	1.70b	0.37a	0a	-	-	17.72/3.32
Wf9xH99	0.34a	0.64a	6.33b	-	0a	-	0a	56.38/3.32
B14xWf9	38.46a	5.25b	18.26c	0.32de	7.40b	-	2.86be	463.31/3.02
Wf9xB14	-	3.73a	28.13b	0.44a	0a	-	5.08a	190.50/3.32
Wf9xLH148	-	8.40a	-	0b	-	27.24c	-	113.19/4.61
And44xLH148	1.32a	0.13b	6.85c	3.20c	0.95ab	-	0.07b	28.62/3.02
Wf9xAnd44	0.24a	-	8.32b	-	0a	-	8.25b	103.60/3.78
Average	5.45a	1.32b	6.88c	0.93b	1.08b	2.30d	1.13b	358.5/3.18

* - Data for one genotype with the same letter are not significant at the level 0.01. ** F - the criterion of Fisher for the given genotype, F0.01 - standard criterion of Fisher at the level 0.01.

The data testify to the significant influence of such factors as " year " on both parameters of induction in anther culture of corn. Each genotype was studied within 3-6 years, and for each genotype, except B14xWf9, and for the average of genotypes the year 1995 was outstanding among the years investigated. For hybrid B14xWf9 the most positive was the year 1993, though the other genotypes had not shown high results that year. We designed coefficients of pair correlation between parameters of androgenic induction and such weather characteristics as average annual temperature of air, average monthly temperatures of air in May, June and July, sums of average monthly temperatures of air in May - June, May - July, maximal temperatures of air in May, June, July, humidity of air in May, July, the sum of rainfalls in September - December of a previous year, sums of rainfalls in January - April, May, June, July, May - June, May - July of a current year, and the changes of these characteristics in comparison with long-term norms. The majority of such coefficients had small values (<0.7) and were non-significant. For further analysis we have selected only those coefficients, which even for one of the genotypes were at the level of 0.7 and higher. There were coefficients of pair correlation between parameters of androgenic induction and maximal temperature of June, the sum of rainfalls in January - April and the sum of rainfalls in May - July (Table 3).

The coefficients of pair correlation between parameters of androgenic induction and maximal air temperature of June for And44 and And44xLH148 were approximately +0.7 and were significant at the level of probability 0.1. For other genotypes,

Table 3. The coefficients of pair correlation between the parameters of androgenic induction in corn anther culture and weather characteristics .

Genotype	Number of years	Coefficient of pair correlation between anther response and			Coefficient of pair correlation between number of embryoids per 100 anthers and		
		max temp of June	sum of rainfall in		max temp of June	sum of rainall in	
			January - April	May-July		January - April	May-July
And44	6	0.720	0.806**	-0.578	0.733*	0.809**	-0.589
H99 x Wf9	5	0.706	0.800*	-0.738	0.729	0.808*	-0.686
Wf9 x H99	5	0.684	0.758	-0.804*	0.706	0.781*	-0.777
B14 x Wf9	6	0.168	-0.408	0.181	0.278	-0.311	0.120
Wf9 x B14	5	0.631	0.834*	-0.506	0.727	0.873**	-0.452
And44 x LH148	6	0.743**	0.469	-0.731*	0.772*	0.709*	-0.680

* - significant at the level 0.1; ** - significant at the level 0.05.

except B14xWf9, the coefficients of correlation were +0.6 - +0.7, but were not significant because of the limited number of years investigated. The pair correlation between parameters of androgenic induction and the sum of rainfalls in January - April for And44 and And44xLH148 was high, positive and significant at the level 0.05 (r = +0.8). The coefficients of the same dependence for other genotypes, except B14xWf9, and the correlation with anther response for And44xLH148 also were +0.7 - +0.8, but were not significant. The coefficients of pair correlation between parameters of androgenic induction and the sum of rainfalls in May - July for all genotypes, except B14xWf9, were negative (r= -0.5 - -0.8) and significant for Wf9xH99 and And44xLH148 for anther response.

The coefficients of multiple correlation between parameters of androgenic induction and joint influence of weather conditions, such as maximal air temperature of June and the sum of rainfalls in January - April, maximal temperature of air in June and the sum of rainfalls in May - July and sums of rainfalls in January - April and May - July, are shown in Table 4. These coefficients for all genotypes, except B14xWf9, were +0.6 - +0.9. Depending on considered connection for And44 they were significant at levels 0.01, 0.05 and 0.1, for H99xWf9 - at the level 0.1. For Wf9xH99 five coefficients of six, and for Wf9xB14 three of six were significant at the level 0.1, for And44xLH148 four coefficients of six were significant at the level 0.05, one - at the level 0.1.

Thus, the cultivation of donor plants in different years under the same conditions of field growing and cultivation in vitro leads to significant distinctions in androgenic induction for the same genotype. The most probable weather factors affecting the androgenesis through a donor plant are maximal temperature of June, the sum of rainfalls in January - April and the sum of rainfalls in May - July. For the first two weather characteristics mentioned above a positive correlation with parameters of androgenesis for a number of genotypes was noticed and for the last one - the negative. The coefficients of multiple correlation show that the interaction of these elements especially maximal air temperature of June and the sum of rainfalls in January - April influences the reaction of genotypes. From these positions it is possible to explain, why the year 1995 was the most favorable for induction. That year the maximal air temperature of June (38 C) and the rainfall amount in January - April (284.3 mm) were the highest for the years investigated, and the rainfall amount in May - July (84.3 mm) - the lowest. Possibly, the increased amount of moisture in soil at the time of sowing, the effect of rainfalls in January - April, ensures favorable conditions for initial and further plant growth and development. The high maximal temperature of June and the lowered rainfall amount in the period of vegetation before cutting tassels in July create stress conditions, which promote transition of pollen grains from the gametophytic to the sporophytic way of development. In June - July meiosis occurs in corn plants, and the effect of stress factors in the period of meiosis was always considered to be decisive for transition on the androgenic way of development in cereals (Batygina, TB, Chlebnoe zerno, Nauka, 1987).

Table 4. The coefficients of multiple correlation between the parameters of androgenic induction in corn anther culture and weather characteristics.

Genotype	Number of years	Coefficient of multiple correlation between anther response			Coefficient of multiple correlation between number of embryoids per 100 anthers		
		max temp of June and		the sum of rainfall in January - April and May-July	max temp of June and		the sum of rainfall in January - April and May-July
		the sum of rainfall in January - April	the sum of rainfall in May-July		the sum of rainfall in January - April	the sum of rainfall in May-July	
And44	6	0.922***	0.824*	0.875**	0.931***	0.837*	0.879**
H99 x Wf9	5	0.882*	0.877*	0.913*	0.899*	0.867*	0.902*
Wf9 x H99	5	0.850	0.902*	0.922*	0.874*	0.894*	0.917*
B14 x Wf9	6	0.527	0.243	0.441	0.672	0.300	0.330
Wf9 x B14	5	0.842	0.615	0.882*	0.896*	0.790	0.899*
And44 x LH148	6	0.760	0.888*	0.796*	0.876**	0.885**	0.854**

* - significant at the level 0.1; ** - significant at the level 0.05.; *** - significant at the level 0.01.

The marked tendencies of the influence of weather conditions on androgenic efficiency of corn donor plants with various degrees of probability are inherent to different investigated genotypes. Only the reaction of B14xWf9 was not typical for donor plant response.

In that way, not only the conditions of anther cultivation in vitro, but also certain weather factors of field donor plant growing are capable of determining the androgenic ability in corn.

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The vascular development in the *na2/na2* mutant

--Cheng, WY, Cheng, PC, Walden, DB

The phenotype of nana2 (*na2/na2*, Fig 1-6) is characterized by its stubby dwarf appearance. As reported earlier, the longitudinal sections of stem of *na2/na2* generally appear similar to the wild-type but with significantly shorter internodes (Cheng et al., Microsc. Microanal. Vol. 6, Supp 2:692-693, 2000). However, in the 2001 summer nursery (an excellent growing season) in London, Ontario, we observed an interesting characteristic (described below) of the mutant in more than 60 homozygous *na2/na2* plants. This phenotypic expression was also observed in two plants previously in our 1999 and 1998 summer crop (relatively poor growing sessions).

The lower portion of the mutant *na2/na2* (Fig. 1-6) stem has a “normal” stem appearance but the internode length is significantly shortened compared to that found in the wild type (Fig. 1-1, 1-2,

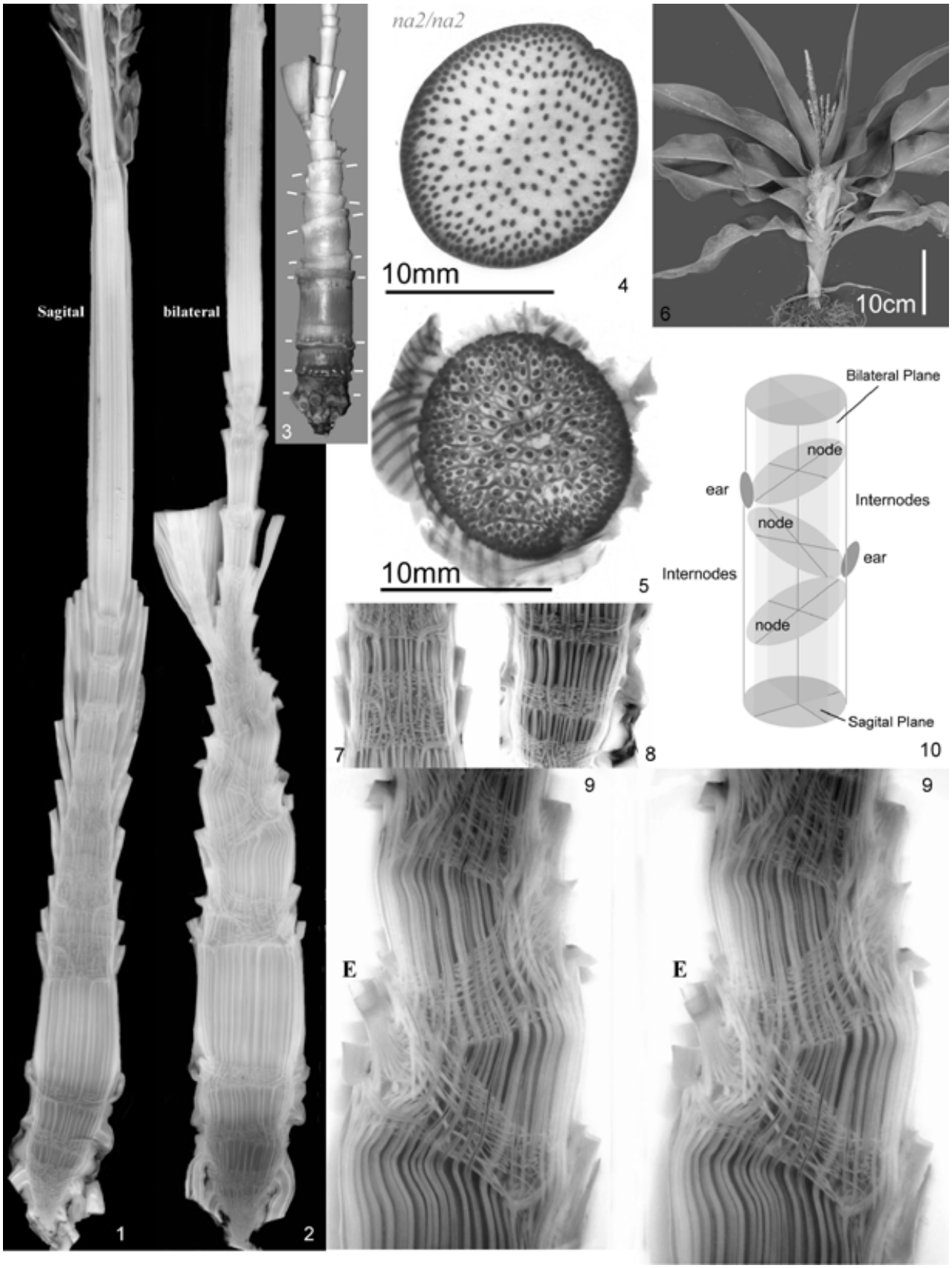

Figure 1. (1 and 2) Longitudinal section of *na2/na2* in sagital (1) and bilateral (2), (3) *na2/na2* plant showing tilted leaf sheaths attachment, (4) Cross-section of internode, (5) Cross-section of a nodal region, (6) *na2/na2* plant, (7 and 8) Digested sagital and bilateral sections, (9) 3D-pair of tilted nodes and ear attachment (E), (10) Diagram representing a *na2/na2* stem.

1-8). The cross-sectional views (Fig. 1-4, 1-5) reveal similar nodal and internodal vascular arrangements, as found in the wild type (Cheng et al., Microsc. Microanal. 7:444-445, 2001). However, at a more apical portion of the plant, one frequently observes a continuous "zig-zag" nodal structure; slanted nodes with wedge-shaped internodes characterize this phenotype. Figure 1-2 shows a longitudinal section cutting through the bi-lateral axis of the stem. Figure 1-1 shows a longitudinal section with a sagital cut plane, perpendicular to Fig. 1-2, 1-8. Sandwiched between the slanted nodes, one finds the initiation of axilary buds apparently supplied by the two joining nodal networks (Fig. 1-9 stereogram). The leaf-sheath insertion on the plant is also tilted (Fig. 1-3, indicated by white-line pairs). These results suggest that the lower internodes may have been initiated in the embryonic stage assuming the expression of the *na2* is not in effect, while the expression of the allele occurs only in the post-germination growth. The tassel internodes in *na2/na2* revert to normal, suggesting that this gene expression is "turned off" in the reproductive organs (Fig. 1-1 and 1-3). A diagrammatic representation of the nodal arrangement is shown in Fig. 1-10.

This article is part of a report by WYC for Siemens Westinghouse Science and Technology Competition (Semi Finalist) and Intel Science Talent Search (2001).

The number of vascular bundles in 18 different cultivars of inbred, hybrid or commercial sweet corn

--Cheng, WY, Cheng, PC, Walden, DB

This article reports a study on the vascular bundles in a number of cultivars. In this study, inbred, hybrid lines and mutants were grown in the nurseries at the University of Western Ontario (London, ON, Canada) in the summers of 1999, 2000, and 2001. The inbred lines used in this study included Oh43, B73, W23, CM48, KYS, Mo17, and Gaspé. Hybrids were Oh43/B73, Oh43/W23, Oh43/CM48, Oh43/Gaspé, Oh43/Mo17, Oh43/KYS, CM48/Oh43, CM48/Gaspé, CM48/W23, Mo17/B73, and B73/Mo17. In addition, commercial sweet corn varieties, including: 'Platinum Lady,' 'Honey and Cream,' 'Seneca Chief,' and 'Odyssey,' were employed. In order to determine the effect of parental influence on stem development, reciprocal F1 hybrid plants where available also were employed. The specimens were harvested and fixed in methanol and/or EtOH/acetic acid (3:1), serially sectioned with a razor blade using a special jig we made. The hand sections, approximately 800 µm in thickness, were decolored in 70% iso-propanol, dehydrated in acetone and cleared in xylene. The number of vascular bundles, cross-sectional area of the immediate (1st) internodes and second internodes (2nd) below the main ear-bearing node, and perimeter of the stem were obtained by using the counting function in ImageAcquire™ software (Williamsville, NY).

A minimum of five samples were collected from each variety at silking. The data were tabulated and a Student T-test was performed. For further analyses, the cross section of an internode was subdivided into three zones: the para-epidermal zone 1 (19% of the total area), zone 2 (56%) and zone 3 (pith) (25%). This zoning was achieved by first detecting the edge of the stem by intensity threshold, followed by edge-detection using an image processing software (Paint Shop Pro, v5.0). This procedure obtained the outline of the stem which could then linearly reduce the outline to 50% and 90%. Finally, the outline was pasted onto the original image to define the zoning (Fig. 1). Cross sectional area (in mm2) and stem perimeter (in mm), as well as the number of vascular bundles, were obtained using ImageAcquire™. Statistical analyses were performed using Origin software (V5.0, Microcal Inc., MA).

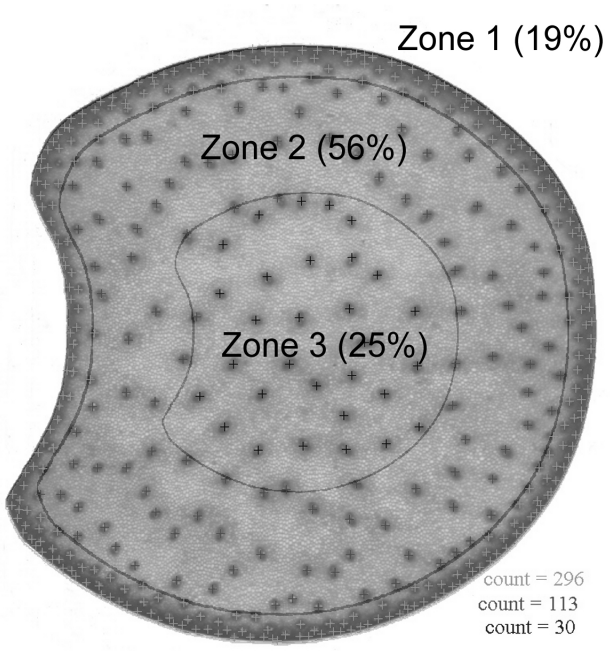

Figure 1. Definition of zoning used in this article. Zone 1: 19%, Zone 2: 56%, Zone 3: 25%

In a systematic study of various inbreds and their F1 hybrids, a number of interesting observations were made. While the general pattern of vascular distribution is similar, the numbers of vascular bundles and cross sectional areas of the stem can be significantly different. For a given cultivar, the standard deviation of the number of bundles is well within 5-10% of the value, while cross-sectional areas show a much larger standard deviation (Fig. 2 and Table 1). These observations suggest that the vascular bundle number is largely determined genetically and may be set in the embryonic phase, while the internode cross sectional area is more influenced environmentally. In B73/Mo17, Oh43/CM48, and their reciprocal F1 hybrids, the number of vascular bundles shows no significant difference (Fig. 2-C to 2-F), suggesting that cytoplasmic factors may have little influence in the determination of vascular numbers. Depending on the parental backgrounds, the F1 hybrids show a significant "hybrid vigor" in increasing the number of vascular bundles and cross sectional area (e.g. Oh43/KYS and B73/Mo17), although the early maturing hybrids, (CM48/Gaspé and Oh43/Gaspé) have bundle counts which resemble one of the parents or are an intermediate between the parents (Fig 2-G, 2-H). However, the results may indicate the degree of genetic similarity in the inbred lines used in this study as far as the vascular development is concerned. For comparison, the data from four commercial sweet corn varieties are also shown in Fig. 2-I, 2-J. It is important to note that for proper comparison, all the samples examined were obtained at the half silk stage; the time required from planting to silking is listed in Table 1.

This article is part of a report by WYC for Siemens Westinghouse Science and Technology Competition (Semi Finalist) and Intel Science Talent Search (2001).

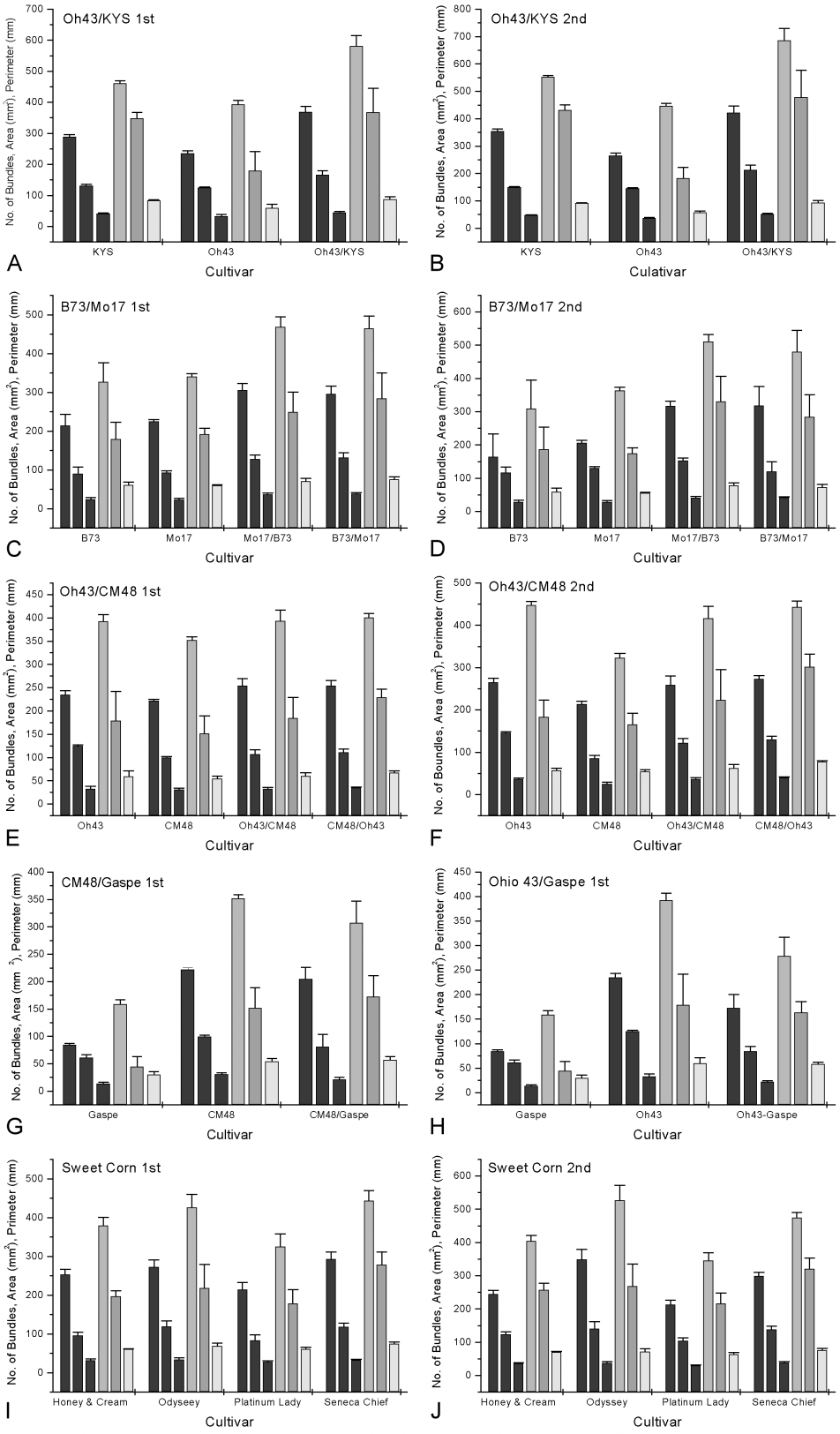

Figure 2. Histograms showing the number of vascular bundles (1st three bars from left) in zone 1, 2, and 3 respectively, number of total bundles (4th bar), cross sectional area of stem (5th bar) and stem perimeter (6th bar) for the two internodes below the top ear node in different cultivars.

Table 1. Comparison of the CS area (mm2), and perimeter (mm), the number of vascular bundles in zones 1, 2, 3 and total, and the number of bundles in zone 1 per mm of stem perimeter (B/mm) from two internodes of various inbreds, hybrids, and commercial sweet corn hybrids. n: sample size.

Cultivars (1st, 2nd internode)	n	Days to half Silking	Cross sect. Area (mm2)	Perimeter (mm)	**Total Bundles**	Zone 1	Zone 2	**Zone 3**	B/mm
Oh43 1st	7	73	178.70 ± 62.93	59.33 ± 12.08	393.7 ± 14.6	235.0 ± 8.5	124.5 ± 2.3	32.7 ± 5.6	3.9
Oh43 2nd	5	73	182.67 ± 40.46	57.07 ± 5.36	447.0 ± 9.6	265.0 ± 9.8	145.8 ± 2.5	36.2 ± 2.7	4.6
Gaspé flint 1st	7	41	44.22 ± 18.98	29.81 ± 5.70	158.8 ± 8.4	84.0 ± 3.1	60.8 ± 5.9	14.0 ± 2.6	2.9
CM48 1st	7	64	151.50 ± 37.74	54.16 ± 5.64	352.0 ± 7.0	222.0 ± 3.3	99.2 ± 3.0	30.8 ± 3.0	4.1
CM48 2nd	7	64	165.55 ± 26.18	54.34 ± 4.3	322.8 ± 10.8	213.0 ± 7.3	85.2 ± 24.6	24.6 ± 4.7	3.9
KYS 1st	4	89	347.44 ± 20.27	82.30 ± 3.41	460.2 ± 9.4	288.5 ± 7.5	130.2 ± 5.1	41.5 ± 2.6	3.5
KYS 2nd	5	89	431.48 ± 19.37	91.88 ± 1.37	552 ± 6.4	354.7 ± 8.3	149.7 ± 2.9	47.5 ± 1.0	3.8
Mo17 1st	5	83	191.62 ± 15.60	59.29 ± 2.03	340.6 ± 7.7	224.8 ± 4.	92.8 ± 5.4	23.0 ± 3.5	3.7
Mo17 2nd	6	83	174.14 ± 17.07	55.75 ± 2.41	363.8 ± 9.9	206.3 ± 7.3	129.5 ± 5.7	28.0 ± 4.4	3.7
B73 1st	7	80	179.01 ± 43.55	60.31 ± 8.22	326.5 ± 49.6	213.5 ± 29.3	89.5 ± 17.7	23.4 ± 5.4	3.5
B73 2nd	8	80	186.47 ± 67.02	58.86 ± 10.84	308.8 ± 86.3	164.2 ± 65.5	116.1 ± 17.3	28.5 ± 5.5	2.7
Mo17/B73 1st	7	74	249.08 ± 51.04	70.59 ± 7.47	464.7 ± 25.8	304.8 ± 17.6	127.4 ± 10.9	36.1 ± 4.4	3.9
Mo17/B73 2nd	7	74	331.02 ± 75.69	78.07 ± 8.31	510.4± 21.8	316.8 ± 15.2	152.4 ± 8.5	41.1 ± 3.6	4.0
B73/Mo17 1st	7	76	284.29 ± 65.33	75.28 ± 7.01	468.4 ± 32.1	295.2 ± 20.6	131.2 ± 12.7	38.1 ± 3.1	3.8
B73/Mo17 2nd	7	76	284.58 ± 66.01	72.29 ± 9.13	480.1 ± 64.1	318.2 ± 57.8	120.1 ± 29.3	41.7 ± 2.5	4.4
CM48/Oh43 1st	5	61	229.16 ± 17.77	67.49 ± 7.25	401.2 ± 9.4	254.4 ± 11.3	111.2 ± 10.3	34.6 ± 3.2	3.7
CM48/Oh43 2nd	7	61	301.72 ± 30.27	77.57 ± 2.91	442.5 ± 14.5	272.8 ± 8.5	130.1 ± 8.0	39.5 ± 1.9	3.5
Oh43/CM48 1st	7	63	184.89 ± 44.08	60.12 ± 7.25	393.2 ± 23.7	254.4 ± 15.0	106.7 ± 10.3	32.1 ± 3.2	4.2
Oh43/CM48 2nd	7	63	223.32 ± 71.98	62.32 ± 9.25	416.1 ± 29.1	258.4 ± 21.9	121.7 ± 10.6	36.0 ± 4.2	4.1
Oh43/Gaspé 1st	5	58	163.60 ± 21.88	57.64 ± 4.0	278.6 ± 38.4	172.6 ± 27.5	84.4 ± 10.0	21.6 ± 3.0	2.9
Oh43/KYS 1st	7	88	367.31 ± 77.69	86.54 ± 8.98	580.4 ± 34.3	369.1 ± 18.0	166.2 ± 13.8	45.0 ± 3.7	4.2
Oh43/KYS 2nd	4	88	477.98 ± 99.00	92.45 ± 9.37	685.0 ± 44.5	421.2 ± 25.5	212.5 ± 18.1	51.2 ± 3.2	4.5
CM48/Gaspé 1st	5	59	172.51 ± 38.76	56.53 ± 6.54	307.1 ± 40.2	204.8 ± 20.9	81.1 ± 22.8	21.1 ± 3.8	3.6
Platinum Lady 1st	7	72	177.63 ± 36.23	60.64 ± 5.45	324.7 ± 33.5	214.2 ± 17.8	83.0 ± 14.4	27.4 ± 2.9	3.5
Platinum Lady 2nd	7	72	215.59 ± 32.57	63.73 ± 4.87	345.7 ± 23.3	212.5 ± 13.4	103.5 ± 9.8	29.5 ± 1.7	3.3
Honey & Cream 1st	7	70	196.29 ± 14.79	60.16± 1.94	379.1 ± 21.6	252.5 ± 14.5	95.5 ± 9.1	31.1 ± 4.3	4.1
Honey & Cream 2nd	7	70	256.35 ± 20.96	69.64 ± 2.45	404.0 ± 16.8	244.5 ± 11.7	123.7 ± 7.1	35.7 ± 2.7	3.5
Seneca chief 1st	7	75	278.38 ± 33.09	74.58 ± 4.89	443.4 ± 25.9	293.0 ± 17.9	118.0 ± 10.2	32.4 ± 2.2	3.9
Seneca chief 2nd	7	75	320.68 ± 32.96	76.15 ± 4.88	474.5 ± 16.1	298.2 ± 11.4	138.1 ± 10.0	38.1 ± 3.7	3.8
Odyssey 1st	7	78	218.30 ± 60.94	67.92 ± 8.84	425.1 ± 34.1	272.5 ± 17.8	119.0 ± 14.5	33.5 ± 4.9	4.0
Odyssey 2nd	7	78	267.83 ± 67.89	71.45 ± 8.62	526.1 ± 45.6	348.4 ± 30.8	140.0 ± 22.1	37.7 ± 4.3	4.8

A visualization of airflow pattern around a maize plant

--Cheng, WY, Cheng, PC, Walden, DB

In order to study the airflow pattern around different organs of a maize plant, a small wind tunnel was constructed (Figure 1a). Three 16-inch box fans were stacked in series to provide different airflow velocities. Laminar flow was achieved by using a honeycomb panel constructed by using 5mm McDonald^{R} plastic beverage straws (Figure 1a). A Kestrel 3000 anemometer (Nielsen-Kellerman, Chester, PA) was used to determine the air velocity. Two 15cm spherical mirrors (f=150cm 1/10λ) were used to construct a Schlieren optical set-up (Edmund Optics Inc., NJ) (Figure 1b). A 100W Hg lamp (XBO) was used as the light source; a Sony^{TM} camcorder operated in night-vision mode with built-in IR illuminator disabled was used to acquire the Schlieren images (Figure 1c). Acetone vapor was generated and ejected from a set of specially made nozzles in the air stream. The acetone vapor has a different reflective index from surrounding air, hence it can be easily delineated from the surrounding air. Plants were placed downstream from the nozzle in the wind tunnel, and rotated to allow a study of multiple wind directions.

At 3 km/h and 4.6 km/h breeze conditions, a significant updraft flow pattern and turbulence were detected when the lamellar airflow encountered leaf blades (Figure 2b, 2c, 2d, and 2e, arrows). The turbulence in the leeward side suggests significant drag acting on the plant. We have also observed an interesting turbulent pattern occurring at the tassel region of the plant

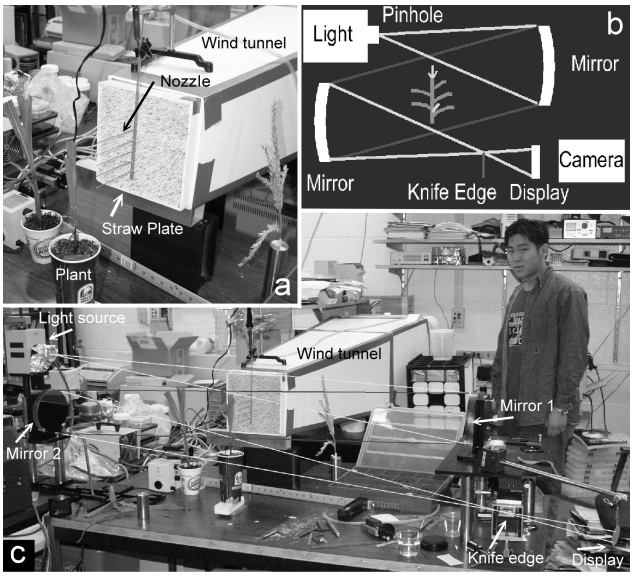

Figure 1. (a) Wind tunnel with straw plate and nozzle. (b) Schlieren optics layout, (c), Schlieren optical setup.

(Figure 2f, 2g, arrows). This turbulence caused the tassel and the dehiscing anthers to vibrate even at low wind velocities and, in conjunction with the leaf-generated updraft, may play an important role in the efficient dispersing of pollen grains. The leaf-generated updraft may also minimize pollen deposition on the sur-

face of the leaf. Downward draft can be observed only at the basal part of a leaf blade; this downward draft may assist the airborne pollen grains attaching to the silk.

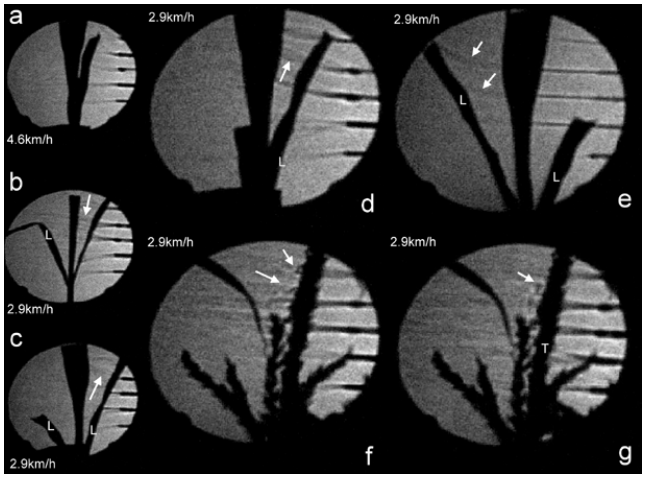

Figure 2. (a-g), Airflow pattern around a plant, L: leaf, T: tassel, white arrows: turbulence flow.

Due to the limitations on the size of the mirrors and the wind tunnel, our Schlieren optical study can only be performed on young plants and isolated plant parts such as the tassel. Nevertheless, the results demonstrate the potential application of Schlieren optics to the study of aerodynamic response and pollen dispersion of maize.

This article is part of a report by WYC for Siemens Westinghouse Science and Technology Competition (Semi Finalist) and Intel Science Talent Search (2001).

Mechanical properties of stem vascular bundle

--Cheng, WY, Cheng, PC, Walden, DB, Shinozaki, DM

Mechanically, the maize plant can be characterized as a "foam stick" with a reinforced outer shell, which resembles a concrete pillar in modern constructions. The peripheral region can be considered as the steel reinforcing bars and cylinder, and the interconnecting nodal networks are the steel bracings ("rebar") found in a concrete pillar. In the early stage of development, the vasculatures act as the tensile element, while the highly turgid parenchyma cells are the compression element in the model. In the later stage while the parenchyma cells become air-filled, the highly lignified para-epidermal bundles become the structural element. The binding between individual para-epidermal vascular bundles by lignified sclerenchyma cells is an important structural development. This binding transforms those loosely parallel arranged vascular bundles into a solid cylindrical structure as depicted in a model built of straws (as vascular bundles) and foam boards (as node (N) and internode (IN)) (Fig. 1a). If the individual straws are not glued together, the "stem" is subject to a "twisting" motion (Fig. 1a-left; arrow), but the stem becomes rigid when the straws are bound together (Fig. 1a-right).

To isolate the vascular bundle, mature stems (Oh43/KYS) were placed in cellulase-pectinase solution to remove parenchyma cells. (Cellulase from *T. longibrachiatum*; Cat. WA18420, and pectinase from *A. niger*; Cat. 18579; Fluka, Switzerland.) This digestion process took 1-6 days, depending on the age of the specimen. Heavily lignified vascular bundles are resistant to the enzymatic digestion and remain intact. The longitudinal vascular bun-

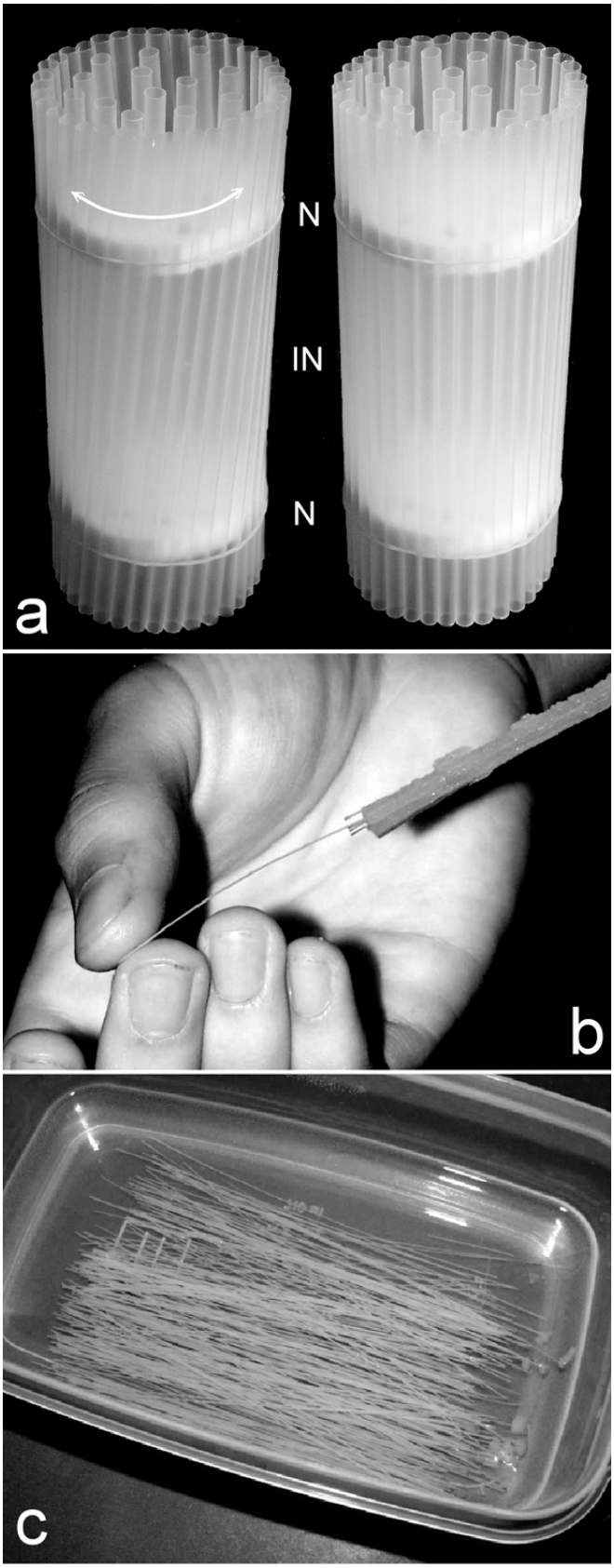

Figure 1. (a) a stem model demonstrating the effect of torsion force (arrow), (b, c) Isolated bundles. N: node, IN: internode.

dles in the stem can be isolated by enzymatic treatment as long fibers (Fig 1b, 1c), which allow us to perform mechanical testing on individual bundles. A tensile load-displacement curve was obtained from individual bundles using a computer-controlled thin-film tensile tester developed by D M Shinozaki (Faculty of UWO).

Preliminary material testing on the isolated vascular bundle reveals the structure is moderately strong (Fig. 2); the individual vascular bundles fractured around 100g (or about 1 N), with an elongation of about 1200µm. For a bundle diameter approximately 0.5mm, the effective stress is about 5MPa at fracture. This is probably lower than the real value since it fractured at the grip in our preliminary experiment. Since the vascular bundle is not a solid rod, the true stress at fracture is much higher. Therefore, effectively the bundle may be stiffer than polyethylene. This test was done wet (in 30% iso-propanol); therefore, the strength probably increases when it is dried.

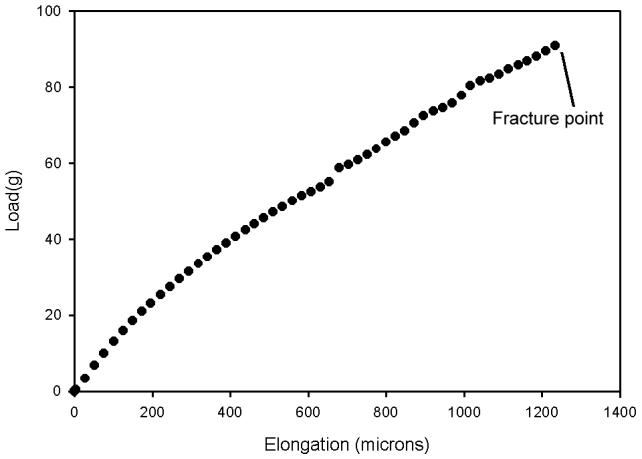

Figure 2. 30mm isolated vascular bundle of Oh43/KYS fractures at approx. 100 g. with an elongation about 1200 µm

This article is part of a report by WYC for Siemens Westinghouse Science and Technology Competition (Semi Finalist) and Intel Science Talent Search (2001).

Distribution of vascular bundles in Ohio43/KYS stem

--Cheng, WY, Cheng, PC, Walden, DB

Throughout the maize plant, most of the longitudinal vascular bundles travel through a number of nodes where side branching occurs (Fig. 1). In other than the nodal region, it seems that no interconnections occur. The longitudinal vascular bundles form branches just before entering the nodal region (Fig. 1); these vascular branches in turn form an interconnecting network. We observed that most of the branches are derived from the cortex of the stem while the vasculatures in pith tend to pass through with few connections. Where the leaf initiates, the vascular bundles form an intense array (Fig 1), which shows as dark radiating structures (A) in the MRI image (Cheng et al., MGCNL 75:8, 2000)

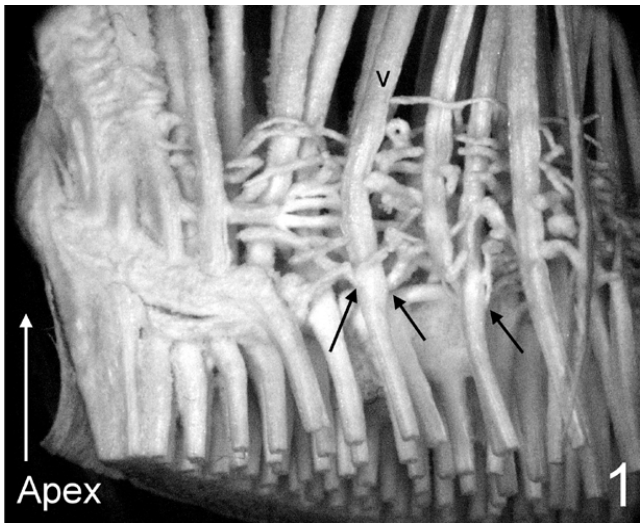

Figure 1. Vascular bundles below and through a node. Note the branching before the vasculatures enter the node (black arrow). V: vascular bundles, White Arrow: Direction of Apex.

Figure 2 shows the internode cross sectional area and the distribution of bundles in cortex (the para-epidermal zone 1 is 19% of the total cross sectional area) and pith (89%) in four Ohio43/KYS plants at the stage of silking. The numbering system adapted places the top ear above the internode #12 (Fig. 2a). The tassel begins above internodes #2-3 and the top crown roots initiate at the node above internodes #19-20. The results indicate a maximum bundle number at the ground level (#19) and demonstrate a general exponential reduction in the number of vascular bundles and cross-section area toward the apex of the plant. However, a shoulder in the curve was observed around the ear-bearing node (internodes 12-16) as the large number of vascular bundles supplying the ear (Fig. 2b-e).

This article is part of a report by WYC for Siemens Westinghouse Science and Technology Competition (Semi Finalist) and Intel Science Talent Search (2001).

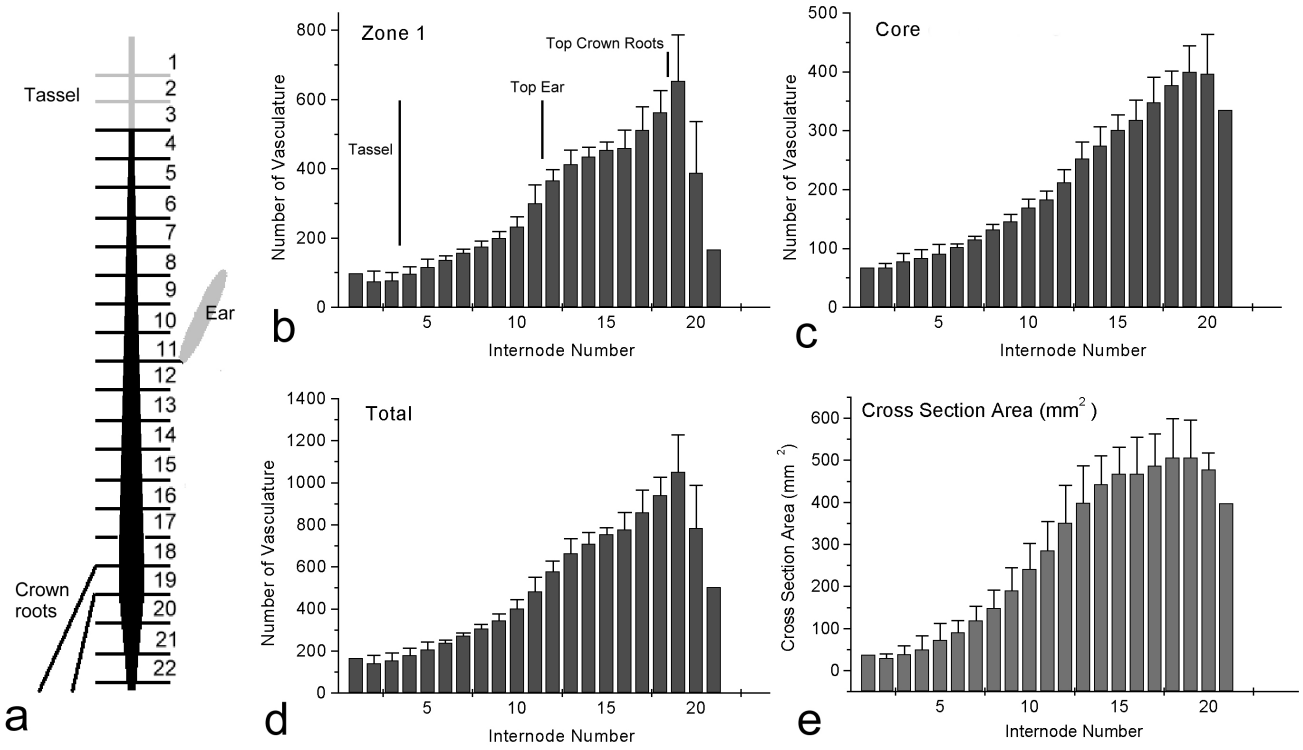

Figure 2. (a) Diagrammatic representation of the using internode numbering system used in this article, (b) number of vasculatures in zone 1, (c) number of vasculatures in pith (core), (d) number of total vasculatures, (e) cross sectional area.

Method for visualizing the vascular bundles in stem

--Cheng, WY, Cheng, PC, Walden, DB

A method was developed to visualize the arrangement of vasculatures in the absence of the parenchyma cells. To visualize the vasculature, mature stems were placed in cellulase-pectinase solution to remove parenchyma cells. (Cellulase from *T. longibrachiatum*; Cat. WA18420, and pectinase from *A. niger*; Cat. 18579; Fluka, Switzerland.) This digestion process took 1-6 days, depending on the age of the specimen. Heavily lignified vascular bundles are resistant to the enzymatic digestion and remain intact. This leaves the vasculatures, and the outer "shell" of the plant without any support, which thus collapse.

In order to preserve the vascular architecture, the stem segments when wet, were pre-glued by cyanoacrylate glue in a polystyrene frame prior to enzymatic digestion (Fig. 1-a). This prevents the vascular bundles from collapsing after the removal of parenchyma cells (Fig. 1-b). During the digestion process, tissue fragments were washed away by flushing the specimen gently with a Pasteur pipette. Figure 1-c shows the node region of Ohio43/KYS after digestion. Note the parallel bundles running through the node with branches before entering the node from below.

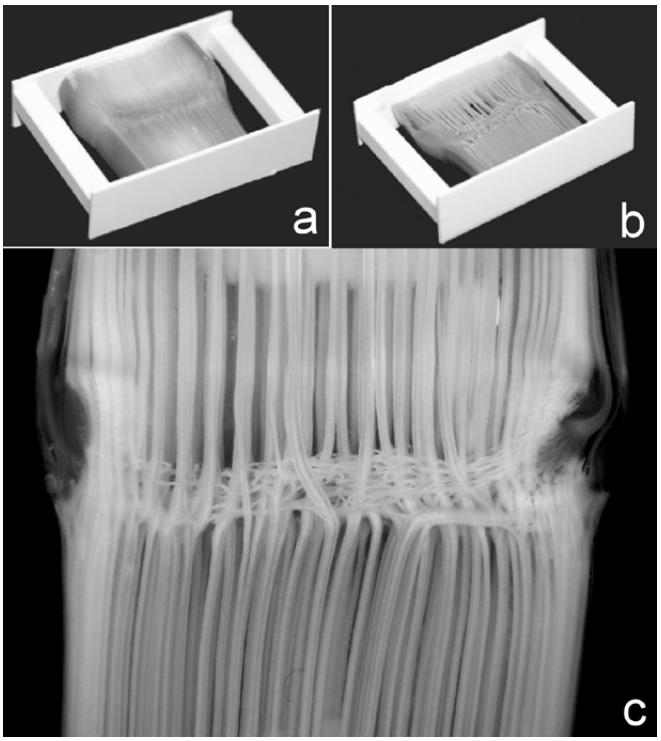

Figure 1. Undigested (a) and partially digested (b) stem sections glued into polystyrene frames. (c) Digested nodal region, note the straight through vasculatures.

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The relationship between synapsis, recombination nodules, and crossing over in maize

--Anderson, LK, Stack, SM

We examined spreads of synaptonemal complexes (SCs) from inbred KYS maize using electron microscopy to determine the pattern of synapsis in relationship to the frequency and distribution of recombination nodules (RNs). In maize, synapsis generally initiates distally (near ends) and proceeds proximally, although there are also a few interstitial SC initiation sites. Segments near centromeres are ordinarily the last to synapse.

Early RNs (ENs), like late RNs (LNs), are proteinaceous ellipsoids found on the central element of SCs, but ENs are found during zygotene and occur six times more frequently than maize LNs, which occur later in pachytene (see below). ENs are thought to have roles in synapsis and/or early recombination events. Evidence from yeast, mammals, and plants indicates that a subset of ENs gives rise to LNs. Maize ENs do not appear to show interference with respect to one another, although the distribution of ENs along the length of bivalents varies. For example, distal SC segments that synapse early in zygotene have nearly two-fold more ENs per unit length of SC than more proximal segments that synapse later. It is interesting that as zygotene progresses, the frequency of ENs does not increase in distal segments, indicating that ENs do not continue to assemble on already formed SC. This, coupled with the observation that the highest frequency of ENs occurs at synaptic forks, suggests that ENs are assembled on SCs at synaptic forks where synapsis is temporarily delayed.

ENs disappear early in pachytene to reveal LNs. In contrast to ENs, LNs show interference just like crossover events, and, indeed, LNs appear to reside at sites of crossing over. The frequency of LNs per m of SC is six-fold higher in distal compared to proximal segments of SCs. Thus, distal chromosomal segments that synapse first have more ENs in zygotene and more LNs in pachytene. These results suggest that (1) synaptic patterns are important in determining crossover patterns in maize and (2) the first ENs to associate with SC are more likely to give rise to LNs (and crossover events) than ENs that associate with the SC later in zygotene.

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Evaluation of international resistance genes against the European corn borer under Central European conditions

--Papst, C, Götze, S, Eder, J

The European corn borer (ECB, *Ostrinia nubilalis*) is a worldwide major pest of maize. Populations with up to four generations per year are observed (http://www.ent.iastate.edu/pest/cornborer/intro/intro.html 2001) in the U.S. corn belt, in contrast to Central Europe, where only one generation per year is usual. Depending on the stage of development of the plant at infestation time, more or less extensive yield reductions are reported. The first generation mainly feeds on the leaves of young plants, the following generations cause stalk breakage and dropped ears. If only one generation appears, in case of shorter growing seasons like in Germany, the plants are mostly damaged by tunneling in stalks and ears. Here the neonate larvae feed only a short time during the first two stages of development (early to mid-July) on the leaves before they begin tunneling. In most cases, the larvae feed from the tassel down to the stem base, where they overwinter as the fifth larval stage (L5) and in spring they pupate. The flight of the moths and egg-laying is observed from mid-June to mid-July.

In the most important maize growing regions, like in the Middle West of the U.S.A. and in Mexico, material with increased resistance to ECB is being developed. Particularly in the case of a resistance to the second generation, this material could be useful for European maize breeding as a source of resistance. But in most cases this material needs far longer growing-seasons than central European material. To determine the resistance of international material under German conditions, experiments with populations and inbred lines were carried out for the present study.

Germplasm. For resistance examination, 18 (1999) to 34 (2000) lines and populations from the U.S.A., Mexico and South Africa were cultivated (Table 1). To compare the resistance and agronomic traits with adapted European material, the two inbred lines D06 und D408 were evaluated in 2000. D06 is regarded as resistant, and D408 as susceptible (Melchinger, AE et al., Euphytica 99:115-125, 1998). The populations 590, 590B, 591 and G1 IST were provided by CIMMYT (Mexico). They have been selected for resistance to the first generation of ECB, and in the case of population 591, also for resistance to the second generation of larvae (Bergvinson, D, unpublished, 1999). In 2000, selfings from these populations were tested and signed with "S" and the respective population number. Diverse MBR/MDR lines (multiple borer resistance/multiple disease resistance) also derive from the populations described above by self-pollination. Material from South Africa has come out of a backcross program of various selections with one experimental line from North American material (verbal information, van Rensburg 1999). The lines Mo45, Mo46 and Mo47 (Barry, BD et al., Crop Science 35:1232-1233, 1995), as well as the Mo-2 ECB population from Missouri is adapted to the climatic conditions in the southern part of the U.S. “Corn Belt” and carries resistance genes to the second generation of ECB. The Mo-2 ECB-2 population is derived from the “Nigerian Composite B” material by a recurrent selection for resistance to the second generation of ECB. Material from Iowa (Hallauer, AR, Crop Science 37:1405-1406, 1997) is reported to be resistant to the first generation of ECB.

In the year 1999, the lines and populations were tested in one-row plots of 23 plants and without replication. The plot size was 4.0 m x 0.75 m with a planting density of 8 plants/m^2. In 2000, the variants were evaluated in a lattice design with two replications in one-row plots of 4.0 m x 0.75 m and 25 plants. The trials were overplanted and later thinned to the necessary plant density of 8 plants/m^2. Because of extremely late maturation of the foreign genotypes in 1999, the trial was covered with a perforated transparent polyethylene foliage for three weeks after sowing in 2000.

For evaluating the level of resistance against ECB, every plant was manually infested at three times in weekly intervals with 20 neonate ECB larvae. The date of application was synchronized with the natural flight of the ECB. For the infestation freshly hatched larvae were mixed with maize-cob grits and placed into the whorl or leaf collar of maize plants with a dispenser (Mihm, JA,

Table 1. Overview of the evaluated genotypes and results of our study.

Origin	genotype	evaluation-year 1999	evaluation-year 2000	DA* days after sawing	PH cm	DME %	LA 1999	LA 2000
Mexico	MBR/MDR F117	x	x	115	245	31.5	0	2
	MBR/MDR F12	x	x	109	190	38.4	0	4
	MBR/MDR F20	x	x	105	240	47.5	0	47
	MBR/MDR F68	x	x	109	240	34.2	7	20
	MBR/MDR F78	x	x	106	210	38.0	7	37
	P. 590†	x	x	118	328	32.7	0	4
	S. 590-1¶		x	106	250	40.5		8
	P. 590B†	x	x	110	323	39.2	7	0
	S. 590B-1¶		x	109	275	40.9		4
	S. 590B-2¶		x	111	305	32.5		23
	S. 590B-3¶		x	110	275	45.7		9
	P. 591†	x	x	116	325	31.3	0	8
	S. 591-1¶		x	113	245	27.1		2
	S. 591-2¶		x	109	260	35.2		11
	S. 591-2¶		x	113	208	19.7		9
	P G1†	x	x	108	290	37.3	8	22
	S G1-1¶		x	109	223	45.1		19
	S G1-2¶		x	112	250	40.8		13
South Africa	4131.P28-16		x	110	270	39.6		16
	4131.P28-6		x	109	260	35.7		46
	609.P28-2		x	106	230	37.3		43
	P6528.P28-2		x	117	210	15.7		20
	P6528.P28-3		x	107	250	38.0		32
	P6528.P28-4		x	109	250	36.2		21
	SM42.P28-7		x	106	260	61.3		27
Missouri	Mo 45	x	x	108	245	41.9	5	29
	Mo 46	x	x	101	205	45.7	29	51
	Mo 47	x	x	102	213	47.9	6	6
	Mo2-ECB†	x	x	100	225	54.0	24	77
	Mo2-ECB2†	x	x	99	185	51.8	18	51
Iowa	B 102	x	x	108	155	34.3	21	36
	B 103	x	x	99	140	45.5	22	78
	B 104	x	x	109	180	42.2	20	43
	B 106	x	x	106	225	49.7	6	55
Europe	D06		x	78	169	57.1		155
	D408		x	76	166	55.5		77

*DA = date of anthesis, PH = plant height, DME = dry matter content of ears, LA = larvae alive per 100 plants
† population
¶ selfings (S1) from the population

CIMMYT, Mexico, D.F., Mexico, 1983). In both years of experimentation, the plants were split longitudinally from the stem base to the tassel at harvesting time in late October to record infestation level and the number of larvae alive (LA). From the number of infested plants per plot, the relative infestation frequency (RIF) was calculated referring to 100 plants. Additionally, in 2000, the stalk damage rating (SDR) was evaluated based on a 1 to 9 scale (1 for intact plant, 9 for dropped ears or breakage below the ear, Hudon, M and Chang, MS, Maydica 36:69-70, 1991).

As agronomic traits, date of anthesis in days after sowing (DA) and plant height (PH) were recorded in both years, and dry matter content of the ears (DME) was determined from 3 ears per genotype in the year 2000.

The two European dent lines had an RIF of 100%, whereas the non-European genotypes were infested in 1999 between 0 and 94%, and in 2000 between 8.3 and 98%. The highest RIF of more than 64% showed the genotypes from Missouri and Iowa, except Mo47 with a relatively low RIF in the year 2000 (Fig. 1). The populations, their progenies as well as the lines from Mexico and South Africa achieved a RIF of less than 64%. The analysis of variance showed in 2000 highly significant differences among the genotypes ($p \leq 0.01$; $r^2 = 15.2\%$).

In the established European dent lines D06 and D408, 155 and 77, respectively larvae could survive per 100 plants in 2000. Whereas in 1999 only between 0 and 29 larvae and in 2000 between 0 and 78 larvae per 100 plants could survive in the international genotypes. The analysis of variance showed highly significant differences ($p \leq 0.01$; $r^2 = 17.6$ %) in 2000. The highest amounts of larvae (52 - 78 larvae/100 plants) were found in two genotypes from Iowa and Missouri, the lowest number in the genotypes from Mexico. The only exception was Mo47 from Missouri with 6 larvae per 100 plants.

The European lines D06 and D408 showed a SDR of 2.2 and 3.8, respectively, whereas the SDR of the foreign genotypes varied between 1.0 and 3.0 ($p \leq 0.05$; $r^2 = 0.4$). Populations from Mexico and lines from South Africa showed no stalk damage, whereas only lines from Iowa and Missouri had higher SDR levels (Fig. 2).

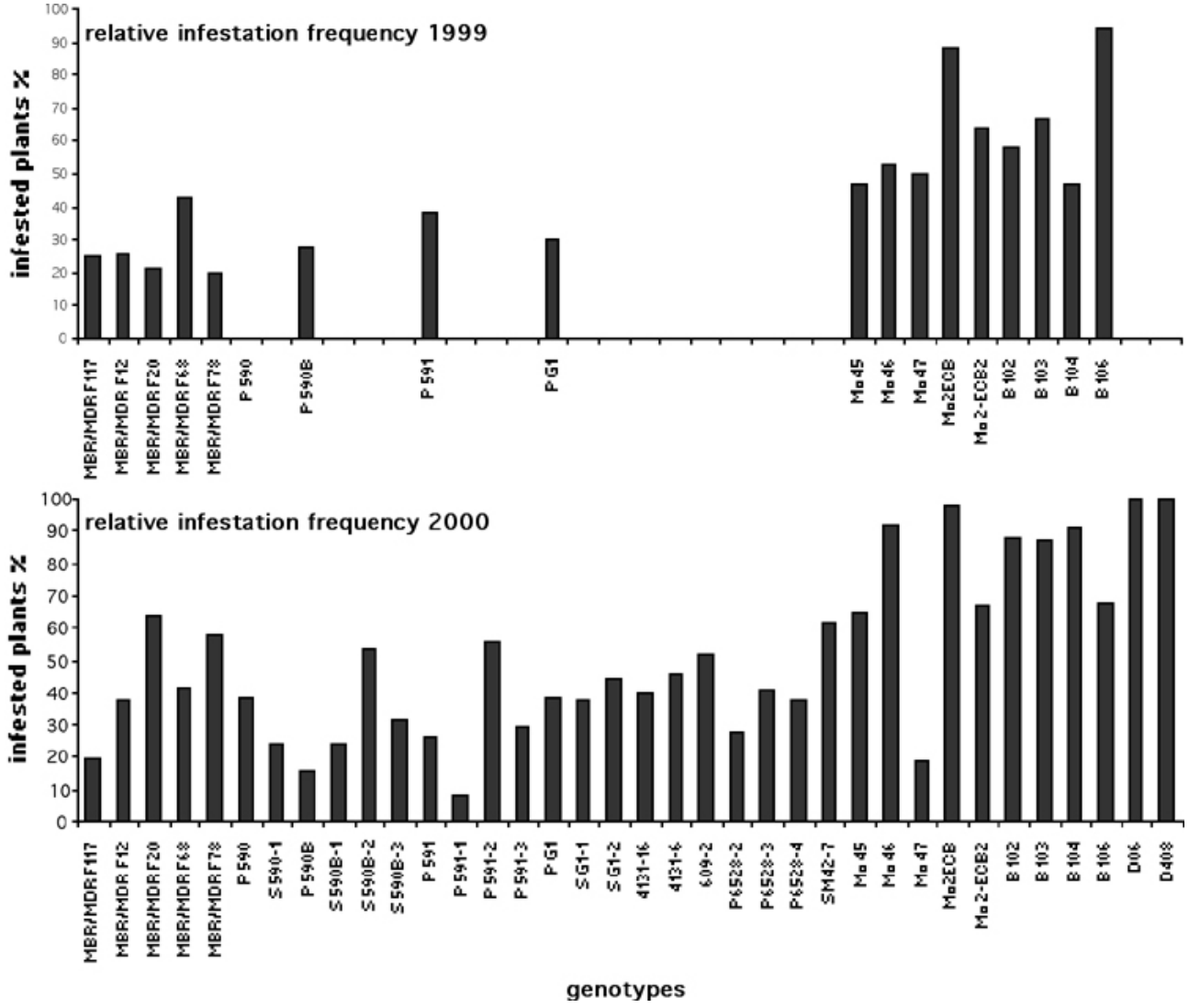

Figure 1. Comparison of relative infestation frequency in % of 1999 and 2000.

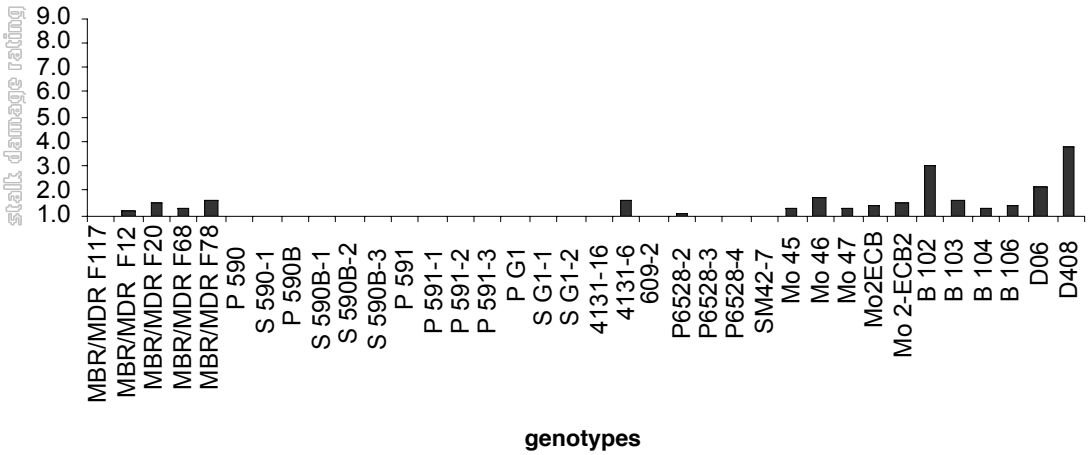

Figure 2. Stalk damage rating in 2000, according to a 1-9 rating scale of Hudon et al. (Maydica 36:69-70, 1991)(1=intact plant, 9=stalk damage below the ear or dropped ears).

For the European dent lines D06 and D408, the DA was after 78 and 76 days, using a foliage for three weeks after sowing. In contrast the DA of the foreign genotypes was much later between 99 and 116 days after sowing (Table 1).

In the year 2000, the DME was 57.1% and 55.5 %, respectively, for lines D06 and D408. In contrast to this, the DME of 19 genotypes mostly originating from Mexico and South Africa was below 40 %. Ten genotypes – mainly from Missouri and Iowa - reached between 40 % and 50 % and only four lines (SM42-7; Mo2-ECB, Mo2-ECB2 and B106) had a DME of 50 % or more (Table 1) and, thus, were comparable to the adapted European lines.

PH of the European lines D06 and D408 was 169 cm and 166 cm, respectively, whereas the foreign genotypes had a PH between 140 cm and 328 cm.

Highly significant correlations among the resistance characteristics were observed in the present study (Table 2). The resistance traits RIF, LA, and SDR showed a positive correlation ($r = 0.5 - 0.9$; $p \leq 0.01$) in both years. In 2000 the correlation between RIF, LA, and SDR was highly significant ($r = 0.5$ and 0.6), so that in the future probably the resistance could only be evaluated by SDR instead of the more labour intensive traits RIF and LA.

A high RIF could be observed in genotypes of Iowa and Missouri. These lines also showed a higher SDR in our evaluation and had the highest number of LA. In contrast the populations and lines from Mexico, had a very low RIF and in most cases no or only very small SDR. The European lines had a substantially higher RIF than all the other genotypes. For these, a very high number of LA and a relatively high SDR were found. Even the line D06, classified as resistant, had a SDR of 2.2, and was with one exception (Line B102 from Iowa) not exceeded by the other genotypes. Already in an earlier study with European material (Melchinger et al., Euphytica 99:115-125, 1998), relatively high SDRs between 2.5 and 7.3 were observed. Many of the genotypes examined in the present study showed an essentially lower infestation level than the established material, and thus, are very promising as probable sources of resistance genes.

A positive relationship between resistance traits and DA as well as maturity (DME) could be observed. Also in earlier studies (Russell, Crop Science 14:725-727, 1974; Kreps, Vortr. Pflanzenzüchtung 38:171-186, 1997), a positive correlation between late flowering time and increased resistance (lower SDR, AL, and RIF) to ECB has been found. Hudon, M and Chang, MS (Maydica 36:69-70, 1991) explained this relationship by an improved stalk stability of late maturing germplasm at harvesting time. For the non-adapted genotypes the DA was on average about 30 days later than for the European dent lines D06 and D408. Because of the late flowering also a bad maturation and therefore low DME, sometimes below 50 %, was observed. As the correlation analysis showed, the more susceptible lines from Iowa and Missouri were quite early in flowering (DA: 99 to 109 days after sowing), whereas the resistant populations from Mexico were later (DA: 110 to 118 days after sowing). So it could be supposed that the extraordinary "resistance potential" of the populations is not only based on genetic tolerance and antibiosis, but also is a consequence of late maturity. To study the influence of late maturity on the ECB resistance, and to use these genotypes commercially the material has to be adapted to Central European climatic conditions. Only a few of the genotypes examined (B106, Mo2-ECB2, Mo2-ECB and SM42.P28-7) reached a DME between 50 and 60% in late October and, thus, are near to the European breeding material in maturity. These genotypes appear to be the most suitable for further studies and practical breeding efforts.

Besides maturity, the PH as a further agronomic trait could also play a role in resistance. The negative correlation, observed between the PH and resistance, has already been described by Schulz, B et al. (Plant Breeding 116:415-422, 1997) and by Magg, T et al. (Plant Breeding 120:397-403, 2000). They showed that bigger genotypes mostly had a lower SDR and RIF. Possibly an increased stem stability of big plants is responsible for a smaller SDR. Additionally high lignin or phenol levels in higher plants are reported (Bergvinson, D et al., Insect resistant maize, CIMMYT, 1994) to have on the one hand an influence on the plant stability, and on the other hand a negative effect on the development and survival rate of larvae. Kreps, R et al. (Vortr. Pflanzenzüchtung 38:171-186, 1997) describe a “diluting effect”

Table 2. Correlations among agronomic and resistance traits.

	LA† 2000	RIF in % 1999	RIF in % 2000	SDR 2000	DME in % 2000	DA in days after sawing 2000	PHT 2000
AL 1999	0.72**	0.61**	0.82**	0.56*	0.44^{s}	-0.61**	-0.62**
AL 2000		0.69**	0.85**	0.54**	0.47**	-0.49**	-0.53**
RIF in % 1999			0.60**	0.35	0.54*	-0.37^{s}	-0.41^{s}
RIF in % 2000				0.63**	0.48**	-0.44**	-0.53**
SDR 2000					0.2	-0.23	-0.55**
DME in % 2000						-0.57**	-0.14
DA in days after sawing 2000							0.24

† AL= number of larvae alive per 100 plants, RIF = relative infestation level, SDR = stalk damage rating, DME = dry matter content of ears, DA = date of anthesis, PHT = plant height

^{s} correlation was significant at the 0.05 probability level
* correlation was significant at the 0.01 probability level
** correlation was significant at the 0.001 probability level

on the larvae by the increased PH, whereby the damage is minimized. But even if a better stalk stability is desirable for a lower SDR, for silage use the digestability should not be disregarded. As t is shown in Bohn, M et al. (TAG 101:907-917, 2000) the in vitro digestability of the organic matter (IVDOM) is negatively correlated with resistance. By splitting the stems for the resistance traits, we observed that the very tall and resistant populations of Mexico just had a great stalk stability in contrast to other lines. Therefore the digestibility of the plants is also to be evaluated for further ECB trials.

The non-European genetic material showed a very low susceptibility to the ECB that could not be reached by most of the Central European lines (see Melchinger, AE et al., Euphytica 99:115-125, 1998). Especially with regard to controversial discussions about transgenic maize and to the increasing importance of the European corn borer in Central Europe, the use of conventional sources of resistance genes is of great importance. Therefore marker assisted selection is promising to avoid high costs for manual infection and evaluation. Because breeding in Central Europe requires early material, above all the earlier flowering genotypes from Missouri and Iowa are of great interest. In these lines the high level of resistance is not only a consequence of late maturity. Nevertheless further breeding processes are necessary to improve the yield characteristics of this genetical material and to eliminate the effect of late maturity on the ECB resistance. The next step will be the selection for early-flowering individuals from the most promising sources, as well as a backcross program with established German material. Because ECB resistance is a quantitative trait, a lot of QTL (quantitative trait loci) studies for certain adapted populations have already been done (Groh S et al., Plant Breeding 117:193-202, 1998; Jampatong, C, Maize Genetics Conference Abstracts 40 (http://www.agron.missouri.edu/Coop/Conf/1998.html), 1998; Bohn M et al., J Econ Entomol 92:1892-1902, 2000), so the combination of different gene pools and their QTL could be of great importance for further genetic analyses and the improvement of the European breeding material.

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Regulation of plant uncoupling protein CSP 310 content in maize mitochondria during cold stress

--Kolesnichenko, AV, Zykova, VV, Voinikov, VK

Previously it was found that cytoplasmatic CSP 310 caused uncoupling of oxidation and phosphorylation in cereals (Voinikov et al., J. Therm. Biol. 23:1-4, 1998) because of its association with mitochondria (Kolesnichenko et al., J. Plant Physiol., 156:805-807, 2000). The presence of cold shock protein CSP 310 and CSP 310-like proteins in maize mitochondria was established (Kolesnichenko et al., J. Therm. Biol. 25:203-209, 2000) and its uncoupling activity in maize mitochondria was shown (Grabelnych et al., MNL 75: 21-22, 2001). Since it was shown previously that in winter cereals such as wheat and rye, CSP 310 uncoupling activity depends on the presence of a cytoplasmic "depot" of a low active form of this protein binding with nucleic acid, the aim of the present work was to study regulation of CSP 310 uncoupling activity in 3-day-old maize shoots during cold stress.

Mitochondria were extracted from three-day-old maize shoots by differential centrifugation as described previously (Pobezhimova et al., J. Therm. Biol. 21:283-288, 1996). Cold stress treatment was performed at 4 C for 1 h. The electrophoresis of native proteins was performed as described previously (Kolesnichenko et al., J. Therm. Biol. 25:203-209, 2000). Protein relative molecular weights were determined using the HMW kit of markers (Pharmacia, Sweden). Western blotting and

ethydium bromide staining of native gels were performed as described previously (Kolesnichenko et al., J. Therm. Biol. 25:203-209, 2000).

Ethydium bromide staining of native maize proteins showed the absence of nucleic acids (NA) binding to 300-310 kD cytoplasmic proteins (Fig. 1A). CSP 310 – like maize cytoplasmic proteins isolated by affinity chromatography as described previously (Kolesnichenko et al., J. Therm. Biol. 24:211-215, 1999) also showed the absence of NA (Fig. 1B). On the other hand, western blotting of the native maize mitochondrial proteins showed an increase of CSP 310 concentration during cold stress (Fig. 2). Therefore, we can suppose that the increased CSP 310 content in maize mitochondria depends on the synthesis of this protein de novo during cold shock or on possible structural changes in cytoplasmatic CSP 310-like proteins with different molecular mass but not on the presence of non-active CSP 310 "depot" in cytoplasm.

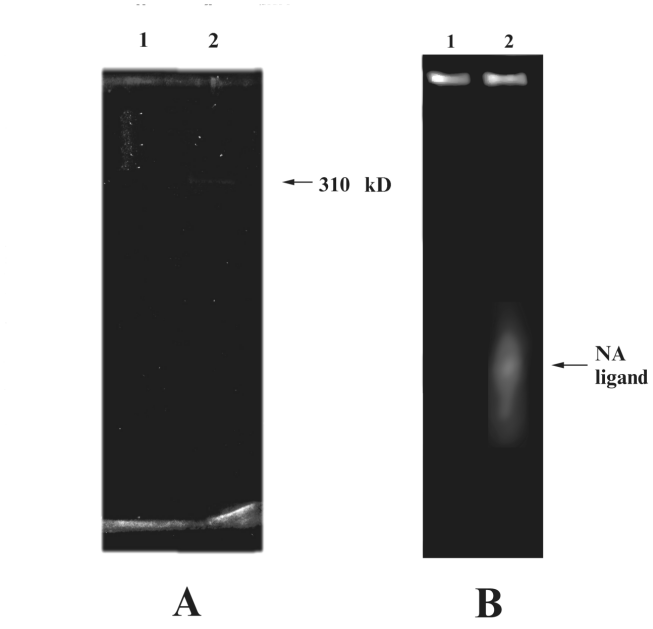


Figure 1. A. Ethydium bromide staining of native electrophoresis of control (1) and stressed (2) at 4 C, 1h maize cytoplasmic proteins; B. Ethydium bromide staining of cytoplasmic CSP-310-like proteins isolated by affinity chromatography from maize (1) and winter wheat (2).

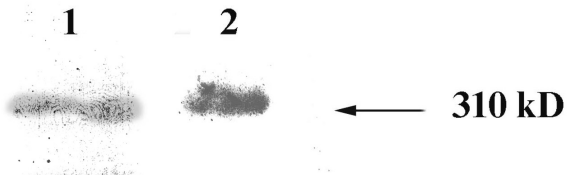


Figure 2. Western blotting of control (1) and stressed (2) at 4 C, 1h maize native mitochondrial proteins with antibodies toward CSP 310 from winter rye.

Based on the data obtained we can conclude that in maize the defense mechanisms against cold stress connected with uncoupling in mitochondria caused by cold stress protein CSP 310 differ from those in winter cereals.

Acknowledgements: The work has been performed with the support of the Russian Foundation of Basic Research (project 01-04-48953).

Metabolism DL-C^{14}-tryptophan in the maize root cells at low temperature

--Akimova, GP, Sokolova, MG, Nechaeva, LV, Luzova, GB

One of the factors regulating the synthesis of endogenous indolylacetic acid (IAA) may be the amount of available tryptophan (Trp), which is known to convert into IAA in plants, with the acid level regulated by the content of free Trp inside the tissue.

The present research was aimed at the demonstration of temperature impact on the content of free Trp in the roots of maize seedlings in connection with the change of IAA level at low positive temperature, and to determine the impact of a temperature of 10 C on metabolism of DL-C^{14}-tryptophan.

The study was carried out on the primary roots of 2 day old seedlings of maize varieties differing in resistance to low temperature - Omskaya-2 (resistant to low temperature) and Uzbeksksya tooth-like (non-resistant). Quantitative determination of free tryptophan was conducted using an amino acid analyser. Growth zones of maize root seedlings (meristem, commencement and termination of cell expansion) with the view to determine the impact of a temperature of 10 C on DL-C^{14}-Trp metabolism were cut immediately prior to the introduction of DL-C^{14}-Trp into the incubation medium. 0.05 ml of DL-C^{14}-Trp were placed into small Petri dishes with 5 ml of H2O; incubation was conducted at 27 C for 4 hours. The plant material was separated from the medium, washed three times with distilled water and put into Petri dishes on wet filter paper. Some of the dishes were transferred in a thermostate with the temperature of 10 C (test), the other part was left at the temperature 27 C (control) for 20 hours. The cut pieces were thoroughly ground, 10-fold volume of boiling ethanole was poured onto them; the samples were used to C^{14}-IAA and its metabolites.

Table 1 demonstrates a gradual increase of free Trp in the course of cell growth. At a temperature of 10 C Trp content in the growth zones of maize seedling roots of Uzbekskaya non-resistant variety goes through the following changes: it increases in the expanding cells and reduces in the cells, which have terminated expansion. The resistant variety Omskaya-2 shows Trp content close to the control.

Table 1. Free tryptophan content in maize root growth zones in optimal growth conditions and at low temperature.

Variety	Root growth zones	Tryptophan, mcg10-6/cell	
		27 C	10 C
Omskaya-2	Meristem	0.240.01	0.160.00
	Expansion commencement	1.660.03	1.760.04
	Expansion termination	3.860.15	3.770.06
Uzbekskaya tooth-like	Meristem	0.600.01	0.420.01
	Expansion commencement	5.800.09	12.360.25
	Expansion termination	21.600.22	14.900.30

Radio-isotope analysis showed that meristem cells and expansion zones actively adsorb Trp (Table 2). Marked Trp is incorporated into water and ether fractions and in the zones of chromatograms with Rf IAA (Table 3). Radioactivity of chromatogram zones with Rf IAA in percentage of radioactive ether fraction in the options with a temperature of 10 C in the cells of resistant variety reduces, and increases in the cells of non-resistant variety.

The data acquired allow the supposition that in the resistant variety IAA metabolism at 10 C takes place with a higher speed,

Table 2. DL-C ^{14}-tryptophan absorption speed by root growth zones.

Variety	Impulse/min per 1 g of raw material	
	Meristem	Expansion commencement
Omskaya-2	5952120	597297
Uzbekskaya tooth-like	438392	437487

Table 3. Radioactivity of water residue (1), ether fraction (2) and chromatogram zones with Rf IAA (3) (103 impulse/min/g of the raw material).

Growth zones	Control, 27 C			Test, 10 C		
	1	2	3	1	2	3
Omskaya - 2 (resistant)						
Meristem	55.51.1	27.70.6	0.440.09	66.41.2	31.40.6	0.310.06
Expansion commencement	48.01.0	24.00.5	0.240.06	66.51.2	52.91.6	0.310.06
Uzbekskaya tooth-like (non-resistant)						
Meristem	62.21.3	32.10.6	0.270.06	64.41.3	26.40.5	0.460.01
Expansion commencement	58.11.2	42.20.8	0.320.07	94.421.9	47.51.2	0.540.01

that is why C^{14}-IAA content amounts to a lower percentage as compared to control. In the non-resistant variety C^{14}-IAA metabolism slows down at 10 C, which is likely to account for IAA accumulation.

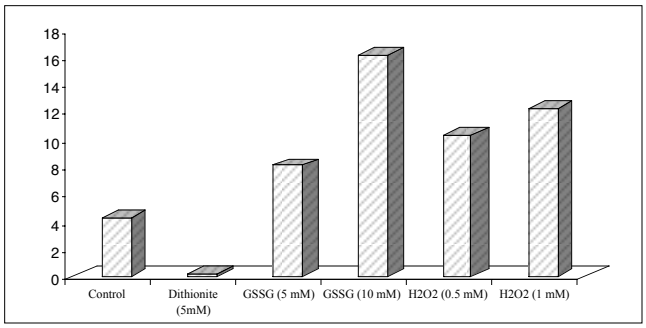
Therefore, the results of the investigation show a certain dependence between Trp content and the level of endogenous IAA in the maize root cells: low temperature increases tryptophan levels and IAA content compared to controls. At the same time, at 10 C the proportion between the rate of synthesis and IAA metabolism changes, and is obviously one of the critical factors of growth regulation in maize varieties differing in cold-resistance.

The effects of hydrogen peroxide on the translational activity in mitochondria

-- Konstantinov, YM, Subota, IY, Arziev, AS, Katyshev, AI

Recent findings suggest that at moderately high concentrations certain forms of reactive oxygen species (ROS) such as H_2O_2 may act as signal transduction messengers (Sen and Packer, FASEB J 10:709-720, 1996) and/or participate in redox regulation of gene expression, particularly by means of redox regulation of appropriate transcription activators (Sun and Oberley, Free Radic Biol Med 21:335-348, 1996). We showed that chemical and physiological redox agents influence significantly transcriptional and translational activities in isolated mitochondria (MNL 72:33, 1998). Substantial activation of both transcription and translation in mitochondria under oxidizing conditions and its strong repression under reducing conditions suggest potential multi-level redox control of mitochondrial gene expression. These data showed also that the translational activity of mitochondria is subjected to the presumably regulatory influence of the redox state of glutathione. Little is known about the effects of hydrogen peroxide as a potential regulatory molecule on the activity of protein synthesis in mitochondria.

In this work we investigated the influence of hydrogen peroxide on the translational activity of mitochondria to compare effects of this physiological oxidant with other redox agents. Mitochondria were isolated from 3-day-old etiolated maize seedlings (hybrid VIR42 MV) by a standard method of differential centrifugation. Mitochondrial protein was determined by the Lowry method. Protein synthesis in mitochondria was measured according to the method of Bhat et al. (Biochemistry 21:2452-2460, 1982) with the use of [^{35}S]-methionine.



The results obtained show that hydrogen peroxide (0.5 mM and 1 mM) caused the significant increase of translational activity in maize mitochondria (Figure 1). There were no substantial differences between activation effect of the oxidized form of glutathione (GSSG) and hydrogen peroxide. These data suggest that a physiological oxidant such as hydrogen peroxide may be one of the regulatory factors involved in multi-level redox control of mitochondrial gene expression.

Redox modulation of activity of DNA-binding protein factors in mitochondria

-- Konstantinov, YM, Tarasenko, VI, Kobzev, VF, Subota, IY, Katyshev, AI, Kirichenko , KA

We have previously reported (MNL 72:33, 1998; MNL 75:23-24, 2001) that transcriptional and translational systems of isolated mitochondria are under redox control, which involves presumably the glutathione redox system. It is well known that DNA topoisomerase I is an important part of the transcription machinery. We showed recently also (MNL 75:30-31, 2001) that mitochondrial DNA topoisomerase I is involved in in organello RNA synthesis. Our previous study of redox conditions' impact on the activity of mitochondrial DNA topoisomerase I in maize, showed that under oxidizing conditions, in the presence of GSSG, a significant decrease of topoisomerase activity was observed, whereas under reducing conditions, in the presence of GSH, enzyme activation was observed (MNL 73:39-40, 1999). Based on these data we suggested that DNA topoisomerase I is involved in redox regulation of transcription in mitochondria in in vivo conditions.

A recent report (M.I. Ikeda and M.W. Gray, Mol Cell Biol 19:8113-8122, 1999) suggests that transcription in wheat mitochondria also involves special DNA-binding protein. There is a great body of evidence that eukaryotic transcriptional activators (c-Jun, c-Fos, c-Myb, Egr-1, AP-2) and their DNA-binding activity may be the target for special redox regulation (Y. Sun and L.W. Oberley, Free Radic Biol Med 21:335-348, 1996). It is clear now that redox status plays an important role in the control of gene expression, both in the nucleus and in mitochondria. Alterations of the redox status in the cell can lead to changes in DNA binding and trans-activating abilities of many transcription factors, and thus lead to changes in gene expression which may affect cellular metabolism and adaptive responses (Y. Huang, F.E. Domann, Bioch Biophys Res Commun 249: 307-312, 1998). But little is known about DNA-binding proteins in maize mitochondria and the sensitivity of the DNA-binding activity of these proteins to redox conditions. The aim of the present work was to investigate the influence of redox conditions on DNA-binding activity in mitochondrial protein extracts.

The mitochondria were isolated from 3-day-old etiolated maize

seedlings of hybrid VIR42 MV by a standard method of differential centrifugation. Mitochondrial protein was determined by the Lowry method. Mitochondrial protein extracts were prepared by a salt lysis procedure performed in a buffer containing 25 mM Tris-HCl (pH 7.8), 600 mM, 1 mM EDTA, 10% glycerol, 13.5 mM 2-mercaptoethanol, 0.5 mM PMSF, 100 µg/ml BSA under stirring for 15 minutes at +4 C. The lysate was centrifuged for 15 minutes under 15,000 rpm. The supernatant obtained was subjected to dialysis against EMSA buffer. DNA-binding activity in mitochondrial extracts was detected in a gel mobility shift assay (EMSA). A DNA probe containing the yeast DNA topoisomerase I binding site was prepared as described (Kagoshima et al., J Biol Chem 271:33074-33082, 1996).

The effects of chemical and physiological redox agents on DNA-binding activity in mitochondrial protein extracts is shown in Figure 1. The mitochondrial DNA-binding activity is seen to decrease in the presence of sodium dithionite. In contrast, the treatment of potassium ferricyanide and the oxidized form of glutathione (GSSG) leads to a significant increase in DNA-binding activity. At the moment we could not explain similar effects both oxidized and reduced forms of glutathione on DNA-binding activity in mitochondrial protein extracts. Possibly, GSH converted rapidly in GSSG form as a result of high oxidant content in non-purified mitochondrial extract.

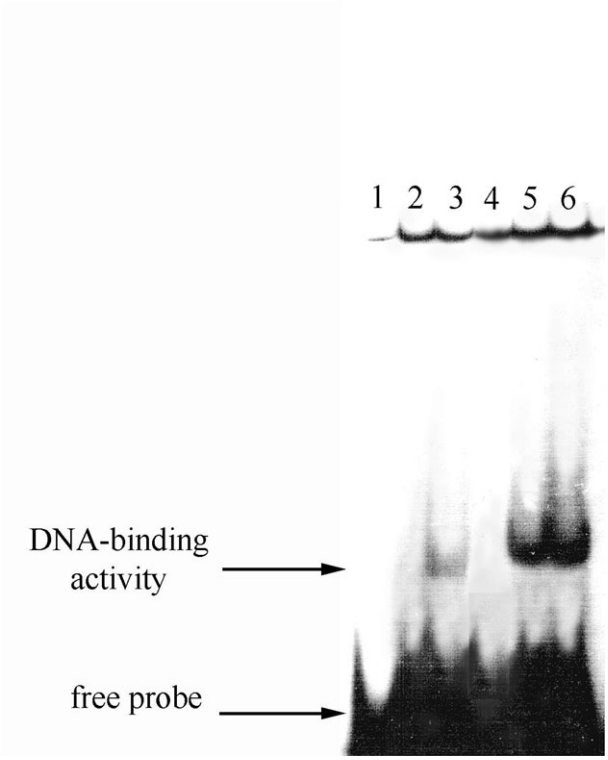


Figure 1. The DNA binding activity of mitochondrial extracts is modulated by reduction-oxidation with chemical and physiological redox agents in vitro. 1, free probe; 2, mitochondrial extract; 3, mitochondrial extract + potassium ferricyanide (5 mM); 4, mitochondrial extract + sodium dithionite (5 mM); 5, mitochondrial extract + glutathione in oxidized form (GSSG) (5 mM); 6, mitochondrial extract + glutathione in reduced form (GSH) (5 mM).

The results obtained suggest that DNA binding activity of proteins potentially implicated in transcription in maize mitochondria is redox-modulated in vitro, presumably by GSSG-mediated oxidation of the appropriate regulatory cysteine residues in transcription factors.

Financial support from the RFBR (Project Number 01-04-48162) is acknowledged.

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Male-sterile mutant *ms24* mapped to chromosome 10

--Fox, TW, Trimnell, MR, Albertsen, MC

Male-sterile mutant *ms24* was first described by DP West and MC Albertsen in MNL 59:87. Until recently, we did not have a chromosome arm map location for this male-sterile mutant. This meant that any new male-sterile mutants that were found would have to be test crossed to *ms24* to verify that they were not allelic to it. For several years, we have been trying to chromosome arm map *ms24*, and finally we have been successful.

In the 1999 Johnston, IA, nursery, we planted an *ms24* F2 segregating family to determine the chromosome arm map location of this mutant. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers, dispersed throughout the genome, were used to genotype these samples. Three markers on chromosome 10 showed linkage with the *ms24* phenotype. Using markers *bngl1655* and *phi059*, four recombinant alleles were found for each in 19 mutant plants (38 alleles) and 1 recombinant allele was found with marker *bngl1079* in 17 mutant plants (34 alleles). This data indicates that *ms24* maps to chromosome 10 near the centromere.

We made a mistake! *ms35* is allelic to *ms23*, but what is the correct map location?

--Trimnell, MR, Fox, TW, Albertsen, MC

During conducting some cytology work in our lab, Shannon Stenejhem found a reference in a 1985 MNL article written by DP West and MC Albertsen (MNL 59:87) that *ms23* was allelic to a male-sterile mutant designated *ms*-Bear7* by the late Earl Patterson and who later designated it as *ms*-6011* (MNL 69:126-128). In 1999, we identified *ms*-6011* as being a new male-sterile mutant, and we designated it as *ms35* (MNL 73:49-50). For whatever reason, we did not consider all the available information when we gave it the *ms35* designation. One of the reasons this may have occurred is that *ms23* had been reported to be located on chromosome 3L (MNL 62:71); whereas *ms35* had been reported to be located on chromosome 9L (MNL 69:126-128).

We decided that we should first find out if the 1985 allele test data was correct. We made test-crosses between *ms23* and *ms35* in the summer of 1999 and grew the progeny in the 1999 Hawaii winter nursery. The two male-sterile mutants were found to be allelic (data shown below):

Female	Male	Progeny		X2(1:1, P>0.050=3.84)
ms23 Hom	*ms35* Het	19 Fertiles	20 Steriles	0.03
ms35 Hom	*ms23* Het	18 Fertiles	16 Steriles	0.12

We then planted the original *ms35* seed that Earl gave us and test-crossed it to both *ms23* and the later developed *ms35* material, to make sure seed stocks had not been “mixed up”. These were grown in our 2001 Johnston, IA, nursery and also were found to be allelic (data shown below):

Female	Male	Progeny		X2(1:1, P>0.050=3.84)
ms35-Bear7 Hom	*ms23* Het	13 Fertiles	25 Steriles	3.79
ms23 Hom	*ms35-Bear7* Het	23 Fertiles	15 Steriles	1.68
ms35 Hom	*ms35-Bear7* Het	21 Fertiles	23 Steriles	0.09

In the 1999 Hawaii winter nursery, we grew an F2 that had been constructed to chromosome arm map *ms35* by using SSR markers. We wanted to compare the SSR-derived map location with the B-A translocation-derived map locations previously assigned to *ms23* and *ms35*. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers, dispersed throughout the genome, were used to genotype this family. No linkage was found with any markers on chromosome 3 or on chromosome 9. Because of this conflicting data, we are now preparing to re-map *ms23* with other SSR markers in the hopes of determining the correct map location.

New chromosome 10 male-sterile mutant: *ms47*
--Trimnell, MR, Fox, TW, Albertsen, MC

A new male-sterile mutant has been identified from among a collection of male-sterile mutants received from the late Dr. Earl Patterson. Earl had designated it as *ms*-6039* (see MNL 69:126-128).

We planted an F2 segregating family of *ms*-6039* in our 1999 Hawaii nursery to determine the chromosome arm map location of the mutant as part of our standard procedure in working with unmapped male-sterile mutants. We first determine the chromosome arm location, then we conduct the appropriate allele test-crosses. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers were used to genotype these samples. Shown below are four markers from chromosome 10 that showed linkage to the mutation.

Marker	Recomb. Alleles	Mutant Plants	% Recombination
bngl1074	9	22	(9/44) 20.5
bngl1079	4	21	(5/42) 11.9
bngl1655	5	24	(5/48) 10.4
phi050	7	24	(7/48) 14.6

The *ms*-6039* mutant appears to map on the short arm of chromosome 10, near the centromere.

After receiving the mapping data, we test-crossed *ms*-6039* with the recessive male-sterile mutants found on chromosome 10 (*ms10*, *ms11*, *ms24*, *ms29*), as well as with the unmapped male-sterile mutants (*ms27*, *ms31*), and those with questionable mapping data (*ms35*). The resultant progeny were grown in our 2001 Johnston, IA, nursery. At least 40 plants were observed for each test-cross. All test-cross progeny were found to be fertile, indicating that *ms*-6039* was not allelic to any of the tested mutants. Our new designation for male-sterile mutant *ms*-6039* is *ms47*.

New chromosome 9L male-sterile mutant: *ms48*
--Trimnell, MR, Fox, TW, Albertsen, MC

A new male-sterile mutant has been identified from among the male-sterile mutants received from the late Dr. Earl Patterson. Earl had designated it as *ms*-6049* (see MNL 69:126-128).

We planted an F2 segregating line of *ms*-6049* in our 1999 Johnston, IA, nursery to determine the chromosome arm map location of the mutant as part of our standard procedure in working with unmapped male-sterile mutants. We first determine the chromosome arm location. Then we conduct the appropriate allele test-crosses. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers were used to genotype these samples. The *ms*-6049* mutant maps to the long arm of chromosome 9, with two SSR markers being linked. Only two recombinant alleles from 19 mutant plants (38 alleles total) were found with either *bngl1012* or *bngl1159*, indicating fairly tight linkage with the mutation.

After receiving the mapping data, we test-crossed *ms*-6049* with the recessive male-sterile mutants found on chromosome 9 (*ms2*, *ms25*, *ms36*, *ms45*), as well as the unmapped male-sterile mutants (*ms27*, *ms31*) and those with questionable mapping data (*ms23*, ms35). The resultant progeny were grown in our 2001 Johnston nursery. At least 40 plants were observed for each test-cross. All test-cross progeny were found to be fertile, indicating that *ms*-6049* was not allelic to any of the tested mutants. Our new designation for male-sterile mutant *ms*-6049* is *ms48*.

New chromosome 10 male-sterile mutant: *ms49*
--Trimnell, MR, Fox, TW, Albertsen, MC

In the 1992 Hawaii winter nursery, Tony Stefani identified male-sterile plants segregating in the public inbred line ND203. He made sib pollinations with these male-sterile plants and gave the seed to us to grow in our 1993 Johnston, IA, nursery. These progenies did not segregate for the male-sterile phenotype, so they were self-pollinated. We did not grow these again until the 1995 Hawaii winter nursery. We planted two selfed ears in our nursery, and they segregated as follows:

Genotype	No. Fertile Plts.	No. Sterile Plts.	X2(3:1, P>0.050=3.84)
ND203 #)1	9	2	0.27
ND203 #)2	9	8	4.41

These male-sterile plants were then crossed with B73 and A632, and these F1 plants were self-pollinated. Their progeny were grown in our 1996 Hawaii nursery, and they segregated as follows:

Genotype	No. Fertile Plts.	No. Sterile Plts.	X2(3:1, P>0.050=3.84)
ND203/A632)1	11	4	0.02
ND203/B73)1	11	5	0.33

We designated this previously unknown male-sterile mutant as *ms*-MB92*.

In the 1998 Hawaii winter nursery, we planted the ND203/B73 F2 listed in the above data to determine the chromosome arm map location of the mutant as part of our standard procedure in working with unmapped male-sterile mutants. We first determine the chromosome arm location. Then we conduct the appropriate allele test-crosses. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers were used to genotype these samples. Shown below are three chromosome 10 markers that show linkage to the mutation.

Marker	Recomb. Alleles	Mutant Plants	% Recombination
bngl1079	1	24	(1/48) 2.1
phi062	6	24	(6/48) 12.5
phi059	5	24	(5/48) 10.4

The *ms*-MB92* mutant is closely linked to *bngl1079* and maps

near the centromere on chromosome 10.

After receiving the mapping data, we test-crossed *ms*-MB92* with the recessive male-sterile mutants found on chromosome 10 (*ms10*, *ms11*, *ms24*, *ms29*, *ms47*), as well as the unmapped male-sterile mutants (*ms27*, *ms31*) and those with questionable mapping data (*ms35*). The resultant progeny were grown in our 2001 Johnston nursery. At least 40 plants were observed for each test-cross. All test-cross progeny were found to be fertile, indicating that *ms*-MB92* was not allelic. Our new designation for male-sterile mutant *ms*-MB92* is *ms49*.

New chromosome 6L male-sterile mutant: *ms50*

--Trimnell, MR, Fox, TW, Albertsen, MC

A new male-sterile mutant has been identified from among the male-sterile mutants received from the late Dr. Earl Patterson.

Earl had designated it as *ms*-6055* (see MNL 69:126-128). We planted an F2 segregating line of *ms*-6055* in our 1999 Johnston, IA, nursery to determine the chromosome arm map location of the mutant as part of our standard procedure in working with unmapped male-sterile mutants. We first determine the chromosome arm location. Then we conduct the appropriate allele test-crosses. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers, dispersed throughout the genome, were used to genotype these samples. Markers *phi445613* and *phi070*, on chromosome 6, were linked to the mutation, with three recombinant alleles out of 20 mutant plants (40 alleles) and four recombinant alleles out of 22 mutant plants, respectively. This linkage indicates that *ms*-6055* maps to the long arm of chromosome 6.

After receiving the mapping data, we test-crossed *ms*-6055* with the recessive male-sterile mutants found on chromosome 6 (*ms1*), as well as the unmapped male-sterile mutants (*ms27*, *ms31*) and those with questionable mapping data (*ms35*). We did not test-cross this with the dominant male-sterile mutant, *Ms42*, nor could we test-cross this with the lost male-sterile mutant, *Ms21*. The resultant progeny were grown in our 2001 Johnston nursery. At least 40 plants were observed for each test-cross. All test-cross progeny were found to be fertile, indicating that *ms*-6055* was not allelic to any of the tested mutants. Our new designation for male-sterile mutant *ms*-6055* is *ms50*.

New male-sterile mutant alleles of known male-steriles

--Trimnell, MR, Fox, TW, Albertsen, MC

As part of our ongoing effort to identify new male-sterile loci in maize, we have identified numerous new alleles of known male-sterile genes. One of these new alleles, designated *ms*-NEMS*, was found in an EMS mutation population established by Stephen Smith, whereas the others (*ms*-TG92A*, *ms*-EA89A*, *ms*-FA92*) arose spontaneously in various breeding materials. Todd Piper, who at the time was the corn breeder at our Eau Claire, WI, Research Station, identified the *ms*-EA89* mutant and sent it to Marc Albertsen. Marc identified the other three mutations. The segregation of these male-sterile mutations in F2 familes is shown below except for *ms*-NEMS*, which also was grown as a sib-pollinated family, segregating 1:1 for the mutation.

Genotype	Year Identified	Year Grown	# Fertile Plants	# Sterile Plants	X2(1:1, P>0.050=3.84)
ms-NEMS* ms# Ear 10	1984	1997	10	6	1.00
ms-NEMS* ms# Ear 2	1984	1997	8	8	0.00
ms-NEMS* ms# Ear 7	1984	1997	5	2	1.29
					(3:1, P>0.050=3.84)
ms-NEMS*/A632)1	1984	1999	12	8	2.40
ms-NEMS*/A632)2	1984	1999	12	6	0.67
ms-TG92A*/A632)1	1992	1995	10	4	0.10
ms-EA89A*/A632)1	1989	1995	14	2	1.33
ms-EA89A*/B73)1	1989	1995	13	5	0.07
ms-FA92*/A632)1	1992	1995	14	4	0.07
ms-FA92*/A632)2	1992	1995	12	4	0.00

In the 1999 Johnston, IA, nursery and the 1999 Hawaii winter nursery, we planted F2 families to determine the map location of these mutants. As part of our standard procedure in working with unmapped male-sterile mutants, we first determine the chromosome arm location for a given mutant, then we conduct the appropriate allele test-crosses. Leaf punches were taken from 24 male-sterile plants and from 24 male-fertile plants for DNA isolation. 96 SSR markers, dispersed throughout the genome, were used to genotype these samples. Results for the SSR mapping are as follows:

Family	Linked Markers	% Recombination*	Mutant Map Position
ms-NEMS*	*phi96342*	2.3	
ms-NEMS*	*bngl1079*	4.8	
ms-NEMS*	*bngl1655*	4.5	
ms-NEMS*	*bngl1071*	23.8	
ms-NEMS*	*phi050*	4.8	10C
ms-TG92A*	*bngl1079*	5.6	10L
ms-EA89A*	*phi090*	19	2L
ms-FA92*	*bngl1065*	2.1	
ms-FA92*	*bngl1056*	23	
ms-FA92A*	*phi015*	29	
ms-FA92*	*phi121*	27.1	8L

* % Recombination was determined using only segregation scores from mutants.

After receiving the mapping data, we made test-crosses with all of the known recessive male-sterile alleles that mapped to the same respective chromosome as these mutants, as well as crossing with the unmapped recessive male-sterile alleles (*ms27*, *ms31*) and those with questionable mapping data (*ms35*). The resultant progeny were grown in our 2001 Johnston nursery. At least 40 plants were observed for most of the test-crosses. The reciprocal test-crosses of these mutants that showed allelism with known male-sterile mutants are shown below, along with their respective allele designations.

Female*	Male*	Progeny	X2(1:1)	Allele Designation
ms-NEMS*	*ms11*	22 Fertiles:20 Steriles	0.10	
ms11	*ms*-NEMS*	18 Fertiles:22 Steriles	0.40	*ms11-NEMS*
ms-TG92A*	*ms29*	21 Fertiles:20 Steriles	0.02	
ms29	*ms*-TG92A*	7 Fertiles: 7 Steriles	0.00	*ms29-TG92A*
ms-EA89A*	*ms33-GC89A*	17 Fertiles:12 Steriles	0.86	
ms33-GC89A	*ms*-EA89A*	14 Fertiles:23 Steriles	2.19	*ms33-EA89A*
ms-FA92*	*ms8*	14 Fertiles:15 Steriles	0.03	
ms8	*ms*-FA92*	18 Fertiles:22 Steriles	0.40	*ms8-FA92*

* All females homozygous for mutation, all males heterozygous for mutation.

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Maize pathogen defenses activated by avirulence gene *avrRxv*

--Simmons, CR, Tossberg, JT, Sandahl, GA, Marsh, WA, Dowd, PF, Duvick, JP, Briggs, SP

The *avrRxv* gene *Xanthomonas campestris* pv. *vesicatoria* is responsible for gene-for-gene resistance interactions in the hosts tomato and pepper, but also in other plant species, including maize, as when the *avrRxv* gene is transferred to *X. campestris* pv. *holcicola* (Whalen et al., PNAS USA 1998 85:6743-6747; Whalen et al., MPMI 1993 6:616-627). The *avrRxv* gene was found to encode a protein related to diverse pathogen genes affecting pathogenesis reactions ranging from plants to animals, including humans, for example *AvrA* of *Salmonella enterica*, *YopJ* of *Yersinia pseudotuberculosis,* and *YopP* of *Yersinia enterocolitica*. *YopJ* has recently been shown to interact with MAPK kinases (MKKs) and interfere with phosphorylation and signal transduction that control apoptosis (Orth et al., Science 1999 285:1920-1923). This is particularly interesting as there is growing indication that MAPK kinases are directly involved in controlling plant defense responses (e.g., Zhang et al., PNAS USA 95:7433-7438; Yang et al., PNAS USA 98:741-746). A recent report also indicates that the YopJ protein may be a cysteine proteinase, and through which control cell death, even HR cell death in plants (Orth et al. 2000 Science 290:1594-1597).

We used a transient expression tungsten particle/helium gun bombardment transformation assay to investigate *avrRxv* function in maize. This assay employed the CRC reporter gene system (a fusion of the *R* and *C1* transcriptional activator genes) that marks cells red due to flavonoid anthocyanin expression. The tissues used were prepared as follows. Mature embryos were derived from germinating scutella and placed face up on an agar medium such that there were 8 scutella in a 2 cm circle (7 peripheral and 1 central), each such octet constituting one bombardment target. Similarly immature embryos were isolated from field-grown ears 9-11 d after fertilization and plated scutellum-side-up on agar medium. Leaves (from stage V5-V6 plants) were obtained from the whorl, unfurled on an agar medium, and covered with a 1.3 cm diameter washer, the exposed center area constituting one target. For more details, see Briggs et al., 1999 PCT WO 99/43923.

When these maize tissues from various genotypic varieties were cotransformed with the *avrRxv* gene coding region and the CRC reporter system, both driven by the ubiquitin promoter, there frequently occurred suppression of the number of red anthocyanin-labeled cells expressing the CRC reporter gene construct. Table 1 provides quantification of this reduction in CRC expression caused by *avrRxv* cobombardment. A positive reaction to *avrRxv* was herein defined as a two-fold or more reduction in the number of red cells. The range in average number of red cells between transformation experiment events could be substantial.

Table 1. The effect of *avrRxv* expression on the CRC reporter gene expression (via anthocyanin-producing red cell phenotype), by tissue and genotype.

Genotype	Tissue [a]	Ratio [b]	DR [c]
PHN46	ME	90.0	+
PHN46	ME	43.6	+
PHN46	ME	5.0	+
PHN46	ME	28.5	+
PHN46	ME	46.0	+
PHN46	IE	4.1	+
PHN46 [d]	IE	47.0	+
B73	LF	2.0	+
B73	LF	6.9	+
B73	LF	2.2	+
B73	LF	1.6	- [e]
1047	ME	3.6	+
B73	ME	49.5	+
PHKM0	ME	46.7	+
CN3K7	ME	162.9	+
PH428	ME	4.4	+
PHHB9	ME	4.0	+
953	ME	10.1	+
PHP02	ME	2.0	+
PHP82	ME	17.1	+
PHK03	ME	2.8	+
3394 [f]	ME	3.2	+
PHK76	ME	0.8	-
PHKE1	ME	1.4	-
PHW52	ME	0.9	-
PHW52	ME	1.1	-
PHP38	ME	0.8	-
PHK46	ME	1.1	-
PH647	ME	0.6	-
PHK56	ME	0.9	-
Mo17	ME	0.7	-

[a]Tissues are: ME, mature embryo; IE, immature embryo; and LF, leaf.
[b]Ratio of average number red transformed cells for CRC only control divided by the average for CRC+*avrRxv* cotransformation.
[c]When the CRC/CRC+*avrRxv* ratio exceeds two, a defense response (DR) is concluded present (+); when less than two, absent (-).
[d]Here only, the *avrRxv* gene DNA amount 10-fold higher than in the other experiments.
[e]Ratio falls just below two-fold threshold. here, but other B73 experiments are positive
[f]3394 is F1 progeny of PHN46 and PHP38.

Nonetheless, multiple experiments for inbred varieties such as PHN46 clearly indicated that *avrRxv* caused a marked reduction in CRC expression. The positive reactions to *avrRxv* varied from 2.0 to 162.9-fold reductions in CRC expression.

Twenty maize varieties were used in these transient transformation studies, 19 inbreds and one hybrid, of which a majority of 12 showed reduction in CRC expression, and 8 did not. The variety 3394 is the F1 progeny of inbred PHN46, which shows the response, and inbred PHP38, which does not. The *avrRxv* caused a 3.2-fold reduction in CRC expression in 3394, indicating that the response is dominant, or at least semi-dominant. Most of these experiments were done for mature embryos, especially when surveying response in different varieties. However, experiments with PHN46 indicated that this *avrRxv* response was present in tissues ranging from leaves, immature embryos and mature embryos. In both embryos and leaves, the reduced CRC expression caused by *avrRxv* in these experiments was correlated with an increase in PR1 protein expression on western blots, a commonly used marker of plant defense system activation.

Maize immature embryo-derived cells were transformed with the ERE::*avrRxv* construct, which contains an estrogen-regulated promoter (ERE) to drive *avrRxv* gene expression. This ERE::*avrRxv* construct contains three tandem plant gene expression units; the estrogen receptor, the estrogen response elements controlling *avrRxv*, and the selectable marker PAT

(phosphinothricin acetyltransferase). This system allows for a tightly controlled, high dynamic range of transgene *avrRxv* expression, with basal expression permitting recovery of healthy transgenic callus. In ERE::*avrRxv* transgenic HiII callus, estradiol treatment was demonstrated by northern blots to rapidly (within an hour) induce *avrRxv* mRNA expression, with a peak by 12-24 hours at 21-fold above baseline. Following estradiol treatment of ERE::*avrRxv* callus or cell suspensions, there occurred visible and biochemical changes consistent with a precipitant defense response, such as: 1) tissue browning and necrosis; 2) induced expression of methanol-soluble metabolites with an absorbance between 230-350 nm - these likely including phenolics, well-documented plant defense response compounds; 3) induced PR1 and chitinase expression on western blots; and 4) induced cationic peroxidase expression on native IEF gels. The cell death associated with the elevated accumulation of pathogenesis-related proteins and molecules are interpreted as indications that a defense response was chemically-induced in the ERE::*avrRxv* system. The induction of a cationic peroxidase species by *avrRxv* was of special interest, because a cationic peroxidase has been shown to be induced in incompatible interactions between rice and *Xanthomosas oryzae* pv *oryzae* (Reimers et al., Pl. Phys. 1992 99:1044-1050).

A high density Affymetrix GeneChip® microarray of some 1500 maize gene sequences (est. 1441 unique genes) representing diverse physiological processes, was next used for surveying mRNA expression changes following chemically-induced *avrRxv* expression in the ERE::*avrRxv* callus. Protocols for preparing in vitro-transcribed biotinylated cRNA probes from poly-A+ mRNA for Affymetrix GeneChip® gene expression analysis were carried out according to the manufacturer's recommendations (Affymetrix, Santa Clara, CA). It was observed that estradiol treatment of ERE::*avrRxv* callus caused a three-fold or more (range 3.5 to 33.2-fold) induction in the expression (mRNA abundance) of nine genes represented on this microarray (Table 2). None of these genes were induced three-fold or more by estradiol treatment of control callus. All of the induced genes are known or suspected plant defense-related genes. In fact, most of these nine have been independently confirmed to be defense inducible in various defense-related experiments, including those with live pathogens, using northerns and other profiling technologies. While one should be aware that promoter systems related to the ERE-estrogen response system could alter gene expression (see Kang et al., Plant J. 1999 20:127-133), these mRNA profiling results, along with the transient bombardment assay above and the previous studies by Whalen et al., appear to support that a maize defense reaction follows induced *avrRxv* expression. Of the 1500 gene sequences on the microarray, only these nine were induced three-fold or more by these criteria. None of the 1500 genes were repressed three-fold or more. Maize is variously estimated to have between 40,000-80,000 genes, so these 1441 genes represent only about 1.8-3.6% of the total. We can assume by extrapolation that there exist many other co-regulated maize genes not assayed in this study.

Table 2. Affymetrix 1500 GeneChip® Microarray Expression Profiling: mRNAs induced three-fold or more in transgenic ERE:: *avrRxv* cells

Gene Name or Description[a]	Fold Change[b] Ave	SE
Proteinase inhibitor WIP1 (Bowman-Birk) (=emb|X71396)	33.2	5.9
Chitinase (Class I type) (=EST gi|8273121)	15.1	3.6
Old yellow enzyme homolog (=EST gi|6672185)	11.7	0.2
Proteinase inhibitor (Bowman-Birk) (=EST gi|7031219)	8.7	1.1
Proteinase inhibitor (Subtilisin-chymotrypsin) (=emb|X69972)	7.8	1.1
Major latex protein, root allergen protein (=EST gi|3157104)	4.9	1.7
Antifungal zeamatin-like protein (=gb|U06831)	4.7	0.4
Pathogenesis-related protein (nearly=EST gi|5398728)	4.0	0.3
Metallothionein (=EST gi|5499507)	4.0	0.9

[a]Gene name or general description derived from blast search of public NR, NT and EST databases. Equal signs (=) indicate essentially exact nucleotide match to the Pioneer Hi-Bred EST gene sequence used on the microarray.

[b]Fold change in mRNA abundance as measured by relative fluorescent intensity of hybridizing signal. Average and SE were calculated from 3 or 4 replicate comparisons between callus samples ERE::*avrRxv* estradiol-treated versus control not estradiol-treated.

By extension from previous related studies involving avirulence gene expression in plants, it would appear that *avrRxv* elicits an incompatible-like HR response in maize possessing a cognate functional resistance gene (i.e. *Zm-Rxv*). This putative *Zm-Rxv* gene appears to be dominant or semi-dominant, like most *R* genes, and it functions in diverse tissues, namely leaves, mature and immature embryos, and callus/cell suspensions. It is also possible however that the *avrRxv* defense response is not specifically *R*-gene or HR-related. Alternatively, at least in maize, AvrRxv could be operating more as a pathogenicity factor that elicits a defense or stress response. Its relationship to *YopJ* and other pathogenicity or virulence factors, suggests a common and conserved molecular mechanism, one that may have just come to light (see Orth et al. 2000 Science 290:1594-1597).

Of general significance is the observation that an avirulence gene *avrRxv*, plucked from a specific host-pathogen system, can elicit defense reactions in non-host plants, even those as diverged as monocots and dicots. Together with other transgenic and conventional breeding studies, this appears to affirm an extensive portability of functional resistance and avirulence genes that bodes well for their application in crop improvement. Nonetheless, many avirulence gene types may not be so widely recognized as the group containing *avrRxv,* which by including *YopJ*, control defense responses in hosts as far apart as plants and animals.

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Influence on seed size of chromosomal abnormalities in maize cells resulting from irradiated pollen

--Viccini, LF, de Carvalho, CR

The production of plants with chromosomal variations has evolved as a valuable cytogenetic tool for gene identification, isolation and mapping using molecular approaches. Considering the difficulty of identifying these plants, the determination of indicators of the existent level of alteration is important to facilitate the selection of abnormal plants. With the objective of verifying the relationship between seed size and chromosomal abnormalities in maize meristematic and meiotic cells resulting from irradiated pollen, maize tester line L-869 of the Federal University of Viÿosa pollen was exposed to 36 and 72 Gy of gamma irradiation and soon thereafter used for pollination. The seeds originating from those two doses were ranked in size classes in order to relate with the percentage of chromosomal alterations. For mitosis study, seeds

were germinated in Petri dishes with a film of distilled water in the dark at 28-29 C. Root tips ranging from 0.5 to 1.0 cm in length were fixed in fresh cold methanol-acetic acid (3:1). Slides were prepared by the air drying technique with enzymatic maceration. After drying on a hot plate, the slides were stained with Giemsa solution. The percentage of abnormal anaphase cell carriers chromosomal bridges was evaluated. In the meiosis study, seeds were germinated in a greenhouse so that the immature tassels were collected at the appropriate stage. Slides were also prepared by the air drying technique. Deficiencies, heteromorphic pairs, partial pairing, laggards, bridges, fragments, fusions, translocations and cells with altered chromosome number due to structural anomalies were observed. The statistical analysis was carried out splitting the sum of the squares for treatments in orthogonal contrasts. Considering the 35.7 Gy dosage-irradiated meristematic cells, no relation was detected between size of the seeds and percentage of abnormal anaphases, while for the 71.4 Gy dosage it was possible to verify an increase of the percentage of abnormal anaphases with the decrease of seed size. Regarding the meiotic cells, the relationship between seed size and levels of chromosomal variations was verified for both radiation doses (36 e 72 Gy). That is to say, small seeds presented a larger number of chromosomal variations. The analysis of M1 plants confirmed this relationship, with smaller-sized plants being observed when small seeds were germinated.

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Meiotic analysis of the artificial hybrids between *Zea luxurians* and the subspecies of *Zea mays*.

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Meiotic behavior in *Zea luxurians* and three subspecies of *Zea mays* belonging to Section *Zea* (*Z. mays* ssp. *parviglumis*, *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *mays*) and their artificial F1 hybrids have been analyzed to investigate their meiotic configurations and to detect chromosomal rearrangements acting as reproductive isolation mechanisms. Poggio et al. (Genome 49:993-1000, 1999) showed that the hybrids between *Zea luxurians* and the other species of the Sect. *Luxuriantes* (*Z. perennis* and *Z. diploperennis*) have irregular meiotic configurations and low fertility.

The F1 hybrid *Z. luxurians* x *Z. mays* ssp. *parviglumis* showed pachytene heterozygosis in the knob regions (Fig. 1A), meiotic asynchrony of two groups of 5 II each (diplotene-diakinesis) (Fig. 1B), heteromorphic bivalents and univalents of different size (diplotene-diakinesis, metaphase I) (Fig. 1C), bridges and fragments (anaphase I), and laggard chromosomes (anaphase I and II). These phenomena were also observed in the F1 hybrid *Z. luxurians* x *Z. mays* ssp. *mexicana* (Fig. 1D, E, F)). In the F1 hybrid *Z. luxurians* x *Z. mays* ssp. *mays* heteromorphic bivalents and 1-2 bridges and fragments (anaphase I) were observed (Fig. 1 G-K).

In these hybrids 8 II + 4 I was the most frequent configuration, although the range of univalents were 0-20 per cell (Fig. 1D).

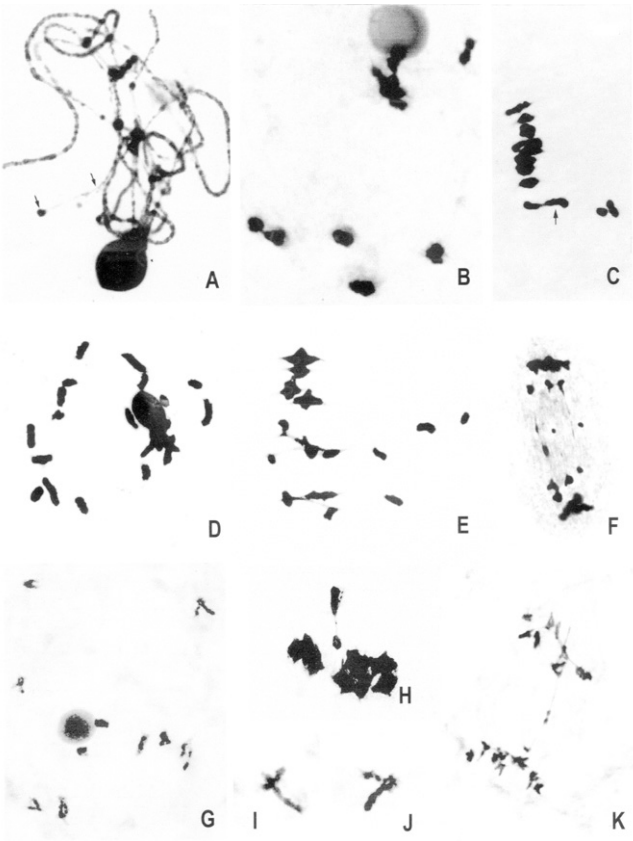


Figure 1. A-C: *Z. luxurians* x *Z. mays* ssp. *parviglumis*. D-F: *Z. luxurians* x *Z. mays* ssp. *mexicana*. G-K: *Z. luxurians* x *Z. mays* ssp. *mays*.
A: Pachytene: the arrows show irregular pairing in the knob regions.
B: Diakinesis: meiotic asynchrony of two groups of 5 II each.
C. Metaphase I: the arrow shows a heteromorphic bivalent, univa lents of different size are observed off the plate.
D: Diakinesis: 20 univalents.
E: Metaphase I: heteromorphic bivalent and univalents of different size off the plate.
F: Anaphase I: Two bridges and two fragments.
G: Diakinesis: 10 heteromorphic bivalents.
H: Metaphase I: heteromorphic bivalent.
I - J: Open heteromorphic bivalents.
K: Anaphase I showing one bridge.

Moreover, they were highly sterile (pollen stainability: 0-12%) and showed loss of pairing in heterozygotes for knobs in pachytene. The genome size varies between 2C= 5.8 - 6.8 pg in the subspecies of *Zea mays*, while *Z. luxurians* has 2C= 9 pg. The difference in the genome size between the hybrid's parental species could explain the presence of heteromorphic bivalents. The presence of two asynchronic groups of 5 II was also frequent.

The bridges and fragments, observed in anaphase I, in the studied hybrids would indicate that the parental species differ in 2-3 paracentric inversions between them.

The presence of two groups of 5 II suggests that the genomes of these hybrids maintain structural and functional independent domains, despite the existence of intergenomic rearrangements revealed by molecular data.

New maize inbreds in Argentina. I: Heat unit requirements.

--Corcuera, VR, Bernatené, EA, Naranjo, CA

During the last growing season 2000/2001 a field trial was sown at the Inst. Fitotécnico de Santa Catalina to evaluate different maize inbreds developed during the last decade for several

agronomic traits. The list of materials is shown in Table 1. The inbreds studied can be divided by their endosperm type into the following categories: 1) waxy maize, 2) opaque-2 maize, 3) +/wx maize and 4) opaque-2/wx maize.

Table 1. List of materials evaluated by their HUR in a field trial during 2000/01.

PEDIGREE	ENDOSPERM	GENERATION	PEDIGREE	ENDOSPERM	GENERATION
2000-3098	OPAQUE2	INBRED	2000-3092	OPAQUE2	INBRED
2000-3088	OPAQUE2	S7	2000-3130	OPAQUE2	S1
2000-3141	NOR/O2	S1	2000-3142	NOR/O2	S1
2000-3132	NOR/O2	S1	2000-3133	NOR/O2	S1
2000-3134	O2/wx	S1	2000-3135	O2/wx	S4
2000-3096b	O2/wx	S3	2000-3096c	O2/wx	S4
2000-3096a	O2/wx	S3	2000-3136b	O2/wx	S3
2000-3137	NOR/O2	S1	2000-3138	NOR/O2	S1
2000-3139	OPAQUE2	S1	2000-3022c	NOR/wx	S7
2000-3022b	NOR/wx	S7	2000-3022a	NOR/wx	S7
2000-3072	NOR/wx	S3	2000-3003	NOR/wx	S3
2000-3024	NOR/wx	S4	2000-3014	NOR/wx	S4
2000-3074a	NOR/wx	S2	2000-3074b	NOR/wx	S3
2000-3074c*	NOR/wx	S4	2000-3074d*	NOR/wx	S3
2000-3016a	WAXY	S7	2000-3016b	WAXY	S4
2000-3002a	WAXY	S4	2000-3002c	WAXY	S6
2000-3078a	WAXY	S4	2000-3078b	WAXY	S4
2000-3136a	O2/wx	S2	2000-3078d	NOR/wx	S4

***:** irradiated materials, 150 Gy Co60.

Different evolutive cycle traits were measured and analyzed, but in this article we shall only refer to Heat Unit (C) requirements necessary for silking (stage R1, Hanway scale). Heat Units (C) were calculated according to the formula proposed by the US Weather Bureau (USWB, 1958):

$$HUR\ (C) = \Sigma DHU$$

DHU = (min temp C + max temp C) / 2 - 10 C[1]
HUR = Heat Unit Requirements during the whole cycle
DHU = Daily Heat Units
[1]: 10 C was taken as the growing base temperature of these materials.

We prefer to base the discussion on HUR instead of number of days to silking because, as is well known, it makes it possible to compare amongst genotypes evaluated in different years and locations.

Most of the waxy maize selected as "foundationals" at the beginning of this breeding programme in 1990 were precocious (418 C – 518 C for silking). On the other hand, while the "foundationals" opaque-2 type showed a long evolutive cycle (750 C – 870 C for silking), some normal endosperm maize denoted medium or short evolutive cycle (484 C – 568 C). All data collected since 1990 up-to-date through the inbreeding and selection process point out that whether inbreeding tends to lengthen evolutive cycle traits, it was possible to obtain new short evolutive cycle (472 C – 535 C) opaque-2 maize inbreds (93.75 % homozygosity) (see Table 2 and Graphs 1 and 4). Then selection by precocity was successful in these materials. In a similar way, the different S3 – S7 waxy inbreds (87.5 % to 99.2% homozygosity) complete their thermal requirements to R1 with 451 C-609 C (see Table 2 and Graphs 2 and 3).

Inbred 3074 merits separate analysis. It was developed from the original backcross (SCV1 x A255) x SCV1. During the growing season 2000/01, the S2 and S3 generations of this inbred were evaluated, as were an irradiated sample of the S3 and S4 generations. Gamma irradiation consisted of a dose of 150 Gy of Co60 using a Gamma-cell. The S2 showed the lower HUR for silking (451.5 C) whilst S3 showed the highest (581.1 C). The differences between values are statistically significant at 1% prob

Table 2. Comparisons of means for Heat Units for silking (C).

a. Opaque-2 maizes

Genotype	Average	Groups *
3098	585.9	A
3130	571.3	AB
3092	562.3	AB
3088	559.2	AB
3141	542.2	ABC
3132	539.0	ABC
3139	535.1	ABC
3142	505.5	BC
3138	471.6	C

b. Opaque-2/waxy maizes

Genotype	Average	Groups *
3136b	608.6	A
3136a	599.1	A
3133	591.8	A
3135	587.0	A
3137	585.0	A
3134	574.9	A
3096b	566.9	A
3096c	490.4	B
3096a	484.8	B

c. +/waxy maizes

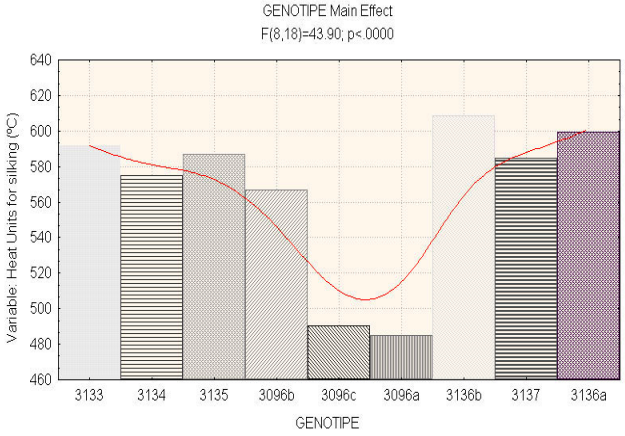
Genotype	Average	Groups *
3078d	615.1	A
3022b	595.0	AB
3074b	580.5	ABC
3022a	578.1	BC
3022c	575.4	BC
3003	552.9	CD
3072	551.6	CD
3024	538.3	DE
3074c	513.1	F
3074d	498.7	F
3014	487.1	F
3074a	451.6	G

d. Waxy maizes

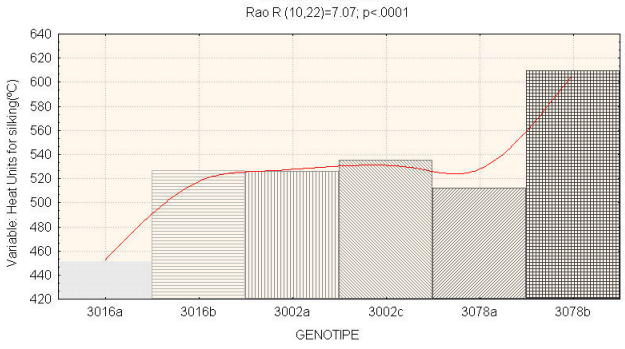
Genotype	Average	Groups *
3078b	609.5	A
3002c	535.6	B
3020	535.6	B
3016b	526.8	B
3002a	525.9	B
3078a	512.1	B
3016a	451.2	C

* Averages followed by the same letter belong to the same group and do not differ significantly at 1% of probability.

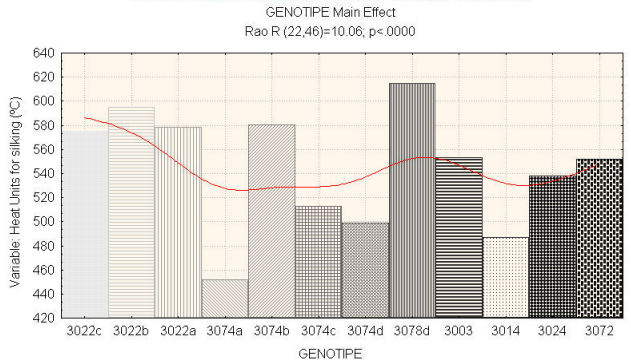
GRAPH 1: Heat units average values for silking in o2/wx maizes



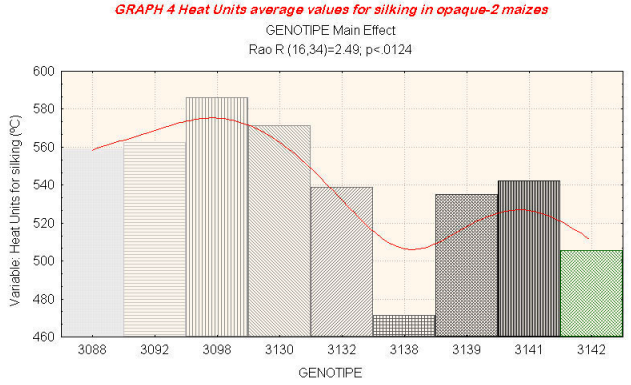
GRAPH 2: Heat units average values for silking in waxy maizes



GRAPH 3: Heat units average values for silking in +/wx maizes



GRAPH 4 Heat Units average values for silking in opaque-2 maizes



ability based on t *Student*. The irradiated generations showed different values: S3 (498.7 C) and S4 (513.5 C). Then, the results are clear that the irradiated generations of the inbred have a shorter cycle than their non-irradiated counterpart. According to the data presented in this article one could state the following:

1. When the inbreeding process progressively lengthens the evolutive cycle, precocious materials can be obtained by selection.
2. Gamma irradiation was a useful tool to generate new precocity in some materials.
3. New precocious inbreds were obtained by quality breeding and are now being tested to produce commercial hybrids.

New maize inbreds in Argentina. II. Reponse to *Puccinia sorghi* (Schw.)

--Corcuera, VR, Sandoval, MC

During the growing season 2000/01 a field trial was conducted at the Inst. Fitotécnico de Sta. Catalina in order to measure the ability and competitiveness of different inbreds developed during the last years within a qualitybreeding programme held at our Institution. Amongst several agronomic traits, respone to "maize common rust" was determined considering: 1) attack *severity* or % of leaf area with pustules (based on the visual scale developed by Peterson et al., 1948 for Wheat orange rust) and 2) type or degree of infection, based on a modification of Cobb´s scale as follows:

No. pustules/leaf	Severity %	Degree of infection	Behaviour to pathogen
<11	<5	I	*Very resistant*
12-19	6-10	II	*Resistant*
20-38	11-25	III	*Mid-resistant*
39-67	26-40	IV	*Mid-susceptible*
68-83	41-65	V	*Susceptible*
>84	66-100	VI	*Very susceptible*

Measurements in the field were taken one month after anthesis and 3 leaves/plant were considered (those of the upper ear and one leaf up and down). Twenty-four plants per inbred (3 repetitions) were observed and their data were averaged. Table 1 summarizes all data collected and gives the respone of each inbred to the pathogen. Attack severity data were transformed into *arcsin* according to Snedecor G(1962) and then an ANOVA test was performed within inbreds with the same type of endosperm (normal or modified). In all the cases analyzed, the differences found amongst averages were due only to variation of the genotype, so no environmental influence exists. In spite of this, the different inbreds can be grouped depending on their average values for attack severity (see Tables 2 and 3). There are 5 different groups in modified endosperm maize and only 3 in normal endosperm inbreds.

Table 1. List of Materials evaluated and their behaviour to *Puccinia sorghi*(Schw.).

Pedigree	Generation	Endosperm	Severity(%)	No. Pustules	Infection Grade	Behaviour*
2000-3098	OPACO2	LE	11a 25	24	III	MR
2000-3092	OPACO2	LE	11 a 25	24	III	MR
2000-3088	OPACO2	S7	6A10	17	II-III	R
2000-3130	OPACO2	S1	41A65	81	IV	MS
2000-3141	NOR/OPAC2	S1	66-100	114	VI	S
2000-3142	NOR/OPAC2	S1	66-100	98	VI	S
2000-3131	NOR/OPAC2	S1	5	10	II	R
2000-3132	NOR/OPAC2	S1	11 A 25	36	III-IV	MR
2000-3133	NOR/OPAC2	S1	6 A 10	17	II	R
2000-3134	o2/cshwx	S1	11 A 25	29	II-III	R
2000-3135	o2/cshwx	S4	<5	8	II	R
2000-3096b	o2/cshwx	S3	26 A 40	43	IV	MS
2000-3096c	o2/cshwx	S4	11 A 25	20	III-IV	MR
2000-3096a	o2/cshwx	S3	11 A 25	30	III-IV	MR
3136b	o2/cshwx	S3	0	0	I	VR
2000-3137	nor/cshwxo2	S1	11 A 25	28	III	MR
2000-3138	nor/o2/o5	S1	41 A 65	60	III-V	MS
2000-3139	o2/o2Oh43	S1	<5	9	II	R
2000-3022c	nor/wx	S7	0	0	0	VR
2000-3022b	nor/wx	S7	<5	6	II	R
2000-3072	nor/wx	S3	26 a 40	60	IV	MS
2000-3002a	w x	S4	65	72	IV	MS
2000-3002c	w x	S6	26 a 40	57	IV	MS
2000-3043a	normal	LE	11 a 25	37	III-IV	MR
2000-3043b	normal	LE	26 a 40	62	IV	MS
2000-3063a	normal	S2	11 a 25	36	III	MR
2000-3063b	normal	S3	41 a 65	75	IV-V	MS
2000-3140	normal	S2	40	67	IV	MS
2000-3165	normal	S2	<11	5	HI	R
2000-3031	normal	S6	26 a 40	46	IV	MS
2000-3008a	normal	S5	<11	5	HI	R
2000-3043a	normal	LE	11 a 25	37	III-IV	MR
2000-3027	normal	S5	<5	8	0-I	VR
2000-3071	normal	S4	10	19	II	R
2000-3011	normal	S3	<5	3	0-I	VR
2000-3028	normal	S3	<5	2	0-I	VR
2000-3057	normal	S4	25	35	III	MR
2000-3136a	o2/cshwx	S2	26 a 40	44	III-IV	MR

*: VR: very resistant, MR: mid-resistant, MS: mid-susceptible, S: susceptible

Table 2. Comparison of means of "attack severity" to *Puccinia sorghi* (Schw.) in modified endosperm maize.

Genotype	Endosperm	Average	Groups(1)
3142	NOR/OPAC2	77.30	A
3141	NOR/OPAC2	75.00	A
3130	OPAQUE2	46.53	B
3096b	o2/cshwx	35.40	BC
3098	OPAQUE2	28.63	BCD
3092	OPAQUE2	28.17	BCD
3096c	o2/cshwx	27.50	BCD
3132	NOR/OPAC2	25.13	CD
3096a	o2/cshwx	24.47	CD
3137	nor/cshwxo2	24.43	CD
3134	o2/cshwx	24.20	CD
3088	OPAQUE2	16.70	CDE
3133	NOR/OPAC2	16.33	CDE
3135	o2/cshwx	11.83	DE
3131	NOR/OPAC2	11.05	DE
3022c	NOR/wx	0.00	E
3022b	NOR/wx	0.00	E
3136b	o2/cshwx	0.00	E

(1) Averages followed by the same letters do not differ significantly at 1% of probability

Table 3. Comparison of means of "attack severity" to *Puccinia sorghi* (Schw.) in normal endosperm maize.

Genotype	Media	
3063b	53.73	A
3043b	34.37	B
3031	33.95	B
3057	29.78	B
3043a	24.82	BC
3071	18.45	BC
3028	13.23	C
3027	12.91	C
3008a	11.97	C

***:** averages followed by the same letter do not differ significantly at 1% probability.

Of all the inbreds studied during 2000/01 under natural infection conditions, 5 inbreds were very resistant to maize common rust, 10 were resistant, 11 were mid-resistant, 10 were susceptible and 2 were very susceptible. Inbreds 3131, 3135, 3136b and 3139 which are very resistant to "maize common rust" attack, are quality protein maizes and they carry in their background the opaque-2 gene from the same progenitor (inbred 7515 Mazoti). Likewise, the inbreds 3022c, 3135 and 3136b which are also very resistant derive from the same progenitor (FW) and normal inbreds 3027 and 3028 came from the progenitor CP27. Finally we want to stress the fact that 10 new inbreds resistant to *Puccinia sorghi* (Schw.) and obtained within a quality breeding programme, are available to develop new hybrids in Argentina.

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Exposure of *Zea mays, Tripsacum dactyloides* and its hybrid *Zea x Tripsacum* to short periods of chilling (5 C)

--Jatimliansky, JR, García, MD, Molina, M del C

Maize and *Tripsacum* are known for their susceptibility to chilling injuries. Maize-*Tripsacum* F1 plants (2n = 56) have shown more tolerance to chilling temperatures in the field than their parents: *Zea mays* (2n = 40) and *Tripsacum dactyloides* (2n = 72). Whilst minimum temperatures of -2 C during 2 days produced irreversible injuries in both parents, no visible damages were observed in the hybrid. A comparative study of the three genotypes was made in controlled conditions to ascertain the different growth behavior of the hybrid relative to its ancestors in the field.

We measured: a) the photochemical efficiency of photosystem (PS) II expressed as the ratio of the variable fluorescence (F_V) to the maximum fluorescence yield (F_M) (F_V/F_M ratio) and b) the injuries of the cell membranes of leaves during growth at 5 C or at 25 C by the electric conductivity of solutions due to electrolyte leakage of leaf slices in distilled water.

Maize seedlings were obtained by germination of seeds while *Tripsacum* and hybrid plants were grown from its rhizomes. When plants were at the four-leaf stage of development, chilling conditions (5 C) were applied in a growth chamber up to 3 days (16 h light - 8 h dark; 400 mol m^{-2} s^{-1} PAR at leaf level; 80% RH). Data were analyzed by ANOVA and differences among individual means were compared by the least significant difference (LSD) test.

Table 1. F_V/F_M ratios in leaves of control (25 C) and chilled (5 C) plants.

	maize	*Tripsacum*	maize x *Tripsacum*
	(2n = 40)	(2n = 72)	(2n = 56)
Control plants	0.796 a	0.727 a	0.764 a
Chilled plants			
5 C 1 day	0.547 b	0.513 b	0.593 b
5 C 2 days	0.297 cd	0.264 d	0.517 b
5 C 3 days	0.150 e	0.173 e	0.380 c

Means followed by the same small letter are not significantly different ($P \leq 0.05$) according to the LSD test.

Table 2. Electric conductivity (μS cm^{-1} g FM^{-1} $hour^{-1}$) of leaf slices suspension in distilled water.

Genotypes	Stressed plants	Control plants
	(5 C; 3 d)	(25 C)
Zea mays (2n = 40)	71.1 ab	36.7 d
Tripsacum dactyloides (2n = 72)	84.9 a	47.0 cd
Zea x Tripsacum (2n = 56)	61.5 c	35.9 d
Mean	72.5 A	39.9 B

Results of both comparisons indicated that the hybrid was less affected than its parents. After the measures, all chilled plants were cultured at 25 C in the same place as the control plants. After one week, stressed maize seedlings died and, also, leaves from chilled *Tripsacum* plants, while stressed hybrid plants remained alive as they did in the field.

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tb1* is epistatic to *ba2

--Hurkman, MM, Tracy, WF

The genes *teosinte branched1* (*tb1*), *barren stalk1* (*ba1*), and *barren stalk2* (*ba2*), affect branch development. Homozygous *tb1* plants lose apical dominance and exhibit many elongated lateral branches at axillary buds along the main culm. In *ba1* plants, tillers, ears, and the concave groove on the culm are absent while the tassels lack branches or spikelets. *ba1* is epistatic to *tb1* and suppresses lateral branching in both the vegetative and inflorescence meristems. *ba2* plants, like *ba1*, lack ears and the concave groove on the culm, but the tassels exhibit bundled branches that have peduncles fused to the culm at the base. The tassels usually develop fertile spikelets. *ba2* plants often have tillers and sometimes develop an ear at the soil surface (Fig. 1). Our objective was to determine the epistatic relationship of *ba2* and *tb1*.

An F2 population segregating for *ba2* and *tb1* was created. In the F2 there was a significant excess of *tb1* individuals. *tb1* homozygotes and putative *ba2/tb1* double mutants were impossible to distinguish. The F2 fit a 9 (wild type): 3 (*ba2*) : 4 (*tb1*) ratio, indicating that *tb1* is epistatic to *ba2* (Shroeck and Tracy, 1998 Maize Genetics Conference Abstracts, p. 81).

In 2001 we grew the same F2 and collected DNA from all the *tb1* plants. We used a SSR marker that was closely linked to *ba2* to determine what proportion of the *tb1* segregates were also homozygous for *ba2*. Of 76 *tb1* plants 25 of them were also homozygous for the *ba2* marker. This was not significantly different from expected (X^2 = 2.51, p<0.10). These *tb1tb1 ba2 ba2* plants were all indistinguishable from *tb1tb1 Ba2-* plants. Thus, *tb1* is epistatic to *ba2*, while *ba1* is epistatic to *tb1*.

Given the phenotype of these mutants it appears that *ba1*

suppresses lateral branching at all nodes including those in the tassel. *tb1* induces branching at both juvenile and adult vegetative nodes but does not affect the development tassel. Since *ba2* plants have tillers, no ears, but relatively normal tassels it appears that *ba2* suppresses branch development in the adult vegetative section of the plant.

Figure 1. The base of a homozygous recessive *ba2* plant, showing the development of an ear from an underground node.

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Isolation and preliminary characterization of a maize low phytic acid mutant

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Phytic acid (phytine), *myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate (IP6), is the major storage compound of phosphorous in plants, accumulating predominantly in seeds (up to 4-5% of dry weight) and pollen. In cereals, up to 80% of P is present as phytate P, which has generally very little bio-availability for monogastric animals due to their lack of phytase activity. As a consequence, it is common to supplement the animals' diet with inorganic phosphate or, in the last decade, with microbial phytase (Pointillart et al., 1987). The latter practice has been shown to increase the utilization of feed P by animals, as well as to decrease the amount of the phytic P excreted with manure, thus significantly reducing eutrophication of surface water. Recently, transgenic seeds were obtained accumulating *Aspergillus* phytase, which can then be included in feeds for animals (Pen et al., 1993).

An alternative strategy to tackle the problem consists in the isolation of cereal mutants accumulating less phytic P and more free phosphate in the seed (Raboy et al. 1990, Rasmussen et al. 1998). We have chosen to apply this approach in maize.

Since normal mature maize seeds contain high phytate phosphate and low free phosphate levels, a screening for high level of free phosphate in seeds provides a quick and inherently sensitive assay for the detection of *lpa* (low phytic acid) mutants. A population of EMS (ethyl methanesulfonate)-induced mutants was thus generated using the pollen-treatment method and approximately 600 M2 families were screened. The first assay was carried out by titrating free phosphate using the molybdate staining method. Putative mutants were then challenged by a TLC (Thin Layer Chromatography) method allowing the simultaneous detection of free phosphate and phytate.

One monogenic recessive mutation (named *lpa 241*), causing approximately a ten fold increase in the amount of free phosphate titratable and a reduction of about 90 % of phytic acid, apparently did not affect normal germination or seedling growth. Genetic analysis of this mutation, as well as its biochemical characterization aimed at identifying the biosynthetic step involved are under way.

Cesium tolerance trait in maize

--Magni, A, Nocito, FF, Pilu, R

Potassium plays a pivotal role in many biochemical and physiological processes. It has been proposed that higher plants have evolved at least two pathways for root potassium uptake, with high and low-affinity transport properties. Although details on the structure and electrical properties of these transporters are beginning to emerge, many aspects related to their function and regulation need to be investigated further. Selection of mutants modified in potassium transport or potassium homeostasis could be a powerful approach to study these topics. For this purpose we are isolating putative maize mutants tolerant to Cs^+, a cytotoxic analogue of potassium which acts as a competitive K^+ uptake inhibitor and as a K^+ channel blocker.

In a preliminary work we isolated 10 putative monogenic recessive Cs-tolerant mutants by screening several hundred M2 families obtained following chemical (EMS) mutagenesis. Selection was carried out detecting the number of seeds (30 for each experiment) able to germinate and grow in the presence of 100 mM Cs^+ (as sulphate) for 10 days. From this initial screening we isolated 10 resistant lines showing a Mendelian segregation ratio expected for a single-gene recessive mutation. These putative Cs-tolerant families were selfed in the last field and the screening of the M3 progenies obtained is now in progress. Preliminary data show that the Cs tolerance trait is maintained at least in two M3 families. Interestingly, one of these putative mutants is able to accumulate more Cs^+ than wild-type in the shoot.

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Agrobacterium tumefaciens –mediated genetic transformation of maize

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In order to enhance the frequency of *Agrobacterium tumefaciens* - mediated transformation of maize, we have modified the method underlying an inoculation of plant explants by *Agrobacterium*. Embryogenic callus tissues of A188 and 91 lines (somaclone obtained from F2 of hybrid Chi31xCateto S. G.) and of the hybrid A188x91 were used in the present work. The genetic transformation of the chosen plants was carried out with the use of the strain of *Agrobacterium* LBA4404 that contained plasmid pBI121 carrying the genes encoding neomycin phosphotransferase II (*nptII*) and β-glucuronidase (*uidA*).

The maize callus was incubated in a saturated *Agrobacterium* culture for 1 h, then cultivated on the MS medium containing cefotaxime to eliminate its contamination by the bacteria. After the formation of shoots, the latter were transferred to the medium supplemented with kanamycin (Kn) to select the plants resistant to this antibiotic. The frequency of producing the Kn-resistant plants by such a method of plant transformation was found to amount to 3.3 and 14.4% for A188x91 and 91 line, respectively. However, our attempts to select the Kn-resistant plants of A188 appeared to be unsuccessful.

In order to accelerate infection of the explants, the callus cultivated with *Agrobacterium* was vortexed for 30 sec or subjected to vacuum infiltration. According to the data reported by Ishida et al. (Nature Biotechnology 14:745-750, 1996), which used immature maize embryos as explants, the vortex treatment of the explants enhanced an efficiency of their genetic transformation and did not bring about any embryo injury. However, the embryonic callus used by us appeared to be more sensitive to mechanic injuries: the vortexed callus inocula grew slowly, and their regeneration capacity was found to significantly decrease. Among the few plants recovered from the use of this procedure, Kn-resistant ones appeared to be absent. The use of vacuum infiltration allowed an increase in the yield of A188x91 green plants of about three-fold, but was found to be ineffective in the case of the A188 line that was more sensitive to such a treatment.

To enhance virulence of *Agrobacterium*, the overnight culture used for co-cultivation with maize callus was supplemented with acetosyringone or tobacco leaf exudate. The application of acetosyringone resulted in the increase of about three-fold of the frequency of the regeneration of the Kn-resistant A188x91 plants, but had only a slight effect on the transformation efficiency of A188 and 91 lines. The exudate-induced activation of *Agrobacterium* was more effective, because in this case the amount of the Kn-resistant plants of 91 line and A188x91 appeared to be 2.4 and 5.4 times, respectively, higher as compared to that of the plants obtained by using inactivated *Agrobacterium*. This may be due to the fact that the tobacco leaf exudate, unlike acetosyringone, contains a wider spectrum of phenols and other compounds capable of affecting the gene transfection.

We have found that incubation of maize callus in a suspension of *Agrobacterium* results in a significant decrease of its morphogenetic capacity. This effect was expressed in a marked decrease in a probability of selection of the transformed plants. For this reason the duration of the co-cultivation was confined, as a rule, to 1 h. Using these findings as the base, we offer a new method of infection of explants that involves cultivation of callus inocula for 15-20 days on the lawn of the *Agrobacterium* activated by tobacco leaf exudate. The advantage of the suggested method is prolonged contact of growing plant tissues with the activated *Agrobacterium*, which provides a high enough probability of plant infection. In addition, it should be noted that in the *Agrobacterium*-containing cultivation medium, both an active growth of the embryogenic callus and regenerative capacity of the latter are still retained.

The above described method of plant transformation was found to be effective upon infection of both immature maize embryos and embryogenic callus. According to our results, the frequency of producing the Kn-resistant plants of A188, 91 and A188x91 was estimated as 17.8, 40.6 and 21.1%, respectively. With the use of fluorometric and histochemical methods, an expression of the inserted reporter GUS gene in tissues of the plants obtained was demonstrated. The resistance to Kn was inherited to the T1 generation.

The designed method of genetic transformation of plants was applied for insertion in embryonic maize callus of plasmid pK22rs containing the genes *nptII* and *rs* from radish, encoding defensin. This transformation was used for the purpose of enhancing plant resistance to some phytopathogenic fungi. The transfer of the plasmid into plant cells was performed with the use of strain LBA 4404 of *A. tumefaciens*. In the transgenic plants thus obtained the insertions of genes *nptII* and *rs* were confirmed by both PCR and Southern blot hybridization.

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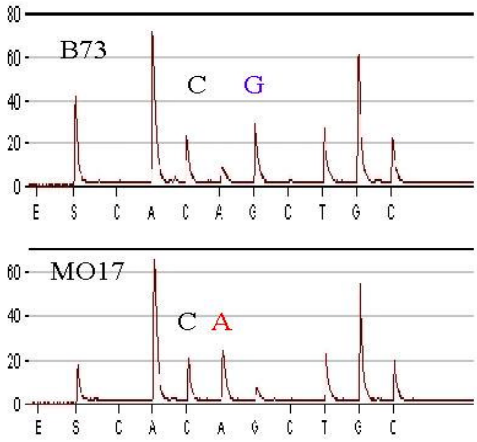
Rapid EST mapping and SNP genotyping of individuals using pyrosequencing

--Ching, A, Rafalski, A

Mapping of a trait or a genetic locus is traditionally done with RFLP, AFLP or SSR markers. Here we described the use of single nucleotide polymorphisms (SNP) for rapid mapping of expressed sequence tags (ESTs). The genotyping of SNPs is performed by pyrosequencing. Other methods of genotyping SNPs can be found in a review by A.C. Syvanen (Nature Reviews Genetics 2:930, 2001).

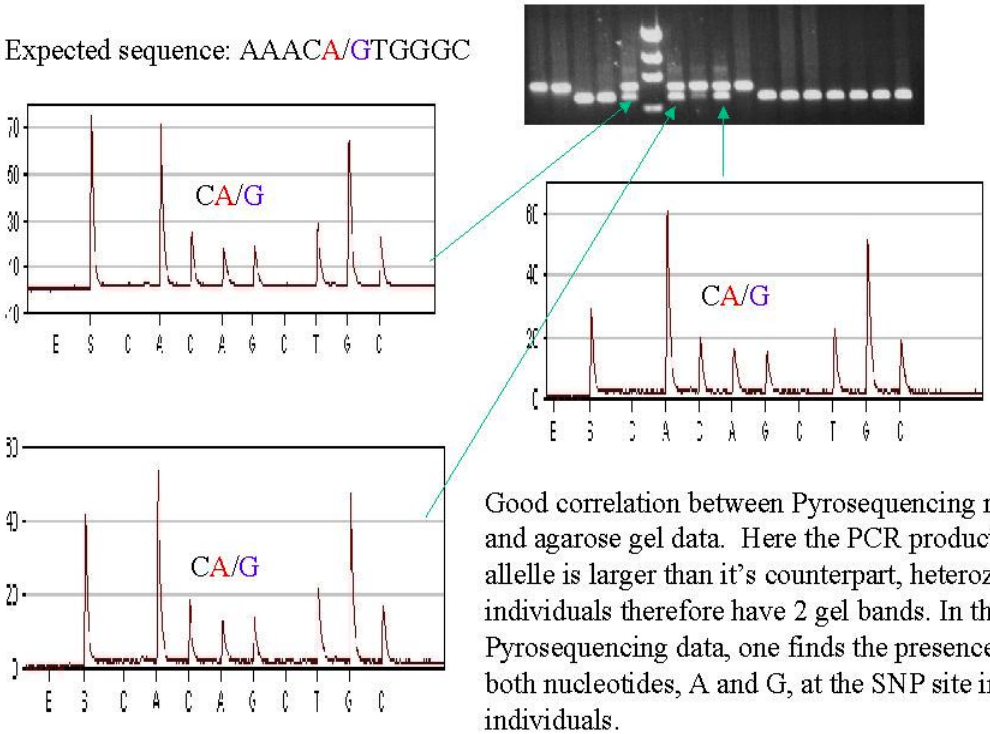
Pyrosequencing is a new method of obtaining short segments of DNA sequence, typically up to 20 nucleotides, simultaneously on 96 different templates. Once the templates have been prepared, 96 templates can be sequenced in 15 minutes. The methodology was developed by P. Nyren (Nyrén, P, Analytical Biochemistry 167:235, 1987; Ronaghi, M. et al, Science 281:363, 1998). It relies on the stepwise elongation of the primer upon sequential addition of the different deoxynucleotide triphosphates. The pyrophosphate released from an incorporated NTP is converted into ATP, which in turn triggers the emission of light from the luciferin – luciferase system. Excess nucleotide triphosphates are degraded



	1	2	3	4	5	6	7	8	9	10	11	12
A	B73	B	M	B	M	M	M	B	B	B	B	B
B	MO17	B	M	B	B	B	B	M	M	-	B	M
C	M	B	B	B	B	M	M	M	B	B	M	M
D	B	B	M	B	B	B	M	M	B	M	M	M
E	M	M	B	B	B	B/M	B	M	M	B/M	B/M	M
F	B	M	M	M	B	M	M	M	M	B	B/M	M
G	M	M	M	M	M	M	M	B	M	M	B	M
H	M	M	B	M	B	M	M	M	M	M	B/M	M

Mapping result for 94 individuals plus parents

Figure 1.



Good correlation between Pyrosequencing results and agarose gel data. Here the PCR product of one allelle is larger than it's counterpart, heterozygous individuals therefore have 2 gel bands. In the Pyrosequencing data, one finds the presence of both nucleotides, A and G, at the SNP site in these individuals.

Figure 2.

by apyrase. As the sequencing reaction progresses, the DNA strand is extended and the sequence is determined from the measured signal output of light upon nucleotide incorporation. The reactions are performed in a 96-well plate in an automated device (PSQ-96, Pyrosequencing, Inc. Uppsala, Sweden). The resulting sequence trace is analyzed automatically by the Pyrosequencing software.

To place an EST on the genetic map, we first identified polymorphisms between the two mapping parents within the EST sequence. This is done by standard dideoxy- terminator fluorescent sequencing of the PCR amplified segments of genomic DNA corresponding to that EST. Sequencing of the 3'-UTR segment of the EST clone increases the likelihood of finding multiple SNPs. SNP frequencies of 1 polymorphism in 60 nt on average are found in a diverse set of maize germplasm (A. Ching, unpublished data). Next, a sequencing primer close to the identified SNP site(s) is prepared. The locus is then PCR amplified from the genomic DNA of the mapping population, this time using one biotinylated and one standard PCR primer. The PCR product is then bound to streptavidin-coated paramagnetic beads, denatured by sodium hydroxide, and a magnetic tool is used to capture the biotinylated template strand for sequencing. Several bases, including the SNP site, are sequenced in the Pyrosequencing machine, using the above-mentioned sequencing primer and the bead-bound template. EST mapping using this approach was tested by mapping the sucrose synthase locus (*sh1*) using the SX19 mapping population (Lee, M et al., Maize Genetics Conference Abstracts 40). The expected sequence read is AAACA/GTGGGC, (SNP position at 4739 GenBank accession X02382, A in B73 and G in MO17, Fig.1). In a heterozygous individual, a half-height peak of both A and G is found. In the case of *sh1*, the genotypes obtained from Pyrosequencing can be further verified by separating the PCR products on an agarose gel. This is because the PCR product of one of the alleles is larger than its counterpart, due to the presence of a 142 bp insertion in B73 (position 4080 in GenBank accession X02382). A heterozygous individual will therefore have two bands per lane (Fig 2). The genotyping scores were placed on the genetic map of the SX19 population using MapMaker software (Lander, E and Green, P, PNAS 84:2363, 1987). As expected, the sucrose synthetase locus co-segregated with *sh1*. We have since placed a number of other EST loci on the SX-19 map using Pyrosequencing genotyping of SNPs, with a success rate of >95% per locus. Failures were due to a poor PCR, and could be rectified by a more careful quantification of the amount of DNA per well before PCR amplification.

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Analysis of genetic relationships among Indian maize lines differing in their response to Brown Stripe Downy Mildew

--Kumar, A, Gadag, RN, *Saxena, SC

Twenty-eight maize genotypes comprising parental lines of elite single cross hybrids, lines derived from inter-specific crosses and North Eastern Hills (NEH) races, were evaluated for their response to Brown Stripe Downy Mildew (BSDM). The pathogen causing this disease in maize in India was first identified in 1967 as *Sclerophthora raysieae* var. *zea* (Payak & Renfro, Phytopath. 57: 394-397, 1967). This disease affects maize crops in the northern part of India. Disease screening was done during Kharif 2000, at Pantnagar, a hot spot for the pathogen, under artificial epiphytotic conditions. Disease was recorded following the standard procedure with a 1-5 scale along with appropriate resistant and susceptible lines as checks. While twenty genotypes had resistance to the BSDM pathogen (score of < 2.0), the remaining eight lines were found to be susceptible (score of > 2.0).

Interestingly, the genotypes in the two groups (resistant and susceptible) were represented by three different categories. A set of six genotypes, three belonging to each of the resistant [N-3, MZD-3, and AH 918 (F)] and susceptible [CM 213, MZP-1C and AH 421 (F)] groups were chosen for analyzing genetic relationship. Particulars of these genotypes are as follows: N-3-NEH race from Nagaland; MZD-3-Interspecifc derivative from *Z. maize* X *Z. diploperennis*; AH 918 (F)-Female parent of an experimental hybrid; CM 213 -Female parent of a released single cross hybrid; MZP-1C -Derivative of inter-specific cross *Z. maize* X *Z. parviglumis*; AH 421(F) -Female parent of an experimental hybrid. Data on morphological characteristics as well as those generated by polymorphism survey using SSR (Simple Sequence Repeats) were used to analyze genetic relationships. Of the 36 SSR markers used, 17 were found to be effective in detecting polymorphism. The similarity matrix was analyzed by NTSYS-PC Version 2.02 (Rohlf, 1992, Exeter Pub. New York) by employing UPGMA with average linkage (Sneath and Sokal, 1973, Numerical Taxonomy, W.H. Freeman & Co., San Francisco).

The clustering pattern obtained individually with a morphological or molecular marker was compared with that obtained by using both in combination. Interestingly, grouping of genotypes followed a common pattern and reflected the response of genotypes to BSDM also (Fig. 1). Thus, a relatively small number of SSR markers (17) in combination with morphological markers enabled meaningful grouping. Improvement in clustering may be expected by analyzing a greater number of polymorphic SSR markers, as there is limited scope for further increasing the number of easily discernible morphological characters.

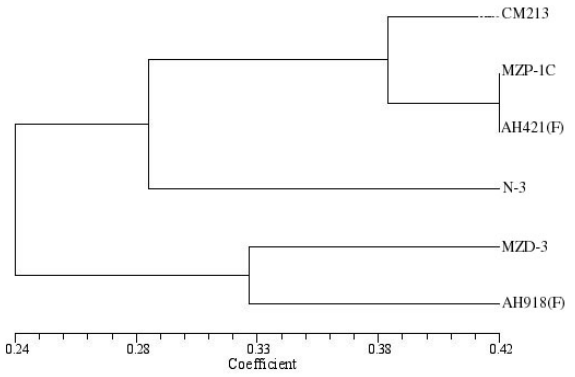

Figure 1. Dendrogram depicting genetic relationships among selected maize lines based on SSR + morphological data.

The present study highlighted the diversity in the material chosen with respect to BSDM disease. For a better understanding of the mechanism of disease response and for increasing the

precision in incorporating disease resistance, molecular markers like SSRs may be effective tools. In this endeavor, considering information regarding the level of heterozygosity and polymorphism, AH 918 (F) and CM 213 are potential lines for further studies.

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Cytoembryological study of maize-*Tripsacum* hybrids

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Information in the literature about the reproductive mechanisms of eastern gamagrass (*Tripsacum dactyloides* L.) and maize-*Tripsacum* hybrids is incomplete. Burson, BL and coworkers (Crop Sci. 30:86-89, 1990) demonstrated that tetraploid (2n=4x=72) and triploid (2n=3x=54) eastern gamagrass reproduced by diplospory and the *Antennaria*-type megagametogenesis, diploid parthenogenesis and pseudogamous endosperm formation. The diploid forms of *Tripsacum* reproduced sexually. Analysis of natural populations and various *Tripsacum* species of different ploidy levels (2n=36, 54, 72, 90,108) confirmed diplosporous apomixis in the polyploids and sexual reproduction in the diploids. Rare cases of meiotically derived dyads and tetrads were observed at very low frequencies (2%) in some apomictic populations (Leblanc, O et al., Am. J. Bot. 82 (1):57-63, 1995).

In the hybrids between tetraploid maize and *T.dactyloides* whose offspring we are continuing to investigate, Laikova reported that 56-chromosome F1 hybrids (20Zm + 36Td) were genetically heterogeneous (In: Petrov, DF (ed.)Apomixis and its role in evolution and breeding, New Dehli, India, 79-87, 1984). Hybrids H416 and H418 (2n=56), developed from backcrossing apomictic F1 hybrid H278 (2n=56) with maize, were also the apomicts and had embryo sacs similar to those observed in the paternal *Tripsacum*. At the same time among F1 hybrid H166 (2n=56) only BII-hybrids with 38 chromosomes (20Zm + 18Td) were recovered in the BC1. Sexual reproduction was confirmed by the presence of meiotically derived linear tetrads of megaspores.

Offspring of the maize-*Tripsacum* hybrids H278 and H32 (2n=56; 20Zm + 36Td), 39-chromosome forms (30Zm + 9Td) retained apomictic reproduction (Sokolov, VA et al., Russian Genetics 34:499-506, 1998). Low female fertility in the F1 hybrids was overcome in subsequent generations by reducing the number of *Tripsacum* chromosomes and selecting the most productive genotypes. However, low seed production remained a problem. The practical use of the hybrids was also restrained by their male sterility. It was important to understand the mechanism of apomixis inheritance when genes of eastern gamagrass were expressed in cytoplasm of maize in the presence of three maize genomes (thirty chromosomes) and only nine *Tripsacum* chromosomes. To determine the causes of decreased kernel set, we examined megasporogenesis, megagametogenesis and pollen tube growth in the maize-*Tripsacum* hybrids. This was in collaboration with scientists from the Komarov Botanical Institute, Saint Petersburg, Russia, and Plant Research International, Wageningen, the Netherlands.

For the study of megasporogenesis and megagametogenesis, ears 3-10 cm long were fixed in FAA [3:7:1 (V:V:V) formalin: 70% alcohol: glacial acetic acid] for 48 hours. The fixed material was stored in 70%-ethanol. The ovules were cleared in methyl benzoate and examined using phase-contrast microscopy and Nomarsky optics (Naumova, TN et al., Sex. Plant Reprod. 12:43-52, 1999).

To study the pollen tube growth, ears were pollinated by maize pollen and then fixed in FAA two days after pollination (DAP). The styles were placed in 10% KOH for 1.5 hours. After maceration the styles were washed with distilled water, placed on slides and stained in a drop of 0.005% aniline blue in 0.15M K2HPO4. The styles were squashed and observed for the presence of the pollen tubes using fluorescent microscopy.

More than 120 ovules from 12 plants of two different lines 4x-6 and W39 with 30 chromosomes of maize and 9 chromosomes of *Tripsacum* were examined. Megasporogenesis and embryo sac formation in both lines proceeded similarly. The subepidermal nucellar cell enlarged and became the archesporial cell and later differentiated into the megaspore mother cell (MMC) (Fig. 1a). Instead of initiating meiosis, characteristic of sexual plants, the elongated MMC enlarged and became vacuolated, the nucleus remained inactive, the chalazal end of the cell became narrow. The thickening of the cell wall was accompanied by degeneration of the adjacent nucellar cells. Integuments enclosed the micropylar part of the ovule (Fig. 1b). After isolation, MMC divided mitotically (Fig. 1c) to produce a 2-nucleate megagametophyte with a huge central vacuole (Fig.1d). The second mitotic division produced a 4-nucleate embryo sac (Fig. 1e) and the third division produced an 8-nucleate embryo sac (Fig. 1f). After cellularization, the embryo sac consisted of two synergids, egg-cell, two polar nuclei and three antipodal cells, which often divided later on to form five or six antipodals (Fig.1g, h). The complete absence of meiosis and the megaspore tetrad formation indicated that the 39-chromo-

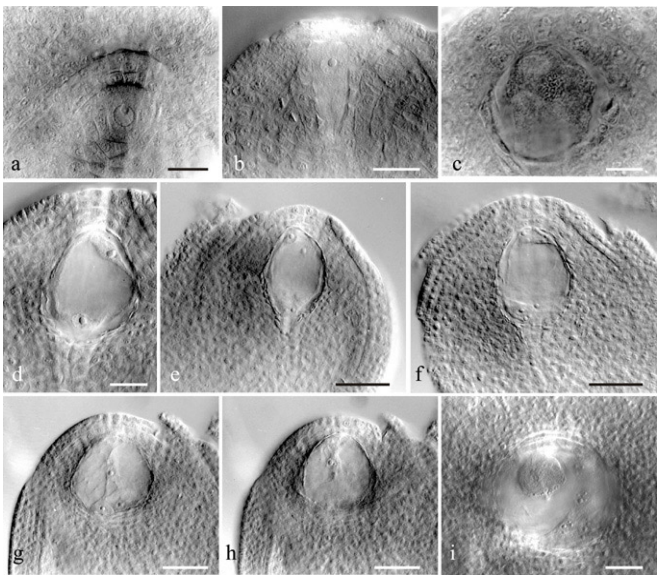

Figure 1 (a) Ovule with archesporial cell. (b) Ovule with big megasporocyte (MMC) showing vacuolization and tendency to dyplospory. (c) Ovule with diplosporous one-nucleate embryo sac of *Antennaria*-type originated directly from MMC. (d) Two-nucleate embryo sac. (e) Four-nucleate embryo sac. (f) Eight-nucleate embryo sac prior to the cell formation. (g, h) Eight-nucleate embryo sac after cellularization and with two synergids, egg cell, central cell with two unfused polar nuclei and three antipodal cells (two successive focus levels of the same ovule). (i) Egg cell at the metaphase stage and 4-nuclei endosperm. Bar: for **a, i**, 50 µm; **c**, 20 µm; **b, d, e, f, g, h**, 100 µm.

some maize-*Tripsacum* hybrids reproduced by mitotic diplospory and the *Antennaria*-type megagametogenesis. In the material studied, neither meiosis nor abnormal embryo sac development were observed.

The embryo in the maize-*Tripsacum* hybrids developed parthenogenetically. Fertilization of polar nuclei is necessary for the formation of the endosperm. In the isolated ears that were not pollinated, only unfertilized embryo sacs with enlarged egg-cell, fused polar nuclei and multiplying antipodals were found on the second to third day after the ejection of stigmata. Thus, pseudogamous apomixis occurred in the 39-chromosome maize-*Tripsacum* hybrids. This is similar to the development of eastern gamagrass as reported by Farquharson, LI (Am.J.Bot.42:737-743,1955), Brown, WV and Emery, WHP (Am.J.Bot. 45:253-263,1958) and Burson, BL et al.(1990).

Cases of embryo development without pollination were not observed in our maize-*Tripsacum* hybrids. Moreover, at 3DAP the endosperm usually overtook the egg cell in the rate of cell divisions (Fig. 1i). Pollination and fertilization of the polar nuclei appeared to be the signal of parthenogenetic embryo development. A similar phenomenon was described in apomictic *Arabis holboellii* (Naumova, TN et al., Sex. Plant Reprod. 2001, in press). Parthenogenesis without pollination was reported by Laikova, LI (1984) and Fokina, ES (In: Petrov, DF (ed.) Inducirovannyi mutagenez i apomiksis, Novosibirsk, USSR, pp. 57-63, 1980).

The absence of embryo and endosperm development in part of the ovules in the pollinated ears suggested that fertilization of polar nuclei did not occur in these. To elucidate the causes of this phenomenon, we examined the pollen tube growth in line 4x-6 (30Zm + 9Td chromosomes) in 1999 and conducted a comparative study of this line with the 38-chromosome line (20Zm + 18Td) in 2000. The investigation of styles of pollinated ears showed that in rare cases, the maize pollen tubes had regular callose plugs and grew toward the ovule (Fig. 2a). More often the pollen tubes stopped growing shortly after germination (Fig. 2b, c) and sometimes within the stylar tissue (Fig. 2d, e, f). Lack and stoppage of growth were accompanied by anomalous callose deposition in the tubes, tube twisting and branching (Fig. 2g, h). In 1999, after the 39-chromosome hybrids were pollinated with the maize Tetraploid Shumnogo (2n=4x=40), pollen tubes grew toward the ovule in 15% of the styles, whereas in the maize control pollen tube growth was 100%. The number of pollen tubes reaching the ovule was also considerably lower in the hybrids when compared to maize. There were 1-2 tubes per style in hybrids as compared to 7-9 tubes in maize. In 2000, we also studied the influence of the pollinator on the pollen tube growth in the 39- and 38-chromosome hybrids. Diploid (Popcorn) and tetraploid (Tetraploid Shumnogo, Purple marker) maize were used as the pollen sources. For each combination, 10 ears were pollinated and 40-50 styles from each ear were analyzed. The percentage of styles with pollen tubes that reached the ovule was calculated. Significant differences for this characteristic between pollinators and the maternal genotypes were not observed. The mean values of pollen tube growth were higher than in 1999: Popcorn - 41%, Tetraploid Shumnogo - 48%, Purple marker - 32% (P>0.05). However when analyzing the material by pollination day it was established that in the last three days of pollination, beginning from 27 August, the percent of styles with tubes and the number of tubes per style increased (Table 1).

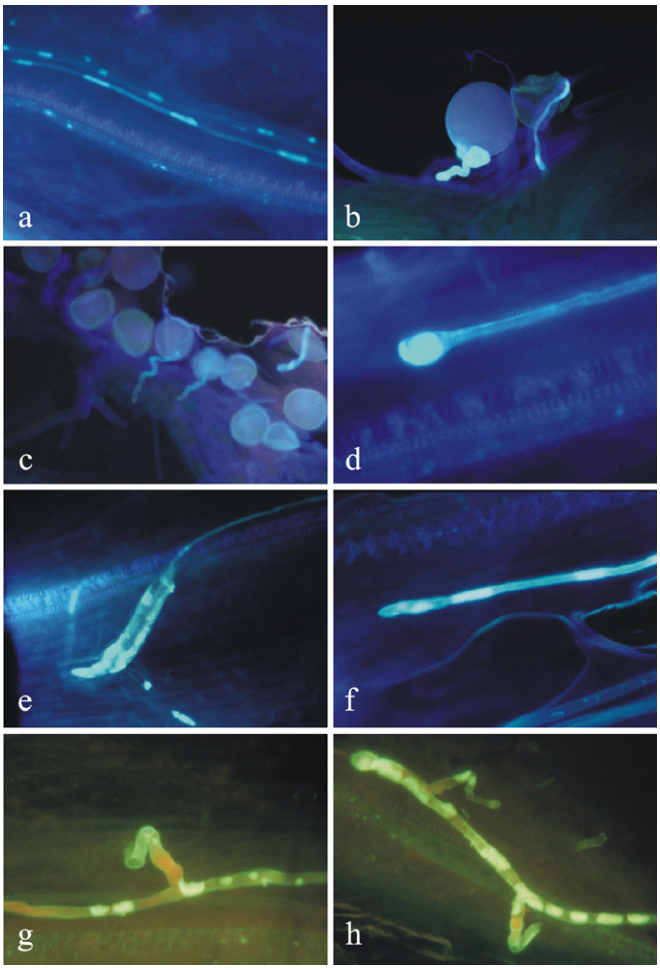

Figure 2. (a) Pollen tube growth is normal, the plugs are equi-distant from each other. (b, c) Pollen tube growth is interrupted at an early stage, soon after germination. (d, e, f) Pollen tube growth is interrupted at later developmental stage, inside the style. (g, h) Abnormality (branching) of the pollen tubes inside the style.

Table 1. Pollen tube growth in the maize-*Tripsacum* hybrids on different days of pollination.

Pollination date	10.08	15.08	24.08	25.08	26.08	27.08	28.08	29.08
Number of styles studied	78	88	116	412	328	550	121	190
Number of styles with pollen tubes	32	12	19	109	53	374	80	149
% of styles with tubes	41	4	16	27	17	***68	***66	***78
Number of tubes found	62	18	34	201	92	769	193	499
Number of tubes per 1 style	0.80	0.20	0.29	0.49	0.28	***1.40	***1.60	***2.63

Note: *** - the differences between days 27-29.08. and the other days of pollination are significant with P<0.001

The weather conditions in the pollination period were characterized by a lack of sharp fluctuations with 15-20C in the day time and 8-9C at night on average. However, on the night of 26-27 August a fall of temperature to 1-2C occurred, so differences between the average and decreased temperature was about 7 degrees. As known from the literature, a change in temperature adversely affects the pollen fertility, the process of fertilization and ovary development (Jakobsen, HB and Martens, H, Annals Bot. 74(5):493-501, 1994; McKee, J and Richards, AJ, Annals Bot. 82(3):359-374, 1998; Tromp, J and Borsboom, O, Scientia

Horticulturae 60(1-2):23-30, 1994). It is possible that the low temperature influenced incompatibility mechanisms in the maize-*Tripsacum* hybrids. Apparently in this case the short-term decrease in temperature weakened the incompatibility of maize pollen tubes and tissues of style of the maize-*Tripsacum* hybrids without having an effect on viability of pollinator-maize pollen. The effect of temperature shock on incompatibility has been reported in *Lolium perenne* (Wilkins, PW and Thorogood, D, Euphytica 64:65-69, 1992).

Thus, one can conclude that abnormal growth of maize pollen tubes in styles of 39- and 38-chromosome maize-*Tripsacum* hybrids was one of the main reasons for poor kernel set. The three maize-pollinator varieties used did not significantly influence the efficiency of pollination. A short-term low-temperature shock promoted the weakening of the incompatibility mechanisms.

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Variations in levels of dehydrodimers of ferulic acid in the pericarp of maize infected by *Fusarium graminearum*

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Phenylpropanoids have been suspected of implication in plant resistance in many different plant-pathogen interactions (Bell, Annu. Rev. Plant Physiol. 32:21-81, 1981). These secondary metabolites are generally induced after elicitation. In maize, different flavones have been found to increase significantly during silk infections in resistant genotypes (Reid et al., Can. J. Bot. 70:1697-1702, 1992). Some other phenolics implicated in resistance are constitutively present in tissues, like 4-ABOA in grain (Miller et al., J. Agric. Food Chem. 45:4456-4459, 1996). We are investigating the implication of cell wall phenylpropanoids in maize resistance to *Fusarium graminearum*. Ferulic acid and p-coumaric acid are the two predominant constitutive forms of phenolics in the cell wall of grasses. These compounds in free forms are known to be fungistatic for different fungi. In vivo, these compounds are ester-linked to cell wall arabinoxylan residues. A positive correlation has been observed between resistance to *Gibberella* ear rot and levels of these compounds at harvest (Assabgui et al., Phytopathology, 83(9):949-53, 1991). Ferulic acid has the ability to be dimerized forming cross-links strengthening the primary cell wall (Fry, Ann. Rev. Plant Physiol. 37:165-186, 1986). Maize genotypes with high levels of diferulates present high levels of resistance to leaf borers (Bergvinson et al., Environ Entomol 23:1516-1523, 1994).

During the 2000 field season, we monitored the level of cell wall phenolics and particularly diferulates in inbred lines of maize developed by Agriculture and Agri-Food Canada with improved resistance to *Gibberella* ear rot (CO432 and CO433). Twelve days after silk emergence, plants of this inbred plus susceptible inbreds were artificially inoculated with a macroconidial suspension of *F. graminearum*. Control plants were inoculated with distilled water. Ears were collected at 0, 2, 4, 6, 8, 10, 15, 20, 30 days and harvesting time. Ergosterol levels were monitored by HPLC (Young, J. Agric. Food Chem., 43(11), 2904-2910, 1995) in whole grains. Pericarp and aleurone layers were separated from the rest of the grain. Cell wall bound phenylpropanoids were analyzed after NaOH digestion of crude cell wall using a HPLC and LC-MS that we developed.

The levels of diferulates were significantly different among genotypes. More interestingly, in CO433, a resistant genotype, diferulates/ferulate ratio was significantly higher in inoculated pericarps than in the controls during the first six days of infection and after the twentieth day (Figure 1). No such differences were found in CO344, a susceptible genotype. High levels of diferulates impede the action of cell wall hydrolases (Grabber et al., J. Sc. Food Agric. 77:193-200, 1996). The high level of dimerization of the cell wall may impede cell wall degradation and cell disruption necessary for the progression of *Fusarium* which is a necrotroph phytopathogen. Cross-links of cell wall proteins in response to fungal elicitors have already been described in bean and soybean cell suspension (Bradley et al., Cell 70: 21-30, 1992) and is considered as a quick and early response of the plant to pathogens.

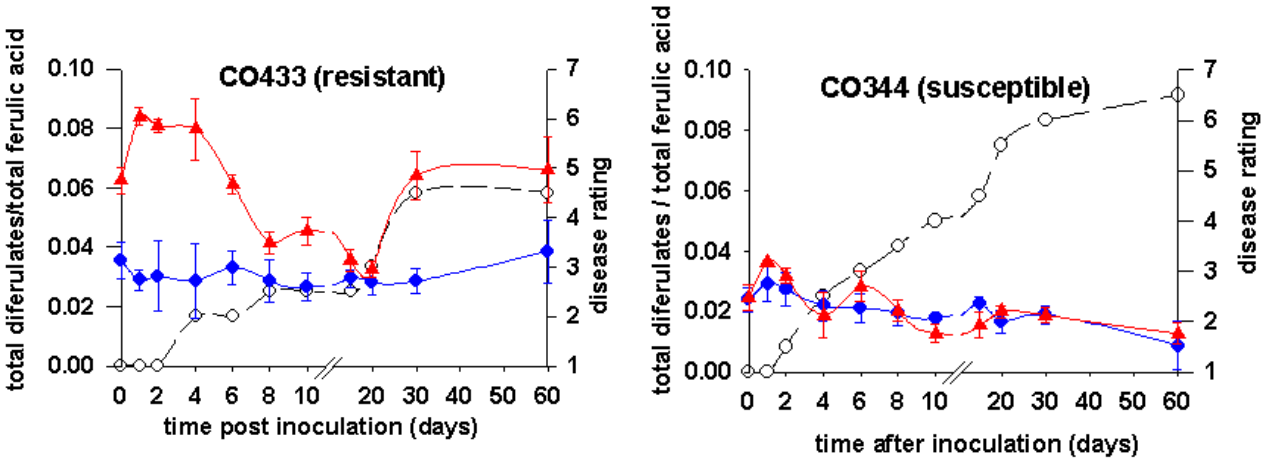

Figure 1. Diferulates/ferulate ratio in maize pericarp of infected (▲) and control (●) pericarp and aleurone layers. Disease ratings (❍) are also shown (lower values indicate less infection).

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A gene for resistance to northern leaf blight is inhibited by CO325

--Zhu, X, Reid, LM, Smith, M

In 1999 and 2000, eight inbreds with different levels of resistance (Table 1) to northern leaf blight [*Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs (teleomorph = *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs; syn. = *Helminthosporium turcicum* Pass.)] were used in a complete 8 x 8 diallel cross study. All genotypes were planted in single-row plots with 15 plants per row and three replicates in a completely randomized design at the Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario. All plants were inoculated twice. At the 6-8 leaf-stage, the whorl was filled with a ground powder of infected leaves collected the previous year. At the 10-12 leaf-stage, three inoculated corn kernels were placed in each whorl. The inoculated kernels were prepared by boiling the kernels for 1 hr, placing them in 1000 ml flasks, sterilizing for 45 min., and adding 6-8 pieces of 1 x 1 cm^2 PDA inoculum into each flask followed by culturing at room temperature (24-28 C) for 14-21 days. After inoculation, plots were irrigated for 10-15 minutes daily if there was no rain.

Table 1. Pedigree and putative alleles affecting northern corn leaf blight reaction in eight inbred lines used in an 8 x 8 diallel study.

Inbred	Source	Pedigree	Putative alleles
73353	USA	FR23 x H111. FR23 = Mo17 x A653, H111 = Mayorbela x B37(2)	*Htm1* from Mayorbela (Robbins and Warren, Maydica 38:209-213, 1993)
73405	USA	B59 x Oh43Ht2, B59 = N32 x B14(2)	*Ht2* from Oh43Ht2, Possibly *Sht1* from B14 (Ceballos and Gracen,Plant Breeding 102:35-44, 1993)
CHN2	China	Chinese inbred x B73(2)	None
CHN1	China	Chinese inbred Yellow early 4	None
CM174	Canada	V3 x B14(2)	Possibly *Sht1* from B14 Ceballos and Gracen, Plant Breeding 102:35-44, 1993)
CO325	Canada	CO256 x CO264 (3). CO256 = BSSS, CO264 = CO109 x 190(2)	None
CO354	Canada	Asgrow RX777	None
CO387	Canada	CO272 x CO266. CO272 = BSTE x [(CO109 x CO106)CO109(2)](2), BSTE = Iowa Two Ear Synthetic CO266 = Pioneer 3990	None

Based on the pedigree of the inbred lines, presumed (or putative) resistance genes were used in this experiment. Four lesion types were recorded 15-20 days after silking: 1) resistant lesion (R) = stripe or narrow elliptical green-yellowish lesion; 2) moderately resistant lesion (MR) = narrow, long, elliptical gray lesion with green-yellowish border; 3) moderately susceptible lesion (MS) = long, elliptical, gray lesion with green-yellowish border; and 4) susceptible lesion (S) = long, elliptical, gray or tan lesion. Table 2 summarizes the lesion types and putative alleles affecting northern leaf blight expression recorded for each genotype in the 8 x 8 diallel cross. Several conclusions can be drawn from this data:

Table 2. Lesion types expressed for resistance to northern leaf blight in an 8 x 8 diallel cross in 1999 and 2000.

Parents[a]	73353	73405	CHN2	CHN1	CM174	CO325	CO354	CO387
73353	R[b]	R	R	R	MR/R	S/MS[c]	MR	R
73405	R	MR	MS	S	S	S	S	S
CHN2	R	MS	MS	S	S	S	S	S
CHN1	R	S	S	S	S	S	S	S
CM174	MR/R	S	S	S	S	S	S	S
CO325	S/MS	S	S	S	S	S	S	S
CO354	MR	S	S	S	S	S	S	S
CO387	R	S	S	S	S	S	S	S

[a]Diagonal are lesion types expressed for eight inbred lines. Above diagonal are F1 hybrid crosses and below diagonal are their reciprocal crosses.
[b]R (resistant lesion) = stripe or narrow elliptical green-yellowish lesion; MR (moderately resistant lesion) = narrow, long, elliptical gray lesion with green-yellowish border; MS (moderately susceptible lesion) = long, elliptical, gray lesion with green-yellowish border; and S (susceptible lesion) = long, elliptical, gray or tan lesion.
[c]Lesion type expressed differently in 1999 and 2000 (types noted are for 1999/2000).

1. Resistant gene(s) appeared to exist in both 73353 and 73405. From the pedigrees (Table 1), the putative resistant gene of 73353 is *Htm1*, which came from Mayorbela (Robbins and Warren, Maydica 38: 209-213, 1993. Maize DB, www.agron.missouri.edu/cgi-bin/sybgw_mbd/mdb3/Locus/65746); and the putative resistant gene of 73405 is *Ht2*, which came from Oh43Ht2.
2. No reciprocal differences exist in the expression of these genes.
3. The presumed resistance gene, *Htm1,* of 73353, did not always express dominance. Its expression was affected by different genotypes and environmental factors. In this diallel cross, *Htm1* expressed dominance in crosses of 73353 x 73405, 73353 x CHN2, 73353 x CHN1, 73353 x CO387 and their reciprocal crosses. *Htm1* expressed partial dominance in 73353 x CM174 and its reciprocal cross in 1999 but complete dominance in 2000. 1999 was much warmer than 2000. *Htm1* only expressed partial dominance in 73353 x CO354 and its reciprocal cross in both years. *Htm1* did not express dominance in the cross 73353 x CO325 and its reciprocal; CO325 may have a gene(s) that inhibits the expression of *Htm1*. This inhibition also was affected by environmental factors, with complete inhibition in 1999, the warmer year, but only partial inhibition in 2000.
4. The expression of the putative resistance gene *Ht2* of 73405 was difficult to explain. Inbred 73405 itself expressed moderate resistance, but when crossed with all susceptible inbred lines (CHN2, CHN1, CM174, CO325, CO354, and CO387) all hybrids were susceptible. This raises several questions. (A) What race (Leonard et al., Plant Dis. 73:776-777, 1989) was used for the inoculations? If it were race 0 (*Ht1, Ht2, Ht3, HtN*/0) or race 1 (*Ht2, Ht3, HtN/Ht1*), why were all hybrids susceptible? If it was race 12 (*Ht3, HtN/Ht1, Ht2*), race 23 (*Ht1, HtN/Ht2, Ht3*), or race 23N (*Ht1/Ht2, Ht3, HtN*), why is 73405 moderately resistant? (B) Because 73405 came from B59 x Oh43*Ht2* and B59 includes a dominant inhibitor gene to *Ht2* called *Sht1* (Ceballos and Gracen, Plant Breeding 102: 35-44, 1989) (Maize DB, Variation *Sht1*, www.agron.missouri.edu/cgi-bin/sybgw_mdb/mdb3/Variation/99275), then if 73405 had genes like *Sht1Sht1/Ht2Ht2*, it should

be susceptible. If 73405 had no *Sht1* gene, then why were all of its F1 hybrids susceptible and not moderately resistant? All of these questions indicate that the relationships among *Ht2, Sht1*, and races are much more complicated than we expected.

5. Both 73405 and CM174 possibly have *Sht1*, which comes from B14 (Ceballos and Gracen, Plant Breeding 102:35-44, 1989. Maize DB, Variation *Sht1*, www.agron.missouri.edu/cgi-bin/sybgw_mdb/mdb3/Variation/99275). Crosses of 73353 to 73405 and CM174 showed resistance, indicating that *Htm1* in 73353 might not be affected by *Sht1*.

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On the timing of the *rbg* excisions from the *o2-hf* allele

--Koterniak, VV

A characteristic feature of the *o2-m(r)*, the mutable receptive allele at the maize *opaque2* locus formed as a result of insertion of the receptor element *rbg* into this locus, is that the excisions of the *rbg* element from the *o2-m(r)* (which are possible in the presence of the regulatory element *Bg*) take place during mega- or microsporogenesis or during endosperm development, but not during embryo or sporophyte development (Montanelli et al., Molecular and General Genetics 197:209-218, 1984). Though this feature is generally confirmed in our stock of instability at the *o2* locus controlled by the *Bg-rbg* system of transposable elements, the possibility of *rbg* excisions before meiosis was not excluded completely (Genetika 31: 950-954, 1995).

The phenotypic expression of the reversion of *o2-m(r)* alleles to the normal depends on its timing. Reversion of the mutable allele occurring after the first division of the primary endosperm nucleus is manifested by the apparition of variegated kernels which are characterized by alternation of opaque and vitreous sectors in the endosperm. If the reversion takes place before the first division of the primary endosperm nucleus, the phenotypically normal kernel (whole endosperm revertants, WER) appear. In addition, if such a reversion occurs during development of the gametes participating in embryo formation, the embryo revertants develop carrying the dominant allele *O2*.

Premeiotic reversion of the mutable *o2-m(r)* alleles should lead to a clustering distribution of WER on ears with the frequency of the embryo revertants in such clusters of 50% (in case of reversion event in one of the homologous chromosomes).

The distribution of WER kernels was analyzed on two selfed ears belonging to earlier obtained strains characterized by a high frequency of WER formation (HFWER strains) which were homozygous for the receptive allele *o2-hf* and the regulatory element *Bg-hf*. A high frequency of WER in indicated strains is conditioned by the presence of the *o2-hf* allele (Maydica 44:195-203, 1999).

The first ear analyzed contained 49 WER and 191 variegated kernels, the second one had 73 WER and 119 variegated. Analysis of ear maps of these ears showed that an overwhelming majority of WER (115 out of 122) were in clusters of contiguous kernels. This was quite unexpected for random WER formation and allowed the suggestion of premeiotic reversion of the mutable allele during ear development. To determine which of the WER were embryo revertants, all WER from indicated ears were planted in field conditions and the resulting plants were selfed or crossed with homozygous recessive *o2-m(r)* tester lacking the regulatory element *Bg*. From 80 progenies of WER kernels tested from both ears only 15 were heterozygous for the *O2* allele. A low fraction of embryo revertants confirms the earlier conclusion that the major part of WER is formed during the period from the fusion of gametes to the first division of the primary endosperm nucleus (Maydica 44:195-203, 1999).

Most of the embryo revertants (13 out of 15) were found on the first ear. Five embryo revertants in this ear formed two clusters consisting of 2 and 3 contiguous revertant kernels. Since the frequency of gametes carrying reversions to the normal *O2* allele on this ear is not less than 0.027 (or 13/(240x2)) the probability of the clusters of 2 and 3 contiguous embryo revertants in this ear should be at least $7.3 \cdot 10^{-4}$ and $2.0 \cdot 10^{-5}$ respectively. Consequently this means that the formation of such clusters through the reversions at the postmeiotic stages is unlikely and indicates the possibility of premeiotic reversion events of the *o2-hf* allele in at least one of the homologous chromosomes. Taking into account the small size of the clusters mentioned, these reversion events occurred at the late stages of ear premeiotic development.

Another phenomenon to explain is the clustering distribution of WER, most of which are non-embryo revertants. One of the possible explanations could be the joint action of two factors at the early stages of endosperm development: 1) diffusion of the additional quantities of transposase from the developing endosperm with its level high enough for *rbg* excision in adjacent developing kernels; 2) high ratio of kernels with above- and sub-threshold excision level of transposase in developing endosperm in HFWER strains. The second cause could be connected both with the possible lower threshold level needed for the *rbg* excision from the *o2-hf* allele and the higher transposase production by the *Bg-hf* element.

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Evidence for genetic variation for phenotypically invariant traits in teosinte

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Many of the plant and inflorescence morphologies in teosinte are discretely different from maize, and vary little or not at all within and among teosinte populations. For example, teosinte ears always have exactly two ranks of cupules that each bear only one spikelet, while maize ears have many ranks of cupules that bear paired spikelets. While the lack of phenotypic variation for such traits in teosinte may suggest an absence of underlying genetic variation, it could also be the case that teosinte harbors genetic variation for such traits that is not phenotypically expressed in the teosinte genetic background.

Among F1 hybrids derived by crossing teosintes to maize inbred lines, we have often observed a broad range of phenotypes for otherwise discrete traits, suggesting that genetic variation for these traits may exist in teosinte. If this variation is genetic,

then F1 hybrids of different teosintes with the same maize inbred should have distinct phenotypes. If this variation is strictly environmental in origin, then all maize-teosinte F1 hybrids with the same inbred should be equivalent. In order to assess to what extent phenotypic variation among maize-teosinte hybrids depends on the teosinte parent (i.e. is genetic), we examined plant and inflorescence morphologies in 12 F1 families derived from crosses between maize inbred W22 and 12 teosinte plants, each of which came from a different natural population in Mexico. So that we could also compare the contributions of the two subspecies of Mexican annual teosinte, we used five *Zea mays* ssp. *mexicana* and seven *Zea mays* ssp. *parviglumis* plants as teosinte parents (Table 1).

Table 1. Accession data for the teosinte parents of the F1 families

Family #	Collector and number	Location
1	Benz 967	El Rodeo, Mexico
2	Beadle and Kato site 1	Mazatlan, Guerrero
3	Beadle and Kato site 2	Chilpancingo, Guerrero
4	Beadle and Kato site 4	Palo Blanco, Guerrero
5	Beadle and Kato site 6	Valle de Bravo, Guerrero
6	Beadle (no number)	El Salado, Guerrero
7	Kato site 4	Palo Blanco, Guerrero
8	Iltis *et al.* 28622	Chalco, Mexico
9	Iltis *et al.* 28620	Texcoco, Mexico
10	Puga 11066	Degollado, Jalisco, Mexico
11	Wilkes 45461	Panindicuaro, Michoachan
12	Beadle (no number)	Nobogame, Chihuahua

The family numbers cross-reference the accession data with the phenotype data in Table 2. The location of the populations where the teosintes came from are listed according to the nearest town/city, state.

Five traits were measured two to three weeks post-anthesis on the uppermost primary lateral branch and on the inflorescence at its tip. These traits were chosen because they define the key morphological differences between maize and teosinte (Doebley and Stec, Genetics 134: 559-570, 1993). Because teosinte has a long lateral branch and maize a short one, we measured the lateral branch length and divided this by the number of internodes in the branch to determine the lateral branch internode length (LBIL). The inflorescence at the tip of the primary lateral branch is a branched male inflorescence (tassel) in teosinte and an unbranched female inflorescence (ear) in maize. To record variation in the sex of this inflorescence, we counted the number of inflorescence internodes with male spikelets and the number with female spikelets, and computed the percentage of the total that were male or staminate (STAM). To record variation in inflorescence branching, we counted the number of branches on the primary lateral inflorescence (LIBN). The ears of teosinte bear single sessile spikelets on each internode, while those of maize have paired spikelets on each internode, one sessile and one pedicellate. To record variation in this trait, the percentage of female internodes lacking the pedicellate spikelet was computed and termed PEDS. Finally, teosinte bears its spikelets in two ranks on opposite sides of the ears, while maize has its spikelet pairs in four or more ranks and thus around the entire circumference of the ear. To record variation in this trait, the number of ranks of spikelets (or spikelet pairs) was counted for the primary lateral inflorescence. Since RANK can vary over the length of the inflorescence in teosinte-maize hybrids, it was calculated as a weighted sum.

There was considerable phenotypic variation among the maize-teosinte F1 families for all five traits (Table 2). Family means ranged from 13.5% to 89% for PEDS, from 20% to 69% for STAM and from 2.5 cm to 5.7 cm for LBIL. It is possible that this wide phenotypic variation is exclusively environmental in origin; however, we think this unlikely, since all twelve families were grown together in the same nursery and the data suggest that there are significant differences among families (Table 2). Since the maize parent of the F1 families was an inbred and therefore isogenic, it could not have been a source of genetic variation for differences among the F1 families. Thus, we suspect that genetic variation affecting these traits existed among the 12 teosinte plants used as parents, even though they each exhibited the same fixed-in-teosinte phenotypes for them.

Table 2. Average phenotypes of inbred maize by teosinte F1 families

Ssp	Family #	n	LBIL	LIBN	PEDS	RANK	STAM
p	1	4	4.2 ± 1.1	1.0 ± 0.6	18.7 ± 6.2	4.0 ± 0.0	27.2 ± 14.6
p	2	4	4.4 ± 0.8	0.5 ± 0.5	78.1 ± 18.5	3.3 ± 0.4	55.0 ± 16.7
p	3	5	2.5 ± 0.3	0.0 ± 0.0	89.1 ± 6.0	3.6 ± 0.2	19.6 ± 5.0
p	4	4	5.5 ± 1.2	0.8 ± 0.5	56.4 ± 10.0	2.7 ± 0.4	69.3 ± 12.2
p	5	6	3.1 ± 0.5	0.0 ± 0.0	45.5 ± 13.8	2.6 ± 0.3	55.6 ± 6.4
p	6	6	3.4 ± 0.9	0.0 ± 0.0	46.0 ± 11.9	3.4 ± 0.3	58.8 ± 7.0
p	7	6	5.7 ± 1.3	0.0 ± 0.0	83.0 ± 4.9	3.2 ± 0.3	66.6 ± 7.6
	Average		4.1 ± 0.5	0.3 ± 0.2	60.2 ± 5.3	3.3 ± 0.1	51.2 ± 4.5
m	8	5	3.6 ± 1.0	1.0 ± 0.8	13.5 ± 6.2	3.1 ± 0.3	35.6 ± 9.6
m	9	7	5.3 ± 1.0	0.4 ± 0.3	49.5 ± 9.2	3.5 ± 0.3	50.3 ± 6.5
m	10	8	5.2 ± 1.4	1.1 ± 0.6	26.9 ± 10.7	2.4 ± 0.2	62.0 ± 11.0
m	11	4	5.4 ± 1.5	0.0 ± 0.0	57.4 ± 20.7	4.0 ± 0.0	34.0 ± 11.2
m	12	7	5.3 ± 1.4	0.3 ± 0.3	32.3 ± 12.1	3.7 ± 0.3	38.7 ± 11.4
	Average		5.0 ± 0.5	0.6 ± 0.2	35.0 ± 5.7	3.3 ± 0.1	46.2 ± 4.8

The subspecies of the teosinte parent is listed as *p* for ssp. *parviglumis* and *m* for ssp. *mexicana*. The family number cross-references the phenotype data with the accession data in Table 1. n is the number of plants per family. The family averages and their standard errors are listed in trait units: cm, branches, %, cupule columns, %, respectively.

We compared the group of 35 F1 plants that had a ssp. *parviglumis* parent to the group of 31 plants which had a ssp. *mexicana* parent in order to investigate whether or not one subspecies consistently harbors alleles that make the plants more maize-like (Table 2). For all traits except PEDS, t-tests indicated that the contributions made by the two classes of parents were statistically equivalent. For PEDS, the F1 plants that had a ssp. *mexicana* parent had a lower percentage of female cupules that lacked the pedicillate spikelet (P=0.002). This difference could be attributable to introgression of maize germplasm, since ssp. *mexicana* plants grow largely as a weed in Mexican maize fields. It could also be the case that these differences simply represent natural differences in allele frequencies that exist between the two subspecies. In either case, our data suggest that teosinte populations contain hidden genetic variation for morphological traits for which they exhibit no phenotypic variation.

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Do transposon sequences in genic regions efficiently participate in recombination?

--Okagaki, RJ, Sullivan, TD, Kamps, TL

Available data suggest that recombination in maize preferentially occurs in genes and other low copy sequences (Xu et al., Plant Cell 7:2151, 1995; Dooner and Martinez-Ferez, Plant Cell 9:1633, 1997). Available evidence in maize also suggests that genes and other low copy sequences are flanked by blocks of

retrotransposons. Within euchromatic DNA, retrotransposons either flank individual genes or small groups of genes (SanMiguel et al., Science 274:765, 1996; Fu et al., PNAS 98:8903, 2001). These results suggest that retrotransposon sequences are refractory to recombination. DNA transposons are another class of repetitive elements, and unlike retrotransposons, DNA transposons are often located in genic regions. Here we present some preliminary results from experiments designed to detect recombination in DNA transposons. The specific question we are asking is whether recombination can occur within a *dSpm* element located inside the *bz1* gene. Recombination between *dSpm* elements could provide a mechanism for the duplication or deletion of genes. Weather and nicking problems prevented us from making the ideal crosses, but the initial results may be of interest.

Alleles used in this work were derived from *bz1-m13* (Fig. 1). The *bz1-m13* allele contains a 2.2 kb *defective-Spm* (*dSpm*) element inserted 38 bp downstream of the *bz1* intron. In the absence of an autonomous *Spm* element, seed carrying the *bz1-m13* allele have a purple aleurone. The *dSpm* element is spliced from the transcript using a cryptic 3' splice acceptor site in the *dSpm*. A partially functional enzyme is made which gives the purple aleurone (Kim, HY et al., PNAS 84:5863, 1987). In the presence of an autonomous *Spm* element, *bz1-m13* is very mutable.

Derivative *bz1-m13* alleles obtained in the presence of an autonomous *Spm* element are easily identified by their phenotype, and a number of derivatives have been isolated and characterized. *bz1-m13:CS6* (Schiefelbein, JW et al., in Plant Transposable Elements pp 261, 1988) and *bz1-m13:CS3* (Okagaki, RJ et al., Plant Cell 4:1453, 1992) are deletion derivatives of *bz1-m13*. *bz1-m13:CS64* (Okagaki, RJ et al., Plant Cell 4:1453, 1992) has a four basepair deletion that disrupts the cryptic 3' splice acceptor site; this allele gives a bronze colored aleurone. In *bz1'-9*, the *dSpm* insertion excised leaving behind a four basepair footprint (Okagaki, RJ et al., MGNL 64:83, 1990).

bz1-m14 was derived from *bz1-m13* and has two *dSpm* insertions. Its structure is consistent with a replicative transposition of the *dSpm* element in *bz1-m13* back into the *bz1* locus (Sullivan, TD unpublished data). The upstream element is unique to *bz1-m14*, and the downstream element is in common with *bz1-m13*. *bz1-m14DII* has lost the downstream *dSpm* element; excision either restored the non-mutant sequences or the transposable element footprint restored the correct reading frame (Sullivan, TD unpublished data).

The first experiment looked for recombination in two heteroallelic combinations where the mutations were separated either by approximately 500 bp or by 2.7 kb; the difference was due to the presence of the *dSpm* insertion (Fig. 2). A recombination event between the *dSpm* insertion in *bz1-m14DII* and the excision footprint in *bz1'-9* would produce a functional *Bz1* allele and give a purple aleurone. Similarly, a recombination event occuring between the upstream *dSpm* insertion in *bz1-m14* and the deletion in *bz1-m13:CS64* would produce a functional *bz1-m13* allele and give a purple aleurone. In the *bz1-m14DII/bz1'-9* combination this recombination event would occur in a region approximately 500 bp in length while the interval in the *bz1-m14/bz1-m13:CS64* combination was approximately 2.7 kb long. If the repetitive *dSpm* sequence does not participate in recombination the reversion frequency should be similar. But if the *dSpm* sequence participates in recombination revertants should be five times more frequent from the *bz1-m14/bz1-m13:CS64* combination assuming that reversion frequency is proportional to the physical distance between mutations.

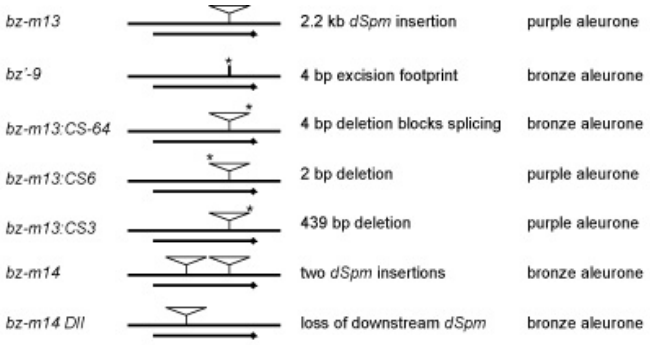

Figure 1. Alleles used in the study and their phenotypes. Asterisks indicate the locations of mutations.

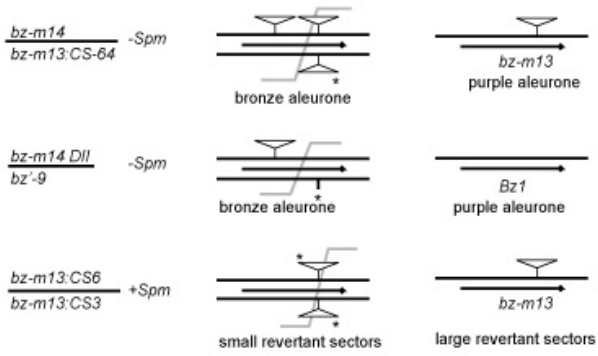

Figure 2. Heteroallelic combinations and crossover products. Genotypes are shown at left. Alleles are shown in the middle; grey lines represent crossovers. Recombinant products are shown on the right.

Problems with nicking prevented us from making crosses with the *bz1* tester. Plants were selfed instead. Twelve purple revertant kernels were found in 65,700 selfed seed from *bz1-m14DII/bz1'-9* plants. Six purple revertant seeds were found in 58,500 selfed seeds from *bz1-m14/bz1-m13:CS64* plants. This result suggests that a *dSpm* element within a gene does not participate in recombination and may even inhibit recombination in adjacent sequences. The two *dSpm* sequences in *bz1-m14* may pair; this could inhibit the recovery of revertants. We hope to isolate new derivative alleles from *bz1-m14* and *bz1-m14DII* that would lack the upstream insertion.

The second experiment looked for recombination within the *dSpm* sequence to generate an intact *bz1-m13* allele that could be identified by its mutable phenotype. The deletion derivative *bz1-m13:CS6* has a 2 bp deletion at the 5' end of the element as it is oriented in *bz1*. *bz1-m13:CS3* has a 439 bp deletion at the 3' end

of the element. A crossover within the 1.8 kb of overlapping *dSpm* sequence would produce the *bz1-m13* structure. In the presence of the autonomous element *Spm*, *bz1-m13:CS3* and *bz1-m13:CS6* give small revertant sectors. *bz1-m13 +Spm* gives early frequent revertant sectors. *bz1-m13:CS3/bz1-m13:CS6* plants were crossed with a *sh bz1* tester homozygous for the autonomous *Spm* element, and ears were examined for kernels with large revertant sectors. Weather and worms hit this field hard, and this experiment will be repeated.

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Identification and characterization of three mutational NILs in maize

--Wang, ZL, Zhang, Y

In developing countries that have limited adapted maize genetic resources for building up good breeding materials, induced mutations may be useful for variability. But, unfortunately theory indicates that chances are low that any new mutant (including adaptively beneficial mutants) will become established in the population and inbred lines in which they occur. On the other hand, practice indicates that mutants for so-called quantitatively inherited traits cause differences of degree (e.g. in yield, plant height, leaf number and seed size etc.) rather than kind. For this we have developed three pairs of near isogenic lines (NILs) derived from inbred female lines 478 (normal-seeded and normal-leaf – 6 leaves above the top ear) and the inbred male is line 340, of the superior performance of hybrid, Yedan 13, which has a large top growing area and a long life span among Chinese major maize hybrids. The mutants were named 488-1 (big-seeded and 6 leaves above the top ear) and 488-2 (big-seeded and 5 leaves above the top ear).

Three crosses were made among 4 inbred lines. The crosses were 478340, 488-1340 and 488-2340. The crosses and 4 in bred lines were planted 2 May 2000 in Shandong, China and 10 May in Columbia, Missouri, USA and harvested on 6 Sept, and 10 Sept respectively. There were three replications in 2000 and two replications in 2001. The three NILs were formed in 1999 (Figure 1).

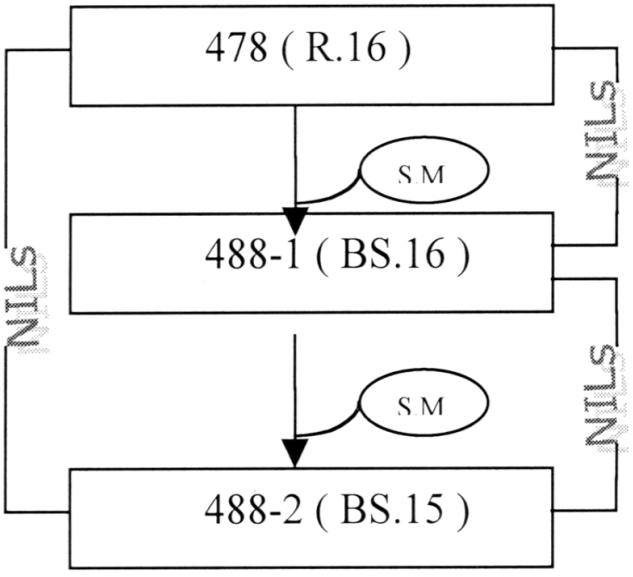

Figure 1. Description of the selection processes of the mutant inbred resulting from spontaneous mutations. R.16=original inbred line, 6 leaves; BS.16= big size seed inbred line, 6 leaves; BS.15=big size seed, 5 leaves; S.M=spontaneous mutations.

Means, two standard deviations of the mean and t-test were calculated for some traits measured (Table 1 and Table 2).

Table 1. Means of NIL traits for two years in China and the U.S.A.

Varieties	Plant Height	Leaf Number	Leaf Length	Leaf Width	Culm. Circum.	100 kernel weight, g	Grainweight per ear, g
478	1802.1	60.8	811.4	12.01.6	7.40.7	24.21.0	98.67.7
488-1	1892.4	6.22.0	801.8	12.32.4	7.51.1	34.11.3	121.88.1
488-2	1822.0	5.11.9	783.2	11.42.8	7.41.5	32.92.2	120.19.3
340	1961.7	5.00.9	791.7	11.51.2	8.50.9	33.0.1.2	124.36.5
478x340	2761.8	6.21.6	93.1.9	11.52.0	8.21.4	34.24.6	197.28.0
488-1x340	2913.1	6.01.9	964.6	12.23.2	8.31.7	36.45.0	242.59.0
488-2x340	2863.4	6.02.1	924.1	12.42.9	8.12.0	36.15.9	2309.8

Table 2. t-test of NIL traits for two years in China and the U.S.A (**p<0.01 *p<0.05, NS = no significant).

Parent	478	488-1	488-2		Hybrid	478x340	478-1x340	478-2x340
t-test for plant height								
478	1	*	NS		478x340	1	**	*
488-1	*	1	NS		488-1x340	**	1	NS
488-2	NS	NS	1		488-2x340	*	NS	1
t-test for leaf length								
478	1	NS	NS		478x340	1	NS	NS
488-1	NS	1	NS		488-1x340	NS	1	*
488-2	NS	NS	1		488-2x340	NS	*	1
t-test for leaf width								
478	1	NS	NS		478x340	1	NS	NS
488-1	NS	1	NS		488-1x340	NS	1	NS
488-2	NS	NS	1		488-2x340	NS	NS	1
t-test for 100 kernel weight								
478	1	**	**		478x340	1	**	**
488-1	**	1	NS		488-1x340	**	1	NS
488-2	**	NS	1		488-2x340	**	NS	1
t-test for grain weight per ear								
478	1	*	*		478x340	1	**	**
488-1	*	1	NS		488-1x340	**	1	*
488-2	*	NS	1		488-2x340	**	*	1

There were only significant differences for plant height between the parent NILs, 478 and 488-1, and their hybrids for 5 replications at two locations from 2000 to 2001. But there were no significant difference for leaf length and leaf width, except for hybrids 478x340 and 488-1x340 for leaf length (Table 2).

The t-test for grain weight per ear and 100 kernel weight shows that the two mutant NILs were significantly different from the original inbred line of 478, but there is no significant difference between the two mutant NILs. However, when those NILs were crossed with paternal parent line 340, 100 kernel weight and grain weight per ear of the hybrids derived from the original inbred line of 478 were significantly different from those derived from the two mutant NILs at the P<0.01 level. For the hybrids derived from the two mutants NILs the grain weight per ear showed a significant difference at the P<0.05 level, but 100 kernel weight does not shown any difference (Table 2).

For maize genetics, mutations are of interest in two different ways. They provide us with new starting material (or building blocks) for the production of new hybrid cultivators and they give us a tool for identifying and cloning new genes. A mapping population has been established for studying the nature of genes and their way of controlling heterosis and biochemical seed size and leaf number.

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Morpho-physiological aspects of seedlings in a local maize population under abiotic stress conditions

--Urechean, V

Local populations of maize represent a source of genetic variability for reducing genetic vulnerability to the biotic and abiotic stress factors.

P21 is a local population collected from the Tia Mare-Corabia area which has been maintained in Agricultural Research Station Simnic`s collection of maize populations for twenty years by multiplication in plots isolated in space. Open pollination assured the genetic variability; both yellow grain and red-purple grain plants were identified in this population.

P21 with yellow grain is the object of this study .The grains were put under abiotic stress conditions in order to germinate:

Five grains/Petri dish x 5 repetitions, covered with 40g dry and well chapped soil and then watered with the following solutions: MT(control)-watered with water, 10 mM NaCl, 100 mM Na Cl, 10 mM PRO (proline), 100 mM PRO, 10 µ M ABA (abscisic acid), 100 µ M ABA, 100 mM MAN(mannitol), 500 mM MAN, 2 ml/Petri dish, every 48 hours, for 14 days, in laboratory conditions (temperature varied between 15 C and 18 C). When temperature attained 22 - 23 C, the watering rate was 10 ml solution/Petri dish (every 48 hours) until transfer of the seedlings into vegetation dishes.

The timing of the coleoptile`s appearance was determined for each treatment variant (Table 1).

Table 1. Timing of coleoptile appearance for a local maize population (P21-yellow grain) under varied stress conditions.

Treatment									
Number of days from the experiment`s beginning	5	6	7	8	9	12	19	% coleoptile`s appearance	Difference from MT (control) %
Mt (control)	1	4	5	10	16	18		72	100
10 mM Na Cl		2	4	6	11	18	19	76	105
100 mM Na Cl		2	2	3	5	17	16	68	94
10 mM PRO		5	6	13	16	20	21	84	116
100 mM PRO	1	1	1	2	5	13	11	52	72
10 µM ABA		2	6	11	13			52	72
100 µM ABA		1	7	13	15			60	83
100 mM MAN		3	3	8	9	12		48	66
500 mM MAN				1	5	14		56	77

At the 5-6 leaf stage the stress becomes visible, the percentage of wilted leaves varying between 3% (MT) and 31% (100 µMABA) (Table 2). At this time, one week, the percentage of wilted leaves increases to 73% in 500 mMMAN treatment variant.

Table 2. Plant height and leaf wilting for stressed seedlings of maize.

Treatment	No. of leaves	No. of wilted leaves I	% dry wilted leaves I	Plant's height cm I	Number of wilted leaves II	Wilted leaves II %	Plant's height cm II	Difference of height (II-I) cm
MT(control)	6.0	0.2	3.0	49.3	1.6	26	53.2	3.9
10 mMNaCl	6.5	1.6	25	57.0	2.7	41	63.1	6.1
100mMNaCl	5.5	1.5	27	37.8	2.6	47	39.8	2.0
10mM PRO	6.0	1.4	23	55.2	2.5	41	61.2	6.0
100µM ABA	4.5	1.4	31	37.8	1.9	42	38.1	0.3
100mM MAN	6.5	1.1	17	52.6	2.8	43	65.0	12.4
500mM MAN	4.5	1.3	28	49.1	3.3	73	59.6	10.5

Plant height appears to be stimulated by high mannitol.

In the 5-6 leaf stage a red purple stalk coloration was obsreved on the first basic internodes in response to stress factors. We used a grading scale of the stalk coloration from zero to 2 where zero=the lack of purple coloration and 2=full coloration.

Mannitol determined the most intensely purple coloration of the stalk, with the color intensity increasing with the mannitol concentration. Seedling survival was highest in the 100 mMMAN (92days) and 10 mMPRO (89days) treatment variants.

The plants selected represent sources of valuable genes for the programs to improve maize response to stress factors.

Interspecific crossing *Zea mays L. x Mamillaria spp.*-- content of amino acids

--Urechean, V

The improvement of intergeneric and interspecific crossbreeding methods (most of the time by using the phytohormones) permitted the crossing of species more and more distant from a taxonomic point of view.

Starting from the idea that the *Cactaceae* family is very well adapted to dry areas, wanting to find out whether there is any gametic compatibility and hoping to obtain a transfer of genetic material to the *Zea mays L.* species, we realized a cross-breeding*: Zea mays L. x Mamillaria spp*.

As a pollen parent we used *Mamillaria* species (cultivated in our country only in green houses and vegetation dishes) which emits a sufficient quantity of easily collectable pollen, and the maternal parent was an inbred maize line very sensitive to drought and heat (Lc 460).

Pollination was made under a bag and a typical maize ear well covered with kernels resulted. The kernels were sowed in the field the next year and, surprisingly, the descendant F1 has the phenotypic aspect (morpho-anatomical characters) of the *Zea mays L.* species, presenting even the heterozygotic vigor specific for the maize hybrids cultivated in that area.

Since the two species were distant from a taxonomic point of view, the elimination of one species genome (in this case genome of *Mamillaria spp*.) was expected, but the hybrid vigor put questions about the homozygotic/heterozygotic aspect of the F1 descendant.

The lack of tests for the analysis of the *Mamillaria* chromosomes didn't permit us to ascertain whether the whole genome of the *Mamillaria* species had been eliminated or a transfer of genetic material had taken place between the two species.

Our research was based on the analysis of the grain's amino acids for the maternal line Lc 460, belonging to *Zea mays L.* species, and also of the hybrid descendant *Zea mays* (Lc 460) x *Mamillaria spp*., comparing with the two simple maize hybrids obtained and cultivated in the same medium conditions (Table 1).

In the interspecific hybrid combination *Zea mays L. x Mamillaria spp*., the content of threonine, serine, and glutamic acid, is a little bit higher than that of the maternal inbred line (Lc 460), being close to the values of the two simple maize hybrids HSS 409 and HSS 49.

The hybrid has the highest content of glycine and a very high content of alanine in comparison to the other genotypes studied. It has, also, the highest content of methionine and leucine which is very high in comparison with the maternal form (Lc 460).

Table 1. The content of amino acid from the kernel at *Zea mays L.x Mamillaria spp.* in comparison with the maternal parent and two simple maize hybrids F1.

Amino acid	Lc 460	Lc460 x M. spp.	HSS409	HSS49
Aspartic acid	0.788	0.762	0.843	0.707
Threonine	0.437	0.456	0.489	0.461
Serine	0.574	0.650	0.601	0.590
Glutamic acid	1.543	1.640	1.695	1.701
Proline	0.693	0.606	0.789	0.738
Cystine	0.345	0.289	0.472	0.384
Glycine	0.401	0.491	0.429	0.390
Alanine	0868	1.054	0.826	0.750
Valine	0.625	0.503	0.550	0.522
Methionine	0.119	0.341	0.187	0.182
Isoleucine	0.479	0.403	0.676	0.559
Leucine	1.121	1.451	1.177	1.169
Tyrosine	0.836	0.652	0.657	0.750
Phenilalanine	0.809	0.847	0.777	0.851
Histidine	0.462	0.509	0.693	0.688
Lysine	0.591	0.408	0.572	0.561
Arginine	0.980	-	-	-
TAA%	11.671	10.062	11.407	11.003
TAAE%	5.625	4.918	5.121	4.993
TAAE/TAA%	48.17	48.87	44.89	45.40
LYS/TAA%	5.06	4.05	5.01	5.09
GLU/TAA%	13.22	16.30	14.86	15.46
ASP/TAA%	6.75	7.57	7.39	6.42
PRO/TAA%	5.93	6.02	6.91	6.70

TAA-total amino acids; TAAE-total essential amino acids; LYS- lysine; GLU- glutamic acid; ASP-aspartic acid; PRO-proline.

The content of tyrosine diminishes considerably in comparison with the maternal form, but stays close to the others hybrids value. The lysine diminishes, having the lowest value related with the total of amino acids (4.05%).

The proline, cystine, valine, and isoleucine have also the lowest values at the hybrid combination *Zea mays x Mamillaria spp*. in comparison with the other genotypes studied, but the quantity of proline related to the total of amino acids (6.02%) is higher than the one obtained at the maternal form (5.93%).

Although the total content of amino acids at the hybrid combination *Zea mays L.* x *Mamillaria spp.* is lower (10.062mg/100g dray matter) there is a favorable relationship between the total content of essential amino acids and the total content of amino acids (48.87%) that is superior to the two simple maize hybrids (HSS 409 and HSS 49).

The glutamic and aspartic acid, as precursors of the other amino acids , also have the highest values.

All these differences between the hybrid combination *Zea mays L.* x *Mamillaria spp.* and the maternal form (the inbred maize line Lc 460) led us to believe that between the two parent plants belonging to two distant species, from a taxonomic point of view, but also geographically isolated, a transfer of genetic material took place.

Subsequent studies, especially an analysis of the chromosomal maps, will be able to show exactly whether these kinds of crossings are recommended for the improvement of maize programs or not.

Local maize populations: sources of genetic variability for maize improvement programs

--Urechean, V, Naidin, C

By cultivating only some varieties and species of plants obtained by improvement methods year after year, a great part of genetic diversity has disappeared. With their reserve of ancestral genes, maize local populations are real sources of genetic diversity and variability for maize improvement programs.

A population can be improved in order to obtain specific characteristics: precocity, disease resistance, strong stalk (mechanical resistance) or a large grain's content of proteins with implications in the production's improvement on the whole that can be used for the infusion with genes of adaptability to particular environment conditions.

In this research we studied five maize local populations (P21; P28; P68; P71; P106) which have been maintained in the genetic stock collection for twenty years, by propagation in isolated lots. Open-pollination assured the transmission of genetic variability of these populations along different generations.

A proof of this genetic diversity was the identification, as part of the same population, of plants that differ in grain color. In this way we found: P21 - yellow grain; P21 - purple grain; P28 - yellow-lemon grain; P28 - white grain; P68 - yellow-orange grain; P71 - white grain; P71 - yellow-orange grain; P106 - white grain; P106 - yellow-lemon grain; P106-yellow-orange grain. For each of these ten genotypes we analyzed the content of amino acids from the grain (Table 1).

The coefficient of variation (c.v. %) for the ten genotypes analyzed shows a middling variation for tyrosine(15.80%); arginine (15.51%) phenylalanine (12.70%) and glutamic acid (11.38%), the other amino acids having a c.v.<10%. At the level of each population there are differences concerning the amino acid content depending on the grain's color. In this way, at P21, the content of arginine is much higher than that of the genotype with purple-colored grain, which probably assures the favorable ratio between the total essential amino acids and the total amino acids (19.38%). The yellow grain genotype (P21 with yellow grain) has a higher content of aspartic acid/TAA% (7.17%) and tyrosine+phenylalanine (TYR+PHE/TAA=13.11 %).

The white grain genotype (P28 white grain) has a higher content of lysine related with the total of amino acids (4.31%), as well as the tyrosine+phenylalanine (11.43%), the rest of the amino acids having close values. P71 with white grain and yellow-orange grain shows the greatest difference at the level of the tyrosine, the yellow-orange grain genotype hawing 1,088mg/100g dry matter and the white grain genotype 0,641mg/100g dry matter.

The total content of amino acids and essential amino acids is also higher in P71 with yellow-orange grain. The content of lysine is higher for the P21 genotype with white grain, but TYR+PHE/TAA=16.26% for the yellow-orange grain genotype is the highest of all genotypes studied.

The P106 population with P106-white grain; P106-yellow-lemon grain and P106 yellow-orange grain genotypes have the lowest total content of amino acids (TAAmg/100g dray matter.) at the white grain variant (11.449mg/100g dry matter.) but the best TAAE/TAA=49.200%. P106 with yellow-lemon grain has the highest content of glutamic acid (1.612 mg), leucine (1.166 mg) and total essential amino acids TAAE mg/100g dry matter (5.905). P106 with yellow-orange grain genotype has a higher content of proline and tyrosine but the lowest arginine level (0.823mg). Also the total content of essential amino acids is the lowest (46.65%).

All these variations of the content of amino acids reflect the genetic variability of the maize local populations on the whole and the genetic diversity existent at each population's level. Because the quality of the grain is one of our maize improvement programs priorities we tried to establish if there was any correlation be-

Table 1. The variation in content of amino acids of the grains of local maize populations mg./100g.dry matter).

	POPULATION-Intensity of the grain color											
Amino acid	P21 y-	P21 p	P28 y-l	P28 w	P68 y-o	P71w	P71 y-o	P106 w	P106y-l	P106y-o	X	c.v.
	1.2	1.5	1.1	1	1.3	1	1.3	1	1.1	1.3		%
Aspartic acid	0.825	0.788	0.801	0.916	0.634	0.824	0.850	0.794	0.866	0.840	0.814	7.00
threonine	0.417	0.427	0.472	0.462	0.331	0.426	0.470	0.438	0.453	0.472	0.437	4.03
serine	0.547	0.576	0.583	0.631	0.428	0.538	0.527	0.559	0.583	0.610	0.558	5.29
Glutanic acid	1.545	1.533	1.622	1.630	1.236	1.444	1.589	1.475	1.612	1.592	1.528	11.38
proline	0.735	0.624	0.842	0.732	0.722	0.653	0.709	0.704	0.720	0.858	0.730	6.88
cystine	0.372	0.344	0.395	0.328	0.166	0.376	0.364	0.387	0.329	0.456	0.352	7.13
glycine	0.399	0.392	0.395	0.448	0.330	0.411	0.481	0.399	0.425	0.405	0.409	3.73
alanine	0.902	0.963	0.961	0.841	0.611	0.904	0.916	0.896	0.852	0.822	0.867	9.61
valine	0.635	0.613	0.583	0.685	0.535	0.602	0.742	0.574	0.617	0.591	0.618	5.59
methionine	0.140	0.121	0.153	0.129	0.076	0.118	0.133	0.167	0.095	0.098	0.123	2.63
isoleucine	0.564	0.580	0.421	0.631	0.353	0.388	0.470	0.400	0.524	0.467	0.480	8.75
leucine	1.068	1.110	1.147	1.172	0.930	1.056	1.263	0.998	1.166	1.086	1.100	8.99
tyrosine	0.727	0.540	0.577	0.612	0.550	0.641	1.088	0.601	0.747	0.814	0.690	15.80
phenylalanine	0.782	0.641	0.606	0.750	0.548	0.750	0.967	0.827	0.917	0.837	0.763	12.70
histidine	0.623	0.445	0.483	0.631	0.623	0.574	0.629	0.624	0.549	0.618	0.580	6.38
lysine	0.519	0.562	0.383	0.513	0.462	0.733	0.638	0.605	0.568	0.602	0.559	9.20
arginine	0.707	1.122	0.913	0.800	0.561	0.797	0.800	1.001	1.016	0.823	0.854	15.51
TAA %	11.507	11.381	11.337	11.911	9.096	11.235	12.636	11.449	12.039	11.991	11.458	
TAAE %	5.455	5.621	5.161	5.773	4.419	5.444	6.112	5.634	5.905	5.594	5.512	
TAAE/TAA%	47.40	49.38	45.52	48.46	48.58	48.45	48.36	49.2	49.04	46.65	48.10	
GLU/TAA,%	13.43	13.47	14.31	13.68	13.59	12.85	12.58	12.88	13.39	13.28	13.33	
LYS/TAA,%	4.51	4.94	3.38	4.31	5.08	6.52	5.05	5.28	4.72	5.02	4.87	
ASP/TAA,%	7.17	6.92	7.07	7.69	6.97	7.33	6.73	6.94	7.19	7.01	7.10	
TYR+PHE/TAA	13.11	10.38	10.43	11.43	12.07	12.38	16.26	12.47	13.82	13.77	12.67	

TAA -total amino acids; TAAE - total essential amino acids; GLU - glutamic acid; LYS - lysine; ASP - aspartic acid; TRY+PHE-tyrosine+phenylalanine

tween grain's content of amino acids and the color of the grain. We used a grade scale of the grain color's intensity:1=white grain; 1.1=yellow-lemon grain; 1.2=yellow grain; 1.3 yellow-orange grain; 1.5=purple-coloured grain. For the genotypes studied we calculated: total content of amino acids (TAAmg/100g dry matter) and color of the grain, where r = -0.128; total content of essential amino acids (TAAE mg/100g dry matter) and color of the grain, where r= -0.110. The genotypes were grouped by intensity of grain colors: genotypes with light colored grain (1 and 1.1 grade) and genotypes with dark colored grain (1.3 and 1.5 grade.

We analyzed the correlation between every essential amino acid and the light color of the grain. We found negative correlations between the level of essential amino acids and the light color of the grain (Table 2).

Table 2. Correlation between the grain's content of essential amino acids and the light color of the maize grain.

Amino acid	Color grain	coefficient of correlation
threonine	1 and 1.1	0.666
valine	1 and 1.1	- 0.375
methionine	1 and 1.1	-0.666
isoleucine	1 and 1.1	-0.002
leucine	1 and 1.1	0.562
phenylalanine	1 and 1.1	-0.125
histidine	1 and 1.1	-1.0
lysine	1 and 1.1	-0.666
arginine	1 and 1.1	0.50

SLIPPERY ROCK, PENNSYLVANIA
Slippery Rock University

Maize crosses to illustrate epistatic ratios of kernel colors
--Shotwell, MA

Epistasis, the nonreciprocal interaction between nonallelic genes, is an important concept in genetics, but one that students often struggle to master. Understanding of this concept is greatly aided by laboratory exercises in which examples of epistasis are examined. Kernel color in maize is an ideal phenotype for illustrating epistasis in the teaching lab. Not only are there a variety of independently assorting genes that influence pigment accumulation, but the large number of kernels on each ear allows for the calculation of statistically meaningful phenotype ratios.

One lab in the genetics course I teach is devoted to epistasis, with the exercise centering on kernel color in maize. Over the years, I grew dissatisfied with the quality of the ears I acquired from commercial sources. The colors were often indistinct, and in some cases the phenotype ratios were not as advertised. I therefore decided to do my own crosses to produce ears that illustrated four epistatic interactions: (1) dominant, (2) recessive, (3) duplicate recessive, and (4) dominant and recessive.

In planning these crosses I was assisted by Phil Stinard of the Maize Genetics Cooperation Stock Center. Of the 12 crosses that I completed, only four will be described here. These involved the parental lines listed in Table 1, all of which were acquired from the Maize Genetics Cooperation Stock Center.

Table 1. Parental lines used in crosses to illustrate epistasis.

Stock No.	Genotype	Phenotype
ACRY	*A1 A2 C1 R1 Pr1 Y1*	purple
506B	*A1 A2 C1 R1 pr1 y1*	red
507A(*Y1*)	*A1 a2 C1 R1 Pr1 Y1*	yellow
507A(*y1*)	*A1 a2 C1 R1 pr1 y1*	white
906D	*A1 A2 Cl-1 R1 Pr1 y1*	white
910D	*A1 A2 c1 R1 Pr1 Y1*	yellow

The parental lines were crossed in 1999, and the F1 plants were self-fertilized in 2000 to give F2 ears. Owing to the small size of these ears, new F1 crosses were re-done in 2001; these are the F2 ears that are reported here. Table 2 shows the results of the four crosses, and Figure 1 shows representative F2 ears from each cross. In each cross (except for cross 4; see below), there were two genes segregating that affect kernel color.

Table 2. Phenotypes in the F1 and F2 generations of crosses to illustrate epistasis.

Cross no.	Parental lines	F1 phenotype	F2 phenotypes*		
1	906D x 506B	white	white	purple	red
			1809	448	166
2	906D x ACRY	yellow	yellow	white	purple
			1836	574	772
3	507A(*Y1*) x 910D	purple	purple	yellow	
			2901	2195	
4	906D x 507A(*y1*)	white	white	purple	red
			3530	657	69

*7, 6, 12, and 9 F2 ears were examined for crosses 1, 2, 3, and 4 respectively.

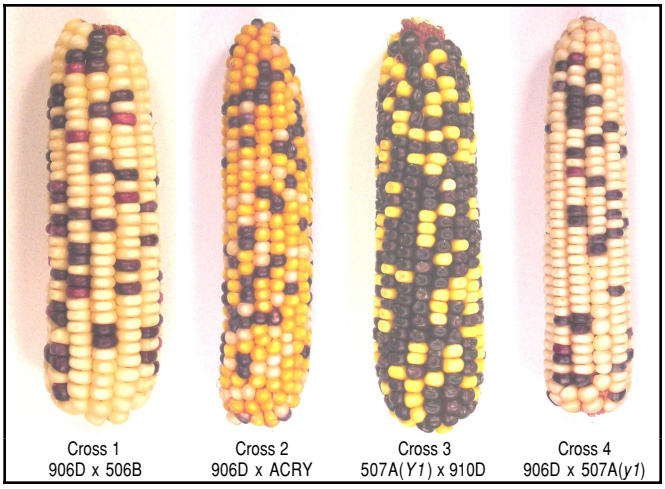


Figure 1. Representative F2 ears from crosses to illustrate epistasis.

In cross 4, only white and purple kernels were expected in the F2 generation, but a small number (69 = 1.6%) of red kernels appeared. These red kernels are the result of the segregation of *Pr1* alleles. The red kernels were unexpected because parental line 507A(*y1*) was believed to be *Pr1*, not *pr1*. There were thus three genes segregating in cross 4. When the red kernels are combined with the purple kernels, however, the expected F2 phenotype ratio for dominant and recessive epistasis is approximated.

As shown in Table 3, in all four crosses the observed ratio of F2 kernel phenotypes was very close to the expected ratio based on an assumption of recessive epistasis, dominant epistasis, duplicate recessive epistasis, and dominant and recessive epistasis between the two segregating genes (ignoring the third segregating gene in cross 4). These F2 ears are superior to those commercially available, and I have begun using them in my genetics labs. Future crosses will be aimed at getting a better example of dominant and recessive epistasis (the 13:3 ratio). In the meantime, I will make the F1 seeds available to anyone who would like them.

Table 3. Ratio of phenotypes in the F2 generation of crosses to illustrate epistasis.

Cross no.	Epistatic interaction	Expected F2 ratio	Observed F2 ratio
1	Dominant	12 white	11.95 white
		3 purple	2.96 purple
		1 red	1.10 red
2	Recessive	9 yellow	9.23 yellow
		3 white	2.89 white
		4 purple	3.88 purple
3	Duplicate recessive	9 purple	9.11 purple
		7 yellow	6.89 yellow
4	Dominant and	13 white	13.27 white
	recessive	3 purple	2.73 purple+red

TAICHUNG, TAIWAN
National Chung Hsing University

The cytological B-breakpoints of seven B-10L translocations

--Cheng, Y-M, Lin, B-Y

The pachytene structure of the B chromosome comprises, from proximal to distal, the short arm, the centromeric knob, the proximal euchromatic region (including five chromomeres), the distal heterochromatic region (H1, H2, H3 and H4), and the euchromatic tip (Fig.1A). Of the 38 B-10L translocations previously isolated by Lin (MNL 46: 193-194, 1972), the cytological B-breakpoint of 31 had been determined based on the pachytene figure of hyperploids (Genetics 90:613-627, 1978; Genetics 92:931-945, 1979; Genetics 100:475-486, 1982 and MNL 60:54, 1986). This article reports the B-breakpoints of an additional five translocations. Also included are two of the previously analyzed breakpoints (TB-10L20 and TB-10L36).

Of the seven translocations, only one (TB-10L16) has a break in the proximal euchromatic region, at the fourth chromomere (Fig.1B). Six others (TB-10L13, TB-10L20, TB-10L32, TB-10L34, TB-10L36 and TB-10L38) have breakpoints in the distal heterochromatic region: TB-10L34 and TB-10L38 at the junction of H1 and H2 (Fig.1C and 1D), TB-10L32 in H3 close to the junction of H2 and H3 (Fig.1E) and TB-10L13 at the junction of H2

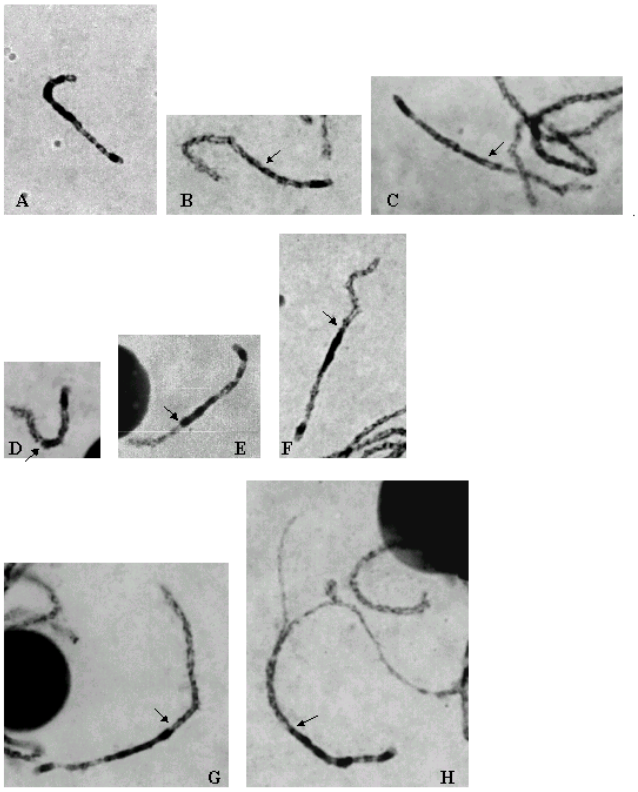


Figure 1. A-H Pachytene figure of the B chromosome and seven B-10 chromosomes. (A) B chromosome. (B) TB-10L16. (C) TB-10L34. (D) TB-10L38. (E) TB-10L32. (F) TB-10L13. (G) TB-10L20. (H) TB-10L36. The breakpoints are marked with an arrow.

and H3 (Fig.1F). TB-10L20 and TB-10L36 break in H3 (Fig.1G and1H), consistent with Lin's earlier observation (Genetics 90: 613-627, 1978). The breakpoint of TB-10L36 is distal but close to that of TB-10L20. The results are summarized in Figure 2.

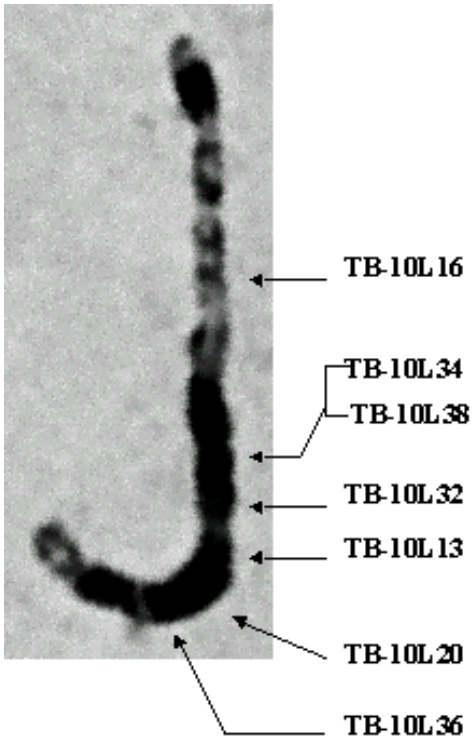


Figure 2. B-breakpoints of seven B-10L translocations.

Cytological 10L-breakpoints of B-10L translocations

--Cheng, Y-M, Lin, B-Y

We reported previously (MNL 75:61, 2001) cytological breakpoints on 10L of ten B-10L translocations. Here, we report the 10L breakpoint of an additional 17 (Table 1). Results of these two studies were combined in Figure 1. Comparison of this map with the previous linkage data (Lin, MNL 48:182-184, 1974) reveals several discrepancies, most of which may be attributable to errors of small sample size associated with the linkage analyses.

Table 1. Cytological breakpoint on 10L of 17 B-10L translocations.

Translocations	Breakpoint on 10L	Average	Standard Error	Lin's data [a]
TB-10L3	0.33, 0.27, 0.35, 0.32, 0.29	0.31	0.03	0.61
TB-10L7	0.50, 0.46, 0.52, 0.54, 0.47	0.50	0.03	0.77
TB-10L8	0.36, 0.35, 0.25, 0.35, 0.46, 0.42	0.37	0.07	0.50
TB-10L5	0.44, 0.51, 0.41, 0.48, 0.41, 0.46	0.45	0.04	0.61
TB-10L10	0.37, 0.50, 0.39, 0.48, 0.33	0.41	0.07	0.65
TB-10L13	0.58, 0.61	0.60	0.02	nd[b]
TB-10L14	0.59, 0.71, 0.69, 0.73, 0.58, 0.67, 0.71	0.67	0.02	0.45
TB-10L18	Centromere	Centromere	-	1.35
TB-10L25	0.44, 0.56, 0.45, 0.46, 0.40, 0.49	0.47	0.05	0.67
TB-10L28	0.53, 0.46, 0.41, 0.36, 0.49, 0.46	0.45	0.06	0.82
TB-10L29	0.43, 0.37, 0.46, 0.40, 0.35, 0.37	0.40	0.04	0.39
TB-10L31	0.65, 0.67, 0.65, 0.71, 0.65, 0.64	0.66	0.03	0.86
TB-10L32	0.69, 0.59, 0.67, 0.73	0.67	0.06	0
TB-10L34	0.59, 0.49, 0.53, 0.46, 0.61, 0.54	0.54	0.06	0.47
TB-10L35	0.58, 0.46, 0.60, 0.56, 0.48	0.53	0.06	0.41
TB-10L37	0.61, 0.64, 0.58, 0.57	0.60	0.03	0.63
TB-10L38	0.60, 0.58, 0.56, 0.63, 0.67	0.61	0.04	0.54

[a]Recombination ratio of breakpoint-*R*/*g*- *R* (from Lin, 1974).
[b]No data.

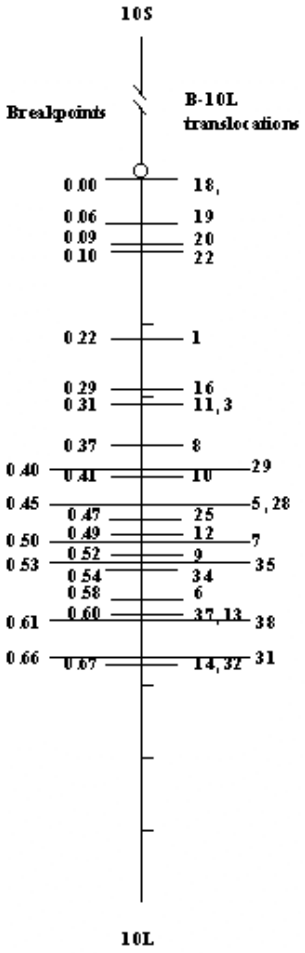


Figure 1. Cytological 10L breakpoints of 26 B-10L translocations.

TALLAHASSEE, FLORIDA
The Florida State University

Pachytene arm ratios for maize chromosome 9 in OMAd9.2, a maize chromosome addition line of oat

--Koumbaris, G, Bass, HW

We are developing a cytogenetic mapping system using a maize disomic chromosome addition line of oat designated OMAd9.2 (origin and use of OMA lines reviewed by Kynast et al., Plant Physiol. 125:1216, 2001). Cytogenetic maps are experimentally useful and biologically informative (reviewed by Harper and Cande, Funct. Integr. Genomics, 1:89, 2000). We have used a newly designed centromeric FISH probe and whole chromosome painting to examine the cytogenetic structure of maize chromosome 9 from OMAd9.2 anthers fixed at the pachytene stage of meiotic prophase. The probe, MCCY, was designed from the junction between two CentC sequence repeats (Ananiev et al., Proc. Nat. Acad. Sci. USA 95:13073, 1998) and used with the 3D acrylamide FISH method as previously described (Bass et al., J. Cell Biol. 137:5, 1997). The MCCY probe is 5'-Cy5-GAAAAACGAAGAAATGGTTCTGGTG-3'. The MCCY probe hybridized to a small discrete spot that appears to co-localize with the primary constriction of the maize 9 centromere. The FISH paint signals from the maize 9 pachytene fibers were im-

aged, traced, and straightened as previously described (Bass et al., J. Cell Sci. 113:1033, 2000). Total DNA from knobless lines of maize provided good whole-chromosome paints. Knobless Tama Flint (KTF, MGCSC stock 11074) DNA painted the entire fiber with fairly uniformly FISH signal intensity along its length. Knobless Wilbur's Flint (KWF, MGCSC stock 11075) DNA provided a good baseline fiber image for cytogenetic mapping because it painted the entire fiber and in the same wavelength revealed two important bonus loci, the 9S telomeric knob and the centromere.

Figure 1 shows a pachytene nucleus displayed as projections of 3D images from separate wavelengths (A, DAPI, total DNA; B, rhodamine, maize 9 FISH signals; C, Cy5, MCCY centromere FISH signals). The computer-straightened chromosome (D) is shown at the bottom (9S, short arm; C; centromere; 9L; long arm) and the FISH probes are indicated at the right. We have found that the arm length ratios are similar to those for the endogenous maize chromosome 9 (in maize) from either meiotic prophase or somatic metaphase cells (Table 1). The maize 9 chromosome in OMAd9.2 maintained a pachytene arm ratio of about 1.8, despite large variations in total length. Previously published arm ratios for maize 9 range from 1.7 to 2.0.

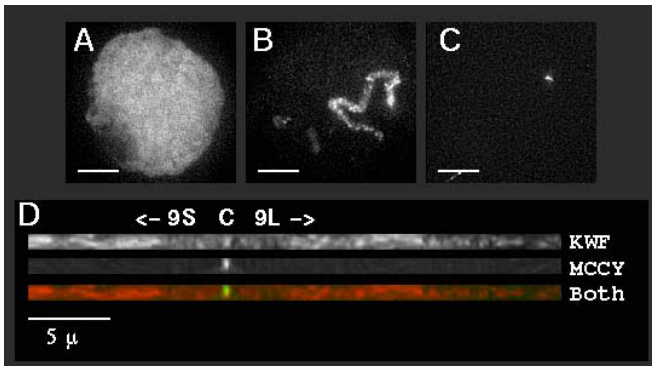


Figure 1.

Table 1. Maize 9 arm length ratios (long/short).

Ratio L/S	maize line	cell type	Reference or source of information
1.8	KYS	mei	Rhoades (1950) J Hered. 61:59.
2.0	KYS	mei	Neuffer et al. (1997) in "Mutants of maize" CSHL Press.
1.93	KYS	mei	Chen et al. (2000) Theor. Appl. Genet. 101:30.
2	KYS	som	Sadder and Weber (2001) Plant Mol. Bio. Reporter 19:117-123.
1.7	Se60	som	Chen, CC, (1969) Can. J. Genet. Cytol. 11:752.
1.8	OM9	mei	this report, total length 22.8 micron.
1.8	OM9	mei	this report, total length 25.2 micron.
1.7	OM9	mei	this report, total length 35.2 micron.

Table Notes: L/S long to short arm ratio; mei meiotic; som somatic; Se60 Seneca60; OM9 OMAd9.2 maize 9 from oat x maize addition line OMAd9.2 (from pollen of Seneca 60).

These numbers suggest that the higher order packaging of meiotic chromosomes, at least with respect to the constancy of arm ratio, may be governed by some intrinsic properties of the chromosome itself. Such properties may involve matrix attachment region sequences, genes, aspects of the chromatin-folding pathway, or the distribution of trans-acting factors. It is interesting to consider that the packing and segregation of maize 9 chromosome in OMAd9.2 probably involves specific and conserved molecular interactions between *Avena* proteins and *Zea* DNA or chromatin elements. Analysis of this material may help unravel some of the complexities of the structure-function relationships operating on meiotic chromosomes. This material should also be suitable for cytogenetic mapping of maize loci by FISH.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center
USDA/ARS/MWA

Three-point linkage data for *du1, inr1* and *g1* on 10L

--Stinard, P

In last year's MNL (Stinard, P, MNL 75:71, 2001), we reported two-point linkage data for *du1* and *inr1* on the long arm of chromosome 10. In that experiment, a three-point linkage test involving the 10L markers *inr1*, *du1*, and *g1* had been set up as indicated in Table 1 (*R1-S* is an *r1* allele that is susceptible to inhibition by *Inr1*; *wx1* facilitates the scoring of *du1*). However, scoring of the progeny for *g1* had not been done by the MNL deadline, so only two-point *du1 inr1* data were presented. This past winter and summer, *Du1 inr1* and *du1 Inr1* crossover kernels from these crosses were planted and the resulting plants scored for *g1* in order to provide partial three-point linkage data. Crosses were made in order to confirm the *du1* and *inr1* scoring as well. Complete scoring of the parental classes for *g1* was not done because of the large populations involved, and because complete scoring was not necessary for determining the gene order since the linkage relation between *du1* and *g1* is already well-characterized (these two loci are 19 centimorgans apart). The partial three-point linkage data are presented in Table 1. These data confirm that *inr1* and *du1* are very tightly linked, with an approximately 0.14 centimorgan distance between them. These data furthermore determine the global order of the three loci on chromosome 10 as *du1 inr1 g1*.

Table 1. Partial three-point linkage data for *du1, inr1* and *g1*.
Testcross: (*Du1 Inr1 G1 R1-S wx1* X *du1 inr1 g1 R1-S wx1*) X *du1 inr1 g1 R1-S wx1*.

Reg.	Phenotype	No.	Totals
0; 2	Du Inr; G or g	4357	
	du inr; G or g	4411	8768
1	Du inr g	6	
	du Inr G	4	10
1 + 2	Du inr G	1	
	du Inr g	1	2

% recombination *du1--inr1* = 0.14 +/- 0.04

The isolation and characterization of *su3* and *su4* duplicate loci

--Stinard, P

In 1992 (Stinard, PS. 1992. MNL 66:4-5), we reported the isolation of a new sugary endosperm mutation, *su3*. At that time, we presented data indicating linkage of *su3* with *wx1* T4-9g (6.5 centimorgans between *su3* and *wx1*), but not with other *wx1*-marked translocations. This seemed to indicate a location of *su3* on chromosome 4. However, linkage tests of *su3* with the chromosome 4 markers *su1* and *gl4* showed no linkage of *su3* with these markers. Since *su1* is tightly linked to *wx1* in the translocation

T4-9g (p ~ .04, Jackson, J and Stinard, P, MNL 72:79-81, 1998; p~.14, Jackson, JD et al., MNL 75:68-71, 2001), these results were inconsistent with our observations of linkage of *su3* with *wx1* T4-9g.

Other anomalies were also observed. F2's of *su3* with inbreds W64A and B73 consistently yielded ears segregating for a low frequency of *su3* mutant kernels, approximating 15:1 ratios. At the time, we thought that this was due to poor penetrance since homozygous *su3* lines often give a range of phenotypes from extreme to near normal. However, the 15:1 F2 ratios held up after several generations of backcrossing. Since the 15:1 ratios were suggestive of the involvement of duplicate loci, both of which need to be homozygous mutant in order to produce a mutant phenotype, we tested this hypothesis.

From the self-pollinated 15:1 segregating ear of a fifth generation outcross of *su3* to W64A, nonmutant kernels were planted in our 1999 winter nursery, and the resulting plants self-pollinated. From the resulting ears, four ears with good 3:1 segregation of nonmutant:mutant kernels were selected and labeled A, B, C, and D. These ears would presumably be from plants homozygous mutant at one duplicate locus, and heterozygous mutant at the second duplicate locus. Nonmutant kernels from these ears were planted in our summer 2000 nursery, and the resulting plants were self-pollinated. Ears segregating 3:1 as well as ears with only nonmutant kernels were obtained. The latter ears would be expected to be homozygous mutant at one duplicate locus and homozygous nonmutant at the other duplicate locus; such ears will be referred to as single factor lines. Kernels from these four single factor lines (A, B, C, and D) were planted in our 2000 winter nursery, and intercrosses were made between these lines in all possible combinations.

During the summer of 2001, kernels from these intercrosses were planted, and the resulting plants were self-pollinated. The following results were obtained: F2's of intercrosses A X C (8 ears) and B X D (8 ears) yielded nonmutant kernels only. F2's of intercrosses A X B (8 ears), A X D (8 ears), B X C (9 ears), and C X D (8 ears) yielded 15:1 ratios of nonmutant: mutant kernels. We can conclude that the *su3* mutant does indeed require two homozygous mutant loci for expression, and that lines A and C are homozygous mutant at one locus, and lines B and D are homozygous mutant at the other locus.

The existence of duplicate loci for *su3* could explain the anomalous linkage results reported in 1992. The linkage experiment involving *wx1* T4-9g might have been tracking one locus, and the linkage experiment involving *su1* and *gl4* might have been tracking the second locus. We have given the name *su3* to the locus linked to *wx1* T4-9g, and the name *su4* to the unlinked locus. We are currently conducting a new round of linkage experiments using the separated factor lines in order to determine which are homozygous for *su3* and which are homozygous for *su4*.

Differences in phenotypic expression between *brn1-R brn2-R* and *brn1-Nelson brn2-Nelson* mutant kernels are attributable to the *brn1* locus

--Stinard, P

Phenotypic variation in mutant kernel expression exists among the various alleles of the brown aleurone duplicate factor pair *brn1 brn2* (Stinard, PS, Maydica 39:273-278, 1994). In the B73 inbred background, homozygous mutant kernels of the reference alleles, *brn1-R brn2-R* are normal sized with normal embryos and dark brown pigmentation of the aleurone. Homozygous mutant kernels of Oliver Nelson's alleles, *brn1-Nelson brn2-Nelson*, are small, with defective embryos and lighter brown aleurone pigmentation (Figure 1). In order to better understand the nature of the phenotypic differences, we decided to isolate the four alleles (*brn1-R*, *brn1-Nelson*, *brn2-R*, and *brn2-Nelson*) and combine them in all possible pairs in order to determine the effect of allelic differences on mutant expression.

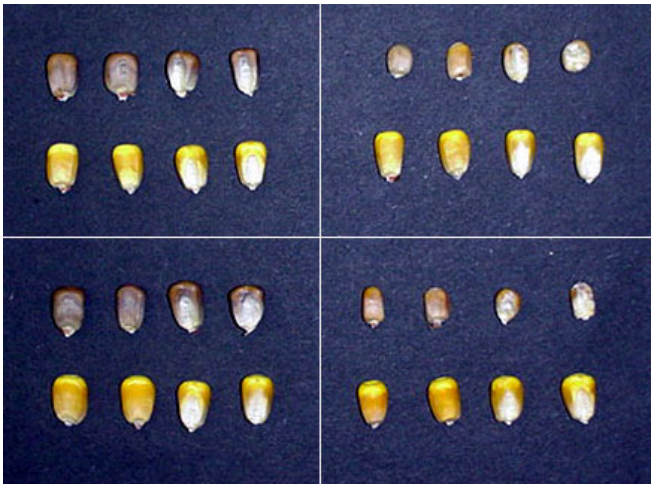


Figure 1. Segregating mutant (upper row) and nonmutant (lower row) kernels from F2 ears of *brn1-R brn2-R* (upper left), *brn1-Nelson brn2-Nelson* (upper right), *brn1-R brn2-Nelson* (lower left), and *brn1-Nelson brn2-R* (lower right). All mutant alleles are in inbred B73 background (six backcross generations).

All four mutant alleles were isolated in the B73 background (six backcross generations) according to the procedure previously outlined by the author (Stinard, P, MNL 74:70, 2000). The nonmutant alleles in these lines are provided by the inbred B73, which is *Brn1 Brn2*. Intercrosses were made between the complementary reference and Nelson alleles to produce the combinations *brn1-R Brn1 brn2-Nelson Brn2* and *brn1-Nelson Brn1 brn2-R Brn2*, and the resulting F1's were self-pollinated to produce segregating F2 ears.

Mutant and nonmutant kernels from these ears are shown in comparison with kernels from segregating ears of *brn1-R Brn1 brn2-R Brn2* and *brn1-Nelson Brn1 brn2-Nelson Brn2* in Figure 1. Mutant kernels of the combination *brn1-R brn2-Nelson* share the normal kernel and embryo size, and dark brown aleurone pigmentation with the reference allele combination *brn1-R brn2-R*. On the other hand, mutant kernels of the combination *brn1-Nelson brn2-R* share the small kernel size and defective embryo, and light brown aleurone pigmentation with the Nelson allele combination *brn1-Nelson brn2-Nelson*. Thus, it is allelic differences at the *brn1* locus that determine the differences in mutant kernel expression between the reference and Nelson alleles.

It should be noted that at least a few inbred and hybrid lines are homozygous mutant at the *brn2* locus. Inbred B73 is the only exception found to date (Stinard, P, MNL 74:70, 2000). Although the *brn2-R* and *brn2-Nelson* alleles have been maintained separately from each other since they were received by the author, it is conceivable that they could share a common ancestry, as could

all lines that "naturally" carry a mutant allele at the *brn2* locus. Only a molecular analysis will resolve this issue.

Failure to replicate the "cytoplasmic glossy" effect

--Stinard, P

In 1983, Dr. George Sprague reported on a "cytoplasmic glossy" line, whereby a line carrying a particular *gl1* allele (*gl1-c*) in a particular cytoplasmic background (*cgl*) produces glossy seedling progeny when crossed as a female by glossy mutants at other specific loci (*gl2*, *gl3*, *gl4*, *gl5 gl20*, *gl6*, *gl7*, *gl11*, and *gl15*), but not when crossed as a male (Sprague, GF. 1983. Maydica 28:189-200). Female crosses by *gl8* and *gl17* lines result in nonglossy seedlings. We have made extensive attempts to reproduce these results, with no success.

The *gl1-c cgl* line used in our experiments was provided to the Maize Genetics Stock Center by Dr. Sprague in 1989. Initial crosses of this line by *gl2*, *gl3*, and *gl6* testers made in 1999 gave 0 to 3 kernels per ear when *gl1-c cgl* was used as a female, but full sets when used as a male. The few kernels produced on female outcross ears gave rise to nonglossy seedlings. The male outcross progeny were also nonglossy. The disparity in ear sets suggested the involvement of a gametophytic factor. If the *gl1-c cgl* line carried a *Ga1-S* allele, then pollination by our glossy tester lines carrying *ga1* would give poor sets since *ga1* pollen shows poor pollen tube growth on *Ga1-S* silks. However, pollination of this line by a *Ga1-S* line would give a full kernel set. We tested this hypothesis by crossing the *gl1-c cgl* line by a *Ga1-S* tester the following winter. Female outcrosses of *gl1-c cgl* produced full sets, confirming that this line carries a *Ga1-S* allele.

In order to get better sets on *gl1-c cgl* females in our experiments, we converted *gl2*, *gl3*, and *gl6* tester stocks to a *Ga1-S* background. We completed these conversions in 2000. During the summer of 2001, we reciprocally crossed Sprague's *gl1-c cgl* line with our *gl2*, *gl3*, and *gl6 Ga1-S* lines. Full seed sets were observed in both directions, and all progeny produced nonglossy seedlings. Thus, we failed to reproduce Dr. Sprague's results and we conclude that the cytoplasmic glossy phenomenon is not real.

The question remains as to how Dr. Sprague obtained the results that he reported. Since his *gl1-c cgl* line is homozygous *Ga1-S* and most genetic stocks are *ga1*, few seeds would set when *gl1-c cgl* is used as a female in crosses with most glossy testers. However, *gl1-c cgl Ga1-S* pollen from self-contamination would be fully functional and capable of out-competing *ga1* tester pollen on *gl1-c cgl Ga1-S* silks, and would result in the production of glossy (homozygous *gl1-c*) progeny. Sprague's observation that the glossy progeny from such crosses are always homozygous for *gl1-c* and never carry the glossy tester allele used in the cross provides support for a self-contamination hypothesis. The fact that female crosses involving *gl8* and *gl17* produced nonglossy progeny could indicate that either these particular tester lines carry *Ga1-S*, or that the crosses were made with no self-contamination by the *gl1-c cgl* parent.

Additional linkage tests of *waxy1* marked reciprocal translocations at the MGCSC

--Jackson, JD, Stinard, P, Zimmerman, S

In the collection of A-A translocation stocks maintained at MGCSC is a series of *waxy1*-linked translocations that are used for mapping unplaced mutants. Also, new *wx1*-linked translocations are being introduced into this series and are in a conversion program to convert each translocation to the inbred backgrounds M14 and W23. These inbred conversions are then crossed together to produce vigorous F1's to fill seed requests. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have been able to sort through the problem ones, and can now supply good sources proven by linkage tests to include the correct translocated chromosomes. Additional pedigree information on bad sources is available should anyone want to check on sources supplied to them previously by the Stock Center.

Previously we reported the linkage results for some of these stocks (MNL72:81-82; MNL73:88-89; MNL74:67-69; MNL75:68-71). Below is a summary of additional translocation stocks we have completed testing. Additional translocation stocks will be tested as time allows.

Table 1. w*x1* T1-9(8389) (1L.74; 9L.13)

A) The F1 source showed linkage of *wx1* with *bm2.*

Three point linkage data for *bm2*-T1-9(8389) *wx1*
Modified backcross: *bm2* N *wx1* x [*bm2* N *Wx1* x *Bm2* T1-9(8389) *wx1*]

source: 93-930-3^F1

Region	Phenotype	No.	Totals
0	+ T wx	95	
	bm N Wx	82	177
1	+ N Wx	36	
	bm T wx	53	89
2	+ T Wx	14	
	bm N wx	4	18
1+2	+ N wx	10	
	bm T Wx	9	19

% recombination *bm2*-T =35.6 $\pm$2.8
% recombination T -*wx1* =12.2$\pm$1.9
% recombination *bm2-wx1* = 47.9$\pm$2.9

Table 2. *wx1* T2-9d (2L.83; 9L.27)

A) The M14 source showed linkage of *wx1* with *ch1.*

Three point linkage data for *Ch1*-T2-9d *wx1*
Modified backcross: *ch1* N *wx1* x [*Ch1* N *Wx1* x *ch1* T2-9d *wx1*]

source: 92-396-1^M14

Region	Phenotype	No.	Totals
0	+ T wx	40	
	Ch N Wx	63	103
1	+ N Wx	36	
	Ch T wx	28	64
2	+ T Wx	3	
	Ch N wx	4	7
1+2	+ N wx	1	
	Ch T Wx	3	4

% recombination *ch1*- T =38.3$\pm$3.6
% recombination T -*wx1*=6.2$\pm$1.8
% recombination *ch1-wx1* =44.4$\pm$3.7

B) The W23 source showed linkage of *wx1* with *ch1.*

Three point linkage data for *Ch1*-T2-9d *wx1*
Modified backcross: *ch1* N *wx1* x [*Ch1* N *Wx1* x *ch1* T2-9d *wx1*]
source: 92-395-1^W23

Region	Phenotype	No.	Totals
0	+ T wx	94	
	Ch N Wx	92	186
1	+ N Wx	27	
	Ch T wx	32	59
2	+ T Wx	0	
	Ch N wx	0	0
1+2	+ N wx	1	
	Ch T Wx	1	2

% recombination *ch1*- T =24.7±2.7
% recombination T -*wx1*=0.8±0.6
% recombination *ch1-wx1* =25.5±2.8

Table 3. *wx1* T3-9(8447) (3S.44; 9L.14)

A) The F1 source showed linkage of *wx1* with *gl6.*

Two point linkage data for *gl6-wx1* T3-9(8447)
Testcross: [*Gl6 wx1* T3-9(8447) x *gl6 Wx1* N] x *gl6 wx1* N

source:93-438-1 x 439^F1

Region	Phenotype	No.	Totals
0	gl Wx	503	
	+ wx	452	955
1	+ Wx	60	
	gl wx	53	113

% recombination *gl6-wx1*=10.6±0.9

B) The M14 source showed linkage of *wx1* with *gl6.*

Two point linkage data for *gl6-wx1* T3-9(8447)
Testcross: [*Gl6 wx1* T3-9(8447) x *gl6 Wx1* N] x *gl6 wx1* N

source: 92-399-1@^M14

Region	Phenotype	No.	Totals
0	gl Wx	437	
	+ wx	448	885
1	+ Wx	28	
	gl wx	30	58

% recombination *gl6-wx1*=6.2±0.8

C) The W23 source showed linkage of *wx1* with *gl6* .

2 point linkage data for *gl6-wx1* T3-9(8447)
Testcross: [*Gl6 wx1* T3-9(8447) x *gl6 Wx1* N] x *gl6 wx1* N

source: 92-397-1 x SIB^W23

Region	Phenotype	No.	Totals
0	gl Wx	1288	
	+ wx	1298	2586
1	+ Wx	111	
	gl wx	105	216

% recombination *gl6-wx1*=7.7±0.5

Table 4. *wx1* T4-9b (4L.90; 9L.29)

A) The F1 source showed linkage of *wx1* with *c2.*

Two point linkage data for *c2-wx1* T4-9b
Testcross: [*C2 wx1* T4-9b x *c2 Wx1* N] x *c2 wx1* N

source: 93W-11392-1 x 1391-1^F1

Region	Phenotype	No.	Totals
0	+ wx	739	
	c2 Wx	929	1668
1	+ Wx	82	
	c2 wx	89	171

% recombination *c2-wx1*=9.3±0.7

B) The M14 source showed linkage of *wx1* with *c2.*

Two point linkage data for *c2-wx1* T4-9b
Testcross: [*C2 wx1* T4-9b x *c2 Wx1* N] x *c2 wx1* N

source: 92-410-1 x SIB^M14

Region	Phenotype	No.	Totals
0	+ wx	1801	
	c2 Wx	2077	3878
1	+ Wx	295	
	c2 wx	206	501

% recombination *c2-wx1*=11.4 ±0.5

C) The W23 source showed linkage of *wx1* with *c2.*

Two point linkage data for *c2-wx1* T4-9b
Testcross: [*C2 wx1* T4-9b x *c2 Wx1* N] x *c2 wx1* N

source: 92H-472-1 x SIB

Region	Phenotype	No.	Totals
0	+ wx	547	
	c2 Wx	568	1115
1	+ Wx	59	
	c2 wx	65	124

% recombination *c2-wx1*=10.0±0.9

Table 5. *wx1* T6-9e (6L.18; 9L.24) (Source from D. Robertson)

A) The original source converted to M14 showed linkage of *wx1* with *y1.*

Two point linkage data for *y1-wx1* T6-9e
Testcross: [*y1 wx1* T6-9e x *Y1 Wx1* N] x *y1 wx1* N

source: 93W-1487-4^M14

Region	Phenotype	No.	Totals
0	y wx	1583	
	+ Wx	1598	3181
1	+ wx	190	
	y Wx	180	370

% recombination *y1-wx1* =10.4±0.5

The following is a correction on data previously reported in MNL74:67-69.

Table 6. *wx1* T2-9c (2S.49; 9S.33)

A) The F1 source showed linkage of *wx1* with *lg1gl2.*

Three point linkage data for *lg1 gl2-wx1* T2-9c
Testcross: [*lg1 gl2 Wx1* N x *Lg1 Gl2 wx1* T2-9c] x *lg1 gl2 wx1* N

source: 93-432-2 x 433 ^F1

Region	Phenotype	No.	Totals
0	lg gl Wx	748	
	+ + wx	1289	2037
1	+ gl Wx	178	
	lg + wx	277	455
2	+ + Wx	115	
	lg gl wx	94	209
1+2	lg + Wx	9	
	+ gl wx	3	12

% recombination *lg1-g12* =17.2±0.7
% recombination *gl2-wx1*=8.1 ±0.5
% recombination *lg1-wx1* = 25.4±0.8 (originally reported to be 24.9±0.8)

B) The W23 source showed linkage of *wx1* with *lg1gl2.*

Three point linkage data for *lg1 gl2-wx1* T2-9c
Testcross: [*lg1 gl2 Wx1* N x *Lg1 Gl2 wx1* T2-9c] x *lg1 gl2 wx1* N

source: 87-838 x 839 ^W23

Region	Phenotype	No.	Totals
0	lg gl Wx	378	
	+ + wx	745	1123
1	+ gl Wx	105	
	lg + wx	159	264
2	+ + Wx	58	
	lg gl wx	64	122
1+2	lg + Wx	4	
	+ gl wx	4	8

% recombination *lg1-gl2* =17.9±1.0
% recombination *gl2-wx1*=8.6±0.7
% recombination *lg1-wx1* = 26.5±1.1 (originally reported to be 25.9±1.1)

Additional linkage tests of non-waxy (*Waxy1*) reciprocal translocations involving chromosome 9 at the MGCSC

--Jackson, JD, Stinard, P, Zimmerman, S

Approximately one acre each year is devoted to the propagation of the large collection of A-A translocation stocks. In this collection is a series of *Waxy1*-linked translocations that are used for mapping unplaced mutants. Each translocation is maintained in separate M14 and W23 inbred backgrounds, which are crossed, together to produce vigorous F1's to fill seed requests. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have been able to sort through the problem ones, and can now supply good sources proven by linkage tests to include the correct translocated chromosomes.

Previously we reported the linkage results for some of these stocks (MNL72:79-81; MNL73:86-88; MNL74:67; MNL75:67). Below is a summary of additional translocation stocks we have completed testing.

Table 1. *Wx1* T1-9(8389) (1L.74; 9L.13)

A) The F1 source showed linkage of *wx1* with *bm2.*

Three point linkage data for *bm2*-T1-9(8389)*Wx1*
Modified backcross: *bm2* N *wx1* x [*bm2* N *wx1* x *Bm2* T1-9(8389) *Wx1*]

source: 87-958-2^F1

Region	Phenotype	No.	Totals
0	+ T Wx	102	
	bm N wx	78	180
1	+ N wx	46	
	bm T Wx	40	86
2	+ T wx	11	
	bm N Wx	6	17
1+2	+ N Wx	13	
	bm T wx	25	38

% recombination *bm2*-T = 38.6±2.7
% recombination T -*wx1* =17.1±2.1
% recombination *bm2-wx1* = 55.8±2.8

Table 2. *Wx1* T2-9d (2L.83; 9L.27)

A) The F1 source showed linkage of *wx1* with *ch1.*

Three point linkage data for *Ch1*-T2-9d*Wx1*
Modified backcross: *ch1* N *wx1* x [*Ch1* N *wx1* x *ch1* T2-9d *Wx1*]

source: 98-842-1^F1

Region	Phenotype	No.	Totals
0	+ T Wx	80	
	Ch N wx	78	158
1	+ N wx	41	
	Ch T Wx	49	90
2	+ T wx	13	
	Ch N Wx	6	19
1+2	+ N Wx	2	
	Ch T wx	21	23

% recombination *ch1*- T =39.0±2.9
% recombination T -*wx1*=14.5±2.1
% recombination *ch1-wx1* =53.4±2.9

B) The M14 source showed linkage of *wx1* with *ch1.*

Three point linkage data for *Ch1*-T2-9d*Wx1*
Modified backcross: *ch1* N *wx1* x [*Ch1* N *wx1* x *ch1* T2-9d *Wx1*]

source: 75-242-2; fr.95W-257^M14

Region	Phenotype	No.	Totals
0	+ T Wx	110	
	Ch N wx	109	219
1	+ N wx	65	
	Ch T Wx	66	131
2	+ T wx	10	
	Ch N Wx	7	17
1+2	+ N Wx	9	
	Ch T wx	9	18

% recombination *ch1*- T =38.7±2.5
% recombination T -*wx1*=9.1 ±1.5
% recombination *ch1-wx1* =47.8±2.5

C) The W23 source showed linkage of *wx1* with *ch1.*

Three point linkage data for *Ch1*-T2-9d*Wx1*
Modified backcross: *ch1* N *wx1* x [*Ch1* N *wx1* x *ch1* T2-9d *Wx1*]

source: 93W-1439-3^W23

Region	Phenotype	No.	Totals
0	+ T Wx	121	
	Ch N wx	97	218
1	+ N wx	35	
	Ch T Wx	59	94
2	+ T wx	21	
	Ch N Wx	2	23
1+2	+ N Wx	3	
	Ch T wx	13	16

% recombination *ch1*- T =31.3±2.5
% recombination T -*wx1*=11.1±1.7
% recombination *ch1-wx1* =42.5±2.6

Table 3. W*x1* T2-9c (2S.49; 9S.33)

A) The F1 source showed linkage of *wx1* with *lg1gl2.*

Three point linkage data for *lg1 gl2-Wx1* T2-9c
Testcross: *lg1 gl2 wx1* N x [*lg1 gl2 wx1* N x *Lg1 Gl2 Wx1* T2-9c]

source: 87-962 x 960 ^F1

Region	Phenotype	No.	Totals
0	lg gl wx	429	
	+ + Wx	617	1046
1	+ gl wx	158	
	lg + Wx	217	375
2	+ + wx	82	
	lg gl Wx	88	170
1+2	lg + wx	12	
	+ gl Wx	12	24

% recombination *lg1-g12* =24.7±1.1
% recombination *gl2-wx1*=12.0±0.8
% recombination *lg1-wx1* =36.7±1.2

B) The F1 source showed linkage of *wx1* with *v4.*

Two point linkage data for *v4-Wx1* T2-9c
Testcross: [*V4 Wx1* T2-9c x *v4 wx1* N] x *v4 wx1* N

source: 87-962 x 960^F1

Region	Phenotype	No.	Totals
0	+ Wx	381	
	v wx	333	714
1	v Wx	212	
	+ wx	120	332

% recombination *v4-wx1*=31.7±1.4

Table 4. *Wx1* T3-9(8447) (3S.44; 9L.14)

A) The F1 source showed linkage of *wx1* with *gl6.*

Two point linkage data for *gl6-Wx1* T3-9(8447)
Testcross: [*Gl6 Wx1* T3-9(8447) x *gl6 wx1* N] x *gl6 wx1* N

source: 87-974 x 972^F1

Region	Phenotype	No.	Totals
0	+ Wx	1688	
	gl wx	1675	3363
1	gl Wx	122	
	+ wx	121	243

% recombination *gl6-wx1*=6.7±0.4

Table 5. *Wx1* T4-9(5657) (4L.33; 9S.25)

A) The F1 source showed linkage of *wx1* with *gl4.*

Two point linkage data for *gl4-Wx1* T4-9(5657)
Testcross: [*Gl4 Wx1* T4-9(5657) x *gl4 wx1* N] x *gl4 wx1* N

source: 87-995 x 992^F1

Region	Phenotype	No.	Totals
0	+ Wx	1724	
	gl wx	1569	3293
1	gl Wx	16	
	+ wx	12	28

% recombination *gl4-wx1*= 0.8 ±0.2

Table 6. *Wx* T6-9b (6L.10; 9S.37)

A) The M14 sources showed linkage of *wx1* with *y1.*

Two point linkage data for *y1-Wx1* T6-9b
Testcross: [*y1 Wx1* T6-9b x *Y1 wx1* N] x *y1 wx1* N

source: 96-840-4^M14

Region	Phenotype	No.	Totals
0	y Wx	2071	
	+ wx	3026	5097
1	+ Wx	397	
	y wx	34	431

% recombination *y1-wx1* = 7.8±0.4

source: 96-839-10^M14

Region	Phenotype	No.	Totals
0	y Wx	1280	
	+ wx	2042	3322
1	+ Wx	251	
	y wx	13	264

% recombination *y1-wx1* = 7.4±0.4

B) The W23 sources gave varying linkage values of *wx1* with *y1* depending on whether Translocation was transmitted through male or female.

Two point linkage data for *y1-Wx1* T6-9b
1) Testcross: [*Y1 wx1* N x *y1 Wx1* T6-9b] x *y1 wx1* N

source: 97-1271-1^W23

Region	Phenotype	No.	Totals
0	y Wx	941	
	+ wx	1404	2345
1	+ Wx	246	
	y wx	29	275

% recombination *y1-wx1* =10.5±0.6

source: 97-1271-2^W23

Region	Phenotype	No.	Totals
0	y Wx	1373	
	+ wx	1822	3195
1	+ Wx	367	
	y wx	37	404

% recombination *y1-wx1* =11.2±0.5

source: 97-1273-1^W23

Region	Phenotype	No.	Totals
0	y Wx	4003	
	+ wx	5294	9297
1	+ Wx	883	
	y wx	99	982

% recombination *y1-wx1* =9.6±0.3

2) Testcross: *y1 wx1* N x [*Y1 wx1* N x *y1 Wx1* T6-9b]

source: 97-1271-1^W23

Region	Phenotype	No.	Totals
0	y Wx	228	
	+ wx	229	457
1	+ Wx	5	
	y wx	6	11

% recombination *y1-wx1* =2.4±0.7

source: 97-1271-2^W23

Region	Phenotype	No.	Totals
0	y Wx	388	
	+ wx	370	758
1	+ Wx	21	
	y wx	15	36

% recombination *y1-wx1* =4.5±0.7

source:97-1273-1^W23

Region	Phenotype	No.	Totals
0	y Wx	1180	
	+ wx	1125	2305
1	+ Wx	40	
	y wx	14	54

% recombination *y1-wx1* =2.3±0.3

VIÇOSA, BRAZIL
Universidade Federal de Viçosa

Maize root tip cell cycle synchronization

-- Carvalho, CR, Saraiva, LS, Otoni, WC

Accumulation of the cells in metaphase implies the use of some cytogenetic strategies. The well-known spindle mitotic inhibitors action, on normal meristematic cycle treatment, leads to metaphasic chromosome numbers appropriated for several cytogenetic approaches. However, this chromosome amount is not suitable for running flow cytometry analysis. This way, chromosome accumulation procedures, previously to the metaphase blocking, must be improved by means of an additional synchronization step. Treatments were optimized in our laboratory to synchronize meristematic root tip cell cycles of germinating maize seeds (test line L-869). Seeds were germinated in Petri dishes containing a film of distilled water, and incubated at 29 C in the dark. Seedlings (1.5 to 2 cm root length) were carefully transferred to a plastic mesh adapted inside a 6 cm diameter plastic vessels containing 100 ml of either 0, 1, 2, 4, and 6 µM hydroxyurea (HU). After two cycles, approximately 18 h, the roots were recovered by washing for 15 min (running tap water) and incubated in distilled water under the same conditions as before. Thereafter, samples of root tips were taken from 0 to 10 h, at 1 h intervals, and fixed in a fresh ice-cold methanol:acetic acid solution (3:1), and kept in a freezer for at least 24 h. Next, root tips were excised at 0.1 cm and macerated with 200 µM of freshly prepared Flaxzyme (NOVO) enzymatic solution plus 1.6 ml distilled water, and incubated at 35 C for 2h 30 min. The macerated cells were dissociated in a clean slide with a freshly fixative solution, air-dried and stained with a Giemsa solution. Meiotic figures were photomicrographed and digitized directly by a microscope-coupled CCD video camera to a computer. Under the experimental conditions, 2 µM HU enabled higher cell synchronization indexes (Figure 1) as compared to higher HU concentrations. By using only the synchronization step, at 2 µM HU, combined with 6-7 h recovering time, an average of 28% of metaphasic cells (Figure 2) was obtained in comparison to 0 µM HU control treatment (Figure 3),

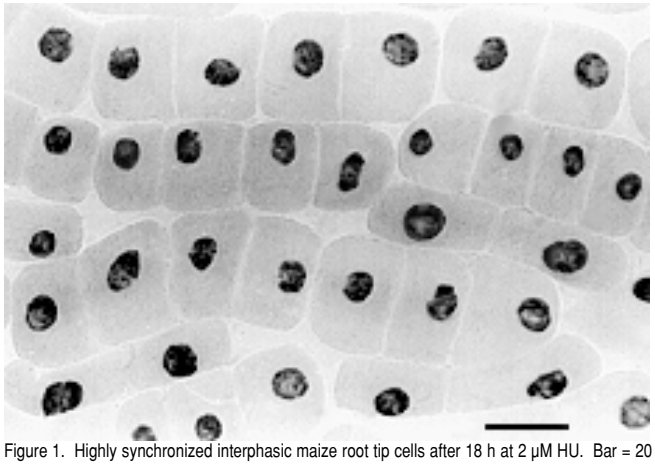


Figure 1. Highly synchronized interphasic maize root tip cells after 18 h at 2 µM HU. Bar = 20 µm.

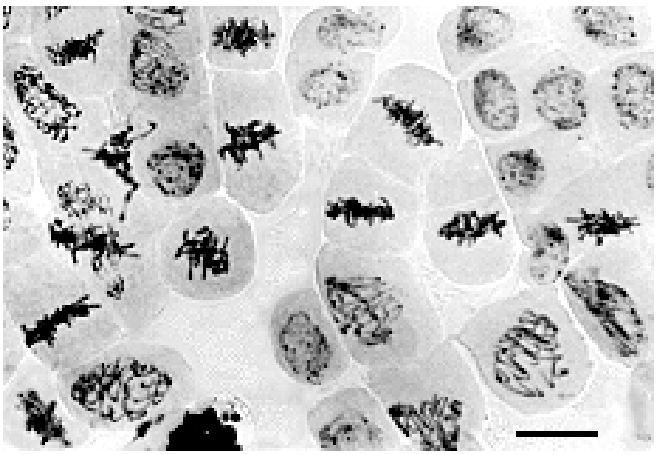


Figure 2. Highly synchronized metaphasic maize root tip cells obtained after 2 µM HU treatment and 6-7 h recovering time without HU. Bar = 20 µm.

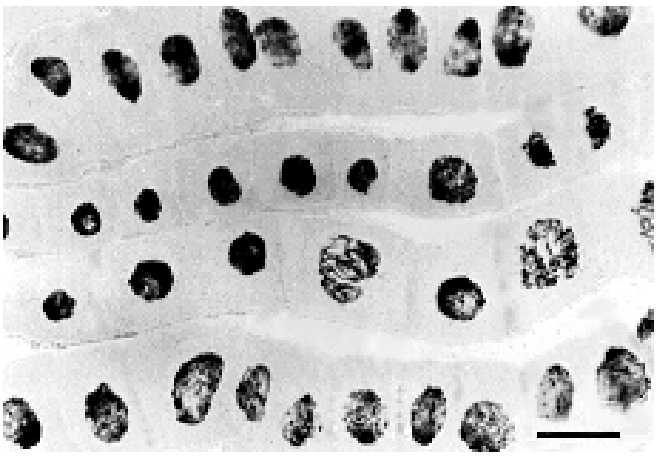


Figure 3. Typical pattern of maize root tip cell cycle from control treatment (0 µM HU). Bar = 20 µm.

that normally displayed less than 1% of metaphasic cells. It was also noted that at 6 µM HU the cells remained in interphase, therefore losing the reversibility. This technique proved to be reproducible, being applied not only to cytogenetic and cytometric purposes, but to a wide range of cell cycle studies.

WOOSTER, OHIO
The College of Wooster

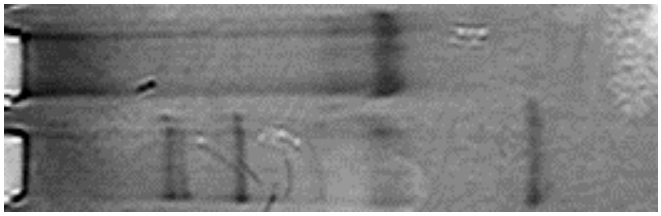
Maize small heat shock protein 18-9 exhibits characteristics of a molecular chaperone *in vitro*.

--Buss, J, Tsacoumangos, AM, Pett, V, Bouchard, RA

Studies of several plant small heat shock proteins have indicated that they exhibit certain properties of molecular chaperones in vitro. Two key aspects have been well characterized for the *Pisum sativum* HSP18.1 (Lee and Vierling, EMBO J. 16:659-71, 1997). One is the formation of dodecameric oligomers under native conditions. The other is the capacity to prevent the formation of denatured aggregates by thermally-sensitive substrate proteins at temperatures where irreversible denaturation would otherwise occur. We now report initial results indicating the presence of both these activities in a bacterially expressed fusion protein comprising a 6-histidine affinity leader peptide and the product encoded by a member of the maize shsp gene family.

Maize shsp18-9 (*uwo 11*) has been previously shown to undergo strong induction during heat shock (Goping et al., Plant Mol. Biol.16:699-711, 1991). It also shows developmentally modulated expression at several stages in the growing anthers and spikelets (Bouchard et al., Maydica 38:135-144, 1993) as well as induction by insult with toxic heavy metals (Yang and Walden, MNL 71:55, 1997). Construction of a plasmid encoding fusion protein TRCHIS18-9 and preparation and affinity-purification of the product under denaturing conditions was as described in Greyson et al. (Dev. Genetics 18:244-253, 1996). Purified TRCHIS18-9 was then dialyzed into 50mM Sodium Acetate buffer, pH 4.5, aliquoted, and frozen until use.

We examined the protein's capacity for dodecamer formation using pore gradient Native-PAGE electrophoresis at temperatures ranging from 4 C and 43 C. Under all conditions, all of the fusion protein ran as a defined band with an estimated molecular weight of 250 kDa relative to native protein standards, consistent with formation of a single dodecameric oligomer. A characteristic example of this oligomer alongside Native-PAGE standards is shown in Figure 1 below.



We also tested chaperone activity of this oligomer using the substrate protein citrate synthase (CS), an enzyme which normally undergoes rapid, irreversible aggregation and denaturation upon incubation at 43 C. In our assays, a solution containing CS was incubated at denaturing temperature and samples withdrawn at regular intervals were then assayed for residual activity at 25 C. In the absence of TRCHIS18-9, CS was rendered totally inactive after 10 minutes of incubation at 43 C. Upon incubation with increasing ratios of TRCHIS18-9, however, higher and higher proportions of the CS activity were preserved. At our highest tested ratio of 15:1 of TRCHIS18-9 dodecamer to CS homodimer, nearly full CS activity was found in samples even after 50 minutes at 43 C, the longest incubation period tested.

These results suggest that maize sHSP18-9 may provide molecular chaperone activity under a variety of stress and developmental conditions where it is expressed. We are currently performing additional experiments to investigate the role of conserved amino acids and structural motifs in oligomer formation and chaperone function.

WUHAN, CHINA
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Physical mapping of the 5S and 45S rDNA in teosinte

--Han, YH, Li, LJ, Song, YC, Li, ZY, Xiong, ZY, Li, DY

Teosinte, the closest relative of maize, is cytologically so similar to maize that it can spontaneously cross with cultivated maize to produce hybrids, resulting in effective introgression of teosinte germplasm into cultivated maize (Wilkes, Bot. Mus. Leaflets, Harvard Univ. 22:297-311, 1970). Therefore, teosinte is an important source of germplasm for maize improvement. But teosinte has for quite some time been considered by many workers to present a number of taxonomic problems. Most modern systems of classification place teosinte into the genus *Zea* (Iltis and Doebley, Am. J. Bot. 67:994-1004, 1980). All annual forms of teosinte have generally been included within *Zea mexicana*, and two perennial species are *Zea diploperennis* and *Zea perennis*. In fact, certain annual teosinte populations are so close to maize that they were ascribed to a subspecies of *Zea mays* as ssp. *mexicana* (Doebley and Iltis, Am. J. Bot. 67:982-993, 1980).

In the genus *Zea*, the chromosome number of all the species is 2n=20 except *Zea perennis* which is 2n=40. For quite some time, *Zea perennis* has been considered an autotetraploid and other species have been thought to be diploid species. But much evidence supporting x=5 as the basic chromosome number of the genus *Zea*, which was proposed by Anderson (Chron. Bot. 9:88-92, 1945), has been obtained (Molina and Naranjo, Theor. Appl. Genet. 73:542-550, 1987). Anderson pointed out that the genus *Zea* could be originated by allopolyploidy from different diploid ancestral species (2n=10), probably extinct at the present time. In these diploid species, the distinct original genomes (x=5) that are present in the actual polyploid may be differentiated. It is most important to know the basic number of the *Zea* genus for clarifying the hypothesis proposed by Anderson.

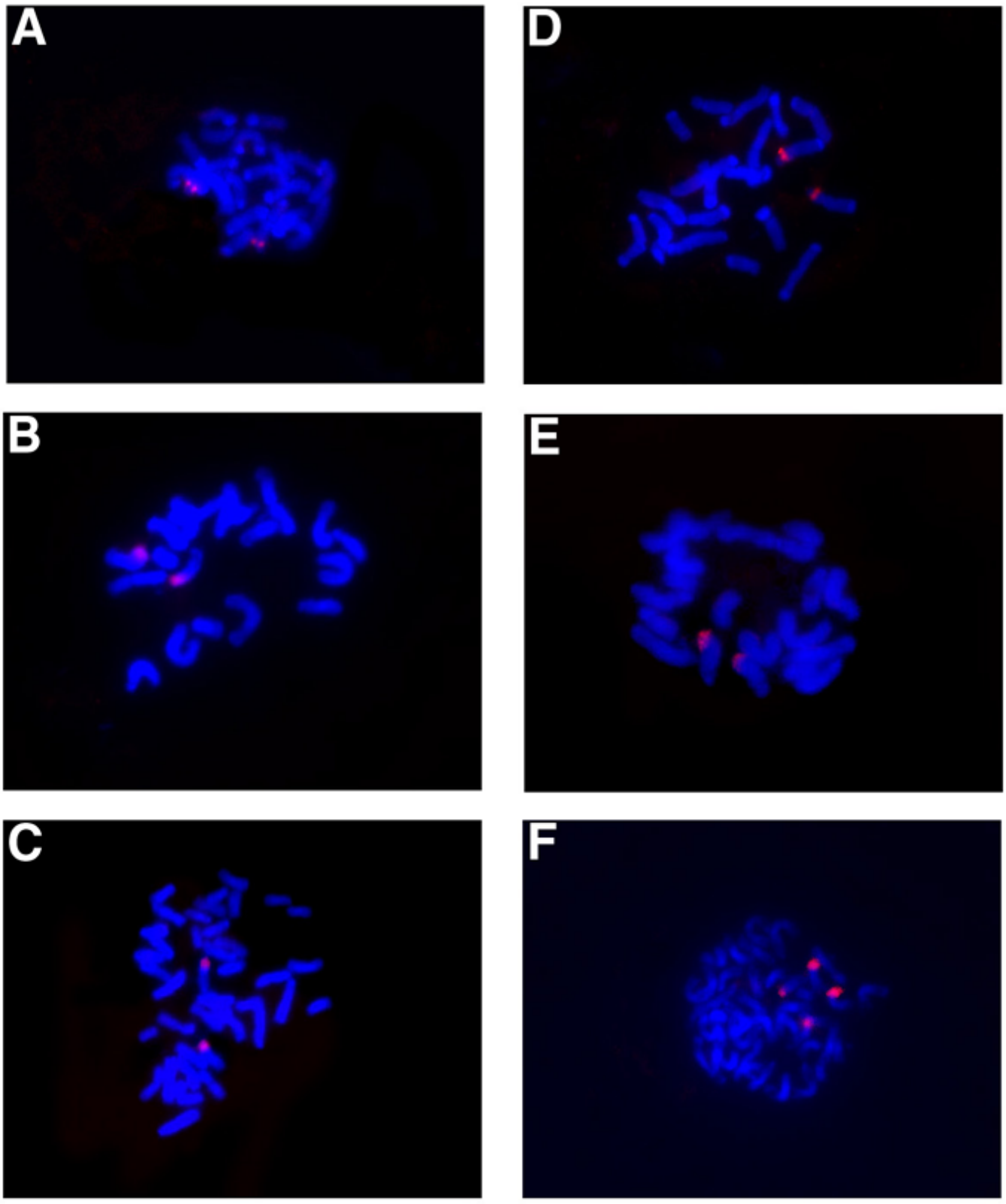
The highly conserved tandem repeats 5S and 45S rDNA sequences are useful chromosome markers for understanding genomic evolution and taxonomic relationships at both the molecular and the chromosomal levels. They have been successfully applied for phylogenetic analysis in many other plants (Jiang and Gill, Chromosoma 103:179-185, 1994; Fukui et al., Theor. Appl. Genet. 96:325-330, 1998; Sang and Liang, Genome 43:918-922, 2000).

In this study, we have detected the number and distribution of 5S and 45S rDNA loci in three teosinte species including *Zea mays ssp. mexicana* (2n=20), *Zea diploperennis* (2n=20) and *Zea perennis* (2n=40), by FISH, and provide additional information for their phylogenetic relationships.

Plant materials. The materials tested include *Zea mays ssp. mexicana* (2n=20), *Zea diploperennis* (2n=20) and *Zea perennis* (2n=40). The materials were kindly supplied by Professor M. G. Gu (Institute of Genetics, Chinese Academy of Sciences, Beijing)

DNA Probes and labelling. The plasmids of 5S and 45S rDNA which were cloned in the vector pUC18 (Arumuganthan et al., Mol. Gen. Genet. 22:551-558, 1994) were kindly provided by K. Arumuganathan, University of Nebraska. The tested probes were labeled with Bio-11-dUTP following the nick translation protocol using the kit (Sino-American Biotechnology Company, China. See also Gustafson and Dille, 1992). Dot Blotting was performed to detect the labelling efficiency.

Chromosome preparation. The root tips of 1-2 mm length from vigorously grown teosinte plants were treated with saturated bromonaphthalene at 25 C for 2.5 h, and fixed with methanol-acetic acid (3:1) overnight. After being fully washed with water, the root tips were treated with a mixture of 2% pectinase and 2% cellulase (SERVA) at 28 C for approximately 3 h. Finally, the treated root tips were squashed and dried in a flame (Song and Gustafson, Genome 36:658-661, 1993). The slides were kept in a -20 C freezer for FISH.


A
D
B
E
C
F

In situ hybridization and fluorescence detection (FISH). For in situ hybridization, the method published by Gustafson and Dille (Natl. Acad. Sci. USA 89:8646-8650, 1992) was adopted with some modifications. Slide preparations were pretreated with 100µg/ml RNase in 2xSSC (0.3 M sodium chloride plus 0.03 M sodium citrate) at 37 C for 1 h, rinsed briefly in 2xSSC, probe DNA was then denatured by immersing the slides in 70% deionized formamide in 2x SSC at 70 C for 3.5 min. After dehydration in an ice-cold ethanol series, 40µl of denatured probe mixture (50% (v/v) formamide, 10% (w/v) dextran sulfate, 0.5% (w/v) SDS (sodium dodecyl sulfate), 0.2% (w/v) sheared salmon sperm DNA, in 2xSSC) was added to each slide and covered with a coverslip. Hybridization was carried out at 37°Ê in a humid chamber overnight. The slides were then washed in 2XSSC, 0.1XSSC, at 42 C for 3-5 min each step, and subsequently washed in 0.1% of TritonX-100, PBS at RT, 5 min each step. Sites of hybridization were detected with streptavidin-Cy3 (Pharmacia) at 37 C for 30 min. Finally the slides were counterstained with 1 µg/ml DAPI. Chromosomes were viewed with an Olympus BX60 fluorescence microscope equipped with a Sensys 1401E CCD camera. Red and blue images were captured in black and white with different filters. The images were combined and pesudocolored in the computer using software V++.

The FISH results showed that the chromosomes were blue, while the signals were red (Fig. 1). Each of the tested materials showed only one hybridization site of 5S rDNA and all of the sites were located on the subterminal regions (Fig. 1A-C). It was detected on the long arm of chromosome 2 in *Zea mays ssp. mexicana* (Fig. 1A), on the short arm of chromosome 5 in *Zea diploperennis* (Fig. 1B), and on the short arm of the detected chromosome pair, which could not be identified, in *Zea perennis* (Fig. 1C). The detected chromosomes for *Zea mays ssp. mexicana* and *Zea diploperennis* were recognized according to the standard karyotypes published by Zhang et al. (J. Wuhan Univ. 1:111-132, 1992) and Pasupuleti et al. (J. Hered. 73:168-170, 1982) respectively. In *Zea mays ssp. mexicana* and *Zea diploperennis*, the signal spot of 45S rDNA was detected on the subterminal region of the short arm of chromosome 6 where it covered the nucleolar organizer (NOR) and no minor 45S rDNA loci were detected (Fig. 1D-E). Whereas in *Zea perennis,* hybridization signals of 45S rDNA were localized on the subterminal regions of the short arms in two different chromosome pairs (Fig. 1F). The signal spots were larger on one pair of them, and smaller on the other. The signal spots for two members of each detected homologous chromosome pair resemble each other in size. It meant that the differences of signal size between the two detected chromosome pairs were not induced by technical reasons. They seem to correspond to the major and minor locus for 45S rDNA respectively.

The signals were observed on each chromatid in the detected chromosomes, which showed separated sister chromatids (Fig. 1A, D and E), while the larger signals combined by two spots were detected in those in which each two sister chromatids had not separated yet (Fig. 1B, C and F).

Our results showed that *Zea mays ssp. mexicana* and cultivated maize (*Zea mays*) (Mascia et al., Gene 15:7-20, 1981) were the same for the position of 5S rDNA, which were located on the long arms of chromosome 2, while *Zea diploperennis* and *Zea perennis* were different and their positions of 5S rDNA were located on the short arms of the unidentified chromosomes. These results support the biochemical, karyotypic, and morphological evidence for the suggested taxonomy in the genus *Zea*.

Based on synapsis data, Molina and Naranjo (1987) deduced that all species of the genus *Zea* were allotetraploid except for *Zea perennis*. RFLP mapping showed that many markers mapped to two or more chromosome locations in the maize genome (Davis et al., Genetics 152:1137-1172, 1999). Actually, different genes in the maize genome could have single, double, triple, and even quadruple locations according to ISH results (Li et al., Hereditas 129(2):101-106, 1998; Ren et al., Hereditas 126(3):211-217, 1997). This means that only a part of the genes were tetraploid in the maize genome. It is evident that synapsis analysis could not tell the site number for a given sequence, nor did the Southern hybridization techniques, the copy number of which could only be inferred. However, FISH technique could not only confirm the site number of the gene on chromosomes, but also map them to a special region. In this study, the FISH results of the location for 5S rDNA showed that all of the tested *Zea* species had only one site in their genomes. For 45S rDNA, all also showed one site except for *Zea perennis*, which had two sites on different chromosomes. All of the sites were located on the subterminal regions of the chromosomes. Compared with the RFLP linkage map, the FISH results were also more precise. Therefore, FISH technique should be a powerful tool for the analysis of structure in a genome.

Zea perennis showed two 45S rDNA hybridization sites correspondingly to two different chromosome pairs (Fig. 1F). This provides evidence that *Zea perennis* is not a simple autopolyploid; it is allotetraploid at least for 45S rDNA. The results were also supported by the results with meiotic and karyotypic analyses (Molina and Naranjo, 1987).

If the genus *Zea* really originated as an allopolyploid from different diploid species (2n=10), that probably are extinct at the present time, as has been postulated (Anderson, 1945), then our results clearly demonstrated that diploidization and evolutionary loss of the 5S rDNA and 45S rDNA have apparently occurred in three teosinte species. This is the case because each diploid ancestral genome must have at least one site of 5S and 45S rDNA in their genome. Such evolutionary reorganization of rDNA loci has been reported in several plant genera in the studies with allopolyploid species including *Scilla* (Araki, Genetica 66:3-10, 1985; Vaughan et al., Heredity 71:574-580, 1993), *Milium* (Bennett and Thomas, Genome 34:868-878, 1991) and *Brassica* (Maluszynska and Heslop-Harrison, Ann. Bot. 71:479-484, 1993). The mechanisms for the diploidization and evolutionary loss of genes remain to be studied.

We would like to thank Prof. K. Arumuganthan, University of Nebraska for kindly providing the 5S and 45S rDNA probes. The work was supported by the National Natural Science Foundation of China (No. 30070376) and the Research Fund for the Doctoral Program of Higher Education (No. 207980112).

YANGZHOU, CHINA
Yangzhou University

Study on male-sterility in Zea mays. XIII. RFLP analysis of mtDNA of Y_{II-1} type maize male-sterile cytoplasm

--Qin, T, Xu, M, Dun, D, Chen, R, Bian, Y

One set of male-sterile lines with the same nuclear back-

grounds in maize, Cms-Tu_{8112}, Cms-Su_{8112}, Cms-Cu_{8112}, Cms-Y_{II-1} U_{8112}, were used in our experiments. MtDNA was prepared according to Kemble, RJ. Hybridization has been described by Sambrook, J. Long-length agarose gel electrophoresis can bring out high resolution for *Eco*RI, *Hin*dIII, *Bam*HI and *Pst*I digests of mtDNA of corn T, S, C groups and new Y_{II-1} type male-sterile cytoplasms. Hybridization of 5 mtDNA specific probes to restricted mtDNA showed that many different hybridized bands existed among T, S and C groups, The Y_{II-1} type was different from the T and S group, but there was little difference to the C group. We would apply the technique of sequence analysis to the division of male-sterile line Y_{II-1} type.

ZHENGZHOU, CHINA
Henan Agricultural University

Mapping the major restorer genes for C-type cytoplasmic male sterility using SSR markers

--Tang, JH, Liu, ZH, Chen, WC, Hu, YM, Ji, HQ, Ji, LY

For a long time, the fertility restoration of cms-C has been found to be a very complex system of inheritance in some analyses, and only the restorer gene *Rf4* on chromosome 8S has been mapped. In this study, restoring line Fengke1 and male sterile lines cms-CMo17 and cms-C237 were used to study the inheritance of restoration of cms-C. The F2 and BC1 progeny were planted in Zhengzhou, China, and scoring was done in the spring and summer 1999-2000. The data indicated that fertility segregation in F2 and BC1 progeny of two crosses suited the theoretical ratio 15:1 and 3:1 (Table 1), and that Fengke1 had two dominant duplicating restorer genes *Rf4* and *Rf5*. This result was the same as Chen WC et al. (1979).

Table 1. The results of restoration of segregating populations of a restoring line and two sterile lines.

Year	Combination	Total numbers	Restorer numbers	Sterile numbers	Theoretical ratio	Chi-square test
1999, Spring	(cms-CMo17 x Fengke1) F2	315	292	17	15:1	0.294
	(cms-CMo17 x Fengke1) BC1	87	70	17	3:1	1.673
	(cms-C237 X Fengke1) F2	195	180	15	15:1	0.692
	(cms-C237 x Fengke1) BC1	118	92	26	3:1	0.544
1999, Summer	(cms-CMo17 x Fengke1) F2	324	292	21	15:1	0.113
	(cms-CMo17 x Fengke1) BC1	148	116	32	3:1	1.01
	(cms-C237 x Fengke1) F2	114	103	7	15:1	0.002
	(cms-C237 x Fengke1) BC1	122	80	41	3:1	4.091*
2000, Spring	(cms-CMo17 x Fengke1) F2	391	365	26	15:1	0.107
	(cms-CMo17 x Fengke1) BC1	83	61	22	3:1	0.100

Note: *, Chi-square test did not suit to theoretical ratio

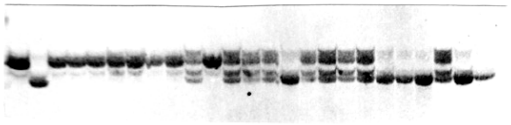
Equal amounts of DNA from 20 restorer plants and 20 sterile plants, from the F2 progeny of the crosses (cms-CMo17 x Fengke1), were mixed randomly to get the bulked pools. One hundred and sixteen pairs of microsatellite primers were used to screen parents and pools. Primers *bnlg1711*, *umc1225*, *bnlg1346*, *umc1072*, *phi058* on chromosome 5L and the primer *bnlg2307* on chromosome 8S produced polymorphic fragments which amplified in one parent or pool and not the other.

The primers, *bnlg1711*, *bnlg1346*, *phi058*, linked tightly with *Rf5*, and *bnlg2307* linked with *Rf4*, amplified successfully for both parents and 141 individuals selected from F2 progeny (Table 2). Microsatellite DNA products amplified by *bnlg1711* and *bnlg1346* are shown in Figure 1 and Figure 2.

Table 2. The statistical results of molecular markers and phenotypes of F2 segregating populations of (cms-CMo17 x Fengke1).

	A		H		B		U	
SSR primers	Sterility	Restorer	Sterility	Restorer	Sterility	Restorer	Sterility	Restorer
bnlg1711	0	32	3	75	2	29	0	0
phi058	3	28	2	77	0	31	0	0
bnlg1346	0	20	5	78	0	36	0	2
bnlg2307	0	24	1	89	4	23	0	0

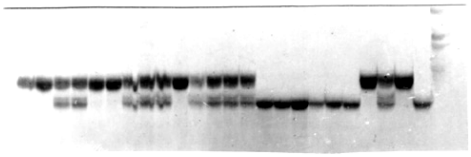
Note: A: Labeled band of Fengke1; B: Labeled band of cms-CMo17; H: Hybrid band; U: Unknown band



P1P2S S S S S S S R R R R R R R R R R R R R R

Note: P1: Fengke1; P2: cms-CMo17; R: F2 restorer individuals; S: F2 sterile individuals

Figure 1. Microsatellite DNA products amplified by *bnlg1711* in parents and some individuals of F2 progeny.



S S S S S S R R R R R R R R R R R R R BSBRP2P1M

Note: M: maker; P1: Fengke1; P2: cms-CMo17; BR: restorer BSA; BS: sterile BSA; R: F2 restorer individuals; S: F2 sterile individuals.

Figure 2. Microsatellite DNA products amplified by *bnlg1346* in parents and some individuals of F2 progeny.

The linkage analysis between *Rf5* and microsatellite markers was performed by JOINMAP version 1.4. The linkage distances between *bnlg1346-Rf5-bnlg1711* were 1.68 cM and 7.51 cM respectively, and for *phi058-Rf5* the distance was 9.87 cM. As the three microsatellite primers tightly linked with *Rf5* are in bin 5.07 of chromosome 5, the restorer gene *Rf5* is also at bin 5.07 of chromosome 5. The locations of the markers, *phi058*, *bnlg1346*, *bnlg1711*, and *Rf5* are displayed in Figure 3.

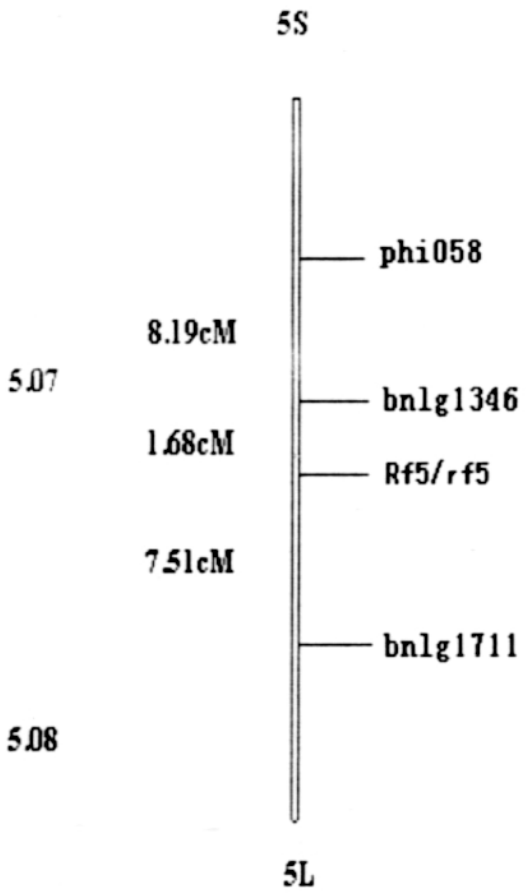


Figure 3. Map location of *Rf5/rf5* and the microsatellite markers.

ALBANY, CALIFORNIA
Plant Gene Expression Center

Regulation of maize inflorescence architecture

--Hake, S, Jackson, D, Kellogg, E, Martienssen, R, Rocheford, T, Schmidt, R, Walbot, V, Brendel, V

Crops that are considered cereals, like maize, rice, wheat, sorghum, barley, millet, and oats, account for the majority of calories consumed in the world. In addition to the cereal crops, there are approximately 10,000 species of wild grasses, which together cover about 11% of the earth's land surface. Genetic information on the regulation of maize inflorescence architecture should be readily transferable between other species, both cultivated and wild. Development of maize as a model system assumes that information from maize should be applicable to other cereals, and indeed to any other plant. Comparisons between maize and the other cereals, and between maize and wild grasses, will test this basic assumption of model system development. Nearly all grasses are characterized by the spikelet, a short branch that contains floral meristems. The arrangement of these spikelets in different grasses, and the branches on which they are home, reflects differing fates of the meristems produced during inflorescence development. Identifying the genes that determine meristem fates and understanding the mechanism by which these genes integrate their activities would be of immense value for developmental biology, evolutionary biology, and applied genetics and breeding. Genomic-based approaches will be integrated with existing genetic and molecular resources developed in maize. Inflorescence genes will be identified that will serve as tools for three different disciplines: investigation of meristem development, quantitative trait analysis, and comparative biology in the grasses. A combination of sequencing and expression analysis will be used to identify a subset of genes expressed at the earliest stages of development, and also correlating with the proliferation of specific meristem types in selected mutants. The function of these genes will be determined through genetics and mapping. Map positions of the inflorescence genes will provide a link to quantitative traits, and to mutations in maize and other grasses. A subset of these genes will be studied in other cereals and wild grasses. These comparisons will provide a valuable data set for the study of evolution and diversity across 60 million years of grass evolution. Whether these genes have been modified over evolutionary time and whether they function in other grasses the same way as they do in maize will be determined.

AMES, IOWA
Iowa State University

Nested deletions: a new tool for plant genomics

--Peterson, T, Zhang, J, Lee, M, Wang, K

Plant genomes are laden with local sequence duplications and clusters of homologous genes. To simplify the analysis of these duplications and gene clusters, this project will develop a new genetic technology to generate chromosomal deletions quickly and efficiently. This approach is based on the finding that transposable elements can participate in alternative transposition pathways that generate novel recombination products, including large deletions and duplications. These products result when transposase acts upon transposon ends in direct orientation as occur in *doubleDs* or certain alleles of the maize *p1* locus (Zhang, J and Peterson, T, Genetics 153:1403-1410, 1999).

The system employed here utilizes a transgene construct containing maize *Ac/Ds* transposon ends in direct orientation within an *I/dSpm* transposon (*Ned1*; *Nested deletions* 1). The *Ned1* construct will be transformed into maize; subsequent crosses will introduce the *En/Spm* transposase to mobilize *Ned1* to various genomic locations, and the *Ac* transposase to activate the deletion process. The action of *Ac* transposase on the *Ac* termini within *Ned1* generates an unlimited set of nested deletions with one end anchored at the transgene locus. The *Ned1* construct contains marker genes for detection of both *Ned1* transpositions and *Ac*-induced deletions, as well as sequences for easy cloning of deletion endpoints via plasmid rescue.

If this demonstration project is successful, this approach could, in the future, be extended to the production of a set of maize lines containing *Ned1* elements at dispersed sites throughout the genome. Researchers could then use these lines to isolate deletions and other rearrangements in specific regions of the genome for a variety of research applications:

1. Nested deletions will facilitate mapping of molecular and genetic markers to defined intervals. Deletions can be used in simple and robust assays for mapping molecular markers; similarly, genetic mutants are easily mapped by test crossing.
2. Deletions will be useful for assigning functions to individual gene copies within complex loci, such as disease resistance loci.
3. Deletions and duplications will be useful in gene dosage studies that seek to examine the effects of gene copy number on expression and silencing.
4. Deletion endpoints can provide landmarks that are critical for genome sequencing projects. Such landmarks can be used for the unambiguous alignment of YAC or BAC clones which may contain multiple repetitive sequence elements.

AMES, IOWA
Iowa State University

Establishment of robust maize transformation systems for the public sector

--Wang, K, Frame, B, Gelvin, S[1], Wang, S-J[1], Kaeppler, H[2], Akula, C[2], Thompson, W[3], Nguyen, T[3], Allen, G[3]
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Recent advancements in molecular biology have enabled the large scale, rapid identification and isolation of plant genes. The determination of the function for thousands of genes, and the application of that knowledge to crop improvement, is now one of the major challenges facing plant biologists. Plant transformation is a key technology for functional analysis of genes via complementation, over-expression, or gene silencing strategies and will be

equally essential in the application of genomic technology to crop improvement.

For most public research groups, maize transformation technology remains non-straightforward because they do not have access to the physical or human resources necessary to establish an efficient transformation effort. In addition, serious limitations exist with the technology, including low efficiency and throughput, difficulty in inbred line transformation, unpredictable transgene copy numbers, gene rearrangements and undesirable levels of transgene silencing through generations.

The long-term goal of this research is to establish a robust maize transformation system to enable the maize research community for future functional genomic research as well as crop improvement. Our specific objectives are:

1) ***Developing a routine Agrobacterium-mediated transformation system.*** We will conduct experiments to systematically optimize transformation and regeneration parameters for *Agrobacterium*-mediated maize transformation using non-super binary vector systems.

2) ***Enhancing transgene integration and expression.*** We will transfer and express the *Arabidopsis* and maize *rat* (resistant to *Agrobacterium* transformation) genes/proteins in maize to evaluate their effects in enhancing transgene integration. We will also evaluate the effect of tobacco and maize MARs (Matrix Attachment Regions) on the efficiency of our transformation protocols and on the frequency of somatic silencing events in maize.

3) ***Investigating germline transformation protocols.*** We will investigate tissue culture-independent transformation protocols such as meristem transformation and female gametophyte transformation.

4) ***Exploring inbred line transformation.*** We will conduct research to improve inbred line transformation on B73, H99, Oh43 and W22.

In addition, we will facilitate transfer of improved protocols to the public sector by providing a more efficient transformation service at the Plant Transformation Facility at Iowa State University and organizing transformation workshops during the course of this program. It is also our intention that any vector systems, reliable transformation protocols and information generated from this research program will be made available for the maize community upon request.

AMES, IOWA
Iowa State University
LINCOLN, NEBRASKA
University of Nebraska

High-throughput mapping and gene discovery tools for maize genomics

--Wen, TJ, Qiu, F, Guo, L, Fu, Y, Liu, F, Lee, M, Russell, K, Ashlock, D, Schnable, PS

EST libraries. Two normalized B73 cDNA libraries with complexities >106 were prepared using mRNA from seedlings and silk (ISUM4-TN), and a wide variety of organs, stages of development, and conditions (ISUM5-RN). More than 27,000 3' ESTs from these libraries have been submitted to GenBank. Mean and modal trimmed sequence lengths are 545 and 575 bp, respectively. Unlike EST projects that are composed of 5' sequences derived from a variety of genetic backgrounds, it is possible for these ESTs to be assembled into a set of unique genes with a very high degree of confidence. Two independent methods have been used to assay the number of genes defined by this collection of ESTs. In the first assay ESTs were contiged using CAP3 (Huang et. al, Genome Res 9:868-877, 1999). Representative contigs were manually QCed using Consed (Gordon et al., Genome Res 8:195-202, 1998). This analysis defined 4,666 singletons and 3,540 clusters for a total of 8,206 genes, resulting in an overall gene discovery rate of 30%. The second assay relies on preliminary grouping via the application of a novel bioinformatic tool developed during this project (the Cosine Homology Tool) followed by local Smith-Waterman alignment. Based on this analysis, these 27,020 3' B73 ESTs define 13,328 genes. Hence, one gene was defined for every two to three ESTs sequenced from these normalized libraries.

An additional B73 library (ISUM6) was prepared using the same mRNAs as ISUM5-RN, but 21 specific 6-bp "bar-code" tags were added to each mRNA prior to cloning (Qiu, et al, submitted). These tags allow the particular mRNA source from which a given EST was derived to be determined, even though this library was prepared using multiple sources of mRNAs. Over 3,600 3' ESTs from ISUM6 have been sequenced and submitted to GenBank.

Over 1,000 3' ESTs from a Mo17 cDNA library (ISUM7) were sequenced and aligned with the 3'UTRs of corresponding B73 alleles. At least one indel is present in 48% (277/572) of these alignments.

Development of IDP Markers and Genetic Mapping of ESTs. IDPs (InDel Polymorphisms) are a class of PCR-based, allele-specific genetic marker that detects insertion and deletions (indels) among maize alleles. Primer pairs were designed based on the 3' UTRs of ESTs and used to PCR amplify B73 and Mo17 alleles from genomic DNA. A useful primer pair can distinguish B73 and Mo17 alleles because it can amplify one allele but not the other, or because the PCR products from the two inbreds display a size polymorphism. A high-throughput primer design tool was developed. 531 out of 7,702 (6.9%) primers designed with this tool yielded validated +/- results or size polymorphisms between B73 and Mo17. About one third of the IDPs are polymorphic between any pair of inbreds tested, demonstrating the widespread utility of these genetic markers. The 531 IDP markers were genetically mapped using a panel of 94 RIs from the Intermated B73 X Mo17 (IBM) population. A set of 22 inbreds has been surveyed with 3,884 primer pairs. About 31% of these primers detect polymorphisms between at least one inbred and B73 or one inbred and Mo17. 88 F1BC populations are being developed and will be used to map an additional 3,000 IDPs and the corresponding cDNAs.

Rescue: a new tool for gene discovery. Because genes are present at equimolar concentrations in genomic DNA, genomic sequencing provides a means to uncover those genes that will be missed via EST projects as a consequence of being expressed only under unusual conditions or at very low levels. Unfortunately, it is not currently feasible/cost-effective to sequence the entire crop genomes. Hence, biochemical approaches need to be developed to filter out the non-coding regions of the genome so that limited sequences resources can be focused on genic DNA. A novel expression vector system has been developed to directly rescue open-reading frames (ORFs) from genomic DNA. In a preliminary experiment, 250 maize genomic fragments cloned into this vector were selected based on a colorimetric screen. Sequence analysis of

these clones revealed that 93.6% (234 out of 250) contain an uninterrupted ORF and 55% (129 out of 234) exhibit significant degrees of sequence similarity to entries in protein and EST databases. Many of the remaining clones are thought to contain ORFs that have not yet been discovered via EST sequencing.

ATHENS, GEORGIA
University of Georgia

Comparative genomics of maize and sorghum
--Paterson, AH

The small genome of sorghum (*Sorghum bicolor*) is valuable in several ways for advancing study of the much more complex genome of maize. Sorghum and maize are thought to have diverged from a common ancestor about 20-25 million years ago and share a high degree of macro-colinearity albeit with localized deviations. Most sorghum and maize chromosomes differ by 1-2 major rearrangements, a much higher level of similarity than either has with rice. Based on outputs and ongoing activities of an NSF-funded comparative genomics project, a growing set of opportunities will soon exist to take advantage of the close relationship between maize and sorghum for genomic research. Highlights of these opportunities include Genbank access to about 50,000 two-pass sorghum ESTs, and BAC hybridization data for genetically mapped probes that are anchor points between sorghum, maize, and rice. The underlying BAC libraries include those being used in the international rice sequencing effort, and the NSF maize genomics effort led by Univ. Missouri -- all BAC libraries have been fingerprinted at CUGI. A host of opportunities in structural, functional, and evolutionary genomics are anticipated through the detailed comparison of the maize and sorghum genomes.

BRISTOL, UNITED KINGDOM
University of Bristol

A small but significant maize functional genomics facility
--Edwards, D, Barker, G, Wilson, I, Grimmer, M, Edwards, KJ

High throughput transposon tagging. We have produced a variety of transposon mutagenesis resources based on Robertson's *Mutator* (*Mu*) transposable elements. Currently over 700 amplified *Mu*-tagged insertion sequences are available on a blast searchable database *Mu*DB and seed harbouring these insertion events are available on request (http://www.cerealsdb.uk.net/). In 1998, we developed a classic PCR screen for mutants within known maize genes; however, this has now been superseded by the *Mu*Array. *Mu*array has been developed for the rapid identification of maize plants harbouring *Mutator* transposon insertions within specific genes. *Mu*-flanking sequences have been amplified from over eight thousand individual *Mu*-active plants (representing over 400,000-insertion events) using an optimised *Mu*AFLP protocol. These transposon-flanking sequences have been arrayed at a high density onto glass slides, with each microarray feature representing an individual *Mu*-active maize plant. Hybridisation of these arrays with Cy dye labelled genes identifies plants with *Mu*-insertions within these genes. Knockout plants are being characterised using a range of physiological, biochemical and molecular methods including gene expression microarrays to provide an insight into gene function. This resource is available for screening by the international maize community by contacting Dave.Edwards@bbsrc.ac.uk or via our web site www.cerealsdb.uk.net

EST-based SNP discovery. We have developed a SNP detection program to identify putative polymorphisms between orthologous and parologous sequences from expressed sequence databases. By calculating SNP scores based on redundancy and co segregation of haplotypes, we can differentiate between errors due to sequencing and true genetic polymorphisms. This has advantages in throughput and accuracy over previous methods, which relied on the analysis of original sequence trace files. This program has been applied initially to the >100 000 expressed maize sequences available in the public databases and will be used to identify putative polymorphisms within expressed sequences derived from the Bristol-based IGF program and other public wheat sequence repositories. Please contact Dave Edwards for further details.

Microsatellite-based SNP discovery. By characterising the flanking regions of 52 sequence tagged microsatellites from 11 *Zea mays* inbred lines we have identified a total of 324 sequence polymorphisms. These polymorphisms consisted of both single nucleotide polymorphisms and indels in a ratio of approximately two to one. Interestingly, the level of sequence variation within the flanking regions of microsatellites linked to expressed sequence tags appears to be lower than microsatellites that were unlinked to expressed sequence tags. Conversion of the SNPs into allele specific oligonucleotides followed by covalent binding to glass slides allowed the sequence polymorphisms to be used in a simple hybridisation-based genotyping procedure. This procedure allowed us to discriminate between different inbred lines and allowed variations within a single inbred to be identified. The sequence information generated and the assay developed could be used as a starting point for the further development of a non-gel based, multi-locus, multi-allele screen for large-scale maize genotyping (Mogg et al. In press).

ZEASTAR: A Functional Blueprint for the *Zea mays* Endosperm Cell factory. In March 2001 the Bristol lab began to co-ordinate a 10 partner EU Framework V programme designed to generate a set of resources for studying Flint-maize grain development. These resources will include numerous cDNA libraries from key stages in endosperm development; high-density EST insert arrays, a proteomics-based description of the endosperm and numerous transposon tagged lines. It is our hope that by characterising the Flint endosperm to this level we will be able to provide both further research materials for the academic laboratories involved and material for European-based plant breeders and food processors.

COLUMBIA, MISSOURI
University of Missouri

Functional genomics of plant polyploids
--Birchler, JA

The NSF genome project "Functional Genomics of Plant Polyploids" seeks to understand the impact of ploidy on gene expression, the genomic changes that accompany newly formed polyploids and the molecular basis of heterosis with special emphasis on its interrelationship with ploidy. The group is composed of Tom

Osborn, PI, University of Wisconsin, Jeff Chen, Texas A and M, Luca Comai, University of Washington, Rebecca Doerge, Purdue University, Rob Martienssen, Cold Spring Harbor, and Jim Birchler, University of Missouri. The species being examined are Arabidopsis, Brassica and Corn (The ABCs).

This report will focus on the maize aspects of the project. Postdoctorate Akio Kato has succeeded in producing tetraploids of diverse inbred lines using a nitrous oxide gas treatment shortly after fertilization of diploids. The gas blocks the first mitosis of development causing a doubling of chromosomes. When the kernels were mature, they were germinated and root tip chromosome counts identified the tetraploid individuals. This procedure allows the production of highly homozygous tetraploids of standard inbred lines. The four tetraploid inbreds that we are focusing on are A188, W22, B73 and Oh43. The generation of other tetraploids is in progress.

Dr. Kato had already developed a technique that is capable of generating triploid embryos that are associated with normal endosperm (Genome 42:154-157, 1999). This involves treatment of developing pollen with trifluralin. This compound blocks mitosis and when applied at the appropriate stage, generates high levels of diploid sperm. Using this technique, it will be possible to generate triploids of these four lines. Using stock 6, material has been generated that will yield maternal haploids of these lines. Thus 1-4x dosage series will soon be available for analysis.

Gene expression will be examined in the 1-4x dosage series to determine the impact of allelic variation on the modulations of gene expression that result from changes in ploidy. These materials will also be useful for examining any epigenetic effects on gene expression or chromatin that might be influenced by ploidy. Lastly, these materials will be used to examine the molecular basis of heterosis. Hybrids and inbreds at the 2x to 4x levels will be examined for global patterns of gene expression and for morphological characteristics to gain an understanding of the relationship of dosage dependent gene regulation and heterosis.

COLUMBIA, MISSOURI
USDA-ARS and University of Missouri
CLEMSON, SOUTH CAROLINA
Clemson University
ATHENS, GEORGIA
University of Georgia

The Maize Mapping Project: Comprehensive genetic, physical and database resources for maize

--Coe, E, Cone, K, Davis, G, Gardiner, J, McMullen, M, Polacco, M, Sanchez-Villeda, H, Wing, R, Soderlund, C, Chen, M, Paterson, A

The objective of this project, supported by the National Science Foundation Plant Genome Research Program, grant DBI 9872655, is to produce an integrated genetic and physical map, with a high-resolution genetic map joined to fingerprinted and assembled BAC contigs. A comprehensive and systematic set of genetic and physical map resources will facilitate (1) cloning target genes based on their function and their position in the genome; (2) documenting all genes and their interplay; (3) defining, exploring, and exploiting all the existing genetic diversity in a species; (4) extrapolating to gene functions in crop plants with functional information and syntenic relationships among related species; and (5) sequencing of the maize genome.

The maize genome is comparable in size to that of humans. Its complexity is even higher because of the many families of retroelements distributed throughout the genome, estimated to be over 80% of the genome content. Fragmenting such a genome, fingerprinting the BAC fragments, and assembling the fragments requires deep coverage accompanied by pegging with markers to confirm and to intercalate the assembled contigs. The markers should be anchored to the genetic map insofar as feasible. This is our central strategy.

The Genetic Map. The goal is a densely marked, high-resolution genetic map, constructed for the intermated B73/Mo17 (IBM) population. We have developed a map containing >1850 markers. The parents of the population, B73 and Mo17, represent the two major heterotic groups of U.S. maize germplasm, and B73 is the inbred line used for the BAC libraries. The IBM population consists of 302 recombinant inbred lines that underwent four rounds of random mating at the F2 stage. The additional meioses result in a three-fold expansion of the genetic map (Liu et al., Genetics 142:247-258, 1996). The combination of a large number of lines and the map expansion generate a map resource with ~17 times the order-resolving power of our previous maize maps (UMC 98 map, Davis et al., Genetics 152:1137-1172, 1999; BNL 96 map, Burr et al., Genetics 118:519-526, 1988). The IBM map now contains >1000 RFLP loci, of which 185 are with new *Pst*I probes developed in the MMP, 46 with new Limagrain (lim) probes, and 129 with nonmaize probes. It contains >800 SSR loci, of which 583 were developed in the MMP.

To contribute to map development, we have produced a number of new single copy RFLP probes, SSR primer sets, and markers for Mutator insertion sites. Screening images for RFLP probes, primer and screening information for 1800 maize SSR loci, and mapscore data for the IBM population are being made available in the Maize Genome Database (MaizeDB, http://www.agron.missouri.edu/). Twelve hundred new SSR loci have been defined in this project (Sharopova et al., Plant Mol Biol 48, 2002) on IBM or either of two other mapping populations. Comparison maps with SSR loci for IBM and the two other populations have been constructed and are displayed at http://www.agron.missouri.edu/body/ssr.html#mapfiles. SSRs in process are expected to reach the goal of 2000 public SSR loci, which are compiled and tabulated at http://www.agron.missouri.edu/ssr.html.

Seed of the IBM lines, or of a subset of 94 of them, can be obtained from the Maize Genetics Cooperation Stock Center, Urbana, IL. In addition to making seed available, we have elected to aid and promote the use of the IBM map by distributing DNA of the 94 lines in microtitre plate format for individual investigators to use to map target genes of their choice (see http://www.cafnr.missouri.edu/mmp/dna_kits.htm). Web entry for submission of map score data, from which the resulting locus positions are returned to the investigator, is available at http://www.cafnr.missouri.edu/mmp/ibm_resources.htm. Our intention is to provide the community of maize investigators with resources by which all can contribute to comprehensive information on maize gene map position.

To map unigenes that are pegged to BAC contigs but are not on IBM, SNP and INDEL markers are being developed from unigene sequences (see below). Genomic sequencing for regions corresponding to 480 unigenes across 12 inbred lines has been completed. SNP assay is by primer extension and detection on the

ABI 3700, and the data are automatically genotyped and processed for mapping by the Laboratory Information Management System (LIMS). The target is to add 2000 genetically mapped SNP pegs.

Mapping of MITEs and AFLPs, which will add to pegs on BAC pools (see below), is also in progress. IBM DNA has been prepared and validated, multiplexed genotyping has been optimized, selective amplifications are completed, and data are ready for evaluation.

The LIMS for the project includes automated genotyping and validation of RFLP, SSR, and INDEL scoring, and preparation of mapping files.

A viewer interface for genetic maps, which provides for selection of various maps and the ability to compare and align any two maps between mapping populations or between chromosomes, or with the 3,267-marker RGP maps for rice, has been deployed and is viewable at http://www.agron.missouri.edu/cMapDB/cMap.html. This utility has been derived from INE of the Japanese Rice Genome Program (http://rgp.dna.affrc.go.jp/giot/INE.html), and was made available to modify for maize applications through the cooperation of Drs. Takuji Sasaki and Bal Antonio. Aligning comparisons with sorghum are to be added as available.

A BLAST server is being made available to return a map location if known.

Mutant mapping has placed 412 mutants to bins with high-throughput SSR protocols.

The Physical Map. Our proposed goal was to derive a 10X public BAC library with one enzyme, and to fingerprint and assemble the BACs into contigs to the extent possible. We have succeeded in expanding beyond this goal to genomic DNA libraries derived with *Hin*dIII (12.6X) and *Eco*RI (7X), plus a third library with *Mbo*I (7X), produced in collaboration with Jo Messing. Six-filter sets (6X for *Hin*dIII, 7X for *Eco*RI, 7X for *Mbo*I) are made available by CUGI at http://www.genome.clemson.edu/orders/. BACs of the *Hin*dIII library may be obtained from CUGI, and of the *Eco*RI and *Mbo*I libraries from CHORI at http://www.chori.org/bacpac/home.htm).

Filter hybridizations of complex markers (telomere, centromere, knob, ribosomal probes) have been scored and are being summarized. Data have been incorporated in the FPC presentation at CUGI.

Filter hybridizations of selected single-copy RFLP markers, including the core markers, have been carried out to peg and anchor BAC contigs to the genetic map. The data have been incorporated in the FPC presentation at CUGI.

Filter hybridizations of RFLP markers that have been mapped in sorghum, maize, and rice are being carried out at the University of Georgia with overgo probes to peg and anchor BAC contigs to cross-species maps. The data will be incorporated in the FPC presentation at CUGI and will be incorporated into MaizeDB.

Following the innovations of Klein et al. (Genome Res 10:789-807, 2000) for pegging with mapped AFLP markers in pools of sorghum BACs, we have prepared 288 pools from six dimensions in a 48 x 48 x 48 array (110,592 BACs, 6X) for analysis with AFLPs, MITES, and selected SSRs. Quality checks on the pools with RFLPs and SSRs have been completed and are positive. So far 100 genetically mapped SSRs have been applied to the pools, and successful tests of deconvolutions of the pools have been done.

On a larger scale, pegging of ESTs has been done in a partnership of the Maize Mapping project with DuPont and Incyte, initiated in April 2001 and completed in November 2001. Over 10,000 EST unigene consensus assemblies have been filter-hybridized to 165,000 BACs (~10X) with overgos. The Public Cornsensus and Unigene set developed by Dupont has been submitted to GenBank. The data have been incorporated in the FPC presentation at CUGI. An ongoing summary of EST documentation is presented at http://www.agron.missouri.edu/ests.html.

Because each library is from the inbred line B73, this enables the project to proceed apace with fingerprinting and assembly of the entire robust resource using the functions of FPC. Fingerprinting progress, including pegging with markers, is viewable at http://www.genome.clemson.edu/projects/maize/fpc/. As of December 23, 2001, 164,701 fingerprints (8X) and 7,760 markers form 9,462 contigs and 7,437 singletons at cutoff 10-12.

A viewer interface is prepared to display the IBM map and BAC contigs with popups for dynamic marker links. This utility has been derived from INE of the Japanese Rice Genome Program (http://rgp.dna.affrc.go.jp/giot/INE.html), and was made available to modify for maize applications through the cooperation of Drs. Takuji Sasaki and Bal Antonio. The viewer, termed iMAP, is being populated with data preparatory to deployment.

Educational Links

Interactive Maize Plant is presented at http://www.agron.missouri.edu/IMP/frames_imp2.html.

Methods for Controlled Pollinations of Maize/Corn are presented at http://www.agron.missouri.edu/IMP/WEB/pollen.htm.

Information about the Life Science Undergraduate Research Opportunities Program at Missouri is provided at http://www.lsurop.missouri.edu/PGI.

COLUMBIA, MISSOURI
University of Missouri
ST. LOUIS, MISSOURI
Washington University
SALT LAKE CITY, UTAH
University of Utah

Sequence, expression and evolution of mitochondrial genomes in the genus *Zea*

--Newton, K, Clifton, SW, Fauron, C

Plant mitochondrial genomes are unusual in their diversity of structure and rapidity of change. Although most of the known coding regions are very conserved, large intergenic regions show no sequence similarities. These “non-coding” regions have important roles in the rapid structural evolution seen for plant mitochondrial genomes, and they can become functional as components of chimeric genes that have been described as being responsible for several cases of cytoplasmic male sterility (CMS). In order to understand how non-coding regions of mitochondrial genomes contribute to their structural and functional diversity, it is necessary to examine closely related species. To address these issues, five mitochondrial genomes within the genus *Zea*, including fertile and CMS mtDNAs of maize, *Zea parviglumis* (the probable progenitor of maize), *Zea perennis (*from the most distant section of the teosintes), will be sequenced and compared to the mtDNA sequences of a related grass, *Tripsacum dactyloides* and a more distantly related plant, *Sorghum bicolor*. A direct sequencing ap-

proach will be complemented with the efficient mapping of several *Zea* mitochondrial genotypes. The regions of a fertile maize mitochondrial genome that are functional will be determined by expression analyses using mitochondrial RNAs from different tissues and stages of development. Previous data indicated that some mitochondrial genes are expressed preferentially during specific stages of development. The extent of this diversity of expression will be examined. This NSF-funded genome project will contribute to an understanding of the content, organization and expression of grass mitochondrial genomes.

EAST LANSING, MICHIGAN
Michigan State University

Functional genomics of hemicellulose biosynthesis in cereals

--Hazen, SP, Scott-Craig, JS, Walton, JD

Elucidation of the biosynthetic pathways for the complex polysaccharides of plant cell walls remains one of the major unsolved questions in plant biology. We summarize here a research grant from the NSF Plant Genome program (award #99-75815) to Jon Walton, Natasha Raikhel, Ken Keegstra, and Curtis Wilkerson, Michigan State University. The goals are to use modern genomic and proteomic strategies to identify candidate genes encoding hemicellulose biosynthetic enzymes and to test their function biochemically and genetically. The hemicelluloses are chemically complex and show striking differences between species and between cell types within a plant. Hemicelluloses are metabolically dynamic and influence many important biological and economic properties of cell walls. Because the cell walls of the Poaceae are different from the walls of other plants, we are working on both dicots (cotton, *Arabidopsis*, and *Nasturtium*), and cereals (maize and rice). Here we discuss two of our strategies for identifying hemicellulose genes in cereals.

Analysis of the *CSL* gene family of rice: Cellulose synthase catalytic subunits are encoded by the large *CESA* family in all plants (e.g., Holland, N et al., Plant Physiol 123:1313, 2000). Plants also contain a large super-family of related genes, called "cellulose synthase-like", or *CSL*. Arabidopsis contains six families of *CSL* genes (*CSLA*, *CSLB*, *CSLC*, *CSLD*, *CSLE*, and *CSLG*). On several grounds it appears likely that *CSL* genes encode hemicellulose biosynthetic enzymes (Richmond, TA, Somerville, CR, Plant Physiol 124:495, 2000).

We have analyzed the *CSL* genes of cereals, particularly rice but also maize and sorghum (Hazen, S et al., Plant Physiol, in press, 2002; available on-line at http://www.prl.msu.edu/walton/research-cwb.htm). *CSL* genes are not highly represented in EST collections from any cereal. Our analysis of the rice *CSL* genes has been based on the Monsanto rice genome database (~50% complete), public genomic data, and the public EST collection (~100,000 ESTs). cDNAs corresponding to the ESTs were obtained mainly from the Rice Genome Project (RGP) in Japan and their sequences completed by us. Based on our experience, the rice EST collection at the Rice Genome Project in Japan (RGP) is of very high quality - virtually all of the clones that we have ordered have arrived quickly and been correct.

To date, we have identified 37 *CSL* genes in rice. Of these, we can reliably predict 23 full-length proteins using gene prediction software and manual alignments to the *Arabidopsis* Csl proteins. All of our sequences have been submitted to GenBank and are also available on-line at http://www.prl.msu.edu/walton/research-cwb.htm.

The *CSL* genes of rice and *Arabidopsis* have striking similarities as well as differences, which is consistent with the fact that the hemicelluloses of dicots and cereals are similar in some respects and different in others. For example, rice has Csl proteins that are closely related to the *Arabidopsis* CslA, CslC, CslD, and CslE families. Some *CSL* genes are physically linked in both species, e.g., the *CSLB* genes in *Arabidopsis* and four of seven *CSLF* genes in rice. On the other hand, rice has no members of the *CSLG* or *CSLB* families but instead has two new families, which we have named *CSLF* and *CSLH* (Hazen, S et al., 2002; and see TA Richmond's web page at: http://cellwall.stanford.edu).

As one way to test the function of the *CSL* genes, we are using *Agrobacterium* to transform rice calli with plasmid constructs designed to express double-stranded RNAs (RNAi), which has been shown in rice and other plants to result in post-transcriptional gene silencing of the endogenous message (Wesley, SV et al., Plant J 27:581, 2001). We are now in the process of analyzing regenerated transgenic plants representing twelve *CSL* genes. Since the *CSL* mRNAs are not sufficiently abundant to analyze by RNA blotting, and RT-PCR cannot reliably detect quantitative changes in mRNA levels, our initial screening is being done at the whole plant level. To date, we have found that some RNAi *CSL* plants have no detectable phenotype whereas at least one, a plant designed to silence *OsCSLC9*, is dwarfed and partially sterile and has no or few root hairs.

Natural genetic variation and map-based candidate gene discovery: Hemicelluloses contribute to several complex phenotypic traits in cereals including pest resistance and digestibility of stover and silage in maize (Hedin, PA et al., J Chem Ecol 22:1655, 1996; Brice, RE, Morrison, IM, Carbohydr Res 101:93, 1982), nutritional quality and dough viscosity in wheat and rye (e.g., Vinkx, CJA, Delcour, JA, J Cereal Sci 24:1, 1996), and barley brewing clarity. Hemicellulose content has been shown to be under complex genetic control, and QTLs affecting hemicellulose composition have been identified in at least one case.

With the existing and emerging genetic resources available for cereals, especially maize, it should be possible to identify QTLs affecting hemicellulose composition and thereby identify the responsible genes. We found significant ($p < 0.05$) differences in cell wall monosaccharide composition (arabinose, xylose, and/or galactose) among eleven of eighteen seedling tissue types tested and among six of eighteen juvenile tissue types. Ten inbreds were analyzed. We have found that the inbred lines Mo17 and B73 are significantly different for cell wall arabinose and xylose content. We can therefore take advantage of the high-density genetic linkage map being developed from a cross of B73 and Mo17 (called IBM) by the Missouri Maize Mapping Project (http://www.cafnr.missouri.edu/mmp/) (Vuylsteke, M et al., Theor Appl Genet 99:921, 1999; Davis, G et al. Maize Genetics Conference Abstracts 42:P79, 2000). We have analyzed the walls of a subset of the IBM population with the intent to identify QTLs associated with the genetic variation we have identified. Once the marker data is made available, these efforts should reveal the number and impact of genes contributing to the variation. A similar and unique study identified a major QTL associated with

the ratio of arabinose to xylose in wheat flour (Martinant, JP et al., Theor Appl Genet 97:1069, 1998). Subsequent to mapping one or more QTLs, candidate genes can be identified using comparative mapping databases. Completion of the rice genome will allow the identification of candidate genes controlling hemicellulose composition in rice based on synteny with maize.

EUGENE, OREGON
University of Oregon

NSF plant genome project: Functional genomics of chloroplast biogenesis

--Barkan, A, Stern, D[1], Hanson, M[2]
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Through funding by the NSF Plant Genome Research Program, we are developing a genetic resource in maize that is tailored for the study of chloroplast biogenesis and function (http://chloroplast.uoregon.edu). The goals of the project are to assemble a saturated collection of *Mu*-induced non-photosynthetic mutants, and to develop the collection for use in two ways: (i) as an efficient reverse genetic resource to identify loss-of-function alleles of nuclear genes suspected of influencing chloroplast functions; and (ii) as a forward genetic resource to identify and clone "new" genes involved in chloroplast biogenesis.

The Mutant Collection. Mutants are identified by screening the F2 progeny of high copy *Mu* lines for the segregation of seedlings with chlorophyll-deficient (pale green, yellow, albino, virescent, albescent, striate, zebra-stripe), or high chlorophyll fluorescent leaves. Prior studies with maize and other organisms indicate that these phenotypes report defects in most aspects of chloroplast biogenesis and function, including defects in the expression of chloroplast or nuclear genes encoding components of the photosynthetic apparatus; assembly of photosynthetic enzymes; protein targeting to and within the chloroplast; plastid pigment/ lipid/ prosthetic group synthesis; light signaling; and bundle sheath cell differentiation.

To ensure that mutants in the collection are independently-arising, *Mu*-active F0 founder plants are progeny-tested to determine whether they carry mutations that cause any of our selected phenotypes. If the progeny of a self-pollination segregate any such phenotype, the corresponding F1 outcross is not used for the generation of F2 screening material. Many different founder plants have been used, to maximize coverage of the genome. Complete lineage information for each mutant is stored in a database that is available on-line.

There are currently ~2000 mutants in the collection. We believe that we are nearing saturation because those reverse genetic screens that are successful generally yield multiple alleles. We intend to add ~500 additional mutants in the coming year and anticipate that the collection will be complete at that time.

The Reverse Genetic Screening Service. The reverse-genetic screening service involves PCR screens of pooled mutant DNAs for *Mu* insertions in genes of known sequence. The fact that these screens are limited to mutants with phenotypes offers the advantage that mutant alleles recovered generally have phenotypes, obviating the need to sort through numerous "silent" insertions in introns and UTRs. Of course, if disruption of the targeted gene does not cause one of the collected phenotypes, alleles are unlikely to be recovered. Therefore, we are limiting screens to genes predicted to encode chloroplast-localized proteins, or for which there is genetic or biochemical evidence that the mutant phenotype will be within the spectrum of those in our collection. A more detailed discussion of the advantages and disadvantages of this resource in comparison with various related resources can be found on our web site.

In pilot screens we have recovered numerous useful alleles, including (a) mutant alleles of genes identified in 2-hybrid screens involving genetically-defined chloroplast gene expression factors; (b) new mutant alleles of chloroplast biogenesis genes previously cloned by transposon-tagging; (c) mutant alleles of genes whose sequences are related to known chloroplast biogenesis genes; (d) mutant alleles of genes with bacterial homologs that function in conserved processes in common to bacteria and plastids.

Users design and test primers according to the protocol on our web site. Screens and heritability tests are then done at the University of Oregon and seed from positive families are provided to the user for propagation and analysis. There will be no charge for this service through the end of 2002. Users should anticipate a turn around time of 2-3 weeks.

The Phenotype Database. The phenotype database contains information on leaf pigmentation, and chloroplast protein and RNA deficiencies. For mutants that are not completely lacking in chlorophyll, a snapshot of chloroplast protein deficiencies is obtained by probing immunoblots with an antibody cocktail that simultaneously detects one core subunit of each of the major photosynthetic enzyme complexes. These characterizations are nearing completion. To detect defects in chloroplast RNA metabolism, plastid RNAs encoding missing proteins are being analyzed by Northern hybridization; these data will be added to the database as they become available.

Users can search the database for pigment or protein defects of interest and request mutant seed for further study and/or cloning.

The Web Site. Our web site (http://chloroplast.uoregon.edu) provides an overview of the mutant collection and its potential applications, instructions for using the reverse genetic screening service, and access to the phenotype and lineage databases.

ITHACA, NEW YORK
Cornell University
MYSTIC CONNECTICUT
Monsanto Company

Regional mutagenesis utilizing *Activator* (*Ac*) in maize

--Conrad, L, Hardeman, K, Lewis, P, Lebjko, S, Farmer, P, Ahern, K, Chomet, P, Brutnell, T

As demonstrated in numerous studies, the *Ac/Ds* family of transposable elements can serve as powerful mutagens in the cloning and characterization of genes (see Dellaporta and Moreno in: The Maize Handbook, pp. 219-233, 1994). Nevertheless, the propensity of *Ac* for short-range transposition has limited its use in genome-wide mutagenesis experiments. The comprehensive goal of this NSF-funded Plant Genome Research project is to overcome this limitation and provide the maize research community with

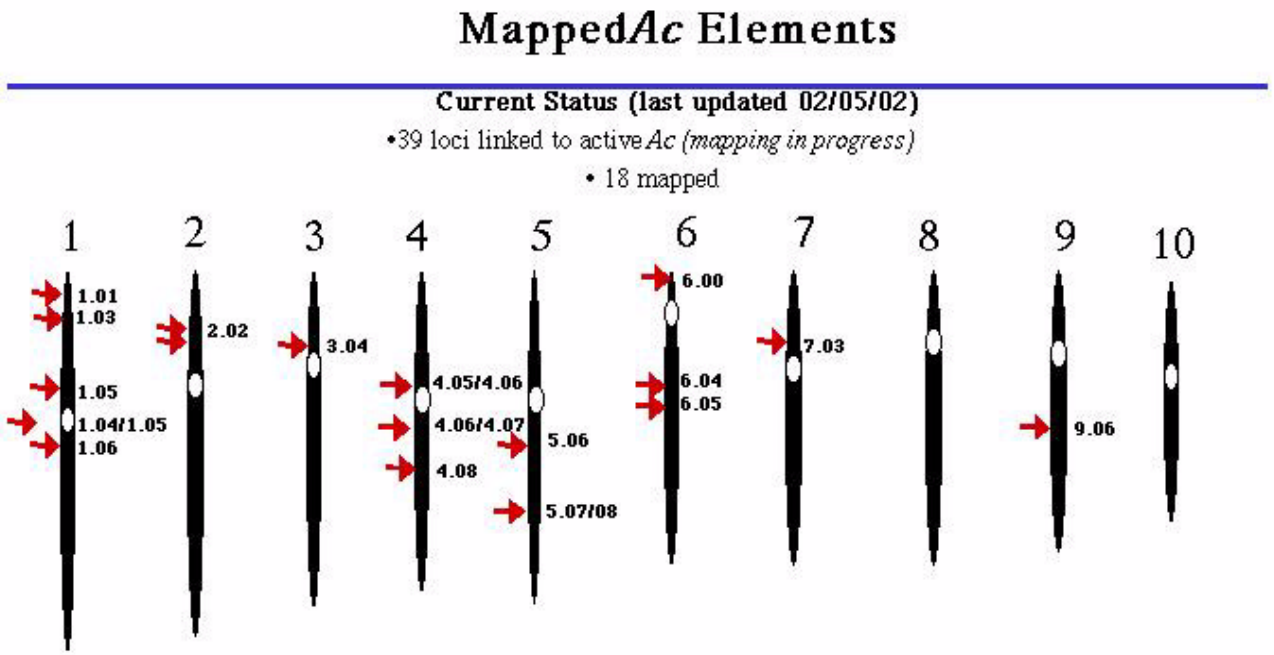


Figure 1.

a series of *Ac*-containing lines for use in regional mutagenesis. We are in the process of creating a collection of approximately 200 near-isogenic lines each containing a single active *anchor Ac* (*aAc*). Each *aAc* will be positioned on publicly available recombinant inbred populations to identify those elements at roughly 10 cM intervals throughout the entire genome. Importantly, the maintenance of this *Ac* population in a well-characterized genetic inbred, W22, will permit screens for subtle phenotypic changes associated with *Ac* insertion. These phenotypes are likely to arise when a single gene contributing to a multigenic quantitative trait is disrupted or when a single member of a multigene family is inactivated. In addition, it will be possible to exploit the tendency of *Ac* to move to linked sites to create an allelic series of a target gene.

To date, we have cloned sequences flanking 64 *Ac* elements and confirmed 39 as linked to an active, germinally-inherited *Ac* element. We have placed 18 on the genetic map, and are continually cloning, mapping, and generating additional active *Ac*'s at unique locations throughout the genome (Figure 1).

In addition to the development of the technology and protocols for regional mutagenesis, we plan to calculate the efficiency of localized *Ac*-mutagenesis. Utilizing several *aAc* lines positioned on chromosome 1, we are generating transpositions and screening the F2 progeny for *Ac*- induced mutations. This will allow us to determine the frequency of *Ac*-induced mutations and to examine the relationship between proximity of a target locus and the donor *Ac* line.

Finally, an important objective of this project is to develop a summer genetics program involving senior researchers, graduate students, undergraduate students, high school teachers, and high school students. This program is designed to facilitate interactions between often isolated groups in both research and education. It is our hope that this will give researchers a chance to familiarize themselves with the use of these *aAc* lines, while at the same time educating young students about maize genetics, and increasing the public understanding of science. The Emerson Summer Genetics Program officially began in June 2001. The participants included: five high school students (local and national), one high school teacher, three undergraduates (local and international), two post-docs, two graduate students, and two senior researchers. The participants cooperated on various projects in the field and lab and have developed a web site detailing the program activities at: (http://bti.cornell.edu

/Brutnell_lab2/Projects/ESGP/ESGP-Program.html). Importantly, the students participating in this program helped generate 3,000 transpositions from *Ac*'s positioned on chromosome 1 that will be used in determining the frequency of *Ac*-induced mutations.

PISCATAWAY, NEW JERSEY
Rutgers University

A set of transgenic maize lines for localized mutagenesis based on the *Ac-Ds* transposon system

--Dooner, HK

The goals of our NSF Plant Genome project are: (a) to demonstrate that the transposon *Ac* (*Activator*) can be used as a gene identification and isolation tool, as well as a mutagen, in the complex maize genome, and (b) to develop the necessary tools to facilitate that use.

In the first phase of the project we confirmed that *Ac* inserts exclusively in or close to genes and is, therefore, an excellent gene-searching engine in the highly repetitive maize genome (Cowperthwaite et al., The Plant Cell 14: in press, 2002). A collection of 1225 independent germinal *Ac* transposants from the mutable allele *wx-m7(Ac)* was generated and deposited in the Maize Stock Center. All transposed *Ac*s were mapped relative to the *wx* donor locus. In parallel, PCR methods were adapted for the isolation of DNA adjacent to the insertion (*tac* sites). Over 40 *tac* sites were isolated, sequenced, and deposited in GenBank, after confirming that they corresponded to bona fide germinal transpositions. All detected either one or two bands in genomic Southern blots and 54% had homology to sequences in the databases. Expression of most of these putative genes was confirmed by Northern blots. *tac* sites from several *Ac*-tagged mutants were also isolated, but most *Ac* insertions did not cause obvious phenotypes, as could have been anticipated from the highly duplicate nature of the maize genome (Gaut, B.S., Gen. Research 11:55, 2001). Besides, *Ac* insertions differed significantly in the subtlety of their mutant phenotypes. At one end was an insertion in a chloroplast ribosomal protein gene, which resulted in a lethal embryo. At the other, was an insertion in a sesquiterpenoid cyclase gene involved in an indirect defense response against insects, which required special assays for detection (Shen, B. et al., Proc. Natl. Acad. Sci. USA 97:14807-14812, 2000). In addition, we began transformation experiments to eventually produce a comprehensive set of transgenic maize lines that will facilitate the isolation of genes from any location in the genome. A highly embryogenic *bz wx* inbred line was developed and transformed with *Ac** and *Ds** constructs that had been modified to facilitate the PCR isolation of the transposon-adjacent sequence.

The main goal of the second phase of this project is to create a comprehensive set of transgenic lines that will serve as starting points for the production of future insertion libraries. First, we will demonstrate the germinal transposition of an engineered *Ac** or *Ds** element and the ready isolation of the DNA adjacent to the transposon. This will be accomplished in transgenic plants transformed with either uniquely marked *Ac** or *Ds** elements. An Agrobacterium-based method is necessary for the former, whereas a biolistics method is sufficient for the latter. Upon deciding on a particular combination of transposon and transformation method, we will proceed to create a set of 124 transgenic lines with a uniquely marked *Ac** or *Ds** element at regularly spaced locations in the genome. In this set, most genes in the maize genome will be within 7 cM of a launching platform and will be, therefore, realistic targets in a localized transposon mutagenesis experiment. These lines will be deposited in the Maize Stock Center and will serve as starting materials for the generation of future insertion libraries by interested scientists.

PISCATAWAY, NEW JERSEY
Rutgers University
WEST LAFAYETTE, INDIANA
Purdue University

Collinearity of maize and sorghum at the DNA sequence level

--Messing, J, Linton, E, Song, R, Lai, J, Tanyolac, B, Kulikowski, C, Muchnik, I, do Lago, AP, Fuks, G, Armhold, G, Ramakrishna, W, Ma, J, Park, Y-J, Emberton, J, SanMiguel, P, Bennetzen, J

Summary. Comparative analysis of plant chromosomal regions has exceptional, but largely untested, potential for the discovery of genes, new classes of mobile DNA's, and the nature, rates and mechanisms of evolutionary change. Moreover, comparative analysis of closely related genomes that differ greatly in size can indicate both the origin(s) of that genome size difference and whether the small genome could be used as a surrogate for map-based cloning of significant genes in the large genome species. By comparing gene clusters in a bi-genomic fashion, better computational programs for predicting gene boundaries and repeat elements will emerge. In a number of cases, gene sequences will be discovered with known phenotypes because of the integration of genetic and DNA sequence data. In this project, the genomes of sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.), two sister taxa from the grass family, have been chosen for such an analysis because of their importance as crop plants and as model genetic systems. Current projects to increase the density of DNA markers and phenotypes on both maize and sorghum maps will also benefit from this analysis because it will provide extensive evidence on the feasibility/difficulty of positional cloning in maize or its relatives. Evolutionary studies of cereal genomes, based on gene islands, will now be enhanced by the analysis of gene clusters and intergenic regions.

Selection of target sites. Since the maize genome (2.7 Gb) has expanded in size because of the recent amplification of eight major retrotransposon families (SanMiguel and Bennetzen, Ann. Bot. 81:27-44, 1998; Meyers et al., Genome Res. 11:1660-76, 2001), a certain length of genomic DNA has to be sequenced to facilitate a comparison between the larger maize and the smaller sorghum genome (0.77 Gb). In the case of sorghum, usually we select a single bacterial artificial chromosome (BAC) clone for each locus. These BACs contained inserts that ranged between 80 and 160-kb. These clones were chosen using maize sequences of a number of selected loci. After sequencing the sorghum clones and subjecting them to gene prediction programs, sorghum sequences were used to select maize BAC clones. Such reverse screening gave us an indication whether orthologous gene sequences are present and closely linked. However, sometimes translocation of paralogous sequences could yield two BAC clones from different chromosomal locations.

A total of 15 maize loci have been used as entry points into the genome. Twelve of these loci represent six duplicate factors (orthologous positions) in the maize genome (Rhoades, Am. Nat. 85:105-110, 1951). The remaining three loci do not represent duplicate factors, but are special gene clusters.

Two examples involve two closely linked orthologous regions. Two BAC clones, Z477F24 and Z178A11, are from chromosome arm 1L and contain the *tbp1* (TATA box-binding protein) and the *tb1* (teosinte-branched) locus at position 190.8 and 197.6, respectively; genetic positions have been taken from the Pioneer composite map, 1999, which has hyperlinks to all references (see MaizeDB). This segment is duplicated on chromosome arm 5S around position 55. Two BAC clones, Z474J15 and Z195D10, contain the *tbp2* and *tb2* loci, respectively. We have already sequenced the orthologous clones from sorghum. SB32H17 contains the TATA box-binding protein gene. Sorghum clone SB45I19 contains the ortholog to maize *tb2* on maize chromosome arm 5S, rather than *tb1*. Another predicted gene linked to *tb2* is present in sorghum, but not maize 1L. It will be interesting to see what function the orthologous genes on 5S might have. In the case of *tb2*, we already know that it is degenerate and probably not expressed, which may explain why *tb1* acts as a single Mendelian factor in crosses with teosinte (J. Doebley, pers. comm.). Interestingly, *tb1* appears to in a gene-poor region, as it is the only gene that we can find with certainty within 130-kb, while *tb2* is contained within a gene-rich region, indicating the divergence of orthologous regions in the maize genome because of retrotranspositions.

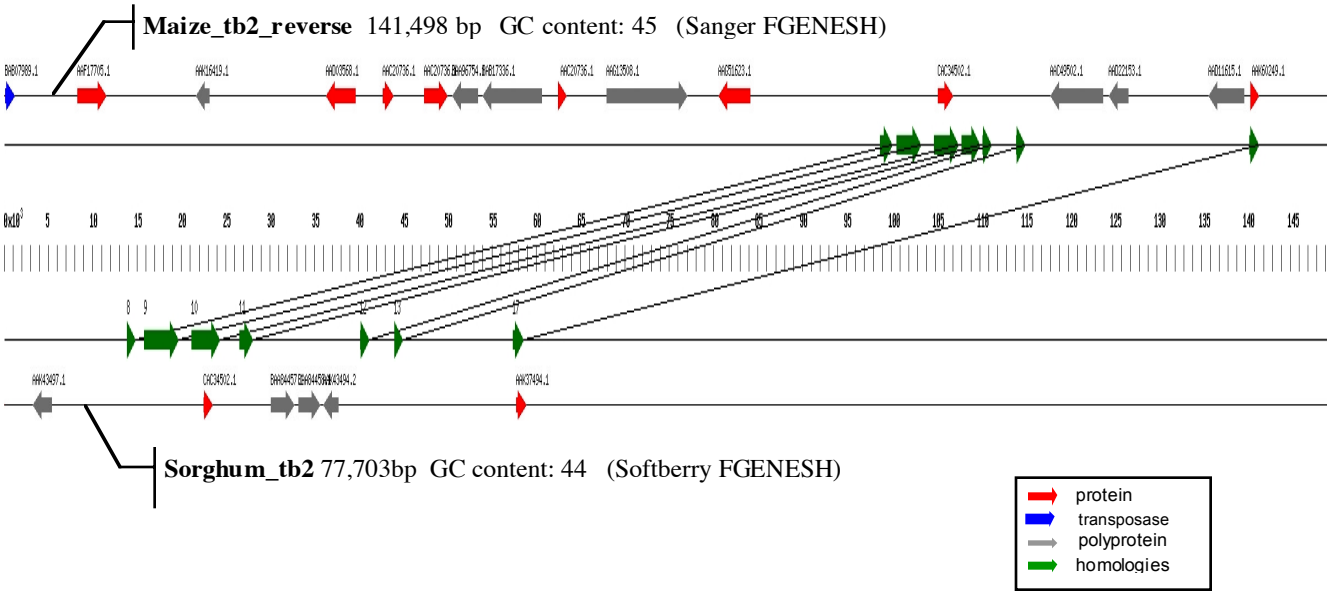
Other closely linked duplicate factors are orange pericarp (*orp*) and fertilization-independent-endosperm (*mfie*), mapping about 1 cM apart on 4S and 10S. We are currently sequencing Z332A24 and Z409L08, which contain the genes for *orp1* (4S, 66.3) and *orp2* (10S, 61). The orthologous clone SB18C08 of sorghum has already been sequenced. The *orp1* and *orp2* loci encode a tryptophan synthase b subunit that is highly conserved from *Arabidopsis* to rice. Z273B07 (*mfie1*) and Z078P04 (*mfie2*) contain the other duplicate factors that are closely linked to the *Orp* genes (Lai and Messing, in preparation). In *Arabidopsis*, the *FIE* gene is important for seed development and is subject to parental imprinting (Luo et al., Proc. Natl. Acad. Sci. USA 97:10637-10642, 2000). In maize, only one of the duplicate factors undergoes imprinting. Since, in mammalian systems, genomic imprinting may involve chromosomal regions that contain several genes (Leighton et al., Nature 375, 34-39, 1995), comparison of the two subgenomes of maize in this region may provide new insights into parental imprinting in plants.

The fifth set of duplicate markers provides an example of regions within two maize chromosomes that have arisen from an ancestral chromosome by a large chromosomal inversion (Devos et al., Genetics 138:1287-1292). RFLP mapping supports this hypothesis because of the reverse order of collinear markers. We are currently sequencing two BAC clones Z438D03 and Z576C20 that contain the *c1* (chromosome 9) and the *pl1* (chromosome 6) loci, respectively, which are positioned in the center of this inversion (*c1*, 33.1 on 9; *pl1*, 75.4). We have also sequenced an orthologous clone from sorghum, SB35P03, to investigate which subgenome is closer to sorghum. The *C1* and *Pl1* genes encode myb-like DNA binding proteins that control pigment synthesis in maize; a closely conserved sequence in rice is located on rice chromosome 2. The duplicate factors in maize differ in their tissue specificity, indicating that gene regulation rather than gene function has diverged. Another duplicate factor that represents transcription factors are *r1* (chromosome 10L, 105.3) and *b1* (chromosome 2S, 65.6). A contig has been formed from two overlapping BAC clones, Z138B04 and Z333J11, from the *r1* region. Z092E12 represents the *b1* locus. The orthologous region from sorghum is on SB20O07. These two loci have been intensively studied because of imprinting and paramutation.

The last three loci are not based on duplicate factors, but on clusters of related genes. One example involves resistance against *Puccinia sorghi* or maize rust. There are several loci, *rp1*, *rp5*, *rp6*, and *rpp9* (resistance to *Puccinia polysora* and *sorghi*) that map to chromosome arm 10S from 8.5 to 10.1. It has been estimated that 11 or more genes are spread over 400 kb (Ramakrishna and Bennetzen, in preparation). We have sequenced two BACs from this interval from a different B73 source. The two BACs contain a 43-kb direct repeat that contains both *rp1* genes and retrotransposons, indicating that amplification of this region occurred in large blocks (Ramakrishna and Bennetzen, in preparation). The orthologous sequence in sorghum differs significantly. We have sequenced SB98N08 and SB95A23, which overlap. This sorghum contig seems to contain most of the orthologous *rp1* genes. The *rp1* genes are all very closely placed in sorghum and other gene sequences were also duplicated, but without the intervening retroelements present in maize. The second region contains members of the DIMBOA pathway. The

Table 1.

Marker	Name	Map	Finished	Size bp	In Production
rp1	rust resistance	8.5 – 10.1 C10	Z163K15	95,078	
			Z238E11	99,156	
			SB98N08	239,785	
			SB95A23	97,616	
r1/b1	red color booster	65.6 C2	Z092E12	147,198	
		105.3 C10	Z138B04	115,734	
					Z333J11 (B04 Ext.)
					SB20O07
tb1/2	teosinte branched		SB45I19	77,947	
		197.6 C1	Z178A11	130,843	
		55 C5	Z195D10	141,939	
					TBD (A11 5' Ext.)
orp1/2	orange pericarp		SB18C08	159,669	
		66.3 C4			Z332A24
		61 C10			Z409L08
tbp1/2	TATA-box binding protein		SB32H17	100,707	
		190.8 C1			Z477F24
		55 C5			Z474J15
c1/pl1	colored aleurone purple plant		SB35P03	144,120	
		33.1 C9			Z438D03
		75.4 C6			Z576C20
bx3	benzoxazin				S106M24
		13.1 – 16.9 C4			TBD
mfie1/2	fertilization independent endosperm development	C 4			Z273B07
		C10			Z078P04
php200725	RFLP marker linked to 22-kDa zein/kafirin cluster	27.3 C4	ZMRS204 ZMRS171 ZMRS072	435,084	
			SB25M18 SB40L16 SB126P21 SB234M12	425,898	
		97.4 C11	L01H19 T16F19	77,605 70,311	



homologies (regions highly similar to other sequences) >800bp are shown

Figure 1. Gene prediction analysis of the maize and sorghum *tb2* regions. Each sequence is first subjected to a gene prediction program as indicated. The predicted proteins are then subjected to BLASTP. The graphic output indicates the polarity of the predicted coding sequence with the accession number of the best hit above it. In addition, each sequence is compared by the new global alignment tool (do Lago et al., in preparation). Arrows on both sides of the bp scale indicate conserved sequences and their polarity. Conserved sequences between the two clones are indicated by the vertical lines.

genes in question are all involved in the biosynthesis of DIMBOA, which inhibits fungal, bacterial and insect damage to aerial parts and roots in *Bx1* plants. Several genes, *bx1*, *bx2*, *bx3*, *bx5*, two cytochrome P450 loci and *rp4* have been mapped to the interval between 13.1 and 16.9 on maize chromosome arm 4S. Again we have isolated orthologous clones from maize and sorghum, but we do not know yet whether the same compactness of the *rp1* genes exists for these genes in sorghum as well. The third region contains a cluster of storage protein genes on chromosome 4S. This example required the construction of a new BAC library of BSSS53 because of our interest in the positional cloning of *dzr1* (Chaudhuri and Messing, Proc. Natl. Acad. Sci. 91:4867-4871, 1994). Two overlapping clones contain 346-kb of maize genomic sequence (Song et al., Genome Res. 11:1817-1825, 2001). This region contains 22 tandem repeated 22-kDa a zein genes that are linked to the *php200725* marker (27.3). Using this marker, orthologous clones have been isolated and sequenced from sorghum and rice (Song, Llaca, and Messing, in preparation).

Table 1 summarizes the current status of our BAC clones. All of the sequences under the heading 'Finished' are being annotated and will be deposited into GenBank. Clones under the heading 'In Production' are either at Phase I or Phase II assembly.

Bioinformatics. Because of the large size and number of BAC clones that were sequenced during this project we found the existing annotation tools to be inadequate for our purposes. Therefore, development of new computational tools for local and global alignments, along with semi-automation of existing methods of gene finding and annotations was undertaken.

Initially we leveraged the existing resources available on the web, FGENESH (www.softberry.com), GenScan (CCR-081.mit.edu/GENSCAN.html), BlastP and BlastN (www.ncbi.nlm.nih.gov), in combination with custom Perl scripts for sequence analyses. After analyses the results were manually reviewed, and a quality annotation of the BAC sequence data was produced. This annotated sequence was produced from reviewed data contained in an Excel spreadsheet. A Perl script converted the spreadsheet data into a graphic JPEG file. At present the descriptive fields for each gene are added manually, though we are working to also automate this final step. Additional work to integrate these processes in an easy-to-use web-based format is underway.

Besides combining existing gene finding methods into an integrated process, new methods of DNA sequence analyses are also being developed. Currently, a new method for comparing two DNA sequences for conserved regions while taking into account events like local inversions has been developed (do Lago et al. in preparation). While the details of this new method are beyond the scope of this report, a brief explanation is possible.

Most pairwise alignment programs use the paradigm of the Longest Common Subsequence (LCS). That is, they try to maximize the overall (global) alignment, but end up sacrificing the best local alignments in their search for the longest single sequence common to both sequences. Our method does not make this sacrifice and identifies local inversions.

Steps in the Alignment Process. 1) Original sequences are decomposed into fragments of equal length that may overlap. 2) Fragments from one sequence are aligned to fragments of the other sequence, based on their alignment scores. 3) Clustering is performed on the obtained assignment of fragments.

An example of the integration of gene prediction and global alignment is shown in Fig. 1. In this example, clone Z195D10 (maize *tb2*) and clone SB45I19 (sorghum *tb2*) are first subjected to gene prediction analysis and homology searches of predicted proteins with the respective databases. The results are presented in two horizontal bars. In addition, sequences are compared by the global alignment methods described above. The results are presented in a collinear fashion with the gene prediction results. The method is more sensitive since it does not rely on ORFs. Interestingly, regions of orthology can be extended beyond gene sequences.

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Evolutionary genomics of maize

--Buckler, E, Doebley, J, Gaut, B, Goodman, M, Kresovich, S, Muse, S, Weir, B

A fundamental challenge for contemporary biologists is to identify those genes that control variation in important traits and to define the effects of diverse alleles at these genes on the traits. To do this, one must understand the distribution of genetic variation in a species and in its genome and the forces that have shaped this distribution. Our project is creating the necessary infrastructure to do this in maize. We are defining where in the maize germplasm pool and where in the maize genome variation is most apt to be found. We are examining nucleotide diversity in a set of candidate genes for agronomic traits and testing whether specific DNA sequence polymorphisms can be associated with phenotypic variation for these traits. Our overall goals are to better understand the distribution of genetic diversity within the maize genome and to facilitate the identification of polymorphisms at the nucleic acid level in candidate genes that control variation at the phenotypic level in agronomic traits.

Our project is composed of three data-generating laboratory groups plus a statistical genetics support group. Group 1, led by John Doebley and Steve Kresovich, is examining microsatellite diversity in maize. Group 1 employs microsatellites in part because they are relatively inexpensive, allowing a large and comprehensive sample of the maize germplasm pool and the entire maize genome to be assayed for genetic diversity. Group 2, headed by Brandon Gaut, is examining DNA sequence diversity in maize with a focus on a limited set of accessions and regions of the genome. Group 3, headed by Ed Buckler, seeks to identify polymorphisms within candidate genes that influence agronomically important traits. To do this, the Buckler group draws on the knowledge of diversity in maize generated by the first two groups since one cannot safely make inferences about the associations between genotypes and phenotypes without incorporating a knowledge of the distribution of diversity into the analysis. The project includes a fourth group headed by Spencer Muse and Bruce Weir. This group provides statistical support for the three data-generating groups and is designing and implementing a database for genetic diversity in maize. Finally, Major Goodman is assisting the data generating groups by selecting and providing appropriate germplasm samples for their analyses and working with Ed Buckler's group on the phenotypic analyses.

Below we summarize the goals and activities of the four groups within our project to date.

Group 1: Microsatellite Diversity In Maize. This portion of the project is investigating the evolutionary dynamics of maize microsatellites and exploiting microsatellites to characterize genetic diversity in maize. We are addressing the following questions.

(1) Do maize microsatellites follow a stepwise mutation model? Microsatellites are simple sequence repeats (SSRs) that in theory evolve in a stepwise manner by changes in the number of repeat units. For example, a trinucleotide (CCG) repeat SSRs should have alleles that differ from each other by multiples of 3 bp. Our results show that maize microsatellites do not follow the stepwise mutation model. Rather, we found that there is a continuous distribution of allele sizes for most maize microsatellites. To answer why this is the case, we sequenced a selection of maize and teosinte alleles at several microsatellites and learned that most of the variation is due to indels in the regions flanking the microsatellite repeat (Matsuoka et al., Theor. Appl. Genet., in press, 2002). Thus, the reason for this violation of the stepwise mutation process is that most variation at maize microsatellite loci is due to indels in the flanking regions and not changes in the number of repeats.

(2) At what rate do microsatellites evolve? We have found that mutation rates for maize microsatellites are similar to those of humans and yeast but higher than those of Drosophila. We determined the mutation rate for SSRs by assaying 100 SSRs for 86 RI lines after 11 generations of selfing (about 80,000 allele-generations). The mutation rate for dinucleotide SSRs was 4.4 x 10-4 for repeat length changes. Among these lines, we observed no indels in the flanking regions, indicating the different dynamics for repeat length changes and indel mutations during short-term evolution. Also, SSRs with trinucleotide repeats or higher produced no mutations in our mutation accumulation experiment showing that these classes of SSRs evolve at a much slower rate (Vigouroux et al., Mol. Biol. Evol., in review).

(3) How is diversity in the maize germplasm pool distributed? Native Americans distributed and adapted maize from Chile to Canada. We are using microsatellites to understand how diversity is structured among native landraces, US breeding lines and maize's wild relative (teosinte). We have screened over 1200 landraces and 200 wild maize (teosinte). An initial analysis of a comprehensive sample of maize landraces revealed that maize genetic diversity is structured largely along eco-geographic lines (Matsuoka et al., Proc. Natl. Acad. Sci. USA, in review) and that maize was domesticated from teosinte only once. This analysis also indicated that the maize exotic germplasm pool can be divided into three supragroups: North American Maize (maize of the Indian tribes of the US and Canada), tropical maize (most maize of Mexico, Central and lowland South America), and Andean maize (maize of the Andes Mountains growing above 2000 meter elevation).

(4) How is microsatellite diversity distributed across the maize genome? We seek to determine how factors such as the domestication bottleneck and proximity to genes under selection during domestication have sculptured SSR genetic diversity in the maize genome. We have found that diversity is not highly patterned across the genome. For example, SSR diversity in regions

of the genome that harbor domestication QTL is not substantially lower than in other regions. Nevertheless, our analyses have identified several dozen SSRs in genic regions that appear to have been targets of selection during domestication. Over all SSR types, maize possesses only 81% of the SSR allelic diversity seen in teosinte, demonstrating that SSRs have yet to fully recover from the domestication bottleneck.

Group 2: Processes that Shape Genetic Diversity in the Maize Genome. Patterns of genetic diversity can provide insight into evolutionary processes that shape genomes, like mutation, recombination, and natural selection. Brandon Gaut's group is studying DNA sequence diversity in maize and its wild relatives to elucidate processes that have shaped the evolution of the maize genome. Thus far, they have characterized single nucleotide polymorphisms (SNPs) in 21 loci along chromosome 1, based on a sample of 25 individuals representing 16 exotic landraces and 9 U.S. inbreds (Tenaillon et al., Proc. Natl. Acad. Sci. USA 98:9161-9166, 2001). These data indicate:

A. Among the organisms that have been well-characterized to date at the DNA sequence level, maize contains the most SNP diversity (Table 1). On average, two randomly chosen maize sequences will vary at 1 out of 104 base pairs.

Table 1. A comparison of sequence diversity among three species. Human estimates are based on two different studies, corresponding to the two columns, that varied in the genes sampled. Values for (a measure of diversity) indicate that maize is 11 times more variable than humans and 1.4 times more variable than *D. melanogaster*.

	Humans		Drosophila	Maize
No. of loci	75	105	24	21
θ_{total}	8.3 ± 1.9	5.3 ± 1.3	70 ± 58	96 ± 32
$\hat{\theta}_{coding}$	8.0 ± 1.9	5.4 ± 1.3	40 ± 31	72 ± 25
$\hat{\theta}_{synonymous}$	15.1 ± 3.6	11.7 ± 2.9	130 ± 92	173 ± 61
$\hat{\theta}_{nonsynonymous}$	5.7 ± 1.4	3.4 ± 0.9	15 ± 14	39 ± 14
$\hat{\theta}_{noncoding}$	8.5 ± 2.0	5.2 ± 1.3	105 ± 80	111 ± 37

B. U.S. inbreds retain only 77% of the SNP diversity of exotic landraces, indicating a loss in diversity due to selective breeding. Surprisingly, the same inbred sample retains >95% of the genetic diversity of landraces at microsatellites (SSRs) within the 21 chromosome 1 loci. Differences between SNP and SSRs may be due to rapid SSR mutation rates that mask historical diversity-reducing events.

C. There is no obvious pattern of SNP diversity along the genetic map of chromosome 1. For example, in Drosophila, it has been shown that loci near the centromere are less variable than those distant from the centromere. This relationship does not appear to occur for SNPs in maize.

Currently, the Gaut laboratory is investigating the relationship between diversity and recombination. Diversity and recombination is correlated in several organisms, but the relationship is not straightforward in maize. We believe the relationship has been obscured by the genetic effects of domestication. This theory will be addressed in our studies of wild relatives.

Group 3: Relating Nucleotide Diversity to Phenotypic Diversity. Ed Buckler's group is pioneering the application of association analyses to plants. Association analyses seek to measure whether polymorphisms in a candidate gene are correlated (or associated) with phenotypic variation for traits that the gene is known to influence. Association approaches are fast and provide high resolution, but they had not been applied to plants due to problems with population structure, linkage disequilibrium (LD), and sequencing costs. We are trying to overcome some of these obstacles by incorporating factors such as population structure and LD into the statistical models used.

An initial issue addressed by the Buckler group was to document the extent of linkage disequillibria in maize. Understanding LD is the key to determining the possible genetic resolution of candidate gene association approaches in maize. Linkage disequilibrium is the correlation between polymorphisms across a sample. By sequencing 18 candidate genes from 102 diverse maize inbred lines, it was found that LD generally decays very rapidly in most loci. LD generally decays within 1500bp (Remington et al., Proc. Natl. Acad. Sci. USA 98:11479-11484, 2001).

Another important issue in association analyses is population structure. If genetic diversity is highly structured among subpopulations, it can lead to false associations between genes and phenotypes. The Buckler group was able to control statistically for this bias by using SSR genotypes provided by our microsatellite group. Essentially, they constructed a model that first factored out associations between genes and phenotypes due to population structure as measured by SSRs before testing for associations between genes and phenotypes.

The Buckler group first investigated candidate genes for flowering time (Thornsberry et al., Nature Genetics 28:286-289, 2001). By sequencing the *Dwarf8* gene from a set of 92 maize lines, they could test whether individual nucleotide polymorphisms are associated with a large effect on flowering time. This research also involved the development of statistics to handle population structure, which had been a major confounding factor in many animal and human studies. They found three important polymorphisms that were significantly associated with flowering time. Of particular interest is the 6bp deletion flanking the SH2 domain. The development of novel statistical approaches was key to our analysis, and is why this research will attract attention in many other organisms.

Group 4: Statistical Genetics and Database Development. The data being generated by the first three groups poses some novel analytical problems and the need for an innovative database that will allow the maize genetics community to make full use of the data and results. Spencer Muse and Bruce Weir are working on these issues.

To assist in data analysis, the Muse group has developed data analysis tools, such as PowerSSR, which is designed for evolutionary analysis using SSR data (see www.stat.ncsu.edu/~panzea/software/software.html). PowerSSR has a variety of attractive features and capabilities. It features an Internet Explorer-like user interface. It performs the following analyses: basic data description, allele frequency estimation, 17 genetic distance measures, phylogenetic analysis, population structure analysis, and linkage disequillibrium analysis. It includes a hierarchy editor that allow up to 4 hierarchical levels for population structure analysis. PowerSSR reads and exports multiple data formats including Excel, Nexus and Arlequin.

The Muse group is also working to develop a genetic diversity database for maize called Panzea (www.stat.ncsu.edu/~panzea/). This database will allow project members and the community in general to create datasets that combine the disparate data types generated by our project, including DNA sequences, SSRs and phenotypes. Web-based data submission tools allow project members to submit data into our databases. The basic scheme and

design of the database have been implemented and the current focus is on developing a variety of graphical search tools targeting "major" categories of work, including genetic diversity, genotype-phenotype association and geography. One tool under development is a genetic map based portal into Panzea. Here, the user could select a chromosome (or set of chromosomal bins) and then view a genetic map of that chromosome with the loci along its length and measures of genetic diversity at each locus. The user could then click on an individual locus to view or down load the data for further analysis. Other tools would allow the user to enter the database by first viewing a geographic map of maize varieties and then view or download diversity data based on geographic criteria. Finally, a phylogenetic tool would show the user a phylogeny for maize varieties and allow the user to view or analyze diversity or phenotypes for the selected varieties.

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University of Minnesota and USDA-ARS

A radiation hybrid system for the genetic and physical mapping of the corn genome

--Okagaki, RJ, Kynast, RG, Odland, WE, Stec, A, Russell, CD, Zaia, HA, Rines, HW, Phillips, RL

Oat-maize chromosome addition lines can be generated by crossing oat with maize and rescuing the developing F1 embryos through in vitro culture. Approximately one-third of the plantlets retained one or more maize chromosomes added to the haploid oat genome. These plants provide the starting material for a project to develop a radiation hybrid system for mapping individual maize chromosomes. The major objectives for this project are:

1) Establish fertile disomic addition lines for each maize chromosome and supply seed and genomic DNA to the scientific community.

2) Produce low and high-resolution radiation hybrid maps.

3) Develop high-throughput technologies for mapping ESTs to chromosomal region.

4) Screen these materials for agronomic and genetic traits that may be of interest to the plant research community.

All 10 maize chromosomes have been successfully introduced into oat. Fertile disomic lines exist for nine of the maize chromosomes. Maize chromosome 10 addition plants have not transmitted the maize chromosome to offspring; these plants are propagated vegetatively. DNA is available for all 10 oat-maize chromosome addition lines. Transmission of the maize chromosome is nearly 100% in a majority of the addition lines. The disomic addition plants carrying chromosome 1, chromosome 5, chromosome 7 and chromosome 8 exhibited irregular transmission of the respective maize chromosome in early generations. Through selection we have obtained offspring for the disomic chromosome 1 and the chromosome 7 addition lines that transmit the maize chromosome nearly 100% of the time. Selection is underway with the chromosome 5 and chromosome 8 addition lines. We have also recovered fertile lines that carry two maize chromosomes, chromosomes 1 and 9, and chromosomes 4 and 6, each in disomic condition.

Radiation hybrid lines are derived from chromosome addition lines by irradiating seed monosomic for the maize chromosome. This treatment can fragment the maize chromosome and produce plants that carry deleted maize chromosomes and translocated maize chromosome segments. After identification and selection of appropriate offspring, maize chromosome-specific panels of radiation hybrid lines are produced and used to map sequences. Low-resolution mapping panels are nearing completion for chromosomes 2 and 9. The low-resolution panels will contain approximately 10 radiation hybrid lines enabling researchers to rapidly map a marker to a chromosomal region. High-resolution panels will then be generated using larger numbers of radiation hybrid lines with a goal to be able to map to a 1-2 Mb region.

Efforts are being made to adapt microarray technology for mapping ESTs with chromosome addition lines. Genomic DNA from maize, oat, and addition lines will be labeled and hybridized to EST sequences immobilized on glass slides. Hybridization with maize genomic DNA provides the positive control, and hybridization with oat genomic DNA provides the negative control. ESTs will be located to chromosome when the hybridization signal from an addition line is significantly above the signal with oat genomic DNA and close to the signal with maize genomic DNA. Preliminary experiments have demonstrated the ability to discriminate between oat and maize sequences with this technology.

An oat-maize addition line represents the introduction of thousands of maize genes into oat. Some of these genes give a visible phenotype. A *liguleless3* (*lg3*) transcript was detected in the chromosome 3 addition lines, and these lines have liguleless upper leaves and frequently a hooked panicle, which is consistent with the variant phenotype seen in maize *Lg3* plants (Muehlbauer, G et al., Genome 43:1055, 2000). The variant phenotype was observed in two independent chromosome 3 addition lines; one addition line was derived from the oat cultivar Preakness and the other from Sun II oat. Other maize chromosomes also have been introduced into different oat cultivars to investigate the effect of the oat background on the expression of maize genes. Efforts are being made to screen for characters such as disease resistance and photoperiod response in this material.

More information about the project, materials available, traits observed, and publications can be found at: //www.agro.agri.umn.edu/rp/genome/. This work was sponsored by NSF Grant No. 9872650. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

STANFORD, CALIFORNIA
Stanford University

2001 Report from the Maize Gene Discovery Project

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The Maize Gene Discovery, DNA Sequencing and Phenotypic Analysis project (NSF DBI-9872657) was initiated in October 1998 during the first year of the Plant Genome Research Program. This large project involves 10 investigators and about 20 staff members. An important lesson from the Maize Gene Discovery Project has been that careful planning and expert

knowledge must be combined with flexibility in exploiting research findings and in modifying methods and goals when new data are in hand. We are interested in furthering maize research through cooperation and collaboration with other projects.

New ZmDB Blast Alert Service. ZmDB introduces a new Blast Service - Blast Alert! ZmDB users can subscribe their blast search query sequences by filling out a simple registration form. Users will store their query sequences and blast specifications at ZmDB. An initial blast search will be carried out against the current database and the blast output will be sent to the user by email. For each registered query, a Query-ID is also sent to the user and is required for unsubscribing. If any new blast hits are found whenever ZmDB updates its sequence database, an email alert will be sent to the corresponding user. ZmDB Blast Alert Service can be accessed by clicking the "Search ZmDB" button at the ZmDB front page.

Table 1. Key Components of the Project

Subproject	Status	Comments
Sequence 50,000 ESTs	Currently ~95,000; ~28,000 Tentative Unique Genes	Exciting discovery that organs express different members of gene families; >2200 complete cDNAs
ZmDB web site	zmdb.iastate.edu key resource for corn	Databases maintained 1. EST assembly 2. Phenotypes of mutants 3. Microarray protocols & data 4. Genomic sequences.
RescueMu site sequencing	~4,000 likely germinal mutations sequenced with goal of 15,000	1 MB per month of gene-enriched maize genomic DNA per month. More than half of the sequences flanking likely germinal insertions of *RescueMu* are readily identified as genes by matches to ESTs. Only 4% of insertions match retrotransposons.
Phenotypes of *Mu*-induced mutants	On-going in the Sachs, Freeling, Schmidt, Smith and Hake labs	Ear, kernel and seedling screening for >20,000 families. Summer 2001 in-depth 3000 adult families screened at the Coop. We plan to repeat the adult screen next year.
Microarraying ESTs	4 array types now, with Unigene set in 2001-2002	Major effort managed by Univ. AZ (David Galbraith, Vicki Chandler). Current arrays cover 11,000 "genes" and the Unigene1.1, 1.2 and 1.3 arrays will cover ~24,000
Postdoctoral training	4 fellows	1 fellow at Stanford (oligo arrays); 1 at Arizona (microarray); 2 fellows at ISU (bioinformatics & intron retention experiments)
Library plates: Immortalized *RescueMu*	Resource for PCR screening to find germinal insertions	Initial plates available from summer 2000 tagging populations with transposed *RescueMu* elements

EST Sequencing. We have deposited nearly twice the original goal of 50,000 high quality ESTs (majority are phred >40, far above the GenBank minimum of 15 = 1 error per 33 bases for ESTs) mainly from inbreds B73, W23, and Oh43. Average length varies per library and has ranged from 380 bp to 520 bp. EST sequencing will continue during 2001-2002 (fourth project year) toward a new goal of 120,000 ESTs. We have been remarkably fruitful in gene discovery with >28,000 likely genes so far from the complete maize database of >114,000 ESTs. If short (100-200 b) and low quality, mainly pre-1998 ESTs are eliminated, ~25,000 genes are defined by ESTs. We attribute our success to libraries prepared from specific developmental stages of different organs. Our current project is BMS tissue culture cells; this library will be deeply sampled as the initial 1000 clones indicate very high diversity.

Our major findings from EST analysis are [1] that maize alleles are quite polymorphic and [2] that specific gene family members are expressed in different organs. In contrast, when such ESTs share >80-85% similarity over ~100 bases, they show a similar pattern of hybridization on microarrays fabricated with the Maize Gene Discovery ESTs (Fernandes et al., 2002; Y. Cho et al., in preparation). Comparisons of ESTs always have the criticism that insufficient sampling prevents detection of rare transcripts, but for gene families with robust EST support, we often find quite different patterns of expression. For example, TUC (Tentative Unique Contig) 09-07-6344.1 and TUC09-07-7818.1 share >99% sequence similarity to maize cytosolic glyceraldehyde-3-phosphate dehydrogenase genes *Gpc3* and *Gpc4*, respectively. Eleven ESTs derived from the mixed late tassel stages library were from *Gpc4* (TUCO9-07-7818.1), but none were found for *Gpc3* (TUCO9-07-6344.1). TUC04-05-8646.1 and TUC07-14-6306 both share ~95% nucleotide sequence and high overall similarity to maize 22kDa alpha zein protein. TUC04-05-8646.1 is expressed in seedling root tissue (library 614) as judged by the presence of 20 ESTs, although there are no matches to TUC07-14-6306. In contrast TUC07-14-6306 has ESTs expressed in the early embryo library (687), but there are no TUC04-05-8646.1 ESTs in the embryo group. It seems very unlikely that there has been contamination with endosperm in either the root or embryo libraries because none of the common zeins dominating endosperm expression were found. A third example of possible tissue-specific expression is provided by TUC01-26-861.2 and TUC07-14-6194.1, which share ~89% sequence similarity to a subunit of the vacuolar proton ATPase. TUC07-14-6194.1 is expressed in root with 23 ESTs from library 614, but no ESTs were recovered from any other source. There are no root ESTs in the likely homeologous TUC01-26-861.2. These data convince us that examination of gene-specific expression patterns using tools with greater resolution than cDNA microarrays will be required to properly annotate the transcript distribution of individual genes.

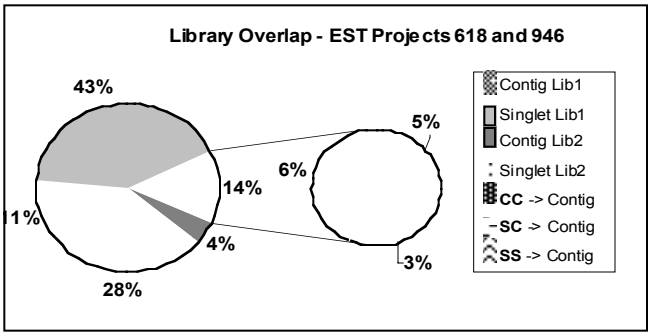

Figure 1. ESTs from 2 cm (618) and 1-2 mm (946) immature tassels overlap by only 14%. More than 5000 ESTs from two stages of tassel development were compared for overlaps of 50 bases, >95% similarity. **C = contig; S = singlet.**

Maize Gene Discovery Microarrays. Approximately 15 months after starting EST sequencing, coPI Galbraith began distributing microarrays: two identical slides for $150. >600 slides have been distributed as of Nov. 2001. PCR yield of cDNA inserts has been ~90% up to 96%, ensuring excellent coverage of the EST diversity. By using leftover plasmid sequencing templates or the consolidated clones from Stanford, we effectively eliminate "tracking problems" in going from sequencing to microarrays. Thus far four EST projects, ~11,000 genes, are available http://gremlin3.zool.iastate.edu/zmdb/microarray/progress.html. The first of three Unigene1 slide types with 8,000

genes each will be available soon. The 24,000 Unigene1 array set should be available by December 2002. We estimate that Unigene arrays will contain about half of the expected number of maize genes.

Sequencing Genomic DNA. The second route to gene discovery is sequencing genomic DNA in a strategy that combines gene discovery with a key step in functional genomics, generating an insertional mutant. *RescueMu*, a 4.7 kb *Mu1* derivative with pBluescript and other markers, inserts preferentially into genes and allows direct cloning of flanking maize DNA into *E. coli* (Raizada et al. 2001). Mutagenized plants are grown in grids of up to 48 rows and 48 columns, DNA is prepared from tissue samples from an entire row or column, and 96 (48 rows + 48 columns) *E. coli* libraries immortalize the *RescueMu* insertion sites. Maize inserts of up to 15 kb are amplified by PCR protocol; given the relatively compact size of maize introns (85% are <200 bp) most *RescueMu* plasmids contain an entire gene and its regulatory regions within the average ~ 6 - 7 kb maize insert. 96-well library plates containing the recovered plasmids organized by rows and columns of the grids are available to the community for PCR screening to find insertions in genes of interest. Heritable (or very rare, early somatic insertions) will be represented in both a row and a column; seed for germinal mutants is obtained from the Maize Coop. To complement ESTs for gene discovery, the Project is sequencing row plasmids (bidirectionally, about 400 bases from each *Mu* end), with a goal of completing 8 grids, about 15,000 likely germinal insertions and 30 MB of genomic sequences enriched for genes. This genomic sequence will identify genes not yet found by EST sequencing. The genomic DNA also contains presumptive introns and promoter regions, recognized after annotation against ESTs by the ISU staff; all of these results are displayed through ZmDB http://zmdb.iastate.edu.

In the Protocols section you will find information for efficiency screening of library plates to determine which plant has a likely germinal insertion mutation of interest to you. Also, the sequencing plan does not identify all germinal insertions (by definition these are sequenced multiple times), hence you may find it useful to do a PCR screen to determine if there is both a row and a column insertion in your favorite gene. Also, the library plates each contain a collection of 10 – 50% of the “gene-rich” part of the corn genome. A somatic plasmid of your favorite gene could contain the regulatory region and other information that interests you. Such plasmids can be found by transforming the DNA samples into *E. coli* to reconstruct a “gene-enriched” library.

Overcoming Transposon Biology to Make Mutants More Efficiently. We encountered a serious problem in scaling from the few dozen plants analyzed initially to the thousands in early grids. *RescueMu* transposition out of the transgene arrays was only 10-30%, compared to the ~100% of *Mu1*. In a massive project coordinated by coPI Vicki Chandler, the team surveyed 1200 *RescueMu* outcross progeny in January 2000 by DNA blot hybridization to identify individuals with transposed *RescueMu* but no transgene array. Fourteen individuals had 2 or 3 transposed *RescueMu*, and these were outcrossed multiple times to generate the tagging populations for summer 2000. In the Stanford 2000 grid, we found 100% *RescueMu* insertion frequency from the once transposed elements; other grids had 150% tagging efficiency. Now we are testing two smaller *RescueMu* elements, *miniMu* (2.2 kb) and *midiMu* (2.7 kb) in a new "cleaner" Mutator background (multiple transposase-encoding *MuDR* but few other *Mu* elements) and in a standard, medium copy *Mu* line. Native 1.4 - 2.2 kb *Mu* elements transpose more frequently than larger elements, and the smaller plasmid size will be more amenable to shotgun sequencing approaches.

Phenotype Database and Direct Access to Transposon-Tagged Mutants. Each plant in a *RescueMu* grid is self-pollinated (or outcrossed if male or female sterile). Because we have systematically selected for the absence of Mutator silencing and new germinal insertions of *RescueMu*, the forward mutation frequency of our materials is about twice the "best lines" reported by Don Robertson. Eight percent of ears have segregating seed mutations, at the seedling stage15 - 28% of families have a visible mutation, and adult families have nearly a 40% mutation frequency (1154 mutants in 3000 families). CoPI Marty Sachs and his staff at the Maize Coop manage the seed and ear screen, and propagate lines as needed. coPI Mike Freeling performs a detailed seedling phenotype screen at UC-Berkeley. Descriptions in a controlled vocabulary and photos are logged into a ZmDB database designed by UC-SD and ISU. During the summer of 2001 we hosted an adult plant phenotype screen at the Maize Coop. 14 Maize Gene Discovery personnel (including four summer REU students) and community researchers annotated 3000 families of 30 individuals each. All phenotypes found in two or more individuals were logged and checked by two scorers. Most new mutants are caused by standard *Mu* elements. Nonetheless, *RescueMu* sequencing identifies likely germinal insertions into many genes, and seed requests based on insertion location have started. Furthermore, our public phenotype database can greatly accelerate maize research. It is difficult and time-consuming to establish effective Mutator populations and to analyze the phenotypes; now, maize researchers can go on line to screen phenotypes: http://zmfmdb.zool.iastate.edu/layout/default.htm and get seed from the Coop. There are "no strings" attached to seed distribution.

Database of Project Information and Analytical Tools. ZmDB is the gateway to Maize Gene Discovery project data, materials, and analytical tools. CoPI Brendel manages ZmDB, and he and his laboratory have written software for gene identification and splice site prediction. As we relate EST data with genomic sequences obtained from the *RescueMu*-tagged sites, we face the same annotation problems as encountered in whole genome sequencing efforts. We have devised a number of computational tools that greatly facilitate our work. The **SplicePredictor** program assigns probabilities to potential splice sites based on species-specific training. (Brendel & Kleffe, 1998; Xing & Brendel, 2000). The **GeneSeqer** program implements a dynamic programming algorithm for spliced alignment of ESTs or proteins to genomic DNA, with scores optimizing both sequence similarity and splice site probabilites (Usuka et al., 2000; Usuka & Brendel, 2000). An upcoming service will be a registered sequence search conducted by ZmDB staff; individuals can post sequences for 3 months and receive notification when a new EST or *RescueMu* genomic sequence is a match.

TUCSON, ARIZONA
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Functional Genomics of Chromatin: a progress report for maize

--Plant Chromatin Consortium*
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Overview. The goal of our NSF-funded project (DBI-9975930) is to identify and functionally analyze the entire complement of genes in maize and Arabidopsis that contribute to chromatin-based control of gene expression. This project will result in the generation and classification of a large set of mutations to facilitate investigations of chromatin-level gene regulation in plants, facilitating a deeper understanding of the complex mechanisms by which plants control the expression of their genes. In this report we summarize the genes that have been identified to date in both Arabidopsis and maize and summarize our progress with the maize experiments.

There are three principle objectives within this project. First, mutations are being generated in genes that can be identified in (a) the Arabidopsis genome sequence and (b) EST collections from maize and other plants by sequence similarity to known chromatin genes, especially those thought to play a role in the control of gene expression. Because certain tests of chromatin gene function require or are more efficiently carried out with dominant mutations, we are producing dominant negative mutations for each target chromatin gene, using RNA silencing triggered by transgenes producing double-stranded RNA molecules homologous to target genes (Smith et al., Nature 407:319, 2000; Wesley et al., Plant J. 27:581, 2001). In the case that dominant negatives are lethal or deleterious, dexamethasone-inducible dominant negative mutations are being generated in Arabidopsis (McNellis et al., Plant J. 14:247, 1998). T-DNA insertional mutations are being generated in Arabidopsis for genes encoding histones, histone acetyltransferases, and histone deacetylases, and other chromatin genes to the extent possible (Krysan et al., Proc Natl Acad Sci U S A 93:8145, 1996; McKinney et al., Plant J. 8:613, 1995). In the case of lethal or deleterious dominant negatives in maize, TILLING will be used to generate mutations (McCallum et al., Plant Physiol. 123:439, 2000). Second, all mutations will be characterized to determine their effects on genetic transmission, plant growth and development, and a comprehensive battery of biochemical and epigenetic tests. These tests include DNA methylation, the processes of epimutation and paramutation, and reactivation of silenced transgenes and transposons. Third, a Plant Chromatin Database is being developed to facilitate dissemination of information on chromatin level control in plants and other organisms.

Chromatin Genes in Arabidopsis and Maize. Extensive sequence similarity searches were performed in Arabidopsis using a variety of queries from well-characterized yeast, Drosophila, mammalian and plant chromatin genes and protein domains. To date 225 genes have been identified and 178 of these genes are targets for RNA silencing (the 45 core histones and two TBP homologs, GTF1 and GTF2 are targets for T-DNA insertions). After accounting for genes sharing greater than 80% nucleotide sequence identity, a minimum of 137 genes or gene pairs need to be targeted for RNA silencing in Arabidopsis. Using Arabidopsis genes as queries, we identified 1,356 maize ESTs that cluster into 184 tentative contigs. One hundred seventeen represent candidates for RNA silencing (core histone genes and several other contigs are not being targeted). After accounting for closely related genes (>80% sequence identity), 109 genes or gene pairs are potential targets for RNA silencing. The numbers of genes identified and their categories are summarized in Table 1.

Table 1. Arabidopsis and Maize Chromatin Genes Identified to Date

Categories of Chromatin Genes	Arabidopsis	Maize
Histone acetyl-transferases (GNAT, MYST, CBP, TafII250 homologs)	12	10
Histone acetyl-transferase complex components (ADA2 homologs)	2	1
Histone deacetylases (RPD3, HD2, SIR2 homologs)	15	11
SNF2 homologs (Chromatin remodelers, not including DNA repair-recombination proteins)	21	22
Components of remodeling complexes	7	1
DNA methyltransferases	7	7
Methyl binding domain proteins	12	7
Global Transcription Factors	6	7
Nucleosome/chromatin Assembly Factors (NAP, CAF, HMG proteins)	25	15
Silencing Genes (ASF1, ARD1 homologs)	5	2
MAR binding filament-like protein	1	0
Core histones	45	ND
Linker histones	5	7
SET domain proteins	35	21
Proteins similar to bromodomain proteins belonging to Ring3 /BDF1/ FSH group of proteins	12	2
Other Bromodomain proteins	13	3
Other Chromodomain proteins (not including retroelement polyproteins)	1	1
TOTAL	225	117

Sequencing, Mapping and Expression Studies With Maize Genes. As there is no genomic sequence currently available for maize, sequences for complete open reading frames will be determined to facilitate phylogenetic comparisons with other organisms. We are isolating full-length cDNA from inbred line B73 for each of the genes being targeted using a combination of sequencing ESTs and RACE/PCR. We continue to acquire and sequence ESTs primarily from the Stanford Maize Gene Discovery project. New ESTs are first verified for insert presence, and then the EST with the largest insert is sequenced to confirm its identity. If the EST is correct, it is sequenced completely. The sequence that is obtained is then immediately used to design primers for PCR amplification. High-quality sequence is being obtained for the full-length cDNA clones isolated by RACE and RT-PCR at Wisconsin. Three independent clones for each full-length cDNA are sequenced on both strands. Sequences are searched for a complete open reading frame that is then compared with the protein sequence in ChromDB. To date full length B73 clones have been obtained for 33 maize chromatin genes and 17 more are in the pipeline. Full-length sequences for 27 clones have been submitted to GenBank.

We are finding many highly similar maize chromatin genes. Mapping the genes is one way to determine whether different contigs and ESTs come from the same or similar genes. Having map positions for chromatin genes may also facilitate matching candidate genes to epigenetic mutants. Furthermore, mapping gives us information about whether similar, recently duplicated genes are parts of segmental duplications. These data are of interest in determining the evolutionary history of plant chromatin genes, and when combined with phenotypic data, will allow us to determine the range of functions performed by different members of chromatin gene families. As of this report, EST clones for nearly all targeted genes have been used as hybridization probes on DNA blots to determine gene copy number and screen for polymorphisms amenable for gene mapping. Images of these screening blots are posted on the project website. All polymorphic bands are being mapped on an abbreviated set (94 lines) of the intermated B73-Mo17 (IBM) recombinant inbred mapping population used in the Maize Mapping Project. When completed, results will be posted on MaizeDB, with links from ChromDB.

Expression of chromatin ESTs is evaluated by hybridization of cDNA clones to a panel of maize RNAs. Chromatin clones are used to probe Northern blots of immature ear, tassel, kernel, seedling leaf (9 days), mature leaf (8-leaf stage), and callus RNA. These experiments have been completed for more than a third of the targeted genes. Results can be viewed at the ChromDB website.

RNA Silencing Mutants in Maize. Ninety-one genes are being directly targeted for dsRNA constructs at this time. Because of the high sequence identity between closely related genes in maize these 91 constructs are expected to silence the 109 maize genes targeted to date. PCR primers have been designed to amplify ~700 bp fragments from the EST in the mid-region of each gene's coding sequence. These products are then each inserted twice into the vector pMCG161 in opposite orientations and flanking an intron. Transcription of this construct will give rise to a dsRNA that should direct the degradation of the target mRNA. The website has details on the vector. Sixty-five dsRNA constructs have been produced and 55 bombarded into immature maize embryos. Multiple transformation events (12-20/construct) have been obtained for 26 constructs. Regenerated plants for six different constructs are currently growing in the greenhouse. Fertile plants will be crossed to B73 to bulk seed, the transgene structure determined via DNA blots and silencing assayed by RNA blots and RT-PCR.

Planned Biochemical and Genetic Assays. The chromatin mutant collection will be characterized functionally with respect to genetic transmission, plant growth and development, DNA methylation, stability of epimutations and paramutation, and stability of silent states of transgenes and transposons.

ChromDB. ChromDB is both a repository for data produced by this project and a research tool to allow users to perform queries of the data contained within it. ChromDB is a relational database built with MySQL and a Perl-CGI interface that displays the contents of the database and allows users to browse through genes in various ways. The public interface to the ChromDB is www.chromdb.org. There are multiple options to access the chromatin genes in ChromDB. Searches can be done using a gene name as query on the welcome page or users can go via a link to a Perl-CGI script that generates a web page with three combinations of choices of how to access data. A local BLAST server is available to search sequences in ChromDB. The query results are displayed with hyperlinks to the genes.

ChromDB holds the following information on the sets of chromatin genes we have identified in Arabidopsis and maize: accession numbers for the sequences in GenBank, the functional class of chromatin gene, sequences, exon models, and corresponding ESTs, as well as experimental data produced by the project that includes the expression profiles and the genomic DNA gel blot images for maize genes to indicate copy number. Arabidopsis exon models incorporate information from EST sequences and project generated RT-PCR sequences so as to provide a better annotation of the gene. Sequence similarity relationships between the chromatin genes in the database have been generated by BLAST comparisons and can be either produced through user selection or viewed along with the detailed information on a gene. The database search for any gene leads to a detailed page containing all the above information for that gene and links from that page go to pages that display the predicted gene, mRNA and protein sequences. Links to GenBank are provided for all sequences. The web site also includes detailed information on the tools and strategies that are being used to silence target genes in Arabidopsis and maize. dsRNA silencing vectors are available for academic research purposes via simple procedures described at the web site.

All data generated in this project will be deposited in the Plant Chromatin Database and mutants will be deposited in public stock centers as soon as they are confirmed to be mutant.

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Maize cDNA microarrays

--Elumalai, R, Gchachu, M, Pierson, E, Chandler, V, Galbraith, DW

Microarray technology has revolutionized gene expression studies by providing a powerful approach to examine gene expression patterns of tens of thousands of genes at a time [M. Schena et al. 1995. Quantitative monitoring of gene-expression

patterns with a complementary-DNA microarray. Science 270:467-470; M.K. Deyholos and D.W. Galbraith 2001. High-density DNA microarrays for gene expression analysis. Cytometry 43:229-238]. One component of the NSF-funded Maize Gene Discovery project led by Virginia Walbot at Stanford University (DBI- 9872657) is to produce cDNA microarrays for maize genes and distribute these to the research community at a reasonable cost. In this report (December 2001), we summarize the status of the maize microarrays. Below we give an overview on the production and summarize the content of each array produced to date. For the latest information, please see the project website at www.zmdb.iastate.edu.

At Stanford University over 100,000 ESTs have been sequenced from cDNA libraries constructed from different tissue types. The Arizona team receives a copy of the sequenced EST libraries for microarray production. The EST sequences are amplified by polymerase chain reaction (PCR) using universal primers in 96 well format. The PCR products are analyzed by gel electrophoresis for the quality of amplification and the approximate size of the PCR products are estimated and cataloged. The PCR products are then purified using the Millipore DNA purification system, dried in a Speed-vac, and re-suspended in buffer for microarray printing. The microarrays are printed onto aminosilane coated microscopic slides using a GeneMachines Omnigridder. For quality control, exemplar slides from each printing are subjected to several procedures prior to distribution. These include staining with DNA intercalating dye propidium iodide to determine the degree of uniformity of spots, and hybridization using targets prepared from mRNA isolated from the same type of tissue that was used for the original cDNA library construction. Information about every EST printed on each microarray is provided on our project web site, http://gremlin3.zool.iastate.edu/zmdb/microarray/ . Included in this information is the size of the PCR amplification product, the sequence annotation, the location of each EST on the microarray and images providing hybridization examples. We also provide details of the primers used for the amplification of each library, and our methods for PCR amplification, slide production, RNA isolation, mRNA purification, mRNA labeling and slide hybridization. We are also available to answer any questions and provide help as needed. Contact information can be found at the website. To date we have distributed 600 slides to 55 laboratories and hosted 15 visitors to the laboratory.

605 Endosperm microarrays. The 605 microarray comprises cDNAs from developing endosperm (14 days post pollination). It contains 8064 ESTs, representing approximately 2000-3000 unique genes. Each EST is spotted twice on the microarray array either in duplicate array format or in duplicate spot format. The 605 microarrays were validated by hybridizing with fluorescent labeled mRNAs isolated from 14 day-old immature endosperm. The reproducibility was high, within a slide the R values ranged from 0.92 to 0.98 for the individual channel intensity values and between the slides R values ranged from 0.77 to 0.95. Details are provided on the web site and in Fernandes et al., 2002, Plant Physiology, in press.

606 Immature ear microarrays. The 606 microarrays comprise cDNAs from developing ear (0.5-2 cm long), and contain 5065 ESTs, representing an estimated 2000 genes. Cherry picking was implemented to reduce the redundancy of cDNAs on this microarray to a maximum of 2 –3 X; also, ESTs that did not produce good quality sequence were not printed. In the 606 microarrays, each EST is printed in triplicate with the three spots immediately adjacent to each other. The 606 microarrays were validated by hybridizing with fluorescent targets produced from mRNA of 2-3 cM long developing ears. The reproducibility was high, within a slide the R values ranged from 0.92 to 0.98 for the individual channel intensity values and between the slides R values ranged from 0.61 to 0.92. Details are provided on the web site and in Fernandes et al., 2002, Plant Physiology, in press.

614 Root microarrays. The 614 microarrays comprise cDNAs from developing roots (3-4 days old), and contain 5065 ESTs, representing 2000 – 2500 unique genes. As for the 606 microarrays, we reduced the redundancy of abundant ESTs to 2 –3 X, removed those ESTs that did not produce good quality sequence, and printed three adjacent spots for each EST. The 614 microarrays were validated by hybridizing with fluorescent target prepared from mRNA of 4 day-old developing roots. The reproducibility was high, within a slide the R values ranged from 0.91 to 0.96 for the individual channel intensity values and between the slides R values ranged from 0.78 to 0.96.

486 Immature leaf microarrays. The 486 microarrays comprise cDNAs from developing leaf primordia (P4/6-P10/11 leaf primordial stage), and contain 4454 ESTs, representing an estimated 2000 unique genes. As for the 606 and 614 arrays, we have reduced the redundancy of abundant ESTs to 2 –3 X, removed those ESTs that did not produce good quality sequence, and printed three adjacent spots for each EST. The 486 microarrays were tested by hybridizing with fluorescent target prepared from mRNA of the P4/6-P10/11 leaf primordial stage. The reproducibility within a slide was high with a R value of 0.94 for the individual channel intensity values.

Unigene 1-1-01 microarrays. The unigene 1-1-01 microarrays are the first component of our Unigene microarray set, which is designed to contain unique genes without redundancy. The unigene 1-1-01 microarrays each contain 5280 unique genes from 3 different cDNA libraries (707&945; mixed adult tissues, 687; developing embryo and 603; salt stressed root). The unigene 1-1-01 array was printed in a triplicate spot format. The Unigene 1-1-01 array has been validated by hybridization with cy5 labeled target from mRNA of husk tissue. The reproducibility within a slide was high with a R value of 0.96 for the individual channel intensity values.

Summary. The microarrays described above are available by ordering from the project website. Our plans for the coming year are to print the rest of the consolidated Unigene library, which will contain a total of 15000 estimated unique genes. As the additional slides become available, this will be announced on the project website.

WEST LAFAYETTE, INDIANA
Purdue University

Assessment of gene content, colinearity and evolution in barley, maize, rice, sorghum and wheat

--Bennetzen, JL

In 1999, the Plant Genome Program (PGP) at the US National Science Foundation funded a two-year project to investigate the structural relationships of orthologous regions of several grass genomes. I was the PI on this project, and the co-PIs were Dr.

Jorge Dubcovsky (UC Davis), Andris Kleinhofs (Washington State Univ.), Phillip SanMiguel (Purdue University), and Bruno Sobral (National Center for Genome Resources). The goals of this project were to sequence a total of twenty bacterial artificial chromosomes (BACs), containing inserts from five orthologous regions of five different grass genomes. The original genomes chosen were barley, maize, rice, sorghum and wheat because they are important grasses with known phylogenetic relationships. At the time of the proposal, some of the BAC libraries were of unproven quality, so we could not be sure that all 25 possible BACs would be recovered. Hence, our proposal to sequence only 20. The proposal was funded at 100% of the requested support.

The chief justification for the proposal related to our nearly complete deficiency in understanding the composition and organization of most grass genomes. Thus, more comprehensive studies of grass genome structure and/or evolution could not be proposed in a reasonable manner until the basal characteristics of these genomes were identified. Moreover, these studies would give a first general impression of the natures, lineages and rates of genome rearrangement in the 50 or so million years of grass diversification from a common ancestor.

At the same time that this proposal was funded, we received word that a similar proposal focused exclusively on maize/sorghum comparisons would be funded by the NSF PGP. This project, "Colinearity of Maize and Sorghum at the DNA Sequence Level" (PI, Dr. Jo Messing), is described elsewhere in this Newsletter issue. Because the depth of the Messing et al. proposal was so much greater than our two-year proposal, we decided to de-emphasize maize and sorghum. Hence, in our final project, we have sequenced six barley, one maize (the *Wx1* region), five rice, two sorghum and eight wheat BACs. We also sequenced one pearl millet BAC, from the *Wx1*-orthologous region, the first large stretch of DNA sequenced from that genome, as part of a collaboration with Dr. Katrien Devos (John Innes Centre). In total, we sequenced 23 BACs from six orthologous regions, for a total of about 3 Mb of completed sequence. These results amount to about 120% of the proposed goals of the project.

Most of the results of this project have not yet been published, although the sequences have been downloaded to GenBank. One paper (Dubcovsky, J et al., Plant Physiol 125:1342, 2001) has been published, two more are in press, an additional four have been submitted, and several others are in preparation. The results of these studies have been quite interesting, and would require several pages of text to summarize. However, I will try to point out a few major observations in the next two paragraphs.

All of the large grass genomes that we have investigated by BAC sequence analysis (barley, maize, pearl millet and wheat) have relatively high gene density compared to their genome size, but still much lower than the gene densities observed for rice and sorghum (about one gene per 8 kb). The greater size of orthologous regions in the large genomes is largely caused by the presence of retrotransposon insertions. Each of the large genomes often has these elements inserted as nested series, although the degree varies between regions and between species. Most retrotransposons appear to be fairly recent insertions (within the last few million years) in each genome, and we find very few cases where the same element is identified at the same location in even our most closely related species (barley and wheat).

Comparisons of gene composition and arrangement between the selected areas has been complicated by the fact that gene-finding procedures are far from perfect. Many small genes may be missed, while pieces of mobile DNAs are often misidentified as genes. Despite these constraints, we have been able to use highly conservative analyses to make an estimate of the degree of conserved gene content and order between the six grasses that we have investigated. The somewhat surprising result is that we see a high frequency of small genic rearrangements. Small inversions encompassing one or a few genes have been seen, while duplication or deletion of tandem gene family members are also frequent events. In maize, the deletion of one or a few genes is fairly common. This loss of genes may be tolerated in maize because of its polyploid origin. Most surprising, translocations of single genes or a few adjacent genes to different chromosomes also appear to be fairly routine events, at least from an evolutionary perspective. In four regions that we have studied in this and other projects, we see that only 80-90% of the same genes are found in the same region in comparisons of maize with rice or maize with sorghum. Even more dramatic, because of the numerous small rearrangements, we see different adjacent genes in 20-60% of the pairwise gene comparisons between maize and rice or between maize and sorghum. We often see better colinearity when comparing rice with sorghum, despite their more ancient divergence, probably because they are closer to true diploids. These genic rearrangement results demonstrate that rice and sorghum will be poor surrogate species for such technologies as chromosome walking. Hence, to understand the genic composition and order of the entire maize genome, we will need to sequence the maize genome.

Perhaps the most important outcome of this project has been the resultant synergy in data generation and (especially) analysis that was gained by assembly of a diverse set of highly trained young scientists. Dr. Wusirika Ramakrishna (Purdue Univ.) was in charge of the overall sequencing project, with exceptional collaborative contributions from John Emberton, Dr. Jianxin Ma, Matt Ogden, Dr. Yong-Jin Park, and Dr. Yinan Yuan at Purdue University. Dr. Nils Rostoks (Washington State Univ.) led all of the barley studies, while Drs. Carlos Busso and Liuling Yan took the lead on the wheat genome analyses. Dr. Bryan Shiloff, working with Drs. Callum Bell and Bill Beavis at NCGR, developed some important annotation tools for the project, and was a vital consultant on all of our genome analyses. In future years, I am sure that this crew of outstanding scientists will continue as highly productive plant geneticists.

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3,797 seed samples have been supplied in response to 306 requests, for 2001. Of these, a total of 67 requests were received from 18 foreign countries. Approximately 90% of our requests were received by electronic mail or through our order form on the World Wide Web.

We received a collection of the 94 IBM (B73 Mo17 intermated population) recombinant inbred lines from Mike Lee, and other families in that population from Georgia Davis. We received enough quality-tested seeds of each line to distribute immediately.

We have listed more stocks to our 'Phenotype Only' category of stocks. These are stocks that have been donated to the COOP over the years, and have been classified according to their mutant phenotype only. For the most part, these stocks have not as yet been allele tested, nor has their gene been located to a chromosome arm. While we expect that most of these will represent new alleles of known loci, some will represent unique, as yet undescribed loci. Over the past few years, some mutants in this class have been mapped and/or allele tested and where appropriate, the now characterized mutant stock was added to our main catalog. We are now listing all of these mutants to give cooperators that are interested in specific traits, easier access to these mutants.

Approximately 9.1 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Cooler than normal weather inhibited germination and seedling emergence in our second nursery planting, but overall growing conditions were excellent. With additional water supplied by irrigation, we obtained good increases of most stocks grown this year.

Special plantings were made of several categories of stocks:

1. In the 'Phenotype Only' collection is a series of stocks donated to the COOP by Dr. Gerry Neuffer upon his retirement. Of the approximately 3000 stocks originally reported we have now been able to list as available approximately 800 of these. This also includes about 50 new mutants found during our summer growouts.
2. Plantings were also made from donated stocks from the collections of Don Auger (translocated Ac lines), Jack Beckett (B-A translocation lines), Ben and Frances Burr (rd4, various transposon-induced mutants), Ed Coe (various genetic stocks), Greg Doyle (inversions), Guiseppe Gavazzi (*lil1* and *Sn1* alleles), Scott Hulbert (*Rp* alleles), Jerry Kermicle (extensive collection of Brink's *R1* alleles), Gaylon Krim of the NCRPIS (shrunken kernel mutant), Don McCarty (*vp14*), Oliver Nelson (*bronze1* alleles and other mutants in his collection), Yong-Bao Pan (*ba3* and *Mn::Uq*), Tom Peterson (*Ufo1*), Mike Scanlon (*ns1 ns2*), Bill Sheridan (*dek* mutants and A-A translocations), J. Torné (salt tolerant line), Bill Tracy (*bilateral coleoptile* mutant), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.
3. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We found additional alleles of *japonica1*, *pink scutellum1*, *yellow endosperm1, yellow endosperm8*, and *albescent1*. In this manner, we hope to move stocks from our vast collection of unplaced uncharacterized mutants and integrate them into the main collection.
4. We conducted linkage tests of several mutants that had been placed to chromosome arm using B-A translocations or *waxy*-marked A-A translocations. More precise locations were determined for *inhibitor of r1* and *inhibitor of r2*.
5. Two acres were devoted to the propagation of the large collection of cytological variants, including A-A translocation stocks and inversions. In this collection is a series of *waxy1*-marked translocations that are used for mapping unplaced mutants. Over the years, pedigree and classification problems arose during the propagation of these stocks. We were able to sort through the problem ones, and we can now supply good sources proven by linkage tests to include the correct translocated chromosomes. Many additional translocation stocks were tested this last year. Results of these tests will be reported in the next issue of the Maize Genetics Cooperation Newsletter.
6. Stocks produced from the NSF project "Maize Gene Discovery, Sequencing and Phenotypic Analysis" (see: http://zmdb.iastate.edu/) were grown this summer. Approximately 30% of these represented plants that originally had to be outcrossed, and needed to be selfed to analyze for mutant segregation. The remaining 70% were seed increases that were planted from those families that originally yielded poorly. These increases help to maintain adequate seed stock to fill future requests. Additionally, we grew 3,000 families of this material to screen for new adult plant mutant traits (see below).

We have received an extensive collection of transposed *Ac* lines from Hugo Dooner and a collection of miniature kernel mutants from Rob Martienssen's Maize Targeted Mutagenesis project. These latter two collections will be increased and made available in future years.

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

Philip continued his work on characterizing modifiers and inhibitors of certain *R1* alleles, inhibitors of *Bn1* alleles, and the inheritance and expression of the duplicate factor pairs *brn1 brn2* and *su3 su4*. He is also testing and pedigree searching problematic stocks and mutants that have been reported to have anomalous inheritance patterns, such as the "cytoplasmic glossy" *cgl1*.

Janet continues with linkage testing and propagation of translocation stocks. She has finished setting up an internal database for the 'Phenotype Only' category of stocks beyond those in the Neuffer collection. Of the original approximately 1600 stocks in the collection, 1200 have been recovered and are now listed on our web page as available for distribution. Over the past years ~140 mutants in this class have been allele tested and moved to our main collection of characterized stocks. This collection continues to grow with accessions from maize cooperators and with new mutations we identify each year in our summer growouts.

The NSF project "Maize Gene Discovery, Sequencing and Phenotypic Analysis" has, to date, generated 11,894 stocks that have been sent to the Stock Center. All of these stocks were screened for ear and kernel mutants at the Stock Center; families with ample seed supplies had two samples removed for additional trait screening. The first sample from each family was sent to UC Berkeley for seedling mutant screening. From the second sample, 3,000 families of the most genetically active grids were planted and screened (by us, other project members and colleagues in the Maize Genetics community) at the University of Illinois for adult plant mutant traits. This was an organized mutant hunt and was very successful in the discovery of novel adult plant mutants. We plan to organize another mutant hunt next summer. The remaining seed generated by this project was placed into cold storage to fulfill requests. Results from the mutant screenings can be found at the ZmDB: Phenotype Database (http://zmfmdb.zool.iastate.edu/). Shane's future work will involve increasing stocks as necessary to maintain seed supply for requests and continue scoring these stocks for kernel and adult plant mutant traits.

Due to a slow down in international airmail service, we have started shipping overseas requests via FedEx. With the prospect of the the US Postal Service irradiating all packages, we will soon need to fill domestic requests via FedEx, as well.

Marty Sachs	Philip Stinard	Janet Day Jackson	Shane Zimmerman
Director	Curator	Senior Research Specialist	Research Specialist

CHROMOSOME 1 MARKER

101A sr1 zb4 p1-ww
101B sr1 P1-wr
101C sr1 p1-ww
101D sr1 P1-rr
101F sr1 ts2 P1-rr
102A Ws4-N1589
102D Blh1-N1593
102F ms28
102G zb3
102H hcf6-N228B
102I hcf7-N1029D
103D vp5
103DA vp5-DR3076
103DB vp5-86GN4
103DC vp5-86GN3
103DD vp5-86GN6
103DE vp5-86GN11
103DF vp5-Mumm-1
103DG vp5-N81
103E zb4 ms17 p1-ww
104A Ts3
104F ms*-6034
104G ms*-6044
105A zb4 p1-ww
105B zb4 P1-wr
105C zb4 p1-ww br1
105E ms17 P1-wr
105F ms17 p1-ww
106B ts2 P1-rr
106C Glb1-0
106D Glb1-0; Glb2-0
107A P1-cr
107B P1-rr
107C P1-rw
107D P1-cw
107E P1-mm
107F P1-vv::Ac
107G P1-or
107H p1-ww
109A gs1-PI228173
109B gs1-PI262495
109C gs1-PI267181
109D P1-rr ad1 bm2
109E P1-wr br1 f1
110A P1-wr an1 Kn1 bm2
110D P1-wr an1 bm2
110E P1-wr ad1 bm2
110F P1-wr br1 Vg1
110H P1-wr br1 f1 bm2
110K P1-wr br1
111B hcf3-N846B
111C hcf3-N1242B
111D hcf44-N1278B
111F Les20-N2457
111G rs2
111H Les5-N1449
112B p1-ww br1 f1 bm2
112E as1
112H p1-ww br1
112I p1-ww br1 gs1 bm2
113B rd1
113BA rd1-Wasnok
113C br1 f1
113E br1 f1 Kn1
113K hm1; hm2
113L Hm1; hm2
114C br1 bm2
114D Vg1
114E Vg1; su1
114F br2 hm1; Hm2
114G br2 hm1; hm2
115C v22-8983
115CA v22-055-4
115E bz2-mVW2::Mu1
115F bz2-mVW4::MuDR
115J bz2-m::Ds; A1 A2 C1 C2 Pr1 R1
116A bz2-m::Ds; A1 A2 Ac C1 C2 Pr1 R1
116C an1 bm2
116D def(an1..bz2)-6923; A1 A2 Bz1 C1 C2 Pr1 R1
116G an1
116GA an1-93W1189
116I bz2 gs1 bm2 Ts6; A1 A2 Bz1 C1 C2 R1
117A br2
117D tb1
117DA tb1-8963
117E Kn1
118B Kn1 bm2
118C lw1
118CA lw1-3108
118CB lw1-6474
118J Adh1-3F1124r53
118K Adh1-1S5657; Adh2-33
118L Adh1-3F1124::Mu3
118M Adh1-3F1124r17
118N Adh1-lL14H; su1
118O Adh1-Cm
118P Adh1-FCm
118Q Adh1-Ct
119A Adh1-1S; Adh2-1P
119B vp8
119C gs1
119D gs1 bm2
119E Ts6
119F bm2
119H Adh1-FkF(gamma)25; Adh2-N
119J Adh1-Fm335::Ds1
119K Adh1-Fm335RV1
119L Adh1-2F11::Ds2
119M Adh1-1F725
120A id1
120B nec2-8147
120C ms9
120CA ms9-6032
120CB ms9-6037
120CC ms9-6042
120D ms12
120E v22-055-4 bm2
120F Mpl1-Sisco
120G Mpl1-Freeling
121A ms14
121AA ms14-6005
121B br2-mi8043
121C D8
121D lls1
121DA lls1-N501B
121E ty*-8446
121G ct2
121GA ct2-rd3
124A v*-5688
124B j*-5828
124C w*-8345
124CA w*-013-3
124CB w*-8245
124D v*-5588
124E w*-018-3
124F w*-4791
124G w*-6577
124H w*-8054
124I v*-032-3
124J v*-8943
125A Les2-N845A
125B Mpl1-Jenkins
125C hcf13-N1097B
125D hcf41-N1275C
125E hcf50-N1481
125F hcf2-N506C
125G hcf31-N1268B
126A bz2 gs1 bm2; A1 A2 Bz1 C1 C2 R1
126B id1-N2286A
126C dek1-N928A
126D dek1-N971
126E dek32-N1322A
126F o13
126H P1-vv::Ac bz2-m::Ds
126I P1-vv::Ac
126J P1-ww-1112
126K P1-ovov-1114
126L P1-rr-4B2
126M P1-vv-5145
126N dek1-N1348
126O dek1-N1394
126P dek1-N1401
127A bz2 zb7-N101 bm2
127B dek1-N792
127C dek2-N1315A
127D dek22-N1113A
127E f1
127F Msc1-N791A
127G Tlr1-N1590
127I gt1
128A ij2-N8
128B l16-N515
128C l17-N544
128D pg15-N340B
128E pg16-N219
128G py2-N521A
128H spc2-N262A
129A w18-N495A
129AA w18-571C
129B wlu5-N266A
129C zb7-N101
129D emp1-R
129E ptd1-MS1568
129F dek*-MS2115
129G dek*-MS6214
130A o10-N1356
130B cp3-N888A
130BA cp3-N888A; mn4-N888C
130C id1-NA972
130D dek1-PB388
130E dek1-DR1129
130F ht4
6502A P1-ww-4Co63
6502C P1-ovov-CFS-29
6502D P1-rr(11)-CFS-33
6502E P1-rr(10)-CFS-36
6502F P1-rr(4-5)-CFS-47
6502G P1-rr(9)-CFS-53
6502I P1-rr(8-9)-CFS-75
6502K P1-vv-CFS-96
6502L P1-vv-CFS-110
6502M P1-vv-CFS-116
6502N P1-ovov-CFS-124
6502O P1-vv-CFS-138
6502P P1-rr(7)-CFS-140
6502Q P1-vv-CFS-155
6502R P1-o-grained-red-CFS-167
6502S P1-r pale(8)-CFS-181
6502T P1-rr(9)-CFS-186
6502U P1-vv-CFS-226
6502V P1-vv-CFS-245
6502W P1-vv-CFS-246
6502X P1-vv-CFS-249
6502Y P1-vv-CFS-252
6502Z P1-vv-CFS-255
6502ZA P1-vv-CFS-256
6502ZB P1-vv-CFS-259
6503A P1-rr(11)-CFS-272
6503B P1-vv-CFS-273
6503C P1-vv-CFS-278
6503D P1-vv-CFS-279
6503E P1-vv-CFS-281
6503F P1-vv-CFS-282
6503G P1-vv-CFS-283
6503H P1-vv-CFS-284
6503I P1-r pale(5)-CFS-285
6503J P1-vv-CFS-286 (Brazil)
6503K P1-mm-CFS-286
6503L P1-mm-CFS-287
6503M P1-mm-CFS-289
6503N P1-mm-CFS-290
6503O P1-mm-CFS-291
6503P P1-mm-CFS-292
6503R P1-mm-CFS-294
6503S P1-mm-CFS-297
6503T P1-mm-CFS-301
6503U P1-rw(9)-CFS-302
6503V P1-rr(11)-CFS-303
6503W P1-rr(10)-CFS-305
6503X P1-rr(10)-CFS-315
6503ZA P1-rr(2)-CFS-319
6503ZB P1-rr(8)-CFS-320
6503ZC P1-rr(7)-CFS-321
6504A P1-rw(8)-CFS-324
6504B P1-rw(6-7)-CFS-325
6504C P1-rr(9)-CFS-327
6504D P1-rw(7)-CFS-330
6504E P1-rw(9)-CFS-332
6504F P1-rw(8)-CFS-334
6504G P1-o-grained-red-CFS-335
6504H P1-rw(5-6)-CFS-336
6504I P1-rw(7-9)-CFS-342
6504J P1-rr(5)-CFS-345
6504K P1-rw(7)-CFS-350
6504L P1-rr-CFS-360
6504M P1-rw(5)-CFS-369
6504N P1-ww(1)-CFS-376
6504O P1-vv-CFS-497
6504Q P1-rr(11)-CFS-548

CHROMOSOME 2 MARKER

201A mrl1-IHO
201B hcf106-Mum1::Mu1; hcf106c
201C hcf106-Mum2::Mu1; hcf106c
201D hcf106-Mum3::Mu1; hcf106c
201F ws3 lg1 gl2 b1
201G sm2-Brawn180
201H sm2-Brawn189
201I sm2-Brawn190
201J sm2-Brawn191
201K sm2-Brawn188
202A lg1-PI200299
202B lg1-PI262493
202C lg1-32TaiTaiTaSarga
202D lg1-ZCXGRB

202E lg1-64-4
202F fl1-o8
202G lg1-56-3037-5
203B al1
203BA al1-Brawn
203BB al1-y3
203C al1-1998-2
203D al1 lg1
203G al1-y3 gl2
204A al1-lty3
204B hcf1-N490B
205A al1 lg1 gl2
205B lg1
205C lg1 gl2
205G al1 gl2 B1
206A lg1 gl2 B1
206C D10-N2428
206D Wrp1-NA1163
206E oro2
207A w3-y11
208B lg1 gl2 B1 sk1
208C lg1 gl2 B1 sk1 v4
208D lg1 gl2 B1 v4
208E lg1 gl2 b1
208H gl2-Salamini
209A gl11-N352A
209E lg1 gl2 b1 sk1
209I gl2-Parker's Flint
210E gl2-3050-3
210F gl2-PI200291
210G gl2-PI239114
210H gl2-PI251009
210I gl2-PI251885
210J gl2-PI251930
210K gl2-PI262474
210L gl2-PI262493
210N gl2-N718
210O gl2-N239
211A lg1 gl2 b1 fl1
211H gl2 wt1
212B lg1 gl2 b1 fl1 v4
212D lg1 gl2 b1 v4
213B lg1 gl2 wt1
213F lg1 B1-v::Bg Ch1
213H lg1 gl2 B1-v::Bg
214A wt1-PI251939
214B lg1 b1 gs2
214C d5
214D gl11 B1
214E B1 ts1
214J sk1
214L lg1 gl2 mn1
215A gl14
215B gl11
215C wt1
215CA wt1-N472A
215CB wt1-N666B
215CC wt1-N178C
215CD wt1-N136A
215D mn1
215E fl1
215EA fl1-o4
215G fl1 v4
215H wt1 gl14
216A fl1 v4 Ch1
216D fl1 w3
216E fl1 v4 w3
216G fl1 v4 w3 Ch1
217A ts1
217B v4
217G v4 Ch1
217H ba2 v4
217I Les10-NA607
217J Les11-N1438
217K Les15-N2007
217L Les18-N2441
217M Les19-N2450
217N cpc1-N2284B
218A w3
218C w3 Ch1
218D Ht1-GE440
218DA Ht1-Ladyfinger
218DB Ht1
218E ba2
218G B1-Peru; A1 A2 C1 C2 r1-r
218GA B1-Peru; A1 A2 C1 C2 R1-r
218H w3-8686
218I w3-86GN12
218J w3-Kermicle-1
219A B1-Peru; A1 A2 C1 C2 r1-g
219B b1; A1 A2 C1 C2 r1-g
219C Ch1
219D Ht1 Ch1
219F B1-Peru; A1 A2 C1 C2 bz2 r1-g
219G B1-Bolivia-706B; A1 A2 C1 C2 r1-g
219H B1-Bolivia; A1 A2 C1 C2 Pl1-Rhoades Pr1 r1-g
219I B1-I; A1 A2 C1 C2 Pl1-Rhoades r1-r
219J B1-I; A1 A2 C1 C2 Pl1-Rhoades r1-g
219K B1-S; R1-g pl1-McClintock
219L B1-S; R1-r pl1-McClintock
220A Les1-N843
220B ws3 lg1 gl2; Alien Addition T2-Tripsacum
220D hcf15-N1253A
220F os1
221A gs2
221AA gs2-0229
221C wlv1-N1860 Ch1
221G wlv1-N1860
224B v*-5537
224H whp1; A1 A2 C1 R1 c2 gl1 in1
224I ws3-7752
224J ijmos*-7335
224K glnec*-8495
224L ws3-8949
224M ws3-8991
224N ws3-8945
226A ws3-N2357
226B b1-m1::Ds1; A1 A2 C1 C2 r1-g
226C b1-md2::Ds1; A1 A2 C1 C2 r1-g
226D b1-Pm5; A1 A2 C1 C2 r1-g
226E b1-Perum216; A1 A2 C1 C2 r1-g
227A dek3-N1289
227B dek4-N1024A
227C dek16-N1414
227D dek23-N1428
227E Les4-N1375
227I nec4-N516B
227K et2-2352
227L et2-91g6290-26
228A l18-N1940
228B spt1-N464
228C ws3-N453A
228CA ws3-N605A
228E B1-Bh
228F ms33-6019
228G ms33-6024
228H ms33-6029
228I ms33-6038
228J ms33-6041
229A rf3 Ch1
229B v24-N424
229BA v24-N576A
229BB v24-N588A
229BC v24-N350
229C w3 rf3 Ch1
229E emp2-MS1047
229F dek*-MS1365
229G dek*-MS4160
229H dek*-MS2159
229J dek*-PIE

CHROMOSOME 3 MARKER

301A cr1
301B bif2-N2354
301C spc3-N553C
301D Wi2-N1540
301E rd4
301F ns1-R; ns2-R
302A d1-6016
302AA d1-N446
302AB d1-N339
302B d1 rt1
302E d1-tall
303A d1 rt1 Lg3-O
303F g2
303FA g2-pg14::I
303FB g2-v19
303FD g2-56-3040-14
303FE g2-59-2097
303FF g2-94-1478
303G g2 d1
304A d1 ys3
304F d1 Lg3-O ys3
304G Lg3-O Rg1
304I d1 h1
305A d1 Lg3-O
305D d1 Rg1
305K d1 cl1; Clm1-4
306A Rp3-A
306F ref1-MS1185
307A Sdw2-N1991
307C pm1
308B d1 ts4
308E ra2
308F ra2 Rg1
308G ra2-D
309A a1-m3::Ds Sh2
309B a1-m1-5718::dSpm
309C a1-m1-5719A1::dSpm
309D a1-m1-5719A1::dSpm; Mod Pr1
309E a1 Sh2; Spm-w
309F a1-m2-8417::dSpm
309G a1-m2(os)-o1
309H a1-m2-7991A-o2
309I a1-m2-7995::dSpm
309J a1-m2-7977B::dSpm
309K a1-m2-8012A-p1
309L a1 Sh2; Spm-s
309M a1-m1-5719A1::dSpm sh2
309N a1-m2-7995B
309O a1-m1-5996-4::dSpm
309P a1-m1-5719A1::dSpm; Spm-i
309Q a1-m5::Spm-w; Spm-s
309S a1-m2-8411A::Spm-w Sh2
309T a1-m2-7981B6::Spm-w
309U a1-m2-8409::Spm-i
309V a1-m5::Spm-w Sh2
309W a1-m2-8011::Spm-w Sh2
309X a1 Sh2; Spm-w-8745
309Y a1 Sh2; Spm-i
309Z a1-m1-5720-o2
310C ra2 lg2
310D Cg1
311A cl1
311AA cl1-N2
311B cl1; Clm1-2
311BA cl1-7716; Clm1-2
311C cl1; Clm1-3
311D cl1-p; Clm1-4
311E rt1
311F ys3
311G Lg3-O ys3
312A Les14-N2004
312B Les17-N2345
312D Lg3-O
312G brn1-R
312H g2 brn1-R
312I brn1-R cr1
312J brn1-R ra2 lg2
312K brn1-Nelson
312L brn1-3071
312M ms23
313A gl6
313AA gl6-gl7
313AB gl6-N672B
313D ms3
313DA ms3-6008
313DB ms3-6009
313DC ms3-6043
313DD ms3-6020
314A gl6 lg2 A1; A2 C1 C2 R1
314C gl6 lg2 a1-m et1; A2 C1 C2 Dt1 R1
314F Rg1 gl6 lg2
314G gl6 lg2
315B Rg1 gl6
315C Rg1
315D A1-b(P415); A2 C1 C2 R1
315I A1-m2(os)-p1
315J A1-m2(os)-r2
315K a1-m2-7991A-o1
315L a1-m2-7991A-p2
315M a1-m2-7991A-p3
315N a1-m2-7991A-p4
315O a1-m2-7991A-p4b
315P a1-m2-7991A-p5
315Q a1-m2-8010A-o2
315R A1-m3-r1a sh2-m1::Ds
315S a1-m5-o1
315T a1-m5-o2
315U A1-m5-r1
315V A1-m5-r4
315W A1-m5-r5
316A ts4
316B a1-N796
316C dek5-N1339A
316D a1-mt2
316E a1-mt3
316F a1-mt4
316G a1-mt5
316H a1-mt6
316I a1-mt7
316J a1-mt8
316K a1-mt11
316L a1-mt13
316M a1-mt15
316N a1-mt16
316O a1-mt18
316P a1-mt19
317F gl6 ts4 lg2
317I a1-m1-5996-4m::dSpm; Spm
317J a1-m2::Spm-s; Spm-w
317K a1-m2-7991A::Spm-s
317L a1-m2-8004::dSpm
317M a1-m2-8010A::Spm-s

317N a1-m2-8011::Spm-w
317O a1-m2-8012A
317P a1-m2-8147
317Q a1-m2-8167::dSpm
317R a1-m2-8414C
317S a1-m2-8549C
317T a1-m5::Spm-w Sh2
317U a1-m5::Spm-w sh2-1
317W a1-m1-5720::Spm
317X a1-m1-6078::dSpm
317Y a1-m2-8409-2
317Z A1 def-1260
318A ig1
318B ba1
318C y10-7748
318D hcf19-N1257A
318E sh2-N391B
318EA sh2-N2307
318F sh2-N2340
318G na1
318H vp1-Mc
318I y10-8624
319A lg2 A1-b(P415) et1; A2 C1 C2 Dt1 R1
319C lg2 a1-m et1; A2 C1 C2 R1 dt1
319D lg2 a1-m et1; A2 C1 C2 Dt1 R1
319F lg2 a1-st et1; A2 C1 C2 Dt1 R1
319G lg2 a1-st et1; dt1
320A lg2
320B lg2-PI184281
320C lg2 na1
320D lg2-podcorn
320E et1
320F A1 sh2; A2 C1 C2 R1 b1 pl1
320K sh2-94-1001-11
320L sh2-94-1001-58
320M sh2-94-1001-1003
320N a3-Styles; B1-b Pl1-Rhoades r1-g
320O a3-Styles; B1-b Pl1-Rhoades R1-nj
321A A1-d31; A2 C1 C2 R1
321B lg2 a1; A2 C1 C2 R1 dt1
321C lg2 A1-b(P415) et1; A2 C1 C2 R1 dt1
321D a1-m4::Ds; A2 C1 C2 R1
321E a1-rUq; A2 C1 C2 R1
321F a1-Mum1; A2 C1 C2 R1
321H a1-Mum3; A2 C1 C2 R1
321I a1-Mum4; A2 C1 C2 R1
321J a1-Mum5; A2 C1 C2 R1
321K a1-rUq; Uq1
321L a1-rUq(flow); Uq1
322A A1-d31 sh2; A2 C1 C2 R1 dt1
322B A1-d31 sh2; A2 C1 C2 Dt1 R1
322C A1-Mum3-Rev; A2 C1 C2 R1
322F a1-m; A2 C1 R1 b1 dt1 pl1
322I et1-24
322J et1-27
322K et1-34
322L et1-2162
322M et1-2320
322N et1-2424
322O et1-2457
322P et1-3191
322Q et1-3328
322R et1-5079
322S et1-84-6013
322T et1-88g-9733
322U et1-43
323A a1-m; A2 C1 C2 Dt1 R1
323D a1-m sh2; A2 C1 C2 Dt1 R1
323E a1-m et1; A2 C1 C2 Dt1 R1
323G a1-m1::rDt (Neuffer); A2 C1 C2 Dt1 R1
323H a1-st; A2 C1 C2 Mrh R1 dt1
323I a1-m1::rDt (Neuffer); A2 C1 C2 R1 dt1
324A a1-st; A2 C1 C2 Dt1 R1
324B a1-st sh2; A2 C1 C2 Dt1 R1
324E a1-st et1; A2 C1 C2 Dt1 R1
324G a1-st; A2 C1 C2 R1 dt1
324H a1 et1; A2 C1 C2 R1 dt1
324I a1-st et1; A2 C1 C2 R1 dt1
324J A2; C1 C2 R1 a1-sh2-del-Robertson
324K a1-Mus1; A2 C1 C2 R1
324L a1-Mus2; A2 C1 C2 R1
324M a1-Mus3
324N a1-Mus4
325A a1-p et1; A2 C1 C2 R1 dt1
325B a1-p et1; A2 B1 C1 C2 Dt1 Pl1 R1
325C a1-x1; A2 C1 C2 R1
325D a1-x3; A2 C1 C2 R1
325E A1 ga7; A2 C1 C2 R1
325G a3
325I a1-p; A2 C1 C2 Dt1 R1
325J a1-p; A2 C1 C2 Pr1 R1 dt1
325K a1-m3::Ds sh2-m1::Ds; A2 Ac C1 C2 R1
326A sh2-Elmore
326AA sh2-Garwood
326AB sh2-60-156
326B vp1
326BA vp1-Mum3
326BC vp1-86N6
326BD vp1-86GN14
326BE vp1-86GN18
326BF vp1-86GN19
326BG vp1-Mum2
326BH vp1-Mum1::Mu
326C Rp3
326D te1-1
326DA te1-Forester
326DB te1-Grogan
329A v*-9003
329B v*-8623
329C w21-022-15
329D yd2
329E w*-8336
329F yg*-W23
329G w*-062-3
329H v*-8609
329HA v*-8959
329I pg2
329K yel*-8630
329L yel*-5787
330A h1
330G a1-mrh; A2 C1 C2 Mrh R1
330H A1-b(P415) Ring 3; A2 C1 C2 R1
330I a1-Mum2; A2 C1 C2 MuDR R1
330J a1-Mum2; A2 C1 C2 R1
330K a1 sh2; A2 C1 C2 R1 dt1
330L a1-mrh; A2 C1 C2 R1
332B dek5-N874A
332C dek24-N1283
332D Wrk1-N1020
332F gl19-N169
332G dek6-N627D
332H dek17-N330D
332I Lxm1-N1600
332M Spc1-N1376
332N wlu1-N28
332S Mv1
333A dek5-25
333AA dek5-MS33
333B te1-Galinat
333C dek5-Briggs-1998-1

CHROMOSOME 4 MARKER

401A Rp4-A
401AB Rp4-B
401C Ga1 su1
401D Ga1-S
401E Ga1-S; y1
401I ga1 su1
401J Ga1-M
401K Ga1-S su1
402A st1
402D Ts5
402E ms30-6028
402F hcf23-N1261A
403A Ts5 fl2
403B Ts5 su1
403C su1-F37
403D su1-PI228183
404A su3-5081; su4-5081
404B su3-89-1303-18; su4-89-1303-18
404C su3-94-4079-6; su4-94-4079-6
404D su3-85-3113-11; su4-85-3113-11
404E su3-87-2340-36; su4-87-2340-36
405B la1-PI239110
405BB la1-Funk:2232
405BC la1-N2020
405BD la1-N2276B
405BE la1-PI184284
405D la1-R su1 gl3
405G la1-R su1 gl4
406C fl2
406CA fl2-DR9234
406D fl2 su1
407D su1
407DA su1-N86
407DB su1-N2316
407DC su1-BKG489-13
407DD su1-Pl
407DE su1-R2412
407DF su1-N896A
407DG su1-N1161A
407DH su1-N2313
407DI su1-N2314
407DJ su1-N959
407DK su1-N1968
407DL su1-N1994
407E su1-am
407F su1-am; du1
408B bm3-Burnham su1
408C su1 zb6
408E bm3-91598-3
408J su1 ra3
408K su1; se1
408L su1 zb6 Tu1
409A su1-st
409B su1-66
409C su1-P
409D su1-5051
409F su1-28510
409G su1-28511
409H su1-28512
409I su1-28513
409J su1-28515
409K su1-28516
409L su1-28517
409M su1-28518
409N su1-28519
409O su1-28520
409P su1-30394
409Q su1-30397
409R su1-30398
409S su1-30399
409T su1-30400
409U su1-30401
409V su1-Bn2
410D su1 zb6 gl3
410E su1-A3
410F su1-4582::Mu1
410G su1-8064
410H su1-2401
410I su1-3837
410J su1-7110
410K su1-2857
410L su1-2859
410M su1-90-1101.1
410N su1-83-3383-4
410O su1-87-2046-27
410P su1-85-3217-10
410Q su1-84-5167-6
410R su1-84-5267-18
410S su1-85-3436-29
411A su1-8908
411B su1 gl4 o1
411F gl7 su1 v17
412C su1 gl3
412G su1 gl4 Tu1
413A su1 o1
413B su1 gl4
413D su1 C2-Idf1(Active-1); A1 A2 C1 R1
413F su1 de*-414E
413G v23 Su1 gl3; bm*-COOP
414A bt2
414AA bt2-Williams
414AB bt2-60-158
414AC bt2-9626
414AD bt2-5288
414B gl4
414BA gl4-Stadler
414BB gl4-gl16
414BD gl4-N525A
414C gl4 o1
414E de*-414E
415A j2
415B o1-N1243
415C o1-N1478A
415D bt2-8132
416A Tu1-A158
416B Tu1-l(1st)
416C Tu1-l(2nd)
416D Tu1-d
416E Tu1-md
416F Tu1 gl3
417B v8
417C gl3
417D o1 gl3
417E gl3-N531
418A gl3 dp1
418B c2; A1 A2 C1 R1
418D C2-Idf1(Active-1); A1 A2 C1 R1
418E dp1
418F o1
418G v17
419A v23-8914
419E gl7
419F Dt6 gl3 C2; A2 C1 R1 a1-m
419G Dt6 C2; A2 C1 R1 a1-m
419H c2-m1::Spm; A1 A2 C1 R1

419I c2-m2::dSpm c2-m3::Mpi1
419J c2-Mum1
419K c2-m2::dSpm; Spm-s
419L c2-m881058Y::IRMA; En Mod wx1-m8::Spm-I8
419M c2-m3::Mpi1
420A su1 Dt4 C2; A2 C1 R1 a1-m
420C nec*-rd
420CA nec*-016-15
420D yel*-8957
420F dp*-4301-43
420G w*-9005
420H Dt4 C2; A2 C1 R1 a1-m
424C gl3-64-4
424D gl3-56-3120-2
424E gl3-56-3129-27
424F gl3-60-2555
424G gl3-PI183683
424H gl3-PI251928
424I gl3-PI251938
424J gl3-PI254858
424K gl3-PI267180
424L gl3-PI267219
424M gl3-PI311517
424N gl3-15
426A Gl5 Su1; gl20
426B gl3-PI251941
426D cp2-N1324A
427A cp2-o12
427AA cp2-N211C
427AB cp2-N1875A
427AC cp2-MS2608
427AD cp2-N912
427B dek25-N1167A
427C Ysk1-N844
427D orp1-N1186A; orp2-N1186B
427E dek8-N1156
427F dek10-N1176A
427G Ms41-N1995
427H dek31-N1130
427I Sos1-ref
428A gl5 Su1; gl20
428C nec5-N642
428D spt2-N1269A
428E wt2-N10
428F lw4; Lw3
428G bx1
428H gl5 su1; gl20
428L dsc1-MS2058

CHROMOSOME 5 MARKER

501A am1 a2; A1 C1 C2 R1
501B lu1
501D ms13
501E gl17
501F gl17-N260B
501G gl17 a2; A1 C1 C2 R1
501I am1
502B A2 ps1-Sprague pr1; A1 C1 C2 R1
502C D9-N2319
502D A2 bm1 pr1; A1 C1 C2 R1
502E Ms42-N2082
502F Nl2-N1445
502G A2 Bt1 ga10
502H hcf21-N1259A
503A A2 bm1 pr1 ys1; A1 C1 C2 R1
503B hcf43-N1277B
503C a2-mu1::Mu1
503D a2-mu2
503E a2-mu3::Mu3
504A A2 bt1 pr1; A1 C1 C2 R1
504C A2 bm1 pr1 zb1; A1 C1 C2 R1
504E A2 bt1; A1 C1 C2 R1
505B A2 pr1 ys1; A1 C1 C2 R1
505C A2 bt1 pr1 ga*-Rhoades; A1 C1 C2 R1
505D pr1-N1515A
505E pr1-N1527A
506A A2 v3 pr1; A1 C1 C2 R1
506B A2 pr1; A1 C1 C2 R1
506C A2 pr1 v2; A1 C1 C2 R1
506D na2 A2 pr1; A1 C1 C2 R1
506F A2 pr1 v12; A1 C1 C2 R1
506L A2 br3 pr1; A1 C1 C2 R1
507A a2; A1 C1 C2 R1
507AA a2-Mus2; A1 C1 C2 R1
507AB a2-Mus3; A1 C1 C2 R1
507AC a2-Mus1; A1 C1 C2 R1
507F a2 bm1 bt1 ga*-Rhoades; A1 C1 C2 R1
507G a2 bm1 bt1; A1 C1 C2 R1
507H A2 bv1 pr1; A1 C1 C2 R1
507I a2-m4::Ds; wx1-m7::Ac7
508A a2 bm1 bt1 pr1; A1 C1 C2 R1
508C a2 bm1 bt1 bv1 pr1; A1 C1 C2 R1
508F a2 bm1 pr1 ys1; A1 C1 C2 R1
508H a2-Mum1
508I a2-Mum2
508J a2-Mum3
508K a2-Mum4
508L bv1 pr1
509G a2-m1::dSpm Bt1
509H a2-m1(II)::dSpm(class II)
509I pr1-m1
509J pr1-m2
509K a2-m1(ps)
509L a2-m1::dSpm; Spm-s
509M a2-m5::dSpm
509N A2-m1(os)-r1
510A a2 bm1 pr1 v2; A1 C1 C2 R1
510D a2 pr1 gl8; A1 C1 C2 R1
510E a2 ae1 pr1 gl8; A1 C1 C2 R1
510G a2 bm1 pr1 eg1; A1 C1 C2 R1
511C a2 bt1 pr1; A1 C1 C2 R1
511F a2 bt1 Pr1 ga*-Rhoades; A1 C1 C2 R1
511H a2 bt1; A1 C1 C2 R1
512C a2 bt1 pr1 ga*-Rhoades; A1 C1 C2 R1
512D vp2-N1136B
512E Wi4-N2445A
512F pb4
512G gl8-N166A
512H v13
512I lw2-vp12
513A a2 pr1; A1 C1 C2 R1
513C a2 pr1 v2; A1 C1 C2 R1
513D A2 pr1 sh4; A1 C1 C2 R1
513E a2 pr1 v12; A1 C1 C2 R1
514A a2 bm1 pr1; A1 C1 C2 R1
514B ae1-PS1
514C ae1-PS2
514D ae1-PS3
514E ae1-PS4
514F ae1-PS5
514G ae1-PS6
514H ae1-PS7
514I ae1-PS8
514J ae1-PS9
514K ae1-PS10
514L ae1-PS11
514M Ae1-5180-r4
514N bt1-m1::dSpm
514O bt1-m2
514P bt1-m3::dSpm
514Q bt1-m4::Ds
514R Bt1-m1-r1
515A vp2
515AA vp2-DR5180
515AB a2 vp2-green mosaic; A1 C1 C2 R1
515C ps1-Sprague
515CA ps1-8776
515CB ps1-881565-2M
515CC ps1-N80
515CD ps1-8205
515D bm1
515E bt1-N1992
515F bt1-N2308
515G bt1-N2309
516B bt1-R
516BA bt1-Elmore
516BB bt1-C103
516BC bt1-Singleton
516BD bt1-sh3
516BE bt1-sh5
516BF bt1-Eldridge
516BH bt1-6-783-7
516BI bt1-Vineyard
516BJ bt1-T
516BK bt1-W187R
516BL bt1-3040
516BM bt1-N797A
516C ms5
516D td1 ae1
516DA td1-Nickerson
516G A2 bm1 pr1 yg1; A1 C1 C2 R1
517A v3
517AB v3-8982
517B ae1
517BA ae1-EMS
517BB ae1-PS12
517BC ae1-PS13
517BD ae1-PS14
517BE ae1-PS15
517BF ae1-PS16
517BH ae1-Elmore
517E ae1 pr1 gl8
518A sh4
518AA sh4-Rhoades
518AB sh4-o9
518B gl8-Salamini
518BA gl8-R
518BB gl8-6:COOP
518BC gl8-6:Salamini
518BD gl8-10:COOP
518BE gl8-PI180167
518C na2
518D lw2
519AA ys1-W23
519AB ys1-5344
519AC ys1-N755A
519AD ys1-74-1924-1
519B eg1
519C v2
519D yg1
519E A2 pr1 yg1; A1 C1 C2 R1
519F A2 pr1 gl8; A1 C1 C2 R1
519H zb1
519I zb1-2
520A hcf38-N1273
520B v12
520C br3
520F A2 Dap1; A1 C1 C2 R1
520G A2 pr1 Dap1; A1 C1 C2 R1
520H Dap1-2
520I ae1-1979-7
520J ae1-MOEWS
520K ae1-1981-MuT
521A nec3-N409
521B Nec*-3-9c
521C nec*-8624
521D nec*-5-9(5614)
521E nec*-7476
521F nec*-6853
521G nec*-7281
521H nec*-8376
521I v*-6373
521J yg*-8951
521K lw3; lw4
521L w*-021-7
521N lnec*-5931
521NA lnec*-8549
521P lw3; Lw4
524A v*-PI267226
524B les*-3F-3330
527A dek18-N931A
527B dek9-N1365
527C dek26-N1331
527D dek27-N1380A
527E grt1-N1308B
527F nec7-N756B
527G dek33-N1299
527H Msc2-N1124B
527I ppg1-N199
527J nec6-N493
528A Hsf1-N1595
528B wgs1-N206B
528C anl1-N1634
528CA anl1-330C
528E prg1-MS8186
528F ren1-MS807
528H dek*-MS2146
528I dek*-MS1182
529A anl1-N1643
529B anl1-N1645
529C anl1-N1671
529D anl1-N1685
529E anl1-N1691
529F anl1-N1673

CHROMOSOME 6 MARKER

601C rgd1 y1
601D rgd1-N372B
601F po1-ms6 y1 pl1
601H rhm1 rgd1 y1
601I rhm1 y1 l11
601J Wsm1 Mdm1; Wsm2 Wsm3
601K wsm1 mdm1; wsm2 wsm3
601L Mdm1 y1
602A po1-ms6 wi1 y1
602C y1
602D rhm1 Y1
602H y1-N2236
602J y1-w-mut
602K y1-gbl
602L y1-pb1
602M y1-8549
602N y1-Caspar
602O y1-0317
602P y1-129E
603A y1 l10
603AA y1 l10-1359
603B y1 l11-4120
603C y1 l12-4920
603D w15-8896 y1
603H mn3-1184 y1
604D y1 l15-Brawn1
604F y1 si1-mssi
604FA y1 si1-ts8
604FB y1 si1-Sam
604H y1 ms1

604HA y1 ms1-Robertson
604I Y1 ms1
604IA ms1-6050
605A wi1 y1
605C y1 pg11; Wx1 pg12
605E wi1 Y1 Pl1
605F wi1 Y1 pl1
605G l3
605H pg11-M14; pg12-KYS
605I pg11-Oh43; pg12-KYS
606A Y1 pg11-4484; Wx1 pg12-4484
606AA pg11-8925; pg12-8925
606AB pg11-48-040-8; pg12-48-040-8
606AC pg11-8563; pg12-8563
606AD pg11-8322; pg12-8322
606B y1 pg11; pg12 wx1
606C Y1 pg11; pg12 wx1
606E y1 pl1
606F y1 Pl1
606I y1 pg11 su2; Wx1 pg12
607A y1 Pl1-Bh1; A1 A2 C2 R1 c1 sh1 wx1
607C y1 su2
607E y1 pl1 su2 v7
607H y1 Pl1-Bh1; A1 A2 C2 R1 Wx1 c1 sh1
607I y1 Pl1-Bh1; A1 A2 C2 R1 c1 sh1 skb1 wx1
607J sm1-Brawn168
607K sm1-Brawn178
607L sm1-Brawn184
608A gs3-N268
608C sbd1-N2292
608D Les13-N2003
608F y1 pl1 w1
608G Y1 l11
608H y1-m1301::dSpm
608I Rp8-A
608J Rp8-B
609D Y1 su2
609DA Y1 su2-89-1273
609DB su2-PS1
609DC su2-PS2
609DD su2-1979-5
609DE su2-87-2279-12
609DF su2-1981
609DG su2-1982
609DH su2-0203
609DI su2-PI193430
609DJ su2-1979-1
609F ms1-Albertsen
610B Dt2 Pl1; A2 C1 C2 R1 a1-m
610F Y1 pl1 su2 v7
610G hcf34-N1269C
610H Y1 Dt2 pl1; A2 C1 C2 R1 a1-m
610I hcf36-N1271B
610J hcf48-N1282C
610K hcf26-N1263C
610L hcf323
610M hcf5-N510C
611A Pl1 sm1; P1-rr
611D Pt1
611E Y1 pl1 w1
611EA w1-7366
611I sm1 tan1-py1; P1-rr
611K Y1 Pl1 w1
611L w1; l1
611M afd1
611N sr4-N65A
611O o14-N924
612A w14
612B po1
612BA po1-ms6
612C l*-4923
612D oro1
612DA oro1-6474
612I tan1-py1
612J w14-8657
612K w14-8050
612L w14-6853
612M w14-025-12
612N w14-1-7(4302-31)
612O yel*-1-7(4302-31)
613A 2NOR y1; A1 C1 C2 R1 a2 bm1 pr1 v2 wx1
613D vms*-8522
613F w14-8613
613I tus*-5267
613J gm*-6372
613L w*-8954
613M yel*-039-13
613N yel*-7285
613O l*-4-6(4447)
613P yel*-8631
613T pg11-6656; pg12-6656
627A dek28-N1307A
627B dek19-N1296A
627C vp*-5111
627G dek*-MS1104; l*-1104

CHROMOSOME 7 MARKER

701B In1-D
701D o2
701E o2-Mum1
701F Hs1
702A o2 v5
702B o2 v5 ra1-Ref gl1
702I In1-Brawn
703A o2 v5 gl1
703B De*-B30
703C o2-m(r); Bg
703D o2 ra1-Ref gl1
703E o2-R; Bg
703F o2-m12::Spm
703G o2-m2::Ds; Ac
703H o2-m5::Ac
703J Rs1-O
703JA Rs1-1025::Mu6/7
703K Rs1-Z
704B o2 ra1-Ref gl1 sl1
704C o2-NA696
704D o2-NA697
704E gl1-m8
704F ms22-6036
704H o2-orange
704I gl1-PI267186
705A o2 gl1
705B o2 gl1 sl1
705D o2 bd1
706A o2 sl1
706B vp9-Bot100
707A y8 v5 gl1
707B in1; A1 A2 C1 C2 R1 pr1
707C in1 gl1; A1 A2 C1 C2 R1 pr1
707D v5
707E vp9-R
707EA vp9-3111
707EB vp9-86GN9
707EC vp9-86GN15
707F y8 gl1
707G in1 gl1; A1 A2 C1 C2 Pr1 R1
708A ra1-Ref
708AA ra1-PI262495
708AB ra1-PI184279
708AC ra1-PI239103
708AD ra1-PI267181
708AE ra1-PI267184
708AF ra1-63-3359
708B bd1-N2355
708C o15-N1117
708D y8-lty2
709A gl1
709AA gl1-56-3013-20
709AB gl1-56-3122-7
709AC gl1-PI183644
709AD gl1-PI218043
709AE gl1-PI251652
709AF gl1-PI257507
709AG gl1-Istra
709AH gl1-BMS
709AI gl1-7L
709AJ gl1-9:COOP
709AK gl1-N212
709AL gl1-N269
709AM gl1-N345B
709C gl1-m
710A gl1 Tp1
710B gl1 mn2
710E o5 gl1
710I gl1 Bn1
710J gl1-N271
710K gl1-dy
710L gl1-PI218038
711A Tp1
711B ij1-ref::Ds
711C ij1-60-2454-20
711G ts*-br
712A ms7
712AA ms7-6007
712B ms7 gl1
713A Bn1
713E Bn1 bd1
713H Bn1 ij1
713I bd1 Pn1
714A Pn1
714B o5
714BA o5-PS3038
714BB o5-N76B
714BC o5-N874B
714C o5-N1241
714D va1
715A Dt3; A2 C1 C2 R1 a1-m
715C gl1 Dt3; A2 C1 C2 R1 a1-m
716A v*-8647
716B yel*-7748
716C dlf1-N2389A
716D dlf1-N2461
716F Les9-N2008
727A dek11-N788
727B wlu2-N543A
727D v27-N590A
727DA v27-N53B
727DB v27-N413C
727E gl1-cgl
727F Rs4-N1606
727G Rs1-O o2 v5 ra1-Ref gl1
727H ms34-6004
727I ms34-6010
727J ms34-6013
727K ms34-6014
728A Px3-6
728B ptd2-MS3193
728C mn2-cp1
728D sh6-8601
728E sh6-N1295
728F ren2-NS326
728G dek*-MS2082
728H dek*-MS5153

CHROMOSOME 8 MARKER

801A gl18-g
801B v16
801I yel*-024-5
801K v16 ms8
802A rgh1-N1285
802B emp3-N1386A
802C Ht2
802G ms43
802H gl18-PI262473
802I gl18-PI262490
803A ms8
803B nec1-025-4
803D gl18-g ms8
803F nec1-7748
803G nec1-6697
804A v21-A552
804B dp*-8925
804C tb*-poey1013
805A fl3
805C gl18-g v21-A552
805E el1
805G ms8 j1
808A ct1
808B Lg4-O
808C Htn1
810A v16 j1; l1
810B j1
810C j1-JSM
827A dek20-N1392A
827B dek29-N1387A
827C Bif1-N1440
827CA Bif1-N2001
827D Sdw1-N1592
827E Clt1-N985
827F pro1-N1058
827G pro1-N1121A
827H pro1-N1528
827I pro1-N1533
827J wlu3-N203A
827K pro1
827L pro1-Tracy
828A ats1
828C pro1-N1154A
828D pro1-NA342
828E pro1-N1530

CHROMOSOME 9 MARKER

901B yg2 C1 sh1 bz1; A1 A2 C2 R1
901C yg2 C1 sh1 bz1 wx1; A1 A2 C2 R1
901E yg2 C1 bz1 wx1; A1 A2 C2 R1
901H yg2 C1 Bz1; A1 A2 C2 R1
902A yg2 c1 sh1 bz1 wx1; A1 A2 C2 R1
902B yg2 c1 sh1 wx1; A1 A2 C2 R1
902C yg2 c1 sh1 wx1 gl15-Hayes; A1 A2 C2 R1
902D yg2 c1 sh1 Bz1 wx1 gl15 K9S-s; A1 A2 C2 R1
902E C1 sh1 Bz1-McC1; A1 A2 C2 R1
902F bz1-m13::dSpm
902G C1 sh1 bz1 wx1; A1 A2 C2 R1 Spm
902H bz1-m13::dSpm; Spm
902I bz1-m13CS1
902J bz1-m13CS3
902K bz1-m13CS4
902L bz1-m13CS5
902M bz1-m13CS6
902N bz1-m13CS7

903A C1 sh1 bz1; A1 A2 C2 R1
903B C1 sh1 bz1 wx1; A1 A2 C2 R1
903D C1-I sh1 bz1 wx1; A1 A2 C2 R1
903E bz1-m13CS8
903F bz1-m13CS10
903G bz1-m13CS11
903H bz1-m13CS12
904B C1 sh1; A1 A2 C2 R1
904C C1 sh1 wx1; A1 A2 C2 R1
904D C1 wx1 ar1; A1 A2 C2 R1
904F C1 sh1 bz1 gl15 bm4; A1 A2 C2 R1
904G rgo1-Sarkar
905A C1 sh1 wx1 K9S-l; A1 A2 C2 R1
905C C1 bz1 Wx1; A1 A2 C2 R1
905D C1 sh1 wx1 K9S-l; A1 A2 C2 K10-I R1
905G C1 bz1 wx1; A1 A2 C2 R1
905H c1 sh1 wx1; A1 A2 C2 R1-scm2 b1
905I ms45-6040
906A C1 wx1; A1 A2 C2 DsI Pr1 R1 y1
906B C1 wx1; A1 A2 C2 DsI R1 Y1 pr1
906C C1-I Wx1; A1 A2 C2 DsI R1
906D C1-I; A1 A2 C2 R1
906G C1-I Sh1 Bz1 Wx1; DsI
906H C1 Sh1 bz1 wx1; Ac
907A C1 wx1; A1 A2 C2 R1
907E C1-I wx1; A1 A2 C2 R1 y1
907G c1-p; A1 A2 B1-b C2 R1 pl1
907H c1-n; A1 A2 C2 R1 b1 pl1
907I C1-S wx1; A1 A2 C2 R1
908A C1 wx1 da1 ar1; A1 A2 C2 R1
908B C1 wx1 v1; A1 A2 C2 R1
908D C1 wx1 gl15; A1 A2 C2 R1
908F C1 wx1 da1; A1 A2 C2 R1
908G c1-mt13
909A C1 wx1 Bf1-ref; A1 A2 C2 R1
909B c1 bz1 wx1; A1 A2 C2 R1
909C c1 sh1 bz1 wx1; A1 A2 C2 R1
909D c1 sh1 wx1; A1 A2 C2 R1
909E c1 sh1 wx1 v1; A1 A2 C2 R1
909F c1 sh1 wx1 gl15; A1 A2 C2 R1
909G hcf42-N1276B
910B c1 sh1 wx1 gl15 Bf1-ref; A1 A2 C2 R1
910D c1; A1 A2 C2 R1
910G C1 sh1-bz1-x2 Wx1; A1 A2 C2 R1
910H C1 sh1-bz1-x3; A1 A2 C2 R1
910I sh1-bb1981 bz1-m4::Ds
910IA sh1-bb1981 bz1-m4::Ds; Ac
910L yg2-str
911A c1 wx1; A1 A2 C2 R1
911B c1 wx1 v1; A1 A2 C2 R1
911C c1 wx1 gl15-Hayes; A1 A2 C2 R1
911D Fas1
911E sem1-1364
911F def(Bf1..bm4)044-4
912A sh1
912AA sh1-1746
912AB sh1-9026-11
912AC sh1-3-6(6349)
912AD sh1-60-155
912AE sh1-EMS
912AF sh1-4020
912AG sh1-9552
912AH sh1-9626
912AI sh1-3017
912AJ sh1-6
912B sh1 wx1 v1
912E lo2
912H lo2 wx1
913C sh1 l7
913D sh1 l6
913E baf1
913F yg2-Mum1
913G yg2-Mum2
913H yg2-Mum3
913I yg2-Mum4
913J yg2-Mum5
913K yg2-Mum6
913L yg2-Mum7
913M yg2-Mum8
913N yg2-Mum9
913O yg2-DR83-106-3
913P yg2-DR83-106-5
914A wx1 d3-COOP
914B dek12-N1054
914K Wc1-ly; Y1
914L bz1-Mus1
914M bz1-Mus2
914N bz1-Mus3
914O bz1-Mus5
914P bz1-Mus6
914Q bz1-Mus7
914R bz1-Mus10
915A wx1
915B wx1-a
915C w11
915D wx1-N1050A
915F wx1-N1240A
916A wx1 v1
916B wx1 v1-JRL
916C wx1 bk2
916E wx1 v1 gl15
916G Trn1-N1597
916H v31-N828
916I d3-8201
917A wx1 Bf1-ref
917C v1
917D ms2
917DA ms2-6002
917DB ms2-6012
917E gl15-Sprague
917EA gl15-Lambert
917EB gl15-KEW
917F d3-COOP
917FA d3-d2
917FB d3-015-12
917FC d3-072-7
917FD d3-8054
917FF d3-d2-Harberd
917FG d3-d2-Phillips
917FH d3-N660B
918A gl15 Bf1-ref
918B gl15 bm4
918C bk2 Wc1
918D Wc1
918F Wx1 Bf1-ref
918G Wc1 Bf1-ref bm4
918GA Wc1-Wh Bf1-ref bm4
918K bk2 v30
918L wx1 Wc1
919A bm4
919B Bf1-ref bm4
919C l6
919D l7
919G l6; l1
919I Bf1-DR-046-1
919J bz1-Mum9; MuDR
919K bz1-Mum4::Mu1
919L bz1-Mum1
919M bz1-Mum2
919N bz1-Mum3
919O bz1-Mum5
919P bz1-Mum6
919Q bz1-Mum7
919R bz1-Mum8
919S bz1-Mum9
919T bz1-Mum10
919U bz1-Mum11
919V bz1-Mum12
919W bz1-Mum15
919X bz1-Mum16
919Y bz1-Mum18
920A yel*-034-16
920B w*-4889
920C w*-8889
920E w*-8950
920F w*-9000
920G Tp3L-9SRhoades
920L ygzb*-5588
920M wnl*-034-5
920N pyd1
923A wx1-a
923B wx1-B
923C wx1-B1
923D wx1-B2::TouristA
923E wx1-B3::Ac
923F wx1-B4::Ds2
923G wx1-B6
923H wx1-B7
923I wx1-B8
923J wx1-BL2
923K wx1-BL3
923L wx1-C
923M wx1-C1
923N wx1-C2
923O wx1-C3
923P wx1-C4
923Q wx1-C31
923R wx1-C34
923S wx1-F
923T wx1-90
923U wx1-H
923V wx1-H21
923W wx1-I
923X wx1-J
923Y wx1-M
923Z wx1-m1::Ds
923ZA wx1-m6R
923ZB wx1-m6NR
923ZC wx1-m8::Spm-I8
923ZD wx1-P60
923ZE wx1-R
923ZF wx1-Stonor
924A Wd1 wd1 C1 C1-I Ring 9S; A1 A2 C2 R1
924B C1-I Ring 9S; A1 A2 C2 R1
924C yg2
924D wd1
924E wd1 C1 sh1 bz1
924F C1 Sh1 sh1 Bz1 bz1 wx1 tiny fragment 9
924G C1-I Bz1; Ac DsI
924H c1 sh1 bz1 wx1; Ac
925A bz1-m1::Ds wx1-m9::Ac
925B wx1-m9::Ac
925C bz1-m2::Ac
925D Wx1-m9r1
925E bz1-m2(DII)::Ds wx1-m6::Ds
925F C1 sh1 bz1 wx1-m8::Spm-I8
925H bz1-m2(DI)::Ds wx1; R1-sc
925I c1-m2::Ds Wx1; Ac
925J c1-m858::dSpm wx1
925K c1-m1::Ds
926A sh1-m5933::Ds
926B Sh1-r3(5933)
926C Sh1-r6(5933)
926D Sh1-r7(5933)
926E Sh1-r8(5933)
926F Sh1-r9(5933)
926G Sh1-r10(5933)
926H Sh1-r11(5933)
926I sh1-m6233::Ds
926J Sh1-r1(6233)
926K Sh1-r2(6233)
926L C1-I sh1-m6258::Ds
926M Sh1-m6258-r1
926N Sh1-r6795-1
926O bz1-m5::Ac
926P Bz1-wm::Ds1
926Q Bz1-m1-p
926R Bz1-m2-r1
926S Bz1-m2(DII)-r1
926T Bz1-m2(DII)-r2
926U Bz1-m2(DII)-r3
926V sh1-bb1981 Bz1-m4-p1
926W sh1-bb1981 Bz1-m4-r6851
926X sh1-bb1981 Bz1-m4-r7840B
926Y sh1-bb1981 Bz1-m4-r8332
926Z Bz1-m5-p1
926ZA Bz1-m5-r1
926ZB Bz1-m5-r2
927A dek12-N873
927B dek13-N744
927C dek30-N1391
927D Les8-N2005
927E Zb8-N1443
927H C1 Dt7; A2 C2 R1 a1-r
927I G6-N1585
927K Rld1-N1990
927L Rld1-N1441
928A yg2-N27
928AA yg2-N585
928AB yg2-N697
928AC yg2-N610
928B wlu4-N41A
928C ms20
928G c1-m5::Spm wx1-m8::Spm-I8; A1 A2 C2 R1
928H wx1-m7::Ac7
928I C1 bz1-mut::rMut; A1 A2 Bz2 C2 Mut R1
928J C1 bz1-(r)d; A1 A2 C2 R1
928K C1 Sh1 bz1-s; A1 A2 C2 Mut R1
928L ms45-6006
928M ms35-6011
928N ms35-6018
928O ms*-6021
928P ms*-6022
928Q ms35-6027
928R ms35-6031
928S ms*-6046
928T ms*-6047
929E Dp9
930A wx1-Mum1
930B wx1-Mum2
930C wx1-Mum3
930D wx1-Mum4
930E wx1-Mum5::Mu
930F wx1-Mum6
930G wx1-Mum7
930H wx1-Mum8
930I wx1-Mum9
930J wx1-Mum10
930K wx1-Mum11
930L wx1-Mus16
930M wx1-Mus181

930N wx1-Mus215
931A Wx1-m5::Ds
931B wx1-m6::Ds
931C wx1-m6-o1
931D Wx1-m7-i1
931E Wx1-m8-r10
931F Wx1-m9-r3
931G Wx1-m9-r4
931H wd1-Mus1
931I wd1-Mus2
931J wd1-Mus3
931K wd1-Mus4
931L wd1-Mus5
931M wd1-Mus6

CHROMOSOME 10 MARKER

X01A oy1-Anderson
X01AA oy1-yg
X01AB oy1-8923
X01B oy1 R1; A1 A2 C1 C2
X01C oy1 bf2
X01E oy1 bf2 R1; A1 A2 C1 C2
X02C oy1 zn1 R1; A1 A2 C1 C2
X02E oy1 du1 r1; A1 A2 C1 C2
X02G oy1 zn1
X02H Oy1-N1459
X02I Oy1-N1538
X02J Oy1-N1583
X02K Oy1-N1588
X02L Oy1-N1989
X03A sr3
X03B Og1
X03D Og1 R1; A1 A2 C1 C2
X03E oy1 y9
X03F Inr1-Ref
X03G Ufo1
X04A Og1 du1 R1; A1 A2 C1 C2
X04B ms11
X04BA ms11-6051
X04D bf2
X04DA bf2-N185A
X04E du1-8501
X04F du1-8802
X05A Og*-0376
X05B Gs4-N1439
X05E bf2 sr2
X05G bf2 g1 R1-r; A1 A2 C1 C2
X06A bf2 r1 sr2; A1 A2 C1 C2
X06C nl1 g1 R1; A1 A2 C1 C2
X06F bf2 R1 sr2; A1 A2 C1 C2
X07A nl1 g1 r1; A1 A2 C1 C2
X07C y9
X07CA y9-y12
X07D nl1
X08A vp10
X08B vp10-86GN5
X08C vp10-TX8552
X08F li1
X08FA li1-IL90-243Tco
X09B li1 g1 R1; A1 A2 C1 C2
X09EA g1-g4
X09EB g1-56-3005-24
X09EC g1-1-7(X-55-16)
X09ED g1-68-609-13
X09EE g1-ws2
X09EF g1-PI262473
X09F ms10
X09FA ms10-6001
X09FB ms10-6035
X09G li1 g1 r1; A1 A2 C1 C2
X10A du1
X10AA du1-PS1
X10AB du1-PS2
X10AC du1-PS3
X10AD du1-PS6
X10AE du1-PS4
X10AF du1-PS5
X10AG du1-8801
X10AH du1-84-5350-31
X10D du1 g1 r1; A1 A2 C1 C2
X10F zn1
X10FA zn1-N25
X10G du1 v18
X11A zn1 g1
X11D Tp2 g1 r1; A1 A2 C1 C2
X11E g1 R1 sr2; A1 A2 C1 C2
X11F g1 r1; A1 A2 C1 C2
X11H zn1 R1-r; A1 A2 C1 C2
X11I Tp2 g1 sr2
X12A g1 r1 sr2; A1 A2 C1 C2
X12C g1 R1-g sr2; A1 A2 C1 C2
X12E g1 R1; A1 A2 C1 C2
X13D g1 r1-r sr2; A1 A2 C1 C2
X13E g1 r1-ch; A1 A2 C1 C2 wx1
X13G R1-p
X13H R1-b
X14A r1-r lsr1-Ej; A1 A2 C1 C2
X14E r1; A1 A2 C1 C2 wx1
X14F v18 r1; A1 A2 C1 C2
X14I r1-sc:m3::Ds
X14J R1-nj::Ac
X14K r1-Del902
X14L r1-g; A1 A2 C1 C2
X15B l1 r1 sr2; A1 A2 C1 C2
X15C R1-g; A1 A2 C1 C2
X15D r1-ch; A1 A2 C1 C2
X15F lsr1 R1-g Sr2
X15G isr1 r1-g sr2
X15H isr1 R1-r:PI302369
X15HA isr1 R1-r:PI302369 sr2
X15I isr1 R1-nj Mst1
X16B r1 K10-l; A1 A2 C1 C2
X16C R1-ch; A1 A2 C1 C2 Pl1
X16CA R1-ch
X16D r1 sr2; A1 A2 C1 C2
X16E r1 K10-ll; A1 A2 C1 C2
X16F R1 K10-ll; A1 A2 C1 C2
X17B r1-r; A1 A2 C1 C2
X17C R1-mb; A1 A2 C1 C2
X17D R1-nj; A1 A2 C1 C2
X17E R1-r; A1 A2 C1 C2
X18A R1-lsk; A1 A2 C1 C2
X18B R1-sk:nc-2; A1 A2 C1 C2
X18C R1-st; A1 A2 C1 C2
X18D R1-sk; A1 A2 C1 C2
X18E R1-st Mst1
X18G R1-scm2; A1 A2 C1 C2 bz2
X18H R1-nj; A1 A2 C1 C2 bz2
X18I r1; A1 A2 C1 C2
X19A R1-sc:124
X19B w2
X19BA w2-Burnham
X19BB w2-2221
X19C l1 w2
X19D o7
X19E R1-r Lc1-Ecuador; b1
X19F r1 w2
X19G r1-n19 Lc1; b1
X19H r1-g:e Lc1; b1
X20B l1
X20C v18
X20I R1-d:Arapaho
X20J R1-d:Catspaw
X24A cm1
X24B lep*-8691
X24C v*-8574
X25A R1-scm2; A2 C1 C2 a1-st
X25B R1-scm2; A1 A2 C1 c2
X25C R1-sc:122; A1 A2 C1 C2 pr1
X25D R1-scm2; A1 C1 C2 a2
X25E R1-scm2; A1 A2 C2 c1
X26A r1-X1 / R1; A1 A2 C1 C2
X26B R1-scm2; A1 A2 C1 C2
X26C R1-sc:122; A1 A2 C1 C2
X26D R1-sc:5691; A1 A2 C1 C2
X26E R1-scm2; A1 A2 C1 C2 pr1 wx1
X26F R1-scm2; A1 A2 C1 C2 In1-D
X26G R1-scm2; A1 A2 C1 c2-m2::dSpm
X26H R1-scm2; A1 A2 C1 C2 wx1
X27A dek14-N1435
X27B dek15-N1427A
X27C w2-N1330
X27D Les6-N1451
X27E gl21-N478B; gl22-N478C
X27F Vsr1-N1446
X27G Oy1-N700
X27H orp2-N1186B; orp1-N1186A
X27I l19-N425
X27J l13-N59A
X27K v29-N418
X27L Les12-N1453
X28B R1-scm2; a1-m1::rDt (Neuffer)
X28C R1-nj:Cudu; A1 A2 C1 C2
X28D Vsr*-N716
X28E Les3
X28F cr4-6143
X28G R1-nj:Chase; A1 A2 C1 C2
X28I R1-scm2; A2 C1 C2 a1-m1-5719::dSpm
X28J R1-scm2; A1 A2 C1 C2 bz1
X29A ren3-MS1339
X29B dek*-MS2181
X29C cr4-N590C
X29D cr4-N647
X29E cr4-N411
X35A Rp1-A
X35B Rp1-B
X35C Rp1-C
X35D Rp1-D
X35E Rp1-J
X35F Rp1-K
X35G Rp1-M
X35H Rp1-Kr3
X35I Rp1-Kr4
X35J Rp1-Kr1J92
X35K Rp1-Kr1J6
X35L Rp1-G
X35M Rp5
X35N Rp1-D Rp1-J
X35O Rp1-C Rp1-J
X35P Rp1-F Rp1-J
X35Q Rp1-C Rp1-F Rp1-J
X35R Rp1-G Rp1-I
X35S Rp1-F Rp1-G Rp1-J
X35T Rp1-D Rp1-G Rp1-J
X35U Rp5 Rp1-D
X35V Rp5 Rp1-G
X35W Rp5 Rp1-C Rp1-G Rp1-J

UNPLACED GENES

U140A aph1
U140AA Aph1
U140C l4
U140G ms22
U140H ms24
U140I zn2-94-234
U240A Les7-N1461
U240D o11
U240E zn2
U240F zn2-PI251887
U240G zn2-PI236997
U240H zn2-PI239110
U240I zn2-56-3012-10
U340D ws1-COOP ws2-COOP
U340DA ws1-Pawnee ws2-Pawnee
U340H oro4
U340I Mei1-mei025
U440B gl13
U440C hcf49-N1480
U440D ub1-76C
U440E frz1
U440F mg1-Sprague
U540A dv1
U540B dy1
U640A dsy1-Doyle
U640B dsy1-Russian
U640C pam1
U640D pam2
U640E ada1
U640F atn1 Adh1-1S5657
U740A abs1-PI254851
U740C lty1
U740F pi1 pi2
U740G Fbr1-N1602
U740H ad2-N2356A
U740I ba3
U740K Rp7
U840A csp1-NA1173
U840B blc1-Tracy
U840D Les21-N1442
U840F agt1
U840G Wi3-N1614
U840H nld1-N2346
U840I Mc1
U840J hcf16
U940A Ht3
U940B dsy2
U940D hcf11-N1250A
U940E hcf17
U940F hcf73
U940G Glb2-0
U940C v25-N17

MULTIPLE GENES

M141A A1 A2 B1 C1 C2 Pl1 Pr1 R1-g
M141AA A1 A2 B1 C1 C2 Pl1-Rhoades Pr1 R1-g
M141B A1 A2 B1 C1 C2 pl1 Pr1 R1-g
M142A A1 A2 b1 C1 C2 pl1 R1-r
M142B a1 A2 b1 C1 C2 pl1 R1-r
M142C A1 a2 b1 C1 C2 pl1 R1-r
M142D A1 A2 b1 bz1 C1 C2 pl1 R1-r
M142E A1 A2 b1 bz2 C1 C2 pl1 R1-r
M142F A1 A2 b1 c1-p C2 pl1 R1-r
M142G A1 A2 b1 C1-l C2 pl1 R1-r
M142H A1 A2 b1 C1 c2 pl1 R1-r
M142I A1 A2 b1 C1 C2-Idfm pl1 R1-r
M142J A1 A2 b1 C1 C2-Idf1(Active-1) pl1 R1-r
M142K A1 A2 b1 C1 C2 pl1 pr1 R1-r
M142L A1 A2 b1 C1 C2 gl1 in1 pl1 R1-r
M142M A1 A2 b1 C1 C2 In1-D pl1 R1-r
M142N A1 a2 bt1 C1 C2 pr1 R1
M142O C1 sh1 bz1 wx1; A1 A2 C2

R1-r
M142P c1 sh1 wx1; A1 A2 C2 R1-r
M142Q yg2 c1 sh1 wx1; A1 A2 C2 R1-g
M142R A1 A2 C1-I C2 R1-r wx1
M142S su1 c2; A1 A2 C1 R1-r
M142T A1 A2 b1 C1 C2 pl1 r1-g
M142U A1 A2 b1 C1 C2 pl1 r1-r
M142V A1 A2 C1 C2 R1-nj
M142W A1 A2 C1 C2 R1-st
M142X A1 A2 b1 C1 C2 Pl1 r1-g
M142Y A1 A2 B1 C1 C2 Pl1 r1-g
M142Z a1-st A2 b1 C1 C2 pl1 R1-scm2
M142ZA A1 a2 b1 C1 C2 pl1 R1-scm2
M142ZB b1 bz1 C1 pl1 R1-scm2 sh1
M142ZC A1 A2 b1 bz2 C1 C2 pl1 R1-scm2
M142ZD A1 A2 b1 c1-n C2 pl1 R1-scm2
M142ZE A1 A2 b1 c1-p C2 pl1 R1-scm2
M241A A1 A2 B1 C1 C2 Pl1 Pr1 r1-g
M241C A1 A2 B1 C1 C2 Pl1 Pr1 R1-r
M241D A1 A2 b1 C1 C2 Pl1-Rhoades r1-g
M242A A1 A2 b1 C1 c2 pl1 R1-scm2
M242B A1 A2 b1 C1 C2 pl1 pr1 R1-scm2
M242C in1 gl1; A1 A2 b1 C1 C2 pl1 R1-scm2
M242D a1 sh2; A2 b1 C1 C2 pl1 R1-scm2
M242E c1 sh1 wx1; A1 A2 b1 C2 pl1 R1-scm2
M242F su1 c2; A1 A2 b1 C1 pl1 R1-scm2
M242G A1 A2 b1 C1 C2 pl1 R1-scm2
M242H A1 A2 b1 C1 C2 pl1 r1-g
M242I A1 A2 b1 C1 C2 pl1 r1-r
M340A A1 A2 B1 c1 C2 pl1 Pr1 R1-g
M340B A1 A2 B1 c1 C2 Pl1 Pr1 R1-g
M340C A1 A2 b1 c1 C2 pl1 Pr1 R1-g
M341B A1 A2 B1 C1 C2 pl1 Pr1 R1-r
M341C A1 A2 b1 C1 C2 Pl1 Pr1 R1-r
M341CA A1 A2 b1 C1 C2 Pl1-Rhoades Pr1 R1-r
M341D A1 A2 B1 c1 C2 Pl1 Pr1 R1-r
M341F A1 A2 b1 C1 C2 pl1 Pr1 R1-r
M441B A1 A2 B1 C1 C2 pl1 Pr1 R1-r wx1
M441D A1 A2 B1 C1 C2 Pl1 Pr1 r1-r
M441F A1 A2 b1 C1 C2 pl1 Pr1 R1-g wx1
M541B A1 A2 b1 C1 C2 pl1 Pr1 R1-g
M541F a1 A2 C1 C2 R1-nj
M541G A1 a2 C1 C2 R1-nj
M541H A1 A2 c1 C2 R1-nj
M541I A1 A2 C1-I C2 R1-nj
M541J A1 A2 C1 c2 R1-nj
M541K A1 A2 C1 C2-Idf1(Active-1) R1-nj
M541L A1 A2 bz1 C1 C2 Pr1 R1-nj
M541M A1 A2 Bz1 C1 C2 pr1 R1-nj
M541N A1 A2 C1 C2 gl1 in1 R1-nj
M541O A1 A2 C1 C2 In1-D R1-nj
M541P ae1 wx1
M641C A1 A2 b1 C1 C2 pl1 Pr1 R1-r wx1
M641D A1 A2 C1 C2 Pr1 r1 wx1 y1
M641E A1 A2 C1 C2 r1-g wx1 y1
M641F r1-g y1; A1 A2 C1 C2
M741A A1 A2 b1 C1 C2 pl1 Pr1 r1-g wx1
M741B Stock 6; A1 A2 B1 C1 C2 Pl1 R1-r
M741C Stock 6; A1 A2 B1 C1 C2 pl1 R1-r
M741F Stock 6; A1 A2 C1 C2 pl1 R1-g y1
M741G Stock 6; A1 A2 C1-I C2 pl1 R1-g wx1 y1
M741H Stock 6; A1 A2 B1 C1 C2 Pl1 R1-nj
M741I Stock 6; A1 A2 C1 C2 R1
M841A A1 A2 C1 C2 pr1 R1 su1
M841B f1 wx1
M841C v4 wx1
M841D v2 wx1
M841F A1 A2 bz2 C1 C2 R1-scm2 wx1
M841G A1 A2 C1 c2 R1-scm2 wx1
M841H gl6 wx1
M841I su1 wx1
M841J v16 wx1
M841K gl4 wx1
M841L gl2 lg1 wx1
M941A A1 A2 c1 C2 Pr1 R1 wx1 y1
M941B Mangelsdorf's tester; a1 bm2 g1 gl1 j1 lg1 pr1 su1 wx1 y1
M941BA Mangelsdorf's tester + R1-nj
M941C a1 Dt1 gl2 lg1 wt1
M941D gl1 wx1 y1
M941E gl8-R wx1 y1
M941F sm1; wx1
MX40A A1 A2 C1 C2 P1-vv::Ac r1-sc:m3::Ds
MX40B A1 A2 Ac2 bz2-m::Ds C1 C2 R1
MX40C A1 A2 C1 C2 r1-sc:m3::Ds Ac8168-9
MX40D P1-vv::Ac r1
MX41A A1 A2 C1 C2 gl1 pr1 R1 wx1 y1
MX41B A1 A2 C1 C2 gl1 pr1 R1 su1 wx1 y1
MX41C a1 a2 bz1 bz2 c1 c2 pr1 r1 wx1 y1
MX41D a1 A2 C1 C2 gl1 pr1 R1 su1 wx1 y1
MX41E a1-m1-n::dSpm A2 C1 C2 R1 wx1-m8::Spm-I8

B-CHROMOSOME

B542A Black Mexican Sweet; B chromosomes present
B542B Black Mexican Sweet; B chromosomes absent

TRISOMIC

123A trisomic 1
223A trisomic 2
328A trisomic 3
422A trisomic 4
523A trisomic 5
615A trisomic 6
718A trisomic 7
807A trisomic 8
922A trisomic 9
X23A trisomic 10

TETRAPLOID

N102A Autotetraploid; A1 A2 B1 C1 C2 Pl1 Pr1 R1
N102D Autotetraploid; A1 A2 C1 C2 R1
N102E Autotetraploid; B chromosomes present
N102EA Autotetraploid; B chromosomes present
N102F Autotetraploid; A1 a2 C1 C2 R1
N103A Autotetraploid; P1-rr
N103B Autotetraploid; P1-vv::Ac
N103C Autotetraploid; P1-ww
N103D Autotetraploid; P1-wr
N103E Autotetraploid; P1-mm
N104A Autotetraploid; su1
N104B Autotetraploid; A1 A2 C1 C2 pr1 R1
N105B Autotetraploid; wx1 y1
N105D Autotetraploid; A1 a2 bt1 C1 C2 R1
N105E Autotetraploid; bt1
N106C Autotetraploid; wx1
N107B Autotetraploid; W23
N107C Autotetraploid; Synthetic B
N107D Autotetraploid; N6

CYTOPLASMIC STERILE/RESTORER

C736A R213 (N); mito-N Rf1 rf2
C736AB R213 (T) Sterile; cms-T Rf1 rf2
C736B Ky21 (N); mito-N Rf1 Rf2 Rf3 RfC
C736C B37 (N); mito-N rf1 Rf2 rf3 rfC
C736CA B37 (T) Sterile; cms-T rf1 Rf2
C736CB B37 (T) Restored; cms-T Rf1 Rf2
C736E Tr (N); mito-N Rf3 rfC rfT
C736F W23 (N); mito-N rf1 Rf2 rf3 RfC
C736FA W23 (N); mito-N rf1 Rf2 rf3 RfC
C736G B73 (N); mito-N rf1 Rf2 rf3 rfC
C736H L317 (N); mito-N rf3 RfC rfT
C836A Wf9 (T) Sterile; cms-T rf1 rf2
C836B Wf9 (N); mito-N rf1 rf2 rf3 rfC
C836C Wf9 (T) Restored; cms-T Rf1 Rf2 rf3 rfC
C836D Wf9 (S) Sterile; cms-S rf1 rf2 rf3 rfC
C836E Mo17 (T) Sterile; cms-T rf1 Rf2 rf3 rfC
C836F Mo17 (N); mito-N rf1 Rf2 rf3 rfC
C836G Mo17 (C) Sterile; cms-C rf1 Rf2 rf3 rfC
C836H Mo17 (S) Sterile; cms-S rf1 Rf2 rf3 rfC
C936D K55 (N); mito-N Rf1 Rf2 rf3 RfC
C936DA K55 (N); mito-N Rf1 Rf2 rf3 RfC
C936F N6 (N); mito-N rf1 Rf2 rf3 RfC
C936FA N6 (N); mito-N rf1 Rf2 rf3 RfC
C936G N6 (T) Sterile; cms-T rf1 Rf2
C936H N6 (T) Restored; cms-T Rf1 Rf2
C936I SK2 (N); mito-N rf1 Rf2 rf3 rfC
C936J SK2 (T) Sterile; cms-T rf1 Rf2
C936K SK2 (T) Restored; cms-T Rf1 Rf2
C936M 38-11 (N); mito-N rf1 Rf2 rf3 rfC
CX36A N6 (C) Restored; cms-C rf1 Rf2 rf3 RfC
CX36B N6 (S) Sterile; cms-S rf1 Rf2 rf3 RfC
CX36C B37 (C) Sterile; cms-C rf1 Rf2 rf3 rfC
CX36D B37 (S) Sterile; cms-S rf1 Rf2 rf3 rfC

CYTOPLASMIC TRAIT

C337A NCS2
C337B NCS3

TOOLKIT

Kindiger's ig1 Maintainer
T0318AB cms-L; ig1 R1-nj
T0318AC cms-MY; ig1 R1-nj
T0318AD cms-ME; ig1 R1-nj
T0318AE cms-S; ig1 R1-nj
T0318AF cms-SD; ig1 R1-nj
T0318AG cms-VG; ig1 R1-nj
T0318AH cms-CA; ig1 R1-nj
T0318AI cms-C; ig1 R1-nj
T0318AJ cms-Q; ig1 R1-nj
T0940A Hi-II Parent A (for producing embryogenic callus cultures)
T0940B Hi-II Parent B (for producing embryogenic callus cultures)
T0940C Hi-II A x B (for producing embryogenic callus cultures)
T0940D KYS (for chromosome observations in pachytene microsporocytes)
T0940E Mu off; a1-Mum2 A2 C1 C2 R1
T3302A Inv1m; P1-vv::Ac bz2-m::Ds
T3302C T1-2b; P1-vv::Ac bz2-m::Ds
T3302D T1-2(036-7); P1-vv::Ac bz2-m::Ds
T3302E T1-2c; P1-vv::Ac bz2-m::Ds
T3302F T1-3(5883); P1-vv::Ac bz2-m::Ds
T3302G T1-3k; P1-vv::Ac bz2-m::Ds
T3302H T1-3(5597); P1-vv::Ac

bz2-m::Ds
T3302I T1-3(5982); P1-vv::Ac bz2-m::Ds
T3302J T1-4i; P1-vv::Ac bz2-m::Ds
T3302K T1-4(064-20); P1-vv::Ac bz2-m::Ds
T3302L T1-4(4308); P1-vv::Ac bz2-m::Ds
T3302M T1-4(8602); P1-vv::Ac bz2-m::Ds
T3302N T1-4b; P1-vv::Ac bz2-m::Ds
T3302O T1-5(5525); P1-vv::Ac bz2-m::Ds
T3303A T1-5(6899); P1-vv::Ac bz2-m::Ds
T3303B T1-5b; P1-vv::Ac bz2-m::Ds
T3303C T1-5(4613); P1-vv::Ac bz2-m::Ds
T3303D T1-5(5045); P1-vv::Ac bz2-m::Ds
T3303E T1-5(043-15); P1-vv::Ac bz2-m::Ds
T3303F T1-5(5512); P1-vv::Ac bz2-m::Ds
T3303G P1-vv::Ac; T1-6(5495) (6S.80; 1.S.25) bz2-m::Ds
T3303H P1-vv::Ac; T1-6e (6L.21; 1.S.37) bz2-m::Ds
T3303I T1-6(028-13); P1-vv::Ac bz2-m::Ds
T3303J T1-6(7352); P1-vv::Ac bz2-m::Ds
T3303K T1-6(7097); P1-vv::Ac bz2-m::Ds
T3303L T1-7(4405); P1-vv::Ac bz2-m::Ds
T3303M T1-7i; P1-vv::Ac bz2-m::Ds
T3303N T1-7(4837); P1-vv::Ac bz2-m::Ds
T3303O T1-7(010-12); P1-vv::Ac bz2-m::Ds
T3304A T1-8(6591); P1-vv::Ac bz2-m::Ds
T3304B T1-8(4685); P1-vv::Ac bz2-m::Ds
T3304C T1-8(4307-4); P1-vv::Ac bz2-m::Ds
T3304D T1-9(7535); P1-vv::Ac bz2-m::Ds
T3304E T1-9(8302); P1-vv::Ac bz2-m::Ds
T3304F T1-9(6762); P1-vv::Ac bz2-m::Ds
T3304G T1-10g; P1-vv::Ac bz2-m::Ds
T3304H T1-10f; P1-vv::Ac bz2-m::Ds
T3304I bz2-m::Ds
T3304J Inv1m; P1-vv::Ac r1-sc:m3::Ds
T3304K Inv1a; P1-vv::Ac r1-sc:m3::Ds
T3304M T1-2c; P1-vv::Ac r1-sc:m3::Ds
T3305A T1-3(5597); P1-vv::Ac r1-sc:m3::Ds
T3305B T1-4i; P1-vv::Ac r1-sc:m3::Ds
T3305C T1-4(064-20); P1-vv::Ac r1-sc:m3::Ds
T3305F T1-4b; P1-vv::Ac r1-sc:m3::Ds
T3305H T1-5(6899); P1-vv::Ac r1-sc:m3::Ds
T3305J T1-5(4613); P1-vv::Ac r1-sc:m3::Ds
T3305M T1-6(5495); P1-vv::Ac r1-sc:m3::Ds
T3305N T1-6e; P1-vv::Ac r1-sc:m3::Ds
T3305O T1-6(028-13); P1-vv::Ac r1-sc:m3::Ds
T3306C T1-7(4444); P1-vv::Ac r1-sc:m3::Ds
T3306D T1-7(4405); P1-vv::Ac r1-sc:m3::Ds
T3306H T1-8(6591); P1-vv::Ac r1-sc:m3::Ds
T3306L T1-9(8302); P1-vv::Ac r1-sc:m3::Ds
T3306M T1-9(6762); P1-vv::Ac r1-sc:m3::Ds
T3306N T1-10g; P1-vv::Ac r1-sc:m3::Ds
T3307A Ac8178-2S
T3307D Ac8163-3S
T3307F Ac8183-3
T3308A Ac8200-4S
T3308B Ac6076-5L
T3308D Ac8175-5S
T3308E Ac8193-5S
T3308F Ac8179-5L
T3308G Ac8181-5L
T3308H Ac8186-5L
T3309A Ac8196-5L
T3309B Ac6062-6L
T3309C Ac6063-6
T3309D Ac8172-6L
T3309E Ac8184-6
T3310A Ac8161-7
T3310B Ac8173-7L
T3310D Ac8190-7
T3310E Ac8194-7
T3310F Ac8185-7L
T3311A Ac8162-8
T3311B Ac8182-8L
T3311D Ac6059-10S
T3311F Ac8180-10
T3312A Ds-1S1 P1-vv::Ac Dek1
T3312B Ds-1S2 P1-vv::Ac Dek1
T3312C Ds-1S3 P1-vv::Ac Dek1
T3312D Ds-1S4 P1-vv::Ac Dek1
T3312E Ds-1L1 P1-vv::Ac Bz2
T3312F Ds-1L3 Bz2; Ac
T3312G Ds-2S1 B1-Peru; P1-vv::Ac
T3312I Ds-2S3 B1-Peru; P1-vv::Ac
T3312J Ds-2S4; P1-vv::Ac
T3312L Ds-3L1 A1 Sh2; P1-vv::Ac
T3312M Ds-3L2 A1 Sh2; P1-vv::Ac
T3312O Ds-4L1 C2; P1-vv::Ac
T3312P Ds-4L3 C2; P1-vv::Ac
T3312Q Ds-4L4 C2; P1-vv::Ac
T3312S Ds-4L6 C2; P1-vv::Ac
T3312T Ds-4L7 C2; P1-vv::Ac
T3312U Ds-5L1 A2 Pr1 Bt1; P1-vv::Ac
T3312V Ds-5S1 A2 Pr1 Bt1; P1-vv::Ac
T3312W Ds-5S2 A2 Pr1 Bt1; P1-vv::Ac
T3312Y Ds-9S1 C1-I wx1; Ac
T3312Z Ds-10L2 R1-sc; P1-vv::Ac

B-A TRANSLOCATIONS (BASIC SET)

122A TB-1La
122B TB-1Sb
222A TB-1Sb-2L4464
222B TB-3La-2S6270
327A TB-3La
327B TB-3Sb
421A TB-4Sa
423E TB-4Lf
522A TB-5La
522C TB-5Sc
614B TB-6Sa
614C TB-6Lc
717A TB-7Lb
719A TB-7Sc
809A TB-8Lc
922B TB-9Lc Wc1
922D TB-9Sd
X21B TB-10L19
X22A TB-10Sc

B-A TRANSLOCATIONS (OTHERS)

122C TB1-Lc
126G TB-1Sb P1-vv::Ac bz2-m::Ds A1 A2 Bz1 C1 C2 R1
221I TB-2Sa B1-Peru
221J TB-2Sb
225A TB-3La-2L7285
225B TB-1Sb-2Lc
320P TB-1La-3Le
320Q TB-5La-3L(1)
320R TB-5La-3L(2)
320S TB-5La-3L(3)
327C TB-3Lc
327D TB-3Ld
329Z T3-B(La); T3-B(Sb)
331A TB-1La-3L5267
331B TB-1La-3L4759-3
331C TB-1La-3L5242
331E TB-3Lf
331F TB-3Lg
331G TB-3Lh
331H TB-3Li
331I TB-3Lj
331J TB-3Lk
331K TB-3Ll
331L TB-3Lm
420B TB-9Sb-4L6504
420I TB-9Sb-4L6222
421B TB-1La-4L4692
421C TB-7Lb-4L4698
423A TB-4Lb
423B TB-4Lc
423C TB-4Ld
423D TB-4Le
423F TB-1Sb-2L4464-4f
425A TB-4Sg
425B TB-4Lh
425C TB-4Li
428I Dt6 TB-4Sa
522B TB-5Lb
522D TB-5Ld
528D TB-1La-5S8041
614A TB-6Lb
627E TB-6Lc Dt2; A2 C1 C2 R1 a1-m
720A TB-7Lb Dt3; a1-m1::rDt (Neuffer)
806A TB-8La
806B TB-8Lb
921A TB-9La
921B TB-9Sb
921C TB-9Lc
922C TB-9Sb C1-I
929A IsoB9-9 isochromosome Type 1
929B IsoB9-9 isochromosome Type 2
929C T9-B(La); T9-B(Sb)
929D IsoB9-9 isochromosome (original)
929F T9-B (La + Sb)
929G TB-9Sb; T9-8(4453)
929H TB-9Sb; T9-3(6722)
929I TB-9Sb-1866
929J TB-9Sb-1852
929K TB-9Sb-2150
929L TB-9Sb-14
929M TB-9Sb-2010
TX40D TB-1Sb P1-vv::Ac r1-sc:m3::Ds
TX40E TB-3La a1-m Dt1
TX40F TB-8Lc Ac2 bz2-m::Ds
TX40G TB-9Sd a1-m Dt1
TX40H TB-9Lc Ac8168-9 r1-sc:m3::Ds
TX40I TB-10L18 P1-vv::Ac r1-sc:m3::Ds
X21A TB-10La
X21C TB-10Ld
X22B T1La-B-10L18
X22C TB-10Lb
X30A TB-10L1
X30B TB-10L2
X30C TB-10L3
X30D TB-10L4
X30E TB-10L5
X30F TB-10L6
X30G TB-10L7
X31A TB-10L8
X31B TB-10L9
X31C TB-10L10
X31D TB-10L11
X31E TB-10L12
X31G TB-10L14
X31H TB-10L15
X31I TB-10L16
X31J TB-10L17
X32A TB-10L18
X32C TB-10L20
X32D TB-10L21
X32E TB-10L22
X32F TB-10L23
X32G TB-10L24
X32H TB-10L25
X32I TB-10L26
X32J TB-10L27
X32K TB-10L28
X33A TB-10L29
X33B TB-10L30
X33C TB-10L31
X33D TB-10L32
X33E TB-10L33
X33F TB-10L34
X33G TB-10L35
X33H TB-10L36
X34A TB-10L37
X34B TB-10L38

INVERSION

I143A Inv1a (1.S.30; 1.L.50)
I143B Inv1c (1.S.30; 1.L.01)
I143C Inv1d (1.L.55; 1.L.92)
I143D Inv1k (1.L.46; 1.L.82)

I243A Inv2b (2S.06; 2L.05)
I243B Inv2h (2L.13; 2L.51)
I444A Inv2a (2S.70; 2L.80)
I343A Inv3a (3L.38; 3L.95)
I343B Inv3b (3L.21; 3L.70)
I343C Inv3c (3L.05; 3L.95)
I343D Inv3(8582) (3S.55; 3L.82)
I443A Inv4b (4S.10; 4L.12)
I443B Inv4c (4S.89; 4L.62)
I443C Inv4a (4L.30; 4L.90)
I443D Inv4d (4L.40; 4L.96)
I443E Inv4f (4L.17; 4L.63)
I543A Inv4e (4L.16; 4L.81)
I543B Inv5a (5S.05; 5L.72)
I743A Inv5(8623) (5S.67; 5L.69)
I743B Inv6d (6S.70; 6L.33)
I743C Inv6(3712) (6S.76; 6L.63)
I743D Inv6a (6S.76; 6L.63)
I843A Inv6e (6S.80; 6L.32)
I943A Inv7f (7L.17; 7L.61)
I943B Inv7(8540) (7L.12; 7L.92)
I943C Inv7(3717) (7S.32; 7L.30)
I943E Inv7a (7L.05; 7L.95)
IX43A Inv8a (8S.30; 8L.15)
I344A Inv9a (9S.70; 9L.90)
IX43B Inv9b (9S.05; 9L.87)

RECIPROCAL TRANSLOCATIONS (wx1 AND Wx1 MARKED)

wx01A T1-9c (9L.22; 1.S.48); wx1
wx01B T1-9(5622) (9L.12; 1.L.10); wx1
wx02A T1-9(4995) (9S.20; 1.L.19); wx1
wx02AA T1-9(4995) (9S.20; 1.L.19); wx1
wx03A T1-9(8389) (9L.13; 1.L.74); wx1
wx04A T2-9c (9S.33; 2S.49); wx1
wx05A T2-9b (9L.22; 2S.18); wx1
wx06A T2-9d (9L.27; 2L.83); wx1
wx07A T3-9(8447) (9L.14; 3S.44); wx1
wx08A T3-9c (9L.12; 3L.09); wx1
wx09A T3-9(8562) (9L.22; 3L.65); wx1
wx10A T4-9e (9L.26; 4S.53); wx1
wx11A T4-9g (9L.27; 4S.27); wx1
wx12A T4-9(5657) (9S.25; 4L.33); wx1
wx13A T4-9b (9L.29; 4L.90); wx1
wx14A T5-9c (9L.10; 5S.07); wx1
wx14B T5-9(022-11) (9L.27; 5S.30); wx1
wx15A T5-9(4817) (9S.07; 5L.06); wx1
wx16A T5-9d (9L.10; 5L.14); wx1
wx17A T5-9a (9S.17; 5L.69); wx1
wx18A T6-9(4778) (9L.30; 6S.80); wx1
wx19A T6-9a (9L.40; 6S.79); wx1
wx19B T6-9e (9L.24; 6L.18); wx1
wx20A T6-9b (9S.37; 6L.10); wx1 y1
wx21A T6-9(4505) (9ctr.00; 6L.13); wx1
wx22A T7-9(4363) (9ctr.00; 7ctr.00); wx1
wx23A T7-9a (9S.07; 7L.63); wx1
wx24A T8-9d (9S.16; 8L.09); wx1
wx25A T8-9(6673) (9S.31; 8L.35); wx1
wx26B T9-10(059-10) (9S.31; 10L.53); wx1
wx27A T9-10b (9S.13; 10S.40); wx1
Wx30A T1-9c (9L.22; 1.S.48); Wx1
Wx30B T1-9(4995) (9S.20; 1.L.19); Wx1
Wx30C T1-9(8389) (9L.13; 1.L.74); Wx1
Wx31A T2-9c (9S.33; 2S.49); Wx1
Wx31B T2-9b (9L.22; 2S.18); Wx1
Wx31C T2-9d (9L.27; 2L.83); Wx1
Wx32A T3-9(8447) (9L.14; 3S.44); Wx1
Wx32B T3-9(8562) (9L.22; 3L.65); Wx1
Wx32C T3-9c (9L.12; 3L.09); Wx1
Wx33A T4-9e (9L.26; 4S.53); Wx1
Wx33B T4-9(5657) (9S.25; 4L.33); Wx1
Wx33C T4-9g (9L.27; 4S.27); Wx1
Wx34A T5-9c (9L.10; 5S.07); Wx1
Wx34B T5-9(4817) (9S.07; 5L.06); Wx1
Wx34C T4-9b (9L.29; 4L.90); Wx1
Wx35A T5-9(8386) (9S.13; 5L.87); Wx1
Wx35B T5-9a (9S.17; 5L.69); Wx1
Wx35C T5-9d (9L.10; 5L.14); Wx1
Wx36A T6-9(4778) (9L.30; 6S.80); Wx1
Wx37A T6-9(8768) (9S.61; 6L.89); Wx1
Wx37B T7-9(4363) (9ctr.00; 7ctr.00); Wx1
Wx37C T6-9(4505) (9ctr.00; 6L.13); Wx1
Wx38A T7-9a (9S.07; 7L.63); Wx1
Wx38B T8-9d (9S.16; 8L.09); Wx1
Wx38C T8-9(6673) (9S.31; 8L.35); Wx1
Wx39A T9-10(8630) (9S.28; 10L.37); Wx1
Wx39B T9-10b (9S.13; 10S.40); Wx1

PHENOTYPE ONLY

Kernel Mutants

blotched aleurone
Bh*-86-1381-1
Bh-Tu*-Mumm

brittle endosperm
bt*-011-11
bt*-1979-14
bt*-1979-16
bt*-1982
bt*-4380
bt*-4539
bt*-4973
bt*-60-151
bt*-8101
bt*-8102
bt*-83-84-3541-1
bt*-84-4
bt*-84-5
bt*-84-5091-9
bt*-84-5257-1
bt*-84-6
bt*-85-3096-6
bt*-85-3098-15
bt*-85-3099-16
bt*-85-3372-27
bt*-87-2132-39
bt*-87-2297-1
bt*-87-88-2630-28
bt*-88-3177-14
bt*-88-3177-2
bt*-88-3177-7
bt*-8804
bt*-8805
bt*-89-1265-18
bt*-90286
bt*-A4109
bt*-Briggs-1998-1
bt*-F-15
bt*-F-23
bt*-F-31
bt*-F-34
bt*-F-36
bt*-F-8
bt*-F10
bt*-Panzio
bt*-PetersonResHy
bt*-PI200197
bt*-PI251887
bt-gm*-84-5045-39
bt-gm*-85-3017-24
bt-sh*-PI251930

brown endosperm
brn*-1981-1
brn*-1981-2
brn*-1981-3
brn*-1981-4
brn*-84-23
brn-bt*-81-F-24

brown kernel
lt-brn-sml*-86-1302-37
bnk*-N747B

brown pericarp
bp*-Coates
bp*-Lima100
bp*-Lima94
bp*-PI183639

collapsed endosperm
cp*-N1076A
cp*-N1078B
cp*-N1092A
cp*-N1104B
cp*-N1275A
cp*-N1294
cp*-N1311C
cp*-N1313
cp*-N1319A
cp*-N1338
cp*-N1369
cp*-N1379A
cp*-N1385
cp*-N1393A
cp*-N1399A
cp*-N1430
cp*-N1436A
cp*-N2356B
cp*-N524E
cp*-N628
cp*-N863A
cp*-N886
cp*-N918A
cp*-N937A
cp*-N968A
cp*-N991

colored plumule
Pu*-1976-RYDCO

colorless aleurone
cl*-85-86-3559-1
cl*-86-1478-16
cl*-N1345A
cl*-N1346A
cl*-N720E
cl*-N795
cl*-N801
cl*-N818A
cl-crown-pale-base*-85-86-3558-23
r*-86-1590-6

colorless floury
clf*-N2425B

crumpled kernel
crp*-N1429A
crp*-N2207
dnj*-N1534

defective crown
dcr*-N1053A
dcr*-N1176B
dcr*-N1233A
dcr*-N1409
dcr*-N871A
dcr*-N925A

defective kernel
de*-1276
de*-17
De*-1976-RYDCO
de*-2080
de*-2192
de*-2424
de*-2915
de*-2919
de*-3188
de*-4309
de*-5044Hagie
de*-85-86-3567-35
de*-8505
de*-8507
de*-8508
de*-86-1472-6
de*-8808
de*-8809
de*-8810
de*-8811
de*-8818
de*-N1002A
de*-N1007A
de*-N1057B
de*-N1122A
de*-N1136A
de*-N1162
de*-N1166
de*-N1177A
de*-N1196
de*-N1310B
de*-N1336B
de*-N1345B
de*-N1390A
de*-N1400
de*-N1420
de*-N232B
de*-N260D
de*-N279B
de*-N296C
de*-N307D
de*-N400A

de*-N408D
de*-N513B
de*-N528C
de*-N540A
de*-N573A
de*-N660C
de*-N674A
de*-N748B
de*-N760B
de*-N877A
de*-N891A
de*-N902A
de*-N903
de*-N929
de*-N932
de*-N970A
de*-N979A
de-sml*-8813
de-sml*-8814
de-sml*-8815
de-sml*-8816
de-sml*-8817
def*-8101
def*-8102
def*-8103
def*-8104
def*-8105
def*-8106
def*-8107
def*-8108
def*-8109
def*-8110
def*-8111
def*-8112
def*-8113
def*-8114
def*-8116
def*-8118
def*-8119
def*-8120
def*-8121
def*-8122
def*-8123
def*-8125
def*-8126
def*-8127
def*-8128
def*-8130
def*-8131
def*-8132
def*-8134
def*-8136
def*-8137
def*-8138
def*-8201
def*-84-22
def*-84-28
def*-84-29
def*-84-30
def*-84-31
def*-84-37
def*-84-40
def*-84-41
def*-84-45
def*-84-48
def*-84-49
def*-84-53
def*-84-54
def*-84-58
def*-84-60
dek*-1979-32
dek*-1981-1
dek*-74-0060-4
dek*-84-14
dek*-86-1496-35
dek*-8902
dek*-8903
dek*-8904
dek*-99-6273-1
dek*-F-16
dek*-PS602
wrinkled-de*-86-1473-5
wrinkled-gm*-86-1582-32

dented kernel
dnt*-N1185A
dnt*-N1326
dnt*-N884A

dilute aleurone
dil*-N452D
dil*-N524C
dil*-N743A

discolored kernel
dsc*-N1084
dsc*-N749
pig*-84-5080-18
pig*-86-1178-6
pig-gm*-1979-51
pig-gm*-1979-52
pig-gm*-1979-9
pig-gm*-1981-A
pig-gm*-1981-B
pig-gm*-1982-3
pig-gm*-5020-14
pig-gm*-84-5078-10
pig-gm*-86-1200-3
pig-gm*-87-2275-15
pig-gm*-87-2305-22
pig-gm*-Briggs 1998-1
pig-gm*-Briggs 1998-2
pig-gm*-PI251930
ptd-dek*-1976-RYDCO
ptd-dek*-1981
ptd-dsc*-87-2490-22
sml-pig-gm*-88-89-3554-44

dull endosperm
du*-Sprague

etched endosperm
et*-3130
et*-3576
et*-5191
et*-6-9321-1
et*-73-766-1
et*-8-M-4
et*-84-5266-26
et*-84-5270-40
et*-85-86-3518-21
et*-86-1493-6
et*-8616
et*-87-2349-13
et*-88-89-3525-22
et*-88-89-3554-33
et*-89-90-1547-19
et*-89-90-1548-13
et*-Mu1767
et*-Mu2349
et-mutable*-87-2519-31
et*-N1078A0
et*-N1361
et*-N164B
et*-N185B
et*-N357C
et*-N403A
et*-N489A
et*-N509A
et*-N514A
et*-N516C
et*-N518B
et*-N561B
et*-N571A
et*-N586A
et*-N615A
et*-N617
et*-N629F
et*-N643A
et*-N670A
et*-N680C
et*-N701A
et*-N702A
et*-N723A
et*-N724D
et*-N745
et*-N76D
et*-N789
et*-N798A
et*-N818B
et*-N837A
et*-N861
et*-N864A
et*-N868A
Et*-N876A
et*-N953A
et*-N965
et*-Osturana
et-de*-88-89-3526-8
et-gm*-86-1475-34
et-gm*-86-87-1742-38
et-gm*-87-2502-19
granular-o*-84-5274-30
sml-et*-85-3522-29
su-sh-et*-98-1887-1

flint kernel
flint*-87-2126-22

floury endosperm
fl*-67-412
fl*-78-513
fl*-83-3386-19
fl*-84-44
fl*-8515
fl*-Mojo
fl*-N1145A
fl*-N1163
fl*-N1208A
fl*-N1287
fl*-N1308A
fl*-N1333B
fl*-N1426
fl*-N7B-65-1294
fl*-N872A
fl*-shoepeg
fl*-sucaxo
fl-cap*-1981
fl-cap*-66-519-1
fl-de*-8905
sml-fl-cap*-1981

germless
brn-gm*-85-3315-6
brn-gm*-85-86-3587-46
brn-gm*-85-86-3595-3
brn-gm*-86-1161-5
emb*-85-3100-32
emb*-85-3378-8
gm*-1387
gm*-1979-11
gm*-1979-53
gm*-5234
gm*-6372
gm*-84-5087-4
gm*-8510
gm*-86-1011-2
gm*-86-1013-4
gm*-86-1097-3
gm*-86-1335-1
gm*-86-1591-7
gm*-86-87-1742-18
gm*-87-2456-9
gm*-N1303
gm*-N1311B
gm*-N1312
gm*-N1319B
gm*-N1390C
gm*-N198C
gm*-N869A
gm*-N928B
o-gm*-84-44
o-gm*-98-5733-1
pr-gm*-86-1109-1
sh-gm*-84-5045-32
sh-gm*-88-3082-4
sml-o-gm*-86-1323-4
sml-dsc-gm*-95W-240
w-o-gm*-85-3135-4
w-o-gm*-86-1349-1
w-o-gm*-88-3270-10
y-gm*-85-3288-28

glassy endosperm
ae*-6921
ae*-84-7
ae*-92-1365-3
ae*-96-1449-1
ae*-Briggs 1998-1
ae*-Mu32

lemon white
lw*-1979-45
lw*-1979-46
lw*-1981
lw*-1981-10
lw*-1998-1
lw*-73-2548
lw*-82-1
lw*-85-3076-28
lw*-85-3252-5
lw*-86-87-1828-7
lw*-88-3177-2
lw*-89-90-3609-5
lw*-87-2407-36
lw*-B73
lw-y-pg*-1998-4
lw-y-pg*-Funk-81-5
lw-y-pg*-PI200303
pale-y*-83-84-3549-13
pale-y*-84-5082-33
pale-y*-84-5167-48
pale-y*-84-5288-19
pale-y*-85-3005-22
pale-y*-85-3006-30
pale-y*-85-3007-40
pale-y*-85-3010-40
pale-y*-85-3016-15
pale-y*-85-3017-31
pale-y*-85-3065-25
pale-y*-85-3069-6
pale-y*-85-3087-29
pale-y*-85-3377-2
pale-y*-86-1155-3
pale-y*-88-89-3551-35
pale-y*-89-1313-3

pale-y*-89-90-1525-23
pale-y*-90-3220-1
pale-y*-90-3220-26
w*-N677
w*-N70
wh*-BMS-Rhoades
y-pg*-1981-17
y-pg*-84-5275-14
y-pg*-85-3042-7
y-pg*-85-3044-34
y-pg*-85-3078-41
y-pg*-85-3562-31
y-pg*-85-86-3533-9
y-pg*-86-1151-7
y-pg*-86-87-1723-27
y-pg*-87-2160-16

marbled aleurone
Dap*-3
dap*-86-8126-2
Dap*-89-3177.0
Dap*-89-3177.5
Dap*-89-3178.3
Marbled*-Sprague

miniature kernel
de*-N663C
mn*-1981-51
Mn*-866248U
mn*-87-2215-17
mn*-87-2346-20
mn*-87-2347-36
mn*-87-2422-14
mn*-88-3177-2
mn*-88-89-3509-40
mn*-88-89-3564-25
mn*-N1536
Mn*-N273C
mn*-N378C
mn*-PI239110
mn*-PI245132
sml-k*-97-4784-1

mosaic aleurone color
msc*-N593A

mottled aleurone
Mt*-2313
Mt*-65-2238
Mt*-N1343A
Mt*-Sprague

multiple aleurone layer
Mal*-Galinat
Mal*-Nelson
Mal*-PI515052

opaque endosperm
lrg-o-crown*-89-1275-17
o*-1979-54
o*-1981-11-Fox-19
o*-1981-3-Fox-7
o*-1981-5-Fox-9
o*-1981-6-Fox-10
o*-1981-8-Fox-15
o*-1982
o*-1982-2-Fox-13
o*-2-Fox-6
o*-3015
o*-73-798-1
o*-76GH-76
o*-8129
o*-82:288-1
o*-83-84-3549-39
o*-84-5025-15
o*-84-5025-17
o*-84-5025-8
o*-84-5044-35
o*-84-5091-13
o*-84-5094-4
o*-84-5095-23
o*-84-5117-16
o*-84-5261-37
o*-84-5270-40
o*-84-5282-27
o*-84-5295-13
o*-84-5321-28
o*-84-5324-29
o*-84-8a
o*-85-3084-8
o*-85-3088-3
o*-85-3335-35
o*-86-87-1767-10
o*-87-2285-33
o*-87-2350-2
o*-88-89-3550-27
o*-97-4784-6
o*-Briggs-1998-1
o*-BS20-Fox-3
o*-Fox-12
o*-N1008A
o*-N1037A
o*-N1039
o*-N1046
o*-N1065A
o*-N1074A
o*-N1119A
o*-N1189A
o*-N1195A
o*-N1218
o*-N1228
o*-N1244A
o*-N1245
o*-N1298
o*-N1301
o*-N1310A
o*-N1320A
o*-N1355
o*-N1358
o*-N1422
o*-N436C
o*-N696A
o*-N829C
o*-N870
o*-N885A
o*-N895
o*-N899
o*-N906A
o*-N908
o*-N915
o*-N930
o*-N938A
o*-N969A
o*-N973
o*-N989
o*-N995A
o*-N996
o*-PI195245
o*-PI200285
o-de*-1981-9-Fox-18
o-dek*-6
o-dek*-87-2279-12
o-gm*-83-3398-6
o-gm*-84-33
o-sh*-86-1297-2
o-sh*-F1979-19
os*-2162
pro*-Mu1
sh-o*-87-2455-7
sml-o*-87-88-2692-5
sml-o*-PI195243

pale aleurone
pa*-N893A
pa*-N917A
pale-Cl*-86-1476-14
pale-Cl*-LGC65
pale-Cl-gm*-84-5251-1

pale aleurone, with pigmented sectors
pa-Cl*-m-86-1474-39
pa-Cl*-m-86-1478-4
pa-Cl*-m-87-2224-33

pale crown
pa-crown*-85-86-3558-23

pale yellow endosperm
lw*-8509
lw*-8513
lw*-8514
pale-endo*-73-3
pale-endo*-73-4004
pale-y*-83-3382-16
pale-y*-83-3382-18
pale-y*-83-84-3548-25
pale-y*-84-5103-16
pale-y*-85-3016-30
pale-y*-85-3036-38
pale-y*-85-3134-46
pale-y*-85-3374-13
pale-y*-85-3511-18
pale-y*-86-1155-2
pale-y*-87-2339-10
pale-y*-87-2350-2
pale-y*-87-2350-25
pale-Y*-87-2422-14
pale-y*-87-88-2679-1
pale-y-gm*-Rsssc-77-110
pale-y-o*-84-5288-2
pale-y-o*-86-1296-27
y*-84-5272-12
y*-84-5288-1
y*-85-3041-2
y*-85-3087-12
y*-85-3125-7

pitted kernel
ptd*-N1425A
ptd*-N660E
ptd*-N738B
ptd*-N855A
ptd*-N901A
ptd*-N923

purple pericarp
Pl*-CFS-69

red aleurone
pr*-N707A
pr*-N850

red pericarp
r*-ch-Burbank-CFS-80
r*-ch-PI213730

red silk scar
red-silk-scar*-MTC

rough kernel
rgh*-N1060
rgh*-N1524
rgh*-N799A
rgh*-N802
rgh*-N882

shrunken kernel
pale-y-su-sh*-88-3133-28
sh*-1979-10
sh*-1981-14
sh*-1982-2
sh*-2927-Mumm
sh*-2928-Mumm
sh*-83-3328-24
sh*-84-3
sh*-84-5248-20
sh*-84-5317-44
sh*-85-3045-7
sh*-85-3104-27
sh*-85-3112-20
sh*-85-3375-38
sh*-8502
sh*-8503
sh*-8506
sh*-8511
sh*-8517
sh*-86-1565-17
sh*-87-2045-25
sh*-87-2045-6
sh*-87-2050-1
sh*-87-2050-3
sh*-87-2213-19
sh*-87-2215-12
sh*-87-2355-29
sh*-87-2406-3
sh*-87-2496-21
sh*-88-89-3540-1
sh*-8806
sh*-8807
sh*-8906
sh*-8907
sh*-97P-29-5
sh*-F-11
sh*-F-2
sh*-F-25
sh*-KERR
sh*-N1105B
sh*-N1320B
sh*-N1341
sh*-N1366
sh*-N1519B
sh*-N1969
sh*-N208C
sh*-N252B
sh*-N399A
sh*-N627A
sh*-N689
sh*-N741
sh*-N742
sh*-N750
sh*-N785A
sh*-N819
sh*-N849
Sh*-N881A
sh*-N887A
sh*-N911
sh*-N961
sh*-PI596356
sh*-RJL
sh-bt*-85-3392-31
sh-crown*-Briggs-1998-1
sh-de*-6607
sh-de*-RSSSC-117
sh-fl*-9180
sh-fl*-9392

sh-o*-87-2410-24
sh-wx*-F-18
su-sh*-F-5

small kernel
smk*-N1003
smk*-N1057A
smk*-N1168A
smk*-N1203
smk*-N1529
smk*-N215D
smk*-N320
smk*-N433A
Smk*-N845B
smk*-N890A
smk*-N942
smk*-N994A

spotted aleurone
cl-mut*-85-86-3564-1
cl-mut*-97-4782-9
cl-mut*-99-2170
coarse-mutable*-86-1417-7
Dt*-a; a1-m
Dt*-b; a1-m
Dt*-c; a1-m
Dt*-d; a1-m
Dt*-e; a1-m
Dt*-f; a1-m
Dt*-g; a1-m
Dt*-h; a1-m
Dt*-i; a1-m
fine mut*-86-1283-45
spk*-N551A
spk*-N600Ce
spk*-N687A

sugary kernel
su*-1979-8
su*-83-3383-21
su*-84-5350-2
su*-85-3133-32
su*-8504
su*-8803
su*-89-1279-14
su*-L874261
su*-N1040
su*-N236C
su*-N748A
su*-N817
su-sh*-F-22

viviparous kernel
pale-vp*-87-2286-1
pale-vp*-87-2286-18
pale-vp*-87-2286-2
pale-vp*-87-2286-25
pale-vp*-87-2286-3
pale-y*-84-5027-22
pale-y*-84-5032-21
pale-y-vp*-83-3100-31
pale-y-vp*-83-3124-33
pale-y-vp*-84-5266-5
pale-y-vp*-85-3140-15
pale-y-vp*-85-3240-5
pale-y-vp*-85-3267-6
pale-y-vp*-85-3267-9
pale-y-vp*-85-3385-34
pale-y-vp*-86-1316-27
pale-y-vp*-88-3177-14
ps*-85-3288-28
ps*-85-3492-36
ps*-85-86-3567-1
ps*-86-1105-2
ps*-86-1352-4
ps*-86-1499-3
ps*-86-87-1742-18
ps*-89-90-1588-37
ps*-90-3222-27
ps*-90-91-8549-7
ps*-96-5032-6
ps*-98-5691-5
ps*-99-2157-1
ps*-Mu85-3061-21
ps*-Mu86-1105-1
vp(ps)*-86-1449-3
vp(ps)*-86-1565-17
vp*-0118
vp*-0315
vp*-2-8c
vp*-2000PR-1
vp*-73-30173
vp*-8101
vp*-8104
vp*-8106
vp*-8107
vp*-8108
vp*-8109
vp*-8110
vp*-8111
vp*-8112
vp*-8113
vp*-8114
vp*-8115
vp*-8116
vp*-8117
vp*-8201
vp*-8203
vp*-8204
vp*-8208
vp*-8209
vp*-8210
vp*-8211
vp*-84-5079-29
vp*-84-5279-29
vp*-84-5315-29
vp*-8418
vp*-8420
vp*-85-3011-11
vp*-85-3017-9
vp*-85-3040-29
vp*-85-3042-7
vp*-85-3099-16
vp*-85-3135-4
vp*-85-3182-6
vp*-85-3250-1
vp*-85-3339-25
vp*-85-3422-13
vp*-85-86-3567-20
vp*-86-1109-1
vp*-86-1407-15
vp*-86-1573-27
vp*-87-2146-18
vp*-87-2213-19
vp*-87-2224-3
vp*-87-2274-37
vp*-87-2299-1
vp*-87-2339-1
vp*-88-89-3555-1
vp*-88-89-8625-5
vp*-89-1181-8
vp*-89-1279-14
vp*-89-90-1561-18
vp*-93-1017-2
vp*-95-2086-1
vp*-Funk-8101
vp*-N702C
vp*-PI183642
vp*-PI185847
vp*-PI200204
vp*-PI254854
vp*-PI430482
vp-de*-87-2406-23
vp-Y*-86-1267-31
vp-Y*-86-1361-7
w-vp*-84-5020-4
w-vp*-85-3014-6
w-vp*-85-3304-13
w-vp*-91-1859-8
w-vp*-91-2544-7
w-vp*-92-1408-1
y-vp*-0730
y-vp*-1982-1
y-vp*-1982-2
y-vp*-2062-Coop
y-vp*-60-153
y-vp*-6961
y-vp*-73-2656
y-vp*-80-6118
y-vp*-81-5
y-vp*-8102
y-vp*-8103
y-vp*-8105
y-vp*-8206
y-vp*-8207
y-vp*-83-1A
y-vp*-83-3101-36
y-vp*-8336
y-vp*-84-13
y-vp*-8419
y-vp*-85-3572-30
y-vp*-8512
Y-vp*-87-2339-10
y-vp*-87-2340-36
y-vp*-8701
y-vp*-88-89-3563-33
y-vp*-88-89-3613-25
y-vp*-99-2226-1

waxy endosperm
wx*-0208
wx*-98-1406-6
wx*-N66C

white cap kernel
Wc*-1982-1
Wc*-Funk-81-22
Wc*-Funk-81-23
wc*-87-2307-1
Wc*-DC
wc*-N1349

white endosperm
y*-1981
y*-1981-14
y*-1981-18
y*-1982-3
y*-73-2
y*-73-2262-1
y*-73-2262-2
y*-73-2394
y*-73-324-1
y*-73-4035
y*-73-426
y*-84-8b
y*-87-2201-3
y*-Funk-81-12
y*-Funk-81-13
y*-Funk-81-2
y*-Funk-81-20
y*-Funk-81-9
y*-Sprague
y*-syn-DOCI
y*-Williams-60-154

wrinkled kernel
wr*-N1389A
wr*-N156C
wr*-N612A

Seedling Mutants

aberrant seedling
abbt*-N399B
abbt*-N454C
abbt*-N594B
abbt*-N595B
abbt*-N712B

adherent leaf
ad*-87-2285-18
ad*-N111
ad*-N194
ad*-N1958
ad*-N253
ad*-N273B
ad*-N316
ad*-N377B
ad*-N512B
ad*-N551B
ad*-N556D
ad*-N582
ad*-N605B
ad*-N640
ad*-N664
ad*-N682B
ad*-N877B
ad*-N984B

albino seedling
nlw*-85-3357-17
peach-albino-mutable*-87-2209-30
peach-albino*-N1983B
w*-002-12
w*-005-19
w*-009-6
w*-010-4
w*-011-11
w*-017-14-A
w*-017-14-B
w*-020-9
w*-034-16
w*-037-14
w*-039-15
w*-2065
w*-2246
w*-3858
w*-4670
w*-4873
w*-5201
w*-5255
w*-5267
w*-56-3003-12
w*-5602
w*-5622
w*-5787
w*-5863
w*-6293
w*-6504
w*-6575
w*-7165
w*-7219
w*-7281
w*-74-1674-1
w*-78-297-3
w*-8105W

w*-8129
w*-8147
w*-8201
w*-84-5205-46
w*-84-5222-30
w*-85-3359-11
w*-85-3552-25
w*-85-3559-30
w*-8529
w*-8549
w*-8569
w*-86-1078-6
w*-86-1265-30
w*-86-2222-5
w*-8630
w*-8635
w*-8637
w*-8670
w*-87-2215-8
w*-8925
w*-8963
w*-8970
w*-8977
w*-8992
w*-9235
w*-B-75
w*-BYD
w*-Canario Hembrilla Enano
w*-Fino
w*-MontenegrinFlint
w*-N103
w*-N109
w*-N115
w*-N126A
w*-N137D
w*-N145
w*-N147B
w*-N167
w*-N176
w*-N178A
w*-N1834
w*-N1839
w*-N1854
w*-N1865
w*-N1890
w*-N1909
w*-N191
w*-N1915
w*-N192
w*-N21A
w*-N22
w*-N220
w*-N23
w*-N24
w*-N278A
w*-N285
w*-N304A
w*-N318
w*-N332
w*-N335
w*-N346A
w*-N355
w*-N364
w*-N367B
w*-N404
w*-N405A
w*-N413B
w*-N42
w*-N428B
w*-N430B
w*-N436A
w*-N456A
w*-N457
w*-N491B
w*-N5
w*-N509B
w*-N516A
w*-N524A
w*-N532
w*-N536A
w*-N540B
w*-N547A
w*-N558B
w*-N563A
w*-N569B
w*-N574
w*-N58
w*-N587C
w*-N593B
w*-N6
w*-N613C
w*-N621B
w*-N627B
w*-N67A
w*-N682A
w*-N704
w*-N708A
w*-N727A
w*-N729A
w*-N736A
w*-N77
w*-N804B
w*-N829B
w*-N883B
w*-N917B
w*-PI184276
w*-PI193438
w*-PI201543
w*-PI213747
w*-PI228176
w*-PI228179
w*-PI228183
w*-PI232961
w*-PI232965
w*-PI232968
w*-PI232972
w*-PI239103
w*-PI239110
w*-PI251009
w*-PI251885
w*-PI251930
w*-PI251932
w*-PI254851
w*-PI267162
w*-PI267179
w*-PI267204
w*-Singleton-16
w*-Singleton-22
w*-Singleton-24
w*-Singleton-25
w*-Singleton-31
w*-Tama
w*-wh-mut
wh*-053-4
wh*-2083
wh*-89-578-6

bilateral coleoptile
blc*-N743C

blue flourescence-leaves
bf*-99-2070-8

clasping leaf
clsp*-87-2320-9
clsp*-88-89-3522-1

distorted
dst*-N444A

ectopic coleoptile
ect*-N641B

flecked leaf
flk*-N403C
flk*-N527A
flk*-N564B
flk*-N570A
flk*-N630B
flk*-N653A

glossy leaf
gl*-1-3(5476)
gl*-218-1
gl*-32TaiTaiTaSarga
gl*-4339
gl*-5201
gl*-5249
gl*-56-3023-6
gl*-56-3023-9
gl*-56-3036-7
gl*-6
gl*-60-2484-8
gl*-63-2440-8
gl*-85-3095-12
gl*-8654
gl*-87-2215-8
gl*-87-2215-30
gl*-87-2278-34
gl*-88-3142-4
gl*-97P-261-5
gl*-Bizika
gl*-gl12
gl*-LGC-117
gl*-LGC-27
gl*-Loesch
gl*-Manglesdorf
gl*-Moritsa
gl*-N168
gl*-N203C
gl*-N356
gl*-N546C
gl*-N616A
gl*-N656A
gl*-N681A
gl*-N696E
gl*-PI184286
gl*-PI200203
gl*-PI228177
gl*-PI232974
gl*-PI239101
gl*-PI239110
gl*-PI251885
gl*-PI251933
gl*-PI262474
gl*-PI262476
gl*-PI262494
gl*-PI262500
gl*-PI267203
gl*-PI267209
gl*-PI267212
gl-nec*-N516D
gl*-STL

gravity non-responsive primary root
agt*-N491C

high chlorophyll fluorescence
hcf*-88-3005-3

luteus yellow seedling
l*-009-6
l*-017-3
l*-025-4
l*-062-3
l*-2215
l*-2673
l*-4356
l*-4545
l*-4871
l*-5-9b[X-7-39]
l*-549-1 Derived Flint
l*-56-3003-12
l*-570-2 Cincantin
l*-5783-straw
l*-62-489-2
l*-6474
l*-6923
l*-6973
l*-7165
l*-7281
l*-73-563
l*-77-564-2
l*-7748
l*-8321
l*-8376
l*-84-5205-13
l*-84-5225-33
l*-8495
l*-85-3215-2
l*-85-3225-4
l*-85-3457-40
l*-85-3513-1
l*-85-3541-20
l*-86-1112-1
l*-86-1354-9
l*-8613
l*-8634
l*-88-89-3555-13
l*-89-90-1552-10
l*-8966
l*-8970
l*-d-8694
l*-leng
l*-LGC-43
l*-Moritza (Bulgaria)
l*-N104
l*-N113
l*-N119
l*-N124B
l*-N129
l*-N137B
l*-N140
l*-N171A
l*-N175
l*-N1806B
l*-N1838
l*-N1878
l*-N188A
l*-N1908
l*-N1920
l*-N195
l*-N209
l*-N218
l*-N231
l*-N251
l*-N31
l*-N336
l*-N347
l*-N368B
l*-N392A
l*-N416A
l*-N438A
l*-N496B

l*-N52
l*-N523
l*-N606
l*-N612B
l*-N62
l*-N691A
l*-N703
l*-N730
l*-PI183642
l*-PI183643
l*-PI193433
l*-PI193435
l*-PI193436
l*-PI195245
l*-PI213737
l*-PI213745
l*-PI218038
l*-PI228183
l*-PI239110
l*-PI239114
l*-PI251884
l*-PI254854
l*-PI254856
l*-PI262495
l*-PI267215
l*-PI267226
l*-Rumanian Flint
l*-Tama
l-nec*-2001-519
l*-y wx 6-9b
pyg*-N761
y-l*-85-3234-6
y-l*-8910 Briggs
yd*-87-2278-34
yel*-5344
yel*-8721
yel*-8793
yg*-8962

orobanche
oro*-6577
oro*-69-9291-8
oro*-84-5080-15
oro*-85-3087-3
oro*-85-3106-41
oro*-85-3113-11
oro*-88-3237-31
oro*-88-89-3550-32

pale green seedling
pas*-90-3222-13
pg*-2142
pg*-5619
pg*-6372
pg*-69-5079-2
pg*-6923
pg*-7122
pg*-8129
pg*-84-5234-29
pg*-8412
pg*-8911
pg*-8959
pg*-Caspar
pg*-Fino
pg*-N102
pg*-N11
pg*-N12
pg*-N123C
pg*-N124A
pg*-N127
pg*-N1389B
pg*-N146A
pg*-N147A
pg*-N150A
pg*-N155A
pg*-N156B
Pg*-N1604
pg*-N161
pg*-N181
pg*-N1821
pg*-N1822A
pg*-N1866
pg*-N1881
pg*-N1885
pg*-N1983
pg*-N213
pg*-N215B
pg*-N222
pg*-N272C
pg*-N296A
pg*-N338A
pg*-N346B
pg*-N349
pg*-N35
pg*-N357B
pg*-N36A
pg*-N361A
pg*-N362A
pg*-N375B
pg*-N379
pg*-N380
pg*-N381
pg*-N384B
pg*-N40
pg*-N408C
pg*-N417A
pg*-N421
pg*-N429B
pg*-N445
pg*-N45A
pg*-N452C
pg*-N459
pg*-N46
pg*-N469
pg*-N481
pg*-N506A
pg*-N507A
pg*-N511
pg*-N514B
pg*-N524B
pg*-N526C
pg*-N550
pg*-N556B
pg*-N558A
pg*-N570C
pg*-N59B
pg*-N590B
pg*-N596B
pg*-N597B
pg*-N600A
pg*-N603
pg*-N615B
pg*-N618
pg*-N619
pg*-N638
pg*-N639
pg*-N641
pg*-N660A
pg*-N663B
pg*-N673A
pg*-N683A
pg*-N686B
pg*-N701B
pg*-N719C
pg*-N71A
pg*-N724B
pg*-N73A
pg*-N76A
pg*-N816A
pg*-N855C
pg*-N884B
pg*-N896B
pg*-N906B
pg*-PI183648
pg*-PI193424
pg*-PI251930
pg*-PI262473
pg*-PI262495
pg*-PI267162
pg*-PI267215
pg-nec*-RJL-6527

pale pale green seedling
ppg*-N1474B
ppg*-N1963
ppg*-N393B
ppg*-N406A
ppg*-N427A
ppg*-N449
ppg*-N458B
ppg*-N881B

piebald leaf
pb*-2-7-4400
pb*-87-2442-5
pb*-N1386C

ragged seedling
rgd*-N203E
rgd*-N2290C
rgd*-N261B
rgd*-N378B

red seedling leaf
red-leaf*-86-1569-7

small seedling
d*-N155B
d*-N230A
d*-N254
d*-N266B
d*-N293B
d*-N408A
d*-N429A
d*-N526B
smp*-N1956
smp*-N279A
smp*-N630C
smp*-N751B
sms*-N1964
sms*-N1971
sms*-N204B
sms*-N252A
sms*-N311C
sms*-N369B
sms*-N566
sms*-N570B
sms*-N666C
sms*-N680B

translucent leaf
trans-leaf*-56-3122-7
trans-leaf*-68F-958
trans-leaf*-78-314
trans-leaf*-79-6533
trans-leaf*-PI228176

tube leaf
fused-leaves*-N36B
fused-leaves*-N835B
fused-leaves*-PI228170

virescent seedling
l*-N184
pg*-N39
v*-002-17
v*-007-18
v*-022-17
v*-025-4
v*-037-5
v*-1-2(5376)
v*-1-9(5622)
v*-2-9(5257)
v*-388-Sprague
v*-4308
v*-4698
v*-5-10(5355)
v*-5287
v*-5413
v*-5575
v*-56-3012-10
v*-5828
v*-60-151
v*-60-2397-15
v*-65-1433
v*-7230
v*-7281
v*-7312
v*-74-1690-1
v*-74-1873-1
v*-74-1948-1
v*-77-549-2
v*-8070
v*-8129
v*-8201
v*-8339
v*-8522
v*-8613
v*-8654
v*-8743
v*-8806
v*-8957
v*-8958
v*-9026
v*-Funk-84-13
v*-Funk-84-9
v*-leng
v*-LGC-111
v*-LGC-142
v*-LGC-98
v*-N1007B
v*-N110
v*-N114A
v*-N116
v*-N125
v*-N128
v*-N1268A
v*-N131
v*-N133
v*-N134
v*-N135A
v*-N153A
v*-N158
v*-N16
v*-N179
v*-N1806A
v*-N183A
v*-N1836
v*-N187
v*-N1886
v*-N19
v*-N1912
v*-N1966
v*-N201
v*-N206A
v*-N2260

v*-N229
v*-N243
v*-N245
v*-N246
v*-N26
v*-N260C
v*-N280
v*-N289
v*-N29
v*-N298
v*-N303
v*-N330B
v*-N34
v*-N341
v*-N352B
v*-N358C
v*-N376
v*-N378A
v*-N397
v*-N398A
v*-N400B
v*-N41B
v*-N422B
v*-N447B
v*-N463
v*-N467
v*-N470A
v*-N473B
v*-N499
v*-N50
v*-N517
v*-N526A
v*-N529B
v*-N53A
v*-N54A
v*-N54B
v*-N55
v*-N560
v*-N587A
v*-N620
v*-N634A
v*-N64A
v*-N655A
v*-N65B
v*-N660D
v*-N661A
v*-N674B
v*-N678B
v*-N69A
v*-N698B
v*-N710B
v*-N713B
v*-N728
v*-N735
v*-N748C
v*-N75
v*-N779A
v*-N7B
v*-N806C
v*-N826
v*-N829A
v*-N84B
v*-N840
v*-N878B
v*-N891C
v*-N947C
v*-N956C
v*-N972B
v*-pb-3019-16
v*-PI180165
v*-PI180231
v*-PI183640
v*-PI183648
v*-PI185851
v*-PI195244
v*-PI195245
v*-PI200197
v*-PI200201
v*-PI218042
v*-PI228174
v*-PI228176
v*-PI232974
v*-PI236996
v*-PI239105
v*-PI239114
v*-PI239116
v*-PI251883
v*-PI251891
v*-PI251930
v*-PI254856
v*-PI254857
v*-PI262476
v*-PI262487
v*-PI262489
v*-PI267184
v*-PI267209
v*-PI267212
v*-PI270293
v*-Pollacsek
v*-RumanianFlint
v*-Singleton-22
v*-Singleton-34
Vsr*-N1447
wst*-N643B

white luteus seedling
wl*-N1
wl*-N122
wl*-N126B
wl*-N1350B
wl*-N1384B
wl*-N165A
wl*-N18
wl*-N1803
wl*-N1857
wl*-N189
wl*-N1928
wl*-N1930
wl*-N1931
wl*-N1949
wl*-N217A
wl*-N221
wl*-N241
wl*-N255
wl*-N283A
wl*-N290
wl*-N299A
wl*-N311B
wl*-N313
wl*-N315
wl*-N345A
wl*-N358A
wl*-N362B
wl*-N38B
wl*-N4
wl*-N401
wl*-N408B
wl*-N415
wl*-N416B
wl*-N44
wl*-N448
wl*-N466
wl*-N47
wl*-N500
wl*-N502B
wl*-N508
wl*-N538A
wl*-N551C
wl*-N554A
wl*-N567
wl*-N575A
wl*-N60
wl*-N629A
wl*-N636
wl*-N637A
wl*-N646
wl*-N648
wl*-N663A
wl*-N686A
wl*-N698A
wl*-N709B
wl*-N720B
wl*-N758A
wl*-N998C

white margins
whm*-N1462
whm*-N1465B

white striped seedling
ij-mos*-8624
j*-N793
stk*-N359B
str*-2104-4 EBP
str*-2116-1 EBP
str*-5120B-Teo
str*-6-10-4307
str*-78-314-4
str*-78-314-5
str*-84-5222-7
str*-86-1494-27
str*-PI262495
str-et*-PI184276
wst*-N173B
wst*-N190A
wst*-N66B

white tipped leaf
wt*-N308
wt*-N432A
wt*-N580B
wt*-N650A

yellow green leaf
pastel*-1-6-5495
pyg*-N1266A
pyg*-N223
pyg*-N321
yg*-0130
yg*-4369
yg*-4889
yg*-5-8(5575)
yg*-56-3021-18
yg*-6697
yg*-68-1429
yg*-6853
yg*-74-1827-1
yg*-77-585
yg*-8105
yg*-8379
yg*-8622
yg*-8631
yg*-8682
yg*-8692
yg*-8946
yg*-910J
yg*-B73
yg*-Caspar
yg*-N1314B
yg*-N157A
yg*-N1910
yg*-N1948
yg*-N2246
Yg*-N2294
yg*-N38A
yg*-N37
yg*-N706B
yg*-N72
yg*-PI180231
yg*-PI228174
yg*-PI239114
yg*-PI267206
yg*-PI267224
yg*-Singleton-127
yg*-Singleton-23
yg*-Singleton-30
yg-nec*-95-5320-7
yg-nec*-Singleton-29
Yg-str*-Mu

zebra striped seedling
zb*-89-3137-5
zb-gl*-2187

Plant Mutants

absence of leaf blade
bladeless*-87-2406-23

adherent tassel
ad*-N613B

albescent
al*-1479
al*-84-5020-32
al*-PI245132
wh top*-Bauman

barren stalk
ba*-1447
ba*-68-679-8
ba*-74-304-12
ba*-74-369-2
ba*-PI200290
ba*-PI218135
ba*-PI239105
ba*-PI251885
ba-ub*-94-4712

bleached leaf
Blh*-N1455
blh*-N2302B
blh*-N2359
Blh*-N2421
Bh*-SF98-12

blotched leaf
bl*-N1278A
bl*-N43
red leaf blotch*-PI213779
yel-spl*-N152

brachytic plant
br*-2180
br*-78-136KEW
br*-Brawn219-221
br*-Brawn227-229
br*-Brawn230
br*-Brawn231-233
br*-Brawn235-237
br*-Brawn259-260
br*-Brawn261-262
br*-Brawn263-266
br*-Brawn267-268
br*-Brawn269-271
br*-Brawn272-273

br*-Brawn274-275
br*-OSIJEK-Yugoslavia
br*-PI228171
br*-PI239105
br*-Singleton-8
br*-Singleton1969-252
td*-PI262476

brevis plant
bv*-N2283

brittle stalk
bk*-N888D

brown midrib
bm*-PI228174
bm*-PI251009
bm*-PI251893
bm*-PI251930
bm*-PI262480
bm*-PI262485
bm*-PI267186

burned leaf
les*-Funk-4
les*-PI262474

chromosome breaking
Chrom-breaking*-Mu

colored leaf
lc*-PI239110

crinkled leaf
cr*-97P-111
cr*-98-1698

crossbanded leaf
cb*-N1620A
Cb*-N2290B
cb*-N696D
cb*-N719A

defective tassel
Tp*-54-55-Jos
Tp*-PI213734
Tp*-Pk41-Jos
Tp*-T8-Jos
Tp*-Tenn61

dwarf plant
d*-018-3
d*-119
d*-136-220
d*-1821
d*-2108
d*-2201
d*-2447-8
d*-3-eared-JC
d*-3047
d*-5312
d*-56-3037-23
d*-60-2428
d*-64-4156-1
d*-74-1701-5
d*-75-6071-1
d*-76-1304-9
d*-76-2186
d*-78-282-3
d*-78-286-1
d*-78-286-5
d*-85-3081-33
d*-87-2198-36
d*-gl11
d*-Brawn254-258
d*-MarovacWhiteDent
d*-N1352B
d*-N1883
d*-N1895
d*-N197A
d*-N203D
d*-N2295
d*-N282
d*-N299B
d*-N403B
d*-N454A
d*-N518A
d*-N549B
d*-N604
d*-N629B
d*-N699B
d*-N994B
d*-PI180231
d*-PI183644
d*-PI184286
d*-PI213769
d*-PI228169
d*-PI228171
d*-PI239110
d*-PI245132
d*-PI251652
d*-PI251656
d*-PI251885
d*-PI254854
d*-PI262495
d*-PI267219
d*-rosette
d*-shlf-9-436-1
d*-su
d*-su2
d*-Teo
d*-ts1

erect leaf
dge*-N2410

faded leaf
fd*-N1938

gritty leaf
gtl*-N2297

green striped leaf
gs*-98-5700-5
gs*-N359A
gs*-N484A

knotted husks
mwp*-Nelson

lazy plant
la*-N2333B

lesion
les*-2119
les*-74-1873-9
les*-ats
Les*-N1378
les*-N2290A
Les*-N2420
Les*-N502C

liguleless
Lg*-64-36
lg*-PI228170

male sterile
Ms*-2471
ms*-6015
ms*-6025
ms*-6026
ms*-6033
ms*-6039
ms*-6045
ms*-6048
ms*-6049
ms*-6052
ms*-6053
ms*-6054
ms*-6055
ms*-6057
ms*-6058
ms*-6059
ms*-6060
ms*-6061
ms*-6062
ms*-6064
ms*-6065
ms*-6066
ms*-N2415
Ms*-N2474
ms*-N2484
ms*-N352C
ms*-N45B
ms*-PI217219
ms-si*-355

many tillers
tlr*-N2243

nana plant
na*-N1519D

narrow leaf
nl*-5688
nl*-N232A
nl*-N410B
nl*-N462B
nl*-N622B
nl*-N625
nl*-N727B
nl*-N732A
nl*-PI245132
stf*-N601
stk*-N363

necrotic leaf
ll*-N248A
ll*-N417D
nec*-011-7
nec*-017-3
nec*-4871
nec*-4889
nec*-5588 early
nec*-5619
nec*-5876
nec*-77-549-2
nec*-77-574-1
nec*-8624
nec*-8737
nec*-fraz
nec*-N1119B
nec*-N1185B
nec*-N1487
nec*-N193
nec*-N200B
nec*-N215F
nec*-N283B
nec*-N419
nec*-N430A
nec*-N465
nec*-N468
nec*-N490A
nec*-N510A
nec*-N541B
nec*-N545B
nec*-N559
nec*-N562
nec*-N581
nec*-N596C
nec*-N599A
nec*-N650D
nec*-N666A
nec*-N712C
nec*-N814
nec*-PI228174
nec*-PI267184
nec*-Vasco
nec-pg*-PI239116
shootless*-99-677-6

oil yellow plant
oy*-N2360A

pale green plant
pg*-56-3012-10
pg*-8321
pg*-Hy2 Nob 7-5
pg*-LGC-61

patched leaf
ptc*-N238A
ptc*-N444B
ptc*-N611
ptc*-N904B

pigmy plant
py*-N656B
py*-N714

ramosa
ra*-412E
ra*-4889

rolled leaf
rld*-N1405B
rld*-N1525
Rld*-N2465
rld*-N556C

small plant
d*-N1074C
d*-N137C
d*-N149
d*-N164A
d*-N188B
D*-N2023
d*-N208B
d*-N210
d*-N262C
d*-N287B
d*-N305
d*-N328
d*-N394
d*-N524D
d*-N528B
d*-N553D
d*-N707B
D*-N987B
smp*-N121
smp*-N135B
smp*-N153B
smp*-N156A
smp*-N183B
smp*-N1954
smp*-N272A

smp*-N306
smp*-N452B
smp*-N586B
smp*-N600B
smp*-N602
Smp*-N842

speckled leaf
spc*-N112
spc*-N198B
spc*-N357A
spc*-N370

spotted leaf
les*-74-1820-6
spt*-N278B
spt*-N412A
spt*-N513A
spt*-N579B

stiff leaf
stf*-N1092C
stf*-N235B

streaked leaf
stk*-N351
stk*-N368A
stk*-N433B
stk*-N584A
stk*-N587D
stk*-N589
stk*-N670B
stk*-N769C
stk*-N777B
stk*-N812B
stk*-N835A
stk*-N925B

striate leaf
Sr*-N2430
sr*-N675B

stubby plant
stb*-N938C

tassel seed
ts*-0174
ts*-69-Alex-MO17
ts*-Anderson
Ts*-N1374
ts*-N2409
ts*-PI251881
ts*-PI267209
ts*-Sprague

tasselless
tls*-Funk
tls*-Va35

tiny plant
ty*-N215A
ty*-N236B
ty*-N326C
ty*-N702B

torn leaf
Trn*-N2438

white sheath
ws*-N1979
ws*-N537D

white stripe leaf
ij*-N504A
li*-PI262476
str*-PI262474
wst*-N1877
wst*-N248B
wst*-N413A
wst*-N548
wst*-N564A
wst*-N696B

yellow stripe leaf
gs*-68-1354
ys*-1479
ys*-5-8(5575)
ys*-67-2403
ys*-68-1354
ys*-8912
ys*-N326A
ys*-PI228180
ys*-PI262172
ys*-PI262475
ys*-whorled

zebra necrotic leaf
zn*-8637
zn*-N230B
zn*-N342A
zn*-N354A
zn*-N372A
zn*-N451
zn*-N571D

Ear Mutants

distichous ear
distichous*-68-1227

distorted segregation
off-ratio*-85-3255-6
off-ratio*-86-1155-1
wx-off-ratio*-86-1110-4

papyrescent glumes
en*-Sprague

polytypic ear
pt*-McClintock
pt*-Mu
pt*-N868B

reduced pollen fertility
ga*-0188
ga*-0213
ga*-0648
ga*-3615
ga*-91-5197-2
ga*-94-764
Ga*-Yugoslavia

silky
si*-0443
si*-0503
si*-8104
si*-N1967A

tunicate
Tu*-5090B

unpaired rows
up*-Shirer

VI. MAIZE GENOME DATABASE

www.agron.missouri.edu

Overview

MaizeDB contains over 650,000 records, many with one or more links to an external data repository for primary data source. These links now total some 570,000; and we do not count sites mirrored with GenBank, EMBL and DDBJ. We are greatly indebted to the maize community for making their data available, in particular Dr Marty Sachs of the Maize Genetics Cooperation Stock Center for continued data entry and updates of new genetic stocks.

Other data providers this year:

- DuPont - 10,600 Unigenes, aka Cornsensus sequences, based on public with proprietary sequences. These have been entered into the HTC division of GenBank.
- Incyte Genomics - BAC anchor information and Overgo primers designed from the Cornsensus sequences;
- ZmDB and TIGR - public EST assemblies;
- Gramene - BLAT mappings of the Cornsensus sequences to rice;
- Dr. Cari Soderlund - the FPC computation of contigs, entered into MaizeDB as external database links for BACs and anchoring markers;
- Dr. Marilyn Warburton of CIMMYT - SSR polymorphism;
- Dr. Jeanne Romero-Severson - SSR polymorphism;
- Dr. Mike McMullen of the Maize Mapping Project - SSR data, complete with screening images;
- Dr. Georgia Davis, of the Maize Mapping Project - IBM map coordinates, data, screening images; new set of CORE markers to define the bin edges on the IBM;
- Dr. Dave Selinger, of ChromDB - registry of chromatin gene family names;
- GenBank and SwissProt for email updates of new maize sequences.

Other data acquisition**:**
We completed bibliographic entry of references back to 1888, with the aid of bibliographies of J. Cunningham in 1948 (for 1888-1916) and 1941 (for 1917-1936). MNL on-line is now retrospective to 1977. Literature citations within MaizeDB are over 100,000, for some 38,000 references.

Genetic Maps

The high resolution IBM map is now entered and includes 1700 loci, complete with probe information, many with links to BAC contigs (WWW FPC). The map is displayed at www.maizemap.org, complete with probe data and links to the evolving physical map. Other sites that display genetic maps extracted from MaizeDB include:

(1) the NCBI, in collaboration with Drs. Brian Smith-White and Tatiana Tatusov; www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search?chr=mays.inf;
(2) CUGI, in collaboration with Drs. Cari Soderlund and Rod Wing, www.genome.clemson.edu/projects/maize/fpc/WebChrom/;
(3) Gramene, in collaboration with Drs. Doreen Ware and Lincoln Stein, www.gramene.org.

Map-based strategies, and their caveats for finding BAC clones of interest are described under the sidebar at BACS/YACS, or at www.agron.missouri.edu/bacs.html. Strategies rely on the above resources and also (1) the Mapped Sequence Locator, www.maizemap.org/cgi-bin/zmMSL.cgi, where a BLAST search returns map coordinates and links out various data repositories; (2) various BAC and Probe Lookups based on map coordinates. The basic strategy is to find markers on BACs for your sequence of interest, or which are closely linked.

Coming Soon

Genetic Map Integration

Over the years, some 12,526 loci have been placed to chromosome arm or better. Most mapping experiments have less than 20% overlap of markers; see the MaizeDB report, MNL 71 1997, www.agron.missouri.edu/mnl/71/maizedb71.html. Because of the high numbers of markers, and maps involved, it is difficult to represent all on a single page or 10, as was the custom for MNL. For this reason one of the main syntheses of MaizeDB has been the bins maps. Thus while the IBM represents 1700 loci, there are an additional 8016 loci that have been placed to a single bin, many with better resolution than represented by the bin. Map based cloning will be supported by a higher resolution integration which we will be presenting on-line this summer, a map at a time, using the IBM map as the frame and supplying the methods of integration. All supporting data and the original maps will be maintained and accessible from the cMAP comparative map display (button in center of MaizeDB home-page).

CIMMYT mirror

We are collaborating with CIMMYT in establishing a mirror site for MaizeDB at CIMMYT that will further enhance access and data acquisition, with CIMMYT storing data directly in validation-readied tables. Anticipated data flow includes polymorphisms and trait studies. Funding for the mirror has been provided directly to CIMMYT by the USDA-ARS.

Submitted May 1, 2002
Ed Coe and Mary Polacco

VIII. AUTHOR INDEX
(* identifies articles authored in this Newsletter)

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

Send your notes for the 2003 Maize Genetics Cooperation Newsletter now, anytime before January 1. Your MNL Notes will go on the Web verbatim promptly, and will be prepared for printing in the annual issue. Be concise, not formal, but include specific data, tables, observations and methods. Check MaizeDB for the most current information on submission of notes. Send your notes as attachments or as the text of an email addressed to Newsletter@chaco.agron.missouri.edu (we will acknowledge receipt, and will contact you further if necessary). If email is not feasible, please mail a double-spaced, letter-quality copy of your note, preferably with a disk containing the electronic version. Please follow the simple style used in this issue (city /institution title /--authors; tab paragraphs; give citations with authors' initials --e.g., Maizer, BA et al., J Hered 35:35, 1995, or supply a bibliography). Figures, charts and tables should be compact and camera-ready, and supplied in electronic form (jpg or gif) if possible. To separate columns in tables, please tab instead of using spaces, to ensure quality tabulations on the web. Your MNL Notes will go on the Web verbatim promptly, and will be prepared for printing in the annual issue. Mailing address:

Mary Polacco
203 Curtis Hall
University of Missouri
Columbia, MO 65211-7020

SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

MNL 51ff. on line	MaizeDB - http://www.agron.missouri.edu
Author and Name Indexes (and see **MaizeDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeDB**)	
Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue and MaizeDB
Rules of Nomenclature (1995)	MNL69:182 and **MaizeDB** (1996 update)
Cytogenetic Working Maps	MNL 52:129-145; 59:159; 60:149 and **MaizeDB**
Gene List	MNL69:191; 70:99 and **MaizeDB**
Clone List	MNL 65:106; 65:145; 69:232 and **MaizeDB**
Working Linkage Maps	MNL69:191; 70:118; 72:118 and **MaizeDB**
Plastid Genetic Map	MNL 69:268 and **MaizeDB**
Mitochondrial Genetic Maps	MNL 70:133 and **MaizeDB**

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

(1) Send stocks of new factors you report in this Newsletter or in publications, and stocks of new combinations, to the collection.

(2) Inform the Stock Center on your experience with materials received from the collection.

(3) Acknowledge the source, and advice or help you received, when you publish.

MaizeDB needs Cooperators (this means you) to:

(1) Look up "your favorite gene or expression" in **MaizeDB** (see section V in this Newsletter) and send refinements and updates to polaccom@missouri.edu, coee@missouri.edu, or db_request@chaco.agron.missouri.edu.

(2) Compile and provide mapping data in full, including the ordered array of map scores for molecular markers or counts by phenotypic classes; recombination percentage and standard error.

(3) Provide probe or primer information per http://www.agron.missouri.edu/cgi-bin/sybgw_mdb/mdb3/Probe/query; fingerprint data and fragment sizes are significantly useful to colleagues.

May you find a Unique corn in MM!