MAIZE GENETICS COOPERATION NEWSLETTER

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November 1, 2009

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

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NOTE: The 52nd Maize Meeting will be held at Riva del Garda, Italy. March 18-21, 2010.

Check MaizeGDB for more details.

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I. FOREWORD

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

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

This year, we call attention to a number of special reports (see pages 98-113): the B73 Genome Sequencing Project; the B73 optical map; the Mo17 genome sequence; the genome annotation workshop held at the March 2009, Maize Genetics Conference, St. Charles, Illinois; POPCorn, a novel portal to maize projects and resources, including annotation of the genome; a brief synopsis of frequently used maps at MaizeGDB, with a short tutorial. At this time, most past newsletters have been scanned and are available at the staging site, www.agron.missouri.edu. This has been accomplished largely with the help of several students, notably Hannah Fernandez, Erin Broocke, Emily Haghighi, and Beth Welsh with funding by the USDA Agricultural Research Service. We are working to move all to the permanent site at MaizeGDB, with linking of each note to individual reference citations at MaizeGDB.

The Newsletter sponsors a new online journal, called the "*maize gene review*", hosted at www.maizegenereview.org. The inaugural submissions are included here, in map order, following the Table of Contents. It is modeled on the 2000 "Mutants of Maize", eds. MG Neuffer, EH Coe and S Wessler, Cold Spring Harbor, NY. The emphasis is mutants, but reviews will include any gene with experimentally confirmed function. This journal was first described in a poster presentation at the 2009 Maize Genetic Conference in St. Charles, IL and was an invited presentation at the 3rd Int. Biocurator Conference, 2009 Berlin, Germany. We welcome contributions from all cooperators on their favorite genes. The text summaries of each mutant, and any new images, will be supplied to MaizeGDB, as was done in 1995 from the "Mutants of Maize", crediting authors and the review page. Each year, new submissions, and any substantial revisions of previous reviews, will be included in the print copy of MNL. Unlike contributions to the Newsletter, these will be peer-reviewed and may be cited without permission of authors. We plan to assign DOI accessions in the near future. The *maize gene review* adheres to the stipulations of the "Creative Commons License", with normal copyright retained by authors, but not this Newsletter.

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes can be submitted at any time and are entered into MaizeGDB. We set an arbitrary cutoff of January 1, 2010 for the next print copy, volume 84. Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions must require minimal editing to be accepted.

As in the past, Shirley Kowalewski has been responsible for final redaction and layout of the Newsletter copy. She has performed this task with speed, precision and a great sense of humor. The maize community owes her much gratitude for her continued service in this capacity.

Mary Schaeffer
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AMES, IOWA
Iowa State University

A sterilization protocol for field-harvested mature maize seed used for in vitro culture and genetic transformation

--Martinez, JC; Wang, K

In vitro tissue culture techniques require not only an aseptic work environment but also contaminant free starting materials (Dixon, Plant Cell Culture, a Practical Approach, IRL Press, 1985). Two common contaminants affecting in vitro-cultured tissues are bacteria and fungi. Enrichment of the media used for in vitro culture makes explants susceptible to these microorganisms (Leifert and Waites, Int. Soc. Plant Tissue Cult. Newsletter 60:2-13, 1990). Despite careful attention while applying sterilization techniques, contamination still may cause 100% loss from field-grown material due to large numbers of microorganisms present in the explants (Skirvin et al., In Vitro Cell Dev. Biol. Plant 34: 278-280, 1999). One of the first steps in the process of plant genetic transformation is to acquire sterile plant materials for in vitro tissue culture.

For maize, a well-established protocol exists for the sterilization of initial material when immature embryos are used (Frame et al., In Vitro Cell Dev. Biol. Plant 36:21-29, 2000). However, no effective sterilization protocols are reported for mature maize seeds, especially for field-harvested seeds that typically carry large amounts of air- and soil- borne pathogens. One strategy to overcome the contamination problem in mature seed sterilization is simply to start with large quantities of seeds, which increases the chances of obtaining adequate numbers of sterilized seed for in vitro culture. Alternatively, a method that prevents introduction of these contaminants into the laboratory in the first place would be ideal.

Here we report an efficient and reproducible seed sterilization method, which was developed specifically for mature maize seeds harvested from field-grown plants. The method includes three major disinfection stages. First, the maize seed surface is disinfected and soaked in sterile water, then the softened seeds are disinfected and the mature embryos isolated. Finally, the dissected mature embryos are disinfected before being placed on plant media for culturing. Using this step-wise sterilization method, an average of 98% sterile mature embryos are obtained, and plant vigor (measured by Seedling Growth Rate; ISTA, Seed Sci. Technol. 27(Suppl.):27-32, 1999) is not compromised compared to the non-sterilized control (Martinez, 2008).

Materials. 1) Mature maize seeds harvested from field-grown plants. 2) 80% Ethanol (~300 ml for ~200 seeds). 3) 50% bleach solution: mix 450 ml of commercial bleach (5.25% hypochlorite) with 450 ml of Millipore water containing 2 drops of the surfactant Tween-20. Use ~900 ml for ~200 seeds. 4) 15% bleach solution: mix 15 ml of commercial bleach (5.25% hypochlorite) with 85 ml of Millipore water containing 1 drop of the surfactant Tween-20. Use ~100 ml for ~200 dissected embryos. 5) Millipore water (autoclaved). 6) Petri dishes: 60 x 20 mm, 100 x 15 mm, 150 x 15 mm

Methods. First seed surface disinfection and seed softening: 1) Place 50 seeds in a 250 ml beaker along with a stir bar. 2) Add ~ 75 ml of 80% ethanol, cover with the aluminum foil and place the beaker on a stir plate. Stir at medium speed for 3 minutes. 3) Take the beaker to the flow bench and decant the ethanol into a liquid-waste container. 4) Add ~ 75 ml of 50% bleach solution, cover with the aluminum foil and stir for 15 minutes on medium speed. 5) In the flow bench, decant the bleach into the liquid-waste container. 6) Sterilize the seeds a second time by repeating Steps 4 and 5. 7) Rinse the seeds 5 times with sterile Millipore water (~ 75 ml each time). 8) After the last rinse, keep seeds in ~50 ml sterile water (just enough to cover the seeds--do not overfill the container), cover with aluminum foil and leave the beaker inside the flow bench for 24 hours. This treatment serves to soften the seed coat and endosperm for ease of embryo dissection.

Second seed surface disinfection and embryo dissection: 9) After 24 hours, sterilize the softened seeds once with 50% bleach solution for 2 minutes while stirring (covered). 10) Decant the bleach and rinse the seeds 5 times with sterile Millipore water. 11) Leave seeds covered with last wash so they do not dry out. 12) Move sterilized seeds to a 150 x 15 mm petri dish in groups of 3 or 4 for embryo dissection. 13) Dissect the mature embryo using bent nose forceps to hold the middle of the seed with the embryo facing up. First, cut longitudinally along both sides of the embryo to remove the endosperm flanks. Next cut the base of the seed away, including two thirds of the root (tip) portion of the embryo axis. Finally, cut away the remaining endosperm from the top end of the embryo being careful not to injure the apical end of the embryo. The remaining endosperm can then be peeled away from beneath the embryo. Gently lift the embryo away from the seed carcass using the scalpel and place it in a 60 x 20 mm petri dish containing 10 ml sterile distilled water. 14) Repeat Step 13 until you dissect all the seeds.

Embryo sterilization: 15) Using a sterile pipet, remove water from the petri plate containing the dissected embryos. 16) Add ~15 ml of 15% bleach solution to the petri plate, close the lid and place on a table top shaker (low speed) for 5 minutes. 17) Remove the bleach solution using a sterile pipet. 18) Rinse the embryos 4 times (15 ml each time) with sterile Millipore water. 19) Embryos are ready to be placed on a plant medium. Place about 10 embryos per 100 x 15 mm petri plate of medium. 20) Check the plates after 2, 3 and 4 days and if contamination is found, move uncontaminated explants to fresh medium.

Acknowledgements. We want to thank Bronwyn Frame, Susana Goggi and Christian Erik for comments and advice, Diane Luth, Susana Goggi, Bronwyn Frame and Thomas Loynachan for providing supplies, equipment and facilities when needed, and Javier Garcia, Jessica Zimmer and Anita Dutta for technical assistance and Bronwyn Frame for critical review of the protocol.

BEIJING, CHINA
Chinese Academy of Sciences

Studies on Zhuo-Zi No.1, a purple hybrid in maize (*Zea mays* L.)

--Wang, Y; Zeng, M

In this paper, we describe the research status quo on Zhuo-Zi No.1, a purple hybrid in maize (*Zea mays* L.). We have a breeding program for purple maize, using information about genetic loci summarized by Coe et al. (1977).

Parents for Zhuo-Zi No.1 are ZP 99-01 (Female) and ZP 99-02 (male). ZP 99-01 was developed by continued inbreeding and selection from a local variety with purple plants and grain, selecting for ears of superior plants from large populations. ZP 99-02 was developed by continued inbreeding and selection from the improvement population No. 02. Both parents have good or high general combining ability; high specific combining ability; high vigor; normal to high production; resistance to biological stress; late maturity; medium tall plant and ear height; fair stalks; vigorous roots; semi-erect and mid-sized leaves; 1~2 ears per plant and purple-black grain on a deep-purple cob. The main agronomic traits of Zhuo-Zi No.1 are summarized in Tables 1 and 2, and Figure 1. It produces a purple color in the seedling, leaf tip, leaf periphery, leaf ear, leaf sheath, stalk, tassel and its branches, anther surface, silk, husk leaf, ear handle, cob, pericarp, aleurone, and leaf blade of the main plant and in tillers at maturity. It has normal color (pale-yellow or nearly white) endosperm, embryonic bud, shield blade, shoot sheath and root system. The biomass yield and grain yield can reach 75000~7950 kg/ha and 7500~8025 kg/ha respectively. The anthocyanidin-3-monoglucoside (maize morado color) content of purple maize Zhou-Zi No.1 is estimated to be 225~300 kg/ha. Anthocyanin content varies in different tissues and organs. It is 0.023% in the stalk and leaf blade, 1.007% in the tassel, 2.228% in the husk leaf, 0.268% in the silk, 0.728% in the cob, 0.106% in the grain, and 0.869% in the stalk coat (Table 3.). Thus, the husk leaf and tassel have a higher anthocyanin content.

Figure 1. Ear of Zhuo-Zi No. 1.

Table 1. The agronomic traits of Zhuo-Zi No. 1.

No.	Trait name	Average value, character and resistance
1	Plant height cm	313.5
2	Ear height cm	124.7
3	Tassel length cm	42.6
4	Tassel branch number	14.0
5	Leaf number	23.5
6	Ear length cm	18.3
7	Ear diameter cm	4.5
8	Row number per ear	15.7
9	Grain numer per row	37.8
10	Weight of 100 grain g	398.6
11	Ear number each plant	1.6
12	Husk number each ear	14.8
13	Grain type	Semi-dent
14	Ear form	Cylinder
15	Resistance to	
	E. turcicum	HR
	B. maydis	HR
	C. lunada	HR
	C. zeae-maydis	MR
	U. zeae	MR
	F. moniliforme and *P. inflatum*	MR
	R. solani and *R. cerealis, zeae*	R
	S. holci-sorghi	HR
	MRDV	MR
	SCMV-MDR	MR
16	Tolerance lodging	
	drought	

Table 2. The biochemical composition of grain of Zhuo-Zi No. 1.

Component	% (g/100g grain), g/L
Protein	10.86
Lipid	5.02
Starch	74.23
Lysine	0.36
Water	10.7
Unit Weight (g/L)	792

Table 3. The anthocyanin content of different tissues and organs of Zhuo-Zi No. 1.

Tissue name	Anthocyanin % (g/100g)
Stalk and leaf blade*	0.023
Tassel	1.007
Husk leaf	2.228
Silk	0.268
Ear cob	0.728
Grain	0.106
Stalk sheath	0.869

*Sampled at maturity

BERGAMO, ITALY
CRA – MAC

The *opaque2* and *opaque7* mutants reveal extensive changes in endosperm metabolism as revealed by transcriptome-wide analyses

--Hartings, H; Lauria, M; Lazzaroni, N; Pirona, R; Rossi, V; Motto, M

The changes in storage reserve accumulation during maize (*Zea mays*) grain maturation are well established; however, the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and protein are more elusive (Motto et al., Cellular and Molecular Biology of Plant Seed Development, Larkins and Vasil, eds., 1997). The *Opaque-2* (*O2*) gene, one of the best-characterized plant transcription factors, is a good example of the integration of carbohydrate amino acids and storage protein metabolism in maize endosperm development. Evidence also indicates that the *Opaque-7* (*O7*) gene plays a role in affecting endosperm metabolism.

To advance our understanding of the nature of the mutations associated with an opaque phenotype, we used nearly isogenic inbreds for *o2* and *o7* mutants, and for the double mutant combination *o2o7*, to provide genome-scale information about gene expression patterns by cDNA microarray. Classifying genes based on similarities or differences in transcript profile with phenotype can confirm existing knowledge, lead to the dissection and revela-

tion of novel mechanisms determining nutrient partitioning, and generate new unbiased hypotheses.

Microarray slides were assembled using clones obtained from 20-part-normalized cDNA libraries representing the major events in endosperm development. Approximately 22,300 ESTs were sequenced, aligned, assembled into contigs using a similarity score of 80%, and annotated using TBLASTN software. It is notable that the distribution of ESTs across the original cDNA libraries was not uniform. The highest proportion of the sequences could be associated with endosperm tissue, the lowest with 8-day-old embryos. Of the 8,950 ESTs identified, 6,719 were singletons and 2,231 formed contigs. EST sequences were analyzed with the BLAST2GO software (http://www.blast2go.de). In the first phase, homology searches using public domain non-redundant databases identified significantly homologous sequences for 48.4% of the ESTs considered. These ESTs represented 3,090 single hit and 1,240 multiple hit sequences.

In the second phase, an attempt was made to associate biological processes to each of the ESTs showing sequence homology using the gene ontology (G.O.; http://www.geneontology .org) and KEGG databases (http://www.genome.jp/kegg). Approximately 85% of these unigenes could be assigned a functional annotation, with the remainder (ca. 15%) having an obscure or unknown function. Twenty-four distinct patterns of expression were resolved to establish the complex regulatory hierarchies that exist to orchestrate the dynamic metabolic, transport, and control processes occurring in developing endosperm. This classification is consistent with the many functions of maize endosperm and is comparable with that reported by other workers (Verza et al., Plant Mol. Biol. 59:363-374, 2005). It appears that our maize endosperm gene set is rather comprehensive and provides a good representation of the entire transcriptome including genes linked to accumulation of storage products and energy supply. More specifically, most of the transcripts appeared to be involved in carbohydrate metabolism (12.0%), followed by those involved in storage protein synthesis (7.9%), translation (11.2%) and transcription (5.3%), nucleotide metabolism (2.5%), and RNA processing (2.1%). Among physiological processes, those transcripts implicated in protein turnover (5.6%), energy metabolism (3.1%), electron transport (1.2%), amino acid metabolism (4.4%), amino acid and sugar transport (7.8%), the latter being intrinsically linked to the accumulation of storage protein and starch, nucleic acid metabolism (2.5%), lipid (2.1%) and fatty acid metabolism (1.6%), and secondary metabolites (2.0%) were represented in our EST collection. Moreover, genes encoding for protein involved in cell wall (2.8%), cytoskeleton (2.8%), and stress and defense (5.1%) appear to be related to relevant cellular processes assigned in the functional classification. Finally, the assignment of other important classes of transcripts, such as DNA (1.2%) and protein folding (0.5%), transcription regulators (5.3%; mostly representing transcription factors) and signal transducers (13.3%) provides new perspectives for data mining and for studies of coordinated gene regulation in developing maize endosperm. Thus, ESTs corresponding to the majority of genes (or their alleles) are represented in the maize endosperm cDNA libraries constructed, and the use of the maize Zeastar Unigene chip to examine endosperm gene expression appeared feasible.

Microarray slides containing the entire Zeastar unigene set, spotted in duplicate, were hybridized with probes derived from endosperm tissue harvested at 14 DAP - a developmental stage in which synthesis of starch and storage protein is known to begin- of normal, *o2*, *o7*, and *o2o7* A69Y inbreds. To reduce hybridization artifacts, all probes were labelled both with Cy3 and with Cy5 and used in dye-swapping experiments on a series of three independent slides. The expression data obtained were assayed for consistency by performing F-tests at 95% confidence levels. Replicates appeared to be in general agreement; thus, we are confident that the alterations of the transcriptomes described here are consistent with the biology of endosperm development. Moreover, we selected a series of thirty clones, believed to be of particular interest and exhibiting distinct patterns of expression, for detailed analysis, using qRT-PCR to confirm the changes in expression levels determined using the arrays. RNAs isolated from the four genotypes were used as templates for amplification. The relative expression levels determined by qRT-PCR showed good agreement with those determined using arrays with high correlation coefficients.

Gene expression profiling, based on a unigene set composed of 7,250 ESTs, allowed us to identify a series of mutant related up-regulated (17.1%) and down-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to gene encoding enzymes involved in amino acid synthesis, carbon metabolism (TCA cycle and glycolysis), storage protein and starch metabolism, gene transcription and translation processes, signal transduction, and protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrate that the mutants investigated are pleiotropic and play a critical role in several endosperm metabolic processes. Pleiotropic effects were less evident in the *o7* mutant, but severe in the *o2* and *o2o7* backgrounds, with large changes in gene expression patterns, affecting a broad range of endosperm-expressed genes involved in several metabolic pathways. Although more work is required to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized to date give us an intriguing insight into the mechanisms underlying endosperm metabolism.

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BERGAMO, ITALY
CRA-MAC
COLLERETTO GIACOSA, ITALY
ISPA-CNR

The *Zea mays* (L.) b-32 ribosome-inactivating protein efficiently inhibits growth of *Fusarium verticillioides*

--Balconi, C; Lanzanova, C; Giuffrida, MG; Baro, C; Hartings, H; Lupotto, E; Motto, M

Fungi of the genus *Fusarium* are widely distributed pathogens of maize, causing diseases for seedlings, roots, stalks and kernels (Bottalico, J. Plant Pathol. 80(2):85-103, 1998; Reid et al., Phytopathol. 89:1028-1037, 1999). In addition to their effects on yield,

Fusarium species can affect grain quality, producing a number of toxic compounds, including fumonisins (Munkvold, Ann. Rev. Phytopathol. 41:99-116, 2003), involved in human and animal health (CAST, Task force rep. 38. Ames, IA: CAST, 2003). Therefore, the development of maize plants carrying resistance to *Fusarium* ssp. (Lew et al., Cereal Res. Commun. 25:467-470, 1997) as well as resistance to mycotoxin production is highly desired.

In maize endosperm, a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins (Soave et al., Cell 27:403-410, 1981). It was shown that the *b-32* genes form a small gene family (Hartings et al., Genet. Res. Camb. 65:11-19, 1995).

Endosperm-derived native b-32 was shown i) to enzymatically inactivate ribosomes, through its capacity to specifically modify rRNA, inhibiting protein synthesis in vitro (Maddaloni et al., J. Genet. Breed. 45:377-380, 1991; Bass et al., Plant Cell 4:225-234, 1992), and ii) to inhibit the growth of *Rhizoctonia solani* mycelia in in vitro bioassays (Maddaloni et al., Transgenic Res. 6:393-402, 1997). Similarly, Balconi et al. (European J. Plant Pathol. 117:129-140, 2007) found that maize RIP-b-32 protein was effective in wheat transgenic lines as an anti-fungal protein by reducing *Fusarium* head blight (FHB) symptoms.

To verify if maize plants expressing b-32 in various tissues have an increased tolerance to fungal pathogens, transgenic plants were obtained through genetic transformation using a chimeric gene containing the *b-32* coding sequence downstream of a constitutive *35SCaMV* promoter. A set of four independent homozygous progenies expressing b-32 were selected for a detailed analysis of b-32 expression in leaves and for pathogenicity tests.

The integration patterns of the *b-32* transgene were determined by genomic Southern-blots using *Eco*RI-*Hin*dIII double digests as appropriate enzymes to estimate the transgene copy number. Four Basta resistant progenies (SM 3.4; SM 16.1; SM 19.4; SM 20.2), one Basta sensitive progeny (SM 20.4) and the B73 inbred line were analyzed using a *nos-bar* (resistance gene) and *b-32* probe. The *b-32* probe detected the presence of the *b-32* endogenous gene in the control B73 inbred line and in the negative control progeny SM 20.4. A band at the same position is present in transgenic progenies SM 3.4, SM 16.1, SM 19.4, and at a slightly different position in the SM 20.2 progeny, and is most likely due to a recombination event involving the endogenous gene and transgene, indicating that several insertion events have occurred. In addition to the endogenous *b-32* band (native gene), all transgenic progenies contained a few additional bands corresponding to insertions of the transgene. The unique hybridization patterns observed indicated that each progeny resulted from independent transformation events.

Comparison of b-32 expression among various individuals was performed, after immuno-blot imagine scanner acquisition, using IMAGINE MASTER 1D Elite Version 3.01 (NonLinear Dynamyc Ltd) software. A differential b-32 content in leaf protein extracts was recorded in the transgenic progenies. As expected, SM 20.4, i.e. the negative control, showed non-detectable b-32 content (n.d.) in leaf tissues. Proteomic experiments were performed on protein leaf extracts of one of the transgenic lines expressing a high b-32 level (SM 20.2) and were compared to the negative control progeny (SM 20.4). The overlapping of the two-dimensional electrophoresis maps clearly showed the presence of additional spots in SM 20.2 progeny in comparison to SM 20.4 progeny, which was Basta-sensitive and b-32 western negative. These spots were cut from gels and digested with trypsin to allow protein identification by the "peptide mass fingerprinting" (PMF) strategy (Pappin et al., Curr. Biol. 3:327-332, 1993). Both induced b-32 spots and herbicide resistance spots were successfully identified.

Transgenic progenies were tested in bioassays to evaluate the response to *Fusarium* attack in leaf tissues. Preliminary experiments supported the choice of bioassay parameters for a reliable evaluation of transgenic progenies. Results indicated that fungal colony diameters measured on the inoculated leaves of SM 20.4 (the negative control) were, at all detection times, significantly (Student's t test = $P \leq 0.05$) larger than those observed in all four progenies expressing b-32. A good correlation between the b-32 content in the leaves and the level of resistance to *Fusarium* attack was observed. In the case of progenies with high b-32 content in the leaves, in addition to reduced mycelial growth around the cut edges of the leaves, very weak growth on leaf surfaces was observed in comparison with progeny exhibiting the lowest b-32 content in leaves.

The data obtained indicate that maize b-32 is an effective antifungal protein in reducing progression of *Fusarium* infection. Additionally, the reduction in *Fusarium* induced symptoms was related to b-32 concentration in leaf tissues. The expression of antifungal proteins in plants or plant tissues in which they are not normally expressed may be very useful in reducing pathogen colonization and growth; from this perspective, a reduction of *F. verticillioides* infection in maize leaves and stalk could be very useful in limiting the spread of fungal infection to the exposed silks, and consequently, in the reduction of grain fumonisin contamination.

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BHUBANESWAR, INDIA
Regional Plant Resource Centre

Effect of water stress on performance of maize inoculated with *Glomus* sp. isolated from a tea plantation of Keonjhar, Orissa

--Gupta, N; Routaray, S

Arbuscular mycorrhizal (AM) fungi have been reported to help maize grow under drought and other stresses (Gupta and Routaray, Acta Agric. Scandinavica 55(2):151-157, 2005; Subramanian et al., New Phytol. 129:643-650, 1995; Subramanian and Charest, Mycorrhiza, 7:25-32, 1997). We have expanded this work to study the effects of tea plantation arbuscular mycorrhizal (AM) fungi on maize grown under well-watered and water-stressed pot culture conditions. Fungi were isolated from the drought-prone tea (*Camellia sinensis* L.) plantations of the Bhuyanpirh tea estate of M/S Orissa Tea Plantation Limited, which is situated in Tarmakanta about 48 km away from Keonjhar, Orissa, India. The plantation area has an elevation of more than 600 m and was once covered by dry and mixed deciduous sal forests. The soil is red clay-loam and poor in nutrient content. The rhizosphere soil of different tea plants was collected 10 inches below ground in polythene bags for

analysis. Bhuyanpirh tea plantations have not been previously surveyed for AM fungi.

Isolation and characterization of AM spores used published methods (Gerdemann and Nicolson, Trans. Br. Mycol. Soc. 46(2):235-244, 1963; Kormanic and McGraw, Pp. 34-45 in Methods and Principles of Mycorrhizal Research, American Phytopathological Society, 1982; Schenck and Perez, P. 245 in Manual for the Identification of VA Mycorrhizal Fungi, INVAM, 1987). 15-day-old maize seedlings were planted in earthen pots after treatment with or without AM fungi. Roots sampled after 75 days of growth had higher mycorrhizal colonization in water-stressed plants compared to well-watered plants (Fig. 1). Biomass and growth was higher in mycorrhizal than nonmycorrhizal plants irrespective of water treatments (Fig. 2). However, the plants irrigated with alter nate watering schedules showed higher biomass than those treated with daily watering.

Acknowledgements: The authors are grateful to the Department of Forests and Environment, Govt. of Orissa, for various help.

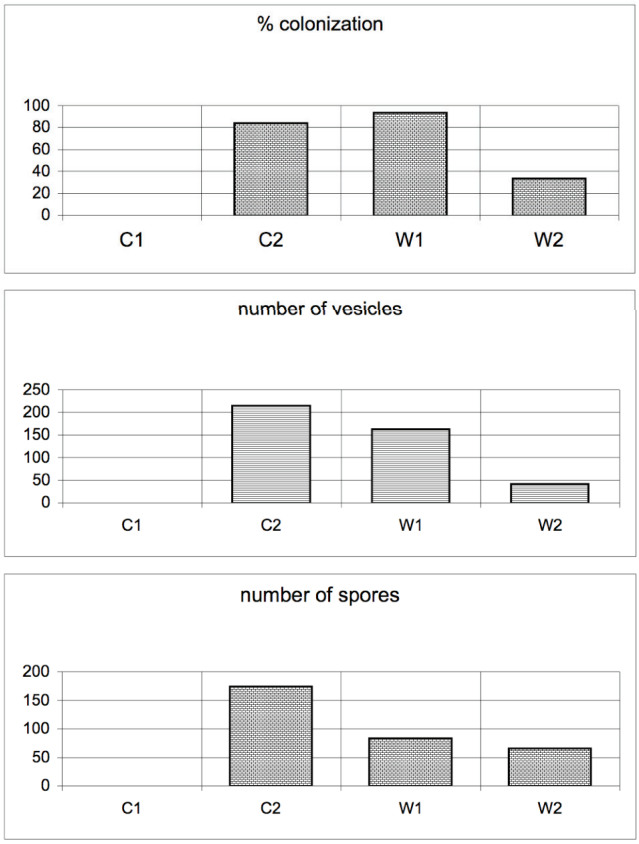

Figure 1. Status of mycorrhization in maize roots inoculated under different treatments. Abbreviations: C1 = daily watering, C2 = AM + daily watering, W1 = AM + alternate day watering , W2 = AM + watering at two day intervals.

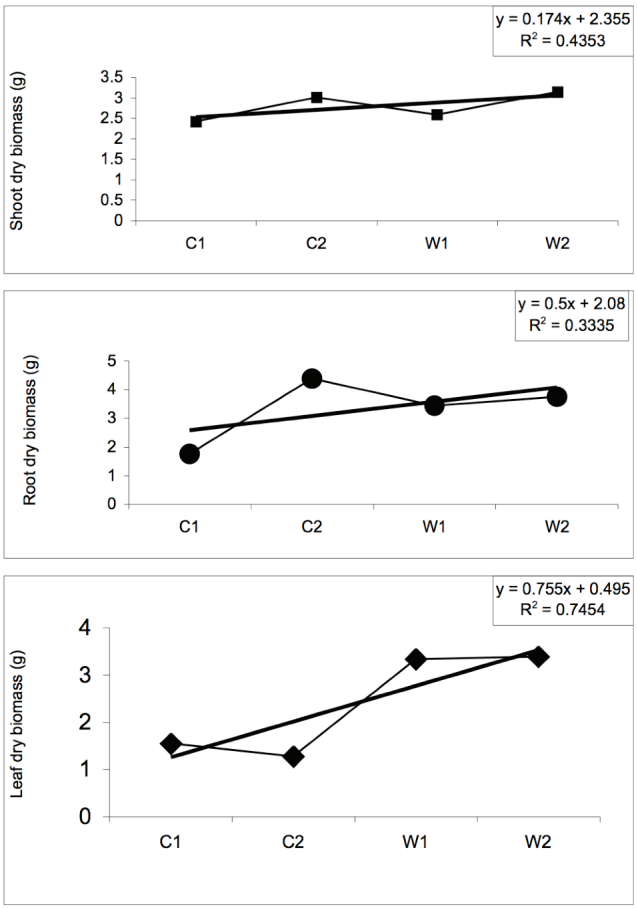

Figure 2. Effect of AM fungi on growth (measured after 75 days of experiment) of maize (host plant) grown under different watering schedules. For abbreviations see Figure 1.

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Analysis of the effect of RAD51 on the spontaneous mutation frequency in maize haploids

--Weber, DF; Liu, S; Li, J; Schnable, PS

Rad51p plays a central role in homologous recombination and the repair of double-strand breaks in *Saccharomyces cerevisiae*. Double mutants of the two *Zea mays* L. *rad51* homologs (*Zmrad51A1* and *Zmrad51A2*) are viable and develop well under normal conditions in diploids. However, they have meiotic abnormalities, are male sterile and have greatly reduced seed set (Li et al., Genetics 176: 1469-1482, 2007). In this article, these alleles will be referred to as *rad51A1* and *rad51A2*.

The purpose of this study was to determine if a higher spontaneous mutation frequency is present in maize plants with both the *rad51A1* and *rad51A2* mutations. For this purpose, the frequencies of mutant sectors on the 5th leaves of haploid plants that were *rad51A1* and *rad51A2* or *Rad51A1* and *rad51A2* were compared. In haploids, all mutant sectors will be detectable because they will not be covered by the non-mutant allele on the normal homolog.

To produce haploids of the two genotypes, a line that produces high frequencies of haploids, RWS, was employed. *Rad51A1/rad51A1; rad51A2/ rad51A2* female parents were crossed with pollen from RWS male parents. The F1s were field planted, and the haploids selected by their distinctive phenotype. Part of the F1 kernels were pre-selected using the *r1-nj* marker allele. Because RWS is *r1-nj/r1-nj* and the pollen parent is *r1/r1*, kernels with colorless embryos can be selected as maternal haploids.

66 haploids were recovered. Each haploid was genotyped to determine if it was *Rad51A1/rad51A2* or *rad51A1/rad51A2.* The number of mutant sectors on leaf 5 of each of the haploid plants was then determined. The leaf sectors were typically chlorotic or necrotic sectors. 46 of the haploids were *Rad51A1/rad51A2* and 20 were *rad51A1/rad51A2*. The reason for the greater frequency of *Rad51A1/rad51A2* plants is not known; perhaps the double mutant ovules function with a reduced frequency. Unfortunately, 2 of the double mutant plants broke off during development and one had half of each leaf missing, and therefore was not evaluated.

The frequency of mutant leaf sectors on the fifth leaf of the 46 *Rad51A1/rad51A2* plants was 1.41±1.26 and the frequency of mutant leaf sectors on the fifth leaf of the 17 *rad51A1/rad51A2* plants was 2.29±2.37. Thus, the frequency of leaf sectors on the two plant types appears to be similar, and we can conclude that the spontaneous mutation frequency is not elevated in somatic cells of double-mutant plants. Also, the two plant types appeared to be indistinguishable from each other, so the double mutants do not appear to have an altered leaf morphology.

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Characterization of maize and teosinte using the variation in their knob sequences

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In maize, the wide variation in nuclear DNA content is mainly caused by differences in heterochromatin amounts (Tito et al., Theor. App. Genet. 83:58-64, 1991; Poggio et al., Ann. J. Bot. 82:115-117, 1998). Knob heterochromatin of maize and teosinte differs from all other classes of heterochromatin due to its cytological appearance and DNA sequence composition (Peacock et al., J. Mol. Evol. 20:341-350, 1981). It is a useful cytological marker because it is polymorphic and was found at 22 different positions within the maize karyotype (Kato, Mass. Agric. Exp. Stn. Bull. 635:1-185, 1976). Knobs consist of thousands to millions of tandem 180- and 350-bp (TR-1) repeats which are present in cytologically detectable knobs in different proportions relative to one another (Ananiev et al., Proc. Natl. Acad. Sci. 95:10785-10790, 1998). Maize knob heterochromatin is also visible in interphase or nondividing somatic cells using a simple DAPI-banding method.

The aim of this work is to analyze the variation in the number of knobs and its sequence composition from different Northwest Argentinean strains of maize and some Mexican teosintes. DAPI banding and fluorescent in situ hybridization (FISH) on interphase cells, using TR-1 and 180-bp repeats as labelled probes, were performed.

Maize materials were kindly provided by Ing. Cámara Hernández from the Vavilov Laboratory of the Facultad de Agronomía (FA) of the Universidad de Buenos Aires (UBA). Mexican teosintes were obtained from the following locations: *Zea diploperennis* from Las Joyas, Jalisco; *Zea luxurians* from Guadalajara; *Zea mays* ssp. *parviglumis* from Mesa Central and *Zea mays* ssp. *mexicana* from Balsas River valley. Plants were cultivated in the greenhouse of FA-UBA. DAPI banding and FISH techniques were done according to the methods of González et al., 2006 (Chrom. Res. 14:629-635).

The experiments showed variations in number and sequence composition of DAPI+ knobs in five maize strains. However, the pattern was recurrent for each race (Table 1).

Table 1. Number and sequence composition of maize knobs by DAPI banding and FISH experiments. Ref: VAV: accession; a.s.l.: about sea level; n/d: no data available.

Maize strain	Cultivation altitude	DAPI+ knobs	180 pb knobs	TR-1 knobs	180 pb + TR-1 knobs
Race Amarillo Chico (VAV6451)	2000 mt a.s.l.	9	5	2	2
Race Orgullo Cuarentón (VAV6482)	910 mt a.s.l.	18	16	n/d	n/d
Race Amarillo Chico (VAV6476)	1690 mt a.s.l.	10	4	0	6
Race Blanco y ocho rayas (VAV6483)	1250 mt a.s.l.	13	9	0	4
Imbreed Line IFSC 13043	00 mt a.s.l.	10	6	0	4

Actually, we are studying the knob sequence composition of different teosintes. We found that the FISH experiments, using the 180 bp repeat as probe, show strong hybridization signals on almost all the DAPI + knobs of *Z. m.* ssp. *parviglumis* and *Z. luxurians*, but on *Z. m.* ssp. *mexicana* knobs these signals are weaker. FISH experiments on *Z. diploperennis* chromosomes using 180-pb and TR-1 probes simultaneously revealed that the two sequences were localized on all DAPI + knobs. These results need to be confirmed for other teosinte accessions.

These experiments demonstrate that the variants of the patterns for number and sequence composition of the heterochromatic knobs, along with their subsequent localization within chromosomes, are useful markers for a proper cytogenetic characterization of maize races and teosintes. The knowledge of these variations will allow further research on the correlation described previously for the presence of knobs and crop altitude (Rosato et al., Am. J. Bot. 85:168-174, 1998). This methodology could then be extrapolated to the cytogenetic characterization of commercial inbreds and maize hybrids.

On the other hand, the cytogenetic characterization of different Argentinean races of maize will contribute to the information about the availability of genetic variability within native materials, useful for its integration in future breeding plans and biodiversity conservation.

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Description of high quality maize single-crosses developed in Argentina

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When completing the process of development, testing and selection of new germplasm specifically designed for commercial use, it is necessary to account with a precise description of the materials. In this sense, the morphological trait descriptors recommended by the IPGRI and UPOV are powerful tools. Accurate morphological descriptions are also indispensable to define the proprietary rights of the new varieties obtained through genetic improvement and facilitate their protection or inscription through utilitarian patents. On November 2007, a three-replicate completely randomized block designed field trial was sown at a density of 71,500 plants/ha in the location of Castelar within the province of Buenos Aires (34º40´00´´S, 58º40´00´´W; 28masl). Twenty-seven high quality single-cross hybrids, generically termed HC, were testcrossed with the dent commercial hybrid ACA 2000 and evaluated through a combination of qualitative and quantitative morphological trait descriptors during the 2007/08 growing season. According to their endosperm characteristics, the materials can be grouped as follows: **I)** *Modified starch (WAXY)*, **II)** *High quality protein (HQP)* and **III)** *Double recessive o2 wx (DR).* Six quantitative morphological traits were used: a) plant height (PH), b) ear insertion height (EIH), c) stem diameter (SD), d) number of total leaves (NL), e) number of leaves above the uppermost ear (NLUE), and f) number of ears per plant (EP). Five qualitative trait descriptors were also considered: g) leaf colour (LC), h) leaf position in relation to the stem (LP), i) sheath pubescence (SP), j) leaf pubescence (LPU) and k) lamina edge curls (LEC). The evaluation was carried out on the basis of individual plants on ten plants per plot. Routine statistical analysis was done according to the classical methodology proposed by Falconer. The information in Table 1 summarizes the evaluation of the quantitative traits of the single-crosses tested in Castelar and reveals their relative behaviour through genotypic means. The maximum PH occurred at silking and the values ranged from 187.6 cm (HC26) to 266.2 cm (HC22). The arrangement of the productive ears in relation to plant height is extremely important, as a lower EIH contributes to a reduction in the tendency to lodging. For that reason, it is preferable that the ears are within the middle third of the PH and this was the case in all the single-crosses tested, as on average, the relationship of EIH/PH was 0.44, ranging from 0.36 to 0.52. Stalk diameter (SD) ranged from 1.4 cm (HC8) to 2.6 cm (HC22), which suggests a satisfactory strength of the stems. On average, the number of total leaves (NL) varied from 11.6 (HC8 and HC16) to 17.6 (HC22), of which 4.4 (HC16) to 7.6 (HC22) were placed above the uppermost ear (NLUE). The data included in Table 1 show that sixteen single-crosses had a PH between the tester (197.0 cm) and the environment mean for the trait (218.4 cm); four hybrids had ears in a lower position than the tester´s EIH (86.6 cm); and fifteen single-crosses showed the same or lower NL than the tester ACA 2000 (13.8). These results permitted us to infer

Table 1. Average of morphological traits evaluated in single-crosses tested in Castelar during the 2007/08 growing season.

Hybrid	Type	PH	EIH	SD	NL	NLUE	EP
HC5	HQP	208.2	89.0	2.2	15.6	6.6	1.6
HC25	HQP	205.2	91.6	1.9	14.2	6.0	1.4
HC26	HQP	187.6	75.8	1.8	13.8	5.8	1.2
HC27	HQP	202.6	88.0	1.6	14.2	5.2	1.2
HC28	HQP	221.6	89.2	2.0	14.0	5.8	1.0
HC29	HQP	217.0	100.4	1.8	13.0	5.8	1.4
HC30	HQP	200.6	78.2	2.2	13.0	6.0	1.4
HC14	DR	205.6	91.0	1.9	13.2	5.0	1.2
HC15	DR	207.4	96.8	1.6	13.2	5.2	1.2
HC8	DR	219.4	80.2	1.4	11.6	4.6	1.4
HC16	DR	209.6	97.4	1.9	11.6	4.4	2.0
HC17	DR	241.2	111.6	2.2	14.6	6.0	1.8
HC18	DR	248.8	127.0	2.1	14.6	5.2	1.4
HC19	DR	208.0	99.8	2.1	13.0	5.0	1.2
HC20	DR	214.8	101.0	2.3	13.8	4.8	1.6
HC21	DR	253.0	108.0	2.5	15.2	6.8	1.8
HC22	DR	266.2	132.2	2.6	17.6	7.6	2.0
HC23	DR	209.2	99.0	2.4	12.6	4.6	1.8
HC24	DR	205.8	80.2	2.3	15.0	7.6	1.4
HC1	WAXY	210.6	97.6	2.0	12.8	5.2	1.2
HC31	WAXY	214.8	97.2	1.9	12.8	4.8	1.4
HC32	WAXY	220.4	103.4	1.7	14.0	5.0	1.2
HC33	WAXY	224.4	108.2	2.1	13.6	5.0	1.4
HC34	WAXY	208.0	90.5	1.7	14.0	6.0	1.6
HC35	WAXY	224.0	103.6	2.3	14.2	5.6	1.6
HC36	WAXY	245.0	114.8	2.5	13.0	5.0	1.8
HC37	WAXY	218.2	107.2	2.2	13.2	5.2	1.6
ACA2000	TESTER	197.0	86.6	2.1	13.8	5.8	1.4
Environment avg.		218.9	98.4	2.0	13.8	5.5	1.5
s.d.		19.1	13.4	0.3	1.2	0.8	0.3
CV%		8.7	13.6	14.8	8.7	14.7	17.9
Min.		187.6	75.8	1.4	11.6	4.4	1.0
Max.		266.2	132.2	2.6	17.6	7.6	2.0
LSD 0,01		3.3	10.1	0.2	0.4	0.2	0.2

that these single-crosses denote a modern architecture which, according to Sangoi et al. (Field Crops Res. 9(1):39-51, 2002), is expressed through a shorter PH, lower EIH, lower NL and more erect leaves. These features result in less interference between adjoining plants, with a better distribution of the light within the canopy, and so, a greater tolerance to high crop density. The hybrid HC22 obtained by crossing an early inbred derived from an Argentine race of maize from the northwestern region by a strongly inbred *wx o2* line produced the tallest plants with the highest number of total leaves.

Although prolificity relies on the genotype, the quality of the environmental conditions around flowering determine the ultimate number of ears per plant. Prolificity is a very important trait for yield determination, as usually only one or two productive ears per plant occur and then individual plant production is slightly elastic (Pedrol et al., IDIA XXI 6(4):141-146, 2004). Most of the commercial hybrids grown in Argentina have 1.0 to 1.5 ears per plant, whilst eleven out of all the single-crosses tested bear more than 1.5 productive ears per plant. Since maize yield decreases when it is grown at high densities due to a marked increase in the number of individuals that undergo ear and kernel abortion, the high prolificity level found in these materials will result in better tolerance to high crop densities.

Significant differences were found for PH (*Student´s t= 2.78*; *p≤ 0.05*) and EIH (*Student´s t= 2.43*; *p≤ 0.05*) among HQP and DR hybrids. Significant differences were also detected for NLUE (*Student´s t= 2.65*; *p≤ 0.05*) among HQP and DR genotypes, and very significant differences for EIH (*Student´s t= 3.27*; *p≤ 0.01*) among HQP and Waxy materials. No significant differences were

found among the different groups of single-crosses for the rest of the morphological traits studied. The ANOVA shows highly significant differences among single-crosses for all the parametric variables measured (Table 2).

Table 2. ANOVA results (mid-squares) for the morphological traits considered in single-crosses evaluated during 2007/08 in Castelar.

Variation source	PH	EIH	SD	NL	NLUE	EP
Hybrid	708.2**	469.9**	0.18**	3.19**	1.21**	0.13**
Replicate	2.16 ns	9.88 ns	0.0006 ns	0.04 ns	0.0001 ns	0.0001 ns

Tables 3, 4 and 5 summarize all of the information collected in relation to the qualitative traits considered in each single-cross. 59.3% of the single-crosses evaluated have their leaves arranged in normal position in relation to the stems, similar to the dent tester ACA 2000. 77.8% of the materials showed abundant sheath pubescence, and in 52.9% of the single-crosses abundant leaf pubescence was observed.

Table 3. Qualitative traits evaluated in HQP single-crosses during 2007/08.

Hybrid	LC	LP	SP	LPU	LEC
3146	dark green	semierect	abundant	abundant	weak
3150a	dark green	semierect	medium	medium	weak
3237	middle green	normal	abundant	medium	weak
3332´´´	middle green	semierect	abundant	medium	weak
3368´´	dark green	semierect	abundant	abundant	weak
3396	dark green	normal	abundant	abundant	weak
3446a	middle green	normal	medium	medium	weak
ACA2000	middle green	normal	medium	medium	strong

Table 4. Qualitative traits evaluated in DR single-crosses during 2007/08.

Hybrid	LC	LP	SP	LPU	LEC
HC14	dark green	normal	abundant	light	weak
HC15	dark green	semierect	abundant	abundant	weak
HC8	dark green	semierect	abundant	light	weak
HC16	dark green	normal	abundant	abundant	strong
HC17	dark green	erect	abundant	abundant	weak
HC18	dark green	normal	abundant	abundant	strong
HC19	middle green	normal	abundant	abundant	weak
HC20	dark green	normal	abundant	abundant	weak
HC21	dark green	semierect	abundant	abundant	strong
HC22	dark green	normal	abundant	abundant	strong
HC23	dark green	normal	abundant	abundant	strong
HC24	dark green	normal	abundant	abundant	weak
ACA2000	verde med	normal	medium	medium	strong

Table 5. Qualitative traits evaluated in waxy single-crosses during 2007/08.

Hybrid	LC	LP	SP	LPU	LEC
HC1	dark green	normal	abundant	light	strong
HC31	dark green	semierect	light	medium	weak
HC32	dark green	normal	abundant	abundant	strong
HC33	dark green	normal	abundant	abundant	weak
HC34	dark green	normal	light	light	weak
HC35	dark green	normal	abundant	abundant	weak
HC36	dark green	semierect	light	light	strong
HC37	dark green	semierect	medium	medium	strong
ACA2000	middle green	normal	medium	medium	strong

Days and heat unit requirements to flowering of quality maize single-crosses developed in Argentina

--Corcuera, VR; Salerno, JC

It is well known that the number of days necessary to complete each phenological phase varies among environments according to changes in relative humidity, air and soil temperature, solar radiation and photoperiod. Most of the disparities in the number of days to flowering and maturity may be explained on the basis of the diverse temperatures recorded among years and locations. The influence of temperature on the length of the different stages of maize development was first mentioned by Lebenhauer in 1914. As temperature is the main factor responsible for the interannual variations observed in the length of growth stages and development, several authors created models to calculate the thermal-time (TT) through linear, exponential or more complex equations.

In November 2007, a three-replicate completely randomized block designed field trial was sown at a density of 71,500 plants/ha at Castelar, in the province of Buenos Aires (34º40´00´´S, 58º40´00´´W; 28masl). Twenty-seven high quality single-crosses, generically named HC, were testcrossed with the dent commercial hybrid ACA 2000 and evaluated last summer (2007/08) for number of days to tasseling (DT), days to silking (DS) and thermal-time, measured as growing-degree days to tasseling (GDDT) and silking (GDDS). The linear method proposed by Gilmore and Rogers in 1958, also known as *modified residual method 10/30*, was used because of its high precision and predictive reliance. This method is based on the amount of energy represented by the sum of degrees centigrade that a plant needs to complete a determined phenological phase and likewise the complete cycle. According to their endosperm characteristics, the materials can be grouped as follows: **I)** *Modified starch (WAXY)*, **II)** *High quality protein (HQP)* and **III)** *Double recessive-o2wx (DR).* The necessary calculations were performed on the basis of information provided by the Climatology Institute of INTA Castelar obtained through field measurements recorded by an automatic station placed in the location.

Because of the poor correlation between the number of days to flowering and plant growth and development it is not possible to get acceptable results when the genotypes are simply classified by their evolutive cycle, although this may be useful as a guideline. Anyway, a well-fitted classification must be based on the proper measurement of the components of the physical environment that promote variations in the number of days necessary to reach a particular phase.

Table 1 summarizes the information obtained for cycle length to flowering evaluated in the single-cross hybrids tested. The average values show that 96.3% of the genotypes evaluated reached silking (R_1; Ritchie & Hanway´s scale, 1993) at 63 days or less from emergence (V_E), with a thermal requirement of ≤733.2 GDD, calculated according to Gilmore & Rogers. These single-crosses showed a shorter evolutive cycle than the tester ACA 2000, and considering their heat unit requirements to silking, belong to FAO classes 100, 200 and 300-400 (Derieux and Bonhomme, Maydica 35:41-46, 1990). They could be subjectively rated as *ultraprecocious* or *precocious.* The DR hybrids HC15 and HC8 reached silking in 55 days and 631.5 GDD, so they could be classified as FAO 100, or *ultraprecocious.* Silking also occurs at 56-57 days from emergence (650.1-670.1 GDD) in the single-crosses HC14, HC18, HC23, HC24, HC26, HC27, HC31 and HC34, which can also be included in the same class FAO 100.

59.3% of the genotypes reached R_1 between 58 and 70 days from emergence, with a 687.8 to 827.8 GDD, so they correspond to classes FAO 200, FAO 300-400, FAO 500 and FAO 600 and can be classiied as *precocious* or *full-season* in relation to their evolutive cycle length. The longest evolutive cycle was observed in the hybrid HC22 (70 days; 771.3 GDD), which was obtained by crossing an early inbred derived from an Argentine maize race

Table 1. Cycle traits measured in different single-crosses tested in Castelar during the summer of 2007/08.

Hybrid	Type	DT	DS	GDDT	GDDS	Interval*	Synchrony
HC5	HQP	56.0	59.0	650.1	702.8	3	good
HC25	HQP	57.0	60.0	670.1	711.2	3	good
HC26	HQP	52.0	56.0	596.6	650.1	4	good
HC27	HQP	54.0	56.0	616.2	650.1	2	very good
HC28	HQP	58.0	62.0	687.8	733.2	4	good
HC29	HQP	54.0	58.0	616.2	687.8	4	good
HC30	HQP	57.0	62.0	670.1	719.2	5	regular
HC14	DR	54.0	57.0	616.2	670.1	3	good
HC15	DR	53.0	55.0	605.7	631.5	2	very good
HC8	DR	52.0	55.0	596.6	631.5	3	Good
HC16	DR	55.0	59.0	631.5	702.8	4	Good
HC17	DR	54.0	58.0	616.2	687.8	4	Good
HC18	DR	55.0	57.0	631.5	670.1	2	very good
HC19	DR	58.0	62.0	687.8	733.2	4	good
HC20	DR	56.0	60.0	650.1	711.2	4	good
HC21	DR	56.0	60.0	650.1	711.2	4	good
HC22	DR	65.0	70.0	771.3	827.8	5	regular
HC23	DR	53.0	56.0	605.7	650.1	3	good
HC24	DR	53.0	57.0	605.7	670.1	4	good
HC1	WAXY	54.0	57.0	616.2	670.1	3	good
HC31	WAXY	53.0	56.0	605.7	650.1	3	good
HC32	WAXY	58.0	63.0	687.8	750.9	5	regular
HC33	WAXY	54.0	58.0	616.2	687.8	4	good
HC34	WAXY	54.0	56.0	616.2	650.1	2	very good
HC35	WAXY	55.0	58.0	631.5	687.8	3	good
HC36	WAXY	57.0	60.0	670.1	711.2	3	good
HC37	WAXY	55.0	58.0	631.5	687.8	3	good
ACA2000	TESTER	57.0	63.0	670.1	733.2	6	wrong
	Env. avg.	55.3	59.0	639.7	694.0		
	s.d.	2.8	3.5	40.0	44.9		
	variance	7.8	12.2	1,603.2	2,015.1		
	CV%*	5.0	5.9	6.3	6.5		
	Min.	52.0	55.0	596.6	631.5		
	Max.	65.0	70.0	771.3	827.8		
	LSD 0,01	1.8	1.9	27.8	31.5		

***=** days between tasseling and silking

from the northwestern region and a highly inbred *wx o2* line. Consequently the single-cross HC22 must be classified as class FAO 600 and must be specially considered for cultivation in the northwestern and northeastern areas of Argentina (≤ 30° S). 57.1% of the HQP single-crosses, 66.7% of the DR hybrids and 75.0 % of the waxy single-cross hybrids are precocious or ultraprecocious (FAO 100, FAO 200 and FAO 300-400). No significant differences were found among HQP, DR and waxy hybrids for cycle traits through Student´s t (see Table 2). The ANOVA detected highly significant differences among hybrids for DT, DS, GDDT and GDDS (see Table 3).

Table 2. Student´s t significance test for cycle traits of single-crosses evaluated in Castelar during the summer of 2007/08.

Contras	DT	GDDT	DS	GDDS
QPM vs DR	0.53 ns	0.34 ns	0.90 ns	0.95 ns
QPM vs Wx	0.00 ns	0.13 ns	0.00 ns	0.20 ns
DR vs Wx	0.57 ns	0.49 ns	0.92 ns	0.77 ns

ns= non significant; ***=** significant at 0.05; ****=** significant at 0.01

Table 3. ANOVA results (mid squares) for cycle traits measured in single-crosses tested in Castelar during the summer of 2007/08.

Variation source	DT	DS	GDDT	GDDS
Hybrid	15.82**	23.26**	3,465.8**	3,964.9**
Replicate	0.23 ns	7.56 ns	196.9 ns	2,454.4 ns

For the hybrids as a group, a highly significant simple correlation index (*r= 0.96*) among days to tasseling and days to silking was found, revealing a good to very good flower synchrony with intervals of 2 to 3 days between both sexes' maturation (see Table 1 for more details) in most of the genotypes.

A cluster analysis using the algorithm UPGMA and the average linkage method developed in 1958 by Sokal and Michener was run with the purpose of grouping genotypes according to their genotype-environment (GE) interaction on the basis of the data recorded in the field for DS (Figure 1). For example, the single-crosses HC5 and HC16, with 59 days to silking, are very similar in their GE interaction and are placed in the lower part of the dendrogram. A similar situation occurs with the genotypes HC19 and HC28, which reach flowering 62 days after emergence, and are situated in the upper portion of the dendrogram. Four groups can be observed throughout the dendrogram: the two central ones limited by HC8-HC26 and HC1-HC29 include precocious and ultraprecocious materials, whilst the longer cycle hybrids are clustered in the upper group. The long cycle length hybrid HC22 appears on the top of the dendrogram, clearly separated from the rest of the genotypes.

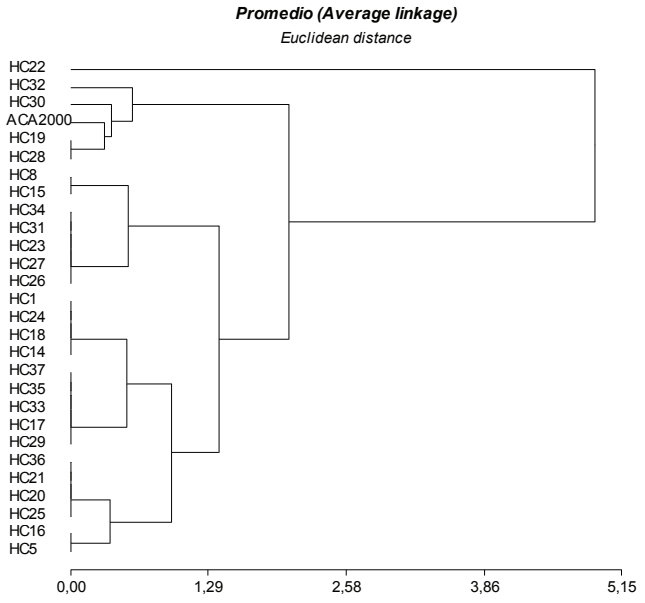

Figure 1. Dendrogram resulting from the cluster analysis of 28 maize genotypes using the UPGMA algorithm on the matrix of Euclidean distances. Only includes data for number of days to silking.

The results obtained reveal the high degree of precocity obtained, even in HQP single-crosses that usually express a long cycle and high thermal requirements to silking. The development of ultraprecocious and precocious materials well-adapted to areas with short summers and a wet autumn will make it possible to increase the economically significant area for corn in Argentina, but in this particular case with high endosperm quality suitable for diverse industrial uses as well as direct feeding. In terms of their degree of precocity, the single-cross hybrids tested could be recommended for late sowing in the northern portion of the Pampeana Area, and in Corn Regions VI, VIII and IX after completion of new field trials.

Yield evaluation of high quality single-crosses in Argentina

--Corcuera, VR; Kandus, M; Salerno, JC

Maize yield is closely related to the number of kernels produced per unit area, which is determined during flowering, and thus a decrease in the aboveground plant growth rate at this phase, promoted for example by shading or diminution of the incident radiation, incites important yield losses. Consequently, the growth rate of the crop at flowering (associated with radiation, temperature, water and nutrient supply) is a reference of the crop capacity to yield kernels. Whilst the number of fertile ears per plant is defined at flowering, the final number of kernels per ear is determined after silking and is associated with the physiological condition of the crop during flowering. If environment or growing conditions are good, a greater number of kernels per unit area can be developed. On the other hand, the morphogenetic processes that end in the determination of kernel rows per ear and spikelets per row are quite insensitive to environment and crop management variations. The number of kernel rows is primarily determined by the plant genotype. So, the number of kernels per unit area relies on the number of kernels per ear, the number of ears per plant and the number of plants per unit area (crop density). Likewise, the average kernel weight results from the combined effect of two coinciding factors: length of filling period and filling rate.

In November 2007, a completely randomized block designed field trial with three replicates was sown at a density of 71,500 plants/ha at Castelar, in the province of Buenos Aires (34º40´00´´S, 58º40´00´´W; 28masl). On the basis of their endosperm characteristics, the hybrids may be grouped as: **I)** *Modified starch (WAXY)*, **II)** *High quality protein (HQP)* and **III)** *Double recessive-o2 wx (DR).* Twenty-seven high quality single-cross hybrids, generically named HC, were testcrossed with the dent commercial hybrid ACA 2000 and evaluated during the summer of 2007/08 according to ear and yield traits. The traits evaluated were *ear length* (EL), *ear diameter* (ED), *number of kernel rows* (KR), *kernel number per row* (KNR), *average ear weight* EW), *average kernel weight per ear* (KWE) and *cob percentage* (%C). Yield estimation was performed as follows: ears from each plot were hand-harvested and only ten representative ears were selected and weighed. After shelling, moisture percentage was recorded using a portable humidimeter (Protimeter model Grainmaster 900) and kernel weight per ear was measured and averaged to obtain KWE. The **minimum potential yield** (MINPY) was calculated as KWE x 71,500 ears/ha, assuming that only one ear is borne by each plant. The **maximum potential yield** (MAXPY) was estimated using the following equation: KWE x crop density x ears per plant (prolificacy).

The average values for ear and yield traits of each quality single-cross hybrid are summarized in Table 1. 29.6% of the materials tested yielded long ears (20 to 25 cm) similar to the tester ACA 2000, and 70.4% yielded middle sized ears (15 to 19.9 cm). The average EW was 185.2 g, including 18.0% cob. The DR hybrid HC17 yielded significantly heavier ears than the tester before and after shelling. 55.6% of the genotypes evaluated showed a higher KWE than the environmental average. Significant differences (*Student´s t*) among the different groups of single-cross hybrids (HQP, DR and waxy) were not found for any of the descriptors considered (see Table 2). The ANOVA detected very significant differences among hybrids for all ear and yield traits measured (see Table 3).

Table 1. Average values for ear and yield traits measured in single-crosses in Castelar during 2007/08.

Hybrid	Type	EL	ED	KR	KNR	EW	KWE	%C
HC5	HQP	19.4	4.1	14.0	41.3	157.0	122.2	22.4
HC25	HQP	17.3	4.2	12.0	43.5	137.5	113.5	17.4
HC26	HQP	18.1	4.1	14.0	35.2	151.0	123.2	18.5
HC27	HQP	19.6	4.0	14.0	39.8	195.4	158.6	18.8
HC28	HQP	18.7	4.3	15.3	40.8	187.5	156.7	16.3
HC29	HQP	21.0	4.3	16.0	37.5	230.5	196.0	15.0
HC30	HQP	20.0	4.4	18.0	49.0	212.0	164.0	22.6
HC14	DR	21.5	4.5	18.0	42.0	231.0	187.5	18.8
HC15	DR	18.0	4.2	15.0	34.0	145.0	119.0	17.9
HC8	DR	20.8	4.3	15.6	42.8	192.4	152.2	20.4
HC16	DR	20.5	4.2	13.0	43.0	191.5	152.5	20.2
HC17	DR	20,5	3.9	13.7	46.5	169.8	146.5	13.9
HC18	DR	17.7	4.4	16.4	36.2	184.4	155.0	16.4
HC19	DR	19.0	4.3	16.0	39.5	190.5	159.3	16.1
HC20	DR	17.7	4.2	16.7	38.0	173.7	149.3	14.1
HC21	DR	19.1	4.1	16.0	41.1	193.1	156.1	19.1
HC22	DR	19.8	4.2	16.0	40.3	212.7	173.3	18.3
HC23	DR	16.5	3.9	13.5	36.3	135.5	111.0	18.2
HC24	DR	17.8	4.2	15.7	37.8	161.7	134.5	17.1
HC1	WAXY	18.9	4.5	16.8	39.0	192.2	161.4	15.9
HC31	WAXY	20.2	4.0	14.4	40.6	190.6	152.8	20.1
HC32	WAXY	18.6	4.3	15.0	34.0	167.0	140.5	15.6
HC33	WAXY	17.0	4.3	17.2	37.6	162.0	132.8	17.4
HC34	WAXY	17.5	4.2	17.3	34.0	153.0	126.0	17.6
HC35	WAXY	17.7	4.3	16.5	35.2	160.7	133.1	16.9
HC36	WAXY	18.6	4.5	17.0	38.3	189.0	160.3	15.2
HC37	WAXY	21.6	4.3	16.5	43.8	217.3	166.0	23.2
ACA2000	TESTER	20.6	4.8	16.8	42.0	261.8	227.6	13.1
Environment avg.		19.1	4.3	15.7	39.6	185.2	151.7	18.0
s.d.		1.6	0.2	1.6	3.9	35.8	30.3	2.6
CV%		8.6	5.4	9.9	9.8	19.3	20.0	14.2
Min.		14.9	3.9	12.0	30.5	133.5	100.5	13.1
Max.		21.6	4.9	18.5	49.0	301.8	246.8	23.2
LSD 0.01		1.1	0.3	0.8	2.6	11.3	6.6	0.9

Table 2. Significance t test (Student´s) for ear traits among the different groups of hybrids.

Contrast	EL	ED	KR	KNR	EW	KWE	%C
QPM vs DR	1.08 ns	0.00 ns	0.39 ns	0.52 ns	1.00 ns	1.07 ns	0.81 ns
QPM vs Wx	0.26 ns	1.25 ns	1.30 ns	0.82 ns	0.32 ns	0.45 ns	0.91 ns
DR vs Wx	0.88 ns	1.05 ns	1.37 ns	1.94 ns	0.87 ns	0.93 ns	0.29 ns

ns= non significant differences

Table 3. ANOVA results for ear and yield traits in single-cross hybrids tested in 2007/08.

Variation source	EL	ED	KR	KNR	EW	KWE	%C
Hybrid	4.98**	0.08**	4.71**	26.91**	2411.39**	1758.15**	12.03**
Replicate	0.08 ns	0.05 ns	0.06 ns	0.05 ns	0.01 ns	1.70 ns	0.0006 ns

ns= non significant; **= significant at 0,01%.

The MINPY and MAXPY of all single-cross combinations tested at Castelar are included in Table 4. The tester ACA 2000, also used in previous years and field trials, expressed a MINPY of 16,273 kg/ha and a MAXPY of 22,782 kg/ha. The general average of the trial was 10,848 kg/ha for MINPY and 15,955 kg/ha for MAXPY. It was observed that the methodology used to estimate the MAXPY produced an overestimation of around 47.1 % in relation to MINPY. In fact, when the plant bears more than one ear, the second one does not have the same size or weight as the older and this promotes the deviation observed. Fifteen genotypes expressed a higher MINPY than the environmental average registered for the trait. It must be remarked that 50% of the DR hybrids, as well as 57.1% of the HQP single-crosses and 62.5% of the waxy genotypes, exceeded the environmental average for MINPY. Considering only MINPY, the hybrid with the lowest yield was HC23, which yielded 51.2% less than the tester and a significant

26.8% less than the trial's combined average. The most common statistics for the MINPY and MAXPY of each group of single-cross hybrids are shown in Table 5. No significant differences (*Student´s t*) could be detected between HQP, DR and waxy hybrids for MINPY and MAXPY (see Table 6).

Table 4. Average yield for single-cross hybrids evaluated at Castelar (2007/2008).

Hybrid	Type	MINPY	MAXPY
HC5	HQP	8,737	13,979
HC25	HQP	8,115	11,361
HC26	HQP	8,809	10,571
HC27	HQP	11,340	13,608
HC28	HQP	11,204	11,204
HC29	HQP	14,014	19,620
HC30	HQP	11,726	16,416
HC14	DR	13,406	16,087
HC15	DR	8,509	10,211
HC8	DR	8,801	12,321
HC16	DR	10,904	21,808
HC17	DR	10,475	18,855
HC18	DR	11,083	15,516
HC19	DR	11,390	13,668
HC20	DR	10,675	17,080
HC21	DR	11,162	20,092
HC22	DR	12,391	24,782
HC23	DR	7,937	14,287
HC24	DR	9,617	13,464
HC1	WAXY	11,540	13,848
HC31	WAXY	10,925	15,295
HC32	WAXY	10,046	12,055
HC33	WAXY	9,495	13,293
HC34	WAXY	9,009	14,414
HC35	WAXY	12,820	20,512
HC36	WAXY	11,461	20,630
HC37	WAXY	11,869	18,990
ACA2000	TESTER	16,273	22,782
Environment avg.		10,848	15,955
s.d.		1,900.5	3,959.5
CV%		17.5	24.8
Min.		7,937	10,211
Max.		16,273	24,782
LSD 0,01		1,593.3	263.9

Table 5. Usual statistics for potential yield of the hybrids tested during 2007/08.

	HQP		DR		WAXY	
Statistic	MINPY	MAXPY	MINPY	MAXPY	MINPY	MAXPY
Average	10,123.6	13,705.3	11,079.2	16,968,7	10,895.6	16,129.7
s.d.	2,141.4	3,673.7	2,212.0	4,132.0	1,288.9	3,404.5
variance	4,585,615.8	13,496,303.9	4,893,031.7	1,707,325.8	1,661,339.0	11,590,372.6
CV%	21.2	26.8	20.0	24.4	11.8	21.1
min	7,186.0	8,623,2	7,937.0	10,210.8	9,009,0	12,055.2
max	14,014.0	19,619.6	17,646.0	24,782.0	12,820.0	20,629.8

Table 6. Significance t test (Student´s) for potential yield among different groups of hybrids.

Contrast	MINPY	MAXPY
QPM vs DR	0.107 ns	2.06 ns
QPM vs Wx	0.93 ns	1.45 ns
DR vs Wx	0.27 ns	0.55 ns

In Argentina, although the nationwide average maize yield is about 5,861 kg/ha, it is not uncommon to obtain 13,000 kg/ha without irrigation. The high yields recorded are due to the incorporation of biotechnology tools during recent years, as well as the use of new crop management practices, that together favour maximum expression of the genetic potential. The quality single-crosses tested at Castelar during 2007/08 exceeded the average national yield by about 85%, when considering the MINPY combined average.

Chemical composition of inbreds and single-crosses developed in Argentina

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The physiochemical constitution of the maize kernel not only defines its nutritional value but also its ability to be used in transformation industries. Kernel quality depends on external factors influenced by the environment, weather, soils, temperature, and rainfall, as well as the management technology used during crop growth and development aimed at obtaining economically sustainable yields. Inherent characteristics of the kernel, such as the genetic background, undoubtedly influence chemical quality and may be modified to improve chemical constitution and so achieve new germplasm with excellent attributes in relation to industrial uses and nutritional value.

Last summer (2007/08), at Castelar, in the province of Buenos Aires (34º40´00´´S, 58º40´00´´W; 28masl), a complete randomized block design field trial with three replicates, which included twenty-seven single-cross hybrids and a tester (ACA2000), was sown at a density of 71,500 plants/ha. Based on their endosperm attributes, the materials, generically named HC, can be grouped as: **I)** *Modified starch (WAXY)*, **II)** *High quality protein (HQP)* and **III)** *Double recessive-o2 wx (DR)*. The materials were evaluated using chemical descriptors, which are better than the morphological ones, in the sense that they are not significantly influenced by the environment. Thus, it is feasible to compare descriptions taken in different locations and years if properly standardized methods are used. The kernels of thirteen inbreds kept in a cold room were also analyzed. The chemical composition of the hybrids and inbreds was determined using an infrared spectrophotometer model Foss Infratec 1241 Grain Analyzer to quantify protein content (%P), starch content (%S), oil content (%O) and kernel density (KD) through a non-destructive assay. Two 60 g samples of each genotype were analyzed and the results were averaged to obtain the final values. In addition, the oil content of the inbreds was also determined through Soxhlet (AOAC, 2000). The simple correlation coefficient (*Pearson*) among the different chemical components was estimated.

Table 1 summarizes all the information relative to the chemical composition of each single-cross hybrid determined via NIRT. Maize is one of the main energy sources of animal dietary rations. On average, oil content is relatively low and usually ranges from 3% to 5%. Bromatological analysis of the maize most commonly produced worldwide indicates that oil content is around 3.0% to 3.5%. According to data published by ILSI (Source = *ILSI Crop Composition Database version 2.0; www.cropcomposition.org*) maize oil content throughout the world varies from 1.74% to 5.56%. If only maize produced in Argentina is considered, the oil content is about 2.68% to 5.56%. According to the previous data, a maize kernel with ≥ 5.6% oil could be considered a high oil content genotype (HOC). Around 40.7% of the HC single-crosses tested at Castelar showed 5.6% to 6.3% oil content. Four hybrids equaled, or even slightly exceeded, the tester´s average oil content.

In general, maize protein content varies greatly depending on the genotype, production environment, sampling and calculation factors used to convert N into protein. According to ILSI, average protein content of maize kernels produced in Argentina is about

Table 1. Chemical composition of single-cross hybrids determined by NIRT.

Hybrid	Type	%Oil	%Protein	%Starch.	Density
HC5	HQP	5.2	11.0	70.3	1.32
HC25	HQP	5.1	12.3	69.7	1.30
HC26	HQP	5.6	11.5	69.4	1.31
HC27	HQP	5.9	11.9	68.9	1.31
HC28	HQP	6.0	12.5	68.0	1.29
HC29	HQP	5.4	11.6	69.7	1.32
HC30	HQP	6.1	11.0	69.4	1.33
HC14	DR	4.3	10.9	71.6	1.30
HC15	DR	4.6	10.9	70.9	1.31
HC8	DR	5.2	11.4	69.8	1.33
HC16	DR	5.4	11.2	69.7	1.32
HC17	DR	5.8	10.4	70.4	1.31
HC18	DR	5.7	10.5	70.7	1.31
HC19	DR	5.2	10.2	70.9	1.30
HC20	DR	5.6	10.5	70.7	1.32
HC21	DR	5.8	10.5	70.1	1.32
HC22	DR	6.3	11.7	68.8	1.32
HC23	DR	6.0	11.1	69.2	1.34
HC24	DR	5.8	10.9	69.9	1.31
HC1	WAXY	4.7	11.7	70.4	1.29
HC31	WAXY	5.1	11.7	69.4	1.28
HC32	WAXY	4.6	10.9	70.6	1.29
HC33	WAXY	5.2	9.9	71.5	1.30
HC34	WAXY	5.3	11.0	70.0	1.29
HC35	WAXY	5.3	11.0	70.0	1.30
HC36	WAXY	4.9	11.1	70.5	1.29
HC37	WAXY	5.1	11.9	70.0	1.31
ACA2000	TESTER	6.0	10.3	69.4	1.32
	Env. avg.	5.4	11.2	69.9	1.31
	CV%	9.5	5.7	1.1	0.8
	Min.	4.3	9.9	68.0	1.28
	Max	6.3	12.5	71.6	1.34

9.5% (estimated on 109 genotypes grown in the provinces of Buenos Aires and Córdoba between 1999 and 2001). This value is in agreement with others published in the Argenfoods database (Universidad Nacional de Lujan, 2002). The HC hybrids showed an average protein content of 11.2% (range = 9.9% to 12.5%) (Table 1). 63% of the single-crosses averaged 11% to 12.5% kernel protein content, exceeding the tester protein content in 7.0% to 21.4%. In addition, kernel starch content averaged 69.9% for the HC hybrids with values ranging from 68.0% (HC28) to 71.6% (HC14). Twenty HC single-crosses exceeded the average starch content found for the tester ACA 2000. The average starch content of HC hybrids corresponds to values published by MAIZAR Association in Argentina after testing 48 commercial hybrids grown in the Argentine Corn Belt or ZMT during the growing season of 2004/05 by NIRT. Significant differences for protein and starch content were only found between HQP and DR single-crosses, as well as between HQP and waxy hybrids (Table 2).

Table 2. Significance test for NIRT values among groups of hybrids.

Contras	%Oil	%Protein	%Starch	Density
QPM- DR	0.51 ns	2.73*	2.34*	0.00 ns
QPM-Wx	1.36 ns	2.26*	2.29*	0.00 ns
DR- Wx	1.00 ns	0.36 ns	0.31 ns	0.00 ns

ns = non significant; * = significant at 0.05

The results of the ANOVA showed highly significant differences among genotypes for oil content ($F_{27\text{-}27}$: *7.77; p: 0.01*), for protein content ($F_{34\text{-}34}$: *2,26; p: 0,01*) and also for starch content ($F_{27\text{-}27}$: *3.09; p: 0.01*). Only significant differences among hybrids were detected for kernel density ($F_{27\text{-}27}$: *1.84; p: 0.05*). When Fisher´s LSD test was used to compare the average oil content, 14 homogeneity groups were distinguished (*D: 0.74; p: 0.01)*. Using the same methodology, 8 homogeneity groups were found for protein content (*D: 0.74; p: 0.01)*, 6 homogeneity groups for starch content (*D: 1.74; p: 0.01)* and only 3 groups for kernel density (*D: 0.03; p: 0.01).* Pearson´s correlation coefficients among starch, protein, oil content and kernel density were estimated (Table 3). All genotypes showed only significant but negative correlations between oil and starch content, as well as between protein and starch content.

Table 3. Relation among the different kernel chemical components.

	Pearson´s simple correlation coefficient (r)			
	%Oil	%Protein	%Starch	Density
%Oil		-0.04 ns	-0.69**	0.17 ns
%Protein			-0.60**	0.05 ns
%Starch				-0.04 ns

ns = non significant;** = significant at 0.01

The data in Table 4 show that the inbreds' oil content averages 5.61% and 5.75% via NIRT and Soxhlet, respectively. The Soxhlet results indicate that oil content varies from 4.4% (CIG29) to 7.7% (CIG6). Six inbreds yielded more than 6.0% oil and could be considered HOC genotypes and used as male progenitors in future crosses. A very high correlation between the results obtained by NIRT and Soxhlet (*r=0.921*) was found.

Table 4. Kernel oil content of inbreds measured by destructive and non-destructive methodologies.

Inbred	Type	NIRT*	Soxhlet*
CIG81	waxy	5.1	4.8
CIG 28	waxy	6.3	6.0
CIG 30	waxy	4.9	5.0
CIG 36	HQP	6.5	6.5
CIG15	waxy	6.8	6.4
CIG35	waxy	3.9	4.5
CIG13	waxy	4.6	5.3
CIG1	waxy	6.1	6.6
CIG6	waxy	7.3	7.7
CIG34	waxy	6.4	7.0
CIG9	waxy	5.5	5.0
CIG29	DR	4.2	4.4
CIG37	HQP	5.4	5.5

* = expressed in percentage on the basis of dry weight.

The results obtained demonstrate that the protein, starch and oil content in most of the HC hybrids analyzed equal or exceed the average values of the commercial hybrids actually grown in Argentina. This fact, along with their grain yield, implies that these genotypes are able to produce important amounts of these components per unit area. As a result, they are very competitive for use in diverse industrial processes. In addition, all these materials carry in their genetic background one or two of the recessive genes *wx*, *o2*, *o5* and *o12* that promote a better response when used in animal or human feeding.

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Polyamine biosynthesis is required for normal plant regeneration from maize callus cultures

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Studies examining the polyamine concentration of light-grown maize callus (Tiburcio et al., Plant Tissue and Organ Cult. 27:27-32, 1991; Torne et al., Plant Cell Physiol. 34:371-374, 1993; Boget

et al., Plant Cell Tissue Organ Cult. 40:139-144, 1995) suggest that plant regeneration is highest from tissues with low levels of polyamines either endogenously present or induced by inhibitors of polyamine biosynthesis. This increased regeneration is greatest when the tissues are pretreated with a polyamine biosynthesis inhibitor prior to, but not during, plant regeneration (Tiburcio et al., Plant Tissue Organ Cult. 27:27-32, 1991; Torne et al., Plant Cell Physiol. 34:371-374, 1993). The most commonly used inhibitors of polyamine biosynthesis are α-difluoromethylornithine (DFMO) and α-difluoromethylarginine (DFMA), irreversible inhibitors of ornithine decarboxylase and arginine decarboxylase, respectively.

Immature embryo-derived maize callus is often grown in the dark and only regenerated in the light (Armstrong and Green, Planta 164:207-214, 1985; Duncan et al., Planta 165:322-331, 1985). Since the previous studies were done using light-grown callus, we examined the role of polyamines in plant regeneration from dark-grown maize callus.

To determine if de novo polyamine biosynthesis is required for plant production from dark-grown callus cultures, type I callus was initiated from immature embryos of the self-pollinated maize *(Zea mays L.)* inbred lines Pa91 and H99. Calluses were maintained in the dark at 28 C on D medium (Duncan et al., Planta 165:322-331, 1985) and sub-cultured at 14 to 28-d intervals.

Plants were regenerated by placing 20 pieces of callus per petri plate (0.01 gfw/piece and 2 to 3 plates per treatment) on H medium (Duncan et al., Planta 165:322-331, 1985) containing 3.5 mg l^{-1} 6-benzylaminopurine (6BA) for 3 d. Calluses were then transferred to H medium without 6BA for an additional 21 d (Duncan and Widholm, Plant Cell Reports 7:452-455, 1988), after which the number of individual regenerated plants were counted. The regenerating cultures were incubated at 28 C in continuous light (approximately 80 μmole photons $m^{-2}s^{-1}$).

Experimental modification of the above media consisted of adding filter sterilized putrescine, spermidine, spermine, DFMO or DFMA to the cooled, autoclaved medium prior to pouring into Petri dishes. Concentrations of medium additives are listed in the Tables. The DFMO and DFMA were obtained courtesy of Merrill Dow Research Institute, Cincinnati, Ohio.

Since it had earlier been shown by Tiburcio et al. (Plant Tissue Organ Cult. 27:27-32, 1991) and Torne et al. (Plant Cell Physiol. 34:371-374, 1993) that polyamines could be synthesized in maize by both arginine decarboxylase and ornithine decarboxylase, both DMFO and DMFA were added simultaneously to the culture media. In the present study, these inhibitors reduced plant production by 4-month-old H99 and Pa91 cultures approximately 62% and 91%, respectively, as compared to untreated controls (Table 1).

Table 1. The effect of polyamine biosynthesis inhibitors added to plant regeneration medium on maize plant regeneration from cultures initiated 4 months earlier.[1]

	Genotype	
Treatment	H99	Pa91
	Shoots gfw^{-1}	
No additions	28. 9 ±10. 2	25.8 ± 6.8
lmM each DFMO and DFMA[2]	11.1 ± 5.5	2.4 ± 1.8

[1]Inhibitors were added to both H medium containing 6-benzyladenine (Duncan and Widholm, Plant Cell Rep. 7:452-455, 1988) and H medium without 6-benzyladenine (Duncan et al., Planta 165:322-331, 1985). The cultures were 4 months old and were subcultured at 28 d intervals. Initial inoculum size was 0.01 gfw $callus^{-1}$ pieces with 20 pieces per petri $dish^{-1}$ and three replicates per treatment.
[2]DFMO = α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase; DFMA = α-difluoromethylarginine, an irreversible inhibitor of arginine decarboxylase.

Regeneration inhibition by DFMO and DFMA was consistent throughout all the experiments; however, the degree by which the combination inhibited plant regeneration was variable. For example, using 8-month-old callus, maintained on a 28 d subculture cycle, H99 and Pa91 were inhibited by the combination of 1 mM DFMO and DFMA approximately 81% and 33%, respectively, as compared to untreated controls (Table 2). Variation in the quantity of preexisting polyamines may explain the variable degree of plant regeneration ability as well as the variation in the inhibition caused by DFMO and DFMA (Tables 1 and 2).

Table 2. The effect of polyamine biosynthesis inhibitors added to plant regeneration medium on maize plant regeneration from cultures initiated 8 months earlier.[1]

	Genotype	
Treatment	H99	Pa91
	Shoots gfw^{-1}	
No additions	4.25 ± 0.75	49.5 ± 2.28
1 MM each DFMO and DFMA[2]	0.83 ± 0.83	33.05 ± 3.32
1 mM each DFMO, DFMA and 1 mM PUT	7.81 ±1.14	46.67 ± 0.95
1 mM PUT	12.52 ± 0.467	40.95 ± 7.62

[1]Inhibitors were added to both H medium containing 6-benzyladenine (Duncan and Widholm, Plant Cell Rep. 7:452-455, 1988) and H medium without 6-benzyladenine (Duncan et al., Planta 165:322-331, 1985). The cultures were 8 months old and subcultured at 28 d intervals. Initial inoculum size was 0.01 gfw $callus^{-1}$ pieces with 20 pieces per petri $dish^{-1}$ and three replicates per treatment.
[2]DFMO = α–difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase; DFMA = α–difluoromethylarginine, an irreversible inhibitor of arginine decarboxylase; PUT = putrescine.

To determine if the inhibitory effect of DFMO and DFMA actually resulted from polyamine biosynthesis inhibition, putrescine (the first polyamine whose synthesis is inhibited by DFMO and DFMA) was added to the DFMO and DFMA treatment medium in an attempt to reverse the inhibition of plant regeneration. In the case of Pa91, putrescine restored plant regeneration to the same level as the untreated control, whereas DFMO and DFMA alone suppressed plant production by about 33% (Table 2). In the case of H99, putrescine alone or in combination with DFMO and DFMA stimulated plant production by about 91% and 81%, respectively, as compared to the untreated control (Table 2). The DFMO and DFMA treatment alone, however, reduced plant production by about 81% as compared to the control (Table 2). These results further demonstrate that de novo polyamine biosynthesis is needed for normal plant regeneration from dark-grown maize callus cultures, especially in the case of H99 where the plant regeneration capacity was low.

Considering the stimulatory effect of putrescine on plant regeneration from H99 callus noted in Table 2, further experiments were conducted to determine if the addition of polyamines to the regeneration protocol would increase plant production. When either putrescine or spermidine were added to regeneration medium, plant production by Pa91 callus was increased compared to the untreated control, with the greatest increase (about 82%) resulting from the 1 mM putrescine treatment (Table 3). No treatment, however, showed a significant effect on plant production of H99 (Table 3). Furthermore, 54-month-old H99 callus which had lost its capacity to regenerate plants did not respond to any polyamine treatment by producing plants (Table 3).

Table 3. The effect of polyamines added to plant regeneration medium on maize plant regeneration.[1]

Polyamine Treatments	Genotype		
	H99 (6 mo. old)[3]	H99 (54 mo. old)	Pa91 (6 mo. old)
	Shoots gfw^{-1}		
No additions	41.3 ± 8.75	0.0	26.1 ± 8.7
1.0mM Putrescine	28.6[2]	0.0	47.4 ± 10.3
0.1mM Putrescine	45.5 ± 5.5	0.0	37.0 ± 2.2
1.0mM Spermidine	47.1 ± 15.1	0.0	33.6 ± 1.7
0.lmM Spermidine	45.7 ± 18.8	0.0	29.6 ± 3.7

[1]Treatments made of H medium containing 3.5 mg l^{-1} 6BA (Duncan and Widholm 1988, Plant Cell Reports 7:452-455) and H Medium (Duncan et al., 1985, Planta 165:322-331) plus appropriate concentration of polyamines. Treatments were randomly inoculated with callus maintained on a 14-21 d subculture routine. Initial inoculum size was 0.01 gfw callus^{-1} pieces, with 20 pieces per petri dish^{-1} and two replicates per treatment.
[2]Only one replicate, the other was contaminated.
[3]Time after culture was initiated.

These accumulated results suggest that, although inhibitor studies indicate that polyamine biosynthesis is required for plant regeneration, under typical culture and regeneration conditions dark-grown maize callus seems to have an adequate supply of polyamines, except in cases where the untreated control plant regeneration capability is low, as with H99 in Table 2 and Pa91 in Table 3. Variability in response to polyamines may possibly be due to variability of the polyamine content of callus prior to regeneration. Also, the variable response to polyamines could possibly be due to uneven and uncontrolled loss of the volatile polyamines from the culture system. Unlike the studies with light-grown maize callus where lower polyamine concentrations stimulated plant regeneration, the dark-grown maize callus seemed in most cases to adequately produce polyamines, and the addition or removal of polyamines to regeneration medium did not increase plant production.

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Induction of maternal haploids in maize

--Rotarenco, V; Dicu, G; Sarmaniuc, M

Recently, haploidy has been used widely in both maize breeding and genetics. Moreover, the technology of chromosome doubling of haploids (DH) is the main method of producing homozygous lines in most maize breeding companies. The creation of inducers of maternal haploids, based on the Stock 6 line (Coe, 1959), has allowed many maize breeders to use haploid methods. However, the mechanism of haploid induction has not been explained, so far. The induction of maternal haploids is considered to occur due to a single fertilization, i.e., one of the sperms fertilizes the central nucleus of an embryo sac and the formation of an endosperm provokes the development of an unfertilized (haploid) egg cell (Enaleeva et al., 1990). Some morphological differences among sperm of a haploid inducer have been revealed (Bylich and Chalyk, MNL 70, 1996). The results of that study have been connected with the hypothesis of single fertilization. This kind of mechanism is known in apomictic development--pseudogamy. However, this ability is specific for female genotypes and, as a rule, results in the development of diploid embryos.

In this work, we would like to present some results which have some contradictions with the mechanism of haploid induction mentioned above. Earlier, it was assumed that the frequency of haploids could be decreased by heterofertilization (Rotarenco and Eder, MNL 77, 2003). Studying the influence of heterofertilization on the induction of maternal haploids was one purpose of this work.

Two inbred lines, A464 and A619, were crossed with two males, a haploid inducer and the X28C line (not a haploid inducer). The males possessed a dominant marker gene *R1-nj* which causes a purple scutellum and a "purple crown" of the aleurone (Nanda and Chase, 1966). Different kinds of pollinations were performed: simple pollinations of females with the pollen of males, pollinations with pollen mixtures (made of the pollen of the males and females in a 50/50 mixture), and repeated pollinations after 24, 48 and 72 hours with the pollen of females (self-pollination). No fewer than 10 ears were used for each kind of pollination.

The following four types of kernels were obtained (Fig. 1): 1) yellow kernels (female type); 2) kernels with the full expression of the *R1-nj* gene; 3) kernels with colored aleurone (endosperm); and 4) kernels with colored scutellum (embryo). The kernels of the third and fourth groups are the results of heterofertilization. However, among the kernels of the third group there were kernels with haploid embryos. All of the kernels of the third group were planted and haploids were identified.

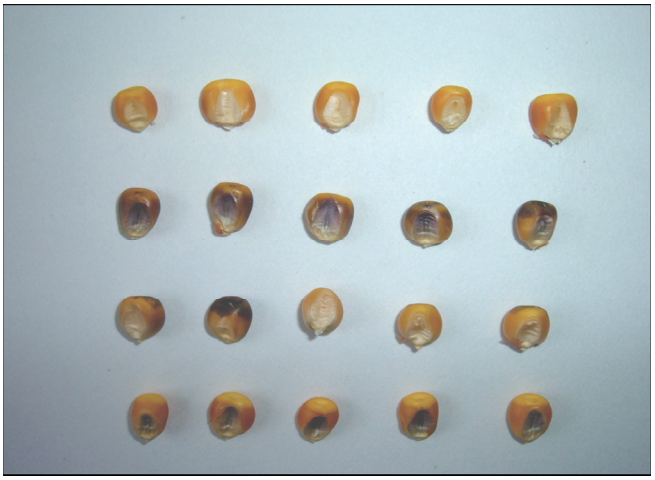


Figure 1. Four types of kernels produced by different methods of pollination.

The results of heterofertilization were more often revealed in the pollinations where the pollen of the inducer was used. In the pollinations with pollen mixtures, 3.9% of such kernels were noticed in the A464 line, and 2% in the A619 line, whereas applying the mixtures with the pollen of the X28C line resulted in 0.32% and 0.42%, respectively. The frequency of haploids in these pollinations decreased from 12.6% to 10.6% in the A464 line, and from 11.8% to 3.9% in the A619 line (Table 1).

The frequency of haploids was almost twice as low in the repeated pollinations after 24 hours. In the A464 line haploid frequency was 5.2% and in the A619 line it was 6%. There were relatively high frequencies of heterofertilization when the pollen of the inducer was used for the first pollination (Fig. 2).

Table 1. Results of haploid induction and heterofertilization.

Pollinators	A464		A619	
	%, haploids	%, heterofert.	%, haploids	%, heterofert.
Haploid inducer	12.6	-	11.8	-
X28C line	-	-	-	-
Haploid inducer (pollen mixture)	10.6	3.9	3.9	2.0
X28C (pollen mixture)	-	0.32	-	0.42
Haploid inducer/selfed after 24h	5.2	1.5	6.0	1.3
X28C/selfed after 24h	-	0.54	-	-
Haploid inducer/selfed after 48h	12.5	0.35	9.5	0.3
X28C/selfed after 48h	-	0.2	-	-
Haploid inducer/selfed after 72h	10.0	0.68	8.5	0.4
X28C/selfed after 72h	-	0.33	-	-

Figure 2. Ears of maternal genotypes in repeated pollinations (first pollination--the haploid inducer or the X28C line; second pollination--self-pollination).

These results indicate that during the induction of haploids a delay of fertilization occurs, and this was noticed both for the egg cells and for the central nuclei. In our opinion, the delay of fertilization was the reason for the high frequencies of heterofertilization in the pollinations where the haploid inducer was used.

Producing haploids under the conditions of repeated pollinations at an isolated plot leads to a high level of heterofertilization or to a complete replacement of early penetrating sperm (carriers of haploid induction) by normal sperm. Thus, the frequency of haploids is reduced in comparison with manual (artificial) pollinations (Rotarenco, MNL 76, 2002). However, the frequency of haploids varies in manual pollinations as well. In this case, a delay of pollination leads to a decrease in the frequency which could also be caused by the phenomenon of heterofertilization (Rotarenco and Mihailov, MNL 81, 2007). The highest yield of haploid kernels per ear was obtained in the manual pollinations of ears with three-day-old silks. In this case, there was a combination of a maximal frequency of haploids and a good seed set (unpublished).

Whatever the reason for the variation in the frequency of haploids, there is a cardinal difference between the induction of maternal haploids and apomictic development--the frequency of maternal haploids is higher in early pollinations, whereas a delay of pollination leads to an increase in the frequency of apomixis (according to the literature).

While creating new inducers, it was noticed that the haploid induction was accompanied by the formation of embryoless and endospermless kernels. It was revealed that the frequency of haploid induction of an inducer positively correlated with the frequencies of both embryoless and endospermless kernels resulting from its self-pollination. By means of the following experiment, it has been found that only the inducers' pollen possessed the ability to induce both embryoless and endospermless kernels, whereas their female inflorescences (ears) were mostly able to form normal kernels.

An inducer with a frequency of haploid induction of about 10% was self-pollinated and crossed with the A464 line. The frequency of embryoless kernels was 10.2% in the self-pollinated ears, and 0.4% as a result of pollination with the A464 line (Fig. 3).

Figure 3. Ears of a haploid inducer. The two ears on the left are the result of self-pollination, the other two ears are the result of pollination with the A464 line.

There were a large number of endospermless kernels in the self-pollinated ears (more than 30%). There were a variety of endospermless kernels in the pollinations with haploid inducers--completely endospermless kernels, kernels with reduced endosperms and endospermless kernels with viable embryos.

Significant variation has been revealed among haploids of an inbred line developed by different inducers. The haploids differed by the speed of germination in a thermostat and phenotype at the early stages of growth (up to 4 leaves). One of the possible explanations for those differences could be the influence of hybrid endosperms. A similar effect was observed by Haskell (1960) in pseudogamous *Rubus* species and called pseudogamous heterosis.

Our next goal was to compare adult haploids of inbred lines produced by different inducers. The A464 line was crossed with three inducers: two of them were homozygous lines (#1 and #2), and the third one was the hybrid between them (1x2). The A619 line was crossed with the second inducer (#2) and the hybrid inducer (1x2).

Haploids were cultivated in three-row plots. By the flowering phase, there were 60 to 80 haploids in each plot. Three plant traits (plant height, leaf length, leaf width) and two ear traits (ear

length and number of kernel rows) have been measured in the haploids. There were significant differences in all the traits among the haploids of the A464 line. The haploids of the A619 line differed significantly in plant height (Table 2). Besides the differences in quantitative traits, it was noticed that the haploids differed in time of flowering.

Table 2. Parameters of quantitative traits of haploids produced by different inducers.

Traits	(n) A464			(n) A619	
	Inducer #1	Inducer #2	Hybrid Inducer 1x2	Inducer #2	Hybrid Inducer 1x2
Plant height, cm.	142.1±3.1	126.2±2.2**	120.2±2.1***	133.4±1.3	125.5±2.0**
Leaf length, cm.	53.6±0.8	51.0±0.6	46.9±0.7***	49.2±0.4	47.7±0.6
Leaf width, cm.	6.7±0.2**	6.7±0.1**	7.1±0.1	7.3±0.6	6.9±0.8
Ear length, cm.	10.2±0.3	8.9±0.2**	9.7±0.2	10.2±0.3	9.2±0.4
Number of kernel rows, no.	13.3±0.2	11.6±0.2***	11.8±0.2***	12.3±0.2	11.7±0.3

It seems unlikely that differences between haploids could have been caused by the influence of their hybrid endosperms. Most likely the reason for the variation was partial hybridization with the inducers, and perhaps, each inducer had a certain degree of partial hybridization. The phenomenon of partial hybridization has been described in rice and found in sunflower (Faure et al., 2002). Every year we find aneuploid plants among haploids (Chalyk et al., MNL 77, 2003). There is much variation among the aneuploids in their phenotype; also, they might be sterile or partially fertile and usually possess the inducer's marker genes. Traces of the marker genes are being revealed in plants considered haploids, too. Some researchers believe that such plants are androgenic haploids. However, these haploids have nothing in common with haploids usually obtained after self-pollinations of inducers, either in phenotype or in the way the marker genes are expressed.

The observations mentioned above can be applied to confirm partial hybridization during the induction of maternal haploids. However, based on all the results presented, we cannot be sure yet that the development of embryos (considered haploid embryos) occurs due to partial hybridization and that maternal haploids actually are aneuhaploids, i.e., possess some genetic information from inducers.

Overall, the results show that the induction of maternal haploids is a rather complex and, at the same time, interesting phenomenon.

COLUMBIA, MISSOURI
University of Missouri

Map locations of the telomeres

--Coe, EH

Tentative positions of the telomeres have been added to the Genetic 2008 maps in MaizeGDB. The names assigned to these loci are Telomere1S, Telomere1L, etc. Each short-arm end is assigned a zero coordinate, while long-arm ends are approximated from available evidence. Wherever possible, placement is inferred in an anchored contig by evidence in silico for localization of telomere-specific sequences (e.g., pMTY9ER, pBF266) at one end of a contig that is oriented correctly. Firmly mapped contigs at the ends often match well by this criterion, but some contigs do not. Telomere9S, in particular, is ambiguously placed because of conflicts in contig order and relationship to knob probes, presumably at K9S. Comments with each telomere locus identify the basis of its placement.

Note: these positions are based on (1) the July 2005 FPC build; (2) sequenced BACs as of October 2008; and (3) genetic mapping of other loci in the same contig, e.g., on IBM2 or NAM maps.

I look forward to receiving feedback on these placements, and hope that a next-generation placement will be possible for persons with direct interest in the telomeres when the sequencing project is completed and a rebuild is done. If matched with a high-density, high-resolution genetic map – e.g., an enhanced NAM, the cross-reinforcement between the physical and the cytological and the genetic map will be substantial.

DEKALB, ILLINOIS
University of Illinois, Urbana

Pollen shed delay, silking anthesis interval (SAI), occurred in a cool, late season

--Troyer, AF

Corn has an imperfect flower. The female flower becomes the ear, and the male flower is the tassel. Darwin pointed out that silk delay after pollen shed is normal in corn varieties to facilitate cross pollination, which increases plant vigor. The term silk delay also explains what happens during drought (moisture stress) at flowering time. Fresh corn silks are 90% water; thus, they are sensitive to water availability. Corn breeders' selection against silk delay at high plant densities has been useful to increase hybrid corn's drought stress tolerance. Growing degree heat units to pollen shed is normally very stable with a much lower coefficient of variation than heat units to silk. Upright leaves have become more popular in commercial hybrid corn during the last 40 years. I noticed some very unusual flowering of corn while pollinating in my nursery during this late, cool, 2008 season in northern Illinois. This is about heat units and flowering in corn.

My breeding starts are typically backcrosses of related, elite inbred lines. I grew 1600 plants each of six backcross pedigrees involving four different elite inbred backgrounds at 60,000 plants per acre, including alleys. I self-pollinated the earliest, strongest silking 10% of the plants. This year many plants silked strongly, and I had to wait a day or two or three or more days for the tassel to shed before pollinating. That's very unusual. The pollen shed delay plants had their tassels tightly encased in the uppermost two leaves of the plant. All of my plants had ligules. When I "unwrapped" these tassels, the tassels felt cool and damp; they were water cooled by plant transpiration. I've never seen plants and felt tassels like these before. This year I saw and handled about a thousand plants with delayed pollen shed.

My nursery was planted April 24 and emerged evenly in about 10 days. We were 30% short of heat units in May and June was normal. We never caught up. We had a cool, late season with timely, ample rainfall. The July 7, 2008 Illinois Weather & Crops,

vol. 29, no. 19, shows corn tied with 2002 for shortest plant height in the last 11 years. August 4, no. 23, shows corn two weeks behind the 5-year average for dough stage. My corn grew very tall. I pollinated inbred plants that appeared to be too tall and too late for northern Illinois; yet, on those same days, I drove by hybrid corn fields on the way to work that had not yet flowered. It was a very unusual season. We had higher than average yields; several experiments on the farm averaged well over 200 bushels per acre.
Is there a lesson? Yes: The delayed, late-shed tassels that were encased in a leaf or two indicate heat units must warm the tassel per se to cause pollen formation and dissemination. The plant is a sufficient enough receptor of heat units to develop the plant and tassel, but the tassel per se must receive heat units to develop and shed pollen.

Spring seasons like our 2008 are probably rare.

HONOLULU, HAWAII
University of Hawaii

Branched tassel (*Brta*) on chromosome 2

--Brewbaker, JL; Yu, H

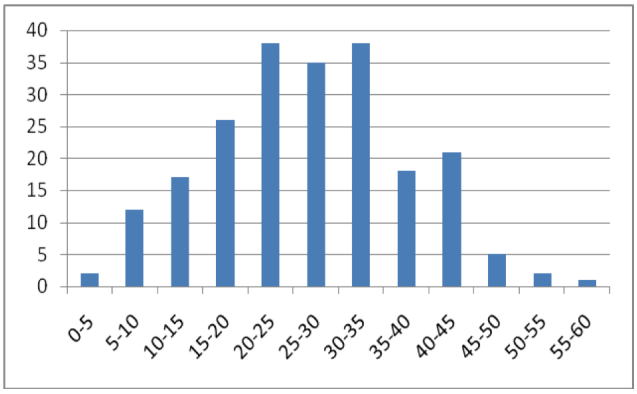
Tassels of inbred Hi27 and most of our 150 NILs (MNL81:15) average ~13 branches (Figure 1). Reduced branch numbers occur only when plants are under biotic or abiotic stress. Several of our Hi27 NILs display more highly branched tassels (Figure 2), of which the most prominent are in stocks with chromosome 2 mutants like *fl* and *v4*. This branching we show to be governed by a single gene designated *Brta*.

Tassel branch numbers were recorded in the classic series of publications on the races of maize. These data are summarized in Table 1, showing an average of 27.0 tassel branches (both primary and secondary) for the 251 races included in our survey. The data were normalized and ranged from 3.6 for Palomero Toluqueño (Mexico) to 50+ for the Piras of Colombia. Tropical breeders are very familiar with the large and impressive tassels of many tropical

Figure 1. Normal Hi27 tassel.

Figure 2. Branched tassel in genotype (*fl Brta*)^Hi27.

Table 1. Tassel branch number in 251 races of maize.



varieties and hybrids. The contrast of a 40-branch tropical tassel with that of B73 (6.0 branches) or of Mo17 (4.7 branches) is most striking. Historically, temperate breeders have selected inbreds with small tassels, reflecting the small but significant energy requirements of tassels. This trend continues for tropical plant breeders. Male-sterile tassels are now seen in many commercial fields.

In a survey of 60 largely tropical inbreds in the collection of Hawaii Foundation Seeds (HFS), branch numbers averaged 15.4. The numbers appeared again to be normally distributed but concentrated around their mean (very similar to Hi27, with ~13 branches). Temperate inbreds were generally at the low end of this range. Environmental effects can be very great. In a trial planted 11/16/07 under severe winter stress (low light, heavy rain, yields reduced 75%), Hi27 averaged only 2.8 branches and the branched NILs averaged only 5.7

Backcross conversions of Hi27 to incorporate the dominant genes *fl* (chrom. 2S-75.7) and *v4* (chrom. 2L-87) began in 1969 with MGC stock 63-2370-5/2367-2 (*lg gl2 fl v4*). Selfing after 6 backcrosses created *fl* and *v4* NILs, each proving to be double mutants *fl v4*. The line selected for *fl* alone had highly branched tassels (20.9 branches), while the line selected for *v4* had normal Hi27 tassels. An additional NIL selected as a floury with white kernels (*y* locus on chrom. 6) was also highly branched, and we've bred a branched floury stock lacking *v4*.

Average branch numbers were collected in a generation mean analysis study planted in February 2008 with parent P1 = Hi27 and parent P2 = *fl v4* (branched). Branch numbers were as follows: P1=11.1, P2=20.9, F1=16.0, F2=16.1, B1=13.1 and B2=16.5. However, seeds were classified as normal or floury before planting, and the data were as follows: F2, 16.6 floury vs. 15.5 normal; B1, 15.3 floury vs. 11.0 normal; B2, 17.3 floury vs. 15.8 normal. A GMA analysis revealed no significant non-additive effects. F2 segregation could generally be interpreted as a 1:2:1 affected by the linkage of floury and the branching locus.

It is inferred that the branched tassel trait is governed by a single locus that we've designated *Brta* ("branched tassel"). We chose to symbolize branched allele as the capitalized *Brta* with normal as *brta*. The locus is on chromosome 2 and suspected to be somewhere between *v4* and *fl*. No other NILs we have on this chromosome show branched, including *sk1* (2-57) and *gs2* (2-50). Inheritance is simple and dominance absent. The brt phenotype bears no resemblance to described loci *ub* (unbranched) and *td* (thick tassel dwarf), nor does it lead to seed-bearing flowers in the tassel as in the highly branched ramosa mutants. Several genes greatly reduce or eliminate tassel branching (*ad1*, *baf1*, *lg1*) but none are in this region. The relevant NILs are now designated (*fl Brta v4*)^Hi27 and (*fl Brta v4*)*y*^Hi27.

Double-cob (*dbcb*) on chromosome 1

--Brewbaker, JL

Conversions of Hi27 to the variegated-pericarp allele *P-vv* were initiated in 1967 using Maize Coop Stock 63-2656-2/2655-5, a stock showing variation at the following loci: *A1, A2, C, et, lg2, R* and *P*. In a somewhat sophomoric way, we began a series of 10 backcrosses to Hi27 together with an extensive series of selfs and sibs aimed at preserving only the *P-vv* (with its *Ac* insertion). The "pure line" *P-vv* inbred has always been uniquely semi-dwarf, narrow-leaved, poor in seed set and irregular in expressivity of variegations.

In 2001, in the 23rd cycle of breeding *P-vv*, we observed four sister lines with a trait we named double-cob (Figure 1). The mutant cobs normally split at the tip into two or three arms, and were not highly competent at filling seed. However, the trait proved to be considerably more stable than *P-vv* and to be inherited as a simple recessive. The linkage of the two loci is inferred from many of these segregations, but mapping has not been done. The double-cob trait has been carried through more backcrosses to Hi27 (which is *P-ww*) and a series of selfs to produce three sub-lines--(*dbcb P-ww)* ^Hi27*, (dbcb P-vv)* ^Hi27 and *(dbcb P-rw)* ^Hi27. All of these NILs are otherwise identical to recurrent parent Hi27 (Brewbaker, Crop Sci. 37:637, 1997) in maturity, color (e.g., bronze tassel), disease resistance, tassel and kernel type, etc. None of the 14 other chromsome 1 mutants among our NILs show the double cobs.

Figure 1. Phenotype of Hi27 near-isogenic line (*dbcb P-ww*)^Hi27.

Floppy tassel (*Flta*) on chromosome 9

--Brewbaker, JL; Yu, H

Tassels of Hi27 and most modern inbreds are relatively erect in appearance (see accompanying article on branched tassels). In contrast, a tassel with lax branches that we characterize as "floppy" is rather common among tropical maize varieties. Breeders of popcorn and of waxy Asian maize ("glutinous" or "sticky" corn) also find such "floppy" tassels to be the norm, as we do also in our breeding of popcorns.

The floppy tassel trait (Figure 1) segregated monogenically in our conversions of inbred Hi27 to the gene *wx* (chrom. 9S-47.9). The mutant originated from MGC stock 70-1000-3/999-3 (*wx-a*), and had 6 backcrosses through 24 generations of breeding to Hi27. Floppy tassel was also observed in our digenic NIL with

Figure 1. Floppy tassel of *wx*^Hi27 near-isogenic line.

genes *bz C* (chrom. 9S-22.5, 16.2) that originated from MGC stock 68-1238-5/1238-4 and had seven backcrosses to the parent.

Branch angle averaged 50.1 degrees in the *wx* and *bz C* NILs (based on branches at center of the tassel). In contrast, the recurrent Hi27 parent had an average branch angle to the central spike of 31.7 degrees. The floppy trait was not accompanied by longer tassel branches, but it did increase the apparent spread or diameter of the tassel. The branched-tassel mutants described in the accompanying article had a much lower branch angle (15 to 20°), as did our Hi27 NILs such as *ra2* and *lg1* (6°).

Hybrids of our *wx* and *bz C* NILs with parent Hi27 both appeared to be intermediate to the parents, with branch angle averaging 44.8 degrees. Preliminary studies of advanced generations verified monogenic segregations and also inferred lack of dominance at the locus. We've designated the locus *flta* and the floppy allele with the capitalized *Flta*. We suspect the locus to be between loci *C* and *wx* on chromosome 9. None of our other NILs for mutants on chromosome 9 (including *bf, bk2, bm4, dt, sh, yg2*) have floppy tassels, nor does our multiple mutant stock *C sh bz wx*.

A very floppy tassel also characterizes one of the major inbreds in our silage-breeding program, Hi58, which we derived from Kasetsart's Thai inbred Ki14 (Brewbaker and Josue, Crop Sci., 2007). Hybrids of Hi58 are always "semi-floppy", as also are hybrids of our Chinese waxy and Indiana popcorns. Since the waxy gene traces to Chinese origin, where waxy maize is a recognized delicacy, the floppy tassel gene may also have its origin in this germplasm. We've a large breeding program for Hawaii of waxy vegetable maize, and all are floppy-tasseled. We continue to evaluate advanced progenies for linkage involving the floppy tassel mutant and crosses with the vegetable waxy and popcorn types.

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Different types of protein phosphatases in inner and outer membranes of mitochondria

--Subota, IY; Arziev, AS; Nevinsky, GA; Konstantinov, YM

The protein phosphorylation/dephosphorylation of maize mitochondrial proteins in organello was investigated. The goal of this study was to compare the level of protein kinase and protein phosphatase activity between intact mitochondria and mitoplasts (organelles without the outer membrane). The mitochondria were isolated from 3-day-old etiolated maize seedlings (hybrid VIR42MV) by a standard method of differential centrifugation. Protein phosphorylation assays were carried out according to Struglics et al. (FEBS Lett. 475:213-217, 2000) with the use of [γ^{32}P] ATP (specific radioactivity was 6000 Ci/mmol). Considerable differences were found in the level of protein phosphorylation between intact mitochondria and mitoplasts (Figure 1). The incorporation of ^{32}P-label was 7261 ± 461 cpm/mg of protein in the case of intact mitochondria, and 106410 ± 16509 cpm/mg of protein in the case of mitoplasts. Thus, the presence of the outer membrane was associated with an extremely low level of phosphorylation activity of mitochondrial proteins.

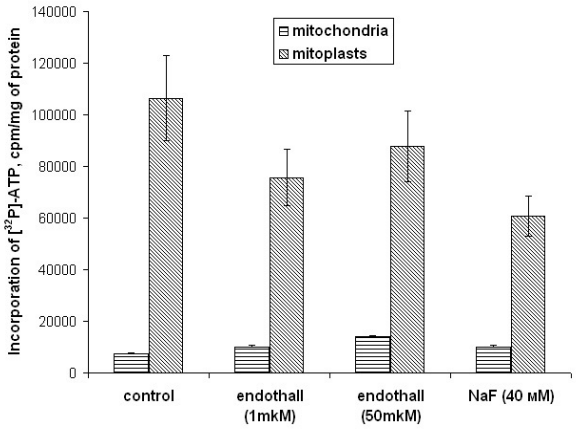

Figure 1. The total activity of protein phosphorylation in maize mitochondria and mitoplasts.

These results could be explained by the presence of different types of protein phosphatases in inner and outer membranes of mitochondria of higher plants. This suggestion was supported by the fact that the effects of inhibitors of protein phosphatases NaF and endothall were different in intact mitochondria and mitoplasts. It was proposed that plant mitochondria possess two types of protein phosphatases. One type is "substrate" phosphatase. The function of substrate phosphatase is to dephosphorylate most of the phosphoproteins. The other type is the phosphatase of protein kinase. Some mitochondrial kinases may exhibit activity only in dephosphorylated form.

The results of our study suggest that the outer membranes of maize mitochondria contain more "substrate" protein phosphatases than the submitochondrial fractions (inner membranes and matrixes). The physiological importance of this phenomenon is not clear.

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Barbara McClintock's contributions to Biological Abstracts: Another Cornell connection

--Kass, LB

I previously published an annotated list of Barbara McClintock's publications in the MNL (Kass, MNL 73:42-48, 1999). Here I supplement the listing with reviews, written by McClintock, covering the latest literature for the innovative new journal Biological Abstracts during her early career at Cornell University and summaries of her pioneering work completed years later at Cold Spring Harbor, Long Island, New York (Table 1).

McClintock is most noted for her discovery of transposable elements in maize for which she was awarded the Nobel Prize in Physiology or Medicine in 1983. Her early contributions to the cytogenetics of maize are often overshadowed by her Nobel

Table 1. Biological Abstracts authored by Barbara McClintock between 1927 and 1956.

1) McClintock, B. 1927. [Abstract #] 2047. KISSER, J. On Kernschwarz and its serviceability for botanical purposes (Uber Kernschwarz und seine Anwendungsmoglichkeit fur botanische Zwecke). Zeitschr. Wiss. Mikrosk. 43(1):116-119, 1926. Biological Abstracts, vol. 1.

2) McClintock, B. 1927. [Abstract #] 2052. NODA, KOI. The chromosomes of *R*[*umex*] *scutatus* (Uber die Chromosomen von *Rumex scutatus*). Jpn. J. Bot. 3(1):21-24, 1926. Biological Abstracts, vol. 1.

3) McClintock, B. 1928. [Abstract #] 106. SCHWEMMLE, J. The hybrid *Oenothera berteriana* X *Onagra* (*muricata*) and its cytology (Der Bastard *Oenothera berteriana* X *Onagra* (*muricata*) und seine Zytologie). Jahrb. Wiss. Bot. 66 (4):579-595, 1927. Biological Abstracts, vol. 2.

4) McClintock, B. 1928. [Abstract #] 8915. LAIBACH, F. Artificial abortions in plants with respect to their importance for hybrid and hereditary investigation (Kunstliche Fruhgeburten bei Pflanzen in ihrer Bedeutung fur die Bastard- und Vererbungsforschung). Naturwissenschaften 15(34):696-700, 1927. Biological Abstracts, vol. 2.

5) McClintock, B. 1933. [Abstract #] 17720. IMAI, YOSHITAKA; TABUCHI, KIYOO. The relative loci of some genes in the variegated chromosome of *Pharbitis nil*. Zeitschr. Indukt. Abstamm. U. Vererbungsl. 58 (1):166-168, 1931. Biological Abstracts, vol. 7

6) McClintock, B. 1934. [Abstract #] 64. FUKUSHIMA, EIJI. Formation of diploid and tetraploid gametes in Brassica. Jpn. J. Bot. 5(3): 273-283, 1931. Biological Abstracts, vol. 8.

7) McClintock, B. 1934. [Abstract #] 5174. KOZHUCHOW, Z. A. Uber die Natur der Extrachromosomen bei *Zea mays* L. Zeitschr. Wiss. Biol. Abt. E Planta 19(1):91-116, 1933. Biological Abstracts, vol. 8.

8) McClintock, B. 1934. [Abstract #] 7687. McCLINTOCK, BARBARA; HILL, HENRY E. The cytological identification of the chromosome associated with the R-G linkage group in *Zea mays*. Genetics 16(2):175-190, 1931. Biological Abstracts, vol. 8. [Biol. Ab. 8(4, April):840, Cytology, Plant 1934].

9) McClintock, B. 1934. [Abstract #] 12787. McCLINTOCK, BARBARA. The order of the genes *C*, *Sh* and *Wx* in *Zea mays* with reference to a cytologically known point in the chromosome. Proc. Natl. Acad. Sci. U.S.A. 17(8):485-491. [2 fig], 1931. Biological Abstracts, vol. 8. [Biol. Ab. 8(6, June/July), p. 1376, Cytology, Plant, 1934].

10) McClintock, B. 1936. [Abstract #] 20257. CHIZAKI, YOSHIWO. Another new haploid plant in *Triticum monococcum* L. Bot. Mag. [Tokyo]. 48 (573):621-628, 1934. Biological Abstracts, vol. 10.

11) McClintock, B. 1941. [Abstract #] 14129. McCLINTOCK, BARBARA. The stability of broken ends of chromosomes in *Zea mays*. Genetics 26 (2):234-282, [1 fig], 1934. Biological Abstracts, vol. 15. [Vol. 15 (August-Dec), p. 1264, Cytology, Plant, 1941].

12) McClintock, Barbara. 1946. [Abstract #] 6165. McClintock, Barbara. (Carnegie Inst. Washington, Cold Spring Harbor, N.Y.) Neurospora. I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. J. Bot. 32(10):671-678, 1945. Biological Abstracts, vol. 20. [Vol. 20 (Jan-July), p. 675, Cytology, Plant, 1946].

13) McClintock, B. 1957. [Abstract #] 6784. McClintock, Barbara. Intranuclear systems controlling gene action and mutation. Brookhaven Symp. Biol. 8:58-74, 1956. Biological Abstracts, vol. 31. [Vol. 31 (Jan-Mar), p. 676, Genetics, Animal, 1957].

awarding winning investigations (Kass, Genetics 164:1251-1260, 2003; Kass, Bonneuil and Coe, Genetics 169:1787-1797; Coe and Kass, PNAS 102(19):6641-6656, 2005). While an instructor in Cornell's Department of Botany (1927-1931), a post-doctoral researcher at Missouri and Caltech (1931-1933) and a researcher in the Department of Plant Breeding (1934-1936) at Cornell University, McClintock was invited to submit summaries of current research in biology for their newly established journal, Biological Abstracts (Table 1). Jacob R. Schramm, Professor of Botany at Cornell University, was editor-in-chief of Botanical Abstracts from 1921-1925 and founder and first editor-in-chief of Biological Abstracts [now BIOSIS] (1924-1937). This is but one of many landmark contributions to American Plant Biology made by Cornellians over the last century (Kass and Cobb, Plant Sci. Bull. 53(3):90-101, 2007; Murphy and Kass, Department of Plant Breeding & Genetics, Cornell University, Ithaca, NY, 2007).

Scientists continue to rely on BIOSIS to gain access to current literature. As a beginning graduate student in the late 1960s, I had used hard copies of Biological Abstracts for my research, and later became familiar with the on-line value of BIOSIS. I used this database to find summaries of the work of McClintock and her contemporaries (e.g., Coe and Kass, 2005; Kass and Chomet, pp. 17-52, in Bennetzen and Hake, The Maize Handbook: Genetics & Genomics, Springer, 2009). Recently, I learned that one may also use this database to find historically recognized papers, summarized by contemporaneous leaders in the field. This was brought to my attention in a note published in Mannifest, the Newsletter of Albert R. Mann Library, Cornell University (Morris-Knower, Mannifest Spring 2007 14(2):3, 2007, http://www.mannlib.cornell.edu/about/news/upload/spring07.pdf). By typing McClintock's name into the "topic" area of BIOSIS Previews one can find a list of abstracts authored by McClintock. The information is not as complete as one would find by examining the original hardbound copies of the journal (i.e., the month of publication and the page on which the abstract appears are not included), yet it provides easy access to the names of authors who summarized research papers, and one can certainly get complete information by seeking out the original source in a library (for example, see Table 1, references 8-9 and 11-13 for the complete source in Biological Abstracts).

It was enlightening to learn of McClintock's contributions to Biological Abstracts and to gain an understanding of the importance of a foreign language requirement for students in the early 20th century. McClintock's comprehension of the German language is reflected by the many papers she read in their original language and summarized for Biological Abstracts. Although most of her publications were encapsulated by others (not listed here), McClintock reviewed five individual investigations for *Biological Abstracts*, the last of which appeared in 1957 (Table 1).

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Evaluation and identification of maize for *turcicum* leaf blight resistance under cold temperate conditions

--Shikari, AB; Zafar, G

In temperate hilly regions, high infestations of *Exserohilum turcicum* (Pass) Leonard and Suggs are encountered, causing *turcicum* leaf blight disease that exceeds economically feasible limits. Disease development is favoured by high relative humidity (75-90%) and moderate temperatures (22-25ºC) during the growing season. The valley of Kashmir, which is a hotbed for this disease, lacks varieties of maize resistant to this disease. In spite of the fact that maize is an important food and fodder crop for the region, chemical control for the disease is not practiced. This results in a need to screen for TLB disease resistance in order to develop high-yielding disease resistant varieties of maize. We

Table 1. *Turcicum* leaf blight disease intensity of maize genotypes under epiphytotic field and controlled pot grown conditions.

S. No.	Name of entry	Disease intensity % under field conditions	Disease intensity % under controlled pot conditions	Log transformed values under field conditions	Log transformed values under controlled conditions	Days to 50% silk emergence	Grain yield (t/ ha)
1	GROP-132	76.29	46.56	1.88	1.67	50.00	1.43
2	GROP-172	66.30	34.10	1.82	1.53	54.00	3.97
3	GROP-165	64.00	41.99	1.81	1.62	56.00	4.24
4	GROP-104	69.76	32.97	1.84	1.52	54.00	1.32
5	GROP-104 wh	68.13	32.26	1.83	1.51	54.00	0.80
6	GRIL-4048	68.12	35.91	1.83	1.56	61.50	2.40
7	GRIL-3714-2	45.31	30.50	1.66	1.48	68.50	3.26
8	GRIL-12-112-1	59.57	43.22	1.78	1.64	64.50	3.10
9	NDSAB(M)C7	49.21	40.88	1.69	1.61	58.00	5.67
10	NDSM(8)WN	52.45	39.95	1.72	1.60	59.00	5.89
11	TL99A 1101-1	34.41	44.44	1.54	1.65	83.00	4.50
12	TL1111 1X2	34.60	47.98	1.54	1.68	84.00	6.15
13	TL99A 1101-3	27.25	40.70	1.44	1.61	84.00	4.27
14	TL99A 1102-6	30.62	38.66	1.49	1.59	91.00	5.07
15	TL00B 6135	33.35	46.39	1.52	1.67	91.00	5.61
16	TL 2000 B 6313	18.18	34.18	1.26	1.53	95.00	4.82
17	TL99 6119 20X19	23.93	38.32	1.38	1.58	81.00	4.07
18	TL99B 6119 6X5	23.77	31.36	1.38	1.50	68.00	4.16
19	Pob-800	41.60	34.03	1.62	1.53	69.00	4.76
20	Pob-845	50.44	40.46	1.70	1.61	58.00	4.16
21	Pob-86 C5	44.30	40.98	1.65	1.61	70.00	4.99
22	Sint-1	41.50	40.40	1.62	1.61	77.00	3.92
23	Sint-2	36.11	35.81	1.56	1.55	73.00	2.77
24	Sint-3	38.73	41.43	1.59	1.62	73.00	2.75
25	Sint-4	46.29	53.87	1.67	1.73	74.00	2.44
26	RS-11	29.17	25.74	1.46	1.41	60.00	2.39
27	RS-12	31.40	15.49	1.50	1.19	60.00	2.67
28	RS-14	25.73	22.84	1.41	1.36	71.00	5.29
29	RS-15	31.88	22.48	1.50	1.35	70.00	4.58
30	Ht-1	12.66	14.91	1.10	1.17	74.00	0.63
31	Ht-2	10.23	12.60	1.01	1.10	75.00	1.76
32	Ht-3	13.23	18.34	1.12	1.26	73.00	0.78
33	Ht-N	28.09	17.61	1.45	1.25	79.00	0.88
34	NIAS-5	29.28	36.79	1.47	1.57	71.00	2.78
35	NIAS-13	42.16	34.28	1.62	1.54	73.00	0.87
36	NZ-3	58.61	57.68	1.77	1.76	63.00	1.26
38	NZ-7	56.83	50.22	1.75	1.70	58.00	2.59
39	NZ-8	45.82	38.38	1.66	1.58	61.00	2.63
40	Po-77	39.30	31.01	1.59	1.49	66.00	3.07
41	Po-89	42.21	33.34	1.63	1.52	63.00	2.66
42	NZ-84	53.75	57.61	1.73	1.76	59.00	2.71
43	MOSSC C15	42.87	51.97	1.63	1.72	70.00	2.75
44	NAC-6004	10.33	31.67	1.01	1.50	99.00	4.45
45	NAC-6002	18.78	29.89	1.27	1.48	80.00	3.75
46	NAI-104	24.84	20.61	1.40	1.31	83.00	1.61
47	NAI-112	7.01	14.99	0.85	1.18	94.00	2.37
48	NAI-147	8.28	12.29	0.92	1.09	95.00	4.44
49	NAI-151	18.07	44.29	1.26	1.65	75.50	4.79
50	NAI-155	15.88	19.73	1.20	1.30	81.50	1.75
51	VL-41	55.54	46.19	1.75	1.66	67.50	2.15
52	VL-16	44.90	45.38	1.65	1.66	64.50	3.68
53	VL-Sk-11	27.50	26.81	1.44	1.43	76.00	3.16
54	VL-88	45.95	35.58	1.66	1.55	58.00	3.12
55	VL-Amb-pop	50.26	55.56	1.70	1.74	77.00	1.49
56	FH-3079	32.28	26.36	1.51	1.42	74.00	6.36
57	FH-3186	28.08	21.93	1.45	1.34	73.00	4.09
58	Him-129	42.64	42.23	1.63	1.63	63.00	4.10
59	Vivek-9	17.65	17.56	1.25	1.24	71.00	6.63
60	Surya	48.52	42.60	1.69	1.63	72.50	3.46
61	Kanchan	46.02	43.15	1.66	1.63	68.50	3.26
62	Girija	2.99	4.26	0.48	0.63	79.00	5.61
63	P7xC6	37.50	30.00	1.57	1.48	72.00	3.13
64	P8xC6	33.94	35.30	1.53	1.55	72.50	3.99
65	QL-1	46.98	37.83	1.67	1.58	72.50	2.72
C	C6	30.06	33.50	1.48	1.52	74.00	5.38
C	C14	38.75	32.98	1.59	1.52	76.50	5.12
C	C15	34.59	36.77	1.54	1.57	71.50	4.79
C	Super-1	42.77	38.13	1.63	1.58	72.50	5.11
	Mean	37.75	34.80	1.52	1.51	71.46	3.47
	SD	16.64	11.63	0.26	0.19	10.82	1.51
	CV (%)	44.08	33.42	17.08	12.76	15.14	43.67

have screened for *turcicum* blight disease resistance in over 43 exotic and 19 indigenous genotypes along with 3 local collections for the consecutive years of 2003 and 2004 at SKUAST-K, Shalimar, Jammu and Kashmir. *Turcicum* blight reaction of genotypes

had no relation to their geographical origin. Ht-monogenic sources, inbred NAI-147 and composite Girija, were among the genoypes that expressed resistance to the disease.

The 65 entries were sown in Augmented Block Design along with 4 checks (viz., Super-1, C_6, C_{14}, and C_{15}) within 3-rowed plots having inter- and intra-row spacing of 70 and 25 cm, respectively. Moderate doses of nitrogen were applied. Nitrogen in too low or too high quantity leads to increased and decreased disease severity, respectively, as cited by Bimla (Ann. Biol. 18(2):137-141, 2002) and Sharma and Mishra (1989). Artificial inoculation in the field was performed at the 6-8 leaf stage per Ivanova (Ras. Nauki 20(6):119-123, 1983). Plantings during the 2 years were altered by one month so as to mitigate the influence of early maturation on disease severity. Similarly, inoculation under controlled plot conditions was done at the 2- and 4-leaf growth stages to rule out the effects of juvenile sensitivity. Disease intensity was calculated according to modified McKinney rapid technique as applied by Horsfall and Heuberger (Phytopathology 32:226-232, 1942). This technique is based on individually scoring the leaves of a plant into 10 grades depending upon the percentage of leaf area infected. The severity (%) was calculated as $\{\sum (nV) / (NG)\} \times 100$, where 'n' is the number of infected leaves in each grade (1-10, which corresponds to 10-100% diseased area); 'V' is the numerical value of each grade; 'N' is the total number of leaves examined; and 'G' is the maximum numerical value of infection grades (i.e., 10). Based on disease intensity, genotypes were categorized into 5 groups as follows (Jeffers, personal communication): 0.1-5% = resistant; 6-25% = moderately resistant; 26-50% = moderately susceptible; 51-75% = susceptible; >75% = highly susceptible.

The results of screening germplasm over the two years indicated that *turcicum* leaf blight (TLB) disease intensity at the field level exhibited very high correlations (0.81** and 0.72**) with those calculated under controlled pot grown conditions. Genotypes at serial numbers 47, 48 and 62 (Girija) showed disease intensity less than 10% at field level. The genotype Girija recorded absolute resistance to the disease under both the screening environments (Table 1) with disease intensity percentages of 2.99 (0.48) and 4.26 (0.63) under field and controlled conditions, respectively. This genotype ranked only 6th for grain yield per hectare with 5.61 tons. The variability ranged from 2.99 to 76.29 and 4.26 to 57.68 percent for disease intensity and from 50 to 99 days for 50% silk emergence. At least 22 genotypes were found superior to check composite C6 with respect to disease log score. The genotypes showing moderate resistance under both the environments included RS-14, Ht-1, Ht-2, Ht-3, NAI-104, NAI-112, NAI-147, NAI-155, and Vivek-9. Smith and Kinsey (Plant Dis. 64:779-781, 1980) suggested the conferring of resistance by Ht-gene backgrounds. These Ht-monogenic sources have expressed resistance under controlled conditions in demonstrations by Leath and Pedersen (Plant Dis. 70:529-531, 1986), and with the exception of Ht-N, are known to display the chlorotic type of resistance (Leonard et al., Plant Dis. 79:776, 1989) observed in the present study. Populations NAC-6002 and NAC-6004, procured from the National Turcicum Leaf blight Nursery, Mysore, were found to be moderately resistant to TLB under field conditions, which has also been reported by Prabhakar et al. (Current Res. 32:63-66, 2003). The land races and most of the exotic materials succumbed to the disease. Disease intensity at the field level was negatively correlated to yield, which corresponds to the findings of Satyanarayana (Madras Agric. J. 82(40):249-251, 1995), and Sharma and Misra (Indian Phytopathol. 36(2):255-256, 1983). As expected, early maturing varieties tend to be more susceptible to disease than full season ones. This is because late summer conditions coincide with the log growth phase of early varieties where 70% or more of the leaf area was infested by the disease. This agrees with the findings of Patil (Mysore J. Agric. Sci. 13(1):1-4, 1979) that indicate genetic linkage between TLB resistance and late maturity traits. Thus there remains a possibility of selecting for early-maturing resistant lines among the recombinant generations of late-maturing resistant and early-maturing susceptible crosses. The varieties Girija, NAI-147, NAI-155 and Vivek-9, showing resistant to moderately resistant reactions to TLB in the present study, are all late season varieties that could be used as parents in backcross breeding to adaptable, high-yielding (average 50 qha^{-1}), susceptible checks C_{15}, C_6, C_{14} and Super-1.

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Evaluation of salinity tolerance at the seedling stage in maize (*Zea mays* L.)

--Collado, MB; Aulicino, MB; Molina, MC; Arturi, MJ

Maize (*Zea mays* L.) is the third most important cereal in the world after wheat and rice, and it grows under a wide range of climatic conditions. It is moderately sensitive to salinity and considered the most salt-sensitive of the cereals (Maas and Hoffman, J. Irrig. Drain Div. ASCE 103:115-134, 1977). Maize contains enormous variability (Paterniani, Crit. Rev. Plant Sci. 9:125-154, 1990) in which salinity tolerance may exist. Based on reports for many crops (Ashraf and McNeally, J. Agron. Crop Sci. 159:269-277, 1987; Ashraf and McNeally, Plant Breed. 104(2):101-107, 1990; Maiti et al., J. Plant Physiol. 148:741-744, 1996), selection for tolerance to salinity at the seedling stage appears useful in selecting for tolerance in saline soils. Previous papers indicate the effects of salinity treatments on the development of the maize coleoptile and radicle were considerable (Cicek and Cakirlar, Bulgarian J. Plant Physiol. 28:66-74, 2002).

This paper examines the presence of genetic variability in salt treatment of maize seedlings in thirteen populations and eighteen inbred lines of maize. Seeds were surface sterilized in 1% sodium hypoclorite solution for 5 minutes, then rinsed with distilled water. Six caryopses of each genotype were germinated between absorbent paper in plastic trays. The paper was moistened with either distilled water (control) or 150 mM NaCl. Each treatment was replicated two times. A completely randomized block design was used. Experiments were carried out in a controlled environmental room at 25ºC, with 16 h day length and with a relative humidity of 60%. After 12 days of treatment, the seedlings were harvested. The length for shoot and radicle (LS and LR, respectively) and the number of leaves (LN) were recorded. Shoot and radicle were separated, and the samples were dried for two days until constant weight, for dry weight determinations (DS and DR, respectively).

The mean values for all traits were compared using the least significance differences test at a 5% level. Five groups of variation were found for LS and DR, four for radicle length, and three for LN and DS (Tables 1 and 2). Length of shoot and dry weight of radicle were useful in identifying a discriminative response to salinity for the genotypes used.

Table 1. Average length of shoot (LS), length of radicle (LR) and leaf number (LN) for each genotype.

LS			LR			LN		
Genotype	Mean	Groups	Genotype	Mean	Groups	Genotype	Mean	Groups
5	14.5	A	5	18.8	A	16	1.2	A
6	14.0	AB	1	18.0	AB	4	0.9	AB
11	13.2	ABC	19	16.4	ABC	23	0.8	ABC
25	12.5	ABCD	28	16.4	ABC	6	0.7	ABC
16	11.4	ABCDE	6	16.4	ABC	22	0.7	ABC
23	11.3	ABCDE	24	16.3	ABC	5	0.6	ABC
29	11.2	ABCDE	2	16.0	ABCD	8	0.6	ABC
8	10.8	ABCDE	22	15.5	ABCD	1	0.4	ABC
28	10.6	ABCDE	13	15.1	ABCD	25	0.4	ABC
10	10.6	ABCDE	25	14.7	ABCD	10	0.3	BC
31	10.5	ABCDE	29	14.6	ABCD	11	0.3	BC
4	10.5	ABCDE	10	13.7	ABCD	26	0.3	BC
13	10.4	ABCDE	26	13.6	ABCD	24	0.2	BC
24	10.4	ABCDE	23	13.6	ABCD	27	0.2	BC
2	8.8	ABCDE	31	13.2	ABCD	20	0.2	BC
1	8.8	ABCDE	27	13.1	ABCD	31	0.2	BC
26	8.6	ABCDE	18	12.9	ABCD	2	0.1	BC
22	8.6	ABCDE	30	12.5	ABCD	7	0.1	BC
19	8.2	ABCDE	8	12.5	ABCD	13	0.1	BC
15	7.8	ABCDE	11	11.8	ABCD	17	0.1	BC
21	7.7	ABCDE	14	11.7	ABCD	3	0.0	C
9	7.3	BCDE	4	11.0	ABCD	9	0.0	C
27	7.1	BCDE	12	10.9	ABCD	12	0.0	C
20	6.8	CDE	20	9.9	ABCD	14	0.0	C
30	6.5	CDE	7	9.8	ABCD	15	0.0	C
17	6.1	CDE	9	9.8	ABCD	18	0.0	C
18	6.0	DE	16	9.1	ABCD	19	0.0	C
14	5.6	DE	21	7.7	BCD	21	0.0	C
7	5.3	E	3	6.7	CD	28	0.0	C
3	4.9	E	15	5.5	D	29	0.0	C
12	4.3	E	17	5.2	D	30	0.0	C

Genotypes with the similar letters are not significantly different at the 5% level

Table 2. Average dry weight of shoot (DS) and dry weight of radicle (DR) for each genotype.

DS			DR		
Genotype	Mean	Groups	Genotype	Mean	Groups
11	544.4	A	4	283.9	A
6	542.4	A	24	198.9	AB
4	528.8	A	31	190.6	ABC
5	494.9	AB	6	184.2	ABC
24	481.9	ABC	28	182.9	ABCD
29	473.5	ABC	14	176.8	ABCD
28	467.7	ABC	8	164.0	ABCDE
8	435.4	ABC	25	154.7	BCDE
23	428.5	ABC	5	148.6	BCDE
13	418.6	ABC	19	146.6	BCDE
10	416.2	ABC	13	144.4	BCDE
16	408.1	ABC	11	140.7	BCDE
25	404.3	ABC	23	135.7	BCDE
31	362.6	ABC	29	128.5	BCDE
21	357.8	ABC	30	127.1	BCDE
2	349.6	ABC	21	125.2	BCDE
1	318.2	ABC	7	119.8	BCDE
26	308.6	ABC	22	111.9	BCDE
19	306.1	ABC	16	102.7	BCDE
20	287.3	ABC	1	95.0	BCDE
30	285.6	ABC	20	90.1	BCDE
7	278.0	ABC	10	87.0	BCDE
15	266.4	ABC	3	86.8	BCDE
27	265.2	ABC	2	85.0	BCDE
9	255.0	ABC	26	78.4	BCDE
22	233.6	BC	27	72.7	CDE
14	214.4	BC	12	72.3	CDE
12	210.1	BC	15	69.6	CDE
17	207.1	BC	9	66.8	CDE
18	203.3	BC	17	58.8	DE
3	197.8	C	18	42.5	E

Genotypes with the similar letters are not significantly different at the 5% level

The technique employed at seedling stages provides a rapid, accurate and inexpensive method for preliminary screening of a large number of accessions. Our results allowed the identification of genotypes with tolerance to saline soils that could be utilized to understand the genetic basis of tolerance and to accelerate a breeding programme in the maize.

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Additional results from candidate-gene-based association mapping in teosinte

--Weber, AL; Doebley, JF

Our previous association mapping study in teosinte was successful in detecting associations between genetic variation in major regulatory genes in maize and trait variation in teosinte (Weber et al., Genetics 177:2349-2359, 2007). Encouraged by this initial success, we have expanded this study by assaying 163 single nucleotide polymorphisms (SNPs) in 68 additional candidate genes in these same individuals. These candidate genes represent genes that have been characterized in maize or genes homologous by sequence to genes characterized in other plant species. We have also included four kernel composition traits (free lysine content, derived starch content, protein content, and oil content) in addition to the 13 traits included in our previous analysis.

Methods were identical to those outlined previously (Weber et al., Genetics 177:2349-2359, 2007). As before, not all marker-trait pairs were tested; instead, prior knowledge was used to determine which marker-trait pairs to test. Supplementary materials including the candidate gene list, trait definitions, and a list of marker-trait pairs tested, as well as all of our data files (genotypes, phenotypes, seed source information, principal components and the kinship matrix) are available for download at http://www.panzea.org.

Of the 1017 marker-trait pairs tested, only 47 (4.6%) had a *P*-value of less than 0.05, similar to the expectation under the null hypothesis (~5%). Of the 47 detected associations, two withstand correction for multiple testing by the false discovery rate ($Q < 0.1$, Table 1). A marker in *waxy1* and a marker in *pbf1* significantly associate with fruitcase weight (FCWT). Both of these associations are biologically plausible given what is known about these candidate genes. *waxy1* is a granule-bound starch synthase that accounts for all amylose production in the kernel (Nelson and Rines, Biochem. Biophys. Res. Commun. 9:297-300, 1962; Shure et al., Cell 35:225-233, 1983). It is possible that the association of *waxy1* and fruitcase weight is due to its role in amylose production. *pbf1* is a prolamin binding factor hypothesized to be a transcriptional activator of storage proteins in maize (Vicente-Carbajosa et

Table 1. List of significant marker-trait pairs after correction for multiple testing.

Trait‡	Gene	Marker	N[a]	R^2	$2a/\sigma_P$	d/a	P	FDR Q value
FCWT	*waxy1*	PZB00547.3	506	0.014	2.15	-0.339	0.0044	0.0770
FCWT	*pbf1*	pbf1.3	483	0.014	0.975	-0.304	0.0077	0.0770

[a]Number of individuals with both trait and marker data.

al., Proc. Natl. Acad. Sci. USA 94:7685-7690, 1997). Recent work in rice and wheat has found that *pbf1* does act as a transcriptional activator of storage proteins in vivo (Hwang et al., Plant Cell Physiol. 45:1509-1518, 2004). The association between *pbf1* and fruitcase weight could be a result of the role *pbf1* plays in the regulation of storage proteins. Although these associations seem biologically plausible given what is known, further work will be needed to validate that these genes do contribute to natural variation of fruitcase weight in teosinte.

Given the small percentage of associations found to be significant after correction for multiple-testing (< 0.2%), we hypothesize that there are many false negatives among our results. It is likely that our model, which was conservative in regard to controlling the false positive rate due to population structure, led to an increase in the number of false negative associations. We have made our datafiles available on http://www.panzea.org, as well as deposited seed from these and other teosinte populations with the U. S. Department of Agriculture North Central Regional Plant Introduction Station in Ames, Iowa, to encourage future teosinte association mapping studies which have the potential to detect genuine biological associations which were not detected in this study.

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Evidence of interaction between mutants of different *emp* genes

--Sangiorgio, S; Gabotti, D; Consonni, G; Gavazzi, G

The symbol *emp* (*empty pericarp*) refers to the phenotype of a group of defective kernel mutants with drastic reduction in endosperm tissue production. Here we report an analysis of the allelic relationship of nine *emp* mutants. They have different origins, thus representing independent mutational events. Originally they were isolated in populations carrying an active *MuDR* or *Spm* and they all behave as single gene mutants. To establish their allelic relationship we made crosses of each mutant with the others. For each of the pairwise combinations of the nine mutants, pollen from 10-20 plants of a given mutant whose heterozygous condition was ascertained by selfing, was applied to the silks of plants representing the selfed progeny of +/emp parents of a different *emp* isolate. The resulting ears were then scored for visual evidence of mutant segregation. If only wild-type seeds are observed in all ears produced by this cross, the two mutants are not considered to be allelic, whereas if some of the ears yield mutants in about one-quarter of the seeds this is taken as evidence of allelism. Wild-type seeds are then tested further in F2 and F3, the expectation being that ears should be recovered segregating 3 to 1 for the mutant, or not segregating in a 3 to 1 ratio. If the F2 obtained by selfing non-mutant plants of the F1 progeny includes ears segregating an excess of mutants (30-40%), this segregation value, approaching a 9 to 7 ratio, is taken as evidence of heterozygosity for two *emp* mutants in the parental F1 plant, thus defining two genes. The results of these tests, presented in Tables 1 and 2, are generally concordant in their conclusions. In two cases, however, where enough data have been collected, the results obtained in F1 and in F2/F3 lead to contrasting conclusions, i.e., one gene as inferred from the lack of complementation observed in F1, and two genes based on the observation of a segregation close to a 9 to 7 ratio, which is expected when the heterozygou *emp* F1 plants identify two genes.

These intriguing results seem to suggest an interaction between different *emp* mutants. Technically similar events are referred to in the literature as second site non-complementation (SSNC). There are 3 possible explanations for these events: interaction between two different mutant proteins leading to a toxic product, the mutant form of one protein sequestering the wild-type form of the other protein into an inactive complex, or combined haplo-insufficiency (Hawley and Gilliland, Genetics 174:5-15, 2006). We will test which of these possibilities applies to the cases reported here.

Table 1. Results of the complementation test involving nine independently isolated *emp* mutants. + and – indicate complementation and non-complementation, respectively. Signs in parentheses refer to dubious results that need further validation.

♀↓ ♂→	*emp4*	*emp8075*	*emp8077*	*emp8300*	*emp8376*	*emp8971*	*emp9106*	*empDAP3*	*emp9475*
emp4	-	+	+	+	+	+	+	+	-
emp8075		-	+	(-)	(-)	+	+	+	(-)
emp8077			-	+	+	+	-	+	+
emp8300				-	+	(-)	-	+	+
emp8376					-	+	+	(+)	+
emp8971						-	+	(+)	-
emp9106							-	+	+
empDAP3								-	+
emp9475									-

Table 2. Segregation in F2 and F3 of double mutants exhibiting non-complementation in the F1. Signs in parentheses refer to dubious results that need further validation.

Cross mode	Non complememtation in F1 (# of ears)	Segregation > 30% in F2/F3 (# of ears)	Inferred number of genes from	
			F1	F2/F3
emp4 x emp9475	9/29	14/78	1	2
emp8075 x emp8300	1/22	6/27	(1)	2
emp8075 x emp9475	4/12	-	1	-
emp8077 x emp9106	14/28	4/35	1	(2)
emp8300 x emp8971	1/15	3/12	(2)	2
emp8300 x emp9106	9/29	5/36	1	2
emp8376 x empDAP3	(1)/13	2/18	2	2
emp8971 x empDAP3	2/20	0/6	1	1
emp8971 x emp9475	4/25	2/67	1	1

Desiccation tolerance of maize viviparous mutants

--Malgioglio, A; Quattrini, E; Della Pina, S; Spini, A; Gavazzi, G

In maize, desiccation tolerance is acquired by the embryo at a precise developmental stage between 20 and 25 DAP (days after pollination) and is probably related to the maturation process char-

acterized by the accumulation of storage products and LEA (late embryogenesis abundant) proteins with a protective role.

Viviparous mutant embryos that are deficient in ABA synthesis or lack an active *vp1* factor do not express the normal set of maturation phase proteins and should not acquire desiccation tolerance. To verify this, we applied premature desiccation to developing *vp* embryos about 25 DAP, and compared their germination capacity to sib embryos not subjected to such treatment (Durantini et al., Heredity 101:465-470, 2008). To this aim, *vp* and normal sibling embryos from a segregating ear were excised and transferred to plant cell culture vessels (Phytatray Sigma) on basal MS medium containing 2% sucrose solidified with 0.8% agar, or subjected to desiccation and a storage period of 60 days at 5°C before transfer to the same medium. For the drying treatment, mutant and normal embryos were laid between two disks of blotting paper within a sterile petri dish and incubated in an oven at 35°C for 48 hours.

At the end of the treatment, the dishes were sealed with parafilm and conserved at 5°C with silica gel at the bottom of the petri dish under the blotting paper. For the germination test, embryos were maintained in a growth chamber at 25°C with a 14/10 h light/dark photoperiod. Germination was determined after 10 days of culture. When cultured immediately after their excision, immature embryos of all mutants tested germinated with a high frequency (95-100%) like their wild-type counterparts (data not shown). On the other hand, if they were cultured following a premature dehydration treatment, only *vp1* and *vp10* maintained a partial desiccation tolerance while the other mutants lost it (Table 1, Fig. 1). In addition, *vp5* showed minimal germination, consisting of primary root protrusion without a shoot.

In contrast to the results presented in this report, White et al. (Plant Physiol. 122:1081-1088, 2000) showed that induction of GA deficiency early in seed development, either genetically or via biosynthesis inhibitors, suppressed vivipary of *vp5/vp5* mutants while maintaining desiccation tolerance. However, since we applied a different protocol to test desiccation tolerance of the viviparous mutants that did not involve the inhibitors of GA synthesis, the results obtained are not strictly comparable. Furthermore, while interpreting these data one should keep in mind that the nature and position of the molecular lesion within the gene might affect the germination test of the mutant under testing, as clearly shown by the analysis of the alleles of *vp7* (*ps1*) obtained by *Ac* insertional mutagenesis (Bai et al., Genetics 175-981-992, 2007). We also found that *vp10-374* mutants exhibiting partial desiccation tolerance are impaired, to a different extent, in their morphogenesis.

These results seem to suggest that acquisition of desiccation tolerance requires the completion of the steps between carotenoid production and the late stage of ABA biosynthesis, suggesting a link between embryo morphogenesis and desiccation tolerance which should be further investigated.

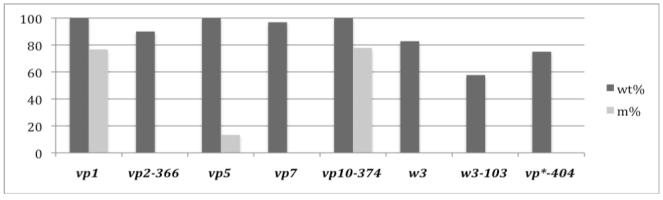

Figure 1. Effect of premature dehydration on germination capacity of wild-type (wt) and viviparous sib embryos.

Table 1. Germination percentage of homozygous *vp* mutant embryos. The germination percentage of the corresponding wild-type sibs is shown in Figure 1.

			+		m	
CODE	DAP	Mutant	No. seeds	germ %	No. seeds	germ %
07.50A-2	26	*vp1*	31	100	30	77
07.01-2	28	*vp2-366*	10	90	9	0
07.62-15	25	*vp5*	30	100	30	13
07.21-2,7	24,26	*vp7*	32	97	30	0
06.182	24	*vp10-374*	24	100	18	78
07.67-7	26	*w3*	29	83	38	0
07.06-1,2	21,24	*w3-103*	33	58	17	0
06.51-N	25	*vp*-404*	20	75	8	0

+ and m refer to wild-type and viviparous siblings

Another case of second site non-complementation

--Galbiati, M; Gavazzi, G

We observed another case of second site non-complementation (SSNC) while analyzing the complementation pattern of *d11**, a dwarf mutant inherited as a monogenic trait. Homozygous *d11** plants, which are drastically reduced in stature, produce andromonecious ears with normal seed set and exhibit a significant increase in their elongation if grown in the presence of 10 µM GA (Galbiati et al., Maydica 47:169-180, 2002). The pattern of complementation of this mutant with recessive *d* mutants reported in the literature (*d1*, *d2*, *d3*, *d5* and *an1*) is unexpected since it indicates that the mutant complements *d3* and *an1* but fails to complement *d1, d2* and *d5*. By further testing each of the double mutants in the F2 generation, we confirmed allelism of *d11** with *d1* and *d5*, a surprising result. On the other hand, the selfed progeny of heterozygous *d11**/+ *d2*/+ dihybrids produceed ears with a 9 to 7 segregation of normal versus dwarfs, a segregation expected if *d2* and *d11** define two separate genes. We hypothesize that the contrasting results observed in the F1 and F2 generation could indicate an interaction of the gene products of two genes as described in our previous report. While germinating seeds of the 9:7 segregating ears, we noticed that a minority of the dwarf seedlings had a more pronounced reduction in their elongation. A similar observation also applied to the F2 progeny of *d11**/+ *an1*/+ parents. In the F2 progeny of these heterozygous double mutants, one-seventh of the dwarfs should be homozygous double mutants and should yield, assuming an additive effect of the two mutations, seedlings with a higher reduction in their length than single gene mutants. This is exactly what we observed and can be taken as evidence that the two genes have an additive effect (Table 1, Figure 1).

Table 1. Stature of wild type, dwarf and severe dwarf seedlings and frequency of the severe dwarf phenotype in the selfed progeny of different double heterozygous combinations. The *d*10/an1* double mutant is included as another example of the detection of severe dwarfs in the F2.

Double heterozygote constitution (F2)	Seedling elongation (cm)			Frequency of severe dwarfs (%)		P-value
	wild type	dwarfs	severe dwarfs	observed	expected	
*d*11* x *d2*	18.2	7.2	4.4	18.7	14.3	0.15
*d*11* x *an1*	22.9	9.4	6.1	15.6	14.3	0.56
*d*10* x *an1*	16.6	8.9	4.5	17.4	14.3	0.38

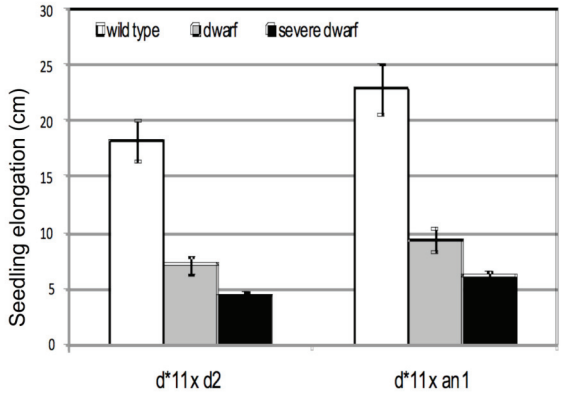

Figure 1. Stature of wild type, dwarf and severe dwarf seedlings in the selfed progeny of *d2/d*11* and *an1/d*11* double heterozygotes. Seedling elongation was determined at day ten after germination by measuring the distance between the scutellar node and the tip of the last leaf. Each value represents the mean (± std. dev) of three independent experiments.

These results seem to suggest that *d11** and *d2* or *an1* affect different biological pathways, both of which contribute independently to seedling elongation. It remains to be explained how *d11** shows non-complementation with *d1* and *d5,* two genes located on different chromosomes and controlling separate steps in GA biosynthesis.

Characterization of a dominant mutation of the *Dwarf8* gene

--Pilu, R; Cassani, E; Bertolini, E; Landoni, M; Gavina, D; Villa, D; Cerino Badone, F; Sirizzotti, A; Casella, L; Lago, C

We have isolated and characterized a new spontaneous dominant dwarf mutation that at maturity shows delayed flowering and reduced stature, ranging from 60-70% in a W23 NIL (Fig. 1A) to a 40-45% in a W23 X B73 F1 hybrid (Fig. 1B), caused by a reduced internode length. This mutant also shows thick broad leaves, that are 25-30% larger than wild type (Fig. 1C), a strong gene dosage effect on phenotype and a less severe phenotype in comparison with the *D8-1* dominant mutant, as shown in Fig. 1D. The dwarf phenotype is also easily detectable in the first stage of plant growth (Fig. 1E) and at maturity shows a tendency to produce tillers (Fig. 1F). In addition, the dwarf mutant is altered in its floral development. In fact, stamens are present in the terminal flowers of the ears (andromonoecious ear); however, they are sterile (Fig. 1G). The dimensions of the silks and anthers in the inflorescence are bigger by about 40% and 17%, respectively, compared to the wild type (Fig. 1H, I). The genetic analysis performed to understand the inheritance of this dwarf mutation demonstrated a monogenic dominant inheritance of this trait, and the map position was established on the long arm of chromosome 1. The results obtained from this analysis showed that *D*-1023* maps where *D8-ref* was located, and thus the mutation was renamed *D8-1023*.

The novel mutant allele was cloned and the alignment with *d8*(+) wild type alleles present in the database has shown a molecular lesion: an insertion of 3bp within the VHYNP domain, located in the 5' of the gene near the DELLA domain, which is responsible for the GA response (Fig. 2). This finding represents the first evidence of a dominant dwarfing mutation that does not involve the DELLA domain but is in the not yet well-characterized VHYNP domain, which is involved in protein degradation. We have found a new and interesting phenotype and we suggest a possible future modification of the VHYNP domain of the *D8* gene to modulate plant growth and to shorten excessively tall germplasm, with the aim of improving crop production.

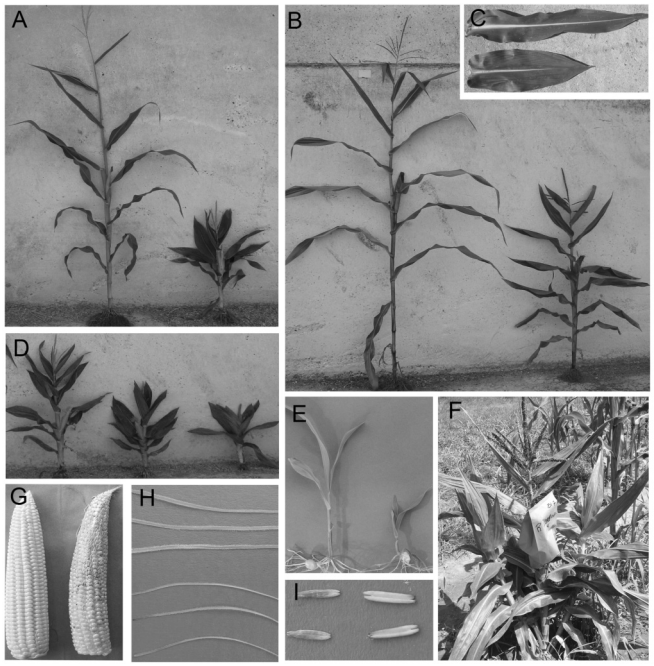

Figure 1. Phenotype of the new dwarf mutant: (A) wild type (left) and *D*-1023/+* mutant (right) whole plants at maturity in a W23 near-isogenic line; (B) wild type (left) and *D*-1023/+* mutant (right) whole plants at maturity in a W23 X B73 F1 hybrid genetic background; (C) leaves, wild type above and mutant below in a W23 near isogenic line; (D) from left to right *D*-1023/+*, *D*-1023/D*-1023*, *D8-1/+* whole plants in a W23 genetic background; (E) wild type seedling (left) and dwarf (right); (F) dwarf tillering growth habit in a B73 near isogenic line; (G) wild type ear (left) and dwarf anthered ear (right); (H) mutant silks (above) and wild type silks (below); (I) wild type anthers (left) and mutant anthers (right).

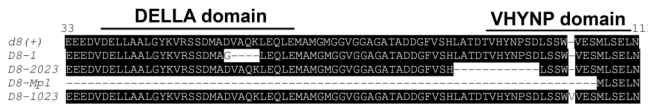

Figure 2. Partial alignment between the *d8* wild type allele and predicted proteins encoded by dominant mutant alleles. The wild type *d8* allele is compared with *D8-1*, *D8-2023*, *D8-Mpl* and *D8-1023* dominant mutant allele-encoded proteins with mutant N-terminals. Differences between wild type and mutant sequences (deletions, insertions and substitutions) are highlighted in white, and the previously identified highly conserved DELLA and VHYNP domains are shown.

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Colegio de Postgraduados

Double kernel fruitcases found in teosinte populations

--Kato Y., TA

Teosinte fruitcases traditionally are known to have only one developed spikelet each because the second one is suppressed during the ontogeny of the female inflorescence. According to Galinat (Corn and Corn Improvement, G. F. Sprague (ed.), pp. 1-47, 1988) the primary step for evolving teosinte to maize was the reactivation of the second spikelet to form the necessary link between these two plant taxa and he states that "The pairing of fe-

male spikelets in wild populations of teosinte occurs at a low frequency. Its significance can be questioned because, rather than being part of the natural variation in teosinte, it could just be a result of introgression from corn."

Recently the author has found these paired female spikelets in populations of teosinte race Chalco (Figure 1). Two types of "double kernel fruitcases", as the author has called them, were found: a) fruitcases with the two spikelets in parallel within the single cupule with an indurated outer glume covering each spikelet, as shown in the middle row of Figure 1; b) two spikelets grown in a different manner, one within the cupule covered by an indurated outer glume in the same way as the normal teosinte fruitcase with a single spikelet, and the second spikelet developing outside the cupule, due to elongation of the pedicel or rachilla, and covered by the floral bracts or glumes with a variously indurated outer glume. Frequently, the outer kernel becomes naked or almost so; the elongated rachilla is grown parallel to the rachis axis, therefore, this spikelet usually is positioned at the top of the fruitcase; and either one of the two spikelets can grow outward from the cupule. No case with the two kernels growing outside the cupule has been observed so far (see bottom row in Figure 1).

Figure 1. Normal and double kernel fruit cases of teosinte from the region of Chalco-Amecameca, State of Mexico-

The parallel orientation of the elongated pedicel of the external kernel in double kernel fruitcases in relation to the rachis axis, seems to indicate that they are not a consequence of introgression from maize into teosinte because the rachilla elongation in maize is perpendicular to the rachis axis. Besides, as Galinat (Univ. Massachusetts, Agric. Expt. Sta. Amherst, Bull. No. 585, 1970) states, "In a hybrid between modern maize and teosinte, the rachilla is shortened and the paired spikelets inclined and partially embedded within the cupule." However, because the present report is based on preliminary observations, the maize introgression hypothesis cannot be discarded completely yet until more detailed studies are made on these paired spikelet female fruitcases from teosinte race Chalco populations. In any event, the evolutionary significance of these findings is that the teosinte populations of 8,000 to 10,000 years ago probably produced the natural variation, which included the paired spikelet female fruitcases with naked kernels among other variants, before man of that time, upon observing this variation was motivated to start domestication of maize from teosinte. This evolutionary process intermediate between teosinte and maize is what Galinat (1988) called the "primary step" toward the origin of maize by means of "a reactivation of the second female spikelet".

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Molecular characterization of selected maize landraces in India using Simple Sequence Repeat (SSR) markers

--Sharma, L; Prasanna, BM; Ramesh, B

Intensive efforts have been initiated in the last few years on phenotypic as well as molecular characterization of the maize landraces in India. Significant variability in plant, ear, and tassel characteristics of maize landraces has been observed in Northeastern and Northwestern highlands of India with relatively less varietal diversity for those collected from the plains (Prasanna and Sharma, Indian J. Plant Genet Resources 18:155-168, 2005).

In the present study, a set of 48 Indian maize landraces was selected for SSR genotyping. These landraces are all important for breeding purposes, since they are mostly early maturing, with excellent yield characters and adaptability, along with biotic and abiotic stress tolerance. The selected germplasm represents diverse agro-ecological zones of India, spanning both NEH (29 landraces) and other regions (19 landraces). Thirty were obtained from the National Gene Bank at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and 18 were collected by the Maize Genetics Unit, IARI, from Sikkim state in the NEH region in November 2005 (Table 1). SSR analysis employed fluorescent-dye labeled SSR primers (CIMMYT Applied Biotechnology Center's Manual of Laboratory Procedures (http://www.cimyt.org/ABC/Protocols/manual ABC.html) (Fig. 1).

Table 1. List of accessions selected for molecular characterization.

S.No.	Accession	State (India)*	S.No.	Accession	State (India)*
1	IML112	HP	25	IML429	Rajasthan
2	IML115	J&K	26	IML436	Rajasthan
3	IML132	Uttarakhand	27	IML452	MP
4	IML181	HP	28	IML454	MP
5	IML196	Manipur	29	IML479	MP
6	IML203	Nagaland	30	IML550	Sikkim
7	IML210	AP	31	IML558	Sikkim
8	IML215	Ar.P	32	IML560	Sikkim
9	IML232	Ar.P	33	IML565	Sikkim
10	IML235	Ar.P.	34	IML567	Sikkim
11	IML255	Meghalaya	35	IML587	Sikkim
12	IML267	Sikkim	36	IML588	Sikkim
13	IML282	Ar.P.	37	IML589	Sikkim
14	IML290	Jharkhand	38	IML590	Sikkim
15	IML293	Bihar	39	IML592	Sikkim
16	IML295	Jharkhand	40	IML594	Sikkim
17	IML297	WB	41	IML595	Sikkim
18	IML298	Meghalaya	42	IML602	Sikkim
19	IML321	Bihar	43	IML608	Sikkim
20	IML415	MP	44	IML610	Sikkim
21	IML420	Rajasthan	45	IML615	Sikkim
22	IML423	Bihar	46	IML616	Sikkim
23	IML427	Rajasthan	47	IML618	Sikkim
24	IML428	Rajasthan	48	IML637	Mizoram

*HP: Himachal Pradesh; J&K: Jammu and Kashmir; AP: Andhra Pradesh; Ar.P.: Arunachal Pradesh; WB: West Bengal; MP: Madhya Pradesh.

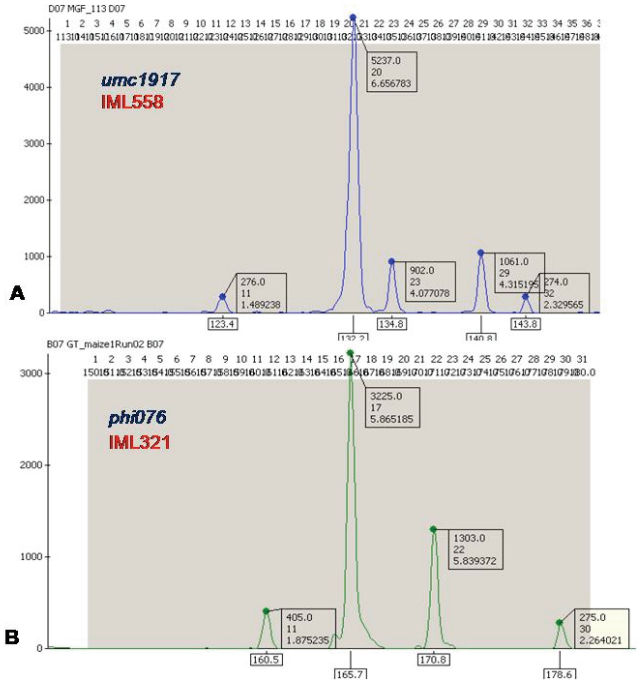

Figure 1. Images of some SSR alleles (seen as peaks) resolved using a MegaBACE DNA Sequencer: (A) *umc1917*; (B) *phi076*.

A total of 547 alleles were detected for 42 SSR markers. We found, on average, a higher number of alleles per marker (13.0) (Table 2) than reported in a study on maize populations from Europe and America using 24 SSR markers (Dubriel et al., Maydica 51:281-291, 2006) where the average number of alleles observed was 7.8. Similarly, Warburton et al. (Crop Sci 42:1832-1840, 2002), in an analysis of seven CIMMYT populations and 57 CML lines, reported mean numbers of SSR alleles of 6.3 and 4.9, respectively. The higher number of alleles per locus observed in this study indicates considerable diversity among the selected landraces in India. The PIC values of the 42 SSR loci in the present study ranged from 0.18 (*phi062*) to 0.85 (*phi083* and *phi331888*), with a high mean PIC value of 0.60, reinforcing the importance and utility of SSR markers in the study of population genetics. The frequency of the major allele at each locus ranged from 0.21 (*phi96100*) to 0.89 (*phi062*). Rare alleles (frequency less than 0.005) were identified at all loci, with an average of 9 alleles/locus.

Genetic relationships among the selected Indian maize landrace accessions are depicted in Figure 2. The distinct feature of the dendrogram is the grouping of 'Sikkim Primitives' into one cluster. 'Sikkim Primitives', first described by N. L. Dhawan in 1964 (MNL 38:69-70), have some unique features, including a complete lack of apical dominance; prolificacy (5-9 ears) with uniformity in ear size; erect leaves; top bearing habit and drooping tassel (Sachan and Sarkar, MNL 56:122-124, 1982). This landrace stays green after maturity, and thus serves well for fodder purpose for the local farmers. The 'Sikkim Primitive' landrace is still grown in remote and isolated villages of Sikkim state in the NEH region. Accessions from other regions in India were found to form different clusters, based on geographical region.

Estimation of Wright's *F*-statistics revealed F_{IS} values ranging from -0.11 (IML602) to 0.13 (IML592), indicating low levels of in-

Table 2. Marker-wise summary statistics for the selected landraces.

S.No.	SSR locus	Observed size range (bp)	No. of alleles	Major Allele		PIC*
				Size (bp)	Frequency	
1	*nc130*	133-148	6	141	0.65	0.46
2	*nc133*	99-120	4	116	0.54	0.51
3	*phi014*	140-172	7	158	0.80	0.31
4	*phi029*	148-162	10	149	0.54	0.57
5	*phi031*	170-230	17	186	0.38	0.79
6	*phi034*	120-150	10	123	0.33	0.79
7	*phi041*	190-220	9	200	0.58	0.59
8	*phi046*	55-70	7	63	0.49	0.58
9	*phi059*	140-180	10	153	0.53	0.60
10	*phi062*	156-162	4	162	0.89	0.18
11	*phi063*	145-191	32	172	0.49	0.67
12	*phi065*	124-152	12	131	0.29	0.77
13	*phi075*	199-245	15	228	0.53	0.57
14	*phi076*	150-180	10	171	0.50	0.55
15	*phi079*	160-210	11	187	0.24	0.78
16	*phi083*	110-140	21	125	0.23	0.85
17	*phi084*	150-162	10	158	0.53	0.65
18	*phi090*	110-150	7	141	0.83	0.24
19	*phi093*	282-291	11	288	0.28	0.82
20	*phi102228*	118-135	9	124	0.66	0.54
21	*phi108411*	112-128	11	123	0.42	0.68
22	*phi109188*	140-181	14	165	0.64	0.56
23	*phi112*	129-170	19	152	0.68	0.52
24	*phi115*	275-320	18	293	0.52	0.52
25	*phi123*	115-185	15	145	0.58	0.62
26	*phi127*	96-135	19	112	0.53	0.67
27	*phi227562*	302-324	8	308	0.38	0.71
28	*phi299852*	99-165	19	122	0.37	0.80
29	*phi308707*	110-160	18	134	0.38	0.76
30	*phi331888*	115-139	16	119	0.25	0.85
31	*phi96100*	245-305	22	297	0.21	0.84
32	*umc1161*	125-160	17	145	0.56	0.64
33	*umc1196*	129-185	14	148	0.63	0.56
34	*umc1266*	130-149	10	134	0.86	0.25
35	*umc1304*	97-141	21	131	0.54	0.62
36	*umc1332*	108-150	14	145	0.40	0.69
37	*umc1367*	130-165	7	159	0.87	0.22
38	*umc1447*	110-127	8	124	0.46	0.56
39	*umc1545*	60-85	16	80	0.36	0.76
40	*umc1917*	121-147	11	132	0.53	0.64
41	*umc2047*	119-140	10	135	0.59	0.59
42	*umc2250*	135-163	18	152	0.75	0.41

*PIC: Polymorphism Information Content.
bp and PIC determined using FreqsR and Fto software (Dubreuil et al., Maydica 51:281-291, 2006) developed under the Generation Challenge Program (GCP) Project titled "Characterization of global maize populations: tracking the maize migration route from the center of origin", coordinated by CIMMYT, Mexico.

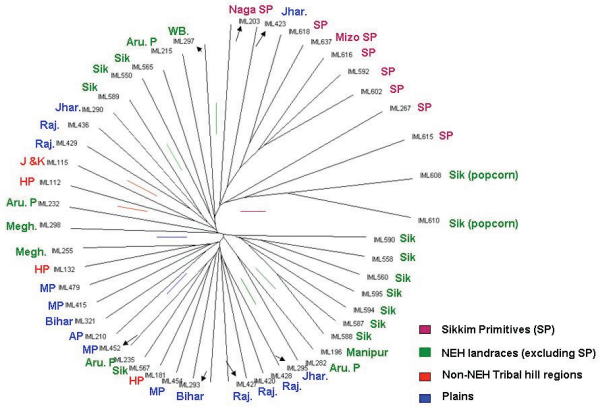

Figure 2. Genetic relationships among selected Indian maize landrace accessions. Pair-wise genetic distances were based on SSR allele frequencies determined using Rogers (1972) distance, followed by cluster analysis using UPGMA; the resulting dendrogram was prepared using TREEVIEW (Page, 1996). Note the occurrence of 'Sikkim Primitive' accessions in a distinct cluster.

breeding and negligible non-random mating within each population. Significant variation in the F_{ST} values was found among accessions, with a mean of 0.38, indicating high genetic differentiation between accessions. AMOVA using Arlequin v2.0 revealed that 60% of the genetic variation in these accessions was within the individuals, while 37% of the variation was among populations within a group.

More extensive efforts are in progress at the Maize Genetics Unit, IARI, New Delhi, with regard to phenotypic and molecular characterization of landraces in India. The goals are to identify 'core collections' with potential utility in breeding programmes, and in mining favourable alleles towards influencing productivity and other target traits.

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Evolutionary divergence of the genes *dek1* and *Agpsem* (*agp1*) of *Tripsacum* and *Zea mays*

--Mglinets, A; Sokolov, V; Blakey, A; Tarakanova, T

Eastern gamagrass (*Tripsacum dactyloides* L.), a perennial cereal, is a distant relative of maize and is found widely throughout South and Central America, and southern and eastern areas of North America. Several cytotypes are encountered in nature, diploid *(2n=36)*, triploid *(2n=3x=54)*, tetraploid *(2n=4x=72)* and hexaploid *(2n=6x=108)* forms. Diploids follow a strictly sexual mode of reproduction, whereas polyploids are apomictic. Hybridisation among these species may occur spontaneously. Thus, *Tripsacum andersonii* J.R. Gray, a *Zea* (maize) x *Tripsacum* hybrid with 54 chromosomes which clonally reproduces through budding, has been found in nature and is considered to be a spontaneous hybrid of the two species *Tripsacum dactyloides* and *Zea mays*.

Modern maize x *Tripsacum* hybridisations are also used in plant breeding, primarily for introgression of various agronomic traits. These hybridisations have made it possible to improve maize resistance to high temperatures, to obtain valuable forms of polyunsaturated fatty acids, and to produce apomictic maize x *Tripsacum* hybrids. The use of both molecular mapping and molecular-cytological methods (FISH) has demonstrated that there is a high degree of homology between maize and *Tripsacum* genomes. Molecular probes derived from maize genes can be used for marking, or tagging, *Tripsacum* chromosomes. However, the precise level of genome homology has yet to be determined.

The goal of this research was to determine the primary sequence structure of some *Tripsacum dactyloides* L. genes and to compare them to candidate orthologous maize genes. A *Tripsacum dactyloides* L. specimen, 4N=72, was obtained from the Tashkent Botanic Garden and used as a representative of the species. This particular specimen is of historic significance in that it originated from the materials of N.I. Vavilov's expedition. In addition, this plant accession has also been used to produce apomictic maize x *Tripsacum* hybrids.

Total DNA extraction was carried out from young leaves using cetyltrimethylammonium bromide (CTAB). The DNA was then quantitated and used in polymerase chain reaction (PCR) amplification of specific fragments. The primer pair used for fragment amplification of gene *dek1* was dek1-F (5'-GGGTGCTTTAACTTCAGTTGCA -3') and dek1-R (5'-GCCANGTTCAAATCCAATAGCTG - 3'). For fragment amplification of gene *Agpsem,* the primers Agp-F (5'- GATATCCCNGTCAGCAACTG T - 3') and Agp-R (5' - TTTTGGTANTCCATACGGTAC - 3') were used. PCR was carried out in 20 µl of reactions using BIOTAQ™ Red DNA Polymerase by Bioline Enterprise and 10 ng of the total DNA. Amplification cycle reactions were as follows: initial denaturation — 95^{0}C, 3 min.; amplification for 35 cycles: 94^{0}C, 30 sec., annealing — 56^{0}C, 30 sec., extension — 72^{0}C, 60 sec.; final extension — 5 min. Amplification products were visualized in a 1% agarose gel. Total maize DNA extracted from accession C435 (VIR Collection, St.-Petersburg) was used as control. Determination of initial PCR product sequences was carried out in the Inter-Institutional Centre for DNA Sequencing, SB RAS, Novosibirsk using a ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit. The same primers were used for the sequencing and amplification reactions. Only part of the initial PCR product sequences were used in the comparative analysis as determined using direct and reverse primers. The maize gene sequences used for comparison were obtained from GenBank (http://www.ncbi.nlm.nih.gov).

Using the primers dek1-F and dek1-R, the expected PCR product size was 886 nucleotides. The fragment of the putative *Tripsacum dek1* gene was determined to be 797 bases long. Direct comparison of initial sequences of the PCR-generated putative *Tripsacum dek1* gene fragment and that of maize demonstrated very high homology, i.e., 99%, and only 1% of differences, for 6 one-nt replacements and 1 three-nt deletion/insertion (Fig. 1). Three one-nt replacements were localised in an exon region, the other three were found in the intron region of maize gene *dek1*. The *Tripsacum* three-nt deletion was found in the gene exon relative to maize.

			27			48			161			264			468			612			739	
T. dactyloides	(1)	..	C	..	T	..	..	A	..	..	G	..	..	A	..	T	..	---	..			
Zea mays	(5066)	..	A	..	C	..	..	G	..	..	C	..	..	C	..	C	..	ACT	..			

Figure 1. Comparison of nucleotide sequences for the *Zea mays dek1* gene (AY061804) and the putative PCR-generated *dek1* gene of *Tripsacum*. Exons of the *Zea mays dek1* gene are shown in grey.

The maize gene *dek1* (*defective kernel1*) is approximately 24k nucleotides long and plays a very important role through its participation in the structural maintenance and functioning of Ca^{2+} ion trans-membrane transport channels. For the maize *dek1* gene, not only is the whole primary structure known, but also intron and exon positions, as well as the structure of its protein product. Therefore, open coding frames may be determined on the primary sequence of the putative *Tripsacum dek1* gene fragment in the areas corresponding to comparative exons of the maize *dek1* gene, and thus it is possible to determine if revealed comparative sequence differences lead to changes at the level of the amino acid sequence. As seen in Fig. 2, the difference in position 161 between maize and *Tripsacum* leads to amino acid replacement. Replacements at nt positions 468 and 612 do not lead to differences in amino acids, i.e., they are synonymous. Absence of an

309
T. dactyloides DWNLGLCSFRFELLKSRMIVLFVAGTSRAFL**I**SFGVHYW

Zea mays (277) DWNLGLCSFRFELLKSRMIVLFVAGTSRAFL**V**SFGVHYW (316)

T. dactyloides YLGHCISYAFVASVLLSAAVSSWLSISNPSVARIDALRSTVIKLREGFRRKGQNSSSNSS

Zea mays (316) YLGHCISYAFVASVLLSAAVSSWLSISNPSVARIDALRSTVIKLREGFRRKGQNSSSNSS (376)

T. dactyloides EGCGSSVKRSSGSVEAGQNGNATDSMYRSNSQSDGVNWSSIPFDRSNSCQEGRSSDKNID

Zea mays (376) EGCGSSVKRSSGSVEAGQNGNATDSMYRSNSQSDGVNWSSIPFDRSNSCQEGRSSDKNID (436)

380
T. dactyloides SARASLAHRSNSCLSAVQDSETAVVSVDRHGDP-TSLVCSSSGLESHGCEPSGS

Zea mays (436) SARASLAHRSNSCLSAVQDSETAVVSVDRHGDP**T**TSLVCSSSGLESHGCEPSGS

Figure 2. Alignment of the amino acid protein sequences coded by the *Zea mays dek1* gene (AAL38188) and the putative PCR-generated *dek1* gene of *Tripsacum*. Positions in amino acid sequence are presented for *Zea mays*.

```
                            39        53            149       202          221       271        285       291
                       30        48            146       183        206          2643       278        288       297
T. dactyloides    (1)    .. T .. T .. A ..  AG ..  .. - .. C .. A .. C ..  G A .. G .. T .. G .. CT .. G .. T .. C .. C ..     (308)

Zea mays     (171646)    .. C .. C .. C .. GA  .. .. T .. T .. G .. T ..  A G .. T .. A .. A .. -- .. A .. C .. T .. A ..     (171953)

                         308       328       335       349                                  403         407       456
                              315       331       348                                  396         405       441
T. dactyloides  (308)    A .. A .. A .. G .. A .. - ..   ----------------    .. G ..  ----  .. C .. C .. A .. --             (456)

Zea mays     (171953)    G .. T .. C .. T .. G .. T .. TTCCTTTTTTTTTTCT      .. A .. GTAG  .. A .. A .. G .. CT             (172124)

                                                       462         501        512                     521         572
                           459                                483         507        516                     552
T. dactyloides  (456)    ..    ---------------------    .. G  ..  A ..  A  .. A .. T ..   -------  ..  A  ..  T  ..  A     (573)

Zea mays     (172124)    ..    GATAATCTAATTAAACCAGTG    .. A  ..  - ..  T  .. G .. C ..   GTCCTGA  ..  G  ..  C  ..  G     (172268)

                                  631          639        666          681        689           720              774
                             618          633        651          674        687          717             744
T. dactyloides  (573)      .. T  ..  T  ..  CT .. C .. T ..  A  .. C .. C ..  C .. C ..   T  ..  A  .. .. A  .. C ..          (776)

Zea mays     (172268)      .. A  ..  C  ..  TC .. A .. C ..  C  .. T .. T ..  A .. T ..  C   ..  T  .. .. G  .. T ..          (172471)
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Figure 3. Comparison of nucleotide sequences for the *Zea mays Agpsemzm* gene and the putative PCR-generated *Agpsem* gene of *Tripsacum*. Exons of the *Zea mays Agpsemzm* gene are shown in grey.

ACT triplet in position 739 of *T. dactyloides* leads to the absence of a threonine amino acid in the putative protein coded by the gene studied compared to the known protein product of the maize gene.

Gene *Agpsemzm* (*agp1*, AGP, small, embryo, *Zea mays*) encodes the small subunit of embryonic ADP-glucosophosphorylase in maize. Comparison of sequence AY032604 (mRNA sequence) and the sequenced maize genome showed that this gene is located on chromosome 2 (BAC_clones: AC177860.4) position 170446 to 181045 (BAC_clones: AC177860.4-Contig127 : 9462 : 20061 : 1). Thus, the total length of the gene coding area is more than 10k bases.

The expected PCR product size was 933 nucleotide pairs with genomic maize DNA and primers Agp-F and Agp-R. Comparison of amplification products obtained using these oligonucleotides and genomic maize and *Tripsacum* DNAs revealed one PCR product with a close molecular weight in each case. The PCR product obtained from genomic *Tripsacum* DNA was determined to be 775 bases long. Bioinformatic analysis of the sequence revealed that it was homologous to genes coding the small subunit of ADP-glucosophosphorylase in different plant species. It manifests the highest homology with the small subunit of embryonic ADP-glucosophosphorylase (AY032604), indicated as *Agpsemzm*. Therefore, one can hypothesize that we have managed to determine the primary sequence of the putative gene fragment encoding the small subunit of embryonic ADP-glucosophosphorylase in *Tripsacum dactyloides* which, in analogy with maize, is indicated as *Agpsem* (AGP, small, embryo).

Comparison of the sequence obtained with the *Agpsemzm* gene showed that the maize sequence is 824 bases long (Fig. 3). Almost 13% of differences are conditioned by one- and two-nt replacements, and deletions/insertions of different lengths (two, four, seven and twenty-one nt long) (Fig. 3).

As intron and exon intra-genic regions have not been determined for the gene *Agpsemzm*, thus it may be possible to determine the open coding reading frames, and to see if nucleotide replacements lead to changes in amino acids in the putative *Tripsacum Agpsem* gene in the comparative regions of maize. Further analysis revealed that all single-nt replacements within the putative *Tripsacum Agpsem* gene fragment corresponding to the second and third maize exons are synonymic (Fig. 4). The two-nt replacement “GA” in maize for that of “AG” in *Tripsacum*

```
                                        149
T. dactyloides              LTQFNSASLNRHLSRAYENNIAGYKNEGFVEVLAAQQSPENPNWFQ

Zea mays         (132)      LTQFNSASLNRHLSRAYGNNIAGYKNEGFVEVLAAQQSPENPNWFQ      (177)

T. dactyloides              QGTADAVRQYMWLFEEH

Zea mays         (177)      QGTADAVRQYMWLFEEH        (193)
```

Figure 4. Protein amino acid sequences coded by the *Zea mays Agpsemzm* gene (NP_001105178) and the putative PCR-generated *Agpsem* gene of *Tripsacum*. Amino acid positions are indicated for *Zea mays*.

leads to the replacement of a glycine (maize) with a glutamine acid (*Tripsacum*).

The major differences between the nucleotide sequences of the maize *Agpsemzm* gene and the putative *Tripsacum Agpsem* gene were observed in the intron regions, whereas the coding sequence remains conserved. However, by comparison, the sequence differences found in both introns and exons between the maize *dek1* gene and putative *Tripsacum dek1* gene were not as varied. This fact is both very interesting and points to the need for further research.

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Studies on secondary traits of maize inbreds, hybrids and composites across environments

--Devi, P; Singh, NK

Yield stability, as a selection trait in plant breeding programmes as well as in evaluation trials, is constantly gaining importance over yield capacity. This is especially important where environmental conditions vary considerably.

The present study was undertaken during the monsoon season of 2007 in three environments: normal conditions, low nitrogen and irrigated conditions, and low nitrogen and rain (non-irrigated) conditions. Five inbred lines, 10 single crosses and two standard checks, namely Surya (composite) and Nath Samrat 1133 (hybrid), were used as experimental materials with the objective of identifying stable genotypes for the secondary traits, anthesis-silking interval (ASI) and days to 75% ear leaf senescence. The evaluation trials were conducted in each environment in a randomized complete block design with three replications at the Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar. The experimental unit was a one row plot 5 m long and 75 cm apart, forming a plot size of 3.75 m^2 and a plant-to-plant distance of 25 cm. The stability of the characters for each genotype was calculated by regressing the mean values of individual genotypes on environmental index and by calculating the deviations of the regression coefficients from unity as suggested by Eberhart and Russell (Crop Sci. 6:36-40, 1966).

The pooled analysis of variance revealed significant differences among genotypes, environments and their interaction for both traits. Inbred lines P_2 and P_3 were found to be the most stable and desirable, whereas single crosses P_1xP_2, P_2xP_3, P_3xP_5 and standard check Surya were identified as ideal in terms of grain yield potential and stability parameters for both the ASI and days to 75% ear leaf senescence (Table).

Table. Stability parameters for anthesis-silking interval (ASI) and days to 75% ear leaf senescence.

Genotypes	Grain yield (kg/ha)	ASI (days)			Days to 75% ear leaf senescence		
	$\overline{X}$ i	$\overline{X}$ i	bi	S^2d_i	$\overline{X}$ i	bi	S^2d_i
Parents							
Pop 31 (P_1)	769.73	4.09	0.877	0.033	80.50	2.539	11.005**
Pop 446 (P_2)	889.29	3.92	0.744	-0.001	78.83	1.735	-0.405
YHP-A (P_3)	1009.72	3.50	0.942	-0.043	80.33	1.312	0.378
Pop 445 (P_4)	737.82	2.67	-0.253**	-0.054	77.00	-0.423*	-0.251
YHP-B (P_5)	858.56	3.17	0.616	2.248**	78.83	-0.375**	-0.546
Crosses							
P_1 x P_2	2184.55	2.50	0.471**	-0.070	80.50	2.253	0.367
P_1 x P_3	1258.50	4.92	1.397	0.140	78.83	3.851	5.318**
P_1 x P_4	1672.48	4.83	1.196**	-0.077	79.83	-0.375**	-0.546
P_1 x P_5	1493.96	4.44	1.341*	-0.040	80.33	0.047*	-0.408
P_2 x P_3	2043.22	4.83	2.429**	0.038	79.94	0.017	0.778
P_2 x P_4	1590.45	3.76	0.418	0.392	79.00	1.126	-0.337
P_2 x P_5	1721.28	2.33	0.725**	-0.074	79.50	3.095*	0.384
P_3 x P_4	1703.05	4.11	1.849**	-0.065	80.17	0.232	0.539
P_3 x P_5	1861.14	5.17	1.921**	-0.077	80.00	-0.142	0.907
P_4 x P_5	1841.12	3.00	0.471**	-0.070	77.33	-2.068*	5.332**
Checks							
Nath Samrat 1133	1530.57	3.17	0.507*	0.018	91.83	2.296	2.341*
Surya	2032.55	4.17	1.341	1.107**	79.33	1.878	0.080
Mean	1482.23	3.798	1.000		80.12	1.000	
SE (±)	219.936	0.373	0.355		1.009	1.430	

Expression of unusual characters in ear shoot and tassel of maize

--Singh, NK; Devi, P; Mishra, P

Maize (*Zea mays* L.) is a monoecious species that produces only unisexual flowers in separate male and female inflorescences. It is one of the most important cereals, with the highest yield potential and diverse uses from staple food and feed to industrial products like starch and biofuels. It is strongly believed that maize is essential for global food security. Maize is largely grown under rainfed conditions where various abiotic and biotic stresses severely affect the genetic yield potential. A global climatic change is now considered to be underway and is expected to result in a long-term trend towards changes in environmental conditions. Congenial environmental seasons support optimal development, however, unfavouable environments influence the genetic architecture of the plant and reduce yield directly by affecting plant growth and development, and indirectly by modifying the normal plant phenotype. Unpredictability of weather conditions has occasionally resulted in many unusual expressions in plant characteristics in general, and ear and tassel characteristics in particular, in maize. Multiple ears on single nodes are one of the environmentally induced oddities widely reported in maize hybrids grown during 2006 in Iowa, Illinois, and Indiana. The expression of multiple ears in inbred lines, populations and experimental hybrids was also recorded in maize grown in the Tarai region of Uttarakhand, India during the monsoon season of 2007. The twin ear expression on

single nodes in maize was observed earlier by Hallauer in 1973 in S_2 and S_5 progenies of two populations (Hallauer, MNL 58:21-22, 1984). Multiple ears on single nodes were also recorded in low frequency in different genotypes of maize grown during the monsoon season of 2008. In addition, this season also experienced some other unusual expressions in maize that have an impact directly or indirectly on grain or green cob yield. The unusual expressions include the expression of silks in tassel, part of the tassel converted into an ear, plants with terminal ears without any tassels, sterile bulky anthers and the induction of many ears from different nodes with rare effective silk emergence. In fact, unisexuality in maize occurs through the selective elimination of stamens in ear florets and by elimination of pistils in tassel florets. The two general classes of sex determining mutants have been identified in maize, including those of masculinized ears and feminized tassels. The endogenous gibberellic acid (GA) has been found to have a feminizing role in sex determination in maize (Tanurdzic and Banks, Plant Cell 16:S61-S71, 2004). Moreover, reversal of sexual expression in maize has been shown to be influenced by environment and heredity (Richey and Sprague, Amer. Nat. 66:433-443, 1932; Heslop-Harrison, Biol. Rev. 32:38-90, 2008). The unusual expressions observed in maize experimental plots planted at the Crop Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar (India) during the monsoon season of 2008 are described in brief on below.

Development of multiple ears on the same ear node. Multiple ears are not unexpected but they typically occur at different nodes, not on the same node. Often one of the double ears is smaller, consisting of a few short husks surrounding a tiny cob with silks. The other ear appears to be full size and not hampered by the double ear. The smaller of the double ears occasionally sets some grain. However, in extreme cases of multiple ears, no seed set will take place and all the ears remain barren (Fig. 1).

Figure 1. Expression of multiple ears on the same node.

Induction of multiple ears on different nodes that lack effective silks. Some plants were found to have many ears on separate nodes. In such cases, ears were generally lacking effective stigmas or silks. As a result, pollination could not take place and ears remained barren or set very few seeds (Fig. 2).

Figure 2. Prolificacy without effective silk development.

Induction of silk in the tassel leading to a lack of anther and pollen development. The tassel is the terminal male organ, consisting of anthers and producing pollen grains for fertilization of the ovule, which is borne in the so-called lateral ear. In some plants, glumes were induced to develop stigmas in the tassel in place of anthers, and therefore, no viable pollen grains were formed.

Figure 3. Tassels with silk development.

Tassels with both anthers and ears. Some plants were found to have both sexual expressions in the tassel. Generally, the tip of the tassel was converted into a small ear that set seed, whereas the remaining lower portions and other branches of the tassel developed anthers with pollen grains of very low viability. (Fig. 4).

Figure 4. Tassel with both anthers and ears.

Induction of terminal ears in place of lateral ears. Maize plants normally consist of terminal tassels as male inflorescences and lateral ears as female inflorescences. In some of the plants,

however, the terminal tassels were entirely modified into small single ears in place of tassels. The ears were small with few seeds (Fig. 5).

Figure 5. Terminal ears in place of tassels.

Compact sterile tassels with swollen glumes. Some of the plants showed compact tassels with bulky glumes and few stigmas emerging. Such types of tassels do not have viable pollen grains. Some of the glumes of the tassel also induced silk in place of anthers, and of these, few were able to pollinate and set individual grain (Fig. 6).

Figure 6. Tassels with bulky sterile glumes.

The unusual expressions in ear and tassel are suspected to be due to environmental factors. The monsoon season of 2008 was peculiar in terms of rainfall at regular intervals starting from the 2nd fortnight of June to mid-September 2008. Due to excess soil moisture, it became difficult to perform inter-cultural operations properly. The plants received less sunlight and also experienced low temperatures due to cloudy weather and frequent rainfall during the cropping season. Richey and Sprague (1932) reported the role of environment, i.e., shorter daylight periods and lower temperatures, and heredity in the development of silks in the tassels. Heslop-Harrison (2008) also shared the viewpoint that low temperatures, particularly when experienced through the dark period of the daily photoperiodic cycle, promote female sexual expression and depress male. The frequencies of the unusual expressions described above were extremely low. In case of widespread occurrence of these kinds of characteristics, the quality as well as the quantity of the maize grain or green cob will certainly suffer.

Influence of low nitrogen and excess soil moisture stress on yield of maize inbreds and their hybrids

--Massey, P; Warsi, MZK

Nitrogen fertilization in agriculture has emerged as a serious matter of world concern. Recent statistics on N fertilizer consumption patterns show the average application of N in developed countries is 250 kg ha^{-1}, while in developing countries it is82 kg ha^{-1}, and in sub-Saharan African it is as low as 5.0 kg ha^{-1} (Sasakawa Africa Assoc. Newsl. 18:4-5, 2002). Indian soils are characteristically low in organic matter and N. In India, water logging is also an important constraint for crop production. Out of a total of 6.55 million hectares of maize, about 2.5 million hectares are affected by an excess soil moisture (ESM) problem that causes an average 25-30 percent loss in national maize production every year (Directorate of Maize, Annual Maize Workshop, Kanpur, India, April 5-9, 2001; Bhan, Indian J. Agric. Res. 11:147-150, 1977; Howell and Hiler, Trans. ASAE 17(2):286-288, 1974). Carter et al. (Trans. ASAE 33(4):1203-1207, 1990) reported that yield reductions from 9 days of ESM during the vegetative and tasseling/silking stages can be as high as 77 and 61 percent, respectively. For June plantings, ESM may coincide with flowering, which may interfere with normal pollination behavior and seed setting (Savita et al., J. Plant Biol. 31(1):29-36, 2004). Therefore, it would be desirable to develop maize cultivars with increased resistance to ESM conditions and with improved N-use efficiency. For purposes of this report, a yield reduction of 25 percent or more is categorized as undesirable. Our results indicate we have ESM tolerant genotypes.

The materials consisted of 12 lines, 4 testers and their 48 crosses and were evaluated under low-N, excess soil moisture and normal conditions in Randomized Block Design during the monsoon season of 2007 at the Crop Research Centre of the University. The experimental plot consisted of 5-meter rows, with between row and within row spacing of 75 cm and 25 cm, respectively. For the low-N trials, 40 kg N ha^{-1} was applied. For the ESM trials, water-logging treatment was given at the knee-high growth stage for 6 days, with continuous submergence to about 5 cm. After 6 days of flooding, water was drained out of the plots.

Analysis of variance for yield under normal, low-N and ESM conditions revealed that mean squares for all genotypes studied were highly significant, clearly indicating the existence of genetic variability in the genotypes.

Estimation of yield loss in low-N conditions. The percent yield reduction varied from 0.37 per cent in L_8T_1 to 83.25 per cent in L_7T_1. While most of the hybrids showed relatively more susceptibility to low-N stress than inbred lines, this is likely due to reduced N-requirements associated with the short plant stature and lower yield potential of the inbreds. Among the lines, the highest reduction was recorded in L_{11} (56.36 percent) with the lowest reduction in L_2 (9.31 percent). Among testers, the highest reduction of 16.26 percent was reported in T_1 and the lowest reduction of 4.3 percent was observed in T_3. The crosses with low yield reduction in comparison to normal were L_1T_4 (0.7 percent), L_4T_2 (1 percent), L_6T_3 (3.8 percent) and L_9T_2 (4 percent). Six lines and 5 single cross hybrids showed yield reduction more than 25%, whereas the remaining test materials exhibited less than 25% yield reduction under low N conditions (Table 1).

Table 1. Yield reduction in genotypes under low-N and ESM conditions.

Genotype	% yield reduction	Response	% yield reduction	Response	Genotype	% yield reduction	Response	% yield reduction	Response
	Low-N	Low-N	ESM	ESM		Low-N	Low-N	ESM	ESM
POB. 33 C_3-12-2-1-1-2-2 (L_1)	39.58	S	59.02	S	L_5T_1	2.55	T	32.71	S
POB. 33 C_3-12-2-1-2-2-5 (L_2)	9.31	T	20.35	T	L_5T_2	3.02	T	30.09	S
POB. 33 C_3-142-1-6-1-1-4 (L_3)	12.16	T	58.29	S	L_5T_3	7.86	T	30.05	S
POB. 45 C_8-86-1-3-7-6-4 (L_4)	13.74	T	22.48	T	L_5T_4	32.39	S	70.63	S
POB. 45 C_8-45-2-6-1-2-7 (L_5)	23.16	T	42.22	S	L_6T_1	15.87	T	27.84	S
POB. 45 C_8-269-2-4-6-3-3 (L_6)	11.64	T	23.08	T	L_6T_2	9.69	T	25.92	S
POB. 45 C_8-86-1-1-7-5-1 (L_7)	20.89	T	44.41	S	L_6T_3	3.8	T	22.16	T
CLG 1708-1-1-9 (L_8)	40.27	S	37.19	S	L_6T_4	6.62	T	3.32	T
POB. 45 C_8-45-2-6-1-1-1 (L_9)	68.57	S	85.88	S	L_7T_1	83.25	S	50.34	S
POB. 45 C_8-86-1-3-4-5-2 (L_{10})	27.18	S	30.88	S	L_7T_2	3.23	T	19.40	T
POB. 45 C_8-86-1-3-2-2-5 (L_{11})	56.36	S	65.98	S	L_7T_3	19.97	T	44.57	S
POB. 45 C_8-269-2-4-6-6-1 (L_{12})	33.11	S	58.63	S	L_7T_4	18.34	T	29.49	S
POB. 445 ⊗ 58-6-3-B-B-B (T_1)	16.26	T	16.22	T	L_8T_1	0.37	T	33.03	S
POB. 446-74-2-B-B-B (T_2)	9.68	T	19.18	T	L_8T_2	17.21	T	19.11	T
CML-421(T_3)	4.3	T	3.87	T	L_8T_3	26.69	S	53.25	S
CML-423(T_4)	7.26	T	80.46	S	L_8T_4	6.05	T	35.52	S
L_1T_1	8.82	T	28.25	S	L_9T_1	27.57	S	27.71	S
L_1T_2	13	T	37.12	S	L_9T_2	4	T	22.88	T
L_1T_3	17.41	T	45.22	S	L_9T_3	8.1	T	43.40	S
L_1T_4	0.70	T	26.52	S	L_9T_4	8.89	T	38.26	S
L_2T_1	6.03	T	45.52	S	$L_{10}T_1$	4.01	T	19.17	T
L_2T_2	23.96	T	55.3	S	$L_{10}T_2$	5.58	T	51.02	S
L_2T_3	8.72	T	52.67	S	$L_{10}T_3$	5.42	T	41.35	S
L_2T_4	5.1	T	39.17	S	$L_{10}T_4$	18.08	T	31.13	S
L_3T_1	7.34	T	41.53	S	$L_{11}T_1$	22.04	T	26.50	S
L_3T_2	6.13	T	20.30	T	$L_{11}T_2$	18.68	T	49.89	S
L_3T_3	20.02	T	55.40	S	$L_{11}T_3$	9.64	T	41.94	S
L_3T_4	5.41	T	45.44	S	$L_{11}T_4$	16.52	T	33.02	S
L_4T_1	12.90	T	17.70	T	$L_{12}T_1$	6.12	T	39.27	S
L_4T_2	1.0	T	50.33	S	$L_{12}T_2$	28.53	S	84.73	S
L_4T_3	20.72	T	48.79	S	$L_{12}T_3$	17.79	T	45.41	S
L_4T_4	4.39	T	42.12	S	$L_{12}T_4$	11.18	T	21.18	T

Note: S = susceptible (greater than 25% yield reduction), T = tolerant (less than 25% yield reduction).

Estimation of yield losses in ESM conditions. The percent yield reduction among the crosses varied from 3.32 percent in L_6T_4 to 84.73 percent in $L_{12}T_2$. Crosses with moderate reductions in yield were L_8T_2 (19.11 percent), $L_{10}T_1$ (19.17 percent) and L_7T_2 (19.40 percent). Among the lines, the lowest reduction in yield was found in L_2 (20.35 percent) and the highest reduction in yield was found in L_9 (85.88 per cent). Among the testers, T_3 showed the least reduction in yield (3.87 percent) and T_4 showed maximum yield reduction (80.46 percent). Excess soil moisture conditions reduced the yield of nine lines, 1 tester and 39 hybrids by more than 25%, whereas the remaining test materials showed less than 25% yield reduction (Table 1).

Kernel carotenoids in 37 maize lines

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Vitamin A deficiency is a global problem. Among the three major cereals, only maize grain contains coloured carotenoid compounds that can be converted into vitamin A in humans and other animals. Maize exhibits considerable natural variability for kernel carotenoids, with some lines accumulating as much as 66 μg/g of dry weight (Brunson and Quackenbush, Crop Sci. 2:344-347, 1962; Buckner et al., Plant Cell 2:867-876, 1990; Harjes et al., *Science* 319:330-333, 2008). The present investigation was undertaken to characterize a set of potential inbred lines and populations for carotenoid content for further analysis and use in development of hybrid with enhanced level of carotenoids.

Thirty inbred lines and 7 improved populations of maize were characterized for kernel carotenoid content using the extraction protocol developed by Torbert Rocheford's Lab (http://www. cropsci.uiuc.edu/faculty/rocheford/quick_carotenoid_analysis_protocol.pdf) and optical density measurement. The total carotenoid content was found to vary from a minimum of 3.54 μg/g dry weight to a maximum of 29.27 μg/g dry weight (Table).

Table. Carotenoid content of different maize lines.

S. No.	Pedigree	Carotenoids (μg/g)	S. No.	Pedigree	Carotenoids (μg/g)
1.	Hyd07R-104-6	18.29	20.	Hyd07R-456-2	20.41
2.	Hyd07R-300-6	12.75	21.	Hyd07R-419-2	24.52
3.	Hyd07R-325-3	17.72	22.	Hyd07R-421-2	17.26
4.	Hyd07R-301-3	22.35	23.	Hyd07R-451-1	27.21
5.	Hyd07R-456-1	18.15	24.	Hyd07R-419-1	27.84
6.	Hyd07R-301-2	23.35	25.	Hyd07R-438-4	19.21
7.	Hyd07R-441-1	21.41	26.	Hyd07R-445-4	29.21
8.	Hyd07R-302-1	21.55	27.	Hyd07R-418-2	22.92
9.	Hyd07R-325-6	23.07	28.	Hyd07R-418-4	22.21
10.	Hyd07R-437-2	19.26	29.	Hyd07R-443-4	27.87
11.	Hyd07R-325-2	22.58	30.	D-131	22.78
12.	Hyd07R-408-2	29.27	31.	D-765	16.55
13.	Hyd07R-438-1	22.29	32.	Kanchan	12.41
14.	Hyd07R-302-5	26.24	33.	Tarun	12.24
15.	Hyd07R-300-4	27.47	34.	Surya	21.24
16.	Hyd07R-407-5	29.10	35.	Amar	24.89
17.	Hyd07R-445-5	26.10	36.	Pragati	14.15
18.	Hyd07R-437-5	25.72	37.	CM-300	3.54
19.	Hyd07R-444-3	26.98			
C.D. (5%)		2.454			2.454

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Clustering methods for determining heterotic patterns using molecular markers

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In hybrid maize breeding programs, efficiency of procedures to identify inbreds used to develop outstanding single crosses strongly affects the success of the program (Hallauer and Miranda, 1988). The best hybrid combinations can be identified using information from diallel (which are prohibitive with large numbers of inbreds) or topcrosses to testers (Terron et al., Agron. Meso. 8:26-34, 1997). When a large number of germplasm exists but no established heterotic groups are available, genetically similar germplasms can be identified with molecular markers. On the basis of this information, field trials can be planned more efficiently (Reif et al., Crop Sci. 43:1275-1282, 2003).

Several studies have been published in the last few years using molecular markers to study genetic divergence with variable results (Dias et al., 2004 Genet. Mol. Res. 3:356-368). According to Reif et al. (Crop Sci. 41:1-7, 2005), the choice of a coefficient for studying divergence depends on the marker system properties involved and on the study objectives, among other conditions. According to these authors, several studies ignore these conditions, especially those related to the coefficient properties, which are connected to the study objective, which are very important for decision making considering the proper coefficient to be used. These studies usually employ the same similarity coefficients for dominant markers, such as RAPDs, and codominant and multiallele markers, such as simple sequence repeats (SSR), even though some of these coefficients are specific for dichotomic variables. Most similarity coefficients are based on comparisons between the occurrence of common and different bands (indicated by ones and zeros in common in a data matrix), while genetic dissimilarity coefficients, such as Roger's modified distance and Nei's distance, make use of information on allele frequency obtained by molecular markers, especially microsatellites (Balestre et al., Genet. Mol. Res. 7:695-705, 2008; Reif et al., 2005).

Pritchard et al. (Genetics 155:945-959, 2000) introduced the software, Structure, which has been used with relative success in maize (Camus-Kulandaivelu et al., Crop Sci 47:887-890, 2007). Given a value for the number of populations (*K*), Structure uses a Bayesian framework to assign lines from the entire sample to clusters in such a way that Hardy-Weinberg disequilibrium and linkage disequilibrium (LD) are maximally explained (Pritchard, et al., 2000). The purpose of this study was to evaluate the reliability of clustering methods based on molecular marker information to replace and/or complement topcross trials in assigning lines to heterotic groups of temperate germplasm.

For the analysis, we used the results of the molecular characterization of 21 microsatellite loci evenly distributed in the genome of 26 inbred lines. All lines except one (B73) were developed by INTA (Instituto Nacional of Tecnologia Agropecuaria) from different sources (mainly landraces) and belong to the Argentine Orange Flint heterotic group. Results were partially published in Morales Yokobori et al. (MNL 79:36-37, 2005). The entire set of 26 lines was previously grouped into four heterotic groups by topcross (Table 1) (Eyherabide et al., Plant Breeding: The Arnel R. Hallauer International Symposium, Blackwell Publishing, pp. 352–379, 2006).

Table 1. Clustering of lines established by topcross (Eyhérabide et al., 2006; Nestares et al., 1999).

Heterotic Group	Inbreds
I	B73, Ip17, Ip32, Ip521, Ip122
II	Ip123, Ip153, Ip22, Ip44, Ip662, Ip70, P1338
III	Ip13, Ip146, Ip147, Ip19, Ip199, ZN6
IV	Ip38, Ip62, Ip103, Ip109, Ip110, Ip138, Ip152, Ip140

Cluster analysis was performed using the Unweighted Pair Group Method using Arithmetic averages (UPGMA) and on the basis of Modified Roger's distance (MRD). According to Melchinger (The Genetics and Exploitation of Heterosis in Crops, pp. 99–118, 1999*)*, heterosis is a function of the dominance effect of the QTL and of MRD between parents. Reif et al. (2005) states that MRD is especially suitable in studies based on (i) the prediction of heterosis with genetic dissimilarities or (ii) the establishment of heterotic groups.

Both distance and clustering were performed using InfoStat/P, v1.1 (Grupo InfoStat, FCA, Córdoba Argentina). Four groups were determined by visual inspection of dendrograms (Table 2). Lines were also subdivided into 4 genetic clusters using **Structure** (Pritchard et al., 2000). We set the parameter K = 4, the number of heterotic populations previously established by topcross. Burn-in time and replication number were both set to 500,000. Results can be seen in Table 3.

Table 2. Clustering of lines based on UPGMA (Unweighted Pair Group Method with Arithmetic average) and Roger's Modified Distance.

Cluster	Inbreds
1	Ip38, Ip44
2	Ip152, p1338, ZN6, Ip199, Ip521,Ip117
3	Ip138, Ip22, Ip32,Ip62,Ip110,Ip19
4	Ip103, Ip122, Ip123, Ip109, Ip13, Ip662, Ip153, Ip70, B73, Ip140, Ip146, Ip147

Table 3. Clustering of lines according to Structure software (Pritchard et al., 2000).

Cluster	Inbreds
A	Ip103, Ip122 , Ip123, Ip22,Ip32 , Ip38, Ip44
B	B73, Ip110, Ip138, Ip140, Ip19, Ip62, Ip662
C	Ip117, Ip152, Ip199, Ip521,p1338, ZN6
D	Ip109, Ip13, Ip146, Ip147, Ip153, Ip70

A script in R language (http://www.r-project.org/) was made in order to determine the best level of agreement between clustering based on molecular data (this work) and clustering based on topcross (Eyhérabide et al., 2006). This allows identification of the best match between molecular and topcross groups. Concordance was measured by Cohen's Kappa coefficient (psy package of R Project). Cohen's kappa measures the agreement between two raters who each classify *N* items into *C* mutually exclusive categories. K< 0 indicates no agreement whereas 1 indicates a perfect match. Kappa values ranged from 0.16 to 0.24 (Table 4), which indicates a fair agreement.

To the present, distance-based methods are most frequently applied (Reif et al., 2005); however, we found that STRUCTURE grouping shows better agreement with topcross data than distance-based methods (Table 4). This could be attributed to: a) the

Table 4. Rate of agreement between topcross grouping and clusters, based on molecular data.

	UPGMA-Modified Rogers Distance	Structure
Topcross	0.1612903	0.3346457

low cophenetic coefficient (0.65) which indicates the goodness of fit of the cluster to the distance matrix, and b) the better performance of Structure per se (Pritchard et al., 2000). All clustering methods, like most reported in the bibliography, combined molecular information under the assumption that loci contribute in similar fashion to heterosis: lines clustered together display similar heterotic perfomance independently of the cross under evaluation. However, it has been reported in testcross trials that QTLs (quantitative trait loci) responsible for grain yield detected with only one tester were not necessarily detected for the rest of the testers (Austin et al., Crop Sci. 40:30-39, 2000; Mihaljevic et al., Crop Sci. 45:114-122, 2005). If so, not only markers associated with loci that positively affect heterosis must be selected for clustering, but more refined clustering algorithms must be designed in order to account for tester effects.

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Fine mapping and gene expression analysis of *de18*, a defective endosperm mutant of maize affecting auxin metabolism

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The *defective endosperm* (*de*) mutants of maize are a class of mutations affecting the final size and shape of the endosperm. On the basis of their pattern of accumulation of dry matter and total proteins in the seed, the mutants are divided into three major classes showing, with respect to the wild type B37: 1) a reduced accumulation rate of dry matter throughout the whole grain-filling period; 2) an initial normal rate followed by an early slowing down, and 3) an initial lag in the accumulation rate coupled with an early termination.

The mutant *de18* drastically reduces the growth rate of the grain throughout the period of development time and, at maturity, the final seed weight is less than one half that of the wild type counterpart. Preliminary histological results indicate a normal number of cells in the endosperm of *de18* seed while the cell dimensions are altered. It is also known that the auxin IAA levels in *de18* endosperm are several times lower with respect to the wild type. In fact, during the five developmental stages covering the most important part of the grain-filling period, namely 12, 15, 20, 30, and 40 DAP, the content of total indole-acetic acid (IAA) in *de18* is at least 15 times lower than in B37. As a consequence, when naftalen-acetic acid (NAA), a synthetic auxin which mimics IAA in its biochemical functions and which is not degraded to such a high extent as IAA, is added to developing seeds, a larger increase in seed weight is observed in the mutant (Torti et al., Theor. Appl. Genet. 72:602-605, 1986).

The *de18* mutant was backcrossed five times with the inbred B37. Segregation data, obtained from F2 ears, were used to evaluate the 3:1 segregating ratio by the chi-squared test. The F2 population was selfed and 16 homozygous wild type and 24 mutant F3 families were selected and used for mapping. The Bulked Segregant Analysis (Michelmore et al., Proc. Natl. Acad. Sci. USA 88:9828-9832, 1991) was applied with 191 *Eco*RI/*Mse*I primer combinations in order to find AFLP (Vos et al., Nucl. Acids Res. 23:4407-4414, 1995) markers linked to the mutation. Only AFLP bands segregating in coupling with the mutant and polymorphic between B37 and *de18* were considered. The polymorphic AFLPs were also tested in B73 and Mo17 in order to permit their use as bridges for their integration into the IBM2 reference map, using the software MapMaker 3.0.

The *de18* locus mapped to chromosome 10 bin 10.03 and was fully linked to the AFLP marker E3443_23, as shown by the absence of recombinants in the F_3 population (Figure 1). Other AFLP markers, E4335_14, E4539_7, E4335_19, E3537_4, E3942_17 (all with three recombinants) and E4445_1 (one recom-

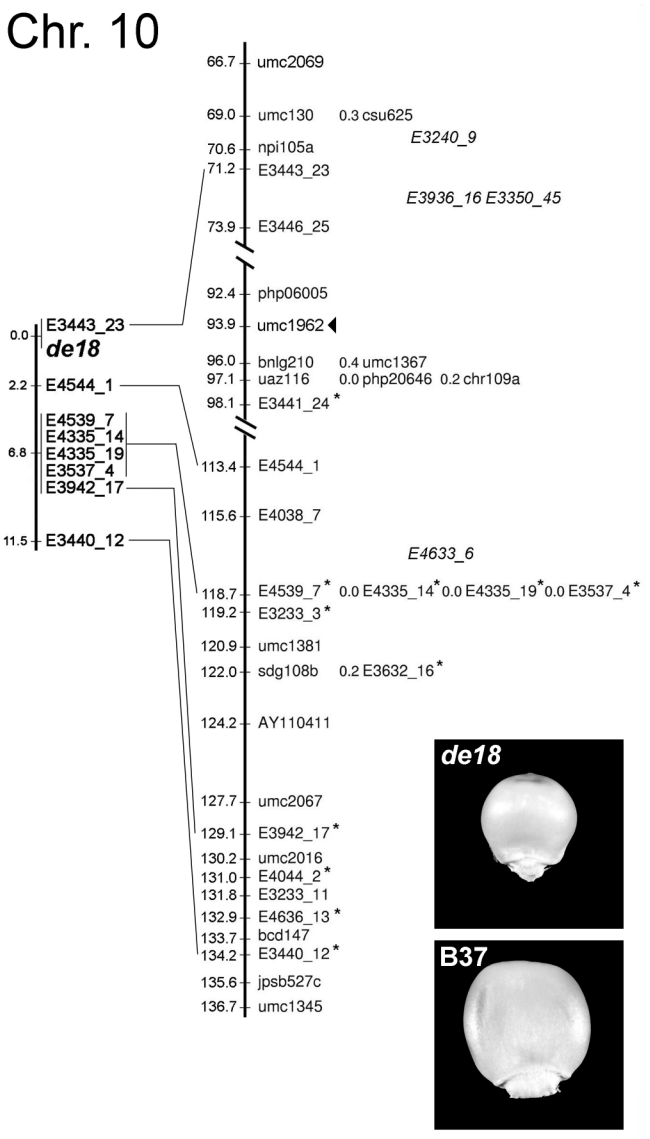

Figure 1. Map position of the *de18* locus on chromosome 10 bin 10.03. The arrow indicates the *umc1962* SSR marker in complete linkage with the mutant. AFLP markers are labelled E; the asterisk indicates the AFLP marker mapped using the NEAR command from MapMaker3.0; italics indicate AFLPs placed with the TRY command from MapMaker3.0, assigned to a map interval and reported on the right of the backbone. Map distances are reported in cM.

binant), resulted in linkage with the mutation. The SSR marker *umc1962*, tested on the same segregating population, resulted in complete linkage with E3443_23, confirming the mapping data previously described. The region containing *de18* was edged by the SSR markers *umc1367* and *umc2069* (Pasini et al., Mol. Breed. 22:527-541, 2008). Furthermore, a QTL for kernel weight was localized in the same bin 10.03 (Austin and Lee, Theor. Appl. Genet. 92: 817-826, 1996). The mutant *orange pericarp* 2 (*orp*2) was placed on chromosome 10 bin 10.03, near the SSR marker *bnlg1712*, at least 38 cM from *de18*. Moreover, it is also known that other candidate genes involved in auxin efflux transports, such as ZmPIN1a, ZmPIN1b and ZMPIN1c (Gallavotti et al., Plant Physiol. 147:1913-1923, 2008), were localized on different chromosomes (chromosome 9, 5 and 4 respectively). In the same manner, the mutant *orange pericarp1* (*orp1*), involved in tryptophan biosynthesis, was mapped to chromosome 4.

In order to build a high resolution map around the *de18* mutant, a large F3 population was developed from the cross A69Y x *de18*. This population, consisting of 391 homozygous wild type, 52 homozygous mutant and 188 heterozygous F3 families, will be screened for recombinants in the interval of flanking SSR markers. Recombinant pools will be screened with AFLP markers to saturate the region.

Maize long oligonucleotide microarrays (Maize Oligonucleotide Array Project, version 1, 45k) were used to determine the differential gene expression between the mutant *de18* and its wild type B37. mRNAs were extracted from seeds at four different stages of development, 7, 14, 21 and 28 days after pollination, and used to perform the hybridization. After normalization and statistical analysis of data groups, differentially expressed genes were detected. We identified many genes involved in the process of endosperm development and linked to auxin metabolism. To confirm the accuracy and reproducibility of the microarray results, 4 differentially expressed genes were selected for confirmation by real-time PCR. The genes selected were: anthranilate phosphoribosyltransferase, auxin response factor 8, *dull2* starch synthase and zein alpha precursor 19kDa. The real-time PCR results showed that the expression trends of these genes were partially consistent with those derived from the microarray analysis. In addition, *DR*5, another gene involved in auxin transport, was tested in quantitative PCR, but no significant differences between *de18* and B37 were detected.

Mutator-induced alleles at the *reduced grain filling1* locus of maize

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The defective kernel mutant *rgf1*, *reduced grain filling1*, induces alterations in both pedicel and transfer layer development, resulting in reduced starch accumulation and a final grain weight of 30% that of the wild type (Maitz et al., Plant J. 23:29-42, 2000). The mutant is dominant, with gene dosage-dependent expression of the phenotype. Selfing of heterozygous kernels gave rise to F2 cobs on which four classes of kernel size were segregating. *rgf1* was mapped to chromosome 2 bin 2.04 within a 0.1 cM interval with the aid of recombinant pools derived from an F3 population of 1406 lines. We report here the isolation of new mutants at the *rgf1* locus that we have obtained by tagging using the *Mutator* (*Mu*) transposable element, in collaboration with the Dipartimento Produzione Vegetale, University of Milan, Italy (F. Salamini).

A *rgf1*/*Rgf1* strain containing *Mu* was used as the female parent in crossing with a wild type *Rgf1*/*Rgf1* line. About 2,500 F1 ears were obtained, for a total of 650,000 seeds; we expected to obtain ears bearing kernels with dosage-dependent reduced size, half of them with the allelic composition *rgf1*/*rgf1*/*Rgf1* and the other half *rgf1*/*Rgf1*/*Rgf1*. Of the 2,500 cobs, 13 carried wild type kernels, probably arising from *Mu* insertion within the *rgf1* locus: 10 ears harboured one wild type kernel, while sectors of wild type kernels were observed on the remaining 3 cobs (Figure 1). Plants

Figure 1. Phenotypes of three F_1 ears derived from the tagging experiment with the putative wild type *Mu*-induced kernels indicated by arrows. The new mutants are named 11508-3, 11508-7 and 11508-10.

were grown from wild type kernels and selfed. In six cases (11508-1, 11508-3, 11508-5, 11508-6, 11508-7 and 11508-10), the segregation data were compatible with the presence of *Mu*-induced alleles. The frequency of these *rgf1-Mu* dependent alleles was 9.2×10^{-6}. In the F2 generation, the 11508-1 *rgf1-Mu* allele yielded a 3 wild type:1 *rgf1* segregation, which is compatible with the presence of a recessive *rgf1* allele generated by *Mu*. This hypothesis will be tested by crossing 11508-1 to the wild type. Another group of four putative mutants (11508-3, 11508-5, 11508-6 and 11508-10) produced F2 cobs with all wild type kernels, suggesting that the insertion of *Mu* into the *rgf1* locus resulted in a wild type allele. F2 progenies will be developed for each mutant and wild type individuals will be screened with the two SSR markers flanking *rgf1*. It is expected to find the dominant pattern at the SSR loci in some wild type kernels. For a third type of mutant, 11508-7, the F2 segregation was compatible with a lethal recessive allele caused by *Mu* insertion. Heterozygous *rgf1*/*rgf1*-lethal individuals and their progenies will be analyzed for germination capability.

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Immunodetection of methylcytosine in maize chromatin by a denaturating protocol

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DNA methylation is an important process in the silencing of genes and mobile genetic elements. Visualization of cytosine methylation at different chromatin states is possible using antibodies against DNA modifications. Lysak et al. (Methods in Molecular Biology, vol. 323: Arabidopsis Protocols) report that the accessibility of the antibodies to modified DNA is improved when the chromatin is denaturated. We have found that using native material, antibodies do not access the heterochromatic GC-rich blocks near centromeres of *Crotalaria juncea* (unpublished). We have applied the protocol described by Lysak et al., with minor modifications, using a Piranão variety of maize with a high content of heterochromatic knobs.

Seeds were germinated on water and transferred to *Sphagnum*. The root tips were harvested when 1 to 2 cm long, treated with 8-hydroxyquinoline for 2 hours 40 minutes, fixed with Carnoy (3 parts of ethanol and 1 part of acetic acid) overnight, and stored at -20°C until use. For slide preparations, the root tips were water-washed, equilibrated in citrate buffer and digested for 1 hour in a mixture of cellulase [9.2 units mL^{-1}] and pectinase [14.7 units mL^{-1}]. Root tips were rinsed in cold citrate buffer to stop the reaction. Single root tips were squashed in 60% acetic acid. For best results, the coverslip was removed, followed by liquid nitrogen and air-drying.

Prior to immuno-detection, the slides were baked at 60°C for 30 minutes, washed 2x5 minutes in 1x PBS, post-fixed 5 minutes in 4% paraformaldehyde in 1xPBS, followed by washing twice, for 5 min each, in 1x PBS, then an alcohol series (70%, 90% and 100%), and finally air-dried. The hybridization mixture was applied as 50 µl to each slide, covered with a glass coverslip and denaturated using a hot plate at 75°C for 10 minutes. Slides were immediately chilled 5 minutes using 1x PBS at 0°C or less, and the process repeated one time. The slides were blocked with 1% BSA in 1x PBS, and incubated1 hour in a moist 37°C chamber. The primary antibody, sheep anti-5' methylcytosine (Fitzgerald), diluted 1:500 in 1x PBST, was applied to each slide, and incubated 1 hour in a moist 37°C chamber. Excess primary antibody was removed with two 5 min washes in 1x PBST, then the second antibody, rabbit anti-sheep-FITC, was diluted 1:250 in 1% BSA, applied and incubated 1 hour in a moist 37°C chamber. The washes were applied as described for the primary antibody. Then, the tertiary antibody, goat anti-rabbit-FITC, diluted 1:125 in 1% BSA, was applied and incubated 1 hour at 37°C in a moist chamber. Slides were finally washed in 1x PBST, air-dried and mounted in Vectashield (Vector) with DAPI. A Zeiss Axiophot-2 epifluorescence microscope with appropriate filter was used for viewing, images were digitalized by a CCD camera, processed using ISIS Metasystems software and Adobe Photoshop.

In the diploid interphase nucleus depicted in Figure 1A, 1B, and 1C, two quenched regions correspond to the nucleus, with heterochromatic blocks appearing as very bright spots. Methylcytosine appears widely distributed and some heterochromatic blocks appear homogeneously labeled, others as negative regions, and others with brighter spots (Fig. 1B). In the merged view (Fig. 1C), the heterochromatic knobs are not clearly labeled, except for a few, observable as small bright spots.

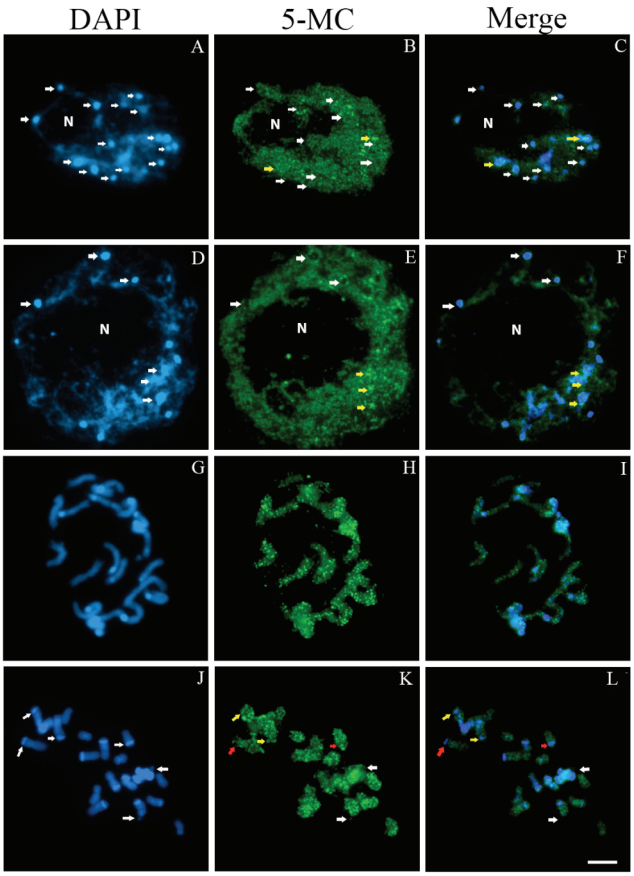

Figure 1. Interphase diploid nucleus (**A, B, C**), endomitotic nucleus (**D, E, F**), prophase (**G, H, I**), and metaphase (**J, K, L**) viewed as DAPI, anti-body treated (5-MC) and merged. **A, D**: Arrows indicate heterochromatic knobs as brighter spots with the nucleolus (N) quenched. **B:** arrows indicate knobs, with yellow arrows for brighter signals. **E**: white arrows indicate quenched regions and yellow arrows brighter regions. **J**: white arrows indicate satellites and heterochromatic knobs. **K**: white arrows indicate satellite methylation, yellow arrows the brighter signals, and red arrows the quenched knob. Bar = 5µm.

In the interphase endomitotic nucleus, the results are similar to those for the diploid interphase nucleus, except that some quenched regions do not correspond to heterochromatic blocks (Fig. 1D, 1E and 1F). Only the biggest knobs showed some small, brighter regions.

Prophase (Fig. 1G, 1H and 1I) and metaphase (Fig. 1J, 1K and 1L) chromosomes showed similar labeling patterns: anti-5MC is distributed over the chromosome arms, with small, brighter regions in interstitial positions. The hetechromatic knobs are either not distinguishable, or, in the central regions, appear as negative or quenched regions. Centromeric heterochromatin is not seen as enriched, in comparison to other heterochromatic or euchromatic regions. The NOR-heterochromatin does not appear differentially brighter, although the satellite of chromosome 6 is labeled. In some cells both satellites were labeled, and in some, only one pair was labeled (data not shown).

We conclude that the detection of methylcytosine by this protocol does not differentiate the heterochromatic blocks of maize

chromatin. There are many possible reasons: heterochromatin has low levels of accessible methylcytosine residues, so that the antibody is unable to distinguish differences; or, the denaturation process was not adequate for the antibody to access all the methylcytosine residues

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α-tubulin-YFP labeled sperm cells for live cell imaging of the fertilization process in maize and relatives such as *Tripsacum dactyloides*

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In vivo imaging of double fertilization processes is a prerequisite to studying and understanding the underlying molecular mechanisms involved in cross-talk between the male (pollen tube) and female gametophyte (embryo sac), as well as sperm cell release, migration and fusion with the two female gametes (egg and central cell). Tremendous progress is currently being made in understanding these fundamental biological processes using *Arabidopsis* as a model system because a large number of tools have been developed over the last couple of years (Berger et al., Trends Plant Sci. 13:437-443, 2008). Comparable tools are missing for maize. In order to visualize these fertilization processes in maize also, we have screened a number of fluorescent protein tagged maize lines generated via the NSF project #0501862 (for review see http://maize.jcvi.org/cellgenomics/) to identify sperm cell marker lines.

One line could be identified displaying YFP-tagged α-tubulin in many cell types of roots, leaves, inflorescences, etc., but exclusive labeling of sperm cells inside mature pollen grains. During microsporogenesis, the YFP signal could be detected earliest after the first mitotic division in the generative nucleus. The generative nucleus then divided to form two sperm cells with similar YFP signal intensity. In the mature pollen grain, only the two crescent-shaped sperm cells displayed YFP signals. This marker line is superior even to *Arabidopsis* marker lines, as the entire sperm cell boundaries are labeled by cortical tubulin strands. Furthermore, the accumulation of thick tubulin bundles at both sides surrounding the sperm cell nucleus allows simultaneous visualization of the nuclei (Fig. 1A). There is no detectable signal in the vegetative cell of the developing pollen grain at any stage of microsporogenesis nor at a later stage during pollen tube growth. Due to its specific expression pattern during microgametogenesis, the endogenous promoter of this *α-tubulin* gene family member, which drives expression of the fusion protein, provides a tool to specifically deposit other proteins or RNAs inside maize sperm cells. Moreover, this marker line enables studies on sperm migration dynamics during pollen tube growth and fertilization. In pollen grains of maize and many other plant species, the two sperm cells lie in immediate proximity to each other and are connected by an unknown mechanism. During tube growth, the two sperm cells leave the pollen grain through the germ pore shortly after germination and migrate back and forth inside the growing pollen tube

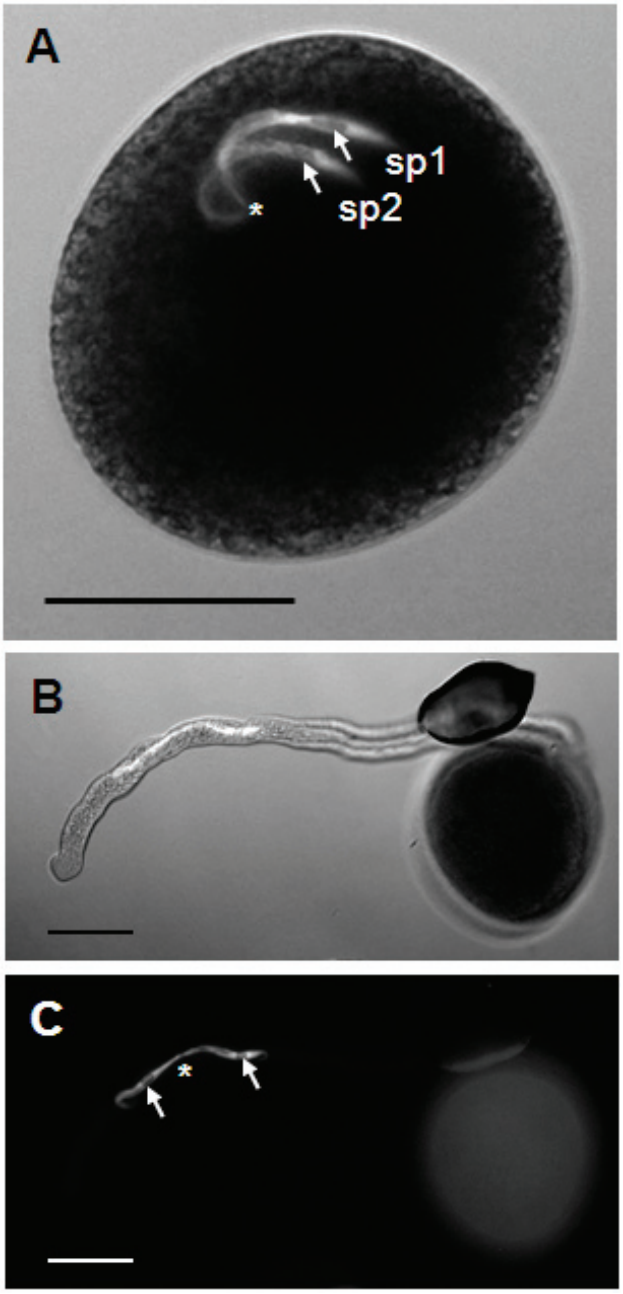

Figure 1. α-tubulin-YFP labeled sperm cells. **A**: Mature pollen grain containing two sickle-shaped sperm cells (sp1 and sp2). Arrows point at sperm nuclei and an asterisk marks the connection between both cells. **B**: Sperm cells migrating towards the tip of a growing pollen tube (merged bright field and fluorescence image). **C**: Same as B but only YFP signal is visible. Arrows and asterisk as in A. Scale bars 50µm.

while still attached to each other (Fig. 1B and C). As soon as the pollen tube reaches the embryo sac, it discharges its contents though interaction with the synergids. These processes occur inside the ovary, which is deeply embedded in maternal tissues, and have never been observed in maize and other grasses. However, a number of labs have already developed methodologies for in vitro fertilization and separation of the female gametophyte from maternal tissues, which will enable study of these fundamental processes using the marker line described above.

Maize sperm cells are also able to fuse with female gametes of related species, which will permit investigation of a number of additional fertilization related questions. In vivo studies using maize pollen and female flowers of maize relatives such as *Tripsacum dactyloides*, for example, showed a very high rate of fertilization events. In the apomict *T. dactyloides*, the unreduced egg cell develops autonomously into an embryo without fertilization, but the unreduced central cell requires fertilization by one of the two male gametes. What is the fate of the second sperm cell? Does it often fertilize a synergid, as a high number of twin embryos are observed in *T. dactyloides* (Bantin et al., Sex. Plant Reprod. 14:219-226, 2001), or does it degenerate? Moreover, this line will allow studies of the fate of sperm-deposited protein/RNA after fertilization, as the *α-tubulin* promoter used to generate this line is switched off after fertilization (Kliwer and Dresselhaus, unpublished). Seeds and promoter sequences of this transgenic line are available from the Jackson lab.

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Selection strategies for tolerance to Mal de Río Cuarto disease in different evaluation environments

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Mal de Río Cuarto (MRC) is the most important viral disease of maize in Argentina The production of maize for grain is greatly affected by MRC disease, which is caused by a virus of the family *Reoviridae*, genus *Fijivirus*, and transmitted by the planthopper, *Delphacodes kuscheli* Fennah (Homoptera: *Delphacidae*) (Nome et al., 1981; Ornaghi et al., 1993).

Characterizing genotype testing sites and identifying environments with negligible genotype x environment crossover interaction is important for plant breeders wishing to identify superior germplasm and (or) genotypes for a wide range of environments. Genotype x environment (G x E) interactions can be an impediment to genetic progress in maize (*Zea mays* L) breeding for Argentina. Therefore, identifying appropriate environments where the selection should be carried out is an important aspect of a plant breeding program in order to maximize the efficiency of selection. Indirect selection efficiency is related to the heritability of the trait and to the genetic correlation between environments.

The objective of this work was to study the effect of different environments on the relative efficiency (*E*) of direct selection in relation to indirect selection for MRC. The trials were conducted with 111 recombinant inbred lines (RILs) derived from a cross between a susceptible inbred line, Mo17, and a tolerant inbred line, BLS14. The trials were conducted during 2004, 2005 and 2006 at Río Cuarto (64º 20'W, 33º 8'S, 334 masl), and during 2004 and 2005 at Sampacho (64º 42'W; 33º 19'S, 510 masl). The year-location combinations were regarded as different environments (Río Cuarto 2004, Río Cuarto 2005, Río Cuarto 2006, Sampacho 2004 and Sampacho 2005). The experimental design at each environment was a randomized complete block design with two replications.

At the beginning of male flowering, 60–70 days after planting, the RILs were evaluated for several traits related to symptoms of MRC disease, which allowed us to estimate the grade of severity of the disease. A four-grade disease severity scale proposed by Ornaghi et al. (1999) (0: no symptoms; 1: enations; 2: enations and "hokey pole" ears; 3: enations, shortened superior internodes and small ears with few or no kernels) was used for each plant and data averaged for a plot.

Indirect selection efficiency is derived from the selection response model using the formula for the ratio of correlation to direct response:

$$E = r_g\, h_Y / h_X$$

where r_g is the genetic correlation for the trait measured in the environment of selection Y and in the environment of interest X, and *h*y and *h*x are square roots of the broad sense heritability of the grade of severity of the disease in Y and X environments, respectively. The efficiency model *E* is based on the assumption that selection intensities *i* of direct and indirect selection are the same. The equation of efficiency of indirect selection states that if $E>1$, indirect selection is more efficient than direct selection, and if $E<1$, direct selection is more efficient.

The genetic correlation between the environment of selection Y and in the environment of interest X to the grade of severity of the MRC, can be estimated from the equation:

$$r_g = \sigma_{g\,(XY)} / \sqrt{(\sigma^2_{g\,(X)}\, \sigma^2_{g\,(Y)})}$$

where $\sigma_{g\,(XY)}$ is the genetic covariance between X and Y, and $\sigma^2_{g\,(X)}$ and $\sigma^2_{g\,(Y)}$ is the genotypic variance component of the X and Y, respectively.

Heritability estimates of the grade of severity of the disease evaluated in each environment were estimated as:

$$h^2 = (\sigma^2_g)/[(\sigma^2_g)+(\sigma^2_e/r)]$$

where *r* denotes the number of replicates and the subscript g indicates variance components associated with genotypes and e, experimental error.

Table 1. Genetic correlation (r_g) for the grade of severity of MRC between environment of selection and environment of interest, and predicted efficiency (*E*) of indirect selection relative to direct selection.

Environment of selection[a]	Environment of interest	r_g	S.E.[b]	*E*
R4	S4	0.30	0.25	0.24
	R5	0.17	0.21	0.15
	S5	0.03	0.19	0.03
	R6	-0.03	0.23	-0.03
S4	R4	0.30	0.25	0.37
	R5	-0.21	0.16	-0.24
	S5	0.24	0.20	0.28
	R6	0.44	0.27	0.53
R5	R4	0.17	0.21	0.19
	S4	-0.21	0.16	-0.19
	S5	-0.02	0.15	-0.02
	R6	0.01	0.19	0.01
S5	R4	0.03	0.19	0.03
	S4	0.24	0.20	0.21
	R5	-0.02	0.15	-0.02
	R6	0.09	0.18	0.10
R6	R4	-0.03	0.23	-0.03
	S4	0.44	0.27	0.36
	R5	0.01	0.19	0.01
	S5	0.09	0.18	0.09

[a]R4 = Río Cuarto 2004, S4 = Sampacho 2004, R5 = Río Cuarto 2005, S5 = Sampacho 2005 and R6 = Río Cuarto 2006
[b]Standard errors of the genetic correlations between environments

Due to the low genetic correlations estimated, genetic mechanisms involved in determining the grade of severity of the disease MRC may not be the same for different environments (Table 1). This complicates selection of genotypes from these RILs.

However, estimated h^2 showed high values (Table 2), which indicates that the grade of the disease is controlled by a high proportion of genes with additive effect and some independence with the medium. Since the *E* had values <1 (Table 1), the direct selection strategy in each environment represents the best alternative and the indirect selection strategy may not have good prospects.

Table 2. Heritability (h^2) in five environments of evaluation for the grade of severity of MRC in 111 RILs of maize.

	Environment [a]				
Parameter	R4	S4	R5	S5	R6
Heritability [b]	0.70	0.45	0.56	0.60	0.66

[a]R4 = Río Cuarto 2004, S4 = Sampacho 2004, R5 = Río Cuarto 2005, S5 = Sampacho 2005 and R6 = Río Cuarto 2006
[b]$h^2 = (\sigma^2_g)/[(\sigma^2_g)+(\sigma^2_e/r)]$

Diallel analysis of Mal de Río Cuarto tolerance and yield components in maize

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Mal de Río Cuarto (MRC) is a devastating disease of maize in Argentina. The disease reduces grain yield (GY) and causes several symptoms, such as enations, reduced root systems, shortened superior internodes, flattened stems, leaves with small leaf areas, atrophic tassels, "hokey pole" ears and small ears with few or no kernels. The identification of heterotic patterns plays an important role in the selection of germplasm to develop hybrids. Analysis of diallel crosses provides preliminary data about heterotic relationships (Hallauer and Miranda Filho, Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, IA, 1981; Hallauer and Miranda, Quantitative Genetics in Maize Breeding, 2nd Edition. Iowa State Univ. Press, Ames, IA, 1988). Our objective for this study was to estimate general (GCA) and specific combining ability (SCA) (Sprague and Tatum, J. Am. Soc. Agron. 34:923-932, 1942) in twelve lines of maize evaluated through diallel crosses, without reciprocals, for tolerance to MRC, grain yield and its components. The maize lines were BLS14, BLS1, BLS16, BLS61, BLS76, BLS91, BLS96, BLS101, BLS104, LP109, LP521 and LP125R.

The lines and their 66 crosses were planted on 21 November 2007 through a complete randomized block experimental design with two replications at Río Cuarto, Argentina (33°8´S 64°20´W). All plants were artificially infested with viruliferous insect vectors of MRC (*Delphacodes kuscheli* Fennah). Data were collected for the grade of severity of MRC disease (GS), number of kernel/m^2 (KN), unit weight of kernels (WK), and grain yield, standardized to 14.5% moisture (GY). Data were subjected to an ANOVA analysis using Griffing´s method 2 model II (Griffing, Aust. J. Biol. Sci. 9:463-493, 1956), by means of a diallel computer program (Magari and Kang, J. Hered. 85:336, 1994). Significance was estimated with *t* tests. The relative importance of general and specific combining ability on progeny performance was estimated as the ratio: $2\sigma^2_{ACG} / (2\sigma^2_{ACG} + \sigma^2_{ACE})$ (Baker, Crop Sci. 18:533-536, 1978) where σ^2_{ACG} and σ^2_{ACE} are the variance components for GCA and SCA. A value of 1 indicates that all genetic variance is additive. Analysis of variance revealed that mean square values for GCA were highly significant ($p \leq 0.01$) for the traits studied, with the exception of grain yield. The variations due to SCA were highly significant ($p \leq 0.01$) for all traits studied. The ratios $2\sigma^2_{ACG} / (2\sigma^2_{ACG} + \sigma^2_{ACE})$ were 0.15, 0.08, 0.19 and 0 for GS, NK, WK and GY, respectively, indicating that non-additive effects predominated in the expression of these traits. Marino and Teyssandier (Congreso Anual de la Sociedad Argentina de Genética, BuenosAires, 1982) reported the same results for tolerance to MRC, and Bhatnagar et al. (Crop Sci. 44:1997-2005, 2004) and Srdic et al. (Maydica 52:261-264, 2007), indicated that SCA effects were highly significant for GY. In our scoring, negative effects on combining ability are associated with tolerance to disease and positive effects with susceptibility. For GS, the highest GCA values were observed for line BLS1 (-0.39), followed by BLS16 (-0.2), and for WK the highest values were observed for line LP109 (0.03), followed by BLS104 (0.02) (Table 1). These parental lines presented highly significant GCA effects

Table 1. General combining ability (GCA) effects of each parental line for different characters.

Line	GS		NK		WK (g)	
BLS61	0.02	ns	57.85	ns	-0.01	ns
BLS91	0.07	ns	-69.54	ns	0.01	ns
BLS101	0.00	ns	-181.68	ns	0.01	ns
BLS76	-0.09	ns	32.95	ns	-0.01	ns
BLS96	-0.08	ns	-36.04	ns	-0.00	ns
BLS104	0.37	**	-74.64	ns	0.02	**
BLS16	-0.2	**	103.85	ns	-0.03	**
BLS14	0.04	ns	195.38	ns	-0.01	ns
BLS1	-0.39	**	119.1	ns	-0.01	ns
LP109	0.08	ns	-29.68	ns	0.03	**
LP521	0.09	ns	-16.93	ns	0.00	ns
LP125R	0.07	ns	-100.61	ns	0.00	ns

* Significant at 5% and ** significant at 1% probability level. GS=grade of severity of MRC disease, NK=number of kernel/m^2 and WK=unit weight of kernel.

for GS and WK, while the effects for NK were not significant. The highest SCA effects were observed for hybrids BLS101 x BLS104, LP109 x LP125R, BLS91 x BLS16 and LP109 x LP125R for GS, NK, WK and GY, respectively. The hybrid that manifested the best behavior for GS also presented good performance for the other traits, in contrast to the hybrid that manifested the greatest SCA for GY, which displayed negative effects for GS. We conclude that the lines with high general combining ability would be a valuable source of germplasm to develop hybrids that combine tolerance to MRC and good yield.

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Discriminant analysis to identify molecular markers associated with Mal de Río Cuarto (MRC) resistance

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In Argentina, the production of maize for grain is highly affected by MRC-disease, which is caused by a virus of the family *Reoviridae*, genus *Fijivirus* and transmitted by the planthopper

Delphacodes kuscheli Fennah (Homoptera: *Delphacidae*) vector (Nome et al., Phytopathologie Zhurnal 101:7–15, 1981). Traditional mapping of QTL for resistance to MRC disease has been reported using early-generation $F_{2:3}$ (Di Renzo et al., J. Agric. Sci., Cambridge 142:289–295, 2004). A series of agricultural applications of discriminant analysis (DA), involving recombinant inbred lines (RILs), is another way to identify meaningful markers associated with phenotypic performance (Capdevielle, MS Thesis, Louisiana State University, Baton Rouge, LA, 2001; Zhang et al., Theor. Appl. Genet. 110:721–729, 2005). Discriminant analysis was first used to identify RAPD markers associated with disease resistance in rice (Capdevielle et al., P. 216 in Proc. Fourth Internatl. Rice Genetics Symp., International Rice Research Institute, Los Baños, Philippines, 2000). It has been extended to other marker types such as SSR markers (Zhang et al., 2005) and AFLP markers (Capdevielle, 2001; McCharo et al., Euphytica 144:125–132, 2005; Miano et al., Euphytica 160:15–24, 2008).

DA has not been hitherto applied in studies for resistance to MRC disease in maize. The objective of this work was to identify an array of SSR markers associated with common symptoms of MRC using maize RILs with distinct reactions to the disease. Identification of molecular markers associated with groups of lines differing in phenotype performance would suggest the localization of genes with small individual effects on tolerance.

Genetic materials. A susceptible dent line, Mo17, and a partially resistant red flint line, BLS14, developed at the Instituto Nacional de Tecnología Agropecuaria, Castelar, were used as parents to produce 144 RILs by self-pollinating a random sample of F_2 plants by the single seed descent method (Burr and Burr, Trends Genet. 7:55–60, 1991). This is the same cross used for studies of traditional mapping of QTL for resistance to MRC disease (Di Renzo et al., 2004).

Field evaluation. For disease evaluations, trials were grown at locations where MRC disease is endemic. The trials were conducted during 2005 at the Sampacho (64º42'W, 33º19'S, 510 masl) location and during 2006 at the Río Cuarto (64º20'W, 33º8'S, 334 masl) location. The year-location combinations were treated as different environments. The experimental design was a randomized complete block design with two plots/RIL at each environment. Each trial included entries of Mo17 and BLS14. At the beginning of male flowering, RILs were evaluated for traits related to common symptoms of MRC disease. Individual plants were evaluated and data averaged at each environment for each RIL. Plants were rated on a discrete scale the values of which increase according to the increase of the disease severity. The following traits and rating scales (in parentheses) were used: superior internodes (0=normal; 1=shortened); presence and type of enations (0=no enations; 1=mild enations; 2=enlarged veins; 3=galls); multiple ears (0=normal; 1=multiple ears; 2=no ear).

Genetic markers. Forty SSR markers described in the Maize Genetics and Genomics Database (MaizeGDB, http://www.maizegdb.org) were used.

Data analysis. RILs were assigned to one of two groups defined to represent low and high values for the traits related to symptoms of MRC disease and representing the 1st and 4th quartile of the trait distribution. Missing marker data, which were around 10-15%, were computed using the multiple imputation procedure of SAS. SSR profile variation among predefined phenotypic groups was ascertained by the AMOVA method. Before performing DA, we ran a marker selection procedure with PROC STEPDISC (SAS Institute ver. 9.1) using the forward option as selection method with the select option set to 0.15. The analytic procedure used here is fully detailed in Zhang et al. (2005). Using the selected markers, a non-parametric method (k-nearest-neighbor) of DA was performed within PROC DISCRIM (SAS Institute ver. 9.1). The linear parametric DA (Fisher 1936) is also recommended because of its high robustness with outliers and non-normal or heteroscedastic data. The percentage of correct classification was calculated from cross-validation error rates by using the cross-validate option within PROC DISCRIM. A high level of correct classification infers an association between molecular markers and agronomic data for a trait expression.

Findings. The maize RILs evaluated in this study exhibited a wide range of phenotypic variation for the three MRC symptoms evaluated. Mean values for the two extreme phenotypic groups for each trait at each environment are shown in Table 1. The phenotypic mean values of the high and low groups were significantly different for the traits in the two environments (P<0.001).

Molecular variance analyses found significant molecular differences between the two extreme groups for each trait. Table 2 shows the number of markers selected by the STEPDISC procedure applied before DA and the percentage of correct classifications of RILs reached with the discriminant function based on the selected markers. For evaluations in S5 environment, a high percentage of correct classifications were obtained using a maximum set of eleven SSR markers. In R6 environment, the same percentage of correct classifications were achieved using between three and nine SSR markers. For internode, a minimum set of four SSR markers were selected by DA, in both S5 and R6 environments. The results suggest an array of markers associated with traits related to symptoms of MRC disease. The rate of correct classification (obtained by cross-validation) was regularly higher than 65%.

SSR markers selected by PROC STEPDISC, which differentiate between low and high trait value groups at each environment, are shown in Table 3 and indicate chromosomes 1, 4, and 8 have regions with significant effects for MRC resistance.

Table 1. Mean scores for traits related to symptoms of Mal de Río Cuarto disease in maize for two groups of RILs at environments where Mal de Río Cuarto is endemic.

Trait	Group*	S5†	R6
Internodes	1	0.46	0.04
	2	0.95	0.74
Enations	1	0.86	0.08
	2	2.43	1.95
Multiple ears	1	0.42	0.00
	2	1.64	1.10

* Group 1: low symptoms, group 2: high symptoms.
† S5, Sampacho 2005 and R6, Río Cuarto 2006.

Table 2. Number of microsatellites pre-selected to classify maize RILs into low and high trait value groups and percent (%) of correct classification of the discriminant function at environments where Mal de Río Cuarto is endemic.

Trait	S5*		R6	
	SSR	%	SSR	%
Internodes	4	65	4	70
Enations	10	65	3	68
Multiple ears	11	81	9	72

* S5, Sampacho 2005 and R6, Río Cuarto 2006.

Table 3. SSR markers pre-selected to classify maize RILs into low and high trait value groups at environments where Mal de Río Cuarto is endemic.

Trait	S5*†	R6
Internodes	*umc1394*, 3.01	*nc004*, 4.03
	nc009, 6.04	*phi021*, 4.03
	umc1086, 4.08	*umc1177*, 1.01
	phi063, 10.02	*umc1220*, 1.11
Enations	*bnlg1371*, 6.02	*nc004*, 4.03
	bnlg1189, 4.07	*bnlg1426*, 6.01
	phi095, 1.03	*bnlg1866*, 1.03
	phi080, 8.08	
	bnlg1225, 2.06	
	bnlg1866, 1.03	
	phi021, 4.03	
	umc1612, 4.08	
	bnlg1627, 1.02	
	umc1177, 1.01	
Multiple ears	*umc1394*, 3.01	*bnlg1225*, 2.06
	nc005, 4.05	*phi076*, 4.11
	umc1086, 4.08	*umc1177*, 1.01
	phi095, 1.03	*nc004*, 4.03
	umc1169, 1.04	*umc1741*, 8.03
	umc1304, 8.02	*nc009*, 6.04
	umc1177, 1.01	*umc1394*, 3.01
	bnlg1866, 1.03	*phi095*, 1.03
	phi021, 4.03	*phi115*, 8.03
	nc004, 4.03	
	umc1021, 1.03	

* S5, Sampacho 2005 and R6, Río Cuarto 2006.

† First name-component is SSR marker, second name-component is chromosome and bin number. SSR marker order corresponds to its relative contribution to the discriminant function.

In the Sampacho 2005 environment, *umc1177*, *phi095*, *bnlg1866*, *umc1394*, *phi021* and *umc1086* SSR markers were associated with two of three traits. In the Río Cuarto 2006 environment, three traits had *nc004* in common and *umc1177* was associated with two traits. To assess consistency across environments, *umc1177*, *phi095*, and *nc004* would be useful.

Our results are consistent with the previously reported MRC-QTL mapping using the $F_{2:3}$ from the same parental cross (Di Renzo et al., 2004) and where QTL for MRC resistance were found on chromosomes 1 and 8. In a separate study, with a different $F_{2:3}$ mapping population, Kreff et al. (J. Basic Appl. Genet. 17:41–50, 2006) found regions on chromosomes 1, 4, 8 and 10 with significant effects for MRC resistance.

Results from this work indicate that it is possible to use DA to select powerful markers that may be useful to breeders. This is a new tool for germplasm improvement providing a discriminant model to integrate the information from markers selected to classify RILs. The model can then be used to facilitate the allocation of new genotypes into groups with distinct performance for MRC resistance, as well as to identify additional markers associated with the trait. Thus far, results suggest that the complementation of DA and QTL analysis in RILs would be a good strategy to identify informative markers.

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Androgenetic, matroclinic, hybrid and semi-lethal plants in progeny from cross-breeding maize and *Tripsacum*

--Zavalishina, AN; Tyrnov, VS

Tripsacum dactyloides (2n = 72) is a wild maize relative capable of apomictic reproduction, and so a cross-breeding of maize and *Tripsacum* is of interest to researchers. In our experiments 4 lines, *W23 igig, W155 fl2fl2*, *CI880 O2O2*, Brown marker *(BM)*, and 6 hybrids of maize, *W155* x *BM; W155 fl2fl2* x *BM; CI880 O2O2* x *BM; W64 fl2fl2* x *BM; (SynA O2O2* x *W64 O2O2*) x *BM; W155 O2O2* x *Tester of Mangelsdorf (TM*), were used as maternal forms, while *Tripsacum* was used as the male parent.

The maize ears were prepared two ways for pollination: 1) the husks of ears were cut, turned down and then the pistils were cut to 4 cm in length; and 2) one-third of the ear was cut together with the husks. Both pollination methods showed high seed production: 40-100%, calculating on pollinated ovaries. Dry weather seed production is better than wet weather, since traumatized ears have rot more often in wet conditions. In total, 186 ears were pollinated and 136 (73%) of them contained hybrid seeds. In all, 4566 seeds were recovered, the majority of which were little and puny. However, 37 were very large. These set on maternal forms, one of the parents of which was the line *BM.*

We did not use nutrient medium for seed germination. We put seeds in tap water for 12 hours, then removed the dense pericarp with sharp tweezers and put seeds on wet filter paper. The seedlings at the coleoptile stage were planted in pots. Then at the 3-leaf stage they were placed in soil in the greenhouse. 943 seedlings were obtained from 4566 seeds; many of them were the hybrids. The average seed germination was 21%. This quantity varied from 0% to 80% depending on genotype. 256 seeds of the maternal form *(W155 x BM)* did not germinate at all. Only one seedling among 302 seeds was found for the hybrid *(W155 O2O2* x *TM)*. The line *W23 igig* showed the highest germination--48% on average for 3 years.

Seeds that did not germinate contained endosperm without an embryo. Three matroclinic haploids were discovered among the seedlings obtained from the different maternal forms. Among the 37 large seeds, only 10 produced hybrids; others did not germinate. 12 polyembryos were discovered among the seedlings obtained from line *W23 igig*. One plant among twin seedlings was identified as an androgenetic *Tripsacum*. It had a *Tripsacum* genome and a maize cytoplasm. It developed more intensively than the hybrids.

778 seedlings among 943 perished at an early stage so they did not form roots. The rest of the 165 seedlings were planted in soil in the greenhouse. It is possible that surviving hybrids depended on maize and *Tripsacum* genome compatibility. When the maternal form was the line *BM*, 203 seedlings were produced. Among them were one matroclinic haploid, three normal hybrids and 199 semi-lethal plants. These had two small leafs during 2-6 months of their life. When a new leaf appeared, the lower one turned yellow and died off. The leaf length was about 5 cm. When the maternal form was used as a hybrid, one parent of which was *BM*, semi-lethal seedlings accounted for 50%.

Only a few of the hybrid plants between maize and *Tripsacum* reached the flowering stage. They had 46 chromosomes. These were large, powerful, bushy plants. Some of them grew some years in greenhouse conditions. They were characterized by full male sterility and partial female fertility.

It has been ascertained also that if the maize has the color genes *A B Pl R*, hybrid kernels and hybrid plants have purple color. Possibly, *Tripsacum* has a gene analogous to the dominant maize gene *A1*. This fact allows the use of genetic markers for discovering apomicts among hybrids of maize and *Tripsacum*.

Megagametophyte investigation of tetraploid maize

--Kolesova, AJ

Tetraploid maize female gametophytes have not been investigated sufficiently. We carried out the analysis of 830 embryo sacs (*ES*) of tetraploid maize form *KrP-1* (population-1 from Krasnodar). *ES* of tetraploids, as a rule, had a structure typical for maize and consisted of a three-celled egg apparatus, the central cell with 2 polar nuclei or one central nucleus and antipodal complex. The characteristic peculiarity of tetraploids in comparison with diploids was the increase of cell, nucleus and, correspondingly, gametophyte sizes. Anomalous *ES* were discovered in 4 of 6 plants examined. The frequency of anomalous *ES* formation in tetraploids varied from 0% to 2.7%. In total, 12 anomalous *ES* were revealed. *ES* with additional polar nuclei (3-4 nuclei) and *ES* with anomalous position of polar nuclei prevailed. *ES* with egg-like synergids, and *ES* with additional nucleoli in the egg and polar nuclei were also discovered. In one ovule, the arrest of *ES* development at a one-celled stage was noted. In tetraploids, in contrast to diploids, the growth of antipodal complex cells was discovered. In one case, cells did not grow so considerably, increasing at a rate of 2-3 times. The structure and morphology of growing cells were similar to the rest of the antipodal cells. In other cases, antipodal complex cells grew considerably more, achieving 2/3 *ES* size. These growing cells were similar to central cells in their morphology. They contained large vacuoles and large nuclei, morphologically similar to polar nuclei. Growing cells always adjoined the antipodal complex. In most cases, growing cells were one-nuclear, and rarely two-nuclear. Cells with 3, 4, 6, 7, 8 and 13 nuclei were also discovered. More often, one cell, rarely two cells grew in the ovule. In one ovule, the growth of three cells was noted. The number of ovules with large growing cells varied from 3.4 to 26.4.

SHALIMAR, INDIA
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Combining ability analysis for *turcicum* leaf blight (TLB) and other agronomic traits in maize (*Zea mays* L.) in the high altitude, temperate conditions of Kashmir

--Rather, AG; Najeeb, S; Wani, AA; Bhat, MA; Parray, GA

Strategies for developing high-yielding cultivars resistant to *turcicum* leaf blight (TLB, *Exserohilum turcicum*; Northern Leaf blight) is one of the major objectives for our high altitude maize breeding programme. Primary breeding objectives also include: (1) earliness, due to the seasonal limitations of high altitude; (2) good performance under low moisture, critical when the temperature drops abruptly in the latter stages of crop growth; and (3) resistance to lodging, as determined by plant height and ear placement.

Three replications each of forty-five half diallel cross combinations were evaluated along with 10 parents (Table 1) at two locations, Larnoo and Khudwani, representing different altitudes with a temperate ecology. Each entry was sown in two 5 m length rows at a spacing of 60 cm. Plantings for each replication and location included 50 plants for each genotype (83333 per hectare basis). Days to 50 percent pollen shed and silking were determined on a plot basis. Plant height (cm), ear height (cm) and moisture content (%) were measured for five randomly selected plants. Grain yield (kg/plot) was adjusted to 14% moisture. The disease severity was recorded for five randomly selected plants from each plot for crosses and 10 plants for parents using a 1-9 rating scale based on the percent of the leaf area affected of adult plants: 0, 1, 10, 20, 30, 40, 60, 80 and >80 percent, respectively, per Payak and Sharma (In: Proc. Twenty Fourth Workshop of All India Coordinated Maize Improvement Project, IARI, New Delhi, 1981). Inoculations were prepared from infected leaf tissue from a farmer's field and made at the mid-silking stage. The first evaluations were made 15 days later, and thereafter, weekly for 4 weeks. Two leaves were evaluated, the ones immediately above and below the ear leaf, as these have impact on yield (Bowen and Pedersen, Plant Dis. 72:952-956, 1988). The percent disease index was calculated by using the formula suggested by McKinney (J. Agric. Res. 26:195-218, 1923). Combining ability analysis was carried out according to Model I, Method II of Griffing (Australian J. Biol. Sci. 9:463-493, 1956).

Table 1. Estimates of GCA effects for TLB and other agronomic traits in inbred lines in maize.

Parents	Pedigree	Disease severity	Grain yield	Days to 50% pollen shed	Days to 50% silking	Moisture content	Plant height	Ear placement
1	PMI-1	-1.52* (17.24)	0.29*	-1.56*	-1.64*	-0.22*	-0.14	-4.77**
2	PMI-26	-1.76** (17.24)	-0.03	-0.45**	-0.12	-0.76**	4.27**	-6.69**
3	PMI-47	-4.47** (19.89)	-0.04	0.06	0.35**	0.44**	6.04**	5.74**
4	PMI-53	2.32** (42.50)	0.26*	-0.10	-0.56**	0.19*	1.98**	-0.70**
5	PMI-83	0.61 (35.32)	-0.14	-0.21*	1.14**	-0.11	-1.01**	4.97**
6	PMI-135	1.38 (36.47)	-0.02	-1.37**	-0.97**	-0.05	8.56**	2.35**
7	PMI-198	2.56** (18.50)	0.05	1.76**	-1.64**	0.58**	1.62**	-3.98**
8	PMI-199	2.17** (40.77)	0.10	2.14**	1.43**	-0.16*	10.46**	1.66**
9	PMI-224	-1.85** (16.32)	0.25*	-0.02	-0.81**	-0.66**	15.02**	9.64**
10	PMI-401	2.48** (36.22)	0.43**	-4.00**	-2.41**	-1.48**	-34.37**	-9.62**
SE gi		0.43	0.11	0.06	0.05	0.08	0.10	0.10
SE gi-gj		0.58	0.17	0.09	0.08	0.13	0.15	0.15

Parents 1, 2, and 6 are indigenous; 9 is a local line; 3, 4, 5, 7, 8, and 10 are CIMMYT lines.
*, **significant at 5% and 1% level, respectively; parentheses (percentage disease score);

The pooled mean squares for combining ability indicated that both GCA and SCA variances were highly significant for all of the traits, with GCA being greater than SCA. Both GCA and SCA were influenced by environment in the case of all traits, with the exception of SCA for grain yield, plant height and ear placement. Parents P1, P2, P3 and P9 were identified as good sources for *turcicum* resistance based on GCA effect. P10 proved an ideal general combiner for all traits followed by P1 and P9. Cross combinations P7 x P9 and P3 x P9 showed resistance to the disease and good performance for other traits, based on the SCA. In general, crosses having at least one parent with negative GCA effect and a resistant reaction showed resistance; however, crosses of most resistant parents gave intermediate to susceptible reactions.

Studies on genetic variability, genotypic correlation and path coefficient analysis in maize under the high altitude temperate conditions of Kashmir

--Najeeb, S; Rather, AG; Parray, GA; Sheikh, FA; Razvi, SM

A number of studies in maize have been conducted to elucidate the nature of association between yield and its components which identify traits like ear length, ear diameter, kernels/row, ears/plant, 100-seed weight and rows/ear as potential selection criteria in breeding programs aimed at increasing yield (Mohan et al., Natl. J. Plant Improve. 4(1):75-76, 2002; Tollenaar et al., Crop Sci. 44:2086-2094, 2004). Hence, an attempt was made to ascertain the influence of different characters on the improvement of grain yield in 3 local and 7 CIMMYT inbred line crosses of maize under the high altitude temperate conditions of Kashmir (7500 ft asl).

The present half diallel material was generated in Kharif 2006 by crossing the inbred lines in all possible combinations, except reciprocals, at the High Altitude Maize Research Station, Larnoo of Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, J&K (India). The parental lines and all forty-five crosses were evaluated at two diverse locations with temperate conditions, namely Larnoo and Khudwani, representing distinct climatic zones during Kharif 2007. All 55 genotypes were replicated twice at each location in CRBD. Each entry was given a plot size of three rows of 3 m length, with row and plant spacing of 60 and 25 cm, respectively. Recommended practices were followed to ensure a good crop. Maturity parameters (days to 50% pollen shed and silking, 75% husk browning), grain weight and straw weight were recorded on a plot basis. For the other 12 traits under study, data were recorded on five randomly selected competitive plants from each replication. The data were statistically analysed for correlation coefficients and path analysis as per the methods of Al-Jibouri et al. (Agron. J. 50:633-637, 1958) and Dewey and Lu (Agron. J. 51:515-518, 1959), respectively.

The analysis of variability parameters revealed the presence of substantial variability for all traits. Relatively higher estimates of GCV for straw weight, grain weight, plant height, ear placement, kernel rows/ear and number of kernels/row along with high heritability (broad sense) suggest that selection can be effective for these traits. Genetic advance was relatively higher for plant height, ear placement and number of kernels/row. The genotypic correlation coefficients revealed positive and significant association with ear length, ear girth, kernel rows/ear, kernels/row, straw weight, plant height and ear placement. The maturity traits recorded significant negative correlation with yield. The path analysis revealed that the traits with the highest direct effect on grain yield are number of kernels/row, ear length, ear girth and kernel rows/ear. Thus, these traits should be used as target traits for tailoring an ideal plant type for higher yield. Other traits exerted positive indirect effects on yield by affecting ear length, ear girth and ear placement (Table 1).

Table 1. Genotypic path coefficients for grain yield and component traits in maize.

Trait	Days to 50% pollen shed	Days to 50% silking	Husk browning	Plant height (cm)	Ear placement (cm)	Moisture content (%)	Ear length (cm)	Ear girth (cm)	Kernal rows/ear	Kernels/ row	Straw weight	Correlation with grain yield/plot
Days to 50% pollen shed	**0.348**	0.163	-0.053	-0.036	-0.22	0.009	0.214	0.126	0.013	-0.212	0.002	-0.342
Days to 50% silking	0.157	**-0.361**	0.052	-0.041	0.280	0.011	-0.072	0.166	0.014	-0.262	0.002	-0.445*
Plant height (cm)	0.196	-0.157	**-0.219**	-0.111	-0.432*	0.013	0.258	0.180	0.025	-0.276	0.035	-0.520*
Ear placement (cm)	0.068	-0.174	-0.074	**-0.086**	0.004	0.002	0.515*	0.338	0.029	-0.555*	0.954	0.886**
Moisture content (%)	0.285	-0.213	-0.076	-0.083	**0.287**	-0.001	0.483	0.314	0.095	-0.520*	0.304	0.875**
Straw weight/plot (kg)	0.042	-0.110	-0.040	-0.004	-0.060	**0.038**	0.195	0.027	0.001	-0.145	0.001	0.008
Days to 75% husk browning	0.056	-0.170	-0.049	-0.070	0.756**	0.011	**0.630****	0.300	0.024	-0.630**	0.004	0.730**
Ear length (cm)	0.052	-0.153	-0.055	-0.074	0.790**	0.002	0.483	**0.392**	0.026	-0.519*	0.003	0.809**
Ear girth (cm)	0.035	-0.079	-0.045	-0.038	0.065	0.000	0.235	0.157	**0.387**	-0.264	0.001	0.384
Kernal rows/ear	0.052	-0.141	-0.049	-0.071	0.767**	0.008	-0.670**	0.304	0.026	**0.593***	0.004	0.69**
Kernels/row	0.056	-0.128	-0.068	-0.058	0.766**	0.010	0.407	0.243	0.107	0.447	**0.006**	0.714**

*, ** Significant at 5% and 1% levels, respectively; R^2 value: 0.841; residual effect: 0.397; diagonal values = direct effect.

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Stability analysis in maize (*Zea mays* L.) for anthesis-silking interval and grain yield

--Lone, AA; Sofi, PA; Warsi, MZ; Wani, SH

An ideal approach in plant breeding is to develop high performing cultivars that have fairly uniform performance (low G[enotype] x E[nvironment] interaction) over a range of environments (Lee et al., Crop Sci. 43:2018-2027, 2003; Worku et al., Seventh Berseen and Southern African Regional Maize Conference, Feb. 11-15, pp. 139-142, 2001; Scapim et al., Genet. Mol. Biol. 23:287-292, 2000; Magari and Kang, Euphytica 70:105-111, 1993; Rasul et al., J. Appl. Sci. Res. 1:307-312, 2005; Signor et al., Crop Sci. 41:663-669, 2001). The present study was undertaken to identify superior performing maize hybrids over a range of environments. Five cultivars, Pop-3118, Pop-3121, YHP-Alm-217, YHP-Pant-45 and Tarun-83, were crossed in 2005 in a half diallel fashion to develop 15 cross combinations. The parent and crosses were evaluated in six environments using two locations (Pantnagar and Gorkhpur), two seasons (*rabi* 2005 and *kharif* 2006) and two growing conditions (normal and submerged), in a completely randomized block design with three replications in each environment. The submergence treatment was given at knee-height stage with a water height of 5 cm for seven days. Anthesis-silking interval (ASI) and grain yield were recorded for 10 competitive plants, randomly selected from each replication. Data were statistically analysed by the Eberhart and Russell (Crop Sci. 6:36-40,1966) model where the variance of regression deviations is a measure of cultivar stability and the linear regression coefficient (β) is a measure of environmental index. In this model, the ideal genotype should have a high mean (μ>X), a unit regression coefficient (βi=1) and no deviation from linearity (S^2di=0).

The pooled analysis of variance for stability revealed significant variability for both traits studied, as well as differential effects of each environment. The estimates of environmental index showed that for ASI, Pantnagar *rabi* (submerged) was the most favourable, whereas Gorkhpur kharif (normal) was poorest. For grain yield, Pantnagar *rabi* (normal) was the best environment for realizing higher yields, whereas Gorkhpur *kharif* (submerged) was the poorest. Analysis of stability parameters in the Eberhart and Russell (1966) model (Table 1) indicated that among lines, Pop-3118 and YHP-Alm-217 had the lowest and highest mean values of ASI, respectively. Among crosses, Pop-3118 x Tarun 83 and Pop-3118 x YHP-Alm-217 had the the lowest and highest values for ASI. For grain yield, YHP-Alm-217 and Pop-3121 were the lowest and highest yielding parents, whereas among crosses, Pop-3121 x Tarun-83 was the highest yielding and Pop-3118 x YHP-Alm-217 was the lowest yielding. Among parents, Tarun-83 was responsive to a better environment (βi>1) for ASI and grain yield, while others were responsive to a poorer environment (βi<1). Most of the crosses were highly responsive to a better environment for both traits. The mean square deviation of regression coefficient (S^2di) deviated significantly from zero for only a few parents and crosses for ASI, but in all parents and crosses for grain yield. The coefficient of determination (R^2) values ranged from 0.374 to 0.990 for ASI and 0.664 to 0.993 for grain yield, suggesting that a large portion of variation in these traits could be attributed to the environmental index. Though many of the parents and crosses exhibited above average performance for both traits, they could not satisfy other parameters of the model to be designated as generally adaptable. Based on the βi estimates for ASI, YHP-Alm-217 was the most stable among parents and YHP-Alm-217 x YHP-Pant-45 among crosses. In the case of grain yield, YHP-alm-217 was the most desirable parent, while the cross Pop-3118 x Po-312 was the most stable.

Table 1. Mean regression coefficient (βi), mean square deviation (S^2di) and coefficient of determination (R^2) for maize genotypes tested in 6 environments.

Genotype	Anthesis silking interval					Grain yield		
	Mean	βi	S^2di	R^2	Mean	βi	S^2di	R^2
Pop-3118	4.055	0.254	-0.199	0.772	1632.9	0.806	37546.2**	0.895
Pop-3121	4.111	0.336	0.807**	0.374	1941.6	0.604	64199.9**	0.755
YHP-Alm-217	5.778	0.947	1.323**	0.732	1479.1	0.945	30473.5**	0.932
Tarun-83	5.500	0.337	0.744**	0.860	1789.9	1.083	132578.0**	0.839
Pop-3118 x Pop-3121	4.611	1.139	0.607*	0.810	1962.6	0.999	37348.2**	0.929
Pop-3118 x YHP-Alm-217	5.444	0.883	-0.198	0.985	1734.2	1.012	18129.2**	0.957
Pop-3118 x YHP-Pant-45	5.277	1.118	0.787	0.938	1890.2	0.624	-9572.1	0.993
Pop-3118 x Tarun-83	3.889	1.826	0.074	0.867	1894.6	1.403	47500.2**	0.954
Pop-3121 x YHP-Alm-217	4.611	1.741	-0.040	0.983	1872.8	1.056	56917.5**	0.912
Pop-3121 x YHP-Pant-45	4.166	0.699	-0.268	0.990	2424.3	1.848	70801.1**	0.963
Pop-3121 x Taru-83	4.611	1.037	0.538	0.864	2472.2	0.973	66849.9**	0.885
YHP-Alm-217 x YHP-Pant-45	5.111	1.002	0.629	0.842	1781.1	0.703	23151.2*	0.902
YHP-Alm-217 x Tarun-83	4.778	1.276	-0.056	0.971	2349.7	1.383	21825.7*	0.973
YHP-Pant-45 x Tarun-83	5.116	1.681	0.067	0.984	1819.1	0.967	26941.6	0.940
Mean	4.763				1920.5			
SE (Mean)	0.343				109.2			

TALLAHASSEE, FLORIDA
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A laboratory and field survey of leaf feeding resistance in diverse maize inbred lines

--Forde, AJ; Applewhite, HS; Bass, HW

Using a common garden plot in Northern Florida (Mission Road Field, FSU) that was established for an unrelated genetic study, we assessed natural levels of insect damage exhibited by a highly diverse population of inbred maize lines (Liu et. al., Genetics 165:2117, 2003). The field contained families for seed propagation from two sets of maize diversity lines developed by E. S. Buckler, J. Holland, M. McMullen and Goodman. The two sets of lines grown in 2008 are referred to by us as the "MDS" set (260 of the 283 lines from Order 185771, Feb 2007) and the "DL25" set (25 of the 26 lines from Order 179535, Apr 2006) originally provided by the USDA, ARS, NCRPIS, ISU, Ames, IA. The MDS lines were planted in families of 16, 12, or 8 plants each and the DL25 lines were planted in two different locations within the same field in families of 24 or 14 plants each.

Cursory inspection of insect damage prior to pesticide treatment revealed potential line-dependent variation in herbivory damage, primarily from lepidoptera exposure. To investigate the possible genotypic basis for this observed variation, we measured field damage in the DL25 lines and carried out laboratory bioassays on a selected subset of them. The DL25 lines, plus a common-parent, B73, have been used to develop a nested association mapping (NAM) population (Yu et al., Genetics 178:539). A general objective of the study described here was to determine which lines and corresponding NAM recombinant inbred lines might be useful for subsequent genetic analyses of complex quantitative traits related to maize-lepidoptera interactions.

Data was collected from our DL25 set, B73, B97, CM103, CML228, CML247, CML277, CML322, CML333, CML52, HP301, Il14H, Ki11, Ki3, Ky21, M162W, M37W, Mo17, Mo18W, Ms71, NC350, NC358, Oh43, Oh7B, P39G-B, Tx303, and Tzi 8. Insect surveys revealed that the damage present on plants in our plot was caused primarily by two species of caterpillars, *Spodoptera frugiperda* (J. E. Smith), the fall armyworm, and *Spodoptera ornithogalli* (Guenée), the yellowstriped armyworm. The amount of damage sustained by plants was quantified using a rating scale of 0-4, with larger ratings indicating that damage was present on a greater proportion of leaves from an individual plant.

Leaf damage varied significantly among lines according to analysis of covariance as shown in Figure 1 ($F=5.48$, $df=25$, $P<0.001$; using MIXED procedure; SAS version 9.2; SAS Institute, 2008). Additionally, planned contrasts found that five lines were significantly more damaged and five were significantly less damaged compared to the population average (Fig. 1). A spatial covariance matrix was incorporated into the ANCOVA to account for autocorrelation because, within each of the two blocks in the plot, all individuals of a maize line were planted in "family groupings" located along contiguous stretches within rows.

Induced and constitutive antibiosis resistance laboratory phenotype data was collected from herbivore growth rate bioassays for nine (B73, CML52, HP301, IL14H, KY21, M162W, M37W, NC350, Tzi 8) of the 26 maize lines present in the field plot, plus

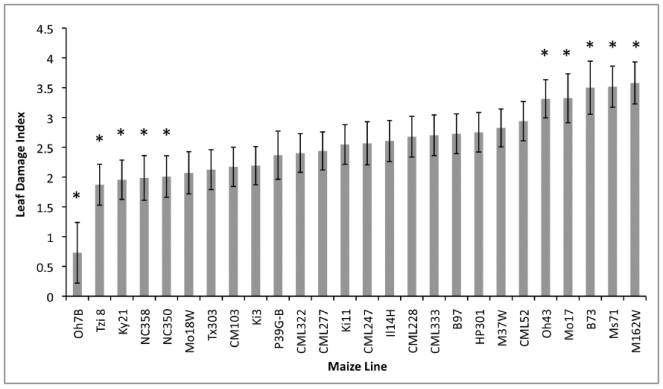

Figure 1. Mean damage index values (±SE) assessed for maize plants (mid to late whorl/V8/8-leaf-collar stage) from different inbred lines in a field in Northern Florida. Damage ratings were assessed visually, and were based on the percentage of leaves exhibiting any chewing damage. The percentage equivalent of each damage index value are as follows: a rating of 0 corresponds to 0-10%, 1 to 11-25%, 2 to 26-50%, 3 to 51-75% and 4 to 76-100%. Asterisks denote lines demonstrating levels of damage that were significantly different when compared to the average amount of damage for all other lines (according to planned contrasts, $P<0.05$). Means represent least square means produced from an ANCOVA model incorporating plant size and spatial location as covariates. Average sample size was 23.92.

Sorghum bicolor and teosinte (Balsas, *Zea mays* ssp. *parviglumis*). The subset of nine from the DL25 lines was chosen in order to encompass as much phenotypic and genetic variation as possible, using our field damage estimates and knowledge of phylogenetic relationships. Second instar fall armyworm caterpillars were fed foliage from herbivore-damaged or undamaged plants (V4/4-leaf-collar stage) grown under greenhouse conditions, and their weight gain over a 48-hour period was measured. An ANOVA on caterpillar growth rates revealed significant variation in constitutive resistance among the lines, as shown in Figure 2 (Main effect of line: $F=2.74$, $df=10$, $P=0.0028$), but provided no obvious evidence of induced resistance (Main effect of leaf damage: $F=0.09$, $df=1$, $P=0.76$; Damage x Line interaction $F=1.21$, $df=10$,$P=0.33$). According to multiple comparisons, caterpillars grew significantly slower when fed NC350, compared to all other lines except Tzi 8. Four of these differences remained significant after a highly conservative Bonferroni p-value correction (Fig. 2). Furthermore,

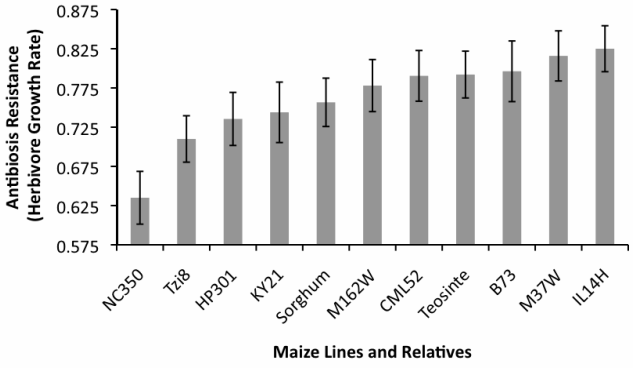

Figure 2. Constitutive antibiosis resistance, assayed by relative growth rates (ln(final mass) – ln(initial mass)) of fall armyworm caterpillars (±SE) fed leaves from different greenhouse/lab-grown maize lines. Faster growth rates denote less resistance. Each caterpillar was allowed to feed on the newest expanded leaf of a plant (excised) for 48 h in a no-choice situation. Plants had been grown in a greenhouse under standard conditions and were stage V4 (4 leaf-collar stage). Means represent least squared means produced from an ANOVA model incorporating a main effect of line. Significant differences were found between NC350 and four lines (B73, CML52, Il14H, M37W) when p-values of multiple comparisons were adjusted with a Bonferroni correction. Average sample size was 39.9.

levels of constitutive resistance in the laboratory were significantly related to the amount of damage that was sustained by lines in the field, as shown in Figure 3 (Likelihood ratio test: deviance=1.267, df=7, P=0.033).

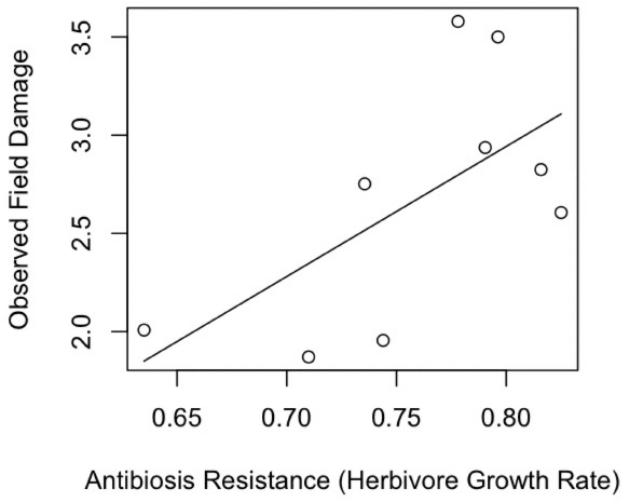

Figure 3. The relationship between average damage index values and average antibiosis resistance (as measured by herbivore growth rates) for the chosen subset of nine lines. Antibiosis resistance demonstrated a significant linear relationship with observed damage levels according to a likelihood ratio test (deviance=1.267, df=7, P=0.033).

The concordance between the patterns of resistance observed in the field versus laboratory suggests that constitutive antibiosis resistance expressed by the lines is important for deterring leaf damage. To the extent that this relationship holds up, these types of herbivore growth rate bioassays may provide an efficient method to pre-screen germplasm for resistance prior to more extensive field trials. Despite the fact that our observations of damage in the field were predicted by laboratory measures of resistance, follow-up studies will still be needed in order to confirm whether levels of resistance in these lines are stably expressed across seasons, locations, and developmental stages. This study confirms our suspicion that these maize diversity lines could be used to learn more about the genetic basis of herbivore resistance and the effect of genetic variation in plant defense on ecological dynamics.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center

Allelism testing of miscellaneous stocks in the Maize COOP phenotype only collection

--Jackson, JD; Harper, C

This report summarizes allele testing of miscellaneous stocks characterized by phenotype only in the Maize Genetics COOP Stock Center collection. Crosses were made between known heterozygotes if possible. Ears were shelled and planted in sand benches to score seedlings for the appropriate phenotypes. Plants from the lazy crosses were scored in the field at maturity. Proposed new designations have been assigned to these alleles. These stocks have been increased and placed on our stocklist. It is expected that with further sorting and allelism testing of mutations characterized by phenotype only, additional alleles of characterized mutants will be discovered and placed in the main collection.

POSITIVE TESTS:

previous designation	allelism test with *spt1*	new designation	MGCSC: stock number
spt-92-3239-53*	positive: (+ / *spt1-N464*) x (+ / *spt**)	*spt1-92-3239-53*	226J

previous designation	allelism test with *oro1*	new designation	MGCSC: stock number
oro-85-3087-3*	positive: (+ / *oro1-6474*) x (+ / *oro**)	*oro1-85-3087-3*	616C
oro-88-89-3550-32*	positive: (+ / *oro1-6474*) x (+ / *oro**)	*oro1-88-89-3550-32*	616D

previous designation	allelism test with *la1*	new designation	MGCSC: stock number
la-05HI-RnjxW22GN-333*	positive: (+/*la1*) x *la**	*la1-05HI-RnjxW22GN-333*	406E
la-MTM4659*	positive: (+/*la1*) x *la**	*la1-MTM4659*	406F

New alleles of *chlorophyll1* found in lemon white endosperm stocks in the Maize COOP phenotype-only collection

--Jackson, JD

This report summarizes allele testing of lemon-white endosperm stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here pale kernels linked to pale-green or albino seedlings characterized all stocks. Many had previously given negative results in tests with *vp*9, *w3* and *y9*. The *cl1 Clm1-3* stock used in crosses here carries a dominant modifier of *cl1* that allows for viable green plants, making crosses with a homozygous stock possible. Crosses were made as follows: [+/*lw**]@ X *cl1 Clm1-3* or +//+/*lw** X *cl1 Clm1-3*. Ears were scored for the segregation of pale yellow kernels.

New designations have been assigned to these alleles and they have been placed in the main collection. Stocks with this same phenotype that were found to complement *cl1 Clm3* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	allelism test with *w3*	New designation	MGCS stock number
5705F *pale- y*-87-88-2679-1*	4 positive	*cl1-87-88-2679-1*	306H
5908Q *y-vp*-1982-1*	3 positive	*cl1-1982-1*	306I
5910M *pale-y*-85-3007-40*	3 positive	*cl1-85-3007-40*	306J
5912P *lw-y-pg*-1998-4*	5 positive	*cl1-1998-4*	306K

New alleles of *white3* found in viviparous stocks in the Maize COOP phenotype only collection

--Jackson, JD

This report summarizes allele testing of various viviparous and lemon-white endosperm stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here pale kernels linked to pale or albino seedlings characterized all stocks. Many had previously given negative results in tests with *vp*9 and *y*9. The *w3-y11* stock used in crosses here is homozygous viable. Crosses were made as follows: [+/*vp**]@ X *w3-y11* and +//+/vp* X *w3-y11*. Ears were scored for the segregation of pale yellow kernels. In most cases, pale-yellow kernels were selected from positive allele test ears and planted in the field for observation. Seed-

lings were pale-green and when self-pollinated these plants gave ears with all pale kernels.

New designations have been assigned to these alleles and they have been placed in the main collection. Stocks with this same phenotype that were found to complement *w3* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	allelism test with *w3*	New designation	MGCSC: stock number
5902D *w-vp*-84-5020-4*	3 positive	*w3-84-5020-4*	211I
5902F *pale-y*-84-5032-21*	3 positive	*w3-84-5032-21*	211J
5903G *pale-y-vp*-85-3385-34*	2 positive	*w3-85-3385-34*	211K
5903J *y-vp*-85-3572-30*	3 positive	*w3-85-3572-30*	211L
5904D *vp*-86-1407-15*	3 positive	*w3-86-1407-15*	211M
5905L *y-vp*-88-89-3563-33*	3 positive	*w3-88-89-3563-33*	211N
5906P *y-vp*-73-2656*	3 positive	*w3-73-2656*	211O
5908H *vp*-8111*	5 positive	*w3-8111*	211P
5909L *y-vp*-84-13*	3 positive	*w3-84-13*	211Q
5910H *pale-y*-84-5082-33*	6 positive	*w3-84-5082-33*	212E
5910L *pale-y*-85-3006-30*	3 positive	*w3-85-3006-30*	212F
5910N *pale-y*-85-3010-40*	3 positive	*w3-85-3010-40*	212G
5911C *lw*-85-3076-28*	2 positive	*w3-85-3076-28*	212H
5911D *pale-y*-85-3087-29*	2 positive	*w3-85-3087-29*	212I
5911H *lw*-86-87-1828-7*	3 positive	*w3-86-87-1828-7*	212J
5911O *pale-y*-90-3220-1*	3 positive	*w3-90-3220-1*	212K
5911P *pale-y*-90-3220-26*	3 positive	*w3-90-3220-26*	212L
5911Q *lw*-89-90-3609-5*	3 positive	*w3-89-90-3609-5*	212M
5912I *y-pg*-85-3044-34*	2 positive	*w3-85-3044-34*	212N
5912N *y-pg*-86-87-1723-27*	3 positive	*w3-86-87-1723-27*	212O
6109G *y-l*-8910 Briggs*	3 positive	*w3-8910*	212P

Mapping data for *enr* factors on chromosome 2

--Stinard, PS

Dominant alleles at the *enr* (*Enhancement of r1*) loci intensify aleurone color conferred by certain pale and near-colorless *r1* haplotypes (Stinard, Kermicle, and Sachs 2009, J. Hered., in press. Electronic version doi: 10.1093/jhered/esn091 http://jhered.oxfordjournals.org/cgi/content/full/esn091). Two *enr* loci, *enr1* and *enr2*, are linked to each other and map to chromosome 2. A third locus, *enr3*, is not linked to the other two.

We report four point linkage data for the *enr1 enr2* combinations *enr1-m594 Enr2-6117a* and *enr1-m694 Enr2-694* with respect to *fl1* and *v4* (Tables 1 and 2) and three point linkage data for *Enr1-628* with respect to *fl1* and *v4* (Table 3). We also report three point linkage data for the partially characterized *enr* factors *Enr*-459A* and *Enr*-459B* (Stinard, MNL 81:33-35, 2007) with respect to *fl1* and *v4* (Tables 4 and 5).

The linkage testcrosses were performed as indicated in the tables. All lines were homozygous for the pale *r1* reporter haplotype *R1-r(Venezuela559-PI302355)*. Kernels from the testcross ears were separated into purple (Enr) vs. pale (enr) vs. sectored (enr-m) as appropriate, and starchy (FI) vs. floury (fl) classes, planted in a cold sand bench, and the resulting seedlings scored for green (V) vs. virescent (v). Linkage values were calculated according to Coe (Pp. 189-197 in Maize Handbook, M. Freeling and V. Walbot eds., New York: Springer-Verlag, 1994). The segregation of two enhancers (*enr1-m* and *Enr2*) in the four point linkage tests (Tables 1 and 2) presented a special problem in that the presence of the *Enr2* allele prevented the scoring for *enr1* vs. *enr1-m* in the purple kernel classes. Therefore, four point linkage data were calculated from *enr2* classes only, and three point linkage data for *fl1 enr2 v4* were calculated from total data.

Table 1. Four point linkage data for *fl1 Enr2-6117a v4 enr1-m594*.

Testcross: [*Fl1 Enr2-6117a V4 enr1-m594* X *fl1 enr2 v4 enr1*] X *fl1 enr2 v4 enr1*

Region	Phenotype	No.	enr2 class
0	fl enr2 v enr1	550	550
0; 3	Fl Enr2 V; enr1 or enr1-m	663	
1; 1 + 3	fl Enr2 V; enr1 or enr1-m	63	
1	Fl enr2 v enr1	54	54
2	fl enr2 V enr1-m	74	74
2; 2 + 3	Fl Enr2 v; enr1 or enr1-m	72	
3	fl enr2 v enr1-m	71	71
1 + 2; 1 + 2 + 3	fl Enr2 v; enr1 or enr1-m	5	
1 + 2	Fl enr2 V enr1-m	5	5
1 + 3	Fl enr2 v enr1-m	6	6
2 + 3	fl enr2 V enr1	4	4
1 + 2 + 3	Fl enr2 V enr1	0	0
Total (n)		1567	764

enr2 data: *fl1 - enr2* = 8.5 +/- 1.1 cM
enr2 - v4 = 10.9 +/- 1.1 cM
v4 - enr1 = 10.6 +/- 1.1 cM
Total data: *fl1 - enr2* = 8.5 +/- 0.7 cM
enr2 - v4 = 10.2 +/- 0.8 cM

Table 2. Four point linkage data for *fl1 Enr2-694 v4 enr1-m694*.

Testcross: [*Fl1 Enr2-694 V4 enr1-m694* X *fl1 enr2 v4 enr1*] X *fl1 enr2 v4 enr1*

Region	Phenotype	No.	enr2 class
0	fl enr2 v enr1	310	310
0; 3	Fl Enr2 V; enr1 or enr1-m	330	
1; 1 + 3	fl Enr2 V; enr1 or enr1-m	34	
1	Fl enr2 v enr1	28	28
2	fl enr2 V enr1-m	32	32
2; 2 + 3	Fl Enr2 v; enr1 or enr1-m	37	
3	fl enr2 v enr1-m	35	35
1 + 2; 1 + 2 + 3	fl Enr2 v; enr1 or enr1-m	13	
1 + 2	Fl enr2 V enr1-m	13	13
1 + 3	Fl enr2 v enr1-m	1	1
2 + 3	fl enr2 V enr1	6	6
1 + 2 + 3	Fl enr2 V enr1	5	5
Total (n)		844	430

enr2 data: *fl1 - enr2* = 10.9 +/- 1.5 cM
enr2 - v4 = 13.0 +/- 1.6 cM
v4 - enr1 = 10.9 +/- 1.5 cM
Total data: *fl1 - enr2* = 11.1 +/- 1.1 cM
enr2 - v4 = 12.6 +/- 1.1 cM

The four point linkage data presented in Tables 1 and 2 establish the gene order *fl1 enr2 v4 enr1* and the linkage values (*fl1* – 8.5 – *enr2* – 10.2 – *v4* – 10.6 – *enr1* and *fl1* – 11.1 – *enr2* – 12.6 – *v4* – 10.9 – *enr1*) are fairly consistent with each other and with previously reported data (*fl1* – 6.2 – *enr2* – 7.8 – *v4* – 10.3 – *enr1*; Stinard, Kermicle, and Sachs, 2009), although the *fl1* – *v4* interval is extended in the present data (18.7 cM and 23.7 cM vs. 14.0 cM reported in Stinard, Kermicle, and Sachs). The *v4* – *enr1* values (10.6 cM, 10.9 cM, and 10.3 cM) are remarkably similar. Differences in the *fl1* – *v4* interval could be due to the fact that the *enr1* and *enr2* alleles used in the two different tests are from different sources and genetic backgrounds (although they have been partially introgressed into W22). It may also be significant that *fl1* and

Table 3. Three point linkage data for *fl1 v4 Enr1-628*.

Testcross: [*Fl1 V4 Enr1-628* X *fl1 v4 enr1*] X *fl1 v4 enr1*

Region	Phenotype	No.	Totals
0	Fl V Enr	425	
	fl v enr	465	890
1	Fl v enr	45	
	fl V Enr	71	116
2	Fl V enr	54	
	fl v Enr	51	105
1+2	Fl v Enr	3	
	fl V enr	8	11
Totals			1122

fl1 - v4 = 11.3 +/- 0.9 cM
v4 – enr1 = 10.3 +/- 0.9 cM

Table 4. Three point linkage data for *fl1 v4 Enr*-459A*.

Testcross: [*Fl1 V4 Enr*-459A* X *fl1 v4 enr*] X *fl1 v4 enr*

Region	Phenotype	No.	Totals
0	Fl V Enr	734	
	fl v enr	768	1502
1	Fl v enr	121	
	fl V Enr	125	246
2	Fl V enr	108	
	fl v Enr	88	196
1+2	Fl v Enr	13	
	fl V enr	6	19
Totals			1963

fl1 - v4 = 13.5 +/- 0.8 cM
*v4 – enr** = 11.0 +/- 0.7 cM

Table 5. Three point linkage data for *fl1 Enr*-459B v4*.

Testcross: [*Fl1 Enr*-459B V4* X *fl1 enr v4*] X *fl1 enr v4*

Region	Phenotype	No.	Totals
0	Fl Enr V	341	
	fl enr v	376	717
1	Fl enr v	24	
	fl Enr V	19	43
2	Fl Enr v	28	
	fl enr V	22	50
1+2	Fl enr V	3	
	fl Enr v	4	7
Totals			817

*fl1 – enr** = 6.1 +/- 0.8 cM
enr - v4* = 7.0 +/- 0.9 cM

v4 flank the centromere of chromosome 2; it is not presently known on which chromosome arm *enr2* resides.

The three point linkage data presented in Table 3 establish the following relationship: *fl1* – 11.3 – *v4* – 10.3 – *enr1*. The *fl1 – v4* interval is shorter in this test, but the *v4 – enr1* interval is similar to other reported data.

For the partially characterized *enr* factors *Enr*-459A* and *Enr*-459B*, the following linkage order and distances (in centiMorgans) were established: *fl1* – 13.5 – *v4* – 11.0 – *Enr*-459A* (Table 4); and *fl1* – 6.1 – *Enr*-459B* – 7.0 – *v4* (Table 5). The *fl1 – v4* distances established by these tests (13.5 cM and 13.1 cM, respectively) agree with each other and are close to that reported on the 1993 genetic map of chromosome 2 (15 cM; Neuffer et al., Mutants of Maize, Cold Spring Harbor Laboratory Press, 1997). The gene order established by these two tests taken together, *fl1 Enr*-459B v4 Enr*-459A*, as well as the map distances, are consistent with those of *enr1* and *enr2* (Stinard, Kermicle, and Sachs 2009; and this report). It is likely that *Enr*-459A* and *Enr*-459B* are alleles of *enr1* and *enr2*, respectively. Direct mapping tests of *Enr*-459A* with *enr1* and *Enr*-459B* with *enr2* are in progress.

Two point linkage data for 3L mutants *w*-5787* and *yel*-8630*

--Stinard, PS; Jackson, JD

We report F2 linkage data for the 3L seedling lethal mutants *w*-5787* and *yel*-8630* with respect to *wx1*-marked A-A translocations. Both mutants are uncovered by TB-3La and therefore located distal to the 3L breakpoint (3L.10) of TB-3La. Plants heterozygous for *w*-5787* were crossed to a line homozygous for *wx1* T3-9c (breakpoints 3L.09; 9L.12). F1 kernels were planted in our summer nursery and the resulting plants self-pollinated. F2 kernels from the selfed ears were separated into starchy (Wx) and waxy (wx) classes, planted in a sand bench, and the resulting seedlings scored for green (W) vs. albino (w). Roughly half the ears segregated for albino seedlings and the data from those ears were pooled and are summarized in Table 1. A similar crossing scheme was used to map *yel*-8630* with respect to *wx1* T3-9c and *wx1* T3-9(8562) (breakpoints 3L.65; 9L.22). Linkage distances were calculated according to the product method (Immer, Genetics 15:81-98, 1930) and are summarized in Table 1.

Table 1. F2 linkage data for *w*-5787* with respect to *wx1* T3-9c and *yel*-8630* with respect to *wx1* T3-9c and *wx1* T3-9(8562).

mutant	translocation	Wx W	Wx w	wx W	wx w
w–5787*	*wx1* T3–9c	1130	464	390	43
yel–8630*	*wx1* T3–9c	1076	497	463	3
yel–8630*	*wx1* T3–9(8562)	407	169	105	26

Map distance *w*–5787 – wx1* T3–9c = 32.1 +/– 2.0 cM
Map distance *yel*–8630 – wx1* T3–9c = 8.3 +/– 2.2 cM
Map distance *yel*–8630 – wx1* T3–9(8562) = 42.7 +/– 3.0 cM

Linkage of *wx1* with chromosome 3 markers in crossings involving 3-9 translocations is dependent upon the linkage of *wx1* and the chromosome 3 markers with the 3-9 cytological breakpoints. The only data that are directly comparable are those involving the same translocation, in this case T3-9c. We conclude that *yel*-8630* is located relatively close, but distal to the 3L.09 breakpoint (separation between *yel*-8630* and *wx1* of 8.3 cM), and that *w*-5787* is located farther out on the long arm of chromosome 3 (separation between *w*-5787* and *wx1* of 32.1 cM).

d4* is allelic to *d1

--Stinard, PS

The Maize Genetics Stock Center recently received a stock of the andromonoecious dwarf plant mutant *d4* from Ron Phillips of the University of Minnesota. *d4* was first reported by Suttle (Cornell Univ. Ph.D. Dissertation, 1924) and appears in Emerson, Beadle, and Fraser's (1935) gene list, but no further information appears in the literature. We figured that it was found to be allelic to some other better characterized dwarf mutant and disappeared from the literature for that reason, but could find no report of allelism. We conducted tests of allelism of *d4* with the andromonoecious dwarfs *d1*, *d3*, *d5*, and *an1* and found it to be allelic to *d1*. We renamed the mutant allele we received from Ron Phillips *d1-4*.

Alleles of *pink scutellum1* with no visible kernel phenotype

--Stinard, PS; Jackson, JD

The Maize Genetics Stock Center has been maintaining two independently isolated seedling mutants (*peach-albino-mutable*-87-2209-30* and *peach-albino*-N1983B*) with a unique peach-tinged albino phenotype. Because of their similar phenotype, tests of allelism were performed and these two mutants were found to be allelic. Rescued seedlings from viviparous alleles of *pink scutellum1* are described as being white with a pink flush. *ps1* mutant alleles are blocked in the production of carotenoids and the pink color is due to the accumulation of lycopene. Many *ps1* mutant alleles are also viviparous due to ABA deficiency. Several dormant alleles of *ps1* have been described which produce pink seedlings with varying degrees of greening (Faludi-Daniel et al., Acta Agron. Hung. 16:1-6, 1967; Bai et al., Genetics 175:981-992, 2007). However, these dormant *ps1* alleles produce kernels with visibly altered endosperm carotenoids that are pinkish in color, also due to the accumulation of lycopene. The *peach albino* mutants do not have visibly altered endosperm carotenoids and mutant kernels are indistinguishable from nonmutant kernels in a *Y1* background. Nevertheless, due to the similar mutant seedling phenotype, allelism tests were performed between the *peach albino* mutants and a viviparous *ps1* allele (*ps1-8205*). From the allelism test crosses, ears were obtained that segregated for pink endosperm kernels with dormant embryos. Seedlings grown from pink kernels had the seedling phenotype of their respective *peach albino* parent (Figure 1). It is interesting to note that although the double heterozygote *peach albino*/*ps1* kernels retained the embryo dormancy of the *peach albino* parent, they retained the endosperm carotenoid expression of the *ps1* parent. We conclude that the *peach albino* mutants are dormant alleles of *ps1* that have a unique nonmutant endosperm phenotype.

Figure 1. Seedlings grown from allelism test cross ears of *peach-albino*-N1983B* (middle row) and *peach-albino-mutable*-87-2209-30* (right) with *ps1-8205*, pink kernels planted. Note the sectors of greening on the plants on the right. They are not revertant sectors but rather represent an allele-specific epigenetic phenomenon. On the left are albino *lemon white1* seedlings for purpose of comparison.

Two point linkage data for *Og1* and *oy1* on chromosome 10

--Stinard, PS

Classic maize genetic linkage maps (e.g., Mutants of Maize, Neuffer et al., Cold Spring Harbor Laboratory, New York, 1997) show a separation of 4 centiMorgans between the *og1* and *oy1* loci on chromosome 10. These data appear to be based on indirect mapping with respect to other markers; no direct mapping between these loci has been reported in the literature. Since dominant *Og1* mutant alleles condition green and yellow/yellow-green striping and there exist dominant mutant alleles at the *oy1* locus that condition yellow-green plants, the possibility exists that *og1* and *oy1* may represent the same locus. We conducted direct mapping experiments between a dominant *Og1* mutant and a recessive *oy1* mutant to try to get at this question.

Homozygous *Og1 Oy1* (*Old gold1* single mutant) plants were crossed to homozygous *og1 oy1* (single mutant *oil yellow1*) plants and the resulting double heterozygotes were backcrossed by a homozygous *og1 oy1* tester. Kernels from testcross ears were planted and the resulting plants scored for *Og1* and *oy1*. 206 green seedling/Old gold striped parental type plants (*Og1 Oy1*) and 186 oil yellow seedling/green parental type plants (*oy1 og1*) were observed. No double mutant *Og1 oy1* plants were observed. Five potential double nonmutant green seedling/green plants were self-pollinated and evaluated one additional generation to confirm genotypes; all five turned out to be single mutant parental class *Og1 Oy1* plants. Thus no crossovers were obtained from a total of 397 plants scored, indicating a separation of less than 0.25 +/- 0.25 centiMorgans. These are not enough data to draw a definitive conclusion, but these two loci are certainly tightly linked if not identical.

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Genetic evidence of an unexpected kind of chromosome 9 aberration induced by the B chromosome in maize

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The maize B chromosome has been associated with induction of late knob replication in A chromosomes resulting in bridge-breakage, loss of acentric fragments and production of terminal-deficient chromosomes. B chromosomes have no effect upon the viability of the organism, inasmuch as they are not essential for growth and development. The B chromosome has been so far best studied in maize, and causes several interesting genetic effects. One of these effects, discovered by Rhoades et al. (1967), is the so-called “high-loss phenomenon”, in which B chromosomes interact with the knobs of A-complement chromosomes, inducing breakage. Since different kinds of chromosome aberrations, besides the single terminal deficiency, have been genetically described and were not included in the Rhoades and Dempsey hypothesis, they demand more investigation aimed at a better understanding of the whole effect of the B chromosome in inducing breakage in chromosomes of the A-complement. With this purpose in mind, this study was conducted in order to genetically analyze an aberrant plant with deficiency induced by B chromo-

somes by searching for chromosomes with more than a single structural variation resulting from several breakages.

In this study, we used the variety Black Mexican Sweet Corn, homomorphic for a large terminal knob on the short arm, as the high-loss stock with B chromosomes to induce breakages in chromosome 9. This stock was used to pollinate a tester stock, homomorphic for a small terminal knob on chromosome 9 and homozygous for all four mutant genes, *yg2, c1, wx and bz1*. *yg2* produces yellow-green seedlings and plants. For statistical analysis, the variance of the recombination frequency between the loci was estimated by the inverse of the Fisher information index, and the confidence intervals were established by approximation with the normal distribution with 95% probability, and by the bootstrap technique with 5,000 simulations (Liu, 1998).

Plant number S-284-7 was a yellow-green exception with a deficient chromosome 9, which, when cytologically analyzed, had the following constitution for the chromosomic pair 9: one chromosome with a small terminal knob on the short arm, and the other knobless. Since the high-loss stock was homozygous for the large knob, the knobless chromosome represented, in fact, a very small deficiency. This plant also presented no pollen abortion. The ratio of *Wx* : *wx* kernels, corresponding respectively to the deficient and the normal chromosomes, in both male (232 *Wx* : 242 *wx*) and female (211 *Wx* : 213 *wx*) test crosses, was essentially 1:1. Genetic tests showed that although the deficiency was very small, *C1*, located at the 5th or 6th chromomere, carried out by the knobbed chromosome 9 of the high loss stock, was changed (mutated) to *c*, inactivated (paracentric inversion with one breakpoint inside the *C* or transposon insertion) or removed (internal deficiency), resulting in the unexpected expression of the recessive phenotype. In a test for the presence of a deficient chromosome 9, half of the resulting seedlings were white or green-white and the other half yellow-green or green-yellow-green striped. This demonstrates that the knobless chromosome 9 in plant S-284-7 is deficient for a very small terminal segment including the *Wd*. It also lacks *C1*, although it is present in the homozygous condition in chromosome 9 of the high-loss stock. Genetic evidence showed that *Bz1*, located at five map units to the right of *C1*, had not been deleted, but this was not a critical test for the internal deficiency hypothesis. Crossing over was therefore studied in the *Bz1*-*Wx* region, which is proximal to *C1*. The *Yg2*-deficient chromosome 9 (Df9) carried the dominant *Bz1* and *Wx* while the normal chromosome 9 (N9) contained the recessive alleles. A plant of Df9 *Bz Wx* / N9 *bz1 wx* constitution, pollinated by a *bz1 wx* tester homozygous for *C1*, supplied the results presented in Table 1. The variance of the recombination frequency (r) between *Bz1* and *Wx* and the confidence intervals (CI) are as follows: r estimation = 0.1698; CI (normal approximation) = 0.1445 - 0.1950; CI (bootstrap) = 0.1214 - 0.1922. The 16.98% of recombination found in the Df9/N9, originating from plant S-284-7, did not differ significantly from the control value of 18.9% observed in a related stock presenting *C1*. A somewhat better test for the hypothesis of internal deletion or paracentric inversion with one breakpoint inside *C1* was done by determining the percentage of recombination between *Wx* and the extremity of 9S, which is genetically marked because it is deficient for *Yg2*. The kernels from this cross were classified for *Wx* (Df9) and *wx* (N9) endosperm, and the resulting seedlings sorted as white or green-white striped (Df9) versus yellow-green or green-yellow-green striped (N9) phenotypes (Table 2). In family S-1012, the average frequency of recombination between *Wx* and the *Yg2* deficiency was 16.9% (Table 2). This value is approximately the same as the 16.98% recombination found between *Wx* and *Bz1* in comparable heterozygotes, indicating that no crossing over took place distally to *Bz1* in the deficient chromosome 9 of plant S-284-7. In plant S-996-7, heterozygous for a deficient chromosome 9, there was 31.5% recombination between *Wx* and the breakpoint in the deficient chromosome. The statistical analysis showed the following results: r estimation = 0.1690; CI (normal approximation) = 0.1381 - 0.1998; CI (bootstrap) = 0.1250 - 0.1866. Because the estimation for the control (0.315) is outside the estimation confidence intervals, one can conclude that there is a difference between the recombination percentage values of the analyzed material and the value of the control, with 5% probability. Since examination at pachynema of S-284-7 heterozygotes revealed no extensive deficiency, the low recombination value of 16.9% cannot be ascribed to a terminal deficiency in 9S, including the *C1* locus.

Table 1. Percentage[1] of crossing over between *Bz* and *Wx* in plants heterozygous for Df S-284-7 (Df9 *Bz1 Wx*/N9 *bz1 wx*).

Phenotype	Number of kernels
Bz1 Wx	360
Bz1 wx	60
bz1 Wx	84
bz1 wx	344
Total	848
Crossing over (%)	16.98
Control	18.90

[1]Data from four years

Table 2. Percentage of recombination between *Wx* and the terminal deficiency in plants heterozygous for the S-284-7 deletion (Df9 *Wx wd*/N9 *wx yg2*).

Plant number	*Wx-wd*	*wx-wd*	*Wx-yg2*	*wx-yg2*	Total	Recombination (%)
S-1012-3	61	14	22	77	174	20.7
S-1012-4	37	5	7	65	114	10.5
S-1012-5	47	7	9	55	118	13.6
S-1012-8	65	17	15	65	162	19.7
Total	210	43	53	262	568	16.9
Control (S-996-7)	70	37	32	79	218	31.5

Wx-wd: white and green-white striped; *wx-wd:* white and green-white striped; *Wx-yg2:* yellow-green and green-yellow striped; *wx-yg2:* yellow-green and green-yellow striped

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Drought tolerant mutant induced by gamma-ray and sodium azide from maize calli

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In this study, our objectives were to develop drought-tolerant mutants and identify an optimum combination of γ ray with NaN_3 to treat embryonic calli derived from immature embryos in maize using the methods of Fu et al. (J. Sichuan Agric. Univ. 18:97-99, 2000; J. Northwest Sci-Tech Univ. Agric. For. (Nat. Sci. Ed.) 31:81-84, 2003). At present, information about workable dosages of gamma-rays and concentrations of NaN_3 to treat plant calli for mutation is limited. In rice, calli differentiation was enhanced with gamma (γ) ray at less than 30 Gy, but inhibited at higher than 40 Gy (Wang et al., Acta Agric. Nucl. Sin. 7:20-28, 1993). However, 1 kR, equivalent to 8.7 Gy of γ ray, was a suitable dosage to treat calli in wheat (Gao et al., Acta Agron. Sin. 20:18-25, 1994). For rice seed, the suitable dosage of γ ray and concentration of NaN_3 could be as high as 200 Gy and 2 mmol/L, respectively (Wang et

al., 1993). In this study, a combination with 20 Gy of γ ray and 1 mmol/L of NaN_3 was identified as suitable for mutation induction from maize calli (Table 1). The γ ray dosage in the present study was much higher than 0.1 kR or 0.87 Gy, the dosage used to mutate wheat anthers by Zheng et al. (Acta Bot. Sin. 35:121-128, 1993). Also, the NaCl concentration for mutant screening used in this study was twice as high as that used to screen mutated wheat anthers by Zheng et al. (1993). Haploid pollen in anthers is more responsive to γ ray treatment and NaCl screening than diploid embryonic calli.

Table 1. Survival percentage of maize calli treated with different doses of gamma-ray and NaN_3 on high osmotic medium.

NaN_3 concentration (mmol/L)	gamma-ray dosage (Gy)				
	0	10	20	30	40
0	0.33 (±0.04)	0.38 (±0.11)	1.04 (±0.06)	0.00 (±0.00)	0.00 (±0.00)
0.5	0.00 (±0.00)	0.24 (±0.05)	0.78 (±0.08)	0.00 (±0.00)	0.00 (±0.00)
1.0	0.00 (±0.00)	0.07 (±0.02)	1.43 (±0.07)	0.23 (±0.01)	-
1.5	0.12 (0.01)	0.34 (±0.13)	0.32 (±0.15)	0.06 (±0.03)	-
2.0	0.00 (±0.00)	0.00 (±0.00)	-	-	-

Maize embryonic calli derived from immature embryos of inbred line 18-599 were treated with gamma (γ) ray and sodium azide (NaN_3), and selected on high osmotic medium containing 1.0% NaCl for drought tolerance. Once plants were regenerated from the selected calli, they were evaluated for drought tolerance in artificial and natural conditions with parental 18-599 and known drought-tolerant line 81565 as checks. Anthesis-silking interval and grain yield were investigated as an estimate of drought tolerance.

Mutagenesis. Immature embryos of 1.5-2.5 mm in length were sampled from inbred line 18-599 13 days after pollination and cultured in the improved N6 medium for callus production. Line 18-599 is not only used as parent in many commercial hybrids, but is also suitable for tissue culture. Embryonic calli (type II) identified by the standard described by Armstrong and Green (Planta 164:207-214, 1985) were subcultured in the dark at 27 C for multiplication. The calli were irradiated with ^{60}Co γ ray at 10, 20, 30 and 40 Gy, respectively when they became stable after three subcultures, three weeks each. One week after stabilizing the cultures, they were treated with NaN_3 (pH3) at 0.5, 1.0, 1.5 and 2.0 mmol/L for 4 h. Relative growth rate used to determine the influence of mutation treatments with γ ray and NaN_3 on callus growth was recorded as [(callus weight after stabilizing cultures - callus weight before mutation treatments) / callus weight before mutation treatments] after stabilizing cultures for two weeks.

Mutant selection. The treated calli were then transferred to high osmotic medium containing 1.0% NaCl and cultured at 27 C twice, three weeks each. After stabilizing the high osmotic cultures for two weeks, these calli were transferred to differentiation medium for regeneration. The regenerated plantlets (M_0) grew in pots for hardening in the greenhouse for 2-3 weeks, and then in the field for selfing to produce M_2 seed. Drought stress was not applied to M_1 generation in the field because the plants were fragile, and the objectives in this generation were to produce enough seed for selection of target mutants and to observe morphological variation from the mutation treatments.

During the screening culture, most of the calli treated with gamma-ray and NaN_3 became brown and died on the high osmotic medium of 1% NaCl. After differentiation, regeneration and transplanting, 20 and 2 fertile plants were obtained from dosage combinations of 20 Gy of γ ray with 1 mmol/L of NaN_3 and 20 Gy of γ ray with 0.5 mmol/L of NaN_3, respectively. A few plantlets regenerated from several other dosage combinations, but failed to survive afterwards due to either poor growth or female sterility of the resulting plants.

The drought tolerance of mutated line 18-599M expressed from M_2 to M_4, as well as hybrids derived from the line, was significantly higher than parental 18-599 (Table 2) in various evaluations conducted in both artificial conditions and naturally water-stressed environments of four provinces (Sichuan, Ningxia, Guangxi and Hainan). Genetic polymorphism between 18-599M and 18-599 was found for 7 of 700 pairs of SSR primers listed at MaizeGDB. (Fig. 1). Among these polymorphic locations, the sequence amplified by SSR primer pair *phi080* located in chromosome bin 8.08 was identified as the 5'-end of glutathione S-tramsferase gene (*GST-1*) (Ulmasov et al., Plant Mol. Biol. 26:1055-1064, 1994). Glutathione S-transferase is one of the key enzymes involved in resistance to oxidation. The relationship of this enzyme with resistance to oxidative, osmotic, heavy metal stress and ultraviolet damage has been demonstrated (McGonigle et al., Plant Physiol. 124:1105-1120, 2000; Ulmasov et al., Plant Physiol. 108, 919-927, 1995). The expression of the glutathione S-transferase gene is up-regulated under drought stress in maize and transgenic *Arabidopsis* (Zheng et al., Plant Mol. Biol. 55:807-823, 2004), and its expression in wheat was up-regulated under polyethylene glycol simulated osmotic stress (Gallé et al., Acta Biol. Szeged. 49:95-96, 2005). More studies are needed for a molecular explanation of drought tolerance associated with genetic mutation in plants.

Nature of the mutations. Based on the sequence of the *GST-1* gene (GenBank accession number: M16900; Shah et al., Plant Mol. Biol. 6:203-211, 1986), we designed a pair of specific primers to amplify exon 1 of this gene: CACCCGATGCAACTTGCGTAGA[1] /GTTCCTACGCTTAGCCCAGAT. Three insertions, three dele-

Table 2. Comparison of anthesis-silking interval and grain yield between hybrids derived from mutant 18-599M and parental 18-599 (CK) under drought environments in three provinces (Sichuan, Ningxia and Hainan).

	Anthesis-silking interval (d)			Grain yield (kg/ha)		
	478	48-2	R15	478	48-2	R15
18-599M	2.63	3.12	2.46	4860*	6940*	6400
18-599 (CK)	2.52	3.56	2.32	4560	6760	6560

* Significantly (P<0.05) higher than its comparative commercial hybrid.

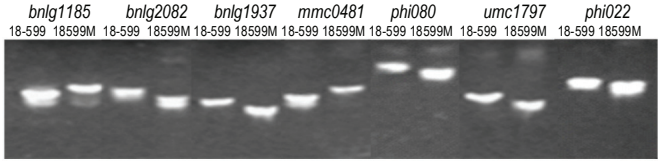

Figure 1. SSR polymorphism between mutated line 18-599M and parental 18-599. Genetic polymorphism amplified by 7 of 700 pairs of SSR primers between mutated line 18-599M and parental 18-599 showed that the genetic differences occurred in different locations of the genome from the mutagen treatments. For each pair of the primers, 18-599M is located in the left lane and 18-599 in the right lane.

tions and seventeen base substitutions were found in exon 1 of the glutathione S-transferase gene by sequence alignment. Base substitutions from A to T at 158 bp (A to T) and (C to G) at 162 bp changed the encoded amino acids from methionine to leucine and from serine to tryptophan, respectively (Fig. 2). The insertion of $(AGAGG)_5$ between the 86 and the 87 bp of 18-599 (Fig. 2) was probably responsible for the larger amplified product in 18-599M (Fig. 1).

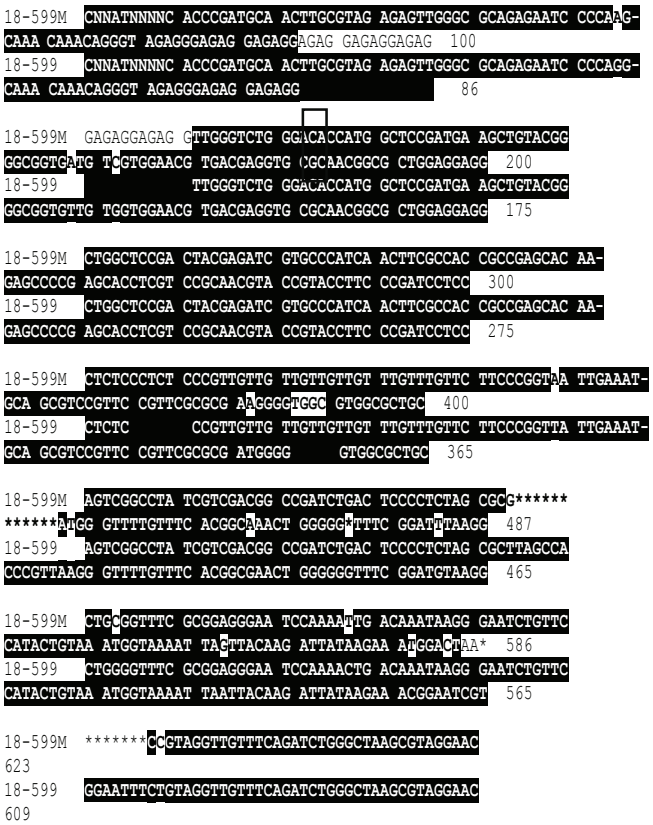

Figure 2. Sequence alignment of exon 1 of *GST-1* gene between 18-599M and 18-599. Three insertions, three deletions and seventeen base substitutions in exon 1 of glutathione S-transferase gene in 18-599M compared to parent allele, 18-599. The black rectangle shows the start codon of the coding sequence.

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[1]The first primer is the same as for *phi080* SSR (MNL Editor note).

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IV. MAIZE GENETICS COOPERATION STOCK CENTER

Maize Genetics Cooperation • Stock Center

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

&

University of Illinois at Urbana/Champaign - Department of Crop Sciences

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32,112 seed samples have been supplied in response to 277 requests for 2008. These include 76 requests received from 23 foreign countries. Popular stock requests include the NAM RIL populations, Hi-II lines, *ig1* lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, transposable element lines, Maize Inflorescence Project EMS lines, and Chromatin stocks.

Approximately 7.2 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Despite frequent heavy rains during the first part of spring, we managed to plant our crossing nurseries on schedule. Smaller than normal stands were observed in parts of the field that were covered with standing water for a day or two. By mid-June, more seasonable rainfall patterns were established and we had a normal pollination season without the need for supplemental irrigation. Moderate temperatures and low plant stress during and following pollination resulted in excellent yields.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of David Braun (*tdy1* and *tdy2* alleles), James Brewbaker (Hi27 near-isogenic mutant lines), Kelly Dawe (abnormal chromosome 10 deficiencies) Patrice Dubois (*phyB1 phyB2* lines), Jerry Kermicle (various *r1* alleles), Gerry Neuffer (recent EMS-induced mutants), the North Central Regional Plant Introduction Station (*r1-cherry* accessions from tribal maize), Ron Okagaki (EMS-induced *wx1* alleles and *Spm* change-of-state mutations), Peter Rogowsky (embryo-lethal mutants), Mark Settles (*vp10* and *vp15* alleles), Margaret Smith (male sterile cytoplasm lines), and others. The dominant *amylose extender1* mutant *Ae1-5180::Mu1* came off patent this year and we have increased it and made it publicly available as well. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

2. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We identified additional alleles of *pink scutelum1, viviparous5, lazy1, chlorophyll1*, *white3*, and *pale yellow9*. In 2009, we plan to test additional members of the viviparous and pale endosperm classes of mutants. In this manner, we hope to incorporate more stocks from our vast collection of unplaced, uncharacterized mutants into the main collection.

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

4. We further characterized the *Enr* (*Fcu*) system of *r1* aleurone color enhancers and published a paper on the results. We are continuing attempts to transposon tag *Enr1* using one of Tom Brutnell's transposed *Ac* lines. We are collecting and characterizing additional alleles of *Enr1* and other *r1* aleurone color enhancers and inhibitors.

5. Samples of 2,004 phenotype–only stocks were sent to the National Center for Genetic Resources Preservation in Fort Collins, Colorado for back-up. Selected samples from the main collection not yet sent were also pulled and sent this year. These represent stocks that had not been previously backed up. Our new inventory system has made selecting ears to be sent and producing a packing list to accompany them a much more efficient procedure.

6. Two acres were devoted to the propagation of the large collection of cytological variants, including A-A translocation stocks and inversions. Additional translocations received from W. R. Findley and Don Robertson marked with *wx1* were checked with linkage tests to confirm the chromosome arms involved. For those where we found no linkage, all sources were discarded. We were also able to add another useful *wx1* marked translocation from Susan Gabay-Laughnan in which the chromosome arms involved have been confirmed. These changes were all entered into MaizeGDB in 2008.

7. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0110189) were grown again this summer. Approximately 250 families of M2 materials that were produced in 2006 and 2007 were grown to increase seed supplies and recover previously observed mutations. Also, 1553 families of 2006 and 2007 M2 EMS materials were grown for adult plant observation; the materials observed include B73 and Mo17 inbred lines and the B73xMo17 hybrid. Visitors from 5 different institutes traveled to Illinois to walk these fields and found many new mutant phenotypes that will be added to the project database.

We grew a winter nursery of 0.5 acres at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico in 2007/2008. We received a good increase of most lines. We did not have sufficient funds in our budget to grow a winter crop in Puerto Rico this year. Critical plantings of a few lines were made in the greenhouse, but the lack of a field grown winter crop represents a set-back to our program, not only because of the loss of a nursery generation, but also because it is easier to transfer mutations out of tropical backgrounds into Midwest adapted backgrounds under winter conditions.

We have received close to 5,000 of the Nested Association Mapping Recombinant Inbred Lines (NAM RILs) consisting of 25 populations, from the Molecular and Functional Diversity of the Maize Genome project (http://www.panzea.org/lit/germplasm.html). Complete sets of this material are available to those willing to increase and redistribute seeds to their company or institution; requests for subsets of this material will be available. There are also additional lines from the Functional Genomics of Maize Chromatin project (see: https://www.fastlane.nsf.gov/servlet/showaward?award=0421619); most of these are unconfirmed lines. The Maize TILLING Project (http://genome.purdue.edu/maizetilling/) has also donated an additional 1,712 lines to our current holdings.

Our IT Specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our collection. The tools are also used for our internal database (e.g., inventory, pedigrees and requests). A new pedigree-entry tool was developed that reduced pedigree data entry time significantly and other tools were rewritten or written from scratch to import many years of unentered pedigree information. A tool to generate field notes conveniently and easily was created and will be modified to also generate harvest tags. Our web site and all other services have been migrated to a new hardware/software arrangement, which is much more reliable and faster than the old setup. Maintenance continues on our web site (http://www.uiuc.edu/ph/www/maize).

The new greenhouse space in Urbana is being used for our third winter crop. The space has proven to be excellent for growing material that doesn't do well under our field conditions. Our new seed storage space presently has 803 seed storage drawers of the 1,584 the room will eventually hold (pending funding). Thanks to the help from the National Program Staff, we finally have enough new storage drawers to unpack and organize the approximately 36,000 Maize Targeted Mutagenesis (http://mtm.cshl.org/) lines we have held, up to now, in boxes in the aisles of our storage rooms.

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

ADDITIONS TO OUR CATALOG OF STOCKS SINCE MNL82
(For a complete list of our stocks, see: http://maizegdb.org/cgi-bin/stockcatalog.cgi)

CHROMOSOME 1 MARKERS
106BA ts2 ^Hi27
107I P1-wr ^Hi27
110EA ad1 ^Hi27
111A bsd2-m1::Mu
115K bz2-s

CHROMOSOME 2 MARKERS
201FA ws3 ^Hi27
214JA sk1 fl1 ^Hi27

CHROMOSOME 4 MARKERS
405DA la1-R fl2 su1 ^Hi27
405DB la1-R fl2 ^Hi27
418FA o1 c2 ^Hi27

CHROMOSOME 5 MARKERS
510B Ae1-5180::Mu1
512A bt1-A
524E tdy2-R

CHROMOSOME 6 MARKERS
604AA y1-Guam
608N su2-ora3
616E tdy1-R

CHROMOSOME 7 MARKERS
703AA o2 v5 ^Hi27
704B o2-Tuxp
705BA sl1 ^Hi27
705BB sl1 sh2 ^Hi27
705DA bd1 ^Hi27
707FA y8 ^Hi27
707GA in1; A1 A2 C1 C2 Pr1 R1 ^Hi27

CHROMOSOME 9 MARKERS
901G Inr2-JD r1-g
903J emb2-8522
903K emb*-8516
903L emb*-g2422
903M bz1-m13CS-C
903N bz1-m13CS-E
906L wx1-EMS14
906M wx1-EMS17
906N wx1-EMS20
906O wx1-EMS28
906P wx1-EMS30
916CA bk2 ^Hi27

CHROMOSOME 10 MARKERS
X03FA Inr1-JD r1-g
X12JA DfK10(L) R1
X237A r1-ch(Kokoma) Pl1
X237B R1-nj:st(n208)
X28EA Les3-NA781
X37A R1-r(spotted dilute2) Inr1-Dil wx1-m8::Spm-I8 Spm
X37B R1-r(spotted dilute2) K10-I Inr1-Dil Spm
X37C R1-r(spotted dilute2) Inr1-Dil wx1-m8::Spm-I8
X37D R1-Randolph

CHROMDB STOCKS
3201-34.1 T-MCG5065.033
3201-34.2 T-MCG5065.033
3201-34.3 T-MCG5065.049
3201-40.2 T-MCG5211.022
3201-41.1 T-MCG6123.017
3201-46 T-MCG3832.023
3201-47 T-MCG4322.006
3201-48 T-MCG4721.010
3201-49 T-MCG4963.024
3201-50 T-MCG4977.011
3201-51 T-MCG5071.014
3201-51.1 T-MCG5071.025
3201-52 T-MCG5641.016
3201-52.1 T-MCG5641.036
3201-53 T-MCG5825.005
3201-53.1 T-MCG5825.011
3201-54 T-MCG6082.002
3201-54.1 T-MCG6082.004
3201-54.2 T-MCG6082.018
3201-54.3 T-MCG6082.019

UNPLACED GENES
U240F zn2-PI251887

MULTIPLE GENES
M341BA B1 C1-I pl1 ^Hi27
M341BB B1 C1 pl1 ^Hi27

RARE ISOZYME
3004-001 Mdh3-16; Mdh5-16
3004-003 Mdh1-5
3004-004 Mdh3-11.5
3004-005 Mdh1-9.2; Mdh3-n
3004-006 Mdh5-15; mmm1-1
3004-007 Mdh5-15.3
3004-008 Cat3-n
3004-009 Mdh1-.05; Mdh2-n; Mdh5-16.5
3004-010 Mdh1-10.5; Mdh2-n; Mdh3-n
3004-011 Mdh1-n; Mdh2-n; Mdh3-16
3004-012 Mdh1-1; Mdh2-n; Mdh3-n
3004-013 Cat3-6
3004-014 Mdh2-n; Cat3-14
3004-015 Mdh4-13; Mdh5-n
3004-016 Mdh1-n; Mdh2-7.4
3004-017 Mdh4-10.5; mmm1-1
3004-018 Mdh1-n; Mdh5-16.7
3004-019 Me1-VF
3004-020 Mdh1-n; Mdh3-n
3004-021 Mdh4-n; Mdh5-n
3004-022 Mdh4-n; Mdh5-12+; Cat3-9+
3004-023 Mdh4-n; Mdh5-12
3004-024 Cat3-n
3004-025 Mdh1-n; Mdh2-9.3; Cat3-n
3004-026 Mdh1-n; Mdh2-n; Me1-VF
3004-027 Mdh4-n; Mdh5-n
3004-028 Mdh4-13; Mdh5-n
3004-029 Mdh1-n; Mdh2-n; Mdh5-n
3004-030 Mdh1-.05; Mdh2-n; Mdh3-n; Gdh1-S
3004-031 Mdh1-n; Mdh2-n; Mdh5-n; Mdh4-D8.5
3004-032 Mdh1-n; Mdh2-n; Mdh3-16.9
3004-033 Mdh1-n; Mdh3-n; Mdh2-B6.1ap
3004-034 Pgd1-3.8; Ep1-6
3004-035 Est8-4.5
3004-036 Cat3-11
3004-037 Mdh1-n; Mdh2-4m
3004-038 Got1-8; Mdh1-n; Mdh2-n; Mdh4-12; Mdh5-16.7
3004-039 Mdh1-n; Mdh2-n; Mdh3-7.3
3004-040 Mdh1-n; Mdh2-n; Mdh3-18
3004-041 Mdh1-n; Mdh2-.2
3004-043 Mdh1-n; Mdh3-n
3004-044 Mdh1-n; Mdh2-n; Mdh4-8; Mdh5-16.7
3004-045 Mdh1-2.8; Mdh2-n
3004-046 Aco1-2; Aco2-6
3004-047 Dia2-4; Pgd1-3.8; Pgd2-n
3004-048 Dia2-6
3004-049 Mdh1-n; Mdh2-n; Mdh5-n; Mdh4-8
3004-051 Mdh1-n; Mdh3-n; Mdh2-6.1
3004-052 Mdh1-n; Mdh3-n; Mdh2-5.6
3004-053 Mdh1-6; Mdh2-n; Mdh3-n
3004-054 Mdh1-n; Mdh2-n; Mdh4-8; Mdh5-16.7
3004-055 Mdh1-n; Mdh2-n; Mdh3-16.8
3004-056 Mdh1-n; Mdh3-n; Mdh2-.03/n
3004-057 Adh1-2; Gdh-S; Amp3-6
3004-059 Mdh1-n; Mdh2-n; Mdh5-n; Mdh4-8.5; mmm1-1
3004-060 Got3-8; Pgd2-8; Est8-8
3004-061 Got3-8; Me1-VS; Mdh1-n; Mdh2-n
3004-062 Aco1-1; Mdh1-n; Mdh2-n; Mdh3-18
3004-063 Pgm2-12; Mdh5-16.4
3004-064 Adh1-2; Phi1-8; Amp3-6
3004-066 Amp1-6; Mdh4-16.7
3004-068 Me1-VS
3004-069 Amp1-6; Mdh4-16.7; Pgm1-5
3004-070 Hex2-6; Idh2-8; Mdh2-.03
3004-071 Idh1-4; Idh2-n
3004-072 Adh1-2; Phi1-8; Gdh1-S
3004-073 Mdh4-n; Mdh5-16.4
3004-074 Mdh1-.4; Mdh2-n; Mdh3-n
3004-075 Tpi4-8; Mdh3-16.9; Me1-VS; Got3-8
3004-076 Amp1-6; Mdh4-16.7; Ep1-12
3004-077 Amp1-6; Mhh4-16.7; Pgm1-5
3004-078 Mdh1-n; Mdh3-n; Mdh2-B5.6s
3004-079 Mdh1-.05; Mdh4-8; Mdh2-n; Mdh3-n; Mdh5-n
3004-080 Dia1-10; Dia2-6
3004-082 Tpi3-2; Mdh1-.65; Idh1-2; Tpi5-8; Aco1-1
3004-083 Acp4-1; Dia2-6; Gdh1-VS; Adh1-2; Phi1-8; Pgm1-18; mmm1-1; Mdh4-8.5
3004-084 Ak1-5; Pgd1-9; Est8-10; Hex2-6; Idh2-8; Mdh2-.03
3004-085 Ak1-5; Est8-10; Pdd1-9; Idh2-8; Mdh2-B.03; Hex2-6
3004-086 Pgd1-9; Ep1-10; Hex2-6; Idh2-8; Mdh2-.03; Adk1-5; Tpi3-2; Mdh1-.65; Idh1-2; Tpi5-8
3004-087 Phi1-8; Gdh1-VS; Dia2-6; Acp4-.5
3004-088 Mdh4-8.5; mmm1-1; Gdh1-VS; Dia2-6; Acp4-.5
3004-089 Mdh4-8.5; mmm-1; Pgm1-18; Adh1-2; Pgd1-8; Gdh1-VS; Dia2-6; Acp4-.5
3004-090 Cat3-5.5
3004-091 Tpi3-2; Est8-8; Mdh1-.65; Idh1-2
3004-093 Amp1-6; Mdh4-8.5; mmm-1; Dia2-6; Acp4-.5
3004-094 Tpi1-2; Tpi2-2; Tpi5-4
3004-095 Pgd2-10
3004-096 Pgm2-12; Amp3-7; Got2-10
3004-097 Cat3-5; Aco1-1; Tpi1-2
3004-098 Amp1-6; Mdh4-8.5; mmm1-1; Pgm1-18; Adh1-2; Phi1-8; Dia2-6; Acp4-5; Gdh seg
3004-099 Pgd1-9; Ep1-10; Hex2-6; Idh2-8; Mdh2-.03; Adk1-5; with Ep12
3004-100 Mdh2-.03; Mdh1-n; Mdh3-n; Mdh4-n; Mdh5-n
3004-101 Sad1-6; Glu1-16
3004-102 Dia1-10; Tpi2-2; Aco1-1; Cat3-5; su1; Tpi1-2
3004-103 Amp1-6; Mdh4-8.5; mmm1-1; Pgm1-18; Adh1-2; Phi1-8; Gdh1-S; Dia2-6; Acp4-5; Pgm2-12; Mdh5-16.4; Got3-2.... + see comment
3004-104 Mdh1-n; Mdh2-n
3004-105 Mdh4-8; Mdh5-n
3004-106 R12 check line

CYTOPLASMIC-STERILE/RESTORER
C437A A619 (N); mito-N
C437B A619 (C); cms-C
C437C A619 (S); cms-S
C437D A632 (N); mito-N Rf3 rfC
C437E A632 (C) Sterile; cms-C Rf3 rfC
C437F A632 (S) Restored; cms-S Rf3 rfC
C437G A634 (N); mito-N Rf3 rfC

C437H A634 (C) Sterile; cms-C Rf3 rfC
C437I A634 (S) Restored; cms-S Rf3 rfC
C437J Hi27 (C) Sterile; cms-C rfC
C437JA Hi27 (N); mito-N rfC
C437K B73 (C) Sterile; cms-C rf3 rfC
C437L B73 (S) Sterile; cms-S rf3 rfC
C437M H95 (N); mito-N Rf3 RfC
C437N H95 (C) Restored; cms-C Rf3 RfC
C437O H95 (S) Restored; cms-S Rf3 RfC
C437P K55 (C) Restored; cms-C rf3 RfC
C437Q K55 (S) Sterile; cms-S rf3 RfC
C437R KYS (C) Sterile; cms-C rf3 rfC
C437S KYS (S) Sterile; cms-S rf3 rfC
C437T Ky21 (C) Restored; cms-C Rf3 RfC
C437U Ky21 (S) Restored; cms-S Rf3 RfC
C537A M14 (N); mito-N rf3 RfC
C537B M14 (C) Restored; cms-C rf3 RfC
C537C M14 (S) Sterile; cms-S rf3 RfC
C537D NY821LE (N); mito-N
C537E NY821LE (C); cms-C
C537F NY821LE (S); cms-S
C537G Oh43 (N); mito-N
C537H Oh43 (C); cms-C
C537I Oh43 (S); cms-S
C537J Oh45 (N); mito-N Rf3 RfC
C537K Oh45 (C) Restored; cms-C Rf3 RfC
C537L Oh45 (S) Restored; cms-S Rf3 RfC
C537M Oh51A (N); mito-N rf3 rfC
C537N Oh51A (C) Sterile; cms-C rf3 rfC
C537O Oh51A (S) Sterile; cms-S rf3 rfC
C537P Tr (C) Sterile; cms-C Rf3 rfC
C537Q Va26 (N); mito-N
C537R Va26 (C); cms-C
C537S Va26 (S); cms-S
C537T Va58 (N); mito-N Rf3 rfC
C537U Va58 (C) Sterile; cms-C Rf3 rfC
C537V Va58 (S) Restored; cms-S Rf3 rfC
C637A W23 (C); cms-C
C637B W23 (S); cms-S
C637C W64A (N); mito-N rf3 RfC
C637D W64A (C) Restored; cms-C rf3 RfC
C637E W64A (S) Sterile; cms-S rf3 RfC
C637F 38-11 (C); cms-C
C637G 38-11 (S); cms-S

RECOMBINANT INBRED
NAM5000 Full set of 5,000 NAM RILs (we presently have 4,835 of these RILs)
Subpopulations of NAM RILs
NAM-Z001 B73 x B97 NAM RILs
NAM-Z002 B73 X CML103 NAM RILs
NAM-Z003 B73 X CML228 NAM RILs
NAM-Z004 B73 X CML247 NAM RILs
NAM-Z005 B73 X CML277 NAM RILs
NAM-Z006 B73 X CML322 NAM RILs
NAM-Z007 B73 X CML333 NAM RILs
NAM-Z008 B73 X CML52 NAM RILs
NAM-Z009 B73 X CML69 NAM RILs
NAM-Z010 B73 X Hp301 NAM RILs
NAM-Z011 B73 X Il14H NAM RILs
NAM-Z012 B73 X Ki11 NAM RILs
NAM-Z013 B73 X Ki3 NAM RILs
NAM-Z014 B73 X Ky21 NAM RILs
NAM-Z015 B73 X M162W NAM RILs
NAM-Z016 B73 X M37W NAM RILs
NAM-Z018 B73 X Mo18W NAM RILs
NAM-Z019 B73 X MS71 NAM RILs
NAM-Z020 B73 X NC350 NAM RILs
NAM-Z021 B73 X NC358 NAM RILs
NAM-Z022 B73 X Oh43 NAM RILs
NAM-Z023 B73 X Oh7B NAM RILs
NAM-Z024 B73 X P39 NAM RILs
NAM-Z025 B73 X Tx303 NAM RILs
NAM-Z026 B73 X Tzi8 NAM RILs

INVERSION
I943D Inv7c (7L.34; 7L.52)

RECIPROCAL TRANSLOCATIONS (wx1 AND WX1 MARKED)
wx24D T8-9(043-6) (8L.17; 9S.34); wx1

STOCKS CHARACTERIZED ONLY BY PHENOTYPE
adherent leaf
3610L ad*-N1945

albino seedling
4510O v*-N1867
6212P w*-92-2440-2

bleached leaf
3612E blh*-N2237

brown kernel
3606H bnk*-N1519C

brown midrib
5803M bm*-PI586725

brown pericarp
5805C bp*-Lima94

collapsed endosperm
3703H de*-N978
4104R cp*-N2376

compressed top
3708P cmpt*-N2378
3912N wrt*-N2384

defective kernel
3703H de*-N978
3704G de*-N1069A

dwarf plant
4406D d*-N2249A
4407F D*-N2468
5505C d*-PI184286

green striped leaf
4009I gs*-N720D

lemon white
5911R lw*-92-1253-80
5911S lw*-92-3240-53
5912Q lw*-RJL

miniature kernel
138-32 mn*-MTM4714; lp*-MTM4714
138-38 mn*-MTM4752; wrk*-MTM4752
238-50 mn*-MTM5888
3807U mn*-N904C
438-45 mn*-MTM16089
438-48 mn*-MTM16165
538-18 mn*-MTM16702

necrotic leaf
4101IA nec*-N249A

opaque endosperm
3904I o*-N1100

patched leaf
4105G ptc*-N888B

reduced pollen fertility
138-40A lp*-MTM4887

rough kernel
3806Q rgh*-N1412

semidwarf
4408L Sdw*-N2433

short plant
4407O Py*-N983

small kernel
4003N smk*-N1238A
4004H smk*-N1946

spotted leaf
4107T spt*-N1620B

streaked leaf
3709G stk*-N208A

striped leaf
6005G str*-Morrow
6009A Og*-65-563

tassel seed
5807M ts*-Ames 17676

virescent seedling
4511K v*-N2250B

white luteus seedling
4108G wl*-N56
4510O v*-N1867

white stripe leaf (japonica-like)
4011J j*-N358B
6005M j*-92-1259-92

wrinkled kernel
138-37C wrk*-MTM4751

Additionally, we received 1,712 stocks from the Maize TILLING project and additional stocks from other maize genome projects.

V. MAIZE GENETICS AND GENOMICS DATABASE
(www.maizegdb.org)

Big news.
(1) MaizeGDB is reported as the fifth most accessed website by lead principal investigator of the National Plant Genome Initiative grants [National Research Council (USA), 2008. Achievements of the National Plant Genome Initiative and New Horizons in Plant Biology. Pp. 144-149, National Academies Press, Washington DC. http://books.nap.edu/openbook.php?isbn=0309114187&page=145].
(2) USDA-ARS 5-Year Project Plan reviewed and accepted with no revisions required. Review process is described here: http://www.ars.usda.gov/research/docs.htm?docid=1503.
(3) Genome Browser release and is continually updated.
(4) Community genome annotation project funded for MaizeGDB and PlantGDB by USDA-ARS & NSF, respectively.

New Staffing.
We welcome Carson Andorf, who joined the core team at Ames, IA in June of 2008. He fills the position vacated by Trent Seigfried in March of 2008. We also welcome Ethalinda (Ethy) Cannon to the group. Ethy is the Solution/Application Architect for the NSF-funded POPcorn project (described below).

Because the genomes of maize inbred lines B73 and Mo17 as well as the Palomero Toluqueño landrace population have been/are being sequenced, sequence data have taken center stage, and MaizeGDB's development has been refocused to allow a sequence-centric perspective linking genome sequences (especially B73) to existing genetic and physical map resources at MaizeGDB. Major endeavors related to creating the sequence-centric perspective include: incorporation of a genome browser into MaizeGDB to visually represent genome sequences; implementation of POPcorn (http://www.maizegdb.org/POPcorn), a portal ancillary to MaizeGDB that offers access to independent maize projects and will allow BLAST similarity searches of participating projects' datasets from a single point; and development of the infrastructure that enables the maize community to be involved in genome annotation.

Genome Browser.
The initiative to implement the MaizeGDB Genome Browser was launched in early 2008, with the browser itself becoming available toward the end of 2008. During the planning stages, the MaizeGDB team considered a variety of genome browser applications and queried the maize community for their inputs. To gauge cooperators' impressions of existing software and to find out what functionalities they would like to have in a maize genome browser, a survey (accessible online at http://www.maizegdb.org/blanksurvey.html) was prepared and distributed on behalf of the Maize Genetics Executive Committee. A summary of the survey results is available online at http://www.maizegdb.org/genome_browser_survey.php and a detailed description of the process is forthcoming (Sen et al., in preparation). Based upon results of the survey, GBrowse [Stein et al., 20002. The generic genome browser: a building block for a model organism system database. Genome Res. 12(10):1599-1610] was selected as the software for the MaizeGDB Genome Browser implementation.

The MaizeGDB Genome Browser was populated using data from: MaizeGDB, the Maize Genome Sequencing Consortium's MaizeSequence.org resource, PlantGDB [cDNAs, PUTs, *Ac*/*Ds* elements, etc.], Pat Schnable's group's MAGI resource, and the UniformMu Group's *Mu* insertions. Five volunteers (P. Balint-Kurti, S. Hake, D. Lisch, M. Muszynski, and V. Walbot) were selected to provide guidance on the MaizeGDB Genome Browser's development and ten were selected (A. Charcosset, O. Dugas, J. Estill, D. Hessel, D. Lisch, M. Muszynski, P. Scott, V. Walbot, R. Wang, and C. Alvarez-Meja) to serve as beta testers. Based upon their inputs and additional feedback from other researchers using the Genome Browser, custom tracks were developed including a 'BIN' track that divides the genome into 90 genetically defined segments familiar to most researchers and a 'Sequenced FPC contig' track that clearly delineates regions of the B73 genome that are not yet sequenced.

Genome Browser Tools.
Guidance and Beta Tester volunteers (1) suggested the implementation of a tool to integrate genetic and genomic coordinates to allow researchers to identify regions of the genome where a genetically mapped locus may lie and (2) asked for mechanisms that would allow them to access genomic locations based upon sequence similarity analyses. Early in 2009, the Locus Lookup Tool was created and the BLAST tool was updated to meet these needs, respectively.

The **Locus Lookup** tool provides a way to find a mapped locus on the B73 sequence if its genomic coordinates are not known. Its algorithm uses the following steps: (1) if the locus has known physical coordinates, those coordinates are returned; (2) else, if there are there are probes that recognize the locus and the probes have known coordinates, then the coordinates of the BAC(s) that contain the probe(s) are returned; (3) else if the locus is on a given genetic map (IBM2 2008 Neighbors [default], NAM, Genetic 2005, or Genetic 2008), then the nearest left and right flanking marker with known physical coordinates are located and the coordinates of the BAC(s) that contain the region from the end position of the left flanking marker and the start position of the right flanking marker are returned; (4) else a

note that the region cannot be identified is returned. The strategy is diagrammed in Figure 1, and the GBrowse result for the *multiple archesporial cells1* (*mac1*) locus is shown in Figure 2.

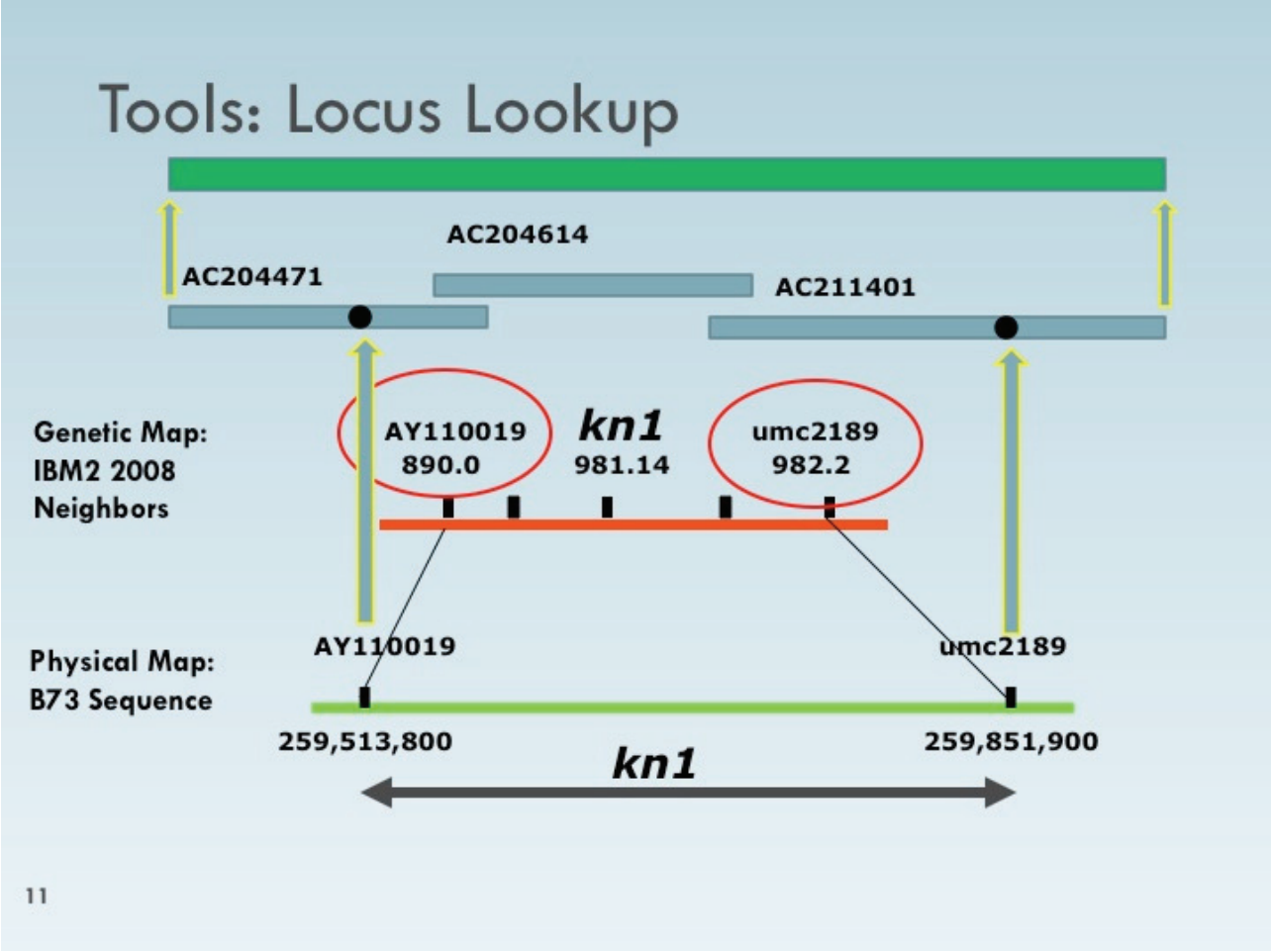

Figure 1. Strategy of Locus Lookup for the *kn1 knotted1* locus.

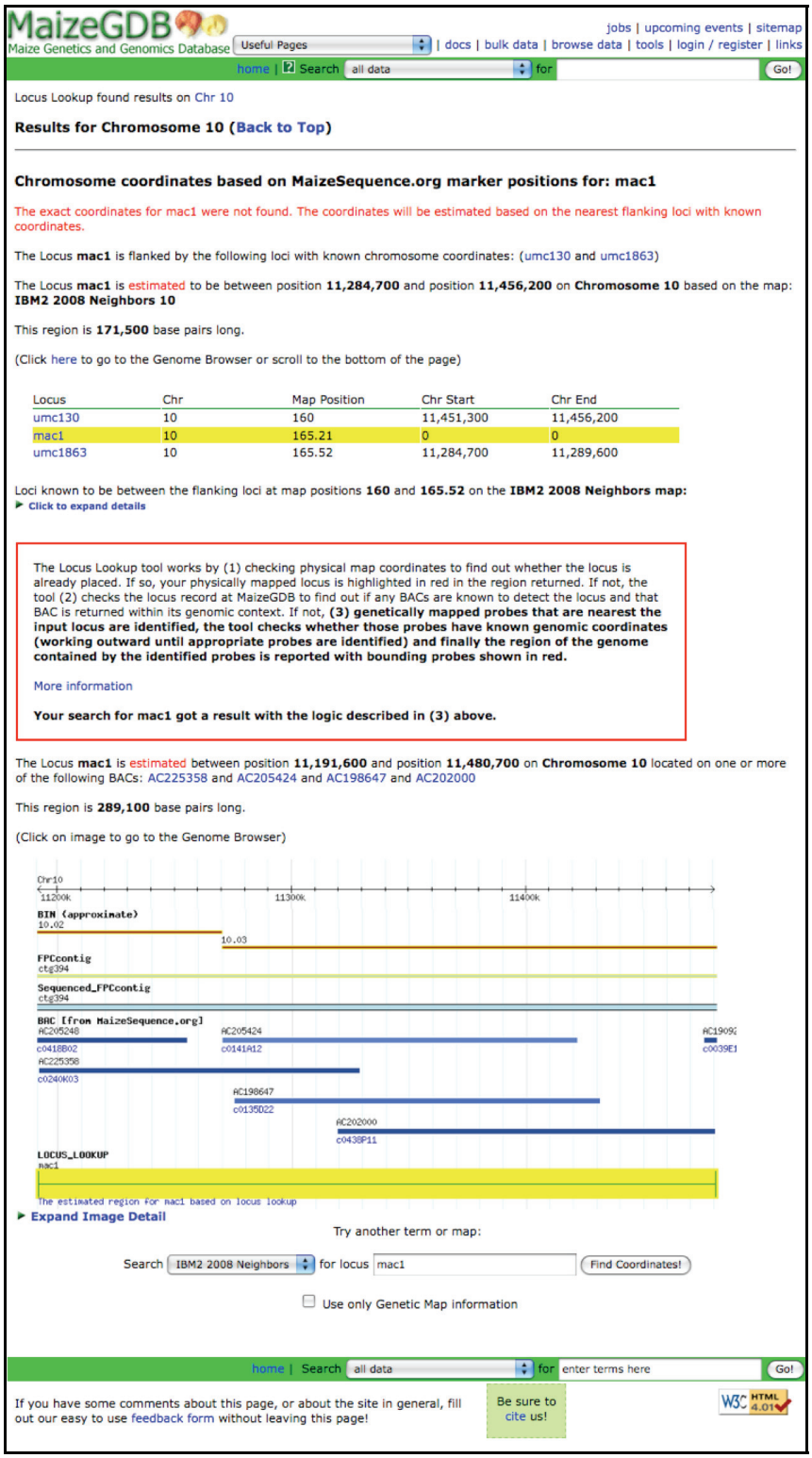

Figure 2. The result page for the approximate genomic coordinates for the *multiple archesporial cells1 (mac1)* locus when the Locus Lookup tool is used.

The MaizeGDB **BLAST tool** has been updated and integrated with the Genome Browser. If you BLAST at MaizeGDB, a thumbnail of the genomic region is returned and the hit itself can be loaded to the MaizeGDB Genome Browser to better visualize how those hits relate to other elements.

In April of 2009, links associated with the Locus Lookup Tool ranked first and sixth and links for BLAST ranked tenth and thirteenth among the top pages accessed at MaizeGDB. THANKS to all of you for directing our development!

New Data Highlights.

Recombination-Based Maps. Some 260 individual NAM maps were added complete with map scores, provided by the "Molecular and Functional Diversity of the Maize Genome Project" (Diversity Project) pre-publication. Map names use the Diversity Project name, along with other specifications to help users. For example the map called "NAM Z002 B73xCML103 RI 1" permits lookups of all NAM maps by typing NAM in the search box; 'Z002' is the name used by the Diversity Project and the Maize Genetics Cooperation – Stock Center for the panel of stocks; "B73xCML103" indicates the parental lines, with B73 as maternal parent; "RI" indicates the panel type is recombinant inbred; "1" is the linkage group for the map. Alleles for each locus were included when the sequences had been submitted to GenBank and named based on the nucleotide position of the SNP used for mapping based on files prepared by the Diversity Project. The other major map set added was the ISU IBM INDEL Map7, which was provided pre-publication, with probe details and related GenBank accessions. Consensus maps have been updated to include: a new Genetic 2008 Map (based on the IBM2 map) to permit ready incorporation into the Neighbors computation; the IBM2 2008 Neighbors maps, with statistically defendable orders on contributing maps (IBM2 Frame) and with additional approximately ordered loci on the contributing maps (IBM2 Neighbors); and Bins maps now include the minimum tiling path BACs, using genomic coordinates provided by MaizeSequence.org.

UniformMu variations, with genomic coordinates defined by the UniformMu project were added, along with phenotypes of the stocks available from the MGCSC.

Outreach, tutorials, and movies.

We plan on up to 3 trips each year to provide a 1-2 hour tutorial, tailored to specific questions or requests with one-to-one sessions after the tutorial. This year tutorials were held at Iowa State and UC Berkeley. Persons interested in scheduling a trip to their location should contact Lisa Harper or Carolyn Lawrence. Online PowerPoint presentations and movies that provide overviews of MaizeGDB, and specific topics are linked to the homepage. New movies describe the genome browser, and caveats in data interpretation. These caveats are expected to change as the sequence and its annotation are updated. We plan to update the movies as required. Cooperators are invited to request tutorials on a particular topic, using the link on the online page for this section.

Meeting Representation 2008 – early 2009.

Sackler Workshop: NPGI's planning meeting for the next 5 years; Biophysical Society Meeting; Genome Informatics Meeting; Plant and Animal Genome Conference (PAG); the Maize Genetics Conference; Corn Crop Germplasm Committee Meeting; MacWorld; 3rd International Biocurators Meeting. At PAG 2009, MaizeGDB hosted an exhibit booth for the Plant Genome DB Outreach Consortium that involved 13 database groups, 3 more than the previous year.

Editorial Board.

We maintain an Editorial Board whose members contribute a paper each month to be highlighted at MaizeGDB. Data from these articles are manually curated and added to the MaizeGDB. The 2009 board includes Jane Dorweiler, Peter Balint-Kurti, Cliff Weil, and Randall Wisser. For more information see, http://www.maizegdb.org/editorial_board.php, where you may now subscribe to an RSS feed.

Contributing your data to MaizeGDB.

If you are developing a project that will generate large datasets and that you would like to submit to MaizeGDB, you need to contact Carolyn Lawrence before you submit the proposal. At this time we have subcontracts from two NSF-funded projects: (1) Construction of Comprehensive Sequence Transposon Resources for Maize with Don McCarty as PI ; and (2) The Grass Regulome Initiative: Integrating control of gene expression and agronomic traits across the grasses", with Erich Grotewold as PI.

You may also contribute data directly. The easiest is very much like a 'wiki', where you simply add a comment using the annotation tool. You will first need to register, using the menu item 'annotation' on the top menu bar of the homepage. Once registered, every time you access MaizeGDB, you will be able to annotate. Annotations will appear in the monthly updates of the database. A second way is to use the community curation tools. Check the box when signing up for an account at MaizeGDB to get access to the curation tools, or ask for access directly using the feedback link at the bottom of any MaizeGDB page.

POPcorn.

POPcorn (Project POrtal for corn), is a new website that addresses the difficulty maize researchers face when searching for and using differing data from multiple sources on the web. The first phase of POPcorn was released in April of 2009. This first release permits researchers to search for maize projects and web resources. The preliminary database contains 42 projects and 76 resources. POPcorn is continuously updated, so send along information on your projects and resources so that we can include them!

The second phase or POPcorn development will permit researchers to search sequences at multiple maize databases by submitting their query via the POPcorn interface. POPcorn will handle querying cooperator databases and returning the results in one place, freeing the researcher from having to make multiple queries at multiple sites.

The third phase will be mostly invisible to users, consisting of behind-the-scenes scripts, pipelines, and procedures for integrating project data into MaizeGDB when those projects come to end and need a permanent home for their data.

POPcorn is a two year, NSF-funded development project which will be folded into MaizeGDB upon completion. POPcorn can be found at http://www.MaizeGDB.org/POPcorn.

Citing MaizeGDB.
Most recent: Lawrence, CJ, Harper, LC, Schaeffer, ML, Sen, TZ, Seigfried, TE, Campbell, DA. (2008) MaizeGDB: The Maize Model Organism Database for Basic, Translational, and Applied Research. Int. J. Plant Genomics 2008:496957.

Stakeholder Input.
In addition to the outreach activity referred to above, we respond quickly, typically within 24 h, to direct inputs by email, phone or comments submitted from each page in the database. The Maize Genetics Executive Committee (MGEC) and our Working Group provide oversight and work with us on developing surveys of the community. In 2008-9, we asked for input from two groups, a Genome Browser Guidance Group, and many Beta testers.

Acknowledgements.
We are deeply grateful to our Working Group for their advice. Our current Working Group includes: Mihail Pop (Chief), Ed Buckler, Mike Freeling, Owen Hoekenga, Anne-Francoise Lamblin, Karen McGinnis, Lukas Mueller, Pat Schnable, Anne Sylvester, Doreen Ware and as *Ex Officio*, Volker Brendel and Marty Sachs. We met most recently April 2009, by teleconference and with all MaizeGDB staff convening at Ames, IA. For more details see http://www.maizegdb.org/working_group.php.

For help in implementing the genome Browser, we greatly appreciate the Guidance Group: Peter Balint-Kurti, Sarah Hake, Damon Lisch, Mike Muszynski, and Virginia Walbot; and the Beta-testers: Alain Charcosset, Olivier Dugas, James Estill, David Hessel, Damon Lisch, Mike Muszynski, Paul Scott, Virginia Walbot, Rachel Wang and Cesar Alvarez-Mejia.

Submitted May 2009 by:
Mary Schaeffer
Taner Z. Sen
Ethyalinda Cannon
Lisa Harper
Carson Andorf
Darwin Campbell
Carolyn Lawrence (Lead Scientist)

Brief Description of Major Genetic Maps in MaizeGDB

(see also Tutorial and the Maps Data Center at MaizeGDB for more details)

MaizeGDB currently stores over 1789 genetic maps created by community members. A list of all maps is here- (http://www.maizegdb.org/cgi-bin/mapadvquery.cgi?query=true&locus1box=&locus2box=&locus3box=&chrom=1&source=15901&panel=981040). In the most general sense, genetic maps are made by crossing two lines with different alleles at a number of loci, and measuring the amount of recombination between those alleles in the resulting progeny. The progeny of a mapping cross is generally called a mapping population, but for a defined set of mapping lines, such as the recombinant inbreds used for IBM, the more precise name is "panel of stocks" or "mapping panel". Most genetic maps stored at MaizeGDB have been calculated from single mapping panels. However, commonly used maps are the "composite" genetic maps, meaning that they have data from many different mapping experiments and panels integrated by various means into one map.

It is useful to think separately about the two stages of genetic mapping: the generation of the mapping panel and the assigning of markers to discreet genetic positions. Understanding how the various mapping panels have been generated will lead to an understanding of the resolution and limitations of any genetic map. In a single meiosis, there is one crossover recombination per chromosome arm (Copenhaver et al., Proc. Natl. Acad. Sci. USA 95:247-252, 1998). To increase map resolution, panels of stocks have been developed that can accumulate detectable recombination per chromosome over several or many generations. Below is a description of the most commonly used genetic maps at MaizeGDB.

Genetic 2008: This is the current version of the composite genetic map developed and maintained by Ed Coe for the several decades. The first version, with some 60 loci, was published in 1935 by Emerson et al. (Cornell Univ Agric Exp Stn Memoir 180:1-83). In the 2008 version, with some 3775 loci, " Genes for which a function or phenotype is known are placed in relation to markers on the high-resolution IBM2 map; with sequenced BACs on the physical map; and by best estimates from other maps and other data where sufficient. The coordinates are approximately those of conventional centiMorgans. For each gene, the basis for map placement is given in a comment. Corrections or additions are invited. *Acknowledgment*: Many placements in this map derive from the fingerprinted contig [FPC] map aligned to the genetic map of the Maize Mapping Project (Wei et al., PLoS Genet. 3:e123, 2007); and from BAC sequence data that have been made available prior to publication, deposited in GenBank by the Maize Genome Sequencing Consortium." (E. Coe, personal communication to MaizeGDB). Thus, this map represents data from both defined mapping panels and crosses between many different inbreds, hybrids, and "mongrels". It also contains information from crosses where recombination occurred in the female parent or in the male parent or both. All recombination information used to place markers on this map can be found by clicking on the locus listed within the MaizeGDB map display. This map is continuously updated.

IBM2 (Intermated B73 x Mo17) Genetic Maps: This map is called "high resolution" because the method used to generate the mapping panel of nearly 300 recombinant inbred lines created more detectable recombinations per chromosome than previous methods [Lee et al. Plant Mol. Biol. 48:453-461, 2002; Sharopova et al. Plant Mol. Biol. 48:463-481, 2002]. To generate the mapping panel (Fig. 1), inbreds B73 and Mo17 were crossed to create the F1 hybrid. This was self-pollinated. F2 progeny were then intermated for four generations, followed by repeated selfing to generate Recombinant Inbred Lines (RILs). This type of panel is often referred to as Intermated Recombinant Inbred Lines (IRILs). The successive intermating crosses following F1 provided increased opportunity for recombination between linked loci. These recombinations remain detectable at any genomic position where B73 and Mo17 are polymorphic for the markers used in the subsequent mapping steps. The fact that these inbreds are so polymorphic relative to each other allows higher detection of crossover events. However, markers cannot be mapped any place in the genome where there is no polymorphism between Mo17 and B73.

After genotyping these IRILs with 2,046 markers, the Maize Mapping Project (MMP) constructed a genetic map (IBM2) that contains 2,026 markers (Coe et al., Plant Physiol. 128:9-12, 2002; Cone et al., Plant Physiol. 130:1598-1605, 2002). Markers that are ordered with strong statistical support are called "FRAME" markers. It is useful to keep in mind that the units on the IBM maps are not really centiMorgans.

IBM Neighbors: This description is taken directly from Cone et al. 2002 (see above). "We are implementing a “neighbors” map approach in which we extrapolate locations of loci from non- IBM maps to their nearest neighbors on the IBM map, such that the framework loci on the IBM serve as a fixed backbone onto which additional loci are added. To extrapolate, we look for shared loci on the two maps that define an interval containing a locus of interest, calculate the distance between the shared and target loci on the non-IBM map as a ratio of the distance for the interval, and use the ratio to estimate a map coordinate for the target locus in that interval on the IBM. In choosing which neighbors to extrapolate, we consider the depth of the genetic data and the confidence levels for locus assignment to the non-IBM map. The new map is called “IBM Neighbors.” "The key distinction between the IBM and IBM Neighbors maps lies in the confidence level of locus order; the IBM has fewer well-ordered loci and IBM Neighbors has more loci, but confidence in the order is lower." IBM Neighbors Frame maps are similar to IBM Neighbors, except that they only include the loci (markers) that were “frame” (or “skeleton” in ISU_IBM Map4 maps, see below) on the original, individual maps.

ISU_IBM Map4 was prepared by Fu et al. (Genetics 174:1671-1683, 2006), using a panel of 91 IBM lines, a subset of the 302 member panel used for IBM2. They mapped 1,329 new gene-based insertion-deletion polymorphism markers (called IDPs or indels) and 2,029 previously developed markers on the IBM map. This groups calls markers "skeleton" if there is excellent statistical support to order them accurately on the map, and "muscle" if support is adequate for approximate placements. Coordinates on these maps were corrected to represent meiotic centiMorgans.

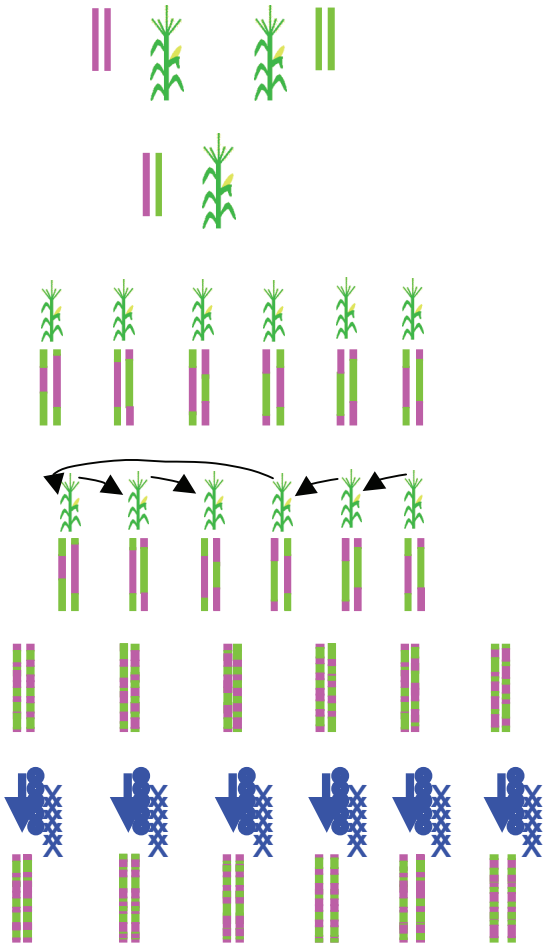

Figure1. Generation of IRILs, a special mapping panel.

LHRF Gnp2004 and IBM GNP2004: Falque et al. (Genetics 170:1957-1966, 2005), generated an IRIL panel from the cross F2 x F252, representing a set of inbreds useful for European maize breeders and permitting mapping a number of markers monomorphic on the IBM panel. In their paper, they state: "We built framework maps of 237 loci from the IBM panel and 271 loci from the LHRF panel. Both maps were used to place 1454 loci (1056 on map IBM_Gnp2004 and 398 on map LHRF_Gnp2004) that corresponded to 954 cDNA probes previously unmapped." Coordinates on these maps were corrected to represent meiotic centiMorgans.

Nested Association Mapping (NAM): The NAM panels are like RILs on steroids. Regarding the IBM map, recall that markers can NOT be genetically mapped in any region that is not polymorphic between B73 and Mo17. To overcome this, Yu et al. (Genetics 178:539-551, 2008) crossed B73 to 26 diverse maize lines (called "founders"). From the 25 F1s that were generated, a total of 5000 RILs where made, with 200 from each F1. The large number of diverse "founders" greatly increases the likelihood that most regions in the genome will be polymorphic in at least one mapping set, allowing markers to be mapped in those genomic regions. Excellent figures describing the process of generating the NAM panels are in the Yu *et al*. paper above. The Diversity Group (panzea.org) is mapping millions of SNP [single nucleotide polymorphism] markers using these lines. This will lead to a very high resolution genetic map and leverages the high throughput genotyping technology available for SNP.

Submitted by:
Lisa Harper
Mary Schaeffer
Taner Sen
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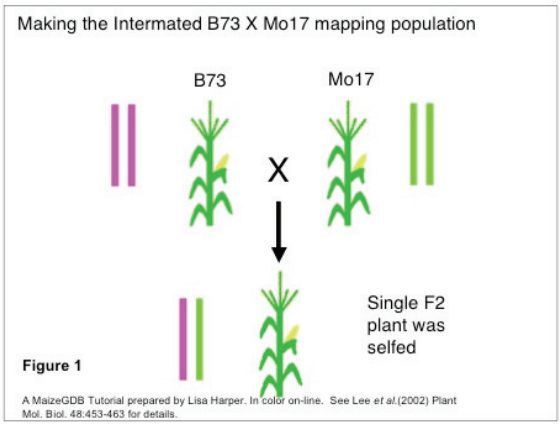
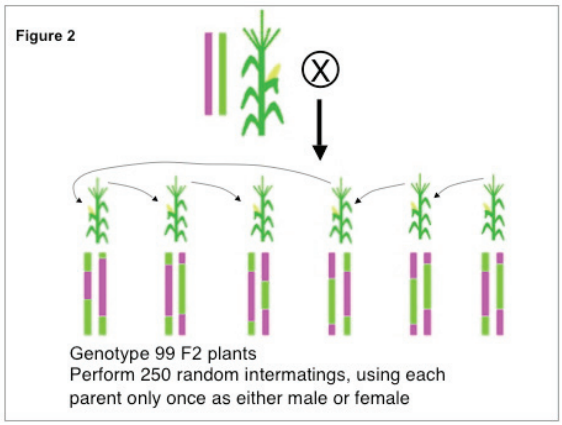
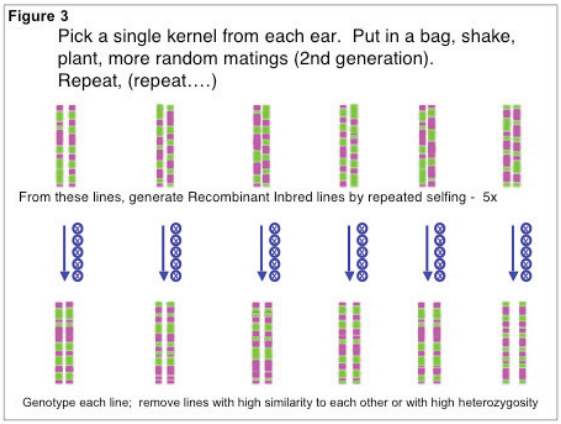
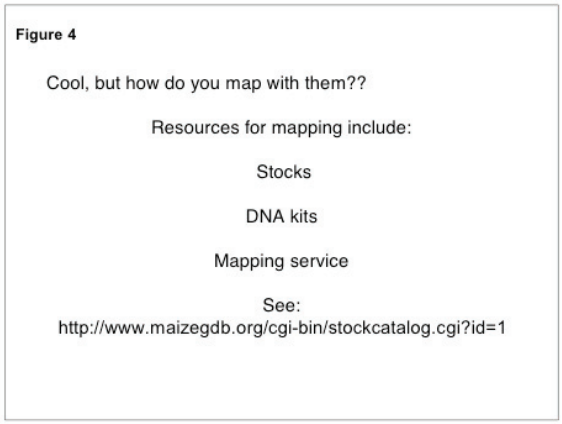
Carolyn Lawrence

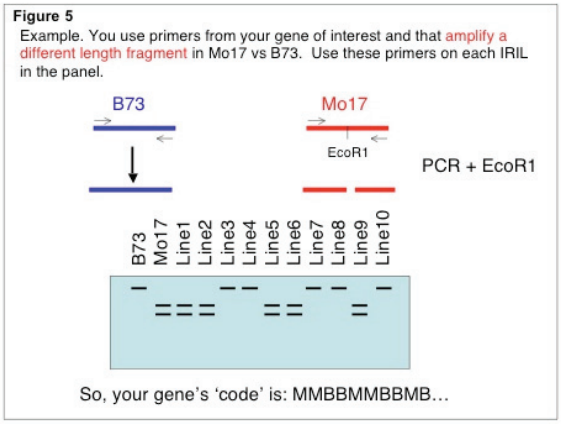
Mapping Tutorial at MaizeGDB

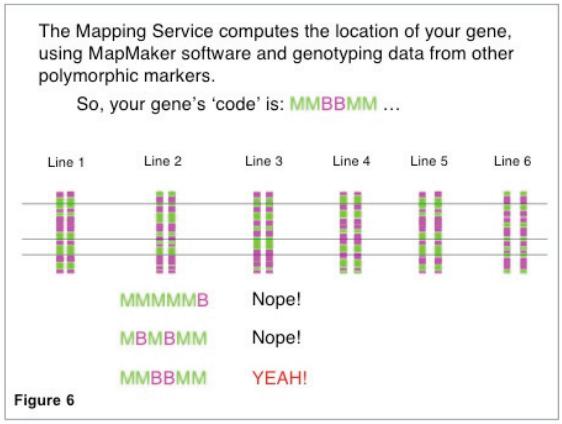












VI. MAIZE GENOME SEQUENCING PROJECTS

B73 Maize Genome Sequencing (www.maizesequence.org)

Washington University, St Louis, MO (lead institution); Cold Spring Harbor Laboratories, Cold Spring Harbor, NY; University of Arizona, Tucson, AZ; and Iowa State University, Ames, IA

As we approach the last year of the Maize Genome Sequencing Project, we are nearing completion of the initial genome sequence and analysis of maize B73. This includes a primary annotation set (working set genes based on repeat masked DNA), along with a secondary annotation set (protein level). These sets include 16,007 Phase 1 BACs of a total of 16, 625 chosen. At present, we may not necessarily know the order and orientation of all contigs within each BAC, but we do know order and orientation of each BAC in the tiling path. Fusheng Wei at the Arizona Genomics Institute has produced 10 pseudomolecules (chromosomes) along with pseudomolecule 0 (unassigned scaffolds). We also have generated compara gene trees with orthologue calls.

The maize browser, available at http://www.maizesequence.org provides public access to maize BACs and their underlying annotations. The website is tightly integrated with Gramene (http://www.gramene.org) and provides cross-linkage for comparative analysis with other cereal genomes. Mature (improved) BACs have been analyzed using an effective evidence-based gene build strategy in collaboration with Gramene that provides higher-quality gene models. Improved sequences have undergone peptide-based analysis, such as InterPro/GO, to provide greater context for gene models. The maize BAC sequence maps have been integrated with the FPC map. This provides a unified view of the physical and sequence map. Other data sets, such as the maize optical map (see below note), generated by the David Schwartz lab, and full-length cDNAs, provided by the Yeisoo Yu lab (http://www.maizecdna.org/) and Ceres (Alexandrov et al, 2009), have been integrated into the browser, as they became available.

There is still work to be done. Several BACs that only recently were chosen to fill gaps in the tiling path have to be finished and added to the annotation pipeline. As of May 8, 2009, 15, 818 BACs of the total of 16, 625 chosen are finished. This work is ongoing at the Genome Center at Washington University School of Medicine and at The Arizona Genome Institute. These data will undergo analysis and will be integrated into the genomic annotations in the browser.

A manuscript describing the preliminary analysis of the maize genome is in progress and, along with several companion papers, will be published in major journals soon. This will give the first comprehensive look from the genomic level at maize B73.

Alexandrov NN, Brover VV, Freidin S, Troukhan ME, Tatarinova TV, Zhang H, Swaller TJ, Lu YP, Bouck J, Flavell RB, Feldmann KA. Insights into corn genes derived from large-scale cDNA sequencing. Plant Mol Biol. 2009 Jan;69(1-2):179-94. Epub 2008 Oct 21.

Submitted by Sandy Clifton
Washington University, St Louis, MO

B73 Optical Map: A single molecule map of the maize genome

Shiguo Zhou[1], John Nugyen[2], Fusheng Wei[3], Shiran Pasternak[4], Doreen Ware[4], Rod Wing[3], Michael Waterman[2], and David C. Schwartz[1]
[1]University of Wisconsin-Madison, Madison, WI 53706; [2]University of Southern California, Los Angeles, CA 90089; [3]Unversity of Arizona,Tucson, AZ 85721; [4]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Maize is one of the most important crops in the U. S., while also being a venerable plant model system for genetic and cytogenetic investigation. Although a substantial body of genetic and cytogenetic studies have provided a global view of maize genome organization, there remains a vast treasure of fine-scaled genomic features awaiting discovery that will surely emerge from analysis of a fully sequenced genome. As part of this effort, we have constructed a genome-wide restriction map for maize using the well-established whole genome shotgun single molecule optical mapping approach [1-13]. Briefly, optical mapping constructs individual restriction maps from millions genomic DNA molecule (300 kb- 2 Mb) that have been stretched on charged surfaces. Automated fluorescence microscopy, coupled to machine vision converts images into data sets comprising high resolution restriction maps. These maps are then assembled in to contigs spanning an entire genome. Although the maize genome is notorious for harboring a complex and extensive panoply of repeats, complicating sequence assembly, such genomic structures are readily characterized by optical mapping because ~500 kb molecules are analyzed. Our optical map of maize genome is facilitating sequence finishing by providing dense restriction marker scaffolds for the ordering and orienting of nascent sequence contigs, the characterization of gaps, and the validation of sequence assemblies.

We constructed a genome-wide optical map of the maize inbred line B73 using SwaI, a methylation insensitive restriction enzyme. SwaI mapped DNA molecules were *de novo* assembled into 68 contigs, each larger than 3 Mb (30.94 Mb, average size) and with a total length of 2103.86 Mb. These optical map contigs span 91.47% of the maize genome (~2300 Mb)[14], with the largest contig spanning more than 100 Mb. A new algorithm was developed in order to utilize the unfinished BAC sequences (http://www.maizesequence.org/index.html release 3a.50) for integration of the maize optical maps with the iMap (FPC physical and Genetic map) [14-21]. This alignment

algorithm includes four steps: *i.* fragment matching – between optical map and sequence contigs; *ii.* BAC alignments – match graph for alignment to optical map contig; *iii.* FPC alignment – dynamic programming for alignment to optical map contig; *iv*. filtering the alignments – based on the colinearity between the alignment order of BACs on optical maps and their order on the FPC map. The algorithm placed 65 of the 68 optical map contigs onto the maize iMap (2082.28 Mb). This result indicates that our optical map is largely congruent with the maize iMap, which in turn cross-validates the BAC physical map resource for maize genome sequencing efforts. Furthermore, as all the anchored optical maps span multiple FPC contigs, gaps between FPC contigs are now estimated. However, we do see multiple regions with conflicting optical map/iMap alignments indicating that some FPC contigs are not correctly placed, or belong to different chromosomes. Further analysis is needed for solving these issues. As most of the BAC sequences in this release comprise of multiple unordered sequence contigs, and some of the BAC sequence contigs can be anchored on optical maps, this also may help finish the BAC sequence assembly. In conclusion, our maize whole genome optical map will be an important resource for finishing maize genome sequence and closing gaps. The finished B73 map/sequence will become a reference for other studies aimed at finding structural differences in other lines, cultivars or varieties of maize.

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2. Lai, Z. et al., *A shotgun optical map of the entire Plasmodium falciparum genome.* Nat Genet, 1999. **23**: p. 309 - 313.
3. Lim, A. et al., *Shotgun optical maps of the whole Escherichia coli O157:H7 genome.* Genome Res, 2001. **11**(9): p. 1584-93.
4. Zhou, S. et al., *A whole-genome shotgun optical map of Yersinia pestis strain KIM.* Appl Environ Microbiol, 2002. **68**(12): p. 6321-31.
5. Zhou, S. et al., *Whole-genome shotgun optical mapping of Rhodobacter sphaeroides strain 2.4.1 and its use for whole-genome shotgun sequence assembly.* Genome Res, 2003. **13**(9): p. 2142-51.
6. Zhou, S. et al., *Shotgun optical mapping of the entire Leishmania major Friedlin genome.* Mol Biochem Parasitol, 2004. **138**(1): p. 97-106.
7. Valouev, A. et al., *Alignment of optical maps.* J Comput Biol, 2006. **13**(2): p. 442-62.
8. Valouev, A. et al., *Refinement of optical map assemblies (original paper).* Bioinformatics, 2006: p. 1217-1224.
9. Valouev, A. et al., *An algorithm for assembly of ordered restriction maps from single DNA molecules.* Proc Natl Acad Sci U S A, 2006. **103**(43): p. 15770-5.
10. Zhou, S. et al., *Validation of rice genome sequence by optical mapping.* BMC Genomics, 2007. **8**: p. 278.
11. Zhou, S., J. Herschleb, and D.C. Schwartz, *A single molecule system for whole genome analysis*, in *New Methods for DNA Sequencing*. 2007, K.R. Mitchelson,Editor, Elsevier B. V.:Amsterdam. p. 269-304.
12. Ananiev, G.E. et al., *Optical mapping discerns genome wide DNA methylation profiles.* BMC Mol Biol, 2008. **9**: p. 68.
13. Kidd, J.M. et al., *Mapping and sequencing of structural variation from eight human genomes.* Nature, 2008. **453**(7191): p. 56-64.
14. Wei, F. et al., *Physical and Genetic Structure of the Maize Genome Reflects Its Complex Evolutionary History.* PLoS Genet, 2007. **3**(7): p. e123.
15. Fang, Z. et al., *cMap: the comparative genetic map viewer.* Bioinformatics, 2003. **19**(3): p. 416-7.
16. Fang, Z. et al., *iMap: a database-driven utility to integrate and access the genetic and physical maps of maize.* Bioinformatics, 2003. **19**(16): p. 2105-11.
17. Cone, K.C. et al., *Genetic, physical, and informatics resources for maize. On the road to an integrated map.* Plant Physiol, 2002. **130**(4): p. 1598-605.
18. Coe, E. et al., *Access to the maize genome: an integrated physical and genetic map.* Plant Physiol, 2002. **128**(1): p. 9-12.
19. Gardiner, J. et al., *Anchoring 9,371 maize expressed sequence tagged unigenes to the bacterial artificial chromosome contig map by two-dimensional overgo hybridization.* Plant Physiol, 2004. **134**(4): p. 1317-26.
20. Nelson, W.M. et al., *Whole-genome validation of high-information-content fingerprinting.* Plant Physiol, 2005. **139**(1): p. 27-38.
21. Yim, Y.S. et al., *Characterization of three maize bacterial artificial chromosome libraries toward anchoring of the physical map to the genetic map using high-density bacterial artificial chromosome filter hybridization.* Plant Physiol, 2002. **130**(4): p. 1686-96.

Mo17 Genome Sequencing (Editors' Note)

Mo17 genome shotgun sequence reads by 454 sequencing have been aligned to the B73 BAC sequence, and are freely available at www.phytozome.org. This project was carried out by the DOE Joint Genome Institute, UC Berkeley. These alignments have been incorporated into the MaizeGDB Genome Browser, representing SNP, insertions and deletions.

VII. MAIZE GENETICS CONFERENCE

Maize Genetics Executive Committee Report at the 51st Maize Genetics Conference, St. Charles, IL March 2009.

Slides 2 and 4 from the online powerpoint http: http://www.maizegdb.org/mgec.php

Activities 2008-2009.
Accepted responsibility for organizing the maize session at PAG (Plant and Animal Genome meetings)
Partnered with the National Corn Growers (Pam Johnson) to ensure funding for the NSF-PGRP
Discussed the future of the NSF-PGRP with James Collins, assistant director for biological sciences at NSF
Corrected public record, in the ASPB Newlsetter, regarding publication productivity of the maize community

Activities Planned 2009-1010
Create an outreach slide set that highlights the impact of public-sector investments in maize genetics research
Prepare meeting report for funding agencies (strategy to collect metrics in the future) [e.g., funding sources for the research to be presented at the annual Maize Meeting, can be entered on forms for submitting abstracts. (editors' note, from MGEC verbal report at Maize Meeting)]
Identify community priorities for future public-sector investment

Please see inside front cover for current membership of the committee, which includes a newly created Asian representative appointed by the committee.

Maize Genome Annotation Workshop and Panel Discussion with Cooperators

Maize Meeting Mar 13, 2009 St. Charles IL

Mike Muszynski chair; Panel Members Carolyn Lawrence, Jeff Bennetzen, Yan Fu, Jim Uphaus, Volker Brendel, and Gernot Presting

www.maizegdb.org/POPcorn/annotation_forum.php

Outline of report

Executive Summary
Meeting's Context of the Panel Discussion
Rough Transcript of the Panel Discussion
Feedback Gathered Subsequent to the Discussion

Executive Summary

The maize genome sequencing projects are complete or nearly complete, and the next order of business is to assign gene structure and functional annotation as well as other data (e.g., cytological positions of centromeres, etc.). For the group sequencing B73 (the reference sequence), part of the deliverables are the annotations (estimated project completion date: February 2010). At the same time, various other groups are also annotating or plan to annotate. A panel of six drawn from broad disciplines was assembled to discuss annotation in the broadest sense, but the discussion focused on annotation of genes (both structure and placement) with emphasis on how individual researchers could contribute to the annotation.

Meeting's Context of the Panel Discussion

Questions to be asked during the panel discussion were submitted by maize cooperators and may be viewed online via MaizeGDB at http://shrimp1.gdcb.iastate.edu/mm2009/question.php.

Genome Sequences: What's New (Chair, Mike Muszynski)
Doreen Ware, Sequence and Analysis of the Maize B73 Genome
Dan Rokhsar, Update on the Mo17 Genome Sequencing Project
Octavio Martinez de la Vega, The Characterization of the Palomero Toluqueño Genome
Dinner
Community Forum on Gene Annotation (Chair, Mike Muszynski)
Pam Johnson from NCGA, Research and the Recession
Volker Brendel, Community Annotation at MaizeGDB/PlantGDB
Discussion with Panel Members Carolyn Lawrence, Jeff Bennetzen, Yan Fu, Jim Uphaus, Volker Brendel, and Gernot Presting

Rough Transcript of the Panel Discussion

Jeff Bennetzen: short introduction suggesting some next endeavors for the community: zeanomics, more genomes (999), hypothesis testing in the form of functional biology, promote maize as the model.

Carolyn Lawrence: MaizeGDB offers coordination. For example, the tracks in the MaizeGDB Genome Browser are generated by the community. A problem, however, is that these are based on different GenBank releases which causes problems with aligning track content relative to a particular BAC. MaizeGDB could house quarterly releases. Would this be helpful?

Audience Member: Will MaizeGDB Genome Browser provide links to NCBI and vice versa?

Brian White-Smith: Links from NCBI's Entrez to MaizeGDB are being worked on now. Notes that 600 out of 20,000 genes from mRNAs are in GenBank. Will use FL-cDNA from B73. All RefSeq entries are being updated to use the B73 sequence as the exemplar.

Dan Rokhsar: Likes the idea of quarterly releases, but isn't maizesequence.org already doing this?

Carolyn: It isn't clear this is happening; if it is, it isn't well advertised. Certainly we would prefer this be handled by maizesequence.org.

Doreen Ware: Releases are generated, trying for quarterly. The process will stay in flux for a few more months. Note on version names: latest release is 3b.50. The 'b' indicates an update of the annotation, '.50' is the Ensembl version. The assembly was not changed. An update is now or shortly will be in progress. The group is trying to maintain mappings across releases where there are no changes but this is usually not possible. Doing 6 month updates with Gramene. Recommends staying with quarterly updates for now.

Carolyn: Need to know when releases are coming up so that annotation groups and the community at large can plan. Currently maizesequence.org announces a release and makes available the GenBank freeze date, but does not publicize the freeze date/data in advance.

John Fowler: Requested a web page of cautions, e.g. outlining the different GenBank releases, flipped contigs, and common gene annotation errors to watch for. Would it be possible for MaizeGDB to put up a page of warnings?

Carolyn: Yes, we can do that. In addition, Lisa Harper can make a movie showing "scary things".

Taner Sen: My talk describing the MaizeGDB Genome Browser functionality included a page of cautions that can be used to seed the MaizeGDB page/movie.

Audience Member: What about gene expression in spatial and temporal scales? Arabidopsis has nice data sets and viewers. What tools are coming for maize?

Volker Brendel: Don't have answers for how to handle all the different types of data. Consider revisiting DAS (Distributed Annotation System). This has been available for a while but has been problematic. Recommends trying to revive this type of data sharing. Some groups are using Google Maps for visualizing. This will help but nothing available now is sufficient.

Fusheng Wei: How often […what?...] given that data changes daily?

Carolyn: Suggests setting up a forum and putting a page on MaizeGDB: if you are interested, get involved.

Pat Schnable: We could have students help with annotation, but will need to test their accuracy and create a set of standards.

Anne Sylvester: Issues are not new; how much communication has there been with other groups tackling the same issues? What about iPlant?

Volker: There is an iPlant meeting on annotation coming up in St. Louis. (http://iplantcollaborative.org/about-ipc/education-outreach-and-training)

Brian: On the subject of community annotation: NCBI has strict requirements before anyone is allowed to contribute annotation, including publications and wet lab experience. If the pseudomolecules were submitted to GenBank, RefSeq could be used to provide first pass on gene models and these could made available to MaizeGDB.

Carolyn: Does updating RefSeq annotation require wet lab experimentation and a peer reviewed publication?

Brian: Yes.

Doreen: Advocates for community annotation. However, other groups have done this but not had much success. How about an "annotation jamboree" right before the Maize Meeting? Success will depend on the maize community. Capturing meta-data is difficult but important. Start making an effort now, use ontologies even if they are imperfect. Meta-data must be computer-readable as well as human-readable. It's important to learn what didn't work in previous efforts at community annotation.

Audience Member: 1) It is difficult to find and keep one's place in the genome browsers. Can we see a genetic map alongside the MaizeGDB Genome Browser's representation of the genome? 2) What happens to community annotation when the build changes?

Carolyn: Keeping the linkage map open while browsing is a technical issue. Regarding the fate of community annotation after a new release, not sure what the answer is.

Dan: An online forum at MaizeGDB is a good idea. Gave history of annotation efforts on human genome: three competing groups with incompatible data finally got together and found a core set of genes they could agree on and publicized a list of those they did not agree on. Errors found in automated annotation helped improve the automated process.

Brian: More history: when there was a new build of the human genome, genes would disappear. NCBI has a web page that highlights genes the three annotation groups can't agree on. GenBank could do the same for maize. Described method for generating RefSeqs for human. Suggests waiting on gene models until sequence assembly is more solid.

Jeff: A tool is needed now, and we can't wait for a perfect assembly. For one thing, we can use community annotation to get an idea of how good the automated process is. Noted that the first release of the rice genome had a 50% error in gene calls, which could have been easily discovered before the release. NCBI requirements for annotation privileges are not compatible with distributed annotation and it is unreasonable to let GenBank create annotation based upon a computational assessment then require that to fix it the researchers to wet lab experimentation. Suggest that the maize community doesn't need to follow NCBI's practices and that creating a RefSeq for maize could be a problem.

Audience Member: Are annotation jamborees feasible? Has this been done?

Jeff: This has been done to provide a 1st round of quality annotation.

Panel Member: Computers can't match human eyes. There needs to be an incentive or enforced requirement to annotate, then deposit the annotation in MaizeGDB. How? Require this for publication? For NSF funding? Cost must have a benefit. Who would coordinate this? Perhaps there could be coordinators by area (transposons, retrotransposons, etc.).

Yan Fu: Regarding gene prediction: numbers (quality scores) should be available to show how reliable the predictions are.

Carolyn: In the real world carrots don't work. Sticks work. Treat annotation like a lab task: clean up after yourself or else. Example is lab worker assigned to keep a scale clean.

Toni Kazic: Quality of annotation will depend on the quality of tools. It should be easy to find and compare annotation. Even the availability of free text typing fields will help.

Jeff: Reading a pre-submitted question: "Will there be a curated repeat database for maize? It is needed soon." Answer: we have this and it will be available soon.

Audience Member: Comment: we could use undergraduates, but they will need training.

Anne: Tutorials exist but need to be advertised.

As Prepared for the Maize Genetics Executive Committee
Notes taken by Ethalinda Cannon
Inputs from Panel Members

Feedback Gathered Subsequent to the Discussion (contributors include Dan Rokhsar, Doreen Ware, Volker Brendel, Mike Muszynski, Carolyn Lawrence)

It should be noted that some were expecting a broad discussion of the future of maize genetics and this panel and the audience focused immediately on gene structure annotation within the context of community annotation. This is not bad, it's just not what everyone expected.

It was noted that PIs on PGRP grants recently discussed similar issues at their annual meeting in Washington DC.

Folks thought the discussion was good, interesting, and they got an idea of the status of genome sequencing. The update and panel discussion was very useful to help inform the general community regarding the context of the genome and how this changes over time. But it was felt no long-term solution(s) would come from the session.

The community appreciates that the B73 project has made sequence data available from the beginning so that the data could be used as soon as possible. With the rapid release there have been some frustrations associated with the instability, but these frustrations are comparatively small relative to the progress made based on immediate access to the data.

The community needs to be better informed and using MaizeGDB as a mechanism for creating a forum makes a good deal of sense. Many of the questions submitted by the community in advance of the discussion (available via MaizeGDB at http://shrimp1.gdcb.iastate.edu/mm2009/question.php) were not addressed, and the forum would be a good place to post those questions and let the sequencing and annotation groups address the questions.

We need to have definitions of what people have already done, both the genomics and materials and methods. For example, it is very important to have more information about the exact accessions for the lines that were sequenced. Researchers work on specific lines and need to know how their work fits in with the sequenced maize genomes.

There is a great deal of good will among those interested in annotating, and having NCBI involved is useful given their central role. However, the fact that researchers would have to do wet lab biology and publish a peer-reviewed manuscript in order to fix a RefSeq annotation by NCBI causes all researchers polled (conversationally) to be against NCBI doing the only annotation. Nonetheless, there is a critical need for subsequent experimental validation that supports both functional and structural annotations.

In order for fixes to the sequences via any group to be incorporated at NCBI, the community database MaizeGDB needs to be listed as authors on the B73 and Mo17 sequence records. Due to NCBI's ownership and update rules, this would allow the community to own and update the sequence prior to the projects' close.

There is a hope that an annotation working group could be formed.

There is a desire expressed after the session by many (but not all) researchers that there be a funded project to annotate the maize genome (B73) via professional curation. This would likely require a RFP and subsequent proposals, etc.

Funding opportunities to develop serious student involvement in annotation are desired. There are a few undergraduate institutions where genome annotation has been incorporated into classes and/or summer research programs (Buckner – Truman State, Gray – Univ. of Toledo). Perhaps these can be a model for encouraging other institutions to annotate the genome. Can increased funding help build a larger network of these institutions to do this in a coordinated fashion?

An annotation jamboree would go a long way toward annotating the genes and gene structures, but it would need to be uncoupled from the Maize Meeting given that the meeting will be in Italy next year. In addition, working with Robin Buell to find out whether annotation jamborees were genuinely useful for rice would be helpful.

> Related detail on rice learned from Pankaj Jaiswal: Three Rice Annotation Project (RAP) jamborees were held in collaboration with the DDBJ (Takeshi Gojobori and Takeshi Itoh). Experts from databases (e.g., GenBank, Uniprot, EBI, Swissprot, Gramene, IRRI and others) and students from local and rice sequencing consortia labs (IRGSP) convened for weeklong workshops to annotate structures for a selected set of genes with known evidence (ESTs and/or FLcDNA from maize and/or known genes from related and angiosperm plant models). The majority of annotations confirmed computational predictions and only a few involved actual changes to the gene structure. DDBJ did similar workshops for human genome annotations. In the case of the rice #1 RAP jamboree, about 23K genes were manually validated by human eyes and brains. For the annotation of *Saccharomyces*, there were jamborees and in addition to annotation, functional assignment projects were in place where PIs were requested to take one/multiple batches of 5 genes through thorough analysis (expression, localization, proteomics, biochemistry and KO/mutant-phenotype). This was quite successful.

Thinking on the rice model of annotation, it should be noted that there were two assemblies and sets of annotations, and those groups were never able to collaborate and consolidate their findings. We do not want to see this happen in maize. The model of three groups working together in human and consolidating their datasets is a much more attractive target.

Related: If various groups are annotating sequence, the Sequence Ontology absolutely must be used by all participating groups.

The various projects absolutely must publish their freeze and release dates and make those available prior to when the freeze and release occur.

Related: Community annotation would be particularly useful to sort out the poorly annotated regions of the genome. However community annotation needs to be focused and coordinated if it is to produce.

Timelines for when the sequencing projects will actually be done and when the data will transition to MaizeGDB would be most helpful.

Related: The B73 Maize Genome Sequencing Project is funded until February of 2010. The Ware group reports that they will continue to work on improving annotations until that time and plans to continue to host the browser at www.maizesequence.org for a short time after the project ends. Their plan is to fold the maize genome into the Gramene resource.

How is data from new transcriptome analyses going to be collected and used to improve the genome? Multiple groups could conceivably take this approach but how can their data be integrated?

There is a critical need to be able to identify paralogs within maize and orthologs with other species. Standardized nomenclature would be needed to underpin this type of information.

While the Gene Ontology (GO) is excellent for getting gene function, it would be also useful to have other functional information captured (that is not handled by GO) - traits and expression data are obvious ones. Long term we need to be thinking about how to integrate this data creating linkages among the various ontologies must be formed (e.g. between GO and the Trait Ontology). This is something that the maize community could really take advantage of.

Question from session chair Mike Muszynski that was not asked:
Is there a mechanism where community annotators can provide the data to help order and orient sub-BAC fragments? Several genes (e.g. *knotted1, ZMM4, ZMM15*) that have been published and are in GenBank are in several pieces within a BAC contig in the wrong order and orientation. If these are identified by a user/annotator, how can the information be relayed to correct the order of sub-BAC fragments?

Related: MaizeGDB is working with Fusheng Wei and the Maize Genome Sequencing Consortium to find out whether and how to create a forum that would allow this via MaizeGDB (similar to the forum used for this session where researchers could submit questions).

Dan Rokhsar has generated a list of items that must be addressed that should be distributed, potentially via MGEC. He plans to follow up with MGEC separately.

VIII. RECENT MAIZE PUBLICATIONS

(This is not intended to be a complete list. Publications listed are primarily from searches at PUBMED, which occasionally misses maize articles even for journals listed as being indexed. Articles from some journals, e.g., Maydica and Crop Science, were independently compiled by the editor.)

Abbott, A; Schiermeier, Q, 2007. Showdown for Europe. Nature 450:928-929. PUBMED id: 18075535.

Adetimirin, VO; Vroh-Bi, I; The, C; Menkir, A; Mitchell, SE; Kresovich, S, 2008. Diversity analysis of elite maize inbred lines adapted to West and Central Africa using SSR markers. Maydica 53:143-149.

Agbaje, SA; Badu-Apraku, B; Fakorede, MAB, 2008. Heterotic patterns of early maturing maize inbred lines in Striga-free and Striga-infested environments. Maydica 53:87-96.

Aguilar-Martinez, JA; Poza-Carrion, C; Cubas, P, 2007. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell 19:458-472. PUBMED id: 17307924.

Aitken, KS; Hermann, S; Karno, K; Bonnett, GD; McIntyre, LC; Jackson, PA, 2008. Genetic control of yield related stalk traits in sugarcane. Theor. Appl. Genet. 117:1191-1203. PUBMED id: 18719878.

Ajala, SO; Odiyi, AC; Thé, C; Olaoye, G, 2008. Population cross diallel of maize genotypes with varying levels of resistance to the pink stalk borer (Sesamia calamistis: Hampson) and the sugarcane borer (Eldana sacharina: Walker). Maydica 53:79-86.

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Alexandrov, NN; Brover, VV; Freidin, S; Troukhan, ME; Tatarinova, TV; Zhang, H; Swaller, TJ; Lu, YP; Bouck, J; Flavell, RB; Feldmann, KA, 2009. Insights into corn genes derived from large-scale cDNA sequencing. Plant Mol. Biol. 69:179-194. PUBMED id: 18937034.

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Aluru, M; Xu, Y; Guo, R; Wang, Z; Li, S; White, W; Wang, K; Rodermel, S, 2008. Generation of transgenic maize with enhanced provitamin A content. J. Exp. Bot. 59:3551-3562. PUBMED id: 18723758.

Amarillo, FI; Bass, HW, 2007. A transgenomic cytogenetic sorghum (Sorghum propinquum) bacterial artificial chromosome fluorescence in situ hybridization map of maize (Zea mays L.) pachytene chromosome 9, evidence for regions of genome hyperexpansion. Genetics 177:1509-1526. PUBMED id: 17947405.

Ananiev, EV; Wu, C; Chamberlin, MA; Svitashev, S; Schwartz, C; Gordon-Kamm, W; Tingey, S, 2009. Artificial chromosome formation in maize (Zea mays L.). Chromosoma 118:157-177. PUBMED id: 19015867.

Andersen, JR; Zein, I; Wenzel, G; Krutzfeldt, B; Eder, J; Ouzunova, M; Lubberstedt, T, 2007. High levels of linkage disequilibrium and associations with forage quality at a phenylalanine ammonia-lyase locus in European maize (Zea mays L.) inbreds. Theor. Appl. Genet. 114:307-319. PUBMED id: 17123062.

Angelini, R; Tisi, A; Rea, G; Chen, MM; Botta, M; Federico, R; Cona, A, 2008. Involvement of polyamine oxidase in wound healing. Plant Physiol. 146:162-177. PUBMED id: 17993545.

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Asakura, Y; Bayraktar, OA; Barkan, A, 2008. Two CRM protein subfamilies cooperate in the splicing of group IIB introns in chloroplasts. RNA 14:2319-2332. PUBMED id: 18799595.

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Badu-Apraku, B; Fakorede, MAB; Fontem Lum, A, 2008. S1 family selection in early-maturing maize populations in striga-infested and striga-free environments. Crop Sci. 48:1984-1994. Doi: 10.2135/cropsci2007.11.0639.

Badu-Apraku, B; Fontem Lum, A; Fakorede, MAB; Menkir, A; Chabi, Y; The, C; Abdulai, M; Jacob, S; Agbaje, S, 2008. Performance of early maize cultivars derived from recurrent selection for grain yield and Striga resistance. Crop Sci. 48:99-112. Doi: 10.2135/cropsci2007.01.0060.

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IX. SYMBOL INDEX

y-vp*-85-3572-30 50
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X. AUTHOR INDEX

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