MAIZE GENETICS COOPERATION

NEWSLETTER

74

April 15, 2000

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

Remembering

Li Jing Xiong (syn. Li Ching Hsiung)

Earl B. Patterson

Cooperators

(see memories following the Table of Contents)

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ISSN 1090-4573

CROP SCIENCE

Volume 39 January–February 1999 Number 1

PERSPECTIVE

Li Jing Xiong (C.H. Li)—Functioning in Adversity

S. S. Chase*

ABSTRACT

Li Jing Xiong (syn. Li Ching Hsiung), member of the Chinese Academy of Sciences (1980), president of the Crop Science Society of China (1984), Chief Scientist, basic agronomy, National Basic Research Program (1992), Cornell University Ph.D. (1948), best known among his American associates as C.H.Li, died on 28 June 1997 at Beijing after a prolonged illness. Dr. Li's direct and personal favorable impact on the food supply of the Chinese people came through two major actions and in a number of contributory ways: first, his sturdy opposition to Lysenkoism and to a compromise with the Chinese Lysenkoists, and second, and only possible with the first contribution, the development of maize (*Zea mays* L.) hybrids of superior yield; third in importance, a series of minor contributions from his early work on wheat-rye hybrids (triticale, × *Triticosecale* Wittmack), Setaria millet [*Setaria italica* (L.) P. Beauv.], sorghum [*Sorghum bicolor* (L.) Moench] hybridization, hybrid maize technology, and specialty maize such as quality protein hybrids. In addition, in his early years, he made contributions to understanding the cytogenetics of maize. And, in spite of formal difficulties, Professor Li was always an honored teacher to his colleagues and associates.

LI JING XIONG (syn. Li Ching-Hsiung), member of the Chinese Academy of Science (1980), president of the Crop Science Society of China (1984), Chief Scientist, basic agronomy, National Basic Research Program (1992), known best among his American associates as C.H. Li., died on 28 June 1997 at Beijing after a prolonged illness. There are few among us who have functioned so well in adversity and made such substantial contributions as this modest, well-beloved man.

Born 20 Oct. 1913 at Fuzhou, Jiangsu Province, China, C.H. Li received his B.A. in 1936 from Zhejiang University where he remained an additional year as a teaching assistant before moving on in April 1937 to join Prof. H.W. Li's team of cytogeneticists at Wuhan University in central China (H.W. Li had been a graduate student of Dr. R.A. Emerson at Cornell in 1929 and was a close friend of Dr. G.W. Beadle). At Wuhan, C.H. Li demonstrated his interest and competence in research by undertaking, as his first cytological study, an investigation of gene-controlled development of abnormal pollen mother cells in maize.

Wuhan University personnel removed themselves westward in advance of invading Japanese troops after the outbreak of the Sino-Japanese War on 7 July 1937. Prof. H.W. Li decided to accept a position in Sichuan Province as chief agronomist in charge of cereal improvement in the war zones and asked his assistants, of whom our C.H. Li was one, to go there as a group. When the group left Wuhan on 1 Dec. 1937, C.H. Li carried with him a key part of his research material, a first generation wheat-rye hybrid seedling dug from the nursery. This progenitor triticale seedling in its small pot was carried by C.H. Li on foot across the upper Yangtze River and the hilly mountain region beyond for over a month before it could be replanted at the Sichuan Agricultural Improvement Institute.

During the Sino-Japanese War and subsequent World War II, the small group of plant cytogeneticists in West China consisting of H.W. Li, C.H. Li, and W.K. Pao played an important role in strengthening agricultural technology. As early as 1938, C.H. Li initiated work on induction of polyploidy in crop plants by use of colchicine.

Li came to the USA in November 1944, holding a research assistantship granted by Dr. L.J. Stadler of the University of Missouri. From Missouri, Li moved first to the University of Minnesota to work with Dr. C.R. Burnham and then to the California Institute of Technology to work with Dr. E.G. Anderson. He then enrolled at Cornell University in August 1945, where he held a research assistantship and pursued his research under the direction of Dr. L.F. Randolph. (There, returning from military service, it was my good fortune to share an office with Li.)

Upon completion of his doctoral research in 1948, he was asked by Dr. Randolph to participate in the USDA research project on the cytogenetic effects of the Bikini atomic bomb radiation on exposed maize.

Before returning to China in September 1948, Dr. Li

P.O. Box 193, Chase Road, Shokan, NY 12481. Received 18 May 1998. *Corresponding author (sschase@aol.com).

Published in Crop Sci. 39:1–3 (1999).

spent two additional months at the California Institute of Technology as a post-doctoral scientist to enable him to study a pericentric inversion of maize chromosome 9 that he had found in his doctoral research material.

Upon his return to China, Dr. Li joined the Agricultural College of Tsing Hua University at Beijing in November 1948, just 6 wk before the Chinese Communist takeover of that city. After "liberation," the several agricultural colleges of the region were reorganized as the Beijing Agricultural University.

Li's expectation upon return to China had been that he would be able to pursue a career in teaching and research as a professor of cytogenetics. However, the period from 1949 to 1956 proved to be a most difficult time for western-trained biologists who wished to pursue studies of genes and chromosomes and to teach genetics. This was the period during which the Russian authoritarian genetic dogma, Lysenkoism, was imposed and prevailed in China, adversely affecting biological sciences in general, and, most severely, those engaged in agriculturally related genetic research.

During the ideological remolding campaign for intellectuals in 1952, biologists, especially western-trained geneticists, were criticized. Li's response was, for the time, to "forget about genes and chromosomes," to avoid formal teaching of genetics, to give up publication, and, without stressing his goal of maize improvement through inbreeding and hybridization of inbred lines, to concentrate on his second choice—practical maize (corn) breeding. (The Lysenkoist dogma held inbreeding to be "bad." Li told me later, with a twinkle in his eyes, "I gave myself permission to inbreed.") Many of his academic colleagues during this period simply discontinued all professional activity. Another Li, the well-known population geneticist, C.C. Li, a conspicuous target, found it prudent to flee China.

In 1956, a break came. From 1949 through 1956 the prevailing policy in China had been to "learn from the Soviet Union." In 1956, Chairman Mao Zedong declared a new policy: "Let one hundred flowers bloom, let one hundred schools of thought contend." A key reason for declaring this new policy was the "genetics question." Mao Zedong and Zhou Enlai appointed a number of party members, not themselves geneticists, to investigate the Russian genetic literature. This committee reported that there were indeed serious problems. The committee suggested that a conference be held, the Qingdao Symposium of 1956, during which the "genetics question" would be fully examined in "free debate."

As a participant in that symposium, Dr. Li, at considerable professional and personal risk, took a key role in the ensuing debate. There had been much argument for effecting a compromise between "the contending schools." According to the report of the Qingdao Symposium, a few of the participants spoke out strongly, and effectively, against any sort of merger. One was Li, the others were Sheng Zujia, the microbiologist, and Wu Zhongxian, the animal geneticist. Dr. C.H. Li said, "there could be no compromise." Freely translated, he asked, in effect, "What precisely do you mean by compromise? That we accept half of Lysenko and let them accept half a gene? In all natural sciences, there is only one language. And there is only one truth. I don't want to fight, but I don't understand what you mean by compromise."

The symposium marked the end, not of Lysenkoism but of the Lysenkoist monopoly in China, and the end of the ban on genetics. Though the "two schools" coexisted for many years after 1956, Lysenkoists individually continued, and continue today, though not as doctrinaires, to hold powerful positions in agriculture and general biology. Soon after the meeting, a withering of direct support for a Lysenkoist "science" occurred.

Dr. Li's highly effective work on improvement of maize for China was both simple in approach and efficient in execution. From the start, he understood the breeding methods and advantages of the inbred-hybrid system of maize improvement undertaken so effectively in the USA; he knew that much of the northeastern agricultural soils and climate of China were very similar to the American "Corn Belt;" he had brought back with him, in 1948, a collection of the then important U.S. inbred lines and in subsequent years was able through friends in the USA to obtain additional elite inbreds and other useful breeding materials. From this base and with the complementary addition of indigenous Chinese corn cultivars from which inbred lines were subsequently developed, Dr. Li was able to breed a series of highly productive maize hybrids for China. Initially, these were double cross hybrids. Then, as in the USA, single cross hybrids came to the fore. Li exploited the Reid-Lancaster heterotic pattern, using first the Connecticut inbred C103 developed by Donald Jones as a key parent and then, when it became available, the Missouri inbred Mo17 developed by Marcus Zuber, crossing these with Chinese Reid-type inbreds of his own breeding.

To a remarkable degree, the initial improvement of maize yields through heterosis breeding in China was due solely to the efforts of Dr. Li and the small group of younger scientists he trained. During these early years, he was often invited to make on-the-spot inspections and to give training courses on breeding and seed production. Meanwhile, Dr. Li generously distributed elite inbred lines and basic breeding materials upon the request of plant breeders in other research institutes. In consequence, superior maize hybrids and efficient breeding and seed production techniques were popularized successfully throughout China.

The first group of Dr. Li's hybrids, double crosses bearing Nuna numbers (Agricultural University numbers) came into use in the early 1960s. Genetic studies on cytoplasmic male sterility were carried on in parallel, leading to gains in hybrid seed production efficiency. By 1965, 333 000 ha of these hybrids were planted in Shanxi Province alone.

In 1966, an epidemic of northern leaf blight blasted the corn crop, affecting both the old open-pollinated cultivars and many of the new hybrids. This led to the second phase of Dr. Li's breeding efforts, with a stronger focus on breeding for disease resistance. It took Dr. Li

and his associates another 8 or 9 yr to develop the elite single cross hybrid, Zhongdan No. 2, which was characterized by its good general performance, wide adaptability, and high resistance to both northern and southern leaf blights as well as head smut, a disease that can be very damaging to maize in cold, dry regions of China. Since its release to farmers during the period 1977 to 1988, Zhongdan No. 2 was widely planted—2 million hectares in 1986 alone. The total increment of maize grain gain over local check varieties for this period amounted to 11.73 million megagrams. A First Class Invention Prize was awarded to Dr. Li and his associates in 1984 for development of this hybrid.

Other phases of Dr. Li's activities, briefly stated, include the following.

1. The development of a high lysine maize hybrid, Zhongdan No. 206, which was planted to about 28 600 ha in 1989, with the grain used primarily as swine feed, and the development of several semi-hard opaque-2 and quality protein hybrids.
2. Organization in 1983 of a highly successful nationwide coordinating program for maize breeding, for study and exchange of information on the use of exotic germplasm, population improvement for special characteristics in conjunction with inbred development, breeding for resistance to newly prevalent maize diseases, and for high oil hybrids.
3. Improvements in seed production technology, including use of cytoplasmic male sterility, and a practical method for use of chromosomal gene male sterility.
4. Initiation, at an early date, of winter-season breeding programs in South China, enabling an increase in the annual rate of breeding progress.

It is not to be thought that all this productive work went forward without being affected by the historical movements of the times. From 1966 through 1976, the impact of the "Cultural Revolution" on science was profoundly destructive. In the early days of the "ten-year turmoil," Dr. Li had been "sent down" to Dazhai Commune in Xiyang County, Shanxi Province, from his university, to receive "re-education" from the peasants, a common experience shared by many other professors during the same period. This was not as trying an experience for Dr. Li as it was for many of his academic colleagues because Dr. Li was used to working with his own hands and with peasants. Though inescapably an intellectual, Dr. Li was totally without arrogance. It is a compliment to both Dr. Li and the peasants of Shanxi that they worked together with mutual respect and admiration, forwarding the genetic improvement and field performance of maize. Dr. Li was lucky in being "sent down" to the Dazhai Commune because it was during that period a leader in utilizing advanced production technology. There is some suggestion in the record that Zhou Enlai played a direct role during the "re-education" period in placing Dr. Li at Dazhai, where his talents would be both utilized and appreciated. For Li "working with the peasants" was nothing new or difficult.

I lost contact with Li during this period of years . For me, corresponding with scientists in China was chilled by McCarthyism. The Chinese likewise were at risk in corresponding with us. However, in 1971, my youngest daughter, Alice, had the remarkable opportunity to participate in a Putney School work-study trip of several months to China. (She was in Beijing when President Nixon came visiting.) I asked her to enquire concerning Dr. Li—she found him at Dazhai Commune. In 1974, consequent to the improvement of intergovernmental relations, a high level U.S. agricultural delegation visited China. Shortly after, a group of Chinese scientists, of whom Dr. Li was a leader, visited here. In 1975, my wife and I went to China to visit Dr. Li, as guests of the Chinese Academy of Agricultural and Forestry Sciences.

Dr. Li's direct and personal favorable impact on the food supply of the Chinese people came through two major actions and in a number of contributory ways: first, his sturdy opposition to Lysenkoism and to a compromise with the Chinese Lysenkoists, and second, and only possible with the first contribution, the development of maize hybrids of superior yield; third in importance, a series of minor contributions from his early work on wheat-rye hybrids (triticale), Setaria millet, sorghum hybridization, hybrid maize technology, and specialty maize such as quality protein hybrids. In addition, in his early years, he made contributions to understanding the cytogenetics of maize. And he was always, in spite of formal difficulties, an honored teacher to his colleagues and associates. It is perhaps as pioneer and teacher of corn breeding that Dr. Li will best be remembered.

Assuming a per capita dietary requirement of about 300 kg of grain per person per year and the key role Dr. C.H. Li played during his professional career in provision of elite maize hybrids to Chinese agriculture, one can estimate that Dr. Li substantially contributed to the feeding of approximately 100 million persons per year in excess of those who could otherwise have been well fed during the period 1952 to 1965. Of himself, Dr. Li said, "I need corn and corn needs me too." Thus, new turmoil or old, strong in his belief that in China improvement of corn production was of key importance, he continued each season until his health failed, to work in his corn nurseries.

Memorial in Honor of
Earl B. Patterson

Earl B. Patterson passed away on Saturday May 1, 1999. He was 75 years old. He is survived by his children, Mark and Anne. His wife Betty passed away August 1, 1999.

His name is synonymous with the Maize Genetics Cooperation Stock Center whose current thriving status is attributable, in large measure, to his unstinting effort in its behalf. His deep imprint also remains with the annual Maize Genetics Conference, which he organized and presided over through the 60s, 70s and early 80s.

Earl Patterson was born on a farm in southeastern Nebraska near the town of Reynolds, on July 21, 1923, the youngest of nine unusually gifted children in a closely-knit family of four girls and five boys. Earl attended the University of Nebraska where, in 1947, after serving three years in the U.S. armed services during WWII, he received his B.S. degree in technical science, graduating first in his class. Dr. Frank Keim, long-time head of the Department of Agronomy at the University of Nebraska, and a genetics teacher who was familiar with Earl's excellent qualifications and interest in the subject, encouraged him to pursue advanced studies with Dr. E. G. Anderson, himself of Nebraska origin, at the California Institute of Technology in Pasadena. Upon Dr. Keim's recommendation, Earl's application was accepted and his graduate years were spent in the Biology Division at Cal Tech with Dr. Anderson as his mentor. He received his Ph.D. degree in genetics at that institution in 1952, and stayed at Cal Tech for another year as a postdoctoral fellow.

In 1953 Earl accepted a position in the Departments of Botany and Agronomy at the University of Illinois in Urbana. Here he was responsible for the Maize Genetics Cooperation Stock Center which had just been moved from Cornell University to Urbana. Two years later, in 1955, he became project leader of that program in the Department of Agronomy. Earlier maintenance of the maize genetic stocks at Cornell led to selection of strains that were adapted to the short growing season at Ithaca but only poorly suited to culture in the Corn Belt and most other corn growing regions. As a result, Earl Patterson's first task in his new position at Illinois was to commence the conversion of these many genetic stocks to inbred and hybrid backgrounds that were better adapted to most corn growing regions. Earl maintained the stock center through these formative years until 1966 when he relinquished his stock center responsibilities to concentrate on research. Earl's research focused on the isolation and characterization of male sterility mutants in maize. He found numerous new nuclear male-sterile mutations. When Southern Corn Leaf Blight, a disease specific to T-type male-sterile cytoplasm, struck the hybrid corn industry, seed companies reverted to manual detasselling. Earl developed a method to use his nuclear male-sterile traits to replace cms-T to avoid detasselling. Combining his male-sterile traits with various chromosomal aberration stocks, he developed a new method for producing hybrid corn seed. This work resulted in the issuing of two patents.

In 1977, Earl stepped up to fill the gap left by the retirement from teaching of the head instructor of the introductory genetics course. He was lead instructor for this course, in collaboration with faculty from the Animal Sciences Department, until 1987. The average enrollment was 80-90 students per semester.

When the Director position at the Maize Genetics Cooperation Stock Center again became vacant in 1986, Larry Schrader, then Head of the Agronomy Department at Illinois, persuaded Earl to resume management of the Stock Center. It was to the great benefit of all maize researchers that Earl returned to that position at a time when future support and direction of the center were uncertain. He continued that effort until his retirement in 1993.

Earl always gave "distribution" of seed stocks very special attention. On each request for seed, he brought to bear his encyclopedic knowledge of maize genetics lore. A request for seeds often resulted in the shipment of more packets than requested because of Earl's uncanny ability to anticipate needs and problems associated with growing and handling the items requested. All manner of useful suggestions were likely to be found in the letters that accompanied the packets of seeds requested. There is no doubt that a collection of letters that Earl has sent in response to seed requests over the years would be a valuable resource for maize geneticists.

While the Maize Genetics Cooperation Stock Center is today well supported and a thriving organization, it was not always so. In its earlier years at Illinois, funds for its operation were uncertain and often meager. With an improved internal status for the Stock Center in recent years has come increased support from the Agricultural Research Service of the United States Department of Agriculture, and in 1992 this agency assumed responsibility for operations and funding of the program. To Earl, whose labors, and sometime frustrations, have been so closely associated with the development of the Stock Center, the strong position that it has recently achieved was a source of great satisfaction and pride.

In 1958, Earl Patterson along with John Laughnan, Ed Coe, and Gerry Neuffer, talked about the possibility of an annual informal get-together of maize geneticists and their graduate students. The first meeting was in January 1959, and took place at Allerton Park, a part of a farm facility owned by the University of Illinois and located just outside of Monticello, Illinois. There were about twelve participants at

that first meeting, so few that it could be held in the quite small Oak Room in Allerton Park House. These maize meetings as they came to be called were delightfully informal and grew in numbers of participants over the years. They were presided over by Earl. He made all the arrangements for use of the facility and dates of the meetings each year. He sent out notices of meetings to potential participants and arranged for ground transportation to Allerton House. There was no prearranged program of speakers; participants would arrive on Friday evening and at that time or early the next morning Earl would talk with people interested in sharing their research experiences and in that way developed a program for the get-together. At first, there was no need for a microphone, even for the most soft-spoken individuals, but as the meetings grew in size it necessarily moved to amplification. Earl introduced the speakers, adjusted the microphone, operated the overhead, arranged for the right kind of soft chalk and erased the blackboard, all with a special finesse that earned for him the position of permanent chair of all sessions. In addition to all these things Earl presided over the gene mapping sessions usually held on Saturday evenings. As the meetings grew in size, it was recognized that some modest level of organization was needed. Earl's suggestion of establishing a steering committee for the annual meetings was approved by the maize group. Today this committee continues to serve an important function in the Maize Genetics community.

After 25 years, the maize meetings grew to such a size that Allerton House could no longer accommodate them and so, regretfully, the maize genetics community was obliged to move the meetings from this treasured site. This past March the 41st annual meeting of maize geneticists, now called the Maize Genetics Conference, was held at the Grand Geneva Convention Center in Lake Geneva, WI, with over 400 teachers and researchers in attendance. Younger members of the maize genetics group are probably not acquainted with Earl Patterson nor aware of the reverence in which the Allerton meetings are still held by their predecessors. However, they should know that it was Earl who established the original format for these meetings and successfully propagated the informal atmosphere that is still recognizable in the present-day meetings, in spite of their size.

(Reprinted from the University of Illinois memorial)

Edward Murray East, '01, M.S. '04, Ph.D. '07
Father of Modern Plant Breeding
And
Co-inventor of Hybrid Corn

Edward Murray East and Harry Houser Love, graduate students in agronomy, shared this office in 1904 and 1905. It became headquarters for the corn chemical composition studies and other plant breeding experiments conducted at the University of Illinois.

East, an only child, was born October 4, 1879 at DuQuoin, Illinois. He was a child prodigy and was graduated from high school at age 15. He received the first Ph.D. awarded in agronomy by the University of Illinois.

After East left Illinois for the Connecticut Agricultural Experiment Station he wrote back to Love and requested the Illinois-developed inbred lines be sent to him. These Illinois inbreds from the Chester Strain of Leaming Corn were used in the first Burr-Leaming double cross hybrid in 1917, and in Copper Cross, the first hybrid commercially grown in Iowa in 1924.

East is credited as the co-inventor of hybrid corn with George Harrison Shull from Princeton. In addition to the distinction as co-inventor of hybrid corn, East continued his career as a famous teacher and geneticist at Harvard University. East provided Mendelian explanations for quantitative inheritance. He directly trained 17 Ph.D. students who in turn became research leaders and mentors for hundreds of the world's leading plant breeders.

Sign to be located outside 328 Davenport Hall

12/16/99

(This copy courtesy of A. Forrest Troyer)

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I. FOREWORD

The Notes in this Newsletter are voluntarily shared "Conversations among Cooperators." This is not a refereed journal -- the data and ideas here are not published but are presented with the understanding that they will not be used in publications without specific consent of the authors. Cooperators provide brief technical notes, updates, mutant descriptions, segregation ratios, tables of mapping data, developmental and anatomical information and techniques, clones, biochemical functions, expression data, and the like. Comprehensive material and analyses are better directed to formal publication. Maize Cooperators have the tradition of sharing information with colleagues, not only in MNL but also in many unheralded conversations, correspondence, and shared stocks and clones. By sharing our research information, we contribute to the advancement of biology and to the power of shared technical knowledge.

Beginning in 1997, MNL became a Virtual Hotletter and Linkletter! Notes submitted at any time go verbatim into MaizeDB as received, flagged as future items for the next issue (http://www.agron.missouri.edu/mnl/). We progressively incorporate figures and tables, and we link the articles to database objects for user access and for the development of summaries and syntheses such as the Genelist, Maps, and Indexes. In parallel, redacting of copy (editing and formatting) in preparation for the press proceeds by desktop processing. After the deadline has passed, the print version of this issue, simply containing the Notes received to that date; the Address List; Stock Center Report and Stock List; and Maize Database, is finalized and sent to the press. Indexes to Symbols and to Authors and Names cited in this MNL issue are included. Assembly of portions of MNL that represent syntheses of information (e.g., Gene Lists and Genetic Maps) will be done periodically insofar as possible, but on a separate schedule from MNL. Syntheses will also be present in MaizeDB (http://www.agron.missouri.edu), where they can be viewed or printed by MaizeDB users.

Gifts to the Endowment Fund for support of the Newsletter have grown to well over $125,000. Please see the listing, in the front of this issue, of donors whose generosity has made this total grow. We are all grateful for the support of our colleagues and of organizations with which we have common interests. Gifts to the Endowment Fund are very much appreciated, to assure that costs of production are met, but more importantly to underwrite distribution to deserving institutions, libraries and individuals. A bequest from Ginny Harrison to the Endowment Fund furthers our emphasis on teaching and education initiatives in MNL and on the net.

The continuity and support necessary for collecting genetic and molecular information from the literature and from individual contributions; evaluating; and preparing gene lists, maps, and similar syntheses, is made possible by the USDA - Agricultural Research Service through the MaizeDB program, of which Mary Polacco is Curator. We urge you with strongest enthusiasm to use, assess, and contribute to the database.

Shirley Kowalewski again refined and redacted the copy; pulled together diverse electronic sources and exotic scripts or performed hand entry; structured indexes; questioned quality or content; and gave the editor a quality technician's creative advice. Lou Butler and Stephanie Moore contributed with diligence and precision to many tasks, including library and literature work, processing of figures, and checking of accuracy and completeness. My colleagues Mary Polacco and Mike McMullen have never yet refused to give invaluable advice and encouragement. At University Printing Services, Yvonne Ball and the printshop staff again efficiently ensured the job was done promptly and well.

Information about the next Maize Genetics Conference, March, 2001, will be on the MaizeDB Web, and information packets will be mailed to former attendees late in 2000; others may request the mailing by addressing coee@missouri.edu, polaccom@missouri.edu, or db_request@teosinte.agron.missouri.edu. The program and abstracts are prepared from electronic submissions; some back copies are available from Coe, or see MaizeDB. Electronic submission, "Webification", and printing of abstracts is done by Mary Polacco. The Steering Committee for the 2000 Maize Genetics Conference is:

Becky Boston, Co-Chair	Ben Bowen	Kelly Dawe
Al Kriz	Tony Pryor	Torbert Rocheford
Neelima Sinha	Cliff Weil	Sue Wessler, Co-Chair
Ex officio: Karen Cone, Treasurer	Marty Sachs, local coordinator	

Preparing notes for the next issue (Number 75, 2001)?
SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME.
See details inside the back cover.

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Editor Coe

May you find a Unique corn in MM!
Prof L.

AMES, IOWA
Iowa State University

Detection of the *p1* expression in vegetative organs

--Cocciolone, SM, Sidorenko, LV, Peterson, T

The *p1* gene encodes a transcriptional regulator of red phlobaphene pigment biosynthesis. Expression of the *p1* gene has long been considered floral-specific, with distinct oral patterns. Two of the best-characterized alleles are *P1-rr* and *P1-wr*. A *P1-rr* allele conditions uniform red pigmentation of the pericarp, cob glumes, husks, silks, and tassel glumes. Plants carrying a *P1-wr* allele lack pericarp pigmentation, but have uniform, dark red cob glumes and pigmented margins on the husks and tassel glumes.

In order to study the tissue-specific regulation of the *P1-rr* allele, maize plants were transformed with various portions of the *P1-rr* upstream regulatory region linked to the GUS reporter gene (P::GUS constructs). Unexpectedly, plants with strong transgene expression in floral organs also had transgene expression in vegetative organs, including the coleoptile, leaves, and roots of seedlings; and the sheath, auricle, and blade of adult leaves. The intensity of GUS staining ranged from light to very dark in seedling organs and from light to moderate in adult leaves. In the coleoptile, GUS activity was primarily localized to the two lateral vascular bundles. For seedling roots, the most intense staining was at the site of lateral branch formation and in the central cylinder (pith and vascular cells) of primary, lateral, and seminal adventitious roots. Leaves of primary regenerants grown in culture boxes stained uniformly blue, while leaves from greenhouse-grown seedlings, which have a thicker cuticle, stained unevenly due to poor substrate penetration. No blue staining was observed in comparable non-transformed plant material. These patterns of vegetative transgene expression were observed in 14 out of 27 independent transformation events, suggesting that such expression is not an artifact of transgene "position effect".

Similarly, when maize plants were transformed with constructs containing the *P1-rr* promoter driving either *P1-rr* or *P1-wr* cDNA sequences (P::P constructs), the transgenes promoted pigmentation in the expected floral organs—pericarp, cob, husk, silk and tassel glumes, as well as in the vegetative organs of plants that had strong transgene expression in floral organs. Non-floral pigmentation was observed in the leaf midrib, sheath, ligule and auricle; in the pith of the culm; and in the lateral veins of the coleoptile. No pigment was discernible in the leaf blade, except for in the midrib. Interestingly, this pattern of transgene expression is comparable to that caused by *Ufo1* (*unstable factor* for *orange*), a dominant allele that induces phlobaphene production in vegetative organs when combined with a *P1-wr* allele (Styles et al., MNL 61:100, 1987). Similar to *Ufo1*, strong transgene expression that results in dark vegetative pigmentation is associated with retarded plant growth.

The expression of P::GUS and P::P transgenes in vegetative organs prompted examination of non-transgenic plants for endogenous *p1*-conferred vegetative pigmentation. Indeed, *P1-rr* plants have a light orange tint to the leaf midrib, auricle and ligule that is absent in *P-ww* plants of the same genetic background. Also, a light orange uniform sheen can be observed on the adaxial surface of the sheath of *P1-rr* plants, especially after the plants have dried. No *P1-rr*-regulated pigmentation was visually discernible in the leaf blade, lateral veins of the coleoptile, or roots. Scrutiny of *P1-wr* plants for pigmentation revealed red sheath and auricle margins; this phenotype is analogous to the pigmented margins of *P1-wr* husk and tassel glumes. Thus, vegetative pigmentation patterns conferred by *P1-rr* and *P1-wr* alleles (uniform vs. marginal, respectively) are consistent with the allele-specific patterns of the floral organs.

Our observation of *p1* expression in vegetative organs is further supported by biochemical and molecular analyses. D. Styles and O. Ceska (Can J Genet Cytol 23: 691-704, 1981) reported detection of *p1*-regulated 3-deoxy flavonoids in coleoptiles, and we reproducibly observe very low levels of *p1* transcript in coleoptiles and auricles by RNA blot analysis. Through the use of a more sensitive rt-PCR method, we were able to detect transcripts in other vegetative organs. The primer set used for the PCR reactions is complementary to *p1*, as well as *p2*, a recently identified *p1* gene homolog (P. Zhang, personal communication). Amplification products of the *p1* and *p2* transcripts can be distinguished by size due to an 80 bp deletion in the 5' untranslated region of *p2*. Amplified products corresponding to *p1* mRNA were detected in coleoptile, seedling leaf, seedling root, and sheath from *P1-rr* and *P1-wr* plants, while no *p1*-specific amplification products were detected in *P1-ww* samples. Taken together, these results indicate that the endogenous *p1* gene is expressed at low levels in vegetative organs, suggesting that *p1* gene expression is floral-preferred rather than floral-specific.

Putative allele test for a Mutator-tagged zebra stripe mutant

--Heck, DA, James, MG, Chitnis, PR

Photosynthetic mutants of maize provide numerous opportunities for analyzing photosynthetic pathways at the biochemical level. We have identified a recessive zebra stripe mutant, *zb*97-2306*, that arose while performing transposon-tagging with Robertson's Mutator. We subsequently performed several allele tests to determine whether or not *zb*97-2306* was allelic to any of the known zebra stripe mutants. We crossed *zb*97-2306* with the following zebra stripe mutants from the Maize Cooperation Seed Stock Center: *zb1* (U340B), *zb3* (519G), *zb4* (105B), *zb6* (408C), and *zb7* (127A). (It has been previously determined that *zb2* (U340C) and *zb3* (504C) are allelic to *zb1* (U340B) (Steve Szalma, Karen Cone, and Phil Stinard, personal communication) and we have subsequently confirmed this). Our zebra stripe mutant was allelic to zb7 but the frequency of expression was low. We are currently testing the influence of environmental factors including variances in temperature and light on the expression of the *zb*97-2306* phenotype.

The authors would like to acknowledge the Chitnis group for helping with the general fieldwork related to this project.

BEIJING, CHINA
Institute of Genetics, Academia Sinica

Studies on types of space induced variations in maize (*Zea mays* L.)

--Zeng, M, Yang, T

In our previous paper it was described that the space flight of seeds has significant influence on photosynthetic pigment contents and leaf ultrastructure in maize (*Zea mays* L.). This paper deals with the different types of space induced variations and their distribution in maize.

Mutants obtained from sp1 (first generation by space treatment) to sp5 showed that amplitude of variability for space induced variations was large enough. Five type mutants for space induced variation were obtained, they are: special high plant mutant; special dwarf plant mutant; mutant of resistance to *H. maydis* and *H. turcicum*; mutant for three-ears with synchronous silking character per plant; mutant for *sh2* kernel type from flint or dent type. The various types of mutants are described as follows:

1. Special high plant mutant was from the inbreds of U8112 and 311. Compared with normal lines, it had a sufficency increase of 71% in plant height; a significant increase of 52% in ear length and 36% in kernel length. Mutation frequency of the mutant is 31%(see Fig. 1).

2. Special dwarf plant mutant was from Yi01-4-1. Mutation frequency of the mutant is 5%.

3. Mutant of resistance to *H. maydis* and *H. turcicum*: Mutation frequency of the mutant is 1%.

4. Mutant for three-ears with synchronous silking character per plant: The three-ears per plant silks at the same time, synchronism for silking and spread pollen of tassel. Mutation frequency of the mutant is 0.4% (see Fig. 2).

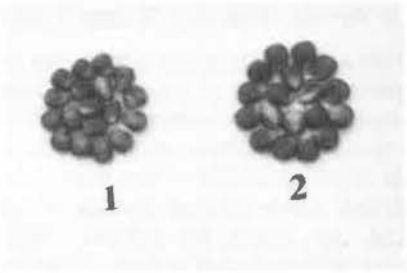


Figure 1. Maize kernels (1) CK U8112, (2) mutant for special high plant.

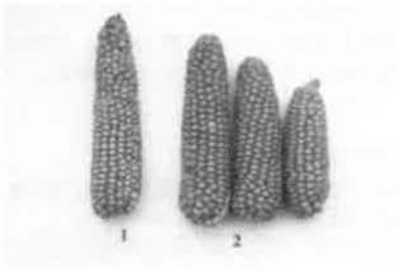


Figure 2. Maize ears (1) CK Yizi24, (2) mutant for three-ears with synchronous silking character per plant.

5. Mutant for *sh2* kernel type from flint or dent type: mutation frequency of the mutant is smaller, about 5%.

Analysis on new CMS types Bao I, Bao II in maize (*Zea mays* L.)

--Yan, Z, Zeng, M, Yang, T

The purpose of this paper is to present the results of the analysis and determination on new CMS types Bao I, Bao II in maize (*Zea mays* L.). Population 157 was used for breeding and selection experiments, 86 inbred lines from various provinces of China and different countries were used for determinative materials in test cross and inoculation experiments with *H. maydis*, *H. turcicum*.

The new types of cytoplasmic male sterility Bao I, Bao II were bred by using the approaches of self pollination, selection and inoculation determination etc. In test-cross of 86 inbred lines to Bao I type, 45 inbred lines were restored to fertility, covering 52.3% of the total tested lines, nine were partly restored to fertility, covering 10.5%, 32 lines kept up sterility, covering 37.2%. In test cross of 80 inbred lines to Bao II type, all tested lines kept up sterility (see Table 1).

	Cytoplasm	
Item	Bao I	Bao II
Full restoration (_)	45 lines	0
Partial restoration (±)	9 lines	0
Full maintenance (_)	32 lines	80 lines

The results obtained from evaluation of resistance to *Helminthosporium maydis* have shown that E157-cms-Bao I is infected with race 0, but resistant to race T, quite resistant to

Table 2. Evaluation of resistance to southern blight disease (*H. maydis*).

	Race O			Race C			Race T		
No.of line	Disease degree	Disease state coefficent	Resistance	Disease degree	Disease state coefficent	Resistance	Disease degree	Disease state coefficent	Resistance
E157-cms-Bao I	4.0	78.71	s	0	0	Q.R	1.0	20.1	R
E157	4.0	77.00	s	0	0	Q.R	1.0	21.1	R
U8112-cms-Bao I	3.0	57.85	m.s	0	0	Q.R	0.5	13.2	H.R
U8112	3.0	57.42	m.s	0	0	Q.R	0.5	13.5	H.R
M017-cms-Bao I	1.0	18.33	R	0	0	Q.R	0.5	8.9	H.R
M017	1.0	18.38	R	0	0	Q.R	0.5	9.2	H.R
F157-cms-Bao II	4.0	78.13	s	0	0	Q.R	1.0	21.2	H.R
F157	4.0	77. 93	s	0	0	Q.R	1.0	22.3	R
U8112-cms-Bao II	3.0	51.03	m.s	0	0	Q.R	0.5	11.5	H.R
U8112	3.0	51.11	m.s	0	0	Q.R	0.5	11.3	H.R
478-cms-Bao II	0.5	11.92	H.R	0	0	Q.R	0.5	9.8	H.R
478	0.5	11.85	H.R	0	0	Q.R	0.5	9.7	H.R
Zhongdan 2(cms-Bao I)	0.5	12.22	H.R	0	0	Q.R	0.5	10.5	H.R
Zhongdna 2(N)	0.5	11.85	H.R	0	0	Q.R	0.5	9.8	H.R

Notes: R...Resistance; H.R...High resistance; S...susceptivity; m.s...mid-susceptivity; Q.R...Quite resistant.

race C; F157-cms-Bao II is mildly infected with race 0, but highly resistant to race T, quite resistant to race C. Resistance of CMS lines and its maintainer is the same (see Table 2).

BERGAMO, ITALY
Istituto Sperimentale per la Cerealicoltura
KÖLN, GERMANY
Max-Planck-Institut für Züchtungsforschung

Developmentally regulated and tissue-specific expression of the *glossy2* gene of maize

--Velasco, R, Korfhage, C, Salamini, A, Tacke, E, Schmitz, J, Motto, M, Salamini, F, Döring, HP

In maize, a large system of genes mapping at 17 separate loci (the *glossy* or *gl* loci) has been identified that affect the quantity and/or composition of cuticular waxes on the surface of the seedling leaves (Bianchi et al., Maydica 30: 179-198, 1985). Mutations at the *Gl2* locus reduce the amount of surface wax to one-fifth of wild-type levels in the first to sixth plant leaves (Bianchi et al., Maydica 20: 165-173, 1975). While waxes of *gl2* plants have a chemical composition similar to those of adult leaves, seedlings homozygous for this mutation accumulate wax compounds two to four carbons shorter compared to wild type. Data available indicate that the *Gl2* gene product could be specifically involved in the chain elongation step from C_{30} to C_{32} (Bianchi et al., 1975). The *Gl2* locus was recently cloned and shown to encode a polypeptide of 426 amino acids of unknown function (Tacke et al., Plant J. 8: 907-917, 1995). Because waxes play a role in resistance of plants to environmental stresses their biosynthesis and secretory processes have relevance both to basic and applied studies. We have considered in this study several regulatory aspects concerning the expression specificity of the *Gl2* gene.

The expression of the gene was analysed on the RNA and protein level. The *Gl2* gene is transcribed in young leaves, in coleoptiles, in husks, in immature ears and in silks of wild type plants. No transcripts were found in roots of wild type plants or in tissues of plants homozygous for a recessive *gl2-ref* allele. Antibodies against the GL2 protein revealed predominant presence in the leaves with a juvenile wax phenotype. Protein detection was consistent with transcriptional activity of the gene in wild type coleoptiles, husks, immature ears and silks but not in roots, anthers and seeds. Sequence data bank analysis revealed homologies of the GL2 protein of maize with eighteen sequences from several plant species.

A domain of 27 amino acids is highly conserved in the proteins of different plant species. This domain, PLx3QxTxFxCGGx8Hx3D, is novel and with a function still unknown. Interestingly, the domain is present in the four proteins found in *Clarika breweri, Cantharanthus roseus, Gentiana triflora* and *Dianthus caryophyllus* which are credited to have a coenzymeA-dependent acyl transferase activity. Based on this finding, already St. Pierre et al. (Plant J. 14: 703-713, 1998) proposed that the GL2 protein belongs to plant proteins which function as acetyl transferases, an enzymatic activity which has still to be demonstrated for *gl2*.

For the *Gl2* gene the highest sequence similarity was observed with the *Cer2 Arabidopsis* gene, having both gene mutant alleles conditioning a defect in wax biosynthesis (Tacke et al., 1995; Negruk et al., Plant J. 9: 137-145, 1996). Their mutant waxes are defective in the accumulation of C_{30} and C_{32} fatty acids, respectively. The fact that both mutants are defective in the last step of fatty acid elongation might indeed support a role of *Cer-2* and *Gl2* genes in acyl-CoA-dependent fatty acid biosynthesis. This putative role of *Gl2* and *Cer-2* as structural genes involved in wax biosynthesis, integrates the current knowledge on this process based on molecular studies of the *Gl1* and *Gl8* maize loci. These genes exhibit sequence similarities to other plant wax synthetic genes coding a putative transfer protein (Hansen et al., Plant Physiol. 113: 1091-1100, 1997) and a ß-ketoacyl reductase (Xu et al., Plant Physiol. 115: 507-510, 1997). It is interesting to note that all the putative proteins with homology to the GL2 protein contain from 420 to 470 amino acids. This detail may indicate their common origin from a precursor gene, which later on evolved acquiring specific functions in different biochemical pathways.

BERGAMO, ITALY
Istituto Sperimentale per la Cerealicoltura
WAGENINGEN, THE NETHERLANDS
Keygene N.V.

Identification of QTLs for grain yield and grain-related traits of maize using an AFLP map, different testers, and cofactor analysis

--Ajmone Marsan, P, Redaelli, R, van Wijk, R, Stam, P, Motto, M

In the last decade the advent of molecular markers has greatly facilitated the systematic dissection of quantitatively inherited traits into their underlying Mendelian factors (QTLs). This has provided the tools to speed up plant improvement for a variety of criteria, including yield, by the generation of fine-scale molecular genetic maps to undertake marker-assisted selection (MAS) and positional cloning (Lee, Adv. Agron. 55: 265-344, 1995). In maize, extensive genome mapping based on DNA restriction fragment length polymorphism (RFLP) markers has been accomplished (Coe et al., Maize Coop. Genet. Newsl. 69: 191-267, 1995). These maps and their associated technology have been used successfully for a number of applications in genetic research and breeding. However, the use of RFLPs in a QTL analysis is an expensive and time-consuming process.

The development of the polymerase chain reaction (PCR) has expanded the repertoire and efficiency of DNA marker systems, which include the AFLP method (Vos et al., Nucleic Acids Res. 23: 4407-4414, 1995). The advantage of AFLP assay over other DNA marker techniques includes the detection of a large number of polymorphisms from a single PCR reaction, within a very short period of time, and the requirement for small amounts of DNA, thus reducing expenses and expediting the construction of high-density linkage maps. Accordingly, as a first step in exploiting AFLPs in a maize genome mapping program, and in the long-term for MAS and positional cloning, we have used AFLPs to identify QTLs for grain yield and grain-related traits in maize F2 lines using different testers and biometrical procedures.

Two-hundred-twenty-nine F3 progenies, each tracing back to an individual F2 plant, derived by crossing the inbred lines B73 and A7 were used. This population has been described previously to construct an RFLP linkage map (Ajmone-Marsan et al., Theor. Appl. Genet. 90: 415-424, 1995). The protocol adopted for the generation of AFLP markers was essentially the same as that described by Vos et al. (Nucleic Acids Res. 23: 4407-4414, 1995) and by Castiglioni et al. (Theor. Appl. Genet. 99: 425-431, 1999).

Basic field experimental procedures were as described earlier (Ajmone-Marsan et al. 1995). Cultural conditions were kept as close as possible to the optimal growth conditions in order to achieve high yield levels. The two series of testcross (TC) progenies were evaluated in field trials for grain yield (t/ha at 15.5% grain moisture), dry matter concentration (% grain dry matter at harvest), and test weight (kg/hl measured at harvest). A linkage map for B73xA7 was assembled by MAPMAKER as previously reported (Castiglioni et al. 1999). Among the 312 RFLP and AFLP markers located on the B73xA7 map, 195 evenly spaced markers belonging to the framework map, and corresponding approximately to a marker every 10 cM, were used for QTL analysis.

QTL analyses were performed on mean values of each trait across environments for each experiment involving the two series of TC progenies and using linkage information. For the analysis of linkage between QTLs and molecular markers the simple interval mapping (SIM) (Lander and Botstein, Genetics 121: 185-199, 1989) and the composite interval mapping (CIM) (Jansen and Stam, Genetics 136: 1447-1455, 1994) statistical methods were used.

The efficiency of generating AFLP markers was substantially higher relative to RFLP markers in the same population, and the speed at which they were generated showed a great potential for application in marker-assisted selection. AFLP markers covered linkage group regions left uncovered by RFLPs; in particular at telomeric regions, previously almost devoid of markers. This increase of genome coverage afforded by the inclusion of the AFLPs revealed new QTL locations for all the traits investigated and permitted mapping of telomeric QTLs with higher precision. The present study has also provided an opportunity to compare SIM and CIM for QTL analysis. Our results indicated that the method of CIM employed in this study has greater power in the detection of QTLs and provided more precise and accurate estimates of QTL positions and effects than SIM.

By the use of selected cofactors, which absorb a major part of the background noise due to other putative QTLs, CIM has allowed the detection of a higher number of QTLs. In some cases also CIM simply increased existing LOD peaks beyond the threshold values. In other situations CIM detected significant QTLs where SIM LOD profiles were almost flat. Furthermore, CIM reduces the significance of QTLs overestimated by SIM. In addition, the R_2 values for the simultaneous fit were always higher with CIM and showed a higher value of substitution effects of unfavourable alleles with favourable ones. Hence, advanced statistical methods promise to make an important contribution for improving the prospects of MAS without any additional cost.

The experimental mating design adopted in our experiment was based on two different tester lines. In this study we found that QTLs revealed by one tester may not be detected with the second one. For all traits and both testers we detected a total of 36 QTLs, of which only 2 were in common between testers. These findings indicate that the allelic compositions of a tester line determine whether a QTL segregating in a population will be detected. In fact, the number of QTL associated with grain yield and yield-related traits detected in this study largely depends on the tester under study. In conclusion, our results suggest that the choice of tester for identifying QTL alleles for use in improving an inbred is critical and expression of QTL alleles identified may be tester specific.

BLACKSBURG, VIRGINIA
Virginia Tech

A β-glucosidase aggregating factor (BGAF) is a member of the small heat shock protein family (sHSP)

--Blanchard, D, Esen, A

In certain maize (*Zea mays* L.) genotypes β-glucosidase occurs as large insoluble or poorly soluble aggregates (Esen and Cokmus, 1990. Biochem Genet 28: pp 319-336). The β-glucosidase zymograms of such genotypes are devoid of enzyme bands after being stained for activity. These genotypes were originally thought to be homozygous for a null allele at the *glu1* locus. However, biochemical data (Esen and Blanchard, 2000. Plant Physiology 122: Feb) from our laboratory clearly establishes that the monogenic inheritance reported for the null alleles at the β-glucosidase gene is actually for the BGAF protein. We also show that BGAF is solely responsible for β-glucosidase aggregation and insolubility, and thus, the apparent null phenotype. The objective of the present study was to clone and identify BGAF.

BGAF was extracted four times from 8 g of 3-d-old H95 etiolated shoots in 50 mM NaAc buffer, pH 5.0, containing 30 % ammonium sulfate (AS). The final pellet containing primarily free BGAF was solubilized in 50 mM NaAc buffer, pH 5.0 and immediately applied to a gel filtration column (Sephacryl HR 200, 90cm x 16mm). Fractions containing BGAF were identified by ELISA and pooled for hydrophobic interaction chromatography. BGAF was then applied to a ToyoPearl-butyl 650M hydrophobic interaction chromatography at 0.8 M AS in 50 mM NaAc buffer, pH 5. The column was washed with 0.8 M AS in NaAc buffer until baseline was reached. Stepwise elution was performed by reducing the ammonium sulfate concentration in 0.1 M increments. Fractions were screened for BGAF by ELISA, pooled, and concentrated 12-fold on a 10 K cut-off spin column (Gelman Sciences).

Purified BGAF (~250 pmoles) was submitted to Commonwealth Biotechnology for N-terminal sequencing. The N-terminal sequence was identified to be: [V, ?] [I, E] [G, P] [N, L] YAPIGIGATV. Therefore, the peptide APIGIGAT was used to design two degenerate primers; BGAF-6, CCNATHGGNATHGGNGCNAC; BGAF-7, CNCCNATHGGNATHGGNGC.

H95 seedlings were germinated in the dark for 2 days at 30 C in wet vermiculate. Whole shoots were harvested with a razor blade and used immediately for RNA isolation. mRNA was isolated using oligo dT coated magnetic beads. An Oligo dT primer (RT-3) was used for first strand cDNA synthesis with AMV-reverse transcriptase. To amplify the BGAF cDNA, BGAF-6 and BGAF-7 were individually paired with RT-3 in separate PCR reactions. The 1 kb PCR product generated was reamplified with pfu Turbo DNA polymerase, gel purified, and blunt-end cloned into pBluescript® II SK (+/-) for sequencing in both directions.

The sequence was used to perform a BLAST search in the maize EST database. The BLAST search exclusively identified heat-shock proteins, which matched with similarities ranging from 56% - 96%. The extreme 5' end of BGAF was obtained by overlapping the BGAF sequence with the highest match (96%) in the EST database. The identity of the 5' end sequence obtained by overlap was corroborated by reamplification of the H95 cDNA using a primer designed from the extreme 5' end of the EST sequence paired with a BGAF specific 3' end primer.

MVSLQVTPTSAFTEWNELKFEGLYLFHTPLGSGANQARVI
DNKAPIGIGATVVNNWTVCDGPGPNAKLIARAQGLHIQAG
NWVNSFSLVFVDQRFSGSPLEVTGIVVESGEWAIVGGTGQ
FAMANGVISKKLHGKTSEGDIIQLTIHAFCPVLGATKRSVT
KVGPWGGSGGSPMDITAEPQRLKSITVATGIAVTSIAFSYV
DSAGQTQSAGRWGGSGGETEPVIQLGDSEVLTELSGTIGN
VDGLTVITSIKFVTSLKTYGPFGAWGNGSDTPFAIPVQQGS
AIVGFFARAGVYLDALGVYVRSL.

Figure 1. Deduced primary structure of the BGAF cDNA. The open reading frame of BGAF encodes a 306-amino acid-long mature protein. The N-terminal protein sequence from which degenerate primers for RT-PCR were designed (arrow, gray box) is shown. The fact that the N-terminal sequence obtained is an internal polypeptide is likely due to an endogenous protease in maize.

The identity of the putative BGAF cDNA was shown following its expression in *E. coli*. Western blots of *E. coli* expression extracts probed with BGAF antiserum showed an immunoreactive band whose electrophoretic mobility and molecular size were identical to BGAF isolated from plant extracts. Additionally, functional assays clearly showed the presence of BGAF in *E. coli* expression extracts, which tested positive for β-glucosidase binding activity in gel-shift assays.

BGAF has a 1143-bp long cDNA sequence, which includes a 918-bp coding sequence, and a 172-bp 3' untranslated region. The incomplete 5' untranslated region is 53-bp long.

The deduced protein has an open reading frame (the 918 bp coding region) encoding a 306- amino acid-long mature protein with a calculated molecular mass of 31.8 kD (Fig. 1). BGAF has a calculated isoelectric point of 6.00, consistent with the experimental isoelectric point.

BUFFALO, NEW YORK
Williamsville North High School
State University of New York
LONDON, ONTARIO
University of Western Ontario

Abnormal node development in *na1/na1*

--Cheng, W, Cheng, P-c, Walden, DB

The phenotype of *na1/na1* is characterized as dwarf plant. The dwarf character is associated with an abnormal development in the stem. Instead of the well-defined nodes and internodes found in the normal wild type stem, *na1/na1* develops a stem lacking organized nodal and internodal vasculature. Different from the parallel longitudinal vascular bundles found in the internode of wild type, *na1/na1* has a poorly structured arrangement. In addition, the interconnecting vascular bundles are not limited to the nodal region as found in the wild type, but can be found at any level of the stem (arrows).

Figure 1 is a longitudinal section of a *na1/na1* stem showing the stem with the aforementioned features. The plant was grown in 1999 summer nursery at the field station of University of Western Ontario, London, Canada. The specimen was fixed in 1:3 acetic acid/ EtOH, stained in diluted Schiff's reagent (10%), washed in 0.1 M metabisulphate solution (with 1 ml of 1 N HCl per liter), dehydrated in EtOH and cleared in methyl salicylate. Note the vascular bundles extend into the leaves but are not connected to a nodal structure (double-arrows).

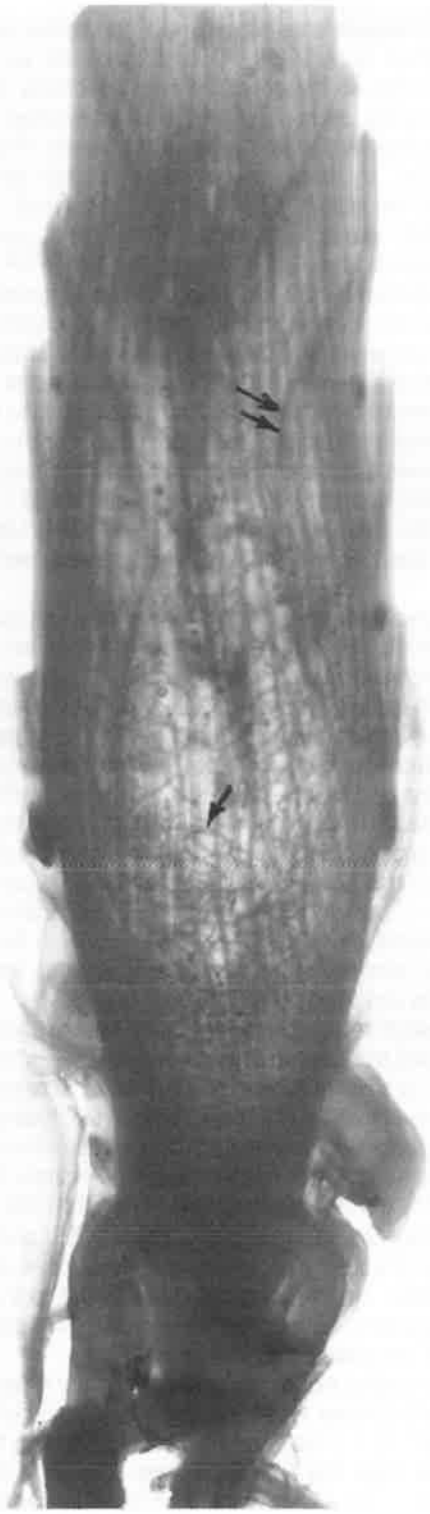


Figure 1.

CEDAR FALLS, IOWA
University of Northern Iowa
EMPORIA*, KANSAS
Department of Biological Sciences

Inflorescence development in a Toluca teosinte

--Orr, AR, Mullen, K, Klaahsen, D, Sundberg*, MD

A previously undiscovered natural wild population of annual teosinte plants in the Valley of Toluca, Mexico, appears similar to a Chalco teosinte population found in the eastern Valley of Mexico (Wilkes and Taba, MNL 67:21, 1993), although no race assignment has been made to date (Vibrans and Estrada Flores. Maydica 43: 45-48, 1998). The wild Toluca population at 2500m-2750m is somewhat unique because it grows 100 m higher than the highest Chalco teosinte at Amecameca in the Valley of Mexico (Vibrans and Flores, 1998), and is higher than the Balsas teosinte at 800 m to 1950 m (Wilkes, Crop Improv. 6: 1-18, 1979). The use of highland maize on approximately 2.8 million hectares in central Mexico and its use as a germplasm for improving maize for tropical highlands (Eagles and Lothrop, Crop Sci. 34: 11-19, 1994) has enhanced an interest in the origin of highland maize races. Toluca teosinte is able to survive to the reproductive stage in, and adjacent to, fields of maize because of its excellent maize mimicry (Wilkes and Taba, 1993) and thus, maize-Toluca teosinte hybrids are common (Vibrans and Flores, 1998). The concept that teosinte and maize hybridization participated in the evolution of maize, and the maize ear, is not a recent discovery (see Wilkes, 1979; Iltis, Science 222: 886-894, 1983). Molecular evidence supports the idea that maize is derived from teosinte and indicates that a low altitude Balsas teosinte, *Z. mays* subsp. *parviglumis*, may be the ancestral taxon (Doebley, Econ. Bot. 44: 6-27, 1990). However, it is not unreasonable to imagine that some highland teosinte population (eg., a Chalco teosinte, *Z. mays* subsp. *mexicana*) also might have contributed to the early evolution of highland races of maize (Galinat, Adv. Agron. 47: 203-231, 1992; Eagles and Lothrop, 1994). Eagles and Lothrop (1994) argue that highland maize probably came from higher altitude populations - an argument that is supported by Wilkes (1979). A fundamental idea in this scenario is that the genetic basis for temperature adaptation existed in wild maize before domestication.

Although the origin of the maize ear is still not unequivocally known, researchers investigating teosinte inflorescence development have revealed a developmental pattern common to all *Zea* (maize and teosinte) inflorescences (Sundberg and Orr, Amer,. J. Bot. 77: 141-152, 1990). However, key developmental features have been uncovered that differentiate ears from tassels and teosinte inflorescences from maize inflorescences (cf. Fig. 25, Orr and Sundberg, Amer. J. Bot. 81: 598-608, 1994). It is especially interesting that one observation in these investigations disclosed a key organogenic event to account for the evolution (distichy to polystichy) of a multiple-ranked maize inflorescence from a two-ranked inflorescence. A key trait in the evolution of the maize inflorescence, acquisition of polystichy, may have arisen from a change in a developmental program that regulates the spikelet pair primordia condition. Spikelet pair primordia in teosinte and maize give rise to two spikelet primordia only. In the "standard exotic" maize (Argentine popcorn) two inflorescence phenotypes were observed: intermediate distichy/polystichy (two-ranked and four-ranked), and wild type four-ranked polystichous inflorescences (Sundberg, LaFargue and Orr, Amer. J. Bot. 82: 64-74, 1995). In the intermediate phenotype the distichy condition was distal to the polystichy condition: the proximal portion of intermediate inflorescences bore eight rows of spikelets in four ranks, and the distal segments produced four rows of spikelets in two ranks. In wild type teosinte and maize inflorescences the apical meristem first produces spikelet pair primordia, each of which bifurcates to produce paired spikelet primordia. In the intermediate inflorescences of Argentine Popcorn a second cycle of bifurcation facilitates a switch from a two-ranked (distichy) condition to a four-ranked (polystichy) condition. Perhaps the origin of polystichy in maize was derived from a change in developmental regulation of meristem determinacy (Sundberg, et al., 1995). Regulation of maize inflorescence meristems that produce an additional number of meristems has been reported (Orr, Haas and Sundberg, Amer. J. Bot. 84: 723-734, 1997; Chuck, Meeley and Hake, Genes Dev. 12: 1145-1154, 1998; Lenhard and Laux, Cur. Opin. Plant Bio. 2: 44-50, 1999).

We used scanning electron microscopy to characterize the organogenesis of Toluca teosinte inflorescence, and to compare the development to other *Zea* inflorescences. Seed from the CIMMYT Maize Germplasm Bank was kindly given to us by Sukitoshi Taba. Plantings were made in a growth chamber under a long day, 15:9 hr light/dark cycle, and at the V5 (fifth vegetative leaf) stage plants were shifted to 8:16 hr light/dark cycle. Congruent with the light/dark cycle, the temperature was maintained at 24:18 C. Light intensity was maintained at 600-700 uE

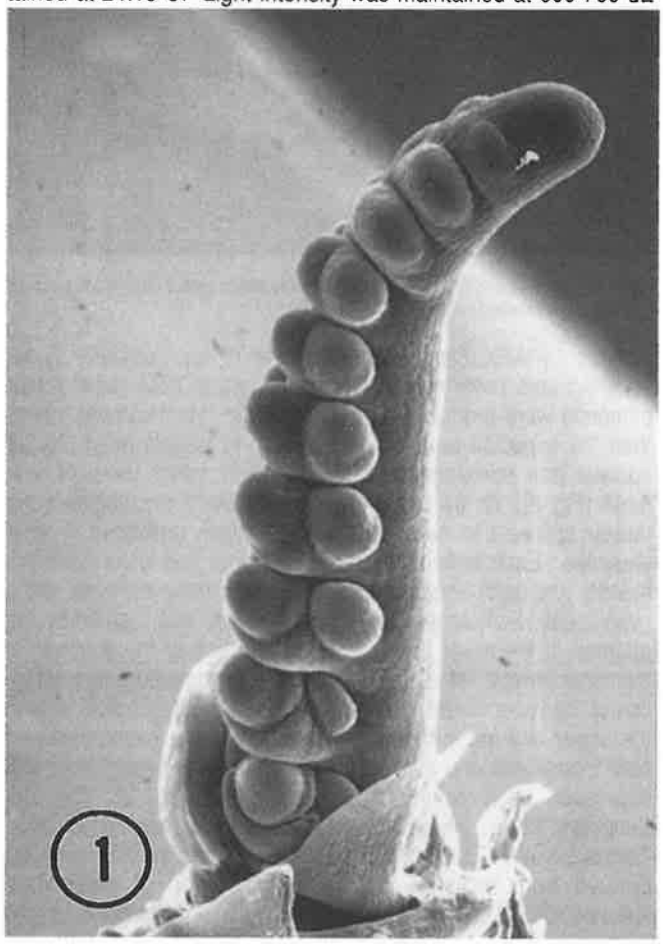


Fig. 1. SEM of developing Toluca teosinte ear with two (distichous) ranks. One rank is hidden behind the central axis. Spikelet pair primordia have divided into paired spikelets. At the base of the right row note the reduction in growth (and eventual abortion) of the pedicellate spikelet.

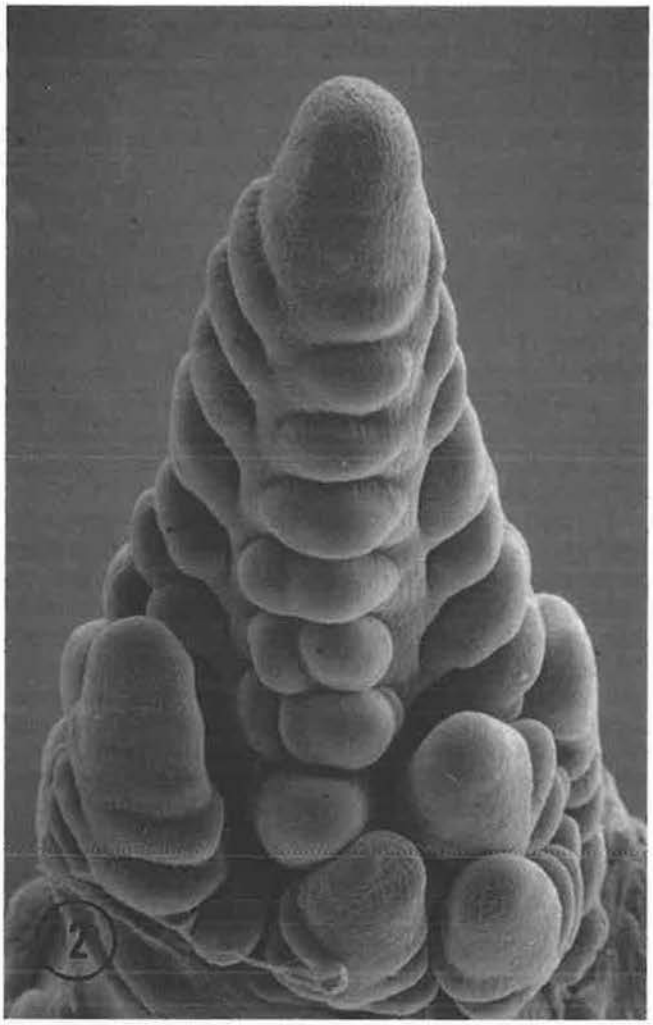


Fig. 2. SEM of developing Toluca tassel with four (polystichous) ranks of spikelet pair primordia. One rank is hidden behind the axis.

m^{-2} sec^{-1} (PAR). Our SEM examination of approximately 35 developing ears confirmed that in most ears (33/35) spikelet pair primordia were produced in two ranks (Fig. 1). However, rather than the expected pure distichy pattern in tassels most (25/30) spikelet pair primordia were produced in either three or four ranks (Fig. 2). As we expected, Toluca spikelet organogenesis in tassels and ears followed a pattern previously described in other teosintes. Each spikelet produced an upper and lower floret primordia, and each floret primordia produced three stamens and a gynoecium. Femaleness in Toluca spikelets was marked by the abortion of the lower florets and the abortion of the stamens in the upper florets. Male Toluca spikelets were marked by a retention of the lower florets, and an abortion of the gynoecium in both the upper and lower florets. This supports the hypothesis that both femaleness and maleness in teosintes are derived from and expressed on a common background (cf. Fig. 25, Orr and Sundberg, 1994). However, unlike the paired spikelet condition in Toluca tassels, Toluca ears displayed solitary sessile spikelets derived from arrested growth and abortion of pedicellate spikelets (Fig. 1). The abortion of pedicellate spikelets in female inflorescences was observed in other teosinte ears (Orr and Sundberg, 1994). This is further evidence that this morphological marker is sufficient to discriminate, at a very young developmental stage, teosinte inflorescences sexually and teosinte ears from maize ears.

The most striking observation in this study of Toluca inflorescences was the occurrence of intermediate (distichous and polystichous) and pure polystichous tassels, and intermediate ears. In the population of 30 tassels we examined ten were intermediate, and 15 were 100 percent, four-ranked polystichous (Fig. 2). In the intermediate state, tassels (Fig. 3) and ears (Fig. 4) exhibited the distichy condition at the distal end of the inflorescence. This morphological intermediate phenotype is similar to that noted above for maize Argentine Popcorn (Sundberg, et al., 1995). In female inflorescences no purely polystichous ears were observed, and only two, three-ranked intermediate ears (Fig. 4) were noticed in the population of 35 we surveyed. It remains unclear why the frequency of additional ranks in the inflorescences of the Toluca population is much lower in the ear than the tassel. Also, it is obscure why an inflorescence meristem that initially produced three or four ranks shifted to make two ranks. We are currently examining additional Toluca inflorescences for evidence of the polystichous condition.

In each of the intermediate ears the distal portion of the inflorescence was distichous with two ranks of lateral primordia. In one ear a third rank arose at the fifth node from a division of a spikelet pair primordium at the fourth node (Fig. 4, arrow), suggesting that a second bifurcation of the original meristem resulted in two ranks of spikelet pair primordia. Thus, one rank with a potential for two rows became two ranks that resulted in four rows. A three-ranked, six rowed female inflorescence resulted. There is some evidence suggesting an additional bifurcation of each original spikelet pair primordia probably was a key event in the switch from distichy to polystichy in the evolution of a maize ear from a teosinte ear (Sundberg, et al., 1995). The other ear that exhibited an intermediate condition produced a partial third rank de novo on the adaxial surface of the inflorescence (Fig. 5). Intermediate and polystichous tassels exhibited both three and four ranks. Pure polystichous, eight rowed tassels arose from the formation of four ranks of spikelet pair primordia arranged in a decussate pattern (Fig. 6). A six rowed tassel or ear produced only three ranks of spikelet pair primordia with primordial ranks shifted toward the abaxial side. Additional rows (ranks?) in the proximal region of intermediate tassels appear to arise from a second bifurcation of lateral primordia (Fig. 3, arrow), or were derived from a de novo event (Fig. 7, arrow). It is also apparent in Fig. 7 that these de novo primordia divide into rows of paired spikelets. It would appear that these de novo primordia function as spikelet pair primordia. Quite possibly, but speculatively, the occurrence of these natural intermediate and pure polystichous inflorescences were the result of introgression between maize and Toluca teosinte. If this view is correct, this is the first developmental inflorescence study of a natural maize-teosinte hybrid and thus, may offer insight into the effect(s) on inflorescence development when different developmental programs are recombined in maize-teosinte hybrids. Interestingly, the first backcross of maize following F1 maize x teosinte hybridization resulted in polystichous ears (see Fig. 11B, Wilkes, Econ. Bot. 31: 254-293, 1977).

Finally, our studies provide evidence that unique phenotypes may arise in a hybrid population. In addition to the de novo spikelet pair primordium we noted above, we also found some tassels with three spikelets, rather than the usual two found in teosinte and

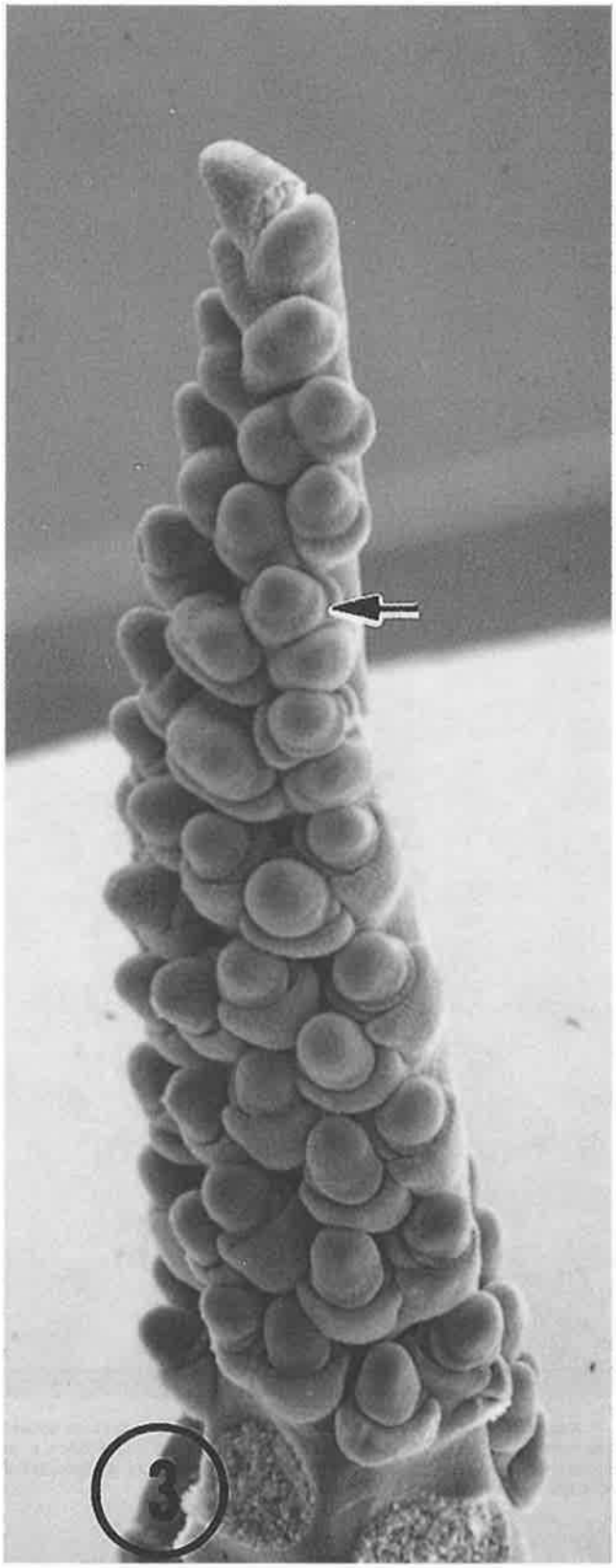


Fig. 3. An intermediate Toluca tassel with four ranks (eight rows) of spikelets on the lower two-thirds of the central axis, and two distal ranks of primordia on the upper third of the inflorescence. Two ranks (four rows) are partially hidden behind the axis. Basal tassel branches were removed to fully reveal the central axis. Proximal spikelets are characterized by outer and inner glumes. Note the basal spikelets include an outer lemma. Additional rows (ranks?) appear to arise from a second division of a lateral spikelet pair primordia (arrow).

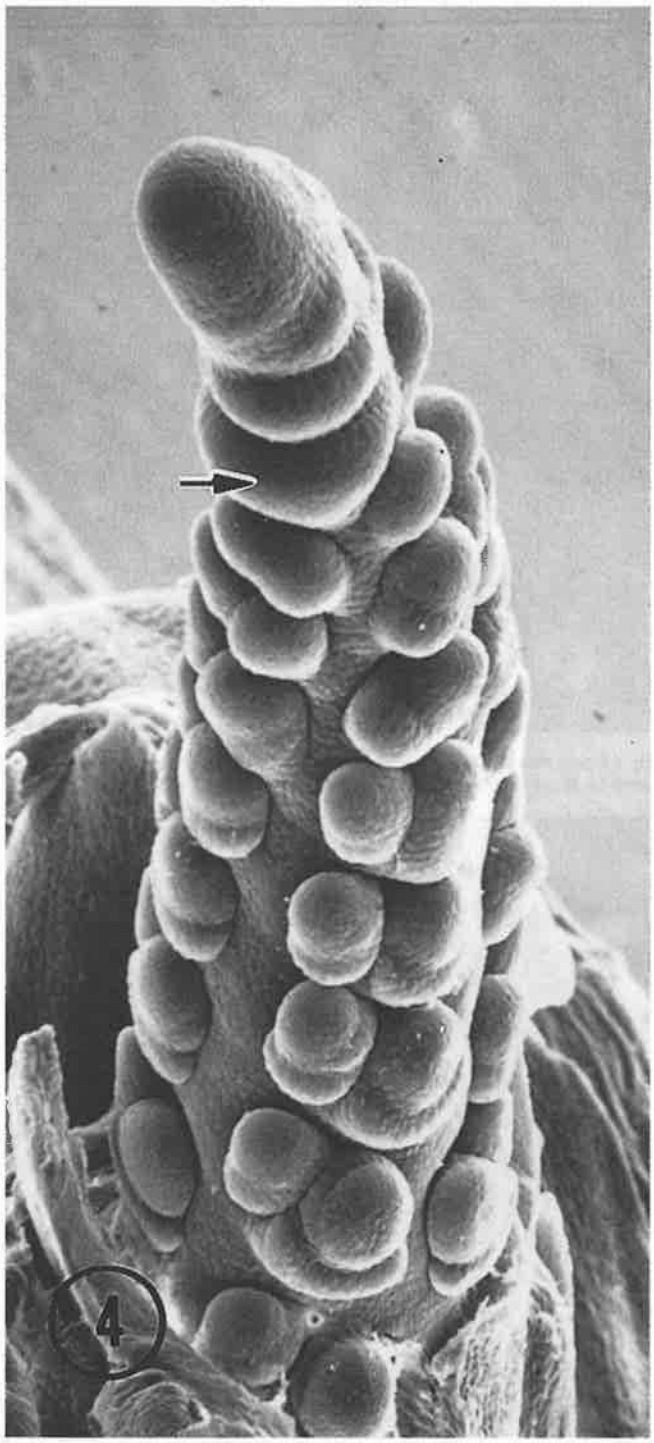


Fig. 4 An intermediate Toluca ear with three ranks. The plane of distichy at the distal end is approximately at a right angle to the photo. A third rank is somewhat visible along the back, right edge of the axis. The central rank appears to arise from a division of a spikelet pair primordium. An older proximal primordium has undergone a second division resulting in two rows of spikelets.

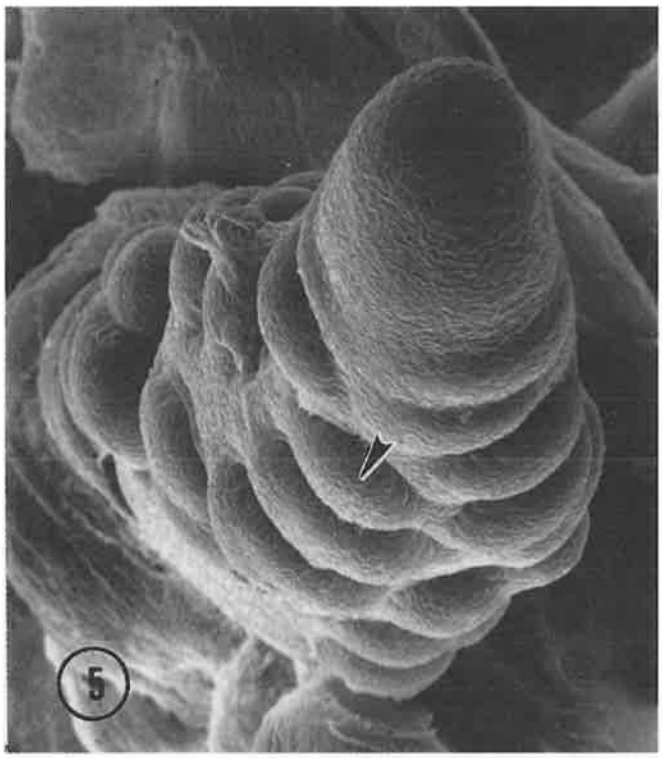


Fig. 5 A polar view of a developing intermediate Toluca ear with three ranks of spikelet pair primordia. Note the apparent de novo production of a third rank (arrowhead).

Fig. 6 A polar view of a pure polystichous Toluca tassel with four ranks of spikelet pair primordia along the central axis. A bifurcation of these primordia is seen at the proximal end of the inflorescence. Basal lateral branches with a distichous arrangement of spikelet pair primordia are apparent

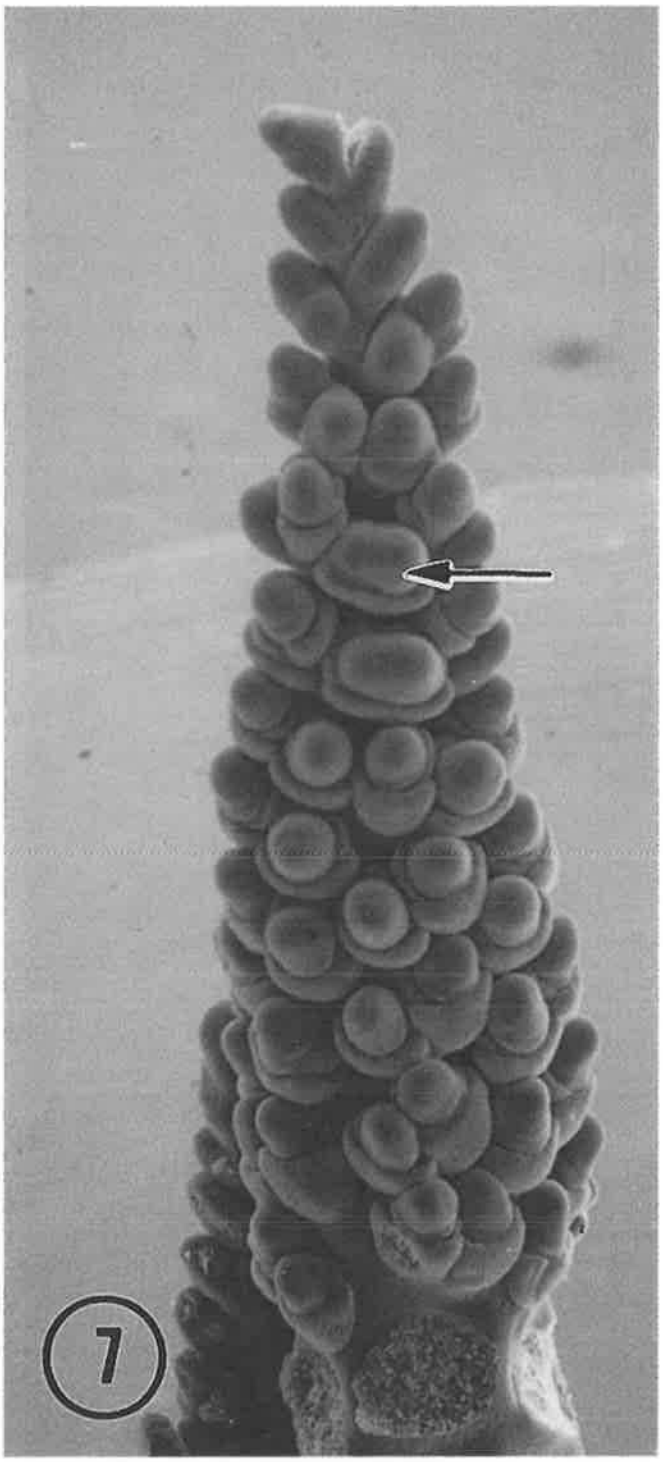


Fig. 7 A central spike of an intermediate Toluca tassel. Basal tassel branches were removed to fully reveal the central axis. This specimen shows an apparent de novo formation of an additional rank of spikelet pair primordia (arrow). Note the bifurcation of this additional rank of spikelet pair primordia in the formation of paired spikelets.

maize. A similar occurrence of the latter was noted in other studies of inflorescence development: F_3 plants derived from a cross of Race Reventador maize by *Z. Mays* subsp. *parviglumis* teosinte (see fig. 5, Doebley, Exp. Mol. Approch. to Plant Syst., Mo. Bot. Gard. 53: 57-70, 1995); the maize mutant *Fascicled ear* (Orr,

Haas and Sundberg, 1997); the primitive maize Chapalote (Sundberg and Orr, Amer. J. Bot. 83: 1255-1256, 1996); and the primitive maize Argentine Popcorn (Sundberg, et al., 1995).

CHESTNUT HILL, MASSACHUSETTS
Boston College

Continued studies on mutable inbred derived from anther culture
--Ting, YC, Tran, L

In the last summer continued studies on the mutable inbred derived from anther culture of maize KH-13 were carried out. This inbred was a descendant from a single self-fertilized plant which was dark green in plant color and normal in fertility for both male and female flowers. A total of 120 kernels was sown in the field and from them 102 plants grew into adult stage. However, it was observed that when the plants were about two-months old, approximately 10 percent, nine plants, appeared slow in growth and short in height. About two weeks later, these short plants became dwarf yellow-green. Apparently their leaf chlorophylls were deficient. All except one, of these plants had barren stalk and sterile male inflorescence. The exceptional plant developed a small ear and a limited amount of pollen. Upon self-pollination, seven defective kernels were obtained. A test on their viability will be made in the next season. As was stated above, nine of 102 plants were phenotypically dwarf yellow-green. According to the Mendelian segregation principle, this was unexpected. It does not fit the expected segregation ratio of either monohybrids or dihybrids. Hence, a hypothesis was once again proposed that the unconventional segregation ratio was evidence of the presence of an *Ac* element in the parental plant. This element was activated through anther culture per se.

Effect of day length on the expression of maize cloning gene
--Ting, YC, Tran, L

Maize cloning gene was previously named tassel plant gene, is one of the apomictic genes in maize and its relatives. In 1946, Singleton (J. Hered. 37) coined this gene *id* due to its indeterminant growth habit. Colasanti et al. (Cell 93:1998), by molecular analysis, identified about 10 *id1*-like genes in maize. The cloning gene reported in the present communication may be one of these genes, because phenotypically they are alike. However, this cloning gene appeared dominant and the plantlets could grow into adult plants and reproduce next generation like the parental plant. With regards to these characteristics which are different from those of the other *id* genes, therefore the name cloning gene, *Clg* for short, was adopted. In view of this, respect for priority of genetic nomenclature is not overlooked.

Regarding the effect of day length on the expression of cloning gene, results of some previous studies (Singleton, J. Hered. 37, Shaver, J. Hered. 58) varied. In order to make further investigation on this effect, two dozen plants from a cross between a plant heterozygous for *Clg* and a plant homozygous for *clg*, were grown in the greenhouse last March. In June all of these plants were transplanted to the field and 22 of them survived. Among them, 10 vigorous plants were selected in July and treated with short-day illumination daily (8-hour light, 16-hour dark) for four weeks. The other plants, 12 in number, grew under long-day illumination (16-hour light, 8-hour dark). One month after the treatment, the treated plants had flower-induction for all of them but there was no evidence of the expression of the cloning gene despite the expected frequency of 50% that should have manifested the gene. On the other hand, three of the control-plants regenerated plantlets on their tassels. This number is much less than the expected 6, if the cloning gene is a responder to long-day illumination. Furthermore, the number of plantlets on the responder plants ranged from three to five per tassel. Comparing with the previous results reported by the senior author, the gene was only weakly activated. With the objective of clarifying this phenomenon, further studies are planned.

CHISINAU (KISHINEV), MOLDOVA
Institute of Genetics, Acad. Sci. Mold. Rep.

The posterior evaluation of maize selection for productivity
--Chernov, AA, Mihailov, ME

The objective of the present study was to establish the efficiency of maize selection for productivity by means of posterior evaluation. The study was carried out on a high-yielding hybrid, Moldavsky 291, that is widely cultivated in Moldova. The parental lines, F1, F2 and F3 plants were estimated for the following quantitative traits:

1) time from emergence to the flowering of panicles;
2) time from emergence to the flowering of top ears;
3) time lag of the onset of flowering between panicle and top ear;
4) time from flowering to the maturation of top ears;
5) time from emergence to the maturation of top ears;
6) number of stems;
7) plant height;
8) stem length;
9) panicle length;
10) top ear position on the stem;
11) diameter of the bottom first internode;
12) number of the above-ground nodes;
13) number of ears with kernels;
14) internode mean length;
15) stem volume parameter;
16) ratio of stem length to bottom first internode diameter;
17) weight of top ear at harvest;
18) weight of the remaining ears at harvest;
19) total weight of ears;
20) the proportion of second top ears in total ear weight;
21) daily increment in ears weight;
22) weight of the cob of top ear;
23) number of kernel rows on the top ear;
24) number of kernels on the top ear;
25) number of kernels in row on the top ear.
26) top ear length;
27) top ear diameter;
28) ratio of top ear length to diameter;
29) weight of 1000 kernels;
30) grain index.

The efficiency of maize selection for productivity was estimated for the correlation of the quantitative traits studied in F2 with the total weight of ears in F3. The correlation analysis has shown three traits to differ with respect to the efficiency of maize selection for productivity: internode mean length, diameter and weight of top ear at harvest. For the internode mean length in F2 statistically significant correlation with total weight of ears in

F3 reached 0.76 (P<0.01), for diameter of top ear: 0.52, for weight of top ear at harvest: 0.49. The correlation between total weight of ears in F2 and F3 reached only 0.22.

The results suggest that the efficiency of maize improvement for productivity may be increased by means of the selection for internode mean length, diameter and weight of top ear at harvest. The results obtained depend on year conditions. Future research is needed to ascertain the nature of the effects observed.

The influence of heterozygosis for marker loci on grain yield in maize

--Mihailov, ME, Chernov, AA

We studied the dependence of productivity in F2 maize plants on heterozygosis for the genome zones marked with 7 mutant and morphological loci. The hybrid combinations Ku123 x 2-9m and UIT-757 x 2-9m were used. The line 2-9m is marked with loci of chromosomes 2 (*ws3*, *lg1*, *gl2*), 6 (*y1*), 9 (*sh1*, *wx1*) and 10 (*R1*).

The F2 plants have been grouped in 8 classes according to number of heterozygous loci. In each class the mean grain yield has been detected. The recessive homozygotes *ws3*, *lg1*, *gl2*, *sh1* and *wx1* were excepted from analysis to avoid the pleiotropic effects.

The results are given in the table. The evident general positive trend of productivity at increase of heterozygosis is shown. The detail analysis has shown that heterozygotes are excellent for the *sh1* and *wx1* loci compared with analogous homozygotes. For the *ws3*, *lg1* and *gl2* loci the heterozygotes have no regular advantage. For the *y1* and *R1* loci the heterozygous plants are intermediate for productivity, exceeding mean value (mean a/d=0.75 for *y1* and 0.92 for *R1*).

Further analysis is needed to test whether the positive influence of heterozygosis can be reduced to the sum of the partial effects of each locus, or if there is a non-allelic interaction between different heterozygous zones of the genome.

Table. The productivity of F2 plants (gm/plant) at different degrees of heterozygosis.

Number of heterozygous loci	Hybrid and year Ku123 x 2-9m 1993	Ku123 x 2-9m 1995	UIT-757 x 2-9m 1993
0	95 (1)	163 (1)	169 (1)
1	132±32 (5)	146±15 (7)	124±20 (6)
2	139±14 (13)	153±24 (8)	133±8 (15)
3	147±6 (26)	164±12 (18)	148±14 (13)
4	148±7 (31)	150±14 (16)	130±10 (31)
5	146±7 (38)	164±16 (16)	135±7 (26)
6	139±10 (30)	175±11 (22)	164±10 (20)
7	155±8 (15)	181±63 (3)	167±14 (10)
Total	145±4 (159)	162±6 (91)	142±4 (122)

Comment: in the brackets the number of plants is given.

The application of treatments of physical and chemical nature in maize radiation mutagenesis

--Ikhim, YG

Radiation mutagenesis as one of the biotechnological directions of radiobiology has encountered a number of problems. The positive correlations between the dose and the radiation effect have been studied by many authors and are well-known. However, the dose increase leads to an enhanced possibility of lethal damages, and the mutation number in the radiated organism shows a loss of changes in genetic plasma. The problem of organism radiation responses, namely regeneration, repopulation and dedifferentiation, also arises at mutagenesis and in the course of studying the repair systems of multicellular plant organisms. Therefore, it is necessary to develop a method, with the aid of which it would be possible to overcome the above problems and keep the maximal possible number of mutagen modifications.

The postradiation (therapeutical) treatment is one of the most appropriate effects for the regulation of all the physiological processes, particularly, repair systems. The exogenic activation of intracellular processes modifies, to a great extent, the radiation damage, increasing the potential of the cell safety system with the help of somatical protection.

Table 1. Biometrical characteristics of plants.

Variances	Plant height	Leaf length	Leaf width
MK-01			
Control	97.93±1.88	56.77±0.78	5.74±0.06
Radiated control	72.84±2.31	51.27±0.85	5.09±0.09
γ + Crossing	79.19±2.16*	52.54±0.77	5.01±0.09
γ + Phytostim	74.77±1.96	51.39±0.81	4.85±0.09
γ+EF	74.11±1.91	50.89±0.82	4.98±0.09
γ+EHF	78.93±1.94*	51.60±068	5.03±0.08
19-3-3			
Control	147.63±2.62	63.82±1.17	7.04±0.15
Radiated control	128.40±2.40	57.25±0.81	6.17±0.11
γ + Crossing	137.29±2.31**	59.84±1.00*	6.23±0.12
γ + Phytostim	118.83±4.52	51.37±1.53	5.66±0.17
γ+EF	127.59±2.97	55.18±1.21	5.90±0.16
γ+EHF	135.40±2.52*	59.42±1.09	6.18±0.12

The difference with the radiated control is significant: * - 5%, ** - 1%.

The studies carried out show a possibility of overcoming the problems of radiation mutagenesis in higher plants. The biometrical characteristics of the first generation plants express in their physiological condition the treatments studied (Table 1). The postradiation treatment with the synthetic growth regulator "Crossing" and exposure to electromagnetic field of an extremely high frequency (EHF) mitigated the damage effect and enhanced plant habitus in the two stocks under study, MK-01 and 19-3-3, while the radiation doses were within the lethal ones (250Gy). The biometrical indices of the plants exposed to postradiation treatment with the biostimulator "Phytostim" and the electric field (EF) of the current at an industrial frequency did not differ from the radiated control.

The findings of the cytological studies are summarized in Table 2. The number of aberrant cells in the control corresponds to the experimental data of other authors and does not exceed 1%, which proves a genetic stability and developed safety system of the 19-3-3 stock. The aberration number of the chromosome material upon radiation made 20.74%, which indicates serious damage of the genetic plasma. Among all the treatments studied, only one contributed to the activation of repair processes; i.e. the postradiation treatment with "Crossing" - 15.94%. However, the percentage of chromosome damage exceeds significantly the control in this treatment, which leads to the suggestion that a high percentage of morphogenetic modifications caused by the chromosome aberrations can be expected. The studies on the elimination of the damaging action of the postradiation treatment for the trait of the number of sterile pollen grains are given in Table 3. The samples of pollen grains were collected on four dates.

The exogenic factors applied after radiation induce the catalysis of repair reactions causing mistakes in the repair system action. The change in the physiological condition of the damaged plant organism must not lead to the elimination of the apical dominance, otherwise it will result in regeneration recovery or dedifferentiation of tissues. But in both cases, these lead to elimination

Table 2. The cytological studies on the action of postradiation treatments. (Ikhim Yu.G., Scorpan V.G., Lysikov V.N., MNL 73, 1999.)

Variances	All of cells	Aberration		Bridges		Fragments	
		number	%	Chromatid %	Chromosome %	Single %	Double %
Control	1414	11	0.78	0.28	0.28	0.07	0.15
Radiated control	1512	314	20.77	6.42	6.94	4.70	2.71
γ + α-tocopherol	1042	291	27.93	8.06	9.79	5.47	4.61
γ + Crossing	1311	209	15.94	4.73	4.73	2.14	4.34
γ + Phytostim	718	142	19.77	2.92	8.91	1.81	6.13
γ + Catolitic	1152	363	31.51	6.68	12.15	5.47	7.21
γ + Anolitic	1521	526	34.58	13.48	7.30	6.77	7.03
γ + t =80 C, 30 min	933	280	30.01	7.72	8.36	6.11	7.82

Table 3. Sterility of pollen grains.

Variances	I date, %	II date, %	III date, %	IV date, %
Control	4.6	4.4	7.4	10.1
Radiated control	17.6	21.3	20.0	23.9
γ + Crossing	12.8	14.7	19.9	22.8
γ + Phytostim	15.4	18.3	21.8	27.6
γ+EF	16.0	19.1	27.2	26.0
γ+EHF	17.7	21.5	18.8	21.7

of mutant population of the apex initials. The observation over M2 showed that this combined effect increased not only the number of specimens in the offspring, but the range and percentage of mutant forms.

Segregation for the marker *ra1* gene in matroclinal haploids of maize

--Rotarenco,VA

The genetic inducers available at present allow the production of matroclinal haploids in mass quantities practically from any maize genotype (Tyrnov and Zavalishina, 1984; Zabirova, 1996; Chalyk, 1999). The absence of a directed elimination of individual genotypes and selection of ovules during the genetic induction of haploids is an important condition of their utilization in breeding and genetic programs.

P. Lashermes et al.(1988) studied the segregation of doubled haploids by isozyme markers and compared the segregation of doubled haploid lines and self-pollinated ones by phenotypical traits. The finding showed that the population of haploid plants represented a randomized set of genotypes. Lashermes showed that the directed elimination of individual genotypes was absent in the experiment. The ovule selectivity was also absent at the induction of haploids.

The goal of our work was to study the segregation for the marker *ra1* gene (conical panicle and branching ear) in haploid plants in order to establish the conformity of its segregation with the theoretically expected one under our conditions and in our material.

The inbred Rf-7 line crossed with the MG line carrying a homozygote for the *ra1* gene was used as initial material. The hybrid produced was crossed with the MHI line, which was an inducer of matroclinal haploids. The MHI line contains the marker *A1, C1, R-nj* gene, the presence of which allows the haploids to be selected reliably enough at both seed and plant level under field condition.

Segregation for the marker gene was studied on the haploid population produced. The table shows the assessment of segregation among haploid plants and the theoretically expected one.

Table. The conformity between the segregation for the marker gene in haploid plants and the theoretically expected one 1:1(χfact)

Haploid population	Total number of haploids	Normal genotype	Mutant genotype	χ-square
Rf-7 x MG	70	38	32	0.5

The theoretical value of the χ-square is 3.84 at the 5% significant level.

Since the haploids originated from the F1 hybrid, heterozygous for the gene under study, the theoretical expected segregation must be 1:1. Our experiment has confirmed that the segregation for the *ra1* gene is in conformity with the theoretically expected one.

The findings of our experiment have led us to the conclusion that in this case the haploids represent a randomly segregating population. The linear elimination of individual genotypes and ovule selectivity were absent during the haploid induction. Haploid plants can be used for the genetic analysis of traits and selection of genotypes with valuable economic traits.

Synchronization of cell cycles as a means of enhancing the efficiency of chromosome doubling in maize

--Rotarenco,VA

Maize haploidy has been more frequently involved in the solution of a wide range of scientific objectives. However, male sterility of maize haploids has remained an urgent issue until present.

Colchicine is known to be an unsurpassed chemical agent used for the polyploidization of plants. Simultaneously, it has a strong mutagenetic property. Possibly this is the cause of its insufficient efficiency during doubling of maize haploids. The haploid chromosome set is known to be more sensitive to mutagenic effect in comparison with the diploid one (Tyrnov, 1970). The other possible reason is an asynchronous division of meristem cells, which causes their varying sensitivity to the polyploidizing action of colchicine. Prophase, metaphase, and anaphase cells duplicate, but the mutagenic action of colchicine results in a significant number of chromosome aberrations, blocking of cell division, death of a part of them. Simultaneously, interphase cells are less sensitive to colchicine and, therefore, they maintain the former level of ploidy and the ability of a normal division (Davoyan, 1972).

It is known that chemical mutagens are characterized by the

capacity to influence directly not only the DNA molecule, but also its precursors. This specific type of mutagenesis is characteristic of the presynthetic (G1) and synthetic (S) phases of the cell cycle, when modified bases or nucleotides may be inserted into the forming DNA molecule (Dubinin, 1968).

In order to overcome the barriers impeding effective chromosome duplication in haploids, we propose the utilization of a method contributing to the increase in the cell number at the phase of mitotic division during the treatment with colchicine.

Seedlings with a root length of about 1.5cm were used for colchicine treatment, as mitosis in the stem meristem proceeds just at this stage of maize development (Gulyaev, 1958; Berlyn, 1972). It has been established that the rootlets reach an appropriate length after 3-day germination of seeds at 26 C. Then the seedlings were placed in a refrigerating chamber and kept at the low temperature (2-4 C) for 72 hours. Further, the seedlings were placed into a thermostat at the same temperature (26 C) and the meristem tissue was examined using a cytological method with equal time intervals. It had been established that all the cells were proceeding with the interphase stage after they were kept in the refrigerating chamber. This condition was maintained for a few hours and the mitotic activity of cells appeared only after seven hours of their storage at the temperature of 26 C. In this case, the mitotic activity was ample in comparison with the meristem tissue of the seedlings which were not exposed to low temperatures.

The effect of mass mitotic activity was studied on three genotypes showing no significant difference among them. The findings of the cytological examination had led us to the conclusion that it was the synchronization of cell cycles we were observing in maize at low temperatures.

The synchronization was employed as a possible means of enhancing the efficiency of haploid diploidization, as well as a possiblity to reduce the mutagenic action of colchicine.

Colchicine treatment was conducted seven hours after the seedlings had been placed into the thermostat. The seedlings were soaked in colchicine solutions at the concentration of 0.02%, 0.04%, and 0.06% supplemented with DMSO(0.5%).The treatment was carried out at the temperature of 18 C and 26 C for 12 hours. The colchicine treated seedlings were planted into sand filled tubs and kept in dark for 24 hours at the temperature of 26 C Further, the seedlings continued to grow for 48 hours at sun light then were planted in the field.

The experimental findings on the duplication of the chromosome number were verified under field conditions. The number of haploid plants producing pollen was estimated.

The effect of the cell cycle synchronization was more apparent in the treatments with the colchicine concentration of 0.02% while flowering plants were absent in the treatment under similar conditions and concentration but without synchronization.

Table. The method of treatment and results obtained

Treatment conditions		Results			
Temperature	Concentration	Number of plant	Haploids	Flowering haploids	%
-	-	404	404	0	0
18 C	0.02%	34	32	2	5.8%
	0.04%	35	25	10	28.5%
	0.06%	45	36	9	21%
Total		114	93	21	18.4%
26 C	0.02%	55	48	7	12.7%
	0.04%	57	38	19	33.3%
	0.06%	31	25	6	19.3%
Total		143	111	32	22.3%

The percentage of flowering haploids in the treatments with the conlchicine concentration of 0.06% was close to that without synchronization. The null influence of different temperatures and synchronization on the duplication result with an increased colchicine dose is likely to be associated with the fact that its diploidizing action, to a larger extent, occurs after soaking, i.e. colchicine, as an alkalyzing chemical mutagen, is characterized by the capacity to remain in tissue for some time. The colchicine action in these cases is likely to take place during at least two cell cycles. This supposition is also linked to the fact that plants supposedly having tetraploid traits were found among the haploids.

The seedlings treated with the concentration of 0.06% colchicine showed a suppressed growth and strong thickening at initial stages.

The highest percentage of flowering haploids was obtained after the treatment with the 0.04% concentration plus synchronization 28.5% at the treatment temperature of 18 C, and 33.3% at 26 C.

A significant difference in the percentage of flowering plants was recorded between the treatments at different temperatures, especially at the concentrations of 0.02% and 0.04%. This is, probably, connected with the highest efficiency of colchicine at high temperatures.

Flowering panicles were not found in the untreated control comprising 404 haploid plants.

Thus, synchronization of cell cycles in combination with low colchicine concentrations and elevated temperature can significantly increase the efficiency of diploidization of maize haploids.

The comparative characteristic of the correlation between the traits of maize diploids and haploids

--Rotarenco,VA

Plant transition from the diploid to haploid level involves quite notable phenotypical modifications. Haploids, as a rule, are similar to the specimens of the initial species for major traits, but in most cases their difference from diploids is of quantitative nature. It is believed that haploids are a reduced copy of parental form, at the same time, the transition to the haploid level involves significant modifications of genotypical medium, gene dose, expression of recessive gene. These peculiar features of haploids cause a definite specificity in gene interaction, as well as in genotype one , or more precisely, that of the genome and the environment. Some researchers (Hollingshead, 1930; Muntzing, 1934; Katayama, 1954) have found among the haploids traits absent in the initial diploids in a number of crops. The correlation between different traits is likely to vary in haploids and diploids, too.

The trait conjunction is a complex property from the genetic and physiologic viewpoints. Much attention is paid to the interrelation of the expression of different traits in both breeding work and genetic studies. Every possible correlation of traits is at the same time a property which hampers the breeding process, as well as an obstacle to developing an expected ideotype.

The goal of our work was to compare the interrelation of some qualitative traits, as well as the comparison of the relations between the indices including plant and earcorn traits at the diploid and haploid level.

The heterogeneous SP population and haploids derived from it were used as initial material for our studies. The plant traits were

measured in diploid and haploid population after the termination of growth. The earcorn traits were measured after their maturation under laboratory conditions. These were followed by the calculation of correlation coefficients and the criteria of their significance.

The correlation coefficients presented in Table 1 show that the pairs of the plant traits have a mean correlation between each other. The comparison of two levels of ploidy with the respect to these traits and their relation shows that the correlation coefficients differ insignificantly. A significant difference between the diploid and haploid levels was recorded for earcorn length and diameter. The correlation coefficient for the earcorn traits was 0.63 in the diploid population and 0.21 in the haploid one.

The earcorn traits are very important for maize haploids as their yielding potential can be predicted from them. This is associated with the dominance of the abnormal meiosis in the haploid chromosome set and the formation of a low number of seeds.

Table 1. The coefficients of correlation between certain plant and ear traits in diploids and haploids.

	Ploidy	
Correlating Traits	2n	n
Plant Height and Height of Attachment of the First Ear	0.53**	0.59**
Leaf Length and Width	0.4**	0.43**
Ear Length and Diameter	0.63**	0.21

*,** The coefficients of correlation are significant at P>0.05 and 0.01 respectively.

Table 2 shows the correlation coefficients between the traits of the earcorn and plant at the diploid and haploid level. The data show a mean correlation of these traits in the diploid plants and actually the absence of the correlation in the haploids.

Table 2. The coefficients of correlation between the ear and plant traits in diploids and haploids.

Traits	Plant Height		Height up to the 1 ear		Leaf Length		Leaf Width	
	2n	n	2n	n	2n	n	2n	n
Ear Length	0.47**	0.17	0.35*	0.13*	0.57**	0.007	0.6**	0.07
Ear Diameter	0.28	0.19	0.020	0.09	0.36*	0.21	0.4*	0.07

*,** The coefficients of correlation are significant at P>0.05 and 0.01 respectively.

In order to provide a general characterization of the correlation between the habitus and earcorn, we calculated the correlation between the indices, combining four traits of the plant and two traits of the earcorn. The indices were calculated in the following way: the value of the trait of an individual plant was divided by the mean value of this trait for all the samples and the data obtained were summed up with those obtained by the same way but for other traits. As a result, each plant in the sampling was characterized by only two figures. One figure characterized the plant traits, the other - the earcorn traits.

We calculated the correlation between the plant index and the earcorn one in the diploid and haploid. The correlation coefficient was 0.53 (significant at P>0.01) at the diploid level, and 0.073 – at the haploid one.

This study has shown that the correlation between the earcorn traits and the plant ones differs significantly at the diploid and haploid levels. This difference is true for both individual pairs of the traits and the indices of plant and earcorn traits. The difference was also significant for the earcorn traits in the diploid and haploid plants. The data suggest that the transition to the haploid level leads to a significant change in the correlation between the maize traits. This fact may have a great importance for breeding work. During the selection at the haploid level, the genetically determined expression of the trait can be selected with a high precision in comparison with the selection at the diploid level. Thus, it is possible to reduce the time of selection work and to increase its efficiency.

Morphological characters variability of male gametophyte of waxy maize regenerants

--Kravchenko, OA, Palii, AF, Kravchenko, AN, Lysikov, VN

In our investigation the 346 and 502 inbred lines and their waxy counterparts, as well as their R1, MR1, MR2 progeny, were taken as the experimental material. For the analysis in the light scanning microscope "Morphoquant" permanent preparations of mature pollen were made using the technique developed by Kravchenko A.N. (In: Recombinogenesis in Evolution and Breeding, Kishinev, p.264-265). Such characters as perimeter (μm), area (μm^2), form factor (relative units), width (μm), diameter (μm), eccentricity (relative units) of pollen grain, as well as those of vegetative and generative cell nuclei were evaluated. The data were processed by two factor analysis of variance.

The results obtained indicate that morphological characters of pollen grain of the 346 and 502 inbred lines and their counterparts varied insignificantly (coefficient of variation (V)<10%). However, the variability range of such pollen grain characters as perimeter, area, and eccentricity in R1, MR1, and MR2 generations was significant (V>20%). In addition, the higher coefficients of variation were noted for waxy counterparts.

Analysis of variance revealed that variability of pollen grain characters studied was either genotype or gamma radiation dependent (Table 1).

Table 1. Share of variability of pollen grain characters, %.

CHARACTERS	DETERMINED BY:		
	genotype	gamma radiation	their interaction
perimeter	65.7***	11.36***	14.11***
area	53.46***	30.65***	-
form factor	9.78*	17.39***	55.18***
diameter	66.73***	-	-
width	39.32***	49.27***	4.63*
eccentricity	15.78***	63.83***	11.24***

*** - P<0.001; * - P<0.05

However, the variability of width and eccentricity was to a large degree gamma radiation dependent. It is worth noting that variability of such a character as form factor proved to be mostly determined by the interaction of factors studied. For waxy counterparts the average values of such characters of pollen grain as perimeter, area, and eccentricity were found to be higher (Table 2).

In generations of regenerants derived from inbred lines studied the average values of perimeter, form factor, and eccentricity proved to be decreasing in comparison with control (Table 3).

Table 2. The average values of morphological characters of pollen grain.

Genotype	Characters					
	perimeter	area	form factor	diameter	width	eccentricity
346+/+	299.16	5178.18	16.11	86.46	72.92	1.173
346wx1wx1	307.69	4935.07	17.98	83.33	71.42	1.360
502+/+	281.37	4447.43	17.93	80.51	67.92	1.206
502wx1wx1	289.99	4685.95	19.95	83.46	67.85	1.340
Least significant difference (LSD 0.001)	7.04	237.28	1.63	2.73	1.93	0.16

Table 3. Pollen grain morphological characters variability in generations of regenerants.

	Characters				
Generation	perimeter	area	form factor	width	eccentricity
Control	304.25	4816.16	18.37	68.62	1.22
R1	296.89	4340.32	17.96	66.79	1.2
MR1	289.66	4546.81	17.55	67.96	1.21
MR2	282.99	5166.75	17.12	73.51	1.15
LSD $_{0.001}$	6.1	205.49	0.41	1.67	0.11

It was also found that morphological character variability of vegetative and generative cell nuclei in MR1 and MR2 generations was higher (14%<V<47%) in comparison with control and R1 generation. Analysis of variance showed the reliable dependence of variability of these characters on genotype, gamma radiation and their interaction. It should also be noted that the variability of characters studied (except eccentricity of generative cell nuclei) was more gamma radiation dependent (Table 4).

Table 4. Morphological character variability of vegetative (V) and generative (G) cell nuclei of pollen.

	the share of variability determined by:		
Characters	genotype	gamma radiation	their interaction
Perimeter V	39.79***	43.32***	11.98***
Area V	40.91***	42.19***	11.75***
Form factor V	35.99***	51.68***	4.89*
Diameter V	35.77***	53.26***	5.79**
Width V	29.39***	35.17***	-
Eccentricity V	-	-	56.71***
Perimeter G	30.01***	61.52***	5.21***
Area G	26.68***	49.35***	10.56**
Form factor G	9.44***	77.68***	5.46*
Diameter G	7.51**	80.69***	-
Width G	6.4***	81.93***	7.06***
Eccentricity G	37.28***	35.36***	-

p<0.05; **p<0.01; ***p<0.001

The average values of such characters as perimeter, width of vegetative cell nucleus and all morphological characters of generative cell nucleus were found to be decreasing for regenerants of waxy counterparts (Table 5).

Table 5. The average values of morphological characters of vegetative (V) and generative (G) cell nuclei in dependence of genotype.

	Genotype				
Characters	346+/+	346wx1wx1	502+/+	502wx1wx1	LSD $_{0.001}$
Perimeter V	43.62	40.33	39.29	37.06	1.74
Area V	92.00	78.24	78.81	69.73	5.92
Form factor V	21.88	19.69	16.18	17.93	1.73
Diameter V	12.25	10.78	8.1	9.62	1.42
Width V	8.71	7.42	8.0	7.4	1.2
Perimeter G	22.00	20.31	22.31	17.58	1.29
Area G	17.24	15.76	18.12	12.39	3.24
Form factor G	26.68	23.31	22.03	18.48	3.18
Diameter G	7.63	5.98	6.27	4.69	1.54
Width G	1.34	1.01	1.73	1.41	0.3
Eccentricity G	3.76	3.2	3..86	2.93	0.54

In MR1 and MR2 generations the average values of characters studied proved to be increasing in comparison with control and R1 generation. In addition the plants of MR2 generation had the highest average values of male gametophyte characters studied (Table 6).

In general, according to the results obtained the pollen of regenerants and their progeny (especially those derived from irradiated embryos) was found to differ from the male gametophyte of inbred lines by the range of morphological characters studied.

Table 6. Morphological character variability of vegetative (V) and generative (G) cell nuclei of pollen among plants of different generations.

	Generation				
Character	Control	R_1	MR_1	MR_2	LSD $_{0.001}$
Perimeter V	36.96	38.51	40.27	42.99	1.5
Area V	69.02	75.37	81.5	88.58	5.13
Form factor V	15.85	17.39	18.96	21.15	1.5
Diameter V	7.15	8.52	9.79	12.48	1.23
Width V	7.24	7.99	9.06	10.65	1.04
Perimeter G	18.35	19.5	20.97	24.33	1.12
Area G	13.66	14.92	15.89	20.07	2.8
Form factor G	19.49	20.89	22.34	31.02	2.76
Diameter G	3.71	5.09	6.7	8.95	1.33
Width G	0.43	1.88	3.26	4.47	1.15
Eccentricity G	3.25	3.49	3.72	3.99	0.17

Studies on the induced variability of maize plants following the radiation of the female gametophyte

--Romanova, IM, Krivov, NV, Lysikov, VN

Radiation mutagenesis one of the promising breeding techniques. Mutations can result from the radiation of maize seed or pollen with different types of radiation. Radiation of female generative structures will make it possible to solve a number of theoretical and practical problems, including the increase of genotypical diversity, the development of the initial genetic stock or donors with improved characteristics. The availability of X-ray units of the Reis type allowed the radiation of plants under field conditions. The power of the exposure dose was 2000 r/min. In our experiments, the radiation was carried out at a distance of the focus objective of 20 mm.

The female gametophyte of maize plants was exposed to the radiation dose ranging between 5 and 70 Gr in 15 treatments. The experimental material produced was sown in the field and the quantitative and qualitative traits of maize plants were studied during the vegetative period in M1. In practical breeding, an appropriate selection of doses, as well as the effect on germinating and producing capacity of plants, are of paramount importance for induced mutagenesis.

Our experiments have shown that the dose curve of the grain content of maize plants exposed to the x-ray radiation of 5-70 Gray at the 3-4 leaf stage has a typically expressed exponential pattern, i.e. the grain content of the maize ear falls with the dose increase by 8-12 times.

The experiments were carried out to analyze the grain content in M2, germinating capacity of the plants in M1.

It has been established that the dose curve of the grain content in M2 changes significantly, acquiring a distinctly expressed peak pattern (Table 1). The one factor dispersion analysis has shown that the grain content per ear in M2 reaches maximum values at the radiation dose of 20 Gray and makes 148.4 ±12.6 and 40 Gray - 135.9 ±10.5 . This increase is statistically significant (P<0.5) and differs from the control values of the grain content making 101.6 ± 9.6. The tendency towards the appearance of peaks on the dose curve is observed in other ranges of x-ray radiation, in particular at the doses of 10 Gray, however, such deviation of the control values is statistically insignificant at the significant level of 95%.

It is noticeable that the dose curve of the M1 germinating capacity is characterised by a trend towards the formation of extrema in the same ranges of the x-ray radiation (Table 1). Thus, one factor dispersion analysis has shown that at the dose of 20 Gray the germinating capacity of plants reaches 62.1 ± 7.3 at 40

Table 2. Studies following the exposure of the female gametophyte to x-ray radiation.

Treatment	Plants			Families			Dwarfs and semidwarfs		Indented and rolled leaves		Male and female sterility		Stripeness		Late ripening	
Doses (Gr)	study	No	%	study	No	%	num. of plants (%)	num. of families (%)	num. of plants (%)	num. of families (%)	num. of plants (%)	num. of families (%)	num. of plants (%)	num. of families (%)	num. of plants (%)	num. of families (%)
Control	84			10												
5	169			16												
10	264	15	5.7	23	6	26.1	7(2.7)	3 (13)	5(1.9)	2(8.7)			3(1.1)	1(4.4)		
15	73	2	2.7	13	2	15.4			1(1.4)	1(7.7)			1(1.4)	1(7.7)		
20	170	5	2.9	14	2	14.3			4(2.4)	1(7.1)	1(0.6)	1(7.1)				
25	220	7	3.2	19	2	10.5			7(3.2)	2(11)			1(0.5)	1(5.3)		
30	271	6	2.2	25	3	12			1(0.4)	1(4)	1(0.4)	1(4)	1(0.4)	1(4)	4(1.5)	1(4)
40	117	2	1.7	10	2	20	1(0.9)	1 (10)	1(0.9)	1(10)						
50	177	4	2.3	22	1	4.6								4(2.3)	1(4.6)	
60	190	21	11.1	17	5	29.4	3(1.6)	2 (11.8)					2(1.1)	1(5.9)	6(2.3)	1(5.9)
							10(5.3)	1 (5.9)								
70	253	23	9.1	20	2	10					1(0.6)	1(7.1)				

Table 1. The grain content and germinating capacity after treating female gametophyte with x-ray radiation

Treatment Doses (Gr)	Grain content per ear	Coefficient variation (%)	Germinating capacity	Coefficient variation (%)
control	101.6 + 9.6	55.6	56.9 + 2.6	14.6
5	97.7 + 11.0	66.7	52.1 + 5.5	41.8
10	129.0+ 8.0	52.7	46.4 + 3.3	34.2
15	84.8 + 9.0	53.1	37.5 + 9.9	95.2
20	148.4 + 12.6	45.0	62.1 + 7.3	43.8
25	131.4 + 12.4	49.1	53.9 + 3.9	36.2
30	115.2 + 8.7	62.9	44.5 + 4.6	51.2
40	135.9 + 10.5	36.3	47.7 + 6.4	42.4
50	105.8 + 12.1	69.8	29.7 + 4.7	73.5
60	99.7 + 11.5	58.9	34.2 + 3.6	43.6
70	80.9 + 10.2	74.7	25.0+ 4.0	69.6

Gray it is 47.7 ± 6.4, while in the control it makes 56.9 ± 2.6. However, it should be mentioned that these data at the significant level of 95% differ from the control ones statiscally insignificantly, though such significance at the dose of 20 Gray is already revealed at the significant level of 95%.

The frequency and mutation type arising due to the action of the x-ray radiation on the female gametophyte have been evaluated (Table 2). The highest number of mutations has been found at the radiation doses of 10 Gray (5.7%) and 60 Gray (11.1%), respectively both in the plants and families. At the radiation doses of 20 and 40 Gray, upon which the peaks of germinating capacity in M1 and grain content in M2 are observed, the number of mutations appears to be significantly lower in plants and families and makes 2.9% and 1.7% respectively. The processes of repair are more intensive under these doses.

The experimental data have led us to the conclusion that the exposure of the female gametophyte to the x-ray radiation within the range of 20 - 40 Gray might significantly intensify repair processes in the maize plant resulting in the increase of seed germinating capacity and grain content of ears. The application of target radiation doses might lead to a maximum number of mutations, which is observed at the doses of 10 and 60 Gray, as well as to a desired type of mutation using experimentally established exact doses of x-ray radiation. All these make it possible to significantly reduce the duration needed to obtain desired results, as well as to carry out a target breeding of plants with a maximal possible efficacy.

Obtaining fertile pollen in maize maternal haploids

--Chalyk, ST (Cealic, ST)

Development of homozygous lines in maize can be significantly accelerated by using maternal haploid plants. However, their rather high male sterility is the limiting factor for selfing of maternal haploids. We have made an attempt to solve the problem by using colchicine. Maternal haploids obtained from MK01y x A619 hybrid were used in the experiment. Haploids were treated by water solution of colchicine with DMSO (dimethyl sulfoxide) 0.5% (by volume). The following three stages of seedlings and germinated seeds were subjected to the treatment: (1) soaked seeds; (2) 2-3 day seedlings and (3) seedlings with 3-4 leaves.

(1) Soaked seeds were treated according to the method of Gayen et al (1994). The seeds were soaked in water 48 hours at 18 C. A small portion of the plumule tip was cut off before treatment. Treatment with 0.06% colchicine concentration was carried out at 18 C. After treatment the seeds were washed in running water, and then kept in the dark at 18 C for two days. Then they were planted in the field nursery.

(2) 2-3 day seedlings were treated with colchicine according to the method proposed by Deimling et al. (1997). The seeds were germinated in a thermostat at 26 C up to the stage when their coleoptile had grown to the length of about 1 cm. The top of the coleoptile was cut, and long roots were shortened to 2-3 cm. The seedlings were placed in a vessel with colchicine. The treatment was carried out in the dark with 0.06% colchicine at 18 C during 12 hours. After treatment the seedlings were washed with running water and kept in the dark for two days. Several modifications of this method were tried. First, the effect of 0.02% colchicine concentration was examined. Second, the two temperatures of post treatment, 18 C and 26 C were tried. The seedlings were kept in the dark for two days at these two temperatures after treatment. Then the seedlings were planted in the field.

(3) Seedlings were treated according to the method of Zabirova et al. (1996) at the stage of 3-4 leaves. The 0.125% colchicine solution was injected approximately 3-5 mm above the apex. After the injection the seedlings were kept in the dark for two days at 18 C and then planted in the field.

During the experiment the effect of colchicine was considered to be successful in each case if several anthers with fertile pollen appeared on a tassel of a haploid plant. Fertility of pollen was

evaluated visually. The Table presents the results obtained. The methods developed by Deimling et al. (1997) and Zabirova et al. (1996) were rather efficient under our conditions. Using the method of Deimling et al. (1997) allowed us to obtain 28.8% and 31.8% of haploids with fertile pollen. Our attempt to modernize the method gave no positive results. Reducing the colchicine concentration from 0.06% to 0.02% resulted in the fact that not a single haploid treated had anthers.

It was difficult to judge on degree of importance of temperature on treatment of the seedlings with colchicine. Unfortunately after planting the seedlings in the field they were attacked by rooks and the number of haploid plants under study was decreased significantly. The small number of plants in the variant with the temperature of the post-treatment of 26 C did not allow determining its difference from the treatment at the temperature of 18 C. Haploids with fertile pollen were obtained with approximately similar frequency in both cases.

Use of injection of colchicine according to the method of Zabirova et al. (1996) resulted in 27.5% haploid plants with fertile pollen. This frequency was quite comparable with the frequency resulting from using the method of Deimling et al. (1997).

In the control, 211 haploid plants were used. Not a single one of these plants had anthers with fertile pollen. We assume that it was the result of rather unfavorable environmental conditions which prevailed in summer 1999 in our region. Strong drought was combined with prolonged high air temperature in the period before tasseling, during tasseling and during flowering. Usually, when environmental conditions are optimal, we obtain, without using any doubling factors, 2-5% haploid plants which have a certain quantity of anthers with fertile pollen. Strong drought and hot conditions led to the fact that in the control all the haploid plants were sterile.

Thus it can be noted that the methods developed by Deimling et al. (1997) and Zabirova et al. (1996) allowed us to obtain fertile pollen in haploid plants even in unfavorable drought and hot conditions. These methods can be successfully used for obtaining doubled haploid lines in a mass quantity.

CLARIFYING NOTE. In the previous article, Chalyk S.T. 1999. Creating new haploid-inducing lines of maize, MNL 73, p. 53-54, it was shown that in our experiments the highest proportion of haploids occurred at the top of an ear. The lowest frequency of haploids was obtained at the bottom of an ear. During the analysis of these data the author referred to the fact that in the article of S. Chase (1969) a similar conclusion was made. However this was a mistake. In his article S. Chase made a directly contrary conclusion. In this connection the author apologizes to Dr. S. Chase and readers for the mistake made.

Table. Frequency of haploid plants obtained with fertile pollen.

Material	Conc. of colchicine, %	Temperature, C		Total haploids	Haploids with fertile pollen	
		Before treatment	After treatment		Number	%
Control	-	-	-	211	0	0
Seeds soaked for 48 hours	0.06	18	18	21	0	0
2-3 day seedlings	0.02	26	18	27	0	0
2-3 day seedlings	0.02	26	26	19	0	0
2-3 day seedlings	0.06	26	18	52	15	28.8
2-3 day seedlings	0.06	26	26	22	7	31.8
Seedlings with 3-4 leaves	0.125	26	18	40	11	27.5

CLEMSON, SOUTH CAROLINA
Clemson University Genomics Institute

Construction and characterization of a maize bacterial artificial chromosome (BAC) library for the inbred line LH132

--Tomkins, JP, Frisch, DA, Byrum, JR, Jenkins, MR, Barnett, LJ, Wicker, T, Luo, M, Wing, RA

The construction of a physical framework for the maize genome requires the use of a large insert genomic library. In order to develop a comprehensive physical framework for maize using fingerprinting and BAC end sequencing technologies, a deep coverage BAC library was developed. Methods used were generally similar to those described previously by Tomkins et al. (Plant Molec. Biol. 41:25-32, 1999).

The library was developed from the maize LH132 Dekalb inbred line and is suitable for constructing a comprehensive physical framework of the maize genome. *Hin*dIII was used as the cloning enzyme because complete digests with maize DNA produced fragments ≤ 30 kb. The library consists of 427,392 clones stored in 1,113 384-well microtiter plates. A negligible amount of clones (0% based on 347 samples) do not contain inserts as judged by random analysis of BACs sampled from the library. A random sampling of 347 BACs taken from the library indicated an average insert size of 118 kb with a range of 50 to 390 kb. Insert size estimates were based on a very conservative molecular weight marker (Midrange I, NEB). Because the corn genome, like most monocots, has a high percentage of *Not*1 sites, many insert fragments migrate below 50 kb. It has been our experience that BAC clones from monocot genomes are likely to have inflated insert size estimates when evaluated with a conventional 50 kb lambda ladder. Therefore, we believe that the insert size estimates in the present study are quite conservative. Based on a haploid genome size of 2,500 Mb (Arumuganathan and Earle, Plant Mol. Biol. Rep. 9:208-218, 1991), the coverage of the library is about 20.2 maize genome equivalents.

To determine the size distribution of BAC clones in the library, the 347 BACs analyzed with *Not*I digests were grouped by insert size and the frequency of each group of clones represented in the library was determined. Based on this analysis, 76% of the clones in the library have an average insert size equal to or greater than 100 kb.

The maize LH132 BAC library is well suited to construct a comprehensive physical framework of the maize genome due to its high redundancy and large average insert size. The physical framework for LH132 will be constructed by *Hin*dIII fingerprinting and BAC end sequencing all clones in the BAC library. Reagent costs for these efforts will be maximized due to a negligible amount of BACs not containing inserts.

Construction of the LH132 maize BAC library was performed at the Clemson University Genomics Institute through funds received from the Monsanto Corp. The library is the property of Monsanto and requests concerning its availability and use should be directed to Joe Byrum (email: joe.r.byrum@nal.monsanto.com).

Construction and characterization of a maize bacterial artificial chromosome (BAC) library for the inbred line B73

--Tomkins, JP, Frisch, DA, Jenkins, MR, Barnett, LJ, Luo, M, Wing, RA

The construction of a physical framework for the maize genome

requires the use of a large insert genomic library. In order to develop a comprehensive physical framework for maize using fingerprinting technology, a deep coverage BAC library was developed. Methods used were generally similar to those described previously by Tomkins et al. (Plant Molec. Biol. 41:25-32, 1999).

The library was developed from the inbred maize line B73 which is suitable for constructing a comprehensive physical framework of the maize genome. The library is also well suited for map-based cloning and other genomics applications. *Hin*dIII was used as the cloning enzyme because complete digests with maize DNA produced fragments ≤ 30 kb. The library consists of 247,680 clones stored in 645 384-well microtiter plates. A neglible amount of clones (0.4 % based on 3/697 samples) do not contain inserts as judged by random analysis of BACs sampled from the library. A random sampling of 697 BACs taken from the library indicated an average insert size of 136 kb with a range of 42 to 379 kb. Insert size estimates were based on a very conservative molecular weight marker (Midrange I, NEB). Because the corn genome, like most moncots, has a high percentage of *Not*I sites, many insert fragments often migrate below 50 kb. It has been our experience that BAC clones from monocot genomes are likely to have inflated insert size estimates when evaluated with a conventional 50 kb lambda ladder. Therefore, we believe that the insert size estimates in the present study are quite conservative. Based on a haploid genome size of 2,500 Mb (Arumaganthan and Earl, Plant Mol. Biol. Rep. 9:208-218, 1991), the coverage of the library is about 13.5 maize genome equivalents.

To determine the size distribution of BAC clones in the library, the 697 BACs analyzed with *Not*I digests were grouped by insert size and the frequency of each group of clones represented in the library was determined. Based on these data, 88% of the clones in the library have an average insert size equal to or greater than 100 kb.

The maize B73 BAC library is well suited to construct a comprehensive physical framework of the maize genome due to its high redundancy and large average insert size. The physical framework for B73 will be constructed by *Hin*dIII fingerprinting. Reagent costs for these efforts will be maximized due to the very low percentage of BACs not containing inserts.

Construction of the maize B73 BAC library was performed at the Clemson University Genomics Institute for the Missouri Maize Project (Ed Coe et al.) through a subcontract as part of a grant received from the National Science Foundation Plant Genome program. Requests concerning availability and use of the library should be directed to David Frisch (email: dfrisch@clemson.edu).

COLUMBIA, MISSOURI
University of Missouri - Columbia

A safe procedure for EMS treatment of pollen in maize

--Kato, A

EMS (ethyl methanesulfonate, methanesulfonic acid ethyl ester) treatment on maize pollen in paraffin oil is known to be very effective to induce mutants. The treatment was originally developed by Neuffer and Coe (Maydica 1978. 23:21-28), and several researchers followed the procedure with successful results (Harper et al. 1995. MNL 69:22 ; Zhao et al. 1998. MNL 72:15-16). Detailed descriptions by Neuffer of the treatment are found in *Mutants of Maize* (pp. 397-398) and in *The Maize Handbook* (pp.212-218.).

Extreme precaution is necessary to handle EMS because the chemical is a volatile carcinogen. Because EMS treatment of pollen is done in an open field, specific safety procedures must be employed to conduct the treatment in a way that protects the experimenter as well as personnel working in the field. Nevertheless, there are no descriptions of safety procedures for this treatment. In the summer of 1999, I had to develop safety procedures for this treatment. The following is a detailed description of the safety procedure for EMS treatment of maize pollen in the field.

Before starting the experiment the following items should be in hand.

A gas mask (Willson chin-style gas mask, and a canister for organic vapors)
Coveralls (Tyvek/Saranex 23-P Coveralls with hood and booties)
Gloves (disposable rubber gloves, size M and L)
Gloves (long gloves that cover the sleeves)
Disposable soft plastic pipets (e.g., Graduated pipets 5ml Fisher Cat. No. 13-711-9A)
Plastic tubes (45 ml disposable plastic tubes with a screw cap)
A pipetter (e.g. Pipetman 20, Rainin)
Spray bottles
Polyethylene bags with zipper
Sodium thiosulfate (500g)
Tween 20
A timer

The mask fit well and was easy to wear and take off. The canister of the gas mask was extended with a rolled towel, and the tip of the towel was sprayed with thiosulfate solution (10%). The coveralls were disposable, and are thin, lightweight and convenient for body movement.

Before doing the following procedures with EMS, it is better to practice without EMS.

Preparation of EMS - oil mixture. In a fume hood deliver 20 ml of paraffin oil (Sigma, mineral oil M8410) to 20 tubes. Pre-mark the 12 microliter volume level on three or four dry 20 microliter tips. This is easily done by pipetting 12 microliters of water with one tip and marking the water level line on the other tips. The wet tip is disposed of. The marked dry tips are attached to a disposable soft plastic pipet. Wearing the gas mask and double disposable rubber gloves, EMS is moved into the hood from a refrigerator, and EMS delivery starts. The materials should be brought into the hood and placed on disposable paper as follows.

An EMS bottle enclosed in a protective can.
Tipped disposable pipets (three to four).
300 ml 10% sodium thiosulfate solution (addition of 1% Tween 20) in a 500 ml plastic beaker.
10% sodium thiosulfate solution (addition of 1% Tween 20) in a spray bottle.
Twenty plastic tubes (45 ml) containing 20 ml paraffin oil.
A thin metal blade to open the lid of the can and the inner cap of the EMS bottle.
Several sheets of paper towels.

The caps of the plastic tubes are removed. The lid of the can and the cap of the EMS bottle are removed very carefully. Twelve microliters of EMS are delivered to each tube using the tipped disposable pipet. The used pipets are dipped into the sodium

thiosulfate solution and the inside of the pipet is washed with the solution after delivery. All contaminated materials are dipped into the sodium thiosulfate solution. Sodium thiosulfate reacts with EMS quickly to produce harmless products. Any undesirable EMS contamination is sprayed with the sodium thiosulfate solution and wiped thoroughly with a piece of paper towel. The EMS bottle is capped and sealed in the can and stored in a refrigerator. The EMS containing tubes are capped, numbered and stored in the hood. Materials that have been decontaminated with the sodium thiosulfate solution can be disposed of as regular waste the next day.

Preparation of maize plants for EMS treatment. An inbred with high fertility should be used (e.g., Oh43). The field should be isolated and have warning signs. Husks of the plants are covered with a polyethylene bag (5 x 15 cm) before silk emergence. A paper bag (5 x 19 cm, Lawson 217) was inserted between the ear and the stalk. Also a paper tag was attached to every plant (Fig. 1).

Transportation of the tubes containing EMS. One to three EMS tubes are transported to the field every day during the treatment. Tubes are covered with a zipped polyethylene bag containing a piece of paper towel soaked with sodium thiosulfate solution. The covered tubes are stood upright in a Styrofoam box and the box is sealed with sticky tape.

Mixing pollen with paraffin oil containing EMS. Wind direction is determined before mixing. A pole with long tape is helpful. Smoke will also work. Once the wind direction is determined, all the materials necessary for the treatment are placed upwind from the EMS field.

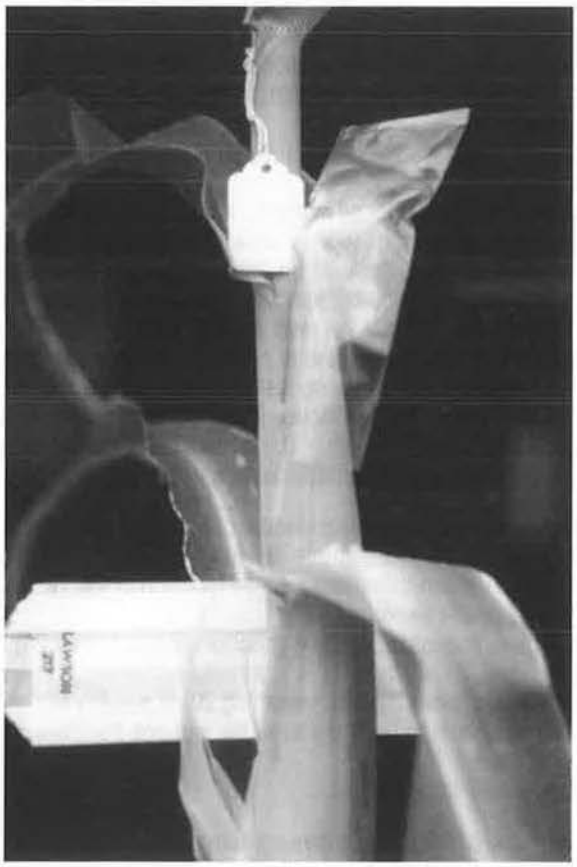


Fig. 1. Preparation of a shoot for EMS pollination. The silks are covered with a bag and another bag is inserted between the sheath and the stalk. A paper tag is attached.

Each tube contains 20 ml of paraffin oil and 12 microliters of EMS. EMS is observed at the bottom of the tube as a translucent droplet. The tube is shaken five to ten times vigorously and placed in the shade. Maize pollen is collected and filtered to eliminate anthers. Collected pollen is poured into a disposable cylinder and 2 ml of pollen is measured. Wearing gloves and the gas mask, the cap of the tube which contains oil and EMS is opened very carefully. The pollen is poured into the tube and the cap is screwed tightly. This tube is shaken vigorously every 3-5 minutes for 30-50 minutes. During shaking it is not necessary to wear the coveralls and the gas mask.

EMS pollination. Near the end of shaking, preparation for EMS-pollination starts, i.e. taking off shoes, putting on coveralls, triplicate gloves (medium disposable glove, long sleeve glove, and large disposable glove), and the gas mask (be sure your chin is fitted to the mask).

After putting on protective clothes, EMS pollination can begin. The polyethylene bag is removed from the ear and 0.5 - 1.0 ml of the EMS-oil/pollen mixture is dropped onto the silks using the disposable pipet (Fig. 2). The pollinated silks are covered with the shoot bag which is already inserted between stalk and the ear (pin or stapler is not necessary). Continuous agitation is necessary during pollination to prevent maize pollen accumulation at the bottom of the tube. 20 ml of oil containing EMS is enough to pollinate 20 - 40 ears and it takes about 10 - 15 minutes. Silks can be cut with a blade at 2 cm height above the husk just before pollination. For this purpose a knife blade can be attached to the wrist with a string.

After each pollination the paper tag on the stalk is bent according to a pattern that indicates the number of the treatment. Bending is easier and faster than writing letters on the label, and it can be done with one hand. Consequently EMS pollination can be done without any assistance.

Neutralization of EMS After pollination, 10% sodium thiosulfate solution (addition of 10% Tween 20) is added (1:1 ratio) to the remaining oil-EMS mixture and shaken. The gloves and coveralls are sprayed with the sodium thiosulfate solution (addition of 1% Tween 20). The gloves are disposed in a polyethylene bag. Coveralls can be used several times; tears can be fixed with sticky tape. The gas mask is put in a cardboard box and other materials are also put in another box. The boxes are covered with the coverall kept at the field. All garbage is treated with sodium thiosulfate solution (addition of 1% Tween 20) and stored in the field for thirty days. After storage, they can be disposed of as regular waste.

Dealing with high temperature during treatment. The person who conducts pollen-EMS treatment may have to deal with high daytime temperatures. The coveralls and the gas mask prevent evaporation of perspiration, and it makes pollination work very difficult. However, about 15 minutes of work can be done without the risk of heat exhaustion. If the temperature is very high and sunshine is strong, the following tips may be helpful.

1. Eating a lot of broken ice before pollination.
2. Bagged broken ice is placed in the gas mask. The ice can be eaten during pollination.
3. Other work, such as covering ears and detasseling in the EMS field should be done in the cooler times of the day.

Fig. 2. EMS pollination with the protective clothes (photo by D. Auger).

Results of pollen-EMS treatment varies according to the quality of pollen, temperature during mixing, and temperature after pollination (personal communication from Dr. Neuffer). I did the pollen-EMS treatment on the inbred line Oh43 in the summer 1999. The results of the trials I have done varied considerably and 80 % ears resulted in no seed set. The remaining ears with seed set suggested that appropriately treated pollen lost fertilizing ability within 10 minutes after the start of pollination and the viability seems temperature dependent. 30 - 50 minutes of shaking seems appropriate to obtain M_1 kernels. Trials involving different

shaking times and different concentrations of EMS would be necessary to determine the appropriate treatment for a particular inbred line.

Mutant photographs on the World Wide Web (Part 2)
--Neuffer, MG

Note: this is an update on the project described earlier (MNL71:24).

My goal was to make the best pictures from my entire photographic collection available on the MaizeDB web site. To this end, digitized images were produced and appended to the appropriate variation form. However, we did not have time to write captions for the photos (over 3,000 images mostly identified only by symbol, lab number, and name), so they were entered essentially as they appeared in our data files. It was fortunate that we did so at the time because we hit the window of favorable conditions (technology, resources, helpers and convenient access to the internet) that made it easy to do in that superficial fashion. With the able assistance of Lou Butler, and support from the Maize Genome Project I am now in the process of correcting that deficiency. Since an image browser is in the works and will most likely be available in the next few months, I believe these images will prove quite useful to researchers. I am also selecting, as appropriate, images to be attached to the "phenotype" form, so that researchers can see a typical expression of a particular phenotype. For example, see the viviparous phenotype (images are posted at the bottom of the page), at the URL:

http://www.agron.missouri.edu:80/cgi-bin/sybgw_mdb/mdb3/Phenotype/11091

The complete collection of images is still listed at:

http://www.agron.missouri.edu/NeufferImages.html

The opportunity to go through the collection one at a time is turning out to be an exceptionally pleasant and rewarding experience with each image being a reminder of some aspect of genetic control of maize biology. There are also many clues to unknown information about maize, as well as facts that were observed but unreported, and reminders of work still unfinished. The following reports are examples of unreported data that have been brought to light and will be reported as we proceed with this effort. Other informed workers who use this collection are invited to view it in the same light and to report their observations with the intent of increasing the field of knowledge of biology.

Location of two EMS mutants, *fl*-N1253B* and *sh*-N1328A*
--Neuffer, MG

The floury, variably collapsed mutant *fl*-N1253B* has been located using TB-4Lf, based on the photo at the URL:

http://www.agron.missouri.edu/db_images/Variation/cd5192-1612-0537/36.jpg

The photo shows two selfed ears from progeny of a cross of *+/fl*-N1253B* x TB-4Lf. The top ear is from a hypoploid plant and shows an excess of mutant kernels, indicating that the normal chromosome 4 carried the mutant. The lower ear is from a normal plant and shows 3:1 segregation for the mutant. These confirm that the mutant is located on chromosome 4, probably between the breakpoints of the two B-A translocations.

The opaque *sh2*-like mutant *sh*-N1328A* has been located to chromosome 5L based on the following photo:

http://www.agron.missouri.edu/db_images/Variation/cd5207-1613-0283/56.jpg

The photo shows a double-pollinated ear crossed by selfed pollen (top half) showing segregation for *sh*-N1328A* kernels and by TB-5La showing shrunken mutant hypoploid endosperms, indicating location on chromosome 5L.

A couple of nice mutants
--Neuffer, MG

In the process of providing image captions, as described above, I have uncovered two mutants that would benefit from further exploration. I do not have the facilities to do so myself, but seed is (or will be) available from the Co-op.

The mutant *sh*-N1544* is not allelic to *sh2* or *bt1*. It is a shrunken (*sh2*-type) kernel, reduced color with ACR. See a picture of the origin ear at URL:
http://www.agron.missouri.edu/db_images/Variation/cd5207-1613-0283/70.jpg
Note that most mutant kernels are nearly colorless. I have a small amount of this seed available to the first person who requests it, if the mutant is not yet available from the Co-op.

The mutant *nec*-N200B* presents an interesting physiology problem. See the photo at this URL:
http://www.agron.missouri.edu/db_images/Variation/cd5207-1613-2875/11.jpg

This picture shows two *nec*-N200B* mutant seedlings with necrotic crossbands and associated rolled-leaf distortion (probably resulting from lack of turgidity in necrotic tissue).

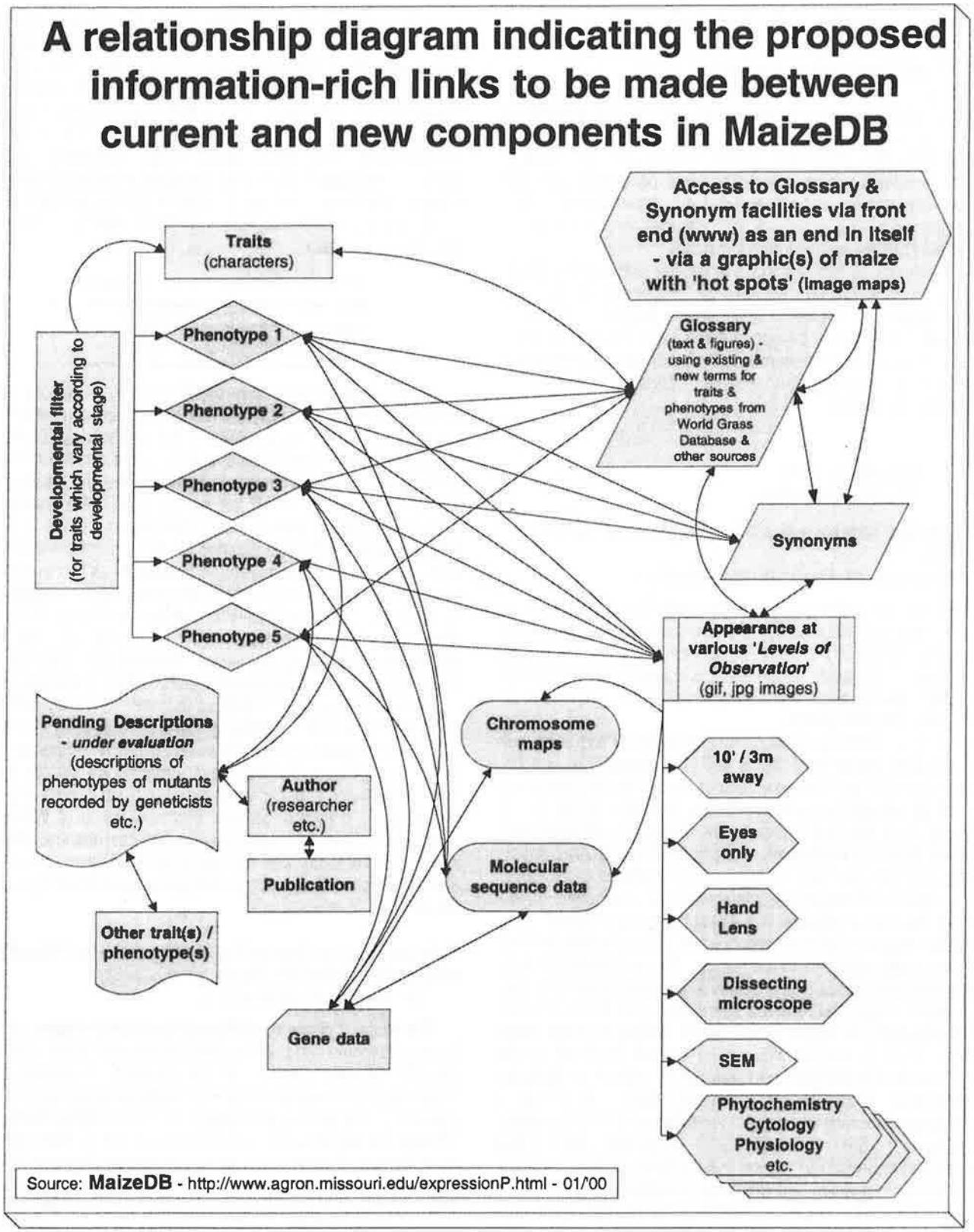
COLUMBIA, MISSOURI
University of Missouri-Columbia
USDA-ARS

The development of systematic descriptors and associated vocabulary for *Zea mays* (maize/corn)
--Vincent, PLD, Coe, EH, Jr.

One of the research areas of the Missouri Maize Project (http://www.cafnr.missouri.edu/mmp/) is the development of systematic descriptors and associated vocabulary for traits and phenotypes for *Zea mays*. These descriptors and associated vocabulary will be for the mutants and normal phenotypes of maize and should greatly assist the searching for and retrieval of information in the Maize database (MaizeDB - http://www.agron.missouri.edu/) by users from all over the world via the world wide web. The curation of the MaizeDB will also be considerably assisted via this set of descriptors and associated vocabulary.

The descriptors and associated vocabulary are based on internationally recognized biological and botanical concepts and terminology and will encompass the breadth of terms generated by maize geneticists, breeders and other researchers working with mutant phenotypes. The descriptors are being developed in an expandable system of 'containment hierarchies' and will accommodate a range of appropriate 'Levels of Observation', from the macromorphological levels through the micromorphological levels and down to the physiological and molecular levels (see diagram).

A relationship diagram indicating the proposed information-rich links to be made between current and new components in MaizeDB



The access to information in MaizeDB via these descriptors and associated vocabulary should accommodate users from a broad spectrum of knowledge-backgrounds. Access to a glossary, a synonym facility, and images associated with terms in the glossary and synonyms will be provided to facilitate understanding of the descriptors and associated vocabulary.

Some of the development of descriptors and associated vocabulary is being assisted by contacts with colleagues at the Royal Botanic Gardens, Kew (K), United Kingdom and at the Missouri Botanical Garden (MO), USA. Through these and other contacts access to a large volume of existing grass descriptors from the World Grass database has been obtained. This information and that obtained from a variety of other sources form the basis of the contents of the 'containment hierarchies'.

Furthermore, to facilitate those unfamiliar with the macromorphology, micromorphology, anatomy etc. of a corn/maize plant an interactive corn plant is being developed for access via the world wide web. This should be a very educational facility. Some of these innovations are represented in the accompanying flow-chart. For further information contact Leszek Vincent – Leszek@missouri.edu.

COLUMBUS, OHIO
Ohio State University

The maize *Mp1* gene encodes a WD-repeat protein similar to An11 and TTG

--Hernandez, JM, Pizzirusso, M, Grotewold, E

Myb domain proteins in conjunction with bHLH proteins regulate the flavonoid accumulation pathway in diverse plant species (Mol et al., TIPS 3:212-217, 1998), as well as trichome formation in *Arabidopsis thaliana*. These proteins share substantial homology across species and in some cases they have been shown to be functionally interchangeable.

The maize anthocyanin accumulation pathway has been well characterized and its regulation is well understood. The Myb domain proteins C1 or PL require the presence of the bHLH proteins R or B to activate transcription of the structural genes of the pathway. It is not clear however how the activity of these transcription factors is modulated. In both *Petunia* and *Arabidopsis* factors that act upstream of the known regulators of flavonoid accumulation and trichome development have been found. In the case of *Petunia*, a cytosolic WD-repeat protein is involved in the regulation of accumulation of anthocyanins, and mutants in *An11* do not accumulate pigments in the corolla. Overexpression of *An2*, the *Petunia* orthologue of *C1*, is able to activate the dfr promoter in an *An11* mutant background suggesting that *An11* acts upstream of *An2* (de Vetten et al., Genes & Dev. 11:1422-1434, 1997). TTG is another WD-repeat protein involved in the accumulation of anthocyanins as well as trichomes in *Arabidopsis* (Walker et al., Plant Cell 11: 1337-1349, 1999). The effects of *ttg* mutations are more pleiotropic than those of *An11* (Koornneef, Arabidopsis Inf. Serv. 18: 45-51, 1981). Lloyd et al. showed that overexpressing the maize *R* gene in *ttg* mutants restored both anthocyanin accumulation and trichome formation, suggesting that TTG is higher in the regulatory hierarchy than the bHLH proteins (Lloyd et al., Science 266: 436-439, 1994).

To determine whether a similar hierarchy of regulator proteins is present in maize, we used degenerate primers to conserved regions of *An11* to generate a PCR from maize seedling polyA+, which was RNA and then used as a probe to screen cDNA and genomic maize libraries. A gene called *Mp1* with no introns and encoding a protein of 410 amino acids (45 kD) was identified. MP1 is also a WD-repeat protein and it shares high sequence identity with AN11 and TTG (Table 1). A northern blot of polyA+ mRNA from various maize tissues indicated that MP1 is expressed throughout the entire plant. This is consistent with the expression patterns of AN11 and TTG (de Vetten et al., Genes & Dev. 11:1422-1434, 1997; Walker et al., 1999). Analysis of the *Mp1* sequence indicates that there is no nuclear localization signal and suggests that it might encode a cytosolic protein, similar to *An11*. Preliminary experiments suggest that *Mp1* is incapable of complementing a *ttg* mutant.

Table 1. Percent identities of MP1 to other WD-repeat proteins.

	TTG	AN11
MP1	58.8	59.8
TTG	-	79.5

Mapping experiments have positioned *Mp1* to the long arm of chromosome 5 close to *Pac1*, a recently identified locus which is involved in anthocyanin accumulation in the aleurone. Examination of the level of expression of the structural genes and the regulators of the pathway in *pac1* mutants revealed that the levels of expression of only the former but not the latter were dramatically decreased (Selinger and Chandler, Plant Cell 11: 5-14). This is a similar situation to the one reported for *An11* in which the levels of *An2*, *Jaf13* and *An1* were not decreased in *an11* mutants (de Vetten et al., Genes & Dev. 11:1422-1434, 1997). In addition to failure to accumulate anthocyanins neither *pac1* or *an11* mutants show any other phenotype which contrasts with the more pleiotropic effects of *ttg* mutations. Whether *Mp1* and *Pac1* are the same gene is an open question that needs further investigation.

Phylogenetic analysis indicates that MP1 is closely related to AN11 and TTG, and that these proteins form a family of proteins that are widespread among animals and plants as suggested by de Vetten et al. Figure 1. WD-repeat proteins are usually part of signal transduction cascades, and are involved in protein-protein interactions. It is possible that this new family of WD-repeat proteins is involved in a signal transduction cascade that ultimately modifies bHLH and/or Myb domain proteins enabling them to activate transcription and switch on the pathways in which they are involved.

Preliminary analysis of green fluorescent compounds induced by ectopic expression of the *P* gene

--Lin, Y, Dong, X, Grotewold, E

The maize *P* gene, an R2R3 myb transcription factor, controls 3-deoxy flavonoid and phlobaphene biosynthesis (Grotewold et al. Cell 76: 543-553, 1994). In the pericarp, *P* regulates the accumulation of a subset of flavonoid biosynthetic genes (*C2*, *Chi1* and *A1*). The ectopic expression of *P* in cultured BMS cells induces the accumulation of distinct classes of flavonoid and phenylpropanoid compounds, as well as orange-fluorescent bodies (Grotewold et al. Plant Cell 10:721-740, 1998). To gain further understanding of *P* function in maize cells, we used BMS cell lines transformed with ERE::P (*P* driven from an estradiol-inducible promoter, Bruce et al. Plant Cell 12, in press). This provides an ideal experimental system to investigate the activation of a branch

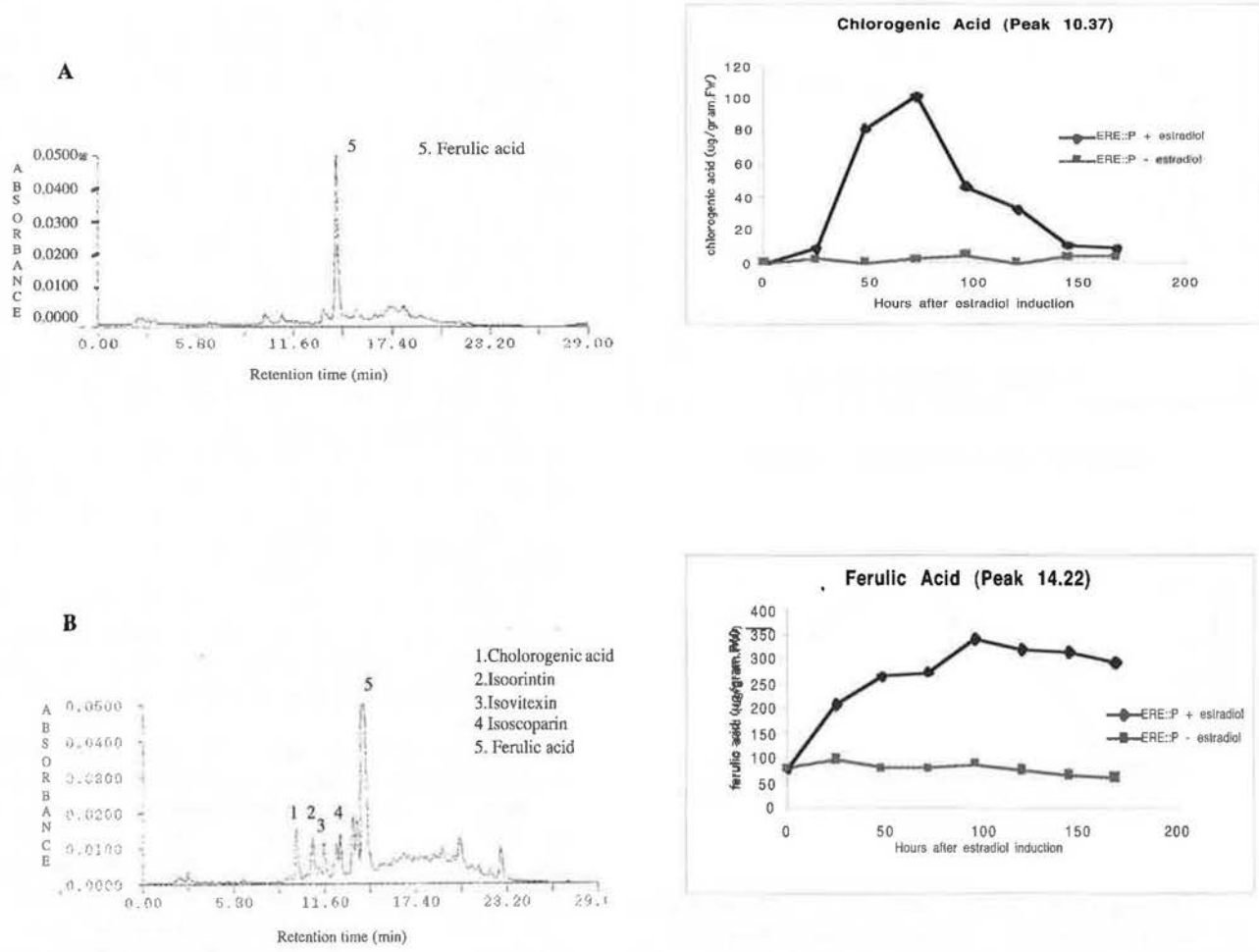


Figure 3. HPLC Chromatograph (340nm) of ERE::P cell line before (A) and after 120 hours induction with estradiol (B). Temporal accumulation of chlorogenic acid (C) and ferulic acid (D) in the ERE::P cell-line induced with estradiol.

of flavonoid biosynthesis, and the distribution and subcellular localization of secondary metabolites. A new type of green fluorescent vacuole-like bodies (GFVLB) was found in BMS cells transformed with ERE::P. These fluorescent vacuoles have different sizes and shapes, and they often localize in the cytoplasm close to the plasma membrane, and appear to be involved in delivering green fluorescent compounds to the cell wall. A GFVLB attached to the cell wall, which shows green fluorescence, was seen in some cells. The GFVLB may originate from SER through vesicles, because a tube-like GFVLB and a GFVLB fused with several small vacuoles were also observed in some cells. The GFVLB were found only in the BMS cells transformed with ERE::P, and were not found in either BMS cells transformed with ERE::Luc (luciferase driven from the estradiol-inducible promoter), or untransformed BMS cells. Although GFVLB exists in both induced and uninduced BMS cells with ERE::P, the number of cells containing GFVLB in the 5-days induced cells is four times higher than in uninduced cells (Fig. 1, page 26).

The compounds responsible for the fluorescence of GFVLB remain to be identified, but preliminary TLC analysis has been initiated. 3-deoxy flavonoids and phenylpropanoids accumulate in ERE::P BMS cells after induction with estradiol (Fig. 2, page 26, and Fig. 3). Accumulation of flavan-4-ols reached a maximum at 6 days after induction, but remained at a very low level in uninduced cells. Of the five identified compounds, only ferulic acid is present at significant levels in the uninduced cells, consistent with previous findings (Grotewold et al. Plant Cell 10:721-740, 1998). After induction, ferulic acid levels stay high for several days (Fig.3D). Thus, ferulic acid and the fluorescent compounds present in GFVLB appear to share similar patterns of temporal expression. In addition, other types of fluorescent bodies were identified in both induced and uninduced BMS cells with ERE::P. They are

Figure 1. Accumulation of GFVLB in BMS cells

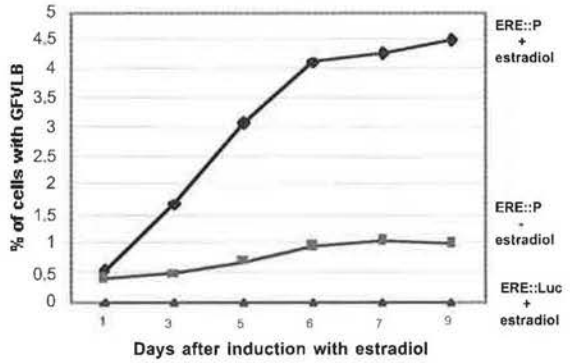
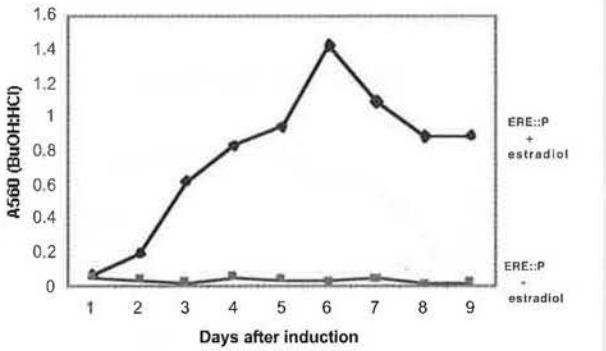


Figure 2. Accumulation of flavan-4-ols in BMS cells



spherical, nonphotobleaching, intensely orange fluorescent bodies very similar in properties to those 35S::P-expressing BMS cells (Grotewold et al. Plant Cell 10:721-740, 1998). The fluorescent compounds present in these bodies also show very strong red fluorescence under a 510-560 nm excision filter. The chemical nature of this second type of fluorescent compound is not known either. We believe that these fluorescent compounds will provide unique tools to investigate transport and subcellular localization of plant secondary metabolites.

DNIEPROPETROVSK, UKRAINE
Institute of Grain Farm, UAAS

Biochemical and morphobiological estimation of the progeny of a maize anther culture regenerant

--Satarova, TN, Savchenko, MP, Pismenetskaya, IY, Chernousova, NM

The level of the efficiency of maize selection is determined by the genofund of inbred lines. The haploid technique shortens the expense of obtaining inbreds. The method of haploid production through anther culture, which has been elaborated since 1975, nowadays has reached great results. Stable regeneration from pollen calli and embryoids has been obtained for different genotypes. However, morphobiological, biochemical and other peculiarities of pollen regenerants and their progeny have been studied only in part (Wan, Widholm, Plant Breed. Rev. 11: 199-224, 1993). The problem of expressing estimation of regenerants' genotypes, and the determination of their resemblance or differences with initial parental forms, are also of great importance. This paper is devoted to the electrophoretical and morphobiological analysis of the maize androgenic regenerant, its progeny and its parental forms.

The seeds of pollen regenerant And44 after the first ($And_1 44$) and the second ($And_2 44$) self-pollinations and the seeds of the donor hybrid H99xWf9 and its parental forms, lines H99 and Wf9, served as a material for electrophoresis. Electrophoretical analysis of storage proteins, zeins, was conducted in flat polyacrylamide gel.

Pollen regenerant And44 was obtained through anther culture of hybrid H99xWf9, colchicinated and planted into the soil. After the self-pollination of this regenerant we had obtained twelve grains of $And_1 44$, eleven of which were analysed electrophoretically, and one was again planted in the field. After the self-pollination of this F1-plant and the getting of $And_2 44$ - grains, part of them (fifteen grains) once more were taken for electrophoresis of storage proteins, and another part was anew planted in the field. The plants of lines H99 and Wf9 were also grown in the field for morphobiological investigations.

Electrophoretical spectra of zeins for all the analysed $And_1 4 4$ grains (the first generation) and all the analysed $And_2 44$ grains (the second generation) were the same. This means absence of segregation on given characteristics in the first and the second generations.

The electrophoretical spectra of the regenerant, its donor hybrid H99xWf9 and its parental lines H99 and Wf9 are shown in the figure. The spectrum of the regenerant was not identical to the spectrum of the donor hybrid. Differences in the intensity of the three lines were observed (1-3). One of the lines which was double in donor hybrid spectrum was single in the regenerant (4). The line in the upper part of the hybrid spectrum was absent in the regenerant one (5). The regenerant has some lines which were absent in the hybrid spectrum (6).

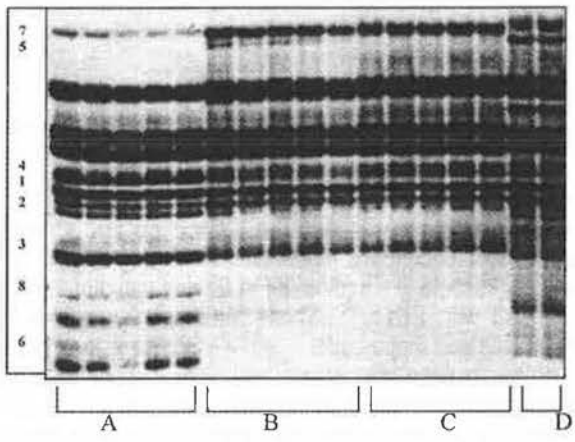


Figure. Electrophoretical spectra of maize androgenic regenerant And44(A), its donor hybrid H99xWf9(B), lines H99(C) and Wf9(D)

The spectrum of the pollen regenerant was close, but not identical to lines H99 and Wf9. The upper and middle part of the spectrum (high-molecular proteins) of the regenerant was similar to line H99, there were only differences in the intensity of some lines (2,7). The lower part of the regenerant spectrum had a resemblance with the parental line Wf9. Besides that, in the zone of

minor components the regenerant had a line which was present neither in paternal nor in maternal lines (8).

The results of the morphobiological analysis of progeny of pollen regenerant and lines H99 and Wf9 are represented in the table.

Table. Morphobiological characteristics of the line of pollen origin And44 and the parental lines of the donor hybrid, H99 and Wf9.

Genotype	Plant height		Number of days from seedlings to tassel flowering		Number of days from seedlings to ear flowering	
	Average, cm	Coefficient of variation, %	Average, days	Coefficient of variation, %	Average, days	Coefficient of variation,%
And44	132.9	6.55	75.1	4.13	76.4	5.66
H99	112.4	8.91	73.8	5.53	73.2	4.03
Wf9	162.7	5.99	70.2	5.45	70.8	6.62

Morphobiological analysis showed that the progeny of And44 had intermediate position between H99 and Wf9 in plant height. As for number of days from seedlings to tassel and ear flowerings it surpassed both of the lines. The coefficient of variation of And44, which may serve as indexes of the evening of a line, for different characteristics were on the level of the inbreds H99 and Wf9.

The evening and the identity of electrophoretical spectra of storage proteins (zeins) obtained from the first and the second self-pollinations of And44 may give evidence of the homozygosis of the androgenic regenerant. The data of electrophoretical analysis are well conformed with the results of the morphobiological estimation. The electrophoretical spectrum of the pollen regenerant differs from the spectra of maternal and paternal lines and donor hybrid and testifies to the obtaining of a new line.

DURHAM, NORTH CAROLINA
Duke University

Pilot study for heritability of enhanced drought tolerance in corn via *Tripsacum-Z. diploperennis* hybrids

--Eubanks, M

Eastern gamagrass, *Tripsacum dactyloides* L., is a native, perennial, warm-season C4 grass that has long been recognized for its remarkable ability to withstand drought. Physiological evidence that shows superior drought resistance in *Tripsacum* is based on high photosynthesis and water use efficiency in leaf gas exchange analysis (P. I. Coyne and J. A. Bradford, Crop Sci. 25:65-75, 1985). In a genetic study, R. G. Reeves and A. J. Bockholt (Crop Sci. 4:7-10, 1964) showed that *Tripsacum* confers increased drought tolerance to corn in maize-*Tripsacum* hybrids, but intergeneric sterility has impeded transfer of the drought resistant trait from *Tripsacum* to corn.

Zea diploperennis, a perennial grass closely related to corn also, exhibits agronomic traits associated with ability to withstand dessication stress. In a study of the linkage and inheritance of the gene for perennialism, W. C. Galinat (MNL 55:107, 1981) found that *Z. diploperennis* and its F1 and F2 hybrids with corn had traits associated with the capacity to withstand drought stress. In a 3-point test population of corn-diploperennis hybrids, P. C. Mangelsdorf and M. E. Dunn (MNL 58:54-55, 1984) demonstrated heterozygous progeny had robust root systems that may impart drought resistance. Such extensive root systems are also found in (corn X *Tripsacum-diploperennis*) plants that exhibit superior resistance to corn rootworm. The root biomass of these plants is approximately 50% greater than that of controls. This signals the possibility that the *Tripsacum-Z. diploperennis* genetic bridge might also be useful for transferring enhanced drought tolerance to corn.

A small-scale pilot study was conducted in a greenhouse at the Duke University Phytotron from March 8-16, 1999, to assess whether corn crossed with two different *Tripsacum-Zea diploperennis* hybrid lines exhibited enhanced resistance to drought when compared to corn. Plants were grown in Peter's professional potting soil in 10-inch diameter pots. Until the drought period, they were watered twice daily, and fertilized with one tablespoon Osmocote 14-14-14, plus they received liquid nutrient (modified Hoagland's solution) three times a week.

The number of treatment plants included seven of 97-1; three of line E; three of (97-5 X 97-1); four of (97-1 X 97-3), and three of B73 corn. Tripsacorn and Sun Star, the two parent drought resistant hybrids in the above lineages, were also included.

Drought stress was induced when the plants began flowering. Treatment plants received no water for five days from March 9-13, during which time controls were watered twice daily according to the normal regimen.

The goal was to achieve 30% reduction in plant available water (% PAW), which was estimated at approximately 20% reduction in pot weight, and not to exceed 50% before the end of the drought treatment. This was monitored gravimetrically by weighing each pot at full saturation when the drought stress was initiated and recording pot weight daily until the end of the drought. The summary of per cent pot weight loss in Table 1 shows that water loss over all the lines tested ranged from 21.7% to 39.4%, indicating there was significant reduction in % PAW during the drought test.

Water use efficiency was monitored by measuring stomatal conductance and net photosynthesis using a Licor 6400 open photosynthesis system. The numbers are reported in Table 1. Leaf rolling and wilting were also observed. These drought symptoms were pronounced in the B73 corn plants, but virtually undetectable in the hybrid lines. At harvest, grain dry weight and shoot biomass dry weight were recorded to provide an index of drought intensity among lines as well as between treatment and control plants within lines.

From the data presented in Table 1, it can be seen that most of the treated hybrid plants had a reduction in stomatal conductance and photosynthesis, signaling that the drought stress induced stomatal closure and consequently reduced levels of carbon dioxide available for photosynthesis. In some cases the differences were dramatic, and in others the reduction in numbers was minimal. Evidence of surprising drought resistance was exhibited by increase of predrought stomatal conductance and photosynthesis measurements in four hybrid plants, numbers 150 and 153 in the 97-1 line and numbers 144 and 146 in the (97-1 X 97-3) line (see Table 1). Table 1 shows that grain yield in drought stressed hybrid plants was greater than the controls except for plant 151 in the 97-1 line and plant 147 in the 97-1 X 97-3 line. This is in striking contrast to the drought stressed W64A corn plants, all of which had significant reduction in grain yield compared to the control.

The results of the drought pilot study revealed evidence of genetic segregation for drought resistance among (*Tripsacum-*

No.	Pedigree Breeding Lines	Predrought SC 8-Mar	Predrought PS 8-Mar	During Drought SC 12-Mar	During Drought PS 12-Mar	Postdrought SC 16-Mar	Postdrought PS 16-Mar	% Pot Wt. Loss 13-Mar	Dry Seed Wt/g 4-Jun
148	97-1	0.21	26.3	0.15	19.9	0.17	21.6	29.7	40
150	97-1	0.12	19.4	0.18	21.9	0.13	21.2	31.5	55.2
151	97-1	0.16	23.4	0.13	20.6	0.13	19.1	21.7	10.5
152	97-1	0.18	24.8	0.15	21.6	0.14	21	34.7	62.8
153	97-1	0.15	21.4	0.2	25.8	0.17	25.5	28.7	43.9
154	97-1	0.14	20.6	0.12	15.6	0.11	15.8	29.3	26.5
155	97-1	0.18	24.7	0.14	20	0.18	24.1	24.4	28.8
158	97-1 (control)	Not rec.						Watered	20.32
129	E	0.4	32.5	0.04	7.27	0.18	24.9	38.8	52.9
130	E	0.24	29.5	0	-0.133	0.14	20.8	39.4	90.9
9099	E	0.25	30.8	0.18	27.5	0.2	28.4	38.3	88.6
9100	E (control)	0.25	31.7	0.22	29.1	0.36	32.4	Watered	45.6
144	97-1 X 97-3	0.17	24.4	0.18	25.2	0.25	27.5	30.5	54.4
145	97-1 X 97-3	0.11	17.6	0.08	13	0.14	21.1	28	54.1
146	97-1 X 97-3	0.13	19.8	0.15	19.7	0.21	24.1	31.7	46.6
147	97-1 X 97-3	0.16	22.9	0.12	20.3	0.23	24.2	27.4	33.1
9064	97-1 X 97-3 (control)	Not rec.						Watered	35.4
	Maize Inbred								
161	W64A	0.13	0.1	0.14	22.5	16.5	21	34.8	27.7
162	W64A	0.14	0.12	0.14	22.2	20.7	21.7	38.9	23.7
165	W64A	0.14	0.11	0.13	22.6	19	19.1	41.7	15.6
166	W64A (control)	Not rec.						Watered	47.4
	Tripsacum-diploperennis Hybrids								
LG4	Sun Star	0.11	16.8	0.1	16.2	Not rec.	18.8	Not weighed	Not coll.
LG3	Tripsacorn	0.13	19	0.11	17.7	Not rec.	17.5	Not weighed	Not coll.

Zea diploperennis X corn) hybrid lines. The findings indicate there is good potential for superior drought resistance to be imparted to corn via a recurrent selection breeding program employing *Tripsacum-Zea diploperennis* in crosses with corn.

1999 growth chamber bioassays to test a natural resource for corn rootworm resistance

--Eubanks, M

T. F. Branson (Ann. Entomol. Soc. Amer. 64:861-863, 1971) reported that *Tripsacum dactyloides* is resistant to corn rootworm. A bridging mechanism for moving *Tripsacum* genes into corn has been achieved through wide cross hybrids between *Tripsacum dactyloides* and *Zea diploperennis* (M. W. Eubanks, Econ. Bot. 49:172-182, 1995). Efficacy of this genetic bridge for conferring natural rootworm resistance to corn has been demonstrated through a series of insect bioassays (M. W. Eubanks MNL 73:30, 1999; Amer. J. Bot. (suppl.):84:116, 1997; MNL 70:22-23, 1996; MNL 68:40-41, 1994). Results of insect bioassays conducted in 1999 that are part of a Phase II recurrent selection program for development of isogenic corn lines with natural rootworm resistance and were completed in 1999 are reported here. Results of Phase II bioassay #1 were reported in MNL 73:30, 1999. The work is supported by NSF grant no. 9801386.

The protocol for each assay reported below included *Tripsacum-Z. diploperennis* X corn hybrid lines and corn inbreds grown in growth chambers under controlled conditions at the Duke University Phytotron. The research design included three replicates of 128 plants per rep in a randomized block. Plants were grown in 4.5-inch diameter pots with nylon cloth covering the bottom of the pots to prevent larval escape out the holes in the bottom of the pots. Each plant was infested with 70 newly hatched first instar diapausing Western corn rootworm larvae at approximately three weeks post germination. The plants were harvested three weeks after infestation. The roots were carefully washed, then scored using the 1-6 Iowa rating scale (Hills and Peters): 1 = no damage or only a few minor feeding scars; 2 = feeding scars evident, but no roots eaten off to within 1.5 inches of the plant; 3 = several roots eaten off to within 1.5 inches of the plant, but never the equivalent of an entire node of roots destroyed; 4 = one node of roots completely destroyed; 5 = two nodes of roots completely destroyed; 6 = three nodes of roots completely destroyed. Plants that have a root rating of 1 or 2 are resistant. After scoring the roots, resistant plants (i.e. those with a root rating of one or two) were repotted in 10-inch diameter pots and transferred to the greenhouse for backcrossing to corn to advance the recurrent selection breeding program for development of rootworm resistant corn lines. Leaf tissue from selected resistant plants was sampled for DNA fingerprinting to identify co-segregating molecular markers.

Bioassay #2 Twelve lines were tested in the second Phase II growth chamber insect bioassay at the Duke University Phytotron. These included five breeding lines (A, C, 97-3 X C, E, 97-1 X 97-3), one corn inbred (W64A), five lines in a *Tripsacum* cytoplasm (TC64, TC64 X TC, TC64 X 97-1, TC64 X A, and TC64 X 97-5). Results are summarized in Table 1.

Table 1. Results of Phase II Insect Bioassay #2

Line	Treated	Controls	Root Ratings 1	2	3	4	5	6
A	50	17	3	4	21	22	0	0
C	15	4	2	5	4	4	0	0
97-3 X C	18	6	3	2	9	4	0	
E	32	8	2	4	17	9	0	0
E reciprocal	24	7	2	7	7	8	0	0
97-1 X 97-3	29	10	2	4	14	9	0	0
97-3 X 97-1	23	2	2	3	10	8	0	0
TC64	17	10	1	1	8	6	1	0
TC64 X TC	13	5	1	3	5	4	0	0
TC64 X 97-1	25	10	1	4	5	13	0	2
TC64 X A	14	6	0	0	3	5	3	3
TC64 X 97-5	14	6	0	1	3	8	0	2
W64A	3	3	0	0	3	0	0	0

After scoring, twenty resistant plants were repotted in 9-inch pots for cross pollinating with corn to advance the recurrent selection program for the development of rootworm resistant corn lines. The resistant families from this bioassay were A, C, 97-3 X C, E, 97-1 X 97-3, 97-3 X 97-1, TC64, TC64 X TC, and TC64 X 97-1.

Bioassay #3 Eleven lines were tested in the third Phase II growth chamber insect bioassay at the Duke University Phytotron. These included two corn inbred lines B73 and W64A, and nine breeding lines including 97-1 X 97-3, 97-1 X 97-5, 97-5 X 97-1, 97-5 X TC, 97-5, 97-1, 97-3, 97-3 X TC, 2023, 2019 X 2023, and E. The results are summarized in Table 2.

Table 2. Results of Phase II Insect Bioassay #3

			Root Ratings					
Line	Treated	Controls	1	2	3	4	5	6
97-1	30	6	0	0	4	10	8	8
97-3	27	6	0	2	9	14	2	0
97-5	21	4	0	0	2	5	8	6
97-1 X 97-3	32	6	0	1	12	14	5	0
97-3 X TC	7	3	0	0	3	4	0	0
97-1 X 97-5	31	4	0	0	4	19	7	1
97-5 X 97-1	30	6	0	0	8	14	7	1
97-5 X TC	30	6	0	0	7	17	5	1
E	30	6	0	2	14	14	0	0
2023	11	3	0	0	2	3	4	2
2019 X 2023	7	4	0	0	2	3	1	1
B73	8	14	0	0	0	1	6	1
W64A	8	12	0	0	0	4	3	1

After scoring, three resistant plants were repotted in 9-inch pots and transferred to the greenhouse for cross pollinating with corn to advance the recurrent selection program for the development of rootworm resistant corn lines. The resistant families from this bioassay were 97-3 and E. In this bioassay with breeding lines that are now greater than 75% corn, resistance appeared to be lost in all but two lines. The two resistant lines were carried forward in the recurrent selection program, and the non-resistant lines were eliminated. These results raised the possibility that it may require more extensive backcrossing of corn lines to the resistant hybrids Tripsacorn and Sun Star than had been anticipated to effectively move the trait into corn.

Bioassay #4 Seventeen lines were tested in the fourth insect bioassay. These included Sun Dance hybrid families from earlier trials that had been backcrossed to corn, then self-pollinated, plus the corn inbred B73. Results are summarized in Table 3.

Table 3. Results of Phase II Insect Bioassay #4

				Root Ratings					
Line	Treated	Resistant	Susceptible	1	2	3	4	5	6
E (97-3-1X97-5-2)	30	9	21	0	9	18	3	0	0
E (97-3-2X97-5-5)	30	3	27	0	3	16	11	0	0
9094 X 7009	30	7	23	0	7	19	3	1	0
7057 X 6088/6021	28	1	27	0	1	12	14	1	0
8089X(4021XA188)	27	3	24	0	3	13	11	0	0
JY X 3029	22	6	16	0	6	9	4	0	3
3024 X W64A self	26	3	23	1	2	15	7	1	0
3024 X 6N615	28	1	27	0	1	15	9	3	0
97-5X97-1 BC B73	30	1	29	0	1	15	12	2	0
7099 X 7101	23	1	22	0	1	10	10	2	0
4021 X A188 self	24	1	23	0	1	8	8	5	2
3029 X A188 self	26	0	26	0	0	7	12	4	3
3028 X 6088	4	0	4	0	0	2	0	1	1
3028 X 7101	5	0	5	0	0	3	2	0	0
3028 X E	6	0	6	0	0	1	0	2	3
3024 X 7099	2	0	2	0	0	0	0	1	1
B73	21	0	21	0	0	4	11	3	3

Ten resistant plants were repotted in 9-inch pots for cross pollinating with corn to advance the recurrent selection program to develop rootworm resistant corn lines. In eleven of the families there was at least one resistant plant. The most promising families selected from this assay for further development in the breeding program were E (97-3-1X97-5-2), 9094 X 7009, and JY X 3029.

Bioassay #5 Ten lines were tested in the fifth bioassay. These included Sun Dance hybrid families from insect bioassay #4 that were crossed to corn then selfed or sib pollinated in the field during the summer of 1999. The lines were 99-1-2 (W64AXE), 99-2-3 (W64AXC), 99-15-4 (1056XW64A), 99-7-19 (7008XA188), 99-7-6 (7008XA188), 99-8-1 (7057XA188), 99-12-19A (7083A188), 99-12-5 X 99-12-3 (7083XA188), 99-16-3 (1091XW64A), and corn inbred W64A. Results are summarized in Table 4.

Table 4. Results of Phase II Insect Bioassay #5

				Root Ratings					
Line	Treated	Resistant	Susceptible	1	2	3	4	5	6
99-1-2	51	12	39	3	9	17	21	1	0
99-2-3	50	10	40	4	6	18	20	1	1
99-15-4	44	5	39	2	3	13	21	5	0
99-7-19	32	13	19	6	7	5	11	2	1
99-7-6	19	3	16	2	1	8	7	1	0
99-8-1	41	12	29	6	6	14	14	1	0
99-12-19A	47	12	25	2	10	16	19	0	0
99-12-5 X 99-12-3	54	30	24	14	16	12	12	0	0
99-16-3	55	20	25	7	13	18	15	2	0
W64A	6	0	21	0	0	4	11	3	3

Fifty-one resistant plants were repotted in 9-inch pots and transferred to tall growth chambers for cross pollinating with corn to advance the recurrent selection program and for selfing or sibbing to increase seed quantities of these resistant families. All hybrid families in this bioassay exhibited resistance to Western corn rootworm. The most promising ones for the breeding

Figure 1. Roots of resistant hybrid.

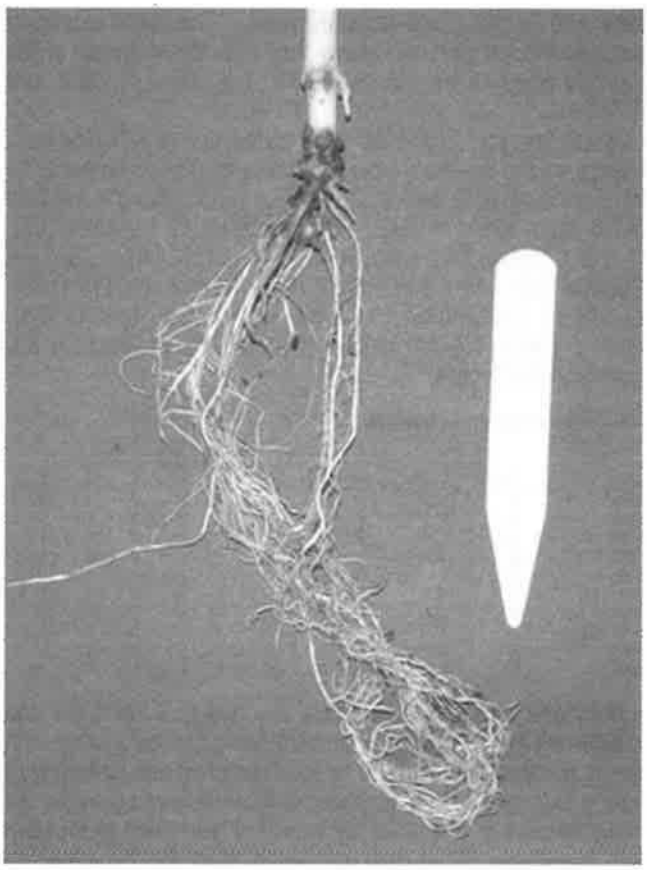


Figure 2. Roots of susceptible corn.

program were (99-12-5 X 99-12-3), 99-12-19A, 99-7-19, and 99-16-3. Compare the roots of the resistant hybrid in Fig. 1 to the roots of a susceptible corn plant in Fig. 2. These F8BC6 breeding lines that are now greater than 90% corn confirm efficacy of moving the Western corn rootworm resistance trait from *Tripsacum* into corn using conventional breeding methods, and establish proof of concept for crop improvement by using the *Tripsacum-Z. diploperennis* genetic bridge to transfer *Tripsacum* genes into corn.

Assay for proteinase inhibition in *Tripsacum-Zea diploperennis* X maize hybrids resistant to Western corn rootworm

--Eubanks, M, Cook, C

A defense response of plants to insect herbivory is production of proteinase inhibitors (PIs) with insecticidal activity. Zhao et al. (Plant Physiol. 111:1299-1306, 1996) demonstated that cysteine proteinase inhibitors have inhibitory activity against gut cysteine proteinases in third instar larvae of Western corn rootworm, *Diabrotica virgifera* LeConte (Coleoptera). Eastern gamagrass, *Tripsacum dactyloides* L., is resistant to Western corn rootworm (T. Branson, Ann. Entomol. Soc. Amer. 64:861-863, 1972), and resistance from *Tripsacum* has been transferred to corn using diploid perennial teosinte, *Zea diploperennis* Iltis, Doebley and Guzmán, as a bridging species (M. W. Eubanks MNL 71: 1999; Amer. J. Bot. (suppl.):84:116, 1997; MNL 70:22-23, 1996; MNL 68:40-41, 1994). In an insect bioassay for selection of rootworm resistant plants, three week old plants were infested with 70 newly hatched first instar diapausing Western corn rootworm larvae per plant. In order to measure the papain inhibitory activity and investigate whether there was a correlation between proteinase inhibition and resistance to corn rootworm, leaf samples were collected from the plants three weeks after infestation just prior to harvesting them for scoring the roots according to the Iowa 1-6 root rating scale.

Bioassay #2 Twelve lines were tested in NSF Phase II insect bioassay #2 conducted at the Duke University Phytotron. The lines included corn inbred W64A, five breeding lines A, C, (97-3 X C), E, and (97-1 X 97-3), and five hybrid lines in a *Tripsacum* cytoplasm TC64, (TC64 X TC), (TC64 X 97-1), (TC64 X A), and (TC64 X 97-5). Root ratings for all lines are summarized below in Table 1. A root rating of 1 or 2 indicates strong resistance to corn rooworm herbivory.

Table 1. Summary of Root Ratings in Insect Bioassay #2

			Root Ratings					
Line	Treated	Controls	1	2	3	4	5	6
A	50	17	3	4	21	22	0	0
C	15	4	2	5	4	4	0	0
97-3 X C	18	6	3	2	9	4	0	0
E	32	8	2	4	17	9	0	0
E reciprocal	24	7	2	7	7	8	0	0
97-1 X 97-3	29	10	2	4	14	9	0	0
97-3 X 97-1	23	2	2	3	10	8	0	0
TC64	17	10	1	1	8	6	1	0
TC64 X TC	13	5	1	3	5	4	0	0
TC64 X 97-1	25	10	1	4	5	13	0	2
TC64 X A	14	6	0	0	3	5	3	3
TC64 X 97-5	14	6	0	1	3	8	0	2
W64A	3	3	0	0	3	0	0	0

Papain Assay - A modification of the technique in Koiwa et al. (Plant J. 14:371-379, 1998) was used to determine papain inhibitory activity in the corn leaves. Four disks of leaf material yielding ~0.1 g/sample or ~7 cm^2/sample were taken from each plant three weeks after infestation with corn rootworm larvae. Leaf disks were frozen at -80 C, then ground to a fine powder using liquid nitrogen in 1.5 ml microcentrifuge tubes. To extract the inhibitor, 1 ml of a solution of 50mM phosphate buffer pH 7.2, with 150 mM NaCl and 2 mM EDTA:2Na, was added to the leaf powder, approximately 10 ml per gram leaf tissue (fresh weight). This was vortexed for 10 s, then centrifuged at 12 000 g for 15 min. To preactivate the papain, 1 volume of 20µg/ml papain in 25mM NaPi pH 7.0, 20 mM 2-mercaptoethanol was incubated for 10 min at 40 C, combined with 2 volumes of 0.25M NaPi pH 6.0, 2.5mM EDTA:2Na, and then kept on ice. Next 0.1 ml of the inhibitor solution and 0.3 ml of the preactivated papain solution were combined and incubated for 5 min at 40 C. The reaction was started by adding 0.2 ml of 2.3mM (final concentration) Na-benzoyl-DL- arginine-b-naphthylamide (BANA). This was incubated for 10 min at 40C, then 1 ml of 2% HCl in ethanol was added to stop the reaction, and finally 1 ml of 0.06% p-dimethylaminocinnamaldehyde in ethanol was added to develop color. The samples were held for at least 30 min at room temperature for full color development. The absorbance of the final mixture was read in a spectrophotometer (Perkin-Elmer Lambda 3B) at 540 nm. The inhibitory activity was expressed as the percentage decrease in absorbance relative to the reaction with no inhibitor.

Statistical Analysis - Although there was considerable range in the inhibitory activity of individual plants for each line tested (see Table 2 below), correlation analysis revealed no relationship between proteinase inhibition and root rating. It is thus concluded that the resistance mechanism conferred via *Tripsacum* does not

involve proteinase inhibition insecticidal activity like the Bt endotoxin that has been engineered into transgenic corn for resistance to European corn borer and is being developed for resistance to corn rootworm.

Table 2. Mean and Coefficient of Variation for Proteinase Inhibition Per Line Tested.

Line	N*	Min	Max	Mean	CV
A	53	31.78	75.73	52.97	19.9
C	19	27.51	62.65	41.99	26.4
97-3 X C	23	33.13	60.94	46.09	16.3
E	40	35.24	73.90	50.70	17.1
E reciprocal	32	26.36	67.65	49.11	24.3
97-1 X 97-3	41	28.52	70.45	50.33	20.4
97-3 X 97-1	29	25.63	73.64	54.26	20.2
TC64	40	25.90	77.24	50.32	26.1
TC64 X TC	18	36.38	75.71	52.19	23.6
TC64 X 97-1	36	27.67	73.00	53.72	23.4
TC64 X A	20	25.35	68.43	45.68	26.4
TC64 X 97-5	20	39.15	74.73	56.51	18.4
W64A	6	46.83	73.21	59.84	17.4

*N is the number of treated and control plants sampled for the papain assay.

DURHAM, NORTH CAROLINA
Duke University
BROOKINGS, SOUTH DAKOTA
USDA ARS

1999 field trial to test natural resource for rootworm resistance

--Eubanks, M, Riedell, W

T. F. Branson (Ann. Entomol. Soc. Amer. 64:861-863, 1971) reported that *Tripsacum dactyloides* is resistant to corn rootworm. A bridging mechanism for moving *Tripsacum* genes into corn has been achieved through wide cross hybrids between *Tripsacum dactyloides* and *Zea diploperennis* (M. W. Eubanks, Econ. Bot. 49:172-182, 1995). Efficacy of this genetic bridge for conferring natural rootworm resistance to corn has been demonstrated through a series of insect bioassays (M. W. Eubanks MNL73:30, 1999; Amer. J. Bot. (suppl.):84:116, 1997; MNL 70:22-23, 1996; MNL68:40-41, 1994). Under the auspices of NSF grant no. 9801386, a field trial testing efficacy of the rootworm resistance trait in crosses between *Tripsacum-Z. diploperennis* and corn under field conditions was conducted at the USDA Northern Grain Insects Research Lab in Brookings, SD, during the summer of 1998 (M. Eubanks and W. Riedell, MNL 73:29-30, 1999). Results of a second field test conducted during the summer of 1999 are reported here.

Thirty seeds of each of two corn inbreds, B73 and W64A, and seven *Tripsacum-Z. diploperennis* X corn breeding line crosses (9015, 9094 X7009, [8089 X (4021 X A188)], 97-5 X 97-1, 97-3 X 97-5, JY X 3029, and 5004X JW2) were planted at the USDA Northern Grain Insects Research Lab in May,1999. The 65 ft X 70 ft plot was flagged out and staked in six rows spaced 40 inches apart on May 6. The plot consisted of eight test rows surrounded with a corn buffer row on both sides of the test rows. On May 13, test rows were infested with 1400 viable Western corn rootworm eggs per linear foot of row. To accomplish infestation, eggs were mixed with room temperature agar and were applied to the soil through an anhydrous ammonia knife at a depth of 3 to 4 inches. Kernels were then hand planted into the furrow to a depth of 2 inches and a spacing of 9 inches. Two biophenometers were placed in the soil to a depth of four inches in two of the buffer rows of the experimental plot to monitor GDD accumulation.

All plants were dug and the roots washed and rated according to the Iowa (a.k.a. Hills and Peters) scale July 12, 1999. Root ratings are: 1 = no damage or only a few minor feeding scars; 2 = feeding scars evident, but no roots eaten off to within 1.5 inches of the plant; 3 = several roots eaten off to within 1.5 inches of the plant, but never the equivalent of an entire node of roots destroyed; 4 = one node of roots completely destroyed; 5 = two nodes of roots completely destroyed; 6 = three nodes of roots completely destroyed. Plants that have a root rating of 1 or 2 are resistant. Results are summarized in Table 1.

Table 1. Results of Phase II 1999 Field Test, Brookings, SD

				Root Ratings					
Line	Treated	Res.	Susc.	1	2	3	4	5	6
97-3-2 X 97-5-5	26	1	25	0	1	2	4	6	13
97-3-1 X 97-5-2	18	3	15	0	3	6	4	3	2
97-5 X 97-1	28	3	25	0	3	7	11	7	0
97-3-1 X 97-5-3	20	2	18	0	2	3	5	5	5
(8009 X 4021) X A188	26	1	25	0	1	8	1	3	13
9015	24	0	24	0	0	4	3	9	8
9094 X 7009	21	14	7	0	14	2	2	2	1
JY X 3029	7	1	6	0	1	3	4	0	0
B73	25	0	25	0	0	3	7	5	10
W64A	27	0	27	0	0	0	5	8	12

Since the corn controls in this field test were severely damaged (Fig. 1), it is clear we had a heavy rootworm infestation in the field in 1999. This indicates that strong natural resistance is present among the segregating progeny of most of the above F6BC4 families (Fig. 1). Two-thirds of the (9094 X 7009) plants were resistant when tested in the field. Seed from this family is being grown and self or sib pollinated to increase this line for further development in the recurrent selection breeding program. Field test results in 1998 and 1999 have confirmed that the rootworm resistance trait selected under controlled experimental conditions in growth chambers is also present under field conditions.

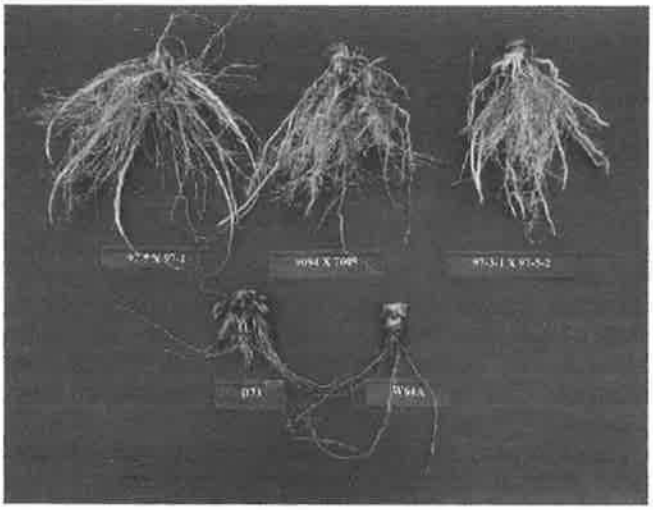


Figure 1. Top row: roots of resistant plants from three F6BC4 *Tripsacum-Z. diploperennis* X corn hybrid families. Bottom row: roots of corn inbreds B73 and W64A. Infestation of Western corn rootworm eggs was at a rate of 1400 eggs per ft of row.

DURHAM, NORTH CAROLINA
Duke University
MANHATTAN, KANSAS
USDA ARS

Bioassays for grain weevil resistance in *Tripsacum* X *Zea diploperennis*

--Eubanks, M, Throne, J

One of the most serious insect pests to corn during grain storage is the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera:Curculionidae). The adult female oviposits eggs into small holes she bores into the kernel. When the larvae hatch they feed on and develop inside the grain. No-choice bioassays were conducted to investigate whether Tripsacorn, a hybrid between *Tripsacum dactyloides* and *Zea diploperennis* that is resistant to corn rootworm, *Diabrotica virgifera*, might also possess resistance to grain weevils.

Three 35 g samples of Tripsacorn seed were equilibrated to 30 C and 75% relative humidity for 6 weeks. There were 3 control 35 g samples of Asgrow RX899 corn. At the end of the equilibration period, 5 *Sitophilus zeamais* adult females age 2-3 weeks were placed in each sample cage for 72 hours to lay eggs. Thirteen days after the ovipositing females were removed. Emerging adult progeny were sieved from the cages daily until no weevils had emerged for 2 weeks. No weevils emerged from the Tripsacorn, and X-ray examination of the kernels indicated that no eggs had been laid in the Tripsacorn seeds. Mean number of adults emerged from the corn controls was 30.3 (SD = 6.43).

Tripsacorn kernels are enclosed in a hard shell-like seedcoat, and it was hypothesized that the hardness of the seed was responsible for lack of weevil oviposition. In order to test this, a second experiment was conducted using ground up Tripsacorn kernels. Since maize weevil development is poor on ground seed, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) was employed in this bioassay. There were twenty replicates of one Tripsacorn kernel each weighing approximately 6 mg and twenty control replicates of 2 wheat kernels each which was about equal to a single Tripsacorn kernel. EachTripsacorn kernel or group of two wheat kernels was individually crushed with an aluminum mortar and pestle, then placed in a 0.4 ml centrifuge tube with a pinhole in the lid, and equilibrated for 1 week at 30 C and 75% RH. A single egg between 0 and 24 hr old was place in each tube. Fourteen days after infestation, tubes were checked biweekly for emerging adults. Emergence rate was 11 out of 20 on the wheat controls and 12 out of 20 on Tripsacorn. This shows that Tripsacorn seed can support insect growth when ground.

Intact Tripsacorn kernels were immune to maize weevil infestation. Given the difficulty in grinding the Tripsacorn kernels and the fact that insect growth occurred on ground Tripsacorn, immunity is probably due to the hardness of the kernels.

FORT COLLINS, COLORADO
Colorado State University
VICTORIA, BRITISH COLUMBIA
University of Victoria

Linkage distance between *whp1* and *ch1*

--Byrne, PF, Styles, ED

Although the loci *whp1* (*white pollen1*) and *ch1* (*chocolate pericarp1*) have both been mapped to the distal end of the long arm of maize chromosome 2 (bin 2.09), the loci apparently had not been mapped in the same population. This region of chromosome 2 became of interest after large QTLs for silk maysin (a C-glycosyl flavone) concentration and corn earworm antibiosis were detected there (Byrne et al., Crop Sci. 38:461-471, 1998). *whp1* is a plausible candidate for the QTLs because it encodes chalcone synthase, which catalyzes the first step in the flavonoid pathway. Chalcone synthase activity is required for maysin synthesis, but it is not clear whether *whp1*, *c2* (which also encodes chalcone synthase), or both contribute to maysin synthesis in silks. Plants that are double homozygous recessive at *whp1* and *c2* produce white, infertile pollen.

Ch1 is a dominant factor for chocolate (tan to dark brown) pericarp and acts independently of *p1*, the transcription activator that controls expression of most other pericarp pigments. The biochemical basis of *ch1* is not known, but because its pigmentation is similar to that produced by other flavonoid pathway mutants (e.g., *bp1*, *A1-b*), we felt that it too should be considered a candidate locus for the maysin and antibiosis QTLs.

Independent of the interest in maysin, Derek Styles had developed the following stocks to obtain estimates of the linkage distance between *whp1* and *ch1*:

(whp1/whp1 Ch1/ch1 P-ww/P-wr R-g/(R-r) A3/a3 c2 b1-/pl1)
x *(Whp1/Whp1 ch1/ch1 P-rr/(P-ww) R-r c2 Pl1 in1 gl1)*

(whp1/whp1 Ch/- (P-wr)/P-ww R c2 b1 -/pl1)
x *(Whp1/Whp1 ch1/ch1 P-rr/(P-ww) R-r c2 Pl1 in1 gl1)*

(whp1/whp1 Ch/- P-ww/(P-rr) R-g/(R-r) c2 b1 -/pl1)
x *(Whp1/Whp1 ch1/ch1 P-rr/(P-ww) R-r c2 Pl1 in1 gl1*

Seed of these stocks were planted in Columbia, MO in 1996 and used to pollinate *c2 whp1 ch1* plants. At harvest, pollen parents with chocolate pericarps were identified and crosses involving those plants were saved.

In 1997, plants from six ears of the crosses were planted in Fort Collins, CO. They were evaluated for pollen color at anthesis and for pericarp color at harvest. Plants arising from parental-type gametes would be either white pollen plus chocolate pericarp, or yellow pollen plus non-chocolate pericarp, whereas recombinant gametes would give rise to plants that were white pollen plus non-chocolate pericarp, or yellow pollen plus chocolate pericarp.

Results were as follows:

		Number of plants per class[a]				
Stock	No. of ears evaluated	W, Ch	W, Cl	Y, Ch	Y, Cl or R	Recombinant fraction
1	1	80	15	16	95	31/206 = 0.150
2	3	46	11	9	49	20/115 = 0.174
3	2	39	14	4	62	18/119 = 0.151
Pooled	6	165	40	29	206	69/440 = 0.157

[a] W, Ch = white pollen, chocolate pericarp
W, Cl = white pollen, clear pericarp
Y, Ch = yellow pollen, chocolate pericarp
Y, Cl or R = yellow pollen, either clear or red pericarp

The recombinant fraction for the three stocks, ranging from 0.150 to 0.174, and the value for the pooled data (0.157) correspond well to the inferred linkage distance of 14 cM in MaizeDB (Genetic Map of chromosome 2). There may have been some confounding of the Y,Ch and Y,R classes, because chocolate pigmen-

tation is reportedly obscured in red pericarps. However, it was generally possible to distinguish between purely red pericarps and those with both red and chocolate pigments. If some Y,Ch plants were mistakenly classified as Y,R, the result would be an underestimation of the true linkage distance.

Based on these results, the position of the QTLs proximal to *whp1*, and the position of *ch1* distal to *whp1*, we conclude that *whp1* is a better candidate than *ch1* for the maysin and antibiosis QTLs. Other strategies are currently being pursued to determine the influence of *whp1* and *c2* on maysin concentration (M. McMullen, personal communication).

IRKUTSK, RUSSIA
Institute of Plant Physiology and Biochemistry

Characterization of nuclear and mitochondrial DNA topoisomerases I

--Konstantinov, YM, Tarasenko VI

It is well known that along with DNA and RNA polymerases DNA topoisomerases are of key importance in the fundamental genetic processes such as replication, transcription, recombination and repair. Once deprived of topoisomerases the cell fails to make up for them and thus perishes. Besides the nucleus, topoisomerases are found in plant mitochondria and chloroplasts. No molecular biological studies of maize topoisomerases I of nuclear and mitochondrial localization have been made up to the present. Neither topoisomerase I gene structure, nor the features of their protein products, is known.

We have previously described (MNL 71:39-40,1997; MNL 73:39-41, 1999) some characteristics of mitochondrial type I DNA-topoisomerase including its sensitivity to different type inhibitors and redox conditions. The aim of the present work was to investigate some characteristics of nuclear topoisomerase I in comparison with the enzyme of mitochondrial localization.

Nuclei were prepared from 3-day-old etiolated maize seedlings of hybrid VIR42 MV generally as described earlier (Chiatante, Bryant, J. Exp. Bot. 45:959-965, 1994). The purification of topo I from isolated nuclei included the stages of organelle solubilization, ammonium sulfate fractionation, chromatography on the column with DEAE-Toyopearl, and chromatography on the column with single-stranded-DNA-cellulose. The mitochondria were isolated by a standard method of differential centrifugation. The method of topo I purification from mitochondria was the same as described earlier (MNL 73: 40-41, 1999). Protein was determined by the Lowry method.

We have previously reported (MNL 73: 39-40, 1999) about the redox modulation of mitochondrial topo I activity under different redox conditions. The study of the influence of redox conditions on the activity of nuclear topo I showed that this enzyme has similar sensitivity to the redox agents used as compared with mitochondrial enzyme. The addition of both oxidising and reducing agents caused changes in the activity of topo I from the nucleus. An activation of topo I was shown in the presence of reduced agents such as sodium dithionite or reduced glutathione and its significant repression following the addition of oxidised agents such as potassium ferricyanide or oxidised glutathione.

We have studied also the effects of different topo I inhibitors (camptothecin, distamycin A, netropsin, bis-netropsines, Hoechst33258, Hoechst33342) on the relaxation activity of this enzyme. There were no significant differences between nuclear and mitochondrial topo I in sensitivity to these inhibitors. However, nuclear topo I can be distinguished from its mitochondrial counterpart by its different affinity to single stranded-DNA-cellulose. In contrast to mitochondrial topo I, which binds only to double-stranded-DNA-cellulose, enzyme from nuclei readily binds to this nucleic acid resin. For plant nuclear and mitochondrial topo I, it is the only known difference between these enzymes, which might point to dissimilarities in their protein structure.

Financial support from the INTAS (Project Number 97-0522) is acknowledged.

DNA synthesis in organello in mitochondria of maize and wild perennial crop *Elymus sibiricus* under different temperature conditions

--Konstantinov, YM, Lutsenko, GN, Zukova, VV, Subota, IY, Arziev, AS

Comparative analysis of DNA-synthesizing systems of mitochondria of maize and *Elymus sibiricus*, a perennial wild crop, is of considerable interest from the point of view of biotechnological creation of new forms of maize with increased cold resistance.

The present work was aimed at the investigation of DNA synthesis in the system *in organello* in mitochondria of maize and *Elymus sibiricus* under various temperature conditions. The mitochondria were prepared from 3-day-old etiolated seedlings of maize (*Zea mays* L. hybrid VIR 42 MV) and 9-day-old etiolated seedlings of *Elymus sibiricus* by a standard method of differential centrifugation. DNA synthesis was measured in mitochondria according to the method of Schegget and Borst (Biochim. Biophys. Acta 95:235-248, 1971) with the use of ^{32}P-dATP (specific radioactivity was 148 PBq mol^{-1}). All kinetic data were obtained from at least 3 independent experiments. Statistical analysis was performed using Students paired t-test.

The crucial peculiarity of DNA-synthesizing system of mitochondria of *Elymus sibiricus* (Fig. 1) is a high level of mtDNA synthesis with a low temperature (+5 C). With the optimal temperature (+30 C) the activity of mtDNA synthesis of *Z.mays* and *E.sibiricus* showed practically no difference. The increase of incubation temperature up to +40 C demonstrated significant growth of mtDNA synthesizing activity of *Z. mays* and a considerable repression of this process in mitochondria of *E. sibiricus*. With the

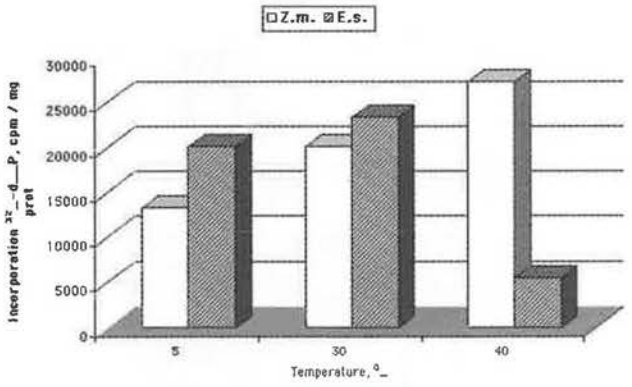


Figure 1. The activity of DNA *in organello* synthesis in mitochondria of maize and *Elymus sibiricus* under different temperatural conditions. Z.m., *Zea mays* ; E.s., *Elymus sibiricus* .

objective to clarify peculiarities of structural and functional organization of *E. sibiricus*, mitochondrial genome cloning of mtDNA sequences of this crop was conducted in bacterial plasmid vectors.

DNA synthesis in mitochondria under model changes of redox conditions

--Konstantinov, YM, Subota, IY, Podsosonny, VA, Arziev, AS

In spite of extensive studies of mitochondrial gene functioning, the molecular mechanisms that govern the expression of plant mitochondrial genomes in response to changes of metabolic conditions in the cell and the whole organism have still remained poorly understood at present. We have previously reported (MNL 69:63-64, 1995; MNL 70:29-30, 1996; MNL 71:40-41, 1997; MNL 72:33, 1998) on the effect of different redox conditions on mitochondrial genome expression regarding DNA, RNA and protein synthesis in organello. Significant activation of transcription and translation in mitochondria under oxidising conditions, created by addition of potassium ferricyanide, and its profound repression under reducing conditions in the presence of sodium dithionite can indicate the existence of a special mechanism of redox regulation of genetic functions in plant mitochondria.

We showed (MNL 69:63-64, 1995) a significant decrease in the DNA-synthesizing activity in the presence of both oxidising and reducing agents being more prominent in the case of dithionite. Apart from the apparent triggering of the genetic regulatory mechanism by potassium ferricyanide and sodium dithionite, the redox agents used can also modify other functional parameters of mitochondria as well, in particular, the activities of energy transformation and utilization.

The aim of the present work was to examine the mitochondrial DNA synthesis in organello under changes of redox conditions by the addition of carbonyl cyanide chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation. It is known that in the presence of CCCP the carriers of the mitochondrial respiratory chain are converted to more oxidising states (Muraoka and Slater, BBA 180:221-226, 1969).

The mitochondria were prepared from 3-day-old etiolated seedlings of hybrid VIR42 MV. The isolation of mitochondria and registration of DNA synthesis in organello were the same as described in our note from this issue.

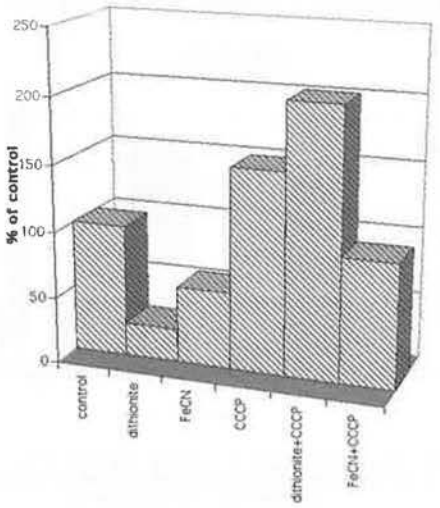


Figure 1. The effects of redox conditions on DNA synthesis in mitochondria. FeCN, potassium ferricyanide; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

Figure 1 shows that the addition of CCCP caused an activation of DNA synthesis in mitochondria. Such an effect of CCCP presumably results from redox states of the carriers of the respiratory chain. The effects of such redox agents as sodium dithionite and potassium ferricyanide on mitochondrial DNA synthesis in organello were shown to be drastically changed in the presence of this uncoupler. We registered even an activation of DNA synthesis in mitochondria when CCCP was added after sodium dithionite. Therefore the uncoupler of oxidative phosphorylation caused the elimination of the inhibitory effect of such a reducing agent as dithionite on DNA-synthesizing activity. The addition of CCCP also eliminated the inhibition of DNA synthesis in oxidised conditions created by addition of ferricyanide. The results obtained suggest that there is an activation of DNA synthesis in mitochondria under uncoupling conditions. We suggest also that this mechanism of redox control of genetic functions in these organelles may operate efficiently in vivo on the level of mitochondrial DNA replication.

JINAN, CHINA
Maize Research Institute, Shandong Academy of Agricultural Sciences

Effect of mass selection on the adaptive improvement of two subtropical maize (*Zea mays* L.) populations

--Liu, Z

Six cycles of mass selection for time to silk, shorter plant height and harvest indices were conducted in two subtropical maize populations in Jinan (long day condition) from 1991 to 1999. One of the populations chosen was CIMMYT's population 68QPM, a white semident kernel, intermediate in maturity, the genetic base of 30% to 40% temperate and 60% to 70% tropical germplasm. Another was CIMMYT's population 70QPM,a yellow dent kernel, intermediate to late in maturity, the genetic background of pool 34QPM and cycle four of temperate tropical yellow dent high oil QPM.

The results of the evaluating experiments showed that average plant heights of improved Pob.68QPM and Pob.70QPM were reduced by 42.0% and 45.0%, 15.2 cm and 16.3 cm per cycle. Times to silk (numbers of day from planting to 50% extruded silks) were decreased 9 d and 10 d, 1.5 d and 1.7 d per cycle. The harvest indices of improved maize materials had increased from about 30% to 45% and 47%, with average grain yield at optimum planting densities increased from about 1800 kg/ha and 2400 kg/ha to 2625 kg/ha (45.8%) and 3588 kg/ha (49.5%) respectively.

In addition to selecting for time to silk, shorter plants to improve adaptability, we have selected for other characters such as reduced tassel size (number of branches). Over the six cycles of selection in these two populations, the results showed that tassel size reduced as grain yields and harvest index were increased significantly. The study also demonstrated the negative correlation between smaller tassel and grain yield, but the high correlation between shorter plant height and time to silk.

Studies on utilization of tropical and subtropical maize germplasm

--Liu, Z

Four self-lines (Ye 107, Ye 8112, Huangying o2 and Qiqun 32) were crossed with tropical and subtropical maize germplasm (pool32, pool33, pool34 and population 70) ever since 1984.

Exotic germplasms were introduced into temperate materials (above self-lines) according to different proportions (from 25% to 75%) for increasing genetic diversity, raising general combining ability (GCA) and resistance ability to disease and stress environments.

Three elite self-lines, YL1732, YL8234 and QIL3233, were bred by the above method in 1998. YL1732, a yellow semident self-line based on 75% subtropical germplasm and 25% temperate germplasm, was selected from 3 generations of inbreeding and 5 generations of self-inbreeding. Better performance was YL8234, an early maturing yellow flint self-line made up of 50% subtropical germplasm and 50% temperate germplasm. QIL3233, an intermediate to early maturing yellow dent material, was based upon inbreeding 4 generations and self-inbreeding 5 generations of temperate population 32 subtropical pool33. At present, these self-lines are used for breeding elite maize hybrids in summer planting corn region in China.

The evaluating experiments of selected self-lines in Jinan showed that disease indices of maize leaf blight (*Helminthosporium turcicum* Pass.) and leaf spot (*Helminthosporium maydis* Nisik.) decreased 61.5% to 78.9% and 53.8% to 69.4% respectively. Plant rates of maize stalk rots (*Fusarium graminearum* and *moniliforme*) and maize rough dwarf decreased 84.1% to 93.2% and 86.7% to 98.2%. Number of green leaves at maturity increased 2.6 to 4.4 times, and average grain yield also increased 20.0% to 55.6% respectively.

JOHNSTON, IOWA
Pioneer Hi-Bred International, Inc.

Use of maize EST databases to identify and isolate homologs for genes with putative function identified from other species and genera

--Helentjaris, TG

With the advent of large collections of ESTs (expressed sequence tags) for maize, it is now possible to "mine" these databases for corn homologs to genes characterized first in other species where some level of functional understanding has been established. While cloning methods based upon hybridization are sometimes successful, they are often frustrated by low degrees of nucleotide similarity, depending upon the availability of a gene sequence from a species of close similarity. "in silico" cloning via computer analyses of EST collections on the other hand can be comparatively straightforward when these collections are available and comprehensive in their gene coverage. Instead of dedicating several person-months to isolate a maize homolog starting from an Arabidopsis or other dicot sequence, this search can take minutes on the computer. Starting with sequences from more distantly-related organisms, such as yeast or mammals, where many of our current gene sequences and understanding are currently archived, isolation by hybridization can be nearly impossible but is again greatly facilitated by analyses of computer-translated EST sequences. Consequently it is no longer a daunting task to isolate homologs for individual genes first characterized in other species and where known, whole pathways can now be identified and isolated in corn for study.

For the last couple of years I have been mining the original Pioneer/HGS EST database, and more recently the Dupont corn EST database, for maize homologs for specific biochemical, hormonal, and gene regulation pathways. My purpose was to acquire corn homologs representing pathways of interest to our team's research in yield stability. This list of putative corn homologs runs into the several hundreds of ESTs and many of these have been characterized by our group at some level, i.e. full length insert sequencing, mapping, expression, in some cases even TUSC-based searches for insertional inactivations. While they have proven invaluable for our purposes, we are still limited by a more detailed understanding of their function in corn and are unable to dedicate enough time to fully explore them. Consequently we would like to make a number of these clones available to interested researchers who wish to characterize them further in corn, emphasizing the placement of groups of clones by pathway with individual investigators. At this point it is relatively straightforward for us to arrange a materials transfer agreement to supply public researchers not only with the clones but also any other associated information and biomaterials we have to facilitate their own studies. Our hope is that as knowledge about these genes grows, it will of course add to our understanding the role of these genes within the context of our own research efforts. I urge researchers who might be interested in any of the genes or pathways in the appended table to contact me directly and discuss their interest. An even larger set of clones with somewhat less characterization is also available from pathways including carbohydrate metabolism and starch biosynthesis, cell cycle genes, and other hormone pathways.

The appended table contains a list of individual maize ESTs/clones which have each been characterized to represent different genes, the first column containing a clone identifier. The next column contains a descriptive name for its closest homolog. Next is a column indicating whether the clone is full length or not. The fourth column indicates a chromosomal bin where the clone has been mapped in the maize genome. The last column indicates those genes where a TUSC search for *Mu* insertions has been initiated.

EST	candidate gene	pathway	size	map location	TUSC?
001	ABA insensitive 1/2 mutant	ABA	no		no
002	ABA insensitive 1/2 mutant	ABA	no		no
003	ABA insensitive 1/2 mutant	ABA	no		no
004	ABA insensitive 1/2 mutant	ABA	no	3.09-10	no
005	ABA insensitive 1/2 mutant	ABA	no	6.07-08	no
006	ABA insensitive 1/2 mutant	ABA	no		no
007	ABA insensitive 4/sun06 mutant	ABA	FL		no
008	ABA-induced protein kinase	ABA	FL	9.04	no
009	ABA-induced protein kinase	ABA	no	7.04	no
010	farnesyl tranferase a-subunit	ABA	FL	2.08	no
011	farnesyl tranferase b-subunit	ABA	FL	3.06	yes
012	ADP-G PPase/bt2-like	carbohydrate metab	FL	2.06	yes
013	ADP-G PPase/sh2-like	carbohydrate metab	FL	6.08	no
014	GRR1	carbohydrate metab	no		no
015	hexose/sugar transporter	carbohydrate metab	no		no
016	hexose/sugar transporter	carbohydrate metab	FL		no
017	hexose/sugar transporter	carbohydrate metab	FL		no

EST	candidate gene	pathway	size	map location	TUSC?
018	hexose/sugar transporter	carbohydrate metab	no	5.04	no
019	hexose/sugar transporter	carbohydrate metab	FL		no
020	hexose/sugar transporter	carbohydrate metab	FL		no
021	SPF1 transcription factor	carbohydrate metab	no	7.04	no
022	sucrose symporter	carbohydrate metab	no		no
023	sucrose symporter	carbohydrate metab	FL		no
024	G-binding protein a-subunit	cytokinin response	FL	1.02	no
025	G-binding protein b-subunit	cytokinin response	FL	1.09	no
026	G-coupled receptor	cytokinin response	FL	6.04	yes
027	HP homolog	cytokinin response	FL		no
028	HP homolog	cytokinin response	FL		no
029	HP homolog	cytokinin response	FL		no
030	AP2-containing/DREB2	gene regulation	FL	8.02	yes
031	AP2-contaning/Tiny-like	gene regulation	no	8.03	no
032	AP2-contaning/Tiny-like	gene regulation	FL	9.04	no
033	N2-regulated protein kinase	gene regulation	no		no
034	N2-regulated protein kinase	gene regulation	FL		no
035	protein phosphatase/GLC7-like	gene regulation	FL		no
036	MAP kinase	signalling	FL	9.06	no
037	MAP kinase	signalling	FL	2.08	no
038	MAP kinase	signalling	FL	4.1	no
039	MAP kinase	signaling	FL		no
040	MAP kinase <aux	signalling	no	5.03	no
041	MAP kinase <aux	signalling	FL	5.04	yes
042	MAP kinase <ga	signalling	FL	9.05	yes
043	MAP kinase <sa	signalling	no	6.01	no
044	MAP kinase <sa	signalling	FL	6.01	no
045	MAP kinase kinase	signalling	FL	9.03	no
046	MAP kinase kinase	signalling	no	5.08	no
047	MAP kinase kinase	signalling	FL	3.07	no
048	MAP kinase kinase	signaling	no	5.05-06	no
049	MAP kinase kinase kinase	signalling	FI	3.07/8.06	no
050	MAP kinase kinase kinase	signalling	no		no
051	MAP kinase kinase kinase	signalling	FL	1.1	no
052	MAP kinase kinase kinase	signalling	no	9.00-01	no
053	MAP kinase kinase kinase	signalling	no	10.04	no
054	MAP kinase kinase kinase CTR1-like	signalling	no	5.06	no
055	MAP kinase kinase kinase epsilon	signalling	FL		no
056	MAP kinase phosphatase	signalling	no	1.08	no
057	MAP kinase phosphatase	signalling	FL	5.07	yes
058	"Os-1p, At histidine kinase"	signalling	no	8.06	no
059	"protein phosphatase, MP2C >STE11"	signalling	no	6.00-01	no
060	MCAF1/POP	sugar sensing	no	7s	no
061	Prl1	sugar sensing	FL	1.09	yes
062	SNF1	sugar sensing	no	2.05	no
063	SNF1	sugar sensing	FL		yes
064	SNF1	sugar sensing	no		no
065	SNF1	sugar sensing	no	1.03	yes
066	SNF1	sugar sensing	FL		no
067	SNF1	sugar sensing	no		no
068	SNF1/WPK4	sugar sensing	FL	3.06	no
069	SNF4	sugar sensing	no	1.08-11	no
070	"spindly-like, TPR-containing"	sugar sensing	no		no

New leaf mutation *shr*-JH87*, shredded leaf

--Trimnell, MR, Albertsen, MC, Noble, SW

In 1987 Steve Noble observed one of his F2 breeding lines segregating for a shredded leaf phenotype. This mutant does not express until after the juvenile phase of plant growth. Then, the leaf blade lamina becomes shredded between the horizontal veins of the adult-phase leaf. The cells between the veins do not undergo visible necrosis before becoming shredded. Depending upon the wind conditions, the phenotype can become very severe, with some leaves reduced to a mass of shredded strands of leaf material that is primarily composed of leaf vein. Figure 1 shows not only the extremely shredded appearance these plants can acquire, but it also shows how the wind can "tie" the shredded leaves into a tangled mass of leaf tissue. Steve gave remnant seed of this line from two different ear rows to us. We decided that the word "shredded" better described this mutant than did "slashed" (as is currently applied to a set of specific leaf mutations) and designated this mutant as *shr*-JH87*. It was planted in our 1990 Johnston nursery. One of the ear rows did not segregate, but the other one did. We made self- and sib-pollinations from this row and put the progeny seed in our cold room.

In 1998 we decided to again plant this mutant and to further characterize it in our Johnston nursery. We planted 2 self rows and 2 sib-pollinated rows. These were crossed with A632 and B73, reciprocally. These F1 crosses were then self-pollinated in our Hawaii winter nursery, and the resulting F2 seed was planted in our nursery this past summer. Segregation data for the previously mentioned plantings are shown:

Genotype	Wild-type	shredded leaf	Corrected X2(3:1)
Original Heterozygote Selfed (F2)	12 Plants	3 Plants	0.023
shr-JH87* Homozygote Selfed Ear #1 (F3)	0 Plants	12 Plants	--
shr-JH87* Homozygote Selfed Ear #3 (F3)	0 Plants	6 Plants	--
shr-JH87*/A632 Selfed Ear #1 (F2)	19 Plants	2 Plants	1.92
shr-JH87*/A632 Selfed Ear #2 (F2)	16 Plants	1 Plant	2.38
A632/*shr*-JH87* Selfed Ear #1 (F2)	13 Plants	4 Plants	0.02
A632/*shr*-JH87* Selfed Ear #2 (F2)	15 Plants	0 Plants*	--
shr-JH87*/B73 Selfed Ear #1 (F2)	13 Plants	2 Plants	0.56
shr-JH87*/B73 Selfed Ear #2 (F2)	18 Plants	1 Plant	2.96

*Oil yellow looking plants segregated in this row.

During the summer of 1998, we also crossed *shr*-JH87* with *sl1* (slashed leaf 1) even though the *shr*-JH87* phenotype is very distinctively different than the slashed leaf mutant phenotype. These test-crosses were grown in our Hawaii winter nursery. A total of 71 plants were observed without any plants showing either the shredded leaf or slashed leaf phenotypes. The leaves of *shr*-JH87* can shred along nearly the entire length of the leaf blade between the parallel veins, although not every interveinal area will shred. With *sl1*, the leaves seem to slit primarily in distinct areas on the leaves, with some areas of the leaf remaining intact both anterior and posterior to a region of slashes. This does not occur in *shr*-JH87* plants. Neuffer described a mutant, *sl*-N1701*, as resulting from a "weakening of midvein tissue (that) results in leaves falling apart longitudinally, like *sl1*". The leaves are further described as slitting following necrotic streaks (Maize DB). We plan to cross our mutant with his and to finish mapping the mutant. Leaf samples were collected for chromosome arm mapping this past summer.

Figure 1

New chromosome 3S mutant *mssi*-DR87A*

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

In 1987, Dan Wilkinson observed male-sterile plants in a *Uq c-ruq/Ht1* F2 line that he was growing in Johnston, IA. Dan sib-pollinated the male-sterile plants and gave the seed to us. We planted this seed in our 1988 Johnston summer nursery where the male-sterile plants were again sib-pollinated. We designated this male-sterile as *ms*-DR87A*. Segregation data for the 1988 nursery are shown:

Genotype	Fertiles	Steriles	X2(1:1)
Uq Sib #1	12 Fertiles	12 Steriles	0.00
Uq Sib #2	13 Fertiles	18 Steriles	0.81
Uq Sib #3	10 Fertiles	10 Steriles	0.00
Original F2 Line	13 Fertiles	4 Steriles	0.01 (corrected X2)(3:1)

In 1992 we test-crossed *ms*-DR87A* with a *Uq* tester line (receptor @ *c1;c-ruq wx*). We scored the ears from this test-cross for *Uq* co-segregation with sterility but did not find consistant co-segregation. While scoring these ears we noticed that there was an unusual number of silks on the male-sterile plants, as well as glume-like structures attached on either side of the kernels. Sometimes these structures are separated from the kernels during the shelling process (using a mechanical sheller), but most of the time they remain joined to the kernel on either side.

During the summer of 1995 in Johnston, we grew the sib-pollinated seed from the 1988 nursery. At that time, male-sterile plants were crossed with A632. The F1 seed was grown in the summer of 1995 and self-pollinated to make F2 ears. The F2 ears were then planted in the 1995 Hawaii winter nursery; their segregation data are shown:

Genotype	Fertiles	Steriles	Corrected X2(3:1)
A632 Ear #1	10 Fertiles	7 Steriles	1.59
A632 Ear #2	11 Fertiles	6 Steriles	0.50

In the Hawaii 1997 winter nursery, *ms*-DR87A* was planted for chromosome arm mapping. Leaf punches from 24 male-fertile and 15 male-sterile plants were taken for DNA isolations. Roughly 60 SSR markers were used to genotype DNA pools of the male-fertile samples and individual male-sterile samples. Four SSR markers on chromosome 3 (*bnlg1035, bnlg1452, phi029, phi053*) showed linkage with the male-sterility phenotype. SSR marker *bnlg1035* (chromosome 3 Bin 5) gave the closest linkage to the trait with only 2 recombinant alleles found in the male-sterile samples.

Test-crosses were made with *ms*-DR87A* and the known male-sterile mutants located on chromosome 3 (*ms3, ms23, ms37*) as well as with the unmapped male-sterile mutants (*ms24* and *ms27*). At least 40 plants were observed for each test-cross, and all test-cross progeny were found to be fertile. We also had test-crossed *ms*-DR87A* with *si1* before we had map data and found that it was not allelic. The *si1* mutant is described as having irregular kernel placement, but there is no description of glume-like structures attached to the kernel. The *ms-si*-355* allele listed in the back of last year's MGN is without description.

Other than the *si1-mssi* allele, none of the other *si*-type mutants identified by Neuffer are described specifically as also being male sterile. This includes *si*-1323* (or *si*-N1323*) that is mapped to chromosome 3S and that is described as "a selfed colorless flinty semi-sterile ear segregating for tiny defective kernels with excess silks" (MaizeDB). Its male fertility is not addressed. Silky mutant *si*-1967*(or *si*-N1967*) is not mapped but is described as being male fertile (MaizeDB). Silky mutant *si*-N815A*, also unmapped, is described as having silks on tassels and ears (MaizeDB).

None of the previously described silky-type mutants have all the features of *ms*-DR87A*. Homozygous mutant plants are 1) distinctly male sterile, 2) have glume-like structures attached on either side of the kernel, and 3) have excess silks on the ears. Because of the silky phenotype of this male-sterile, our designation for this mapped mutant is *mssi*-DR87A*.

New *ra1* allele

--Trimnell, MR, Albertsen, MC

In 1997 in our Johnston nursery, we observed ramosa-like tassels in a segregating row of the *ms26* male-sterile mutant. The ears of these plants also exhibited a ramosa-like phenotype. We designated this mutant as *ra*-ms26*. We carried pollen from the ramosa-like plants and crossed them onto A632. These plants were then grown in our Hawaii winter nursery and self-pollinated.

We grew the F2 ears in our 1998 Johnston nursery. Segregation data are shown below (please note that the low plant numbers are due to a mesocyclone that occurred in Johnston during late June):

Genotype	Wild-type	ramosa	Corrected X2(3:1)
A632/*ra*-ms26* Selfed Ear #1(F2)	5 Plants	2 Plants	0.05
A632/*ra*-ms26* Selfed Ear #2(F2)	3 Plants	0 Plants	--
A632/*ra*-ms26* Selfed Ear #4(F2)	7 Plants	2 Plants	0.04
A632/*ra*-ms26* Selfed Ear #5(F2)	5 Plants	4 Plants	0.92

MCA commented during the 1997 nursery that *ra*-ms26* looked like a *ra1* mutant. Since we did not have any *ra1* planted during that nursery, we made the test-cross in our 1998 Johnston nursery using our *ra1-DEN* allele. Homozygous *ra*-ms26* plants were crossed with homozygous *ra1-DEN* plants. The resultant progenies were grown in Hawaii in our 1998 winter nursery and gave the following results, indicating allelism:

Female	Male	Progeny	
ra-ms26* Homozygote	*ra1-DEN* Homozygote	0 Wild-type	31 ramosa
ra-ms26* Homozygote	*ra1-DEN* Homozygote	0 Wild-type	38 ramosa

Our new designation for this *ra1* allele is *ra1-ms26*.

JOHNSTON, IOWA
Pioneer Hi-Bred Int., Inc.
URBANA, ILLINOIS
University of Illinois

New male-sterile allele of the male-fertility gene *Ms45*

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

A new male-sterile allele of *Ms45* has been identified from a series of male-sterile mutants received several years ago from the late Dr. Earl Patterson of the University of Illinois. Earl had designated this mutant as *ms*-6040* (see MNL 69:126-128). In the 1980's, Earl and we started allelism studies by making some of the test-crosses of *ms*-6040* to the known male-sterile alleles located on chromosome 9 (*ms2*), as well as to unmapped male-sterile alleles that were later found to be located on chromosome 9 (*ms25-6022*, previously *ms*-6022*; and *ms35*, previously *ms*-6011*). Progenies from these test-crosses were fertile, indicating the mutants were not allelic.

To reduce the number of subsequent allelism crosses required, we planted an F2 segregating family of *ms*-6040* in our 1998 Johnston nursery to take leaf samples for chromosome arm mapping. Samples from 24 plants of each phenotypic class were taken for DNA isolation. Approximately 60 SSR markers were used to genotype this line. Linkage of the SSR marker *bnlg619* (chromosome 9, Bin 7 or 8) was found with the male-sterility phenotype.

We then test-crossed *ms*-6040* with the remaining male-sterile alleles on chromosome 9 (*ms36*, *ms45`-9301*) as well as with the unmapped male-sterile alleles (*ms24*, *ms27*). At least 40 progeny plants were observed of these test-crosses. The test-crosses of *ms36* and the unmapped mutants were all fertile, indicating *ms*-6040* was not allelic to any of them. The reciprocal test-crosses of *ms*-6040* with *ms45`-9301* gave the following results, indicating allelism:

Female (homogygous)	Male (heterozygous)	Progeny		X2(1:1)
ms-6040*	*ms45`-9301*	17 Fertiles	15 Steriles	0.13
ms45`-9301	*ms*-6040*	13 Fertiles	21 Steriles	1.88

Our new designation for this *Ms45* allele is *ms45-6040*.

New male-sterile allele of the male fertility gene *Ms30*

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

A new male-sterile allele of *Ms30* has been identified from a series of male-sterile mutants received several years ago from the late Dr. Earl Patterson of the University of Illinois. This mutant had been designated as *ms*-6028* (see MNL 69:126-128).

To reduce the number of test-crosses required to determine allelism, we planted an F2 segregating family of *ms*-6028* in our 1998 Johnston nursery for chromosome arm mapping. Leaf punches were taken from 23 male-sterile plants and 24 male-fertile plants for DNA isolation. Approximately 60 SSR markers, evenly dispersed throughout the genome, were used to genotype these samples. Linkage was found between the SSR marker *phi093* (chromosome 4, Bin 8) and the male-sterile phenotype.

After receiving the mapping data, we test-crossed *ms*-6028* with the recessive male-sterile alleles that are located on chromosome 4 (*ms30*), as well as the unmapped recessive male-sterile alleles (*ms24*, *ms27*). The resultant progeny were grown in our 1999 Johnston nursery. At least 40 plants were observed for each of the test-crosses. The test-crosses with the unmapped male-sterile mutants were all fertile, indicating *ms*-6028* was not allelic to either mutant. The reciprocal test-crosses of *ms*-6028* with *ms30* gave the following results, indicating allelism:

Female (homozygous)	Male (heterozygous)	Progeny		X2(1:1)
ms-6028*	*ms30*	10 Fertiles	16 Steriles	1.38
ms30	*ms*-6028*	19 Fertiles	22 Steriles	0.22

Our new designation for this *Ms30* allele is *ms30-6028*.

JUIZ DE FORA-MG, BRAZIL
Universidade Federal de Juiz de Fora
VICOSA-MG, BRAZIL
Universidade Federal de Vicosa

Image analysis as a tool for chromosome deficiency identification in maize

--Viccini, LF, de Carvalho, CR

Seeds originating from irradiated pollen (maize test-line L-869 of Federal University of Viçosa) were germinated in Petri dishes with a film of distilled water in the dark at 28-29 C. Root tips ranging from 0.5 to 1.0 cm in length were pre-treated with 0.02% colchicine solution for 2h30min. Slides were prepared by the air drying technique with enzymatic maceration.

Image analysis was performed on Macintosh computer using the public domain NHI-Image SXM 1.61 software (Rasband, 1997). Chromosome images were captured by a video camera coupled to the microscope-computer system so as to generate 256 gray value density profile plots.

Chromosome measurements were obtained by the difference of chromosomal area density and that of the background, with the selection tool of the software (profile plot line–width 12) being used. The plot pixel value of relative density was calibrated to the range of 0 (white) to 255 (black). The background picture was set to 0 gray value and the chromosome length spatial was perfomed in micrometers (Carvalho and Saraiva, 1997).

Five metaphases presenting deficiency in chromosome 6 were randomly chosen for analysis (Figure 1). When comparing the chromosome-6 arm length average values, it is observed that the measure of both short arms is about the same, while the long arm difference is 0.67 μm, corresponding approximately to 26% the size of a normal long arm (Table 1).

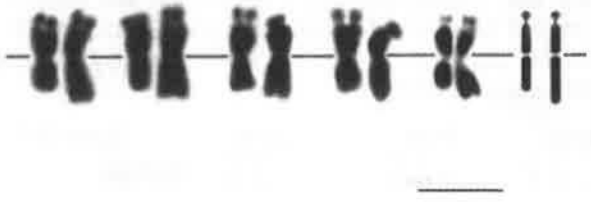


Figure 1. Five pairs of chromosome 6 in root tips of germinating seeds derived from γ-irradiated pollen grains (72 Gy). The chromosomes (left) present a deficiency in the long arm. Bar=5 μm.

Table 1. Measurements (μm) of deficient and normal chromosome 6 originated from five metaphases.

	Chromosome 6					
	Deficient			Normal		
	Arm			Arm		
	Short*	Long	Length	Short*	Long	Length
	1.35	1.88	3.23	1.42	2.78	4.20
	1.53	1.87	3.40	1.59	2.44	4.03
	1.65	1.93	3.58	1.59	2.44	4.03
	1.63	1.99	3.62	1.65	2.73	4.38
	1.59	1.92	3.51	1.48	2.56	4.04
Average	1.55	1.92	3.47	1.55	2.59	4.14

* satellite out.

Normal and deficiency chromosome graphs were overlapped to evidence the alteration (Figure 2). The overlapping of the density graphics of each chromosome of the first homologue pair repre

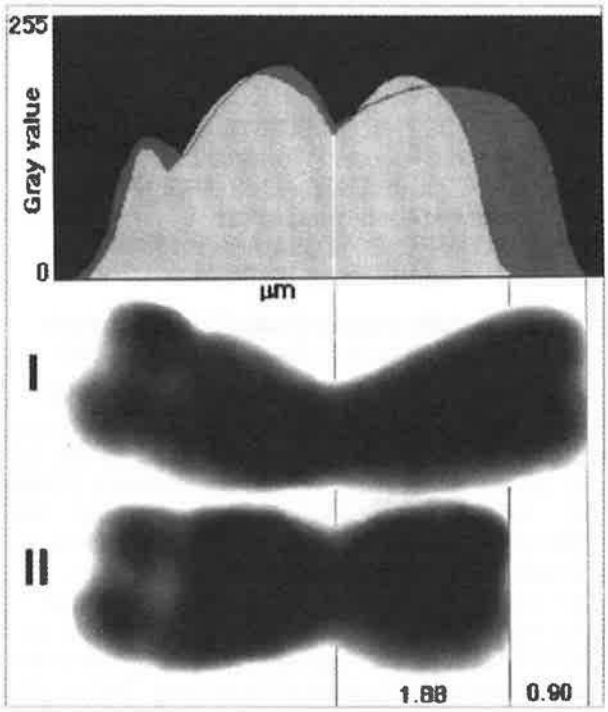


Figure 2. Graph showing density overlap of chromosome 6 pairs. The dark gray area corresponds to gray value of the normal chromosome and the clear gray area of the deficient chromosome.

sented in Figure 1 shows a 0.90 μm chromosome II deficiency. Thus, making it possible to evaluate deficiency length, by considering the difference between chromosome density and that of the background.

It is verified that the image analysis by means of computational resources is a useful tool in studies of this nature.

LAWRENCEVILLE, NEW JERSEY
Rider University

Identification of two cDNAs encoding methylenetetrahydrofolate reductase

--Khan, F, Kite, M, Benner, M

Synthesis of methionine in higher plants involves the net interaction of three processes: 1) formation of an aspartate-derived, four-carbon chain, 2) transfer of a sulfur atom derived from cysteine, and 3) addition of a methyl group donated by 5-methyltetrahydrofolate. Although the enzymes required for these three processes have been identified, the genes encoding them are largely uncharacterized in higher plants. The methyl donor 5-methyltetrahydrofolate is synthesized from 5,10-methylenetetrahydrofolate by the enzyme methylenetetrahydrofolate reductase (MTHFR); this enzyme is critical for the maintenance of an adequate methionine pool in both eukaryotes and prokaryotes. The mammalian genes have been shown to encode two domains - a catalytic domain which binds the substrate and a regulatory domain which contains an allosteric site for S-adenosylmethionine binding (Goyette et al., Nature Genetics 7:195-200). We wish to investigate the role of the regulatory domain in higher plants.

BLAST searches were performed to identify plant ESTs that showed significant homology with previously identified MTHFR sequences. Alignment of the ESTs and comparison with the human cDNA facilitated the design of a degenerate oligonucleotide primer that anneals near the 5' end of the maize MTHFR cDNA and includes the start codon. A primer sequence for the 3' end of the cDNA was designed from a maize EST which contains the putative stop codon. RNA was extracted from maize leaf tissue obtained from two-week old seedlings of W64A using the Qiagen RNeasy protocol. cDNA was prepared with the Qiagen Omniscript RT system. MTHFR cDNA was amplified via 35 cycles of 95 C (30 sec), 50 C (30 sec), and 72 C (2 min). The purified PCR products were ligated into the Stratagene PCR-Script Amp cloning vector. Ligation products were used to transform E. coli strain DH5a; clones were independently sequenced at Rider University and Princeton University.

As predicted, utilization of the above primers in PCR reactions containing maize cDNA resulted in the amplification of a single fragment of approximately 1.8 kb. Sequence analyses indicate that we have isolated two members of the MTHFR gene family in maize. One of our clones is identical to a recently submitted sequence (Roje et al., J. Biol. Chem. 274: 36089-36096), while the other is unreported. Presence of more than one locus has been suggested by Roje et al.; in addition, the RFLP marker *csu134* has been show to be homologous to MTHFR sequences and hybridizes to two *thf* loci, one mapped to chromosome 1 (*csu134a*) and one mapped to chromosome 5 (*csu134b*) (Chao et al., Theor Appl Genet 88:717-721). We hypothesize that one of our clones represents the chromosome 1 locus, while the other represents the chromosome 5 locus. To date, we have ascertained that the unreported cDNA sequence differs with respect to at least three nucleotides. Two of these differences presumably result in divergence at the amino acid level as well (T59A; L132F). In addition, one of the polymorphisms alters a restriction site, giving us a gene-specific difference that will facilitate the confirmation of map location.

LLAVALLOL, ARGENTINA
Instituto Fitotecnico Santa Catalina (FCAyF, UNLP)
CIGEN (UNLP, CONICET, CIC)

Electrophoretic studies on maize endosperm proteins: modifications to Laemmli´s SDS-Page technique

--Corcuera[1], VR, Bernatene[2], E, Naranjo[3], CA
1. Technician of CIC 2. Technician of CONICET 3. Researcher of CONICET

Laemmli´s SDS polyacrylamide gel electrophoresis is based on conditions that assure protein dissociation into their individual polypeptides, minimizing the risk of their aggregation. Electrophoresis is then run, in a discontinuous buffer system using a running buffer with a different pH than the one used to prepare the stacking and resolving gels. These systems were originally developed by Ornstein (1964) and Davis (1964). In our laboratory, electrophoretic studies on maize endosperm proteins were carried out using a Hoeffer Vertical Slab Unit SE 600 and a power source Hoeffer Mighty Slim SX 250. The modifications suggested to the original Laemmli´s technique are listed below:

A. Maize endosperms have to be milled to 100 mesh and the flours defatted with n-hexane using a ratio 1:10 (sample:solvent) at 4 C and stirring during one hour.

B. Several sample:buffer ratios were tested. The ratios examined were 40 mg flour:1000 uL buffer, 70:1000 and 100:1000.

C. Proteins were extracted from meal for 2, 6, 12 and 24 hours at room temperature using SDS-extraction buffer prepared as follows: 18.0 ml water + 7.65 ml 3x buffer + 1.35 ml 2-ME. The 3x buffer contained: 6.25 ml Tris HCl (ph: 6.8) + 12.05 ml tap water + 2.0 g SDS + 10.0 mg gamma pironine + 10.0 ml glycerol. During the extraction time, the samples were vortexed periodically.

D. Protein extracts were heated in boiling water for 10 minutes with later cooling under water flow up to room temperature.

E. The extracts were clarified by centrifugation for 10, 12 or 15 minutes at 12,500 - 13,000 and 14,000 x G.

F. 10 and 12% gels were used, so different monomer concentrations (%T) were analyzed.

G. 50 uL of each extract were loaded onto each lane of the gels. Electrophoresis was run at constant current and variable voltage (20 to 35 ampere/gel).

H. Bands were fixed using a mixture containing 440 ml tap water + 35 ml acetic acid + 25 ml methanol during 3 hours using a shaker. Later on, gel staining was done using a mixture of 500 mg Coomasie Blue R + 250 ml methanol + 200 ml tap water + 50 ml acetic acid during 3 hours using a shaker. Finally, gel decolouring was done employing a mixture of 335 ml tap water + 40 ml acetic acid +125 ml ethanol during 3 to 4 hours using a shaker and with constant changes of the mixture. Between each step, the gels were washed three times with deionized water.

After testing all the modifications listed above, it can be said that for maize endosperm proteins, the best electrophoretic results are obtained using the following procedures:

1. Better results are obtained using fine milling and defatted endosperm flours.

2. The best sample:buffer ratio was 70:1000. When 100:1000 ratio was used, the bands heaped upon themselves producing spots and changing the electrophoretic pattern.

3. The best extracts were obtained after 24 hours extraction, shaking 4 times in a vortex 40 minutes each within the period.

4. Better results are obtained using 12% gels, as they give better definition of the bands. 10% gels are not suitable as some bands do not appear and others join amongst themselves looking like spots or single bands.

5. Better results are also obtained centrifugating 15 minutes at 14,000 x G to clarify the extracts.

The maize samples used belong to different types of endosperm: flint, opaque-2 and waxy. When SDS-PAGE was run according to the above procedures, 19 bands could be observed. The electrophoretic pattern varied with the maize type. Flint maizes present all the bands except band thirteen. Bands 1,2,3,5,6 and 18 are absent in waxy and opaque-2 materials. On the other hand, band 16 is absent in opaque-2 maizes and band 14 was not observed in waxy maizes. It can be said that polypeptide electrophoretic pattern is suitable to differentiate amongst groups of maize with different endosperm natures, though it does not allow distinguishing phenotypes within the group. For this purpose, protein fractionation must be done.

LOMAS DE ZAMORA, BUENOS AIRES, ARGENTINA
Universidad Nacional de Lomas de Zamora
LLAVALLOL, BUENOS AIRES, ARGENTINA
Universidad Nacional de La Plata
RÍO CUARTO, CÓRDOBA, ARGENTINA
Universidad Nacional de Río Cuarto.

Genetic variation in the progeny of maize/*Tripsacum* hybrids.

--García, MD, Bonamico, N, Di Renzo, MA, Molina, MdelC

According to Burson et al. (Crop Sci. 30:86-89, 1990), the form of apomixis in polyploid *Tripsacum* species is diplosporous pseudogamy of the *Antennaria* type, with complete absence of meiosis, which produces progeny genetically identical to the female parent. Nevertheless, Leblanc et al. (Theor. Appl. Genet. 90:1198-1203, 1995) observed the formation of meiotic dyads and tetrads amongst the diplosporic accessions of this genus. Kindiger and Dewald (Crop Sci. 36:250-255, 1996) evaluated the progeny of tetraploid *T. dactyloides* by cytogenetic and molecular (RAPD-PCR) analysis and observed complete absence of sexual development and genetic variation in the progeny. They suggested that an incomplete sexual process could originate this variation.
The objective of this work has been to determine the form of reproduction of the hybrid between maize and *Tripsacum* by means of cytogenetic and molecular analysis of its progeny.

Maize inbred 407B (2n=40) was pollinated with *T. dactyloides* (2n=72). Hybrid embryos (ZT56) were isolated and cultured on the basic medium (MD García et al., Rev. de la Fac. de Agron. de la UNLP 68:15-25, 1992) supplemented with 4 μmol L^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D). Shoots were regenerated by somatic embryogenesis or organogenesis and rooted on the same basic medium without 2,4-D. Progeny was obtained by free pollination with *Zea mays* ssp. *mays* (2n = 20 or 40), *Z. perennis* (2n = 40) and *Z. diploperennis* (2n = 20). Seeds from ZT56 plants were germinated in sand and 8 days later coleoptiles have been cut off and soaked in 50 μl of extraction buffer L-ascorbic, tris-ClH 0,2 M, pH 7. The protein extract was absorbed on Whatman paper N°3 and separation was performed by horizontal starch gel electrophoresis technique. Enzymes analysed were glutamic-oxalacetic transaminase (GOT), endopeptidase (ENP), esterase (EST), alcohol dehydrogenase

Table. Isozyme profile from seedlings of ZT56 hybrid progeny. Polymorphism can be observed in MDH (band 1), ACP (band 4) and ADH (band 1).

Plant	Enzyme															
	MDH							ACP						ADH		
	B1	B2	B3	B4	B5	B6	B7	B1	B2	B3	B4	B5	B6	B1	B2	B3
1	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
3	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
4	-	+	+	+	+	+	+	+		+				+	+	+
5	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
6	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
7	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+

(ADH), malate dehydrogenase (MDH) and acid phosphatase (ACP). The corresponding band pattern was settled for each sample constituted by a single seed taken from the same plant. According to the criteria of absence (-) or presence (+), the bands were enumerated from the anode to the cathode, B1 being the faster band.

Pollen of regenerated ZT56 plants was completely sterile, so some seeds were obtained from free pollination with maize, *Z. perennis* and *Z. diploperennis*. Every progeny plant revealed a chromosome number 2n=56, like the mother plant; consequently, the occurrence of a sexual process could be discarded. But, on the other hand, the molecular analysis showed variations within the progeny. GOT, ENP and EST enzymes showed monomorphism, whilst polymorphism was observed in MDH, ACP and ADH (Table). These results are similar to those published by Kindiger and Dewald (Crop Sci. 36:250-255, 1996) in tetraploid *T. dactyloides*. In the same way, the maize/*T. dactyloides* hybrid showed absence of sexual development, but the apomictic mechanism was able to generate genetic changes in the progeny.

LOMAS DE ZAMORA, BUENOS AIRES, ARGENTINA
Universidad Nacional de Lomas de Zamora
LLAVALLOL, BUENOS AIRES, ARGENTINA
Universidad Nacional de La Plata

Genotype and embryo age affect plant regeneration from maize/*Tripsacum* hybrids

--García, MD, Molina, MdelC, Pesqueira, J

The objective of this work was to evaluate the effects of genotype, embryo age and 2,4-Dichlorophenoxyacetic acid (2,4-D) concentrations on the induction of somatic embryogenesis and plant regeneration from maize/*Tripsacum* hybrids. Plant materials used for this experiment were *Zea mays ssp. mays* (2n=20): i) Inbred A188, from Dr. Phillips; ii) Inbred Santa Catalina 75 (SC75); iii) Cultivar Colorado Klein (CK) and *Tripsacum dactyloides* (2n=72), from Dr. Bird (CIMMYT). The hybrids obtained were i) Inbred A188 x *T.dactyloides* (A188xT); ii) Inbred SC75 x *T. dactyloides* (SC75 x T); iii) Cultivar Colorado Klein x *T. dactyloides* (CKxT); iv) Cultivar SCD x *T. dactyloides* (SCD x T).

Mother plants were grown in the greenhouse during 1998 spring and summer and crossings were practised during December. Maize ears were kept covered with paper bags until silk emergence. Silks were cut immediately before hand pollination with *Tripsacum* pollen and then ears were covered again with paper bags to prevent foreign pollen contamination. Ears were harvested 13, 15 or 19 days after pollination (dap). Developed caryopses were cut off and disinfected with 2,5% sodium hypochlorite solution. Embryos were isolated from the caryopsis, plated on culture media and incubated at 28-30 C with a 16 hour photoperiod. Culture media were composed of García et al. (Rev de la Fac de Agron de la UNLP 68:15-25) basic medium free of plant growth regulators (G0) or with the addition of different 2,4-D concentrations ($\mu mol.L^{-1}$): 2.3 (G2) or 4.6 (G4). Calli were maintained on G4 medium and subcultured monthly. Shoots, obtained on the initiation media, were rooted on G0 medium under the same conditions of temperature and photoperiod. Regenerated plants were transplanted to pots with a plastic cover and three weeks later they were moved to the greenhouse. All the crossings performed gave rise to ears with developed caryopses only in the upper third. Caryopses were turgid with translucent endosperm until 15 dap. Embryo length ranged from 0.3 to 0.5 mm. Caryopses collapsed 19 dap because of endosperm development abnormalities, and by this time embryo length was very heterogeneous, from 0.5 to 2 mm. Only CKxT embryos showed a more uniform size, 1.5 to 2 mm.

Embryos plated on G0 medium germinated precociously (Table 1), but gave rise to weak plants without adventitious roots, which died during the rustication period.

Embryos grown on G4 medium showed low germination percentages but somatic embryogenesis was observed from all the hybrids (Table 2). Although frequencies of organogenic or embryogenic calli did not vary considerably amongst genotypes, the average of plants regenerated per responsive embryo during the culture period (8 months) was very different: 12.5 in A188xT, 2.5 in SC75xT, 2 in CKxT and 0.67 in SCDxT (Table 2).

On G2 medium, germination percentage has also been low and

Table 1: Germination frequencies of Maize/ *Tripsacum* hybrid embryos isolated 13 dap and cultured on G0 medium. Observations were made 2 months after embryo plating.

Genotype	Number of embryos	Germination (%)
A188xT	21	52.4
SCDxT	24	79.2

Germination frequencies did not show significant differences among genotypes (χ^2 = 3.62; P≤ 0.001).

Table 2: Somatic embryogenesis or organogenesis frequencies observed in maize/ *Tripsacum* hybrid embryos isolated 13 or 15 dap and cultured on G4 medium. Observations were made 2 and 8 months after embryo plating.

Genotype	Organogenesis or somatic embryogenesis frequency		Number of regenerated plants		Number of embryos
	2 mos[1]	8 mos[2]	2 mos	8 mos	
A188xT	25.00	11.11	9	50	36
SC75xT	21.87	12.50	4	10	32
CKxT	6.25	6.25	0	2	16
SCDxT	13.64	13.64	4	2	22

Embryogenesis frequencies between genotypes do not differ significantly: [1] χ^2 = 3.13; P≤0.001; [2] χ^2 = 1.38; P≤0.001.

somatic embryogenesis frequencies were 16.6% for A188xT and 20.83% for SCDxT. These values did not differ significantly from those observed in G4 medium (χ^2 = 0.005; P≤ 0.001).

Embryo age notoriously affected somatic embryogenesis frequencies. On G4 medium SC75xT embryos isolated 15 dap showed an induction frequency of 21.87% and 6.15% for those isolated 19 dap (χ^2 = 5.27; P≤ 0.001). No plants were regenerated from calli obtained from embryos isolated 19 dap.
All regenerated plants showed a chromosome number 2n= 46.

In conclusion: Hybrid maize/*Tripsacum* embryos isolated 13 days after pollination and cultured on plant growth regulator free medium germinated but gave rise to weak plants that didn't survive. On the other hand, those embryos plated on medium supplemented with 4.6 µmol.L^{-1} 2,4-D originated, by somatic embryogenesis and organogenesis, an average up to 12.5 vigorous plants/embryo.

The frequency of regenerated plants, of each genotype, was affected mainly by the callus regeneration ability more than the induction frequency of somatic embryogenesis.

Only the embryos less than 1 mm length (isolated 13 to 15 dap) originated calli able to regenerate plants by organogenesis or somatic embryogenesis.

Meiotic pairing in the interspecific hybrid *Zea mays*, *Zea perennis* and *Zea diploperennis*

--Molina, MdelC, García, MD

In this work the following features were analyzed: i) phenotypic and cytogenetic traits as well as fertility and genomic formulae of an hybrid amongst *Zea mays*, *Zea perennis* and *Zea diploperennis* and, ii) the effect of colchicine on the chromosome pairing of the hybrid and parental species.

The plant materials used for these studies were: maize inbreds 2n=40 (Zm40) supplied by the Maize Genetic Coop. Stock Center, Urbana, Illinois, USA, the hybrids DP40 (2n=40) obtained from crossing a non reduced gamete of *Zea diploperennis* (Zd), 2n=20, by *Zea perennis* (Zp), 2n=40, and MDP 2n=40 obtained by crossing Zm40×DP40. Zm40 is annual, 1.8 to 2.0 m height, with one or two fertile ears and indifferent to photoperiod. The hybrid DP40 is perennial, tillering, fertile (80%), seeds scatter at maturity and according to its flowering response it is a qualitative short photoperiod plant with a 13 h critical photoperiod. The hybrid MDP is annual, 2.0 to 2.5 m height, without tillers or at most only one or two. It is prolific, fertile seeds are disposed in 4 to 8 rowed distich ears which do not scatter at maturity and flowering results indifferent to photoperiod.

From the cytogenetic study of the hybrids (Table 1) and their parents it can be deduced:

Zm40 revealed 10iv (30%) and 9iv+2ii (23.94%) with an average of 8.34iv+3.24ii and 33.75 chiasma/cell. According to these results and considering the basic chromosome number for the genus *Zea* as x=5, the following genomic formula can be proposed for Zm40: AmAmAmAm BmBmBmBm.

Zd presents regular meiosis and 10 bivalents could be observed in 73% of the cells studied. The existence of 1iii+8ii+1i or 1iii+7ii+3i in 4% of the cells would point to some homoeologies between A and B genomes and also suggests a probable segmental allotetraploid. The genomic formula proposed for Zd is AdAd BdBd.

Zp has 5iv+10ii (54.47%) and 4iv+12ii (20,15%) with an average of 4.44iv+11.02ii and 34.56 chiasma/cell (15.69 in bivalents and 18.87 in tetravalents). The most frequent chromosomic pairing found in Zp suggests for this species the genomic formula ApApApAp Bp_1Bp_1 Bp_2Bp_2.

The hybrid **DP40** has a low percentage of univalents and trivalents, most of its chromosomes being paired as open ring bivalents and tetravalents, with an average of 31.42 chiasma per cell (15.96 in bivalents and 15.30 in tetravalents). The hybrid DP40 and Zp are very similar in their meiotic configurations as well as in the average of i, ii, iii and iv. According to these results the genomic formula ApApAdAd Bp_1Bp_2 BdBd is proposed.

In **MDP** (Table 2) chromosome pairing is regular forming bi or tetravalents but mono or trivalents were not found. In 43.93 % of the cells 10ii + 5iv pair, in 12.87 % 8ii + 6iv and in 12.87 % of cells 12ii + 4iv. The average number of chiasma/cell is 31.34 (10.24ii + 21.10iv). During anaphase an equal number of chromosomes migrate to each pole and inversion bridges can exceptionally be observed. Pollen fertility ranges from 83% to 95% and seed viability is 90%. The proposed genomic formula is AmAmApAd BmBm BdBp.

Zm40, Zp, DP40 and MDP were treated with diluted solutions of colchicine (0.5 x 10^{-4}M) to analyze chromosome homologies and pairing amongst homoeologous genomes. From the results in Table 3, it can be deduced: a) colchicine does not increase significantly the number of quadrivalents in Zm40, b) in Zp, DP40 and MDP the number of iv results in greatly increased pairing amongst homoeologous chromosomes of genome B, c) homoeologous genomes A pair in all cases, but genomes B only do if there is no other homologous competition during pairing.

Table 1. Meiotic configurations of the species and hybrids

Genotype	2n	Meiotic configurations				Chiasmata/ cell	No of PMCs examined
		i	ii	iii	iv		
Zd	20	0.27 (0-2)	9.73 (8-10)	_	_	14	100
Zp	40	0.16 (0-4)	11.06 (8-18)	0.01 (0-1)	4.44 (1-6)	34.79	134
DP40	40	0.24 (0-4)	11.29 (6-18)	0.06 (0-1)	4.20 (1-7)	31.42	214
Zm40	40	0.04 (0-1)	3.24 (0-10)	0.04 (0-1)	8.34 (3-10)	33.25	142
MDP	40	0.04 (0-2)	10.90 (4-20)	0.01 (0-1)	4.50 (0-7)	31.34	132

Table 2. Meiotic configurations of the hybrid MDP (2n = 40)

Diakinesis metaphase configurations					
i	ii	iii	iv	%	No of cells studied
	4		8	0.76	132
	6		7	4.54	
	8		6	12.87	
	10		5	43.93	
	12		4	12.87	
	14		3	11.36	
	16		2	3.03	
	18		1	6.06	
	20		0	0.76	
2	9		5	1.52	
2	15		2	0.76	
1	10	1	4	0.76	
1	14	1	2	0.76	
Average					
0.04	10.90	0.01	4.50		

Table 3. Number of iv/cell in colchicine (0.5×10^{-4}M) treated (T) and control plants of Zp; Zm40; DP40 and MDP.

Genotype	2n	Number of quadrivalent (%)											No of PMCs Exam.
		0	1	2	3	4	5	6	7	8	9	10	
Zp	40	-	2.24	4.48	7.47	23.88	56.71	5.22		-	-	-	134
Zp (T)	40	-	-	1.36	4.08	12.24	21.76	27.21	22.44	8.16	2.04	0.68	147
Zm40	40	-	-	-	1.40	0.70	4.93	8.45	10.52	18.30	25.34	30.28	142
Zm40 (T)	40	-	-	-	-	0.65	2.35	3.00	7.00	17.00	29.00	41.00	152
DP40	40	-	2.33	4.20	19.62	27.57	34.11	11.22	0.93	-	-	-	214
DP40 (T)	40	-	-	1.47	5.88	7.35	30.88	25.73	16.17	8.86	2.20	1.47	136
MDP	40	0.76	6.06	4.55	11.36	12.87	45.43	12.87	4.54	0.76	-	-	129
MDP (T)	40	-	-	1.43	5.71	7.14	32.14	28.57	12.14	6.42	3.57	2.85	140

LOMAS DE ZAMORA, BUENOS AIRES, ARGENTINA
Universidad Nacional de Lomas de Zamora

Association between yield components, grain morphological traits and volume expansion in popcorn hybrids cultivated in Argentina

--Broccoli, AM, Burak, R

Improvement in quality generally is considered the most important objective of popcorn breeding programs. Expansion volume, defined as the volume of popped corn per gram of unpopped corn, is the quality trait of greatest importance. High expansion volume is associated with increased kernel tenderness (Brunson, 1937). Also, from a commercial standpoint, popcorn genotypes with high expansion volumes will produce more popped corn than genotypes with low expansion volumes.

Compared to dent corn, popcorn kernels contain a high proportion of translucent (hard endosperm) relative to opaque (soft) endosperm. Starch contained in translucent endosperm expands upon heating, while starch contained in opaque endosperm remains relatively unchanged. (Hoseney et al, 1983). Willer and Brunson (1927) concluded that, of all kernel characteristics studied, the proportion of translucent endosperm was most highly correlated with expansion volume.

Popping expansion is a heritable character and the variability of individual ears within an open pollinated variety is similar to other cases of quantitative inheritance controlled by many genes. Significant correlations have been determined between popping expansion and size of kernel and proportion of soft starch, which are quantitatively inherited characters too.

Breeders could achieve popcorn desirable characters including all of the attributes of a good cultivar of flint or dent corn and must include high expansion volume and tenderness. Both characters seem to be closely correlated. The absence of a coarse hull and the presence of good flavor are also desirable.

Because of negative correlation between top yields and superior popping expansion it is difficult to combine both characters in the same genotype. So, some compromise must be made in either yield or popping expansion or both to secure the best results from the utility standpoint.

A smaller inbred depression occurs in popcorn when compared with dent corn. This possibility of finding comparative"per se" productive inbreds makes easy the simple hybrid seed utilization, largely in commercial production. This simplifies the seed production problems and makes possible a most uniform market product.

In Argentina, the traditional popcorns were open-pollinated varieties obtained from a wide range of genetic variability contained in native races.

With the advent of the MERCOSUR (global market of South America) the interest in this crop and the cultivated area increases substantially year by year, but the absence of locally achieved hybrids makes the commercial breeders import these genotypes principally from the USA.

Field trials at six enviroments of fourteen commercially available hybrids were carried out in order to evaluate yield components, popping expansion and genotype by enviroment interaction. To determine the relationships between some kernel characteristics and popping expansion (PE), kernel length (KL), width (KW), thickness (KTH),density of expanded (KED) and not expanded ones (KNED), were determined on a sample taken from the middle sector of the ear. Length (EL) and diameter (ED) of each one was measured. Yield (kg/ha) and prolificacy are positively correlated .

The correlation coefficients of popping expansion with these characters are presented in Table 1.

Table 1. Phenotypic correlations between the investigated traits. (*, **) Significant at the 0.05 and 0.01 probability, respectively.

	PE	YIELD	KTH	KW	KL	ED	EL	KED	KNED
YIELD	* -0.16	-	-	-	-	-	-	-	-
KTH	** 0.46	-0.11	-	-	-	-	-	-	-
KW	0.09	0.02	** 0.32	-	-	-	-	-	-
KL	** -0.18	** 0.52	-0.03	** 0.18	-	-	-	-	-
ED	* -0.15	** 0.51	-0.01	-0.02	** 0.57	-	-	-	-
EL	-0.01	** 0.55	** 0.23	** 0.17	** 0.37	** 0.48	-	-	-
KED	0.02	** 0.21	-0.08	-0.09	0.12	** 0.19	** 0.21	-	-
KNED	** -0.98	* 0.15	** -0.46	-0.11	** 0.19	* 0.16	0.01	-0.014	-
PI	0.03	** 0.32	** 0.19	* 0.17	0.11	0.011	** 0.28	0.004	-0.04

As expected, high positive correlation coefficients of yield with components like KL and KDI exist. PI was also strongly associated because of some hybrids' tendency to produce two ears of uniform type. In agreement with current bibliography, yield components have a negative association with popping expansion, so it is difficult for the breeders to achieve hybrids with both characters simultaneusly.

Analyzing kernel morphological traits, there appears to be some definite association with PE and kernel size and shape. A high positive correlation ($r = 0.46^{**}$) was verified with KTH and a negative one with KL ($r = -0.18^{**}$).

Measuring densities of kernels, initial or without expanding

KED has no correlation with KNED, but final density of the expanded kernels KNED has a strong association (r =0.93**) and would be an effective selection criterion.

These data are in agreement with basic information, even though the hybrids evaluated may be related because of the common origin of some of them, and certainly there is a restricted genetic base that includes some of the best American lines previously evaluated in many Argentine enviroments.

Genotype by environment interaction on popping expansion and yield in popcorn hybrids cultivated in Argentina

--Burak, R, Broccoli, AM

The open-pollinated popcorn varieties traditionally cultivated in Argentina were replaced, during the last years, by introducing popcorn hybrids principally from the USA. Fourteen of these hybrids commercially available were grown in 1997 and 1998 at three locations of the "Milk Belt" (34º 38' South and 58º 48' L , 23m altitude) . The trials were set up in a completely random block design with three replications and the statistical methods applied were AMMI (additive main effects and multiplicative interaction) using biplots in interpreting variety by enviroment interactions, for the variables yield and expansion volume (EV).

AMMI Model

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum \lambda_n \gamma_{gn} \rho_{gn} + \varepsilon_{ge}$$

Y_{ge} = *yield for the* g *genotype in* e *enviroment*
μ = general mean
α_g = genotype effect
β_e = environment effect.
λ_n = n-th axis singular value (eigenvalue)
τ_{gn} and ρ_{en} = corresponding genotype and environment scores (eigenvectors)
ε_{ge} = normally distributed error.

Statistical model for the preliminary ANOVA procedure:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \rho_{k(j)} + \varepsilon_{ijk}$$

Table 1: analysis of variance results for yield

Source	DF	SS	MS
Environments	5	24650	4930**
Rep (Env)	12	23640	1970
Hybrids	13	20540	1580**
Hyb x Env	65	30550	470**
Error	156	40560	260

(*, **) Significant at the 0.05 and 0.01 probability, respectively.

Table 2: analysis of variance results for popping expansion

Source	DF	SS	MS
Environments	5	180.45	36.09**
Rep (Env)	12	100.32	8.36
Hybrids	13	307.84	23.68 **
Hyb x Env	65	716.30	11.02**
Error	156	765.05	5.08

(*, **) Significant at the 0.05 and 0.01 probability, respectively.

The interaction that can be described by two multiplicative components can be represented in a biplot (see biplots I and II). Cultivars are represented by lines, trials by points, both end points are the representation of the end points of vectors starting at the origin. The length of a cultivar line reflects the amount of interaction for that cultivar, thus according with Figure 1, most interaction is due to genotypes 1, 6, 10 and 14. The angle between cultivar lines corresponds to the interaction betwen the interaction residuals. Genotypes 3 and 12, the nearest distributed to the center of the biplot, have more stability. Genotypes 1 and 9, 13 and 8, 2 and 5 are very similar with a high positive correlation. 1 and 10 have a negative correlation. 14 and 6 are very dissimilar with a correlation close to zero. Genotype 14 with a high residual interaction with D environment. The same occurs with 6 in E environment and genotype 1 in A.

In Figure 2, expansion volume, there is less stability for 3, 5 and 6 and the most for genotypes 10 and 14. Similar performance is shown by 7-8, 1-13 and 14. In contrast 5 and 6 are quite alike. There is a particular performance of the genotypes, analysing both figures jointly, that confirms the negative correlation between yield and expansion volume.

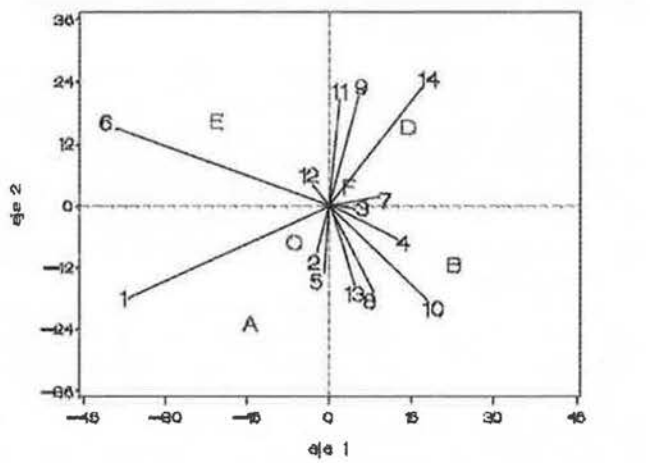


Figure 1. Biplot of the AMMI model for grain yield, with 14 genotypes (1,....14) and 6 environments (A,....F). PCA1 and PCA2 are represented by eje1 and eje2.

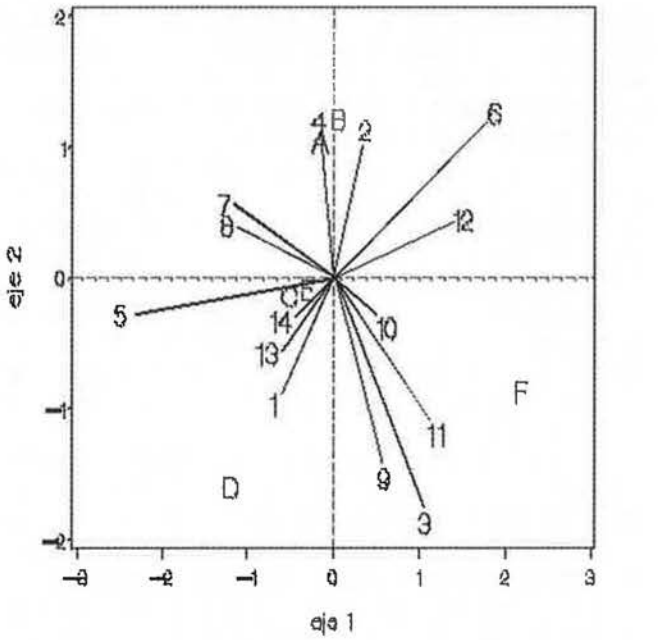


Figure 2. Biplot of the AMMI model for expansion volume, with 14 genotypes (1,....14) and 6 environments (A,....F). PCA1 and PCA2 are represented by eje1 and eje2.

Stability analysis of forage response in maize

--Torrecillas, MG, Bertoia, LM

Knowledge of genotype-environment interaction is of vital importance in breeding programs. The ranking of genotypes changes according to the number of evaluated environments, hindering the task of selection. The aim of the present work was to evaluate the stability of eighteen maize genotypes for silage production, through two methodologies. Four indigenous populations, two partially improved composites and their crosses with inbred lines P1 and P2, were evaluated. These materials were selected on the basis of diversity of cycle, origin, plant architecture, and contribution in yield of ear and stover components.

The studied variables were: whole plant dry matter yield (WPDMY), stover dry matter yield (SDMY) and ear dry matter yield (EDMY). Trials were carried out in four environments and over two years. Analysis of Principal Effects and Multiplicative Interaction (AMMI) and Cruz Medina exact conditional test (ECT) (Heredity 69: 128-132, 1992) were employed. The first two axes of the principal component analysis (PCA) in AMMI model explained 89.7 % of the squares sum of the genotype-environment interaction for WPDMY. In ECT analysis a lack of adjustment of the multiplicative model for the genotype 4 was detected. Genotypes detected as unstable agreed with those obtained in the AMMI analysis. Environments 1 (A1) and 2 (A2) contributed in a greater extent to the genotype - environment interaction. The PCA biplot showed that crosses Population 1 x P2 (genotype 13) and Population 2 x P2 (genotype 14) expressed instability associated with environment 2 (A2), whereas crosses Population 1 x P1 (genotype 7) and Population 2 x P1 (genotype 8) had interaction with environment 4 (A4) (Figure 1). Instability in population 4, population 1 x P1 and population 1 x P2 for SDMY was detected. Population 4 and Population 3 x P2 were unstable for EDMY. In the same manner environment 3 was stable for EDMY whereas environment 4 was stable for SDMY. It can be concluded that high yielding crosses were bound to high instability and that per se materials behaved as stable. This information would be useful for subsequent selection directed towards one or the other component.

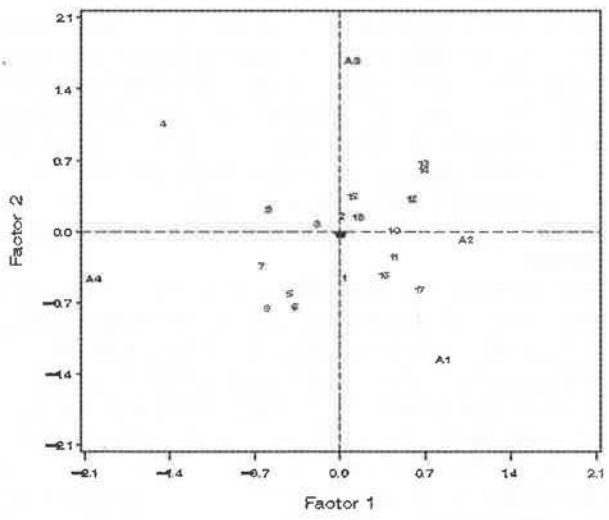


Figure 1. PCA biplot for WPDMY.

Breeding of maize for silage: Yield components in genotypes with different degrees of improvement

--Torrecillas, MG, Broccoli, AM, Bertoia, LM

The objectives of this work were: i) To analyze the importance of morphological characters (plant architecture and size) on ear and stover dry matter yield, and ii) to verify if the behavior of such variables is maintained in germplasm with different origin and degree of improvement.

Twelve genotypes of maize (*Zea mays* L.), representing four groups of different origin and degree of improvement, were evaluated for their aptitude for silage. Trials were carried out in three environments during the 1996/1997 and 1997/1998 growing seasons.

Groups of genotypes were:

G1 = Three commercial hybrids (Three-way hybrids): Cargill Semiden 5, Funk's Tronador and Pioneer 3452.

G2 = Three Argentine Landraces: Accesions 03-056, 14-066 y 16-042.

G3 = Three experimental hybrids (Single hybrids): LZ 2, LZ 14 and LZ 40.

G4 = Three F1 integrated by: subtropical racial composites without any improvement x Synthetic varieties: Composite Cravo x HP3, White Dent composite x SB73 and Yellow Dent composite x SMo17).

The studied variables were:

Leaf number (LN); harvest index (HI) measured as ear dry matter yield : whole plant dry matter yield ratio; stalk diameter (SD), measured in the node of ear insertion; plant height (PH), measured above ground level to basal tassel branch; leaf area (LA); ear dry matter yield (EDMY) and stover dry matter yield (SDMY).

A multiple regression analysis that included LN, HI, SD, PH, LA as independent variables and EDMY, SDMY as dependent variables in the four groups combined among environments was performed.

Absence of multicollinearity among variables was verified. After a stepwise procedure was performed, models with good predictive capacity (adequate values of R2 and cp of Mallows) were selected.

LN and HI were important in all models, explaining EDMY and SDMY. SD was important in G1 explaining EDMY ((4 = 0.61) and SDMY ((4 = 0.06) and in G2 for SDMY ((4 = 0.02). PH contributed significantly ((1 = 0.07) in the model to predict EDMY in G2. The most adequate models that contributed to predict EDMY and SDMY varied according to the involved genetic groups. LA, LN and HI gave models with good predictive behavior, especially for EDMY in three genetic groups (G2, r2 = 0.81; G3, r2 = 0.55; and G4, r2 =0.63). It is important to emphasize that these groups lacked any improvement process defined for grain. Results indicate the feasibility of using leaf area, leaf number and harvest index in selection processes of unadapted germplasm, in order to maximize forage production.

LONDON, ONTARIO, CANADA
University of Western Ontario

Silver staining root-tip sections in maize

--Maillet, DS, Walden, DB

From our ongoing investigation into the heat shock response in

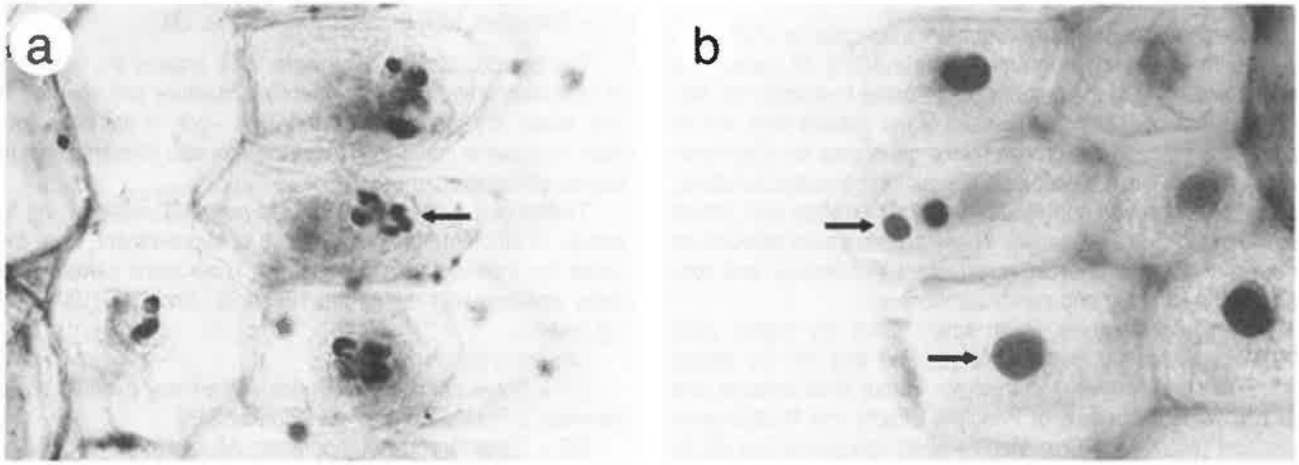


Figure 1.

maize, Greyson et al. (1996 Developmental Genetics 18:244-253) reported that the cells in the root-tip which respond to heat shock by an increase in the expression of HSP 18 are located in the first one to three mm. We have observed (Maillet et al. 1999 MNL 73: 67-68) that heat shock can cause changes in the morphology of nucleoli in several cell types. To test the hypothesis that the cells that respond by altering their protein and mRNA expression patterns also exhibit changes in the morphology of their nucleoli, a silver staining method (Howell and Black 1980 Experientia 36:1014-1015) was adapted to stain root-tip sections. Live heat shocked (shifted from 27 C to 43 C for 3h) and control roots from 5 day old Ohio43 seedlings (8 to 10 cm long) were cut into sections approximately 60 um thick with a hand microtome. Sections at 0.5, 1.0, 2.0, and 4.0 mm from the tip including the root cap were fixed (3:1 ethanol to acetic acid) for 24 h. The sections were rinsed in water for 10 min and stained in a mixture of 50 ul of silver nitrate (0.5 g . ml) and colloidal developer (2 % [w/v] gelatin, 10 % [v/v] formic acid) in the dark for 60 min. The sections were rinsed several times in the developer until all of the silver precipitate was removed. The sections were mounted in developer on a microscope slide under a coverslip. Sections were scored for the number of cortex cells that had nucleoli which demonstrated an altered morphology (Table 1.). In Heat shocked material (0.5 mm from the root-tip) nearly all cells had nucleoli which were altered; sections farther from the root-tip had progressively fewer responding cells. Figure 1 presents a photograph of a section, (a) from a heat shocked root (section taken at 1 mm from the root-tip) and (b) a control section. Cells from heat shocked cells have several small stained nucleolar ovoids, controls have one or two spherical nucleoli.

Table 1. Heat shock (3 hr at 43 C)and control sections from Ohio 43 root-tips. n = number of roots, E = epidermis, C = cortex, ED = endodermis, P = pith. The changes in morphology seen in HS cells were not observed in any of the nuclei in the other experiments where the number of nuclei was counted.

HS		Respond				No Respond			
	n	E	C	ED	P	E	C	ED	P
0.5 mm	6	200	266	15	102	0	2	0	3
1.0 mm	6	249	249	92	87	14	51	1	16
2.0 mm	5	130	140	20	22	20	110	9	7
4.0 mm	5	0	0	0	0	12	137	0	19
C		Respond				No Respond			
	n	E	C	ED	P	E	C	ED	P
0.5 mm	5	0	0	0	0	200	180	113	110

Sequential C-banding and silver staining of interphase cells in maize

--Maillet, DS, Walden, DB

Banding methods greatly improve the accuracy of chromosome identification. C-banded nuclei derived from cold arrested root-tip cells (inbred Mo17) revealed one large C-band on each of 6S and 7L, and a small band on 6L. The large band on 6S included the heterochromatin associated with the NOR. Interphase cells on slides containing root-tip metaphase nuclei had four darkly staining regions (dsr), presumed to be the same material that C-banded in metaphase, and occasionally two smaller dsrs. We reasoned that if other knobs could be seen and distinguished from each other in interphase, they could be used as cytogenetic markers.

The protocols for C-banding (Jewell et al. 1994, Maize Handbook pp. 484:492) and silver staining (Howell and Black 1980, Experientia 36:1014-1015) were modified so that spread cell preparations could be C-banded and then silver stained to examine the arrangement of the knobs on 6S and 7L. Binucleate tapetal cells were used because they are easily identified in interphase, and the stage of the PMCs can be used to ensure that all of the tapetal cells selected are approximately at the same stage of development.

Tassel branches from inbred Mo17 grown in our nursery were fixed for 24 h (3:1 ethanol to acetic acid); anthers were staged by examining propriocarmine stained cells. Anthers with PMCs in diplotene to diakinesis were rinsed in water for 10 min, digested in 5 % [v/v] cellulase (Sigma), 0.5 % betaglucuronidase (Sigma) in 0.01 M citrate buffer, pH4.7 with 10 % [v/v] pectinase (Sigma) for 2 h at 37 C. The anthers were spread in a drop of fixative on a microscope slide, and air dried for 30 min. The slides were stored in 100% ethanol overnight. The slides were immersed in 0.2 M HCl at 60 C for 90 to 120 sec, rinsed in water twice, immersed in filtered 5 % [w/v] BaOH for 7 min at room temperature, rinsed

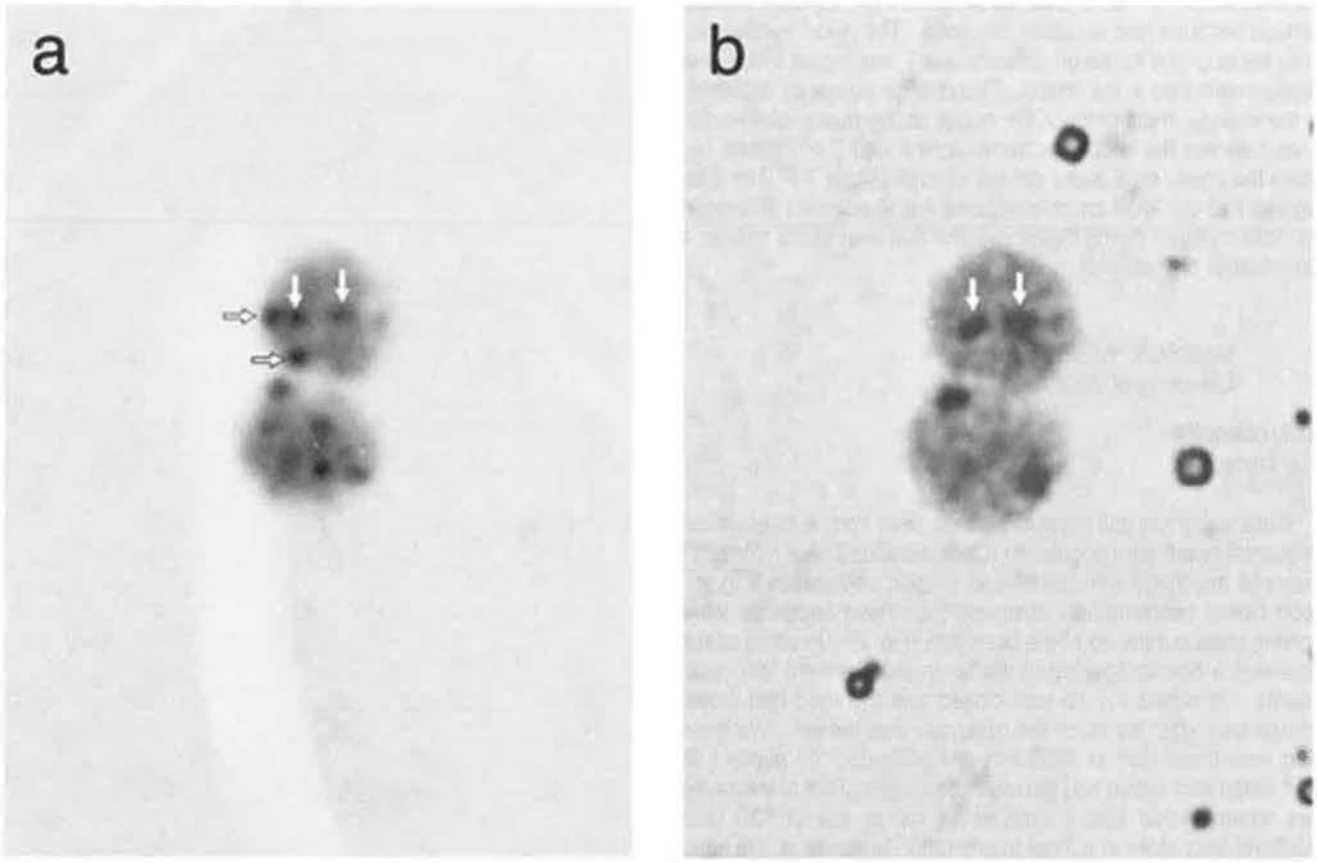


Figure 1.

three times in water and incubated in 2 X SSC for 1 h at 60 C. The slides were stained in 5 % giemsa [v/v] in phosphate buffer for 60 min. Photographs of wet mounted slides were taken at a magnification of 400 with a photomicroscope. The coordinates were recorded at each location where photographs were taken. The C-banding was removed by immersion in 100% ethanol, water, 0.2 M HCl, and water, each for 15 sec and air dried for 30 min. The slides were silver stained with 2 drops of silver nitrate (0.5g / ml) and two drops of colloidal developer (2 % gelatin [w/v], 10% formic acid) under a coverslip for 60 to 90 sec at 70 C on a slide warmer. After staining, the coverslip was removed with warm flowing water, and the slides were air dried overnight. The slides were mounted in permount under a coverslip and photographed again. Figure 1 presents the same binucleate tapetal cell a) C-banded, (four dsrs were observed) and b) silver stained, (two dsrs were present) which allows the two C-bands to be distinguished from each other.

Chromatin affinity in interphase

--Maillet, DS, Walden, DB

Although it has been possible to examine nuclear organization in metaphase cells, interphase is the stage of the cell-cycle where genes are active and chromatin organization may be necessary for cell type expression. Horn (Ph. D. thesis, University of Western Ontario, London Ontario, 1973) observed that the K10 knob could be seen in Feulgen stained interphase nuclei; in the presence of two copies of the K10 chromosome, the knobs were close together. Entire tassels, collected in the morning (ambient temperature 25 C) from inbred Mo17 grown in our nursery, were kept at 27 C or 43 C with the cut end in water for 24 h. Utilizing the methods described in our previous contribution, we were able to examine the arrangement of chromatin in interphase binucleate tapetal cell preparations. By comparing the negatives of cells photographed after C-banding and after silver staining, the relative distance between the knobs on chromosomes 6 (present on both the C-banded and silver stained negatives) and 7 (present on only the silver stained negative) and between chromosomes 6 and 7 could be measured. Measurements were divided by the diameter of the nucleus and compared between 30 heat shocked and 30 control nuclei. From earlier work (Maillet et al. MNL 1999, 73: 67-68) we reasoned that heat shock would disrupt nuclear organization. The

Table 1. Two sample t-test of Mo17 interphase affinities between heat shocked and control and the diameter of the nuclei sampled.

comparison	chromosome affinities HS mean SD	Control mean SD	df	t
chromosomes				
7 to 7	4.08 +/- 1.97	2.44 +/-1.18	58	1.86*
6 to 6	3.86 +/- 2.03	2.94 +/- 1.43	58	0.20
6 to 7	4.34 +/- 2.01	3.12 +/- 1.45	118	0.21
nuclei diameter	8.97 +/- 1.05	6.68 +/- 0.97	58	8.63•

* significant at t(1), 0.05, 58
• significant at t (2), 0.05, 118

diameter of spread nuclei was higher in the heat shocked sample perhaps because less structure remained. The mean relative distance between the knobs on chromosome 7 was higher in the heat shocked cells than in the control. This change cannot be explained by the change in diameter of the nuclei as the mean relative distance between the knob on chromosome 6 and the distance between the knobs on 6 and 7 did not change (Table 1.). The data suggest that the knob on chromosome 7 and adjacent chromatin are close together during interphase and that heat shock causes a disruption of organization.

MADISON, WISCONSIN
University of Wisconsin

Rooty coleoptile
--Tracy, WF

While doing rag doll germinations on seed from ears of an experimental sweet corn population (Cacahuacintle Dulce x Sweet) I observed seedlings with arrowhead shaped coleoptiles (Fig. 1). Upon closer examination I observed that these seedlings were growing roots out the tip of the coleoptile (Fig. 2). In some cases there was a normal opening at the tip so that the third leaf could emerge. In others the tip was closed and the third leaf would emerge only after the tip of the coleoptile was broken. We grew seed from these ears in 1999 and self pollinated the plants. Of eight selfed ears seven had the rooty coleoptiles. Five of the seven ears when pooled had a ratio of 34 rootys out of 120 total seedlings, very close to a three to one ratio. However, of the other two ears, one had most but not all rooty coleoptile seedlings and the other had very few. I have not made enough crosses to make any conclusions on the inheritance of this trait, but it certainly seems heritable.

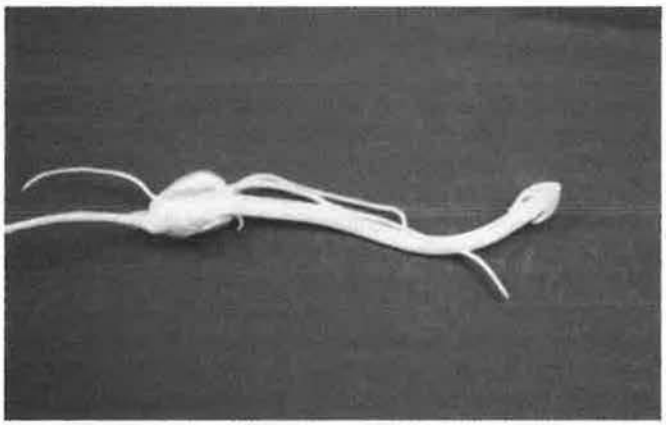


Figure 1.

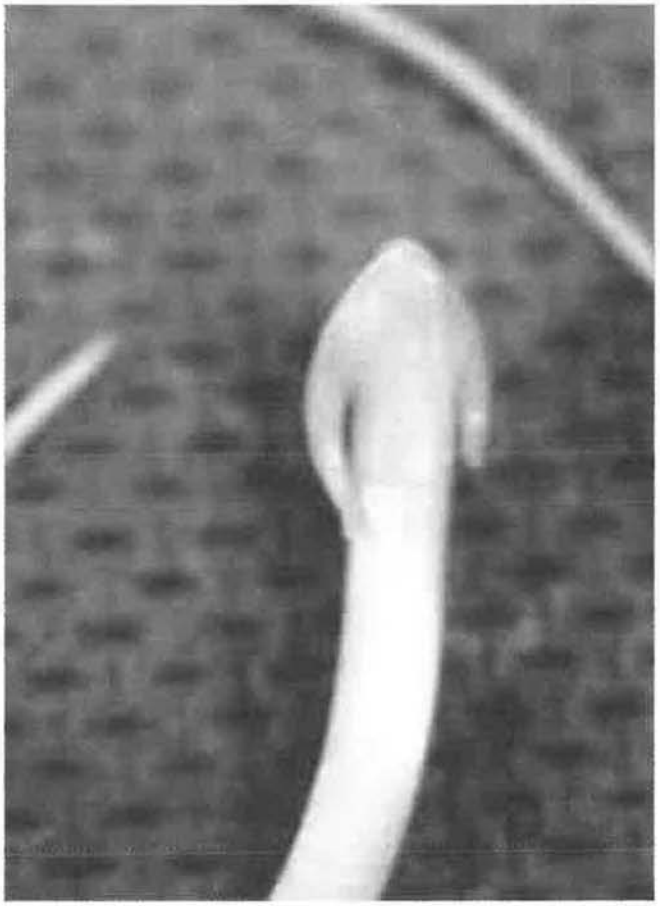


Figure 2.

MARTONVASAR, HUNGARY
Hungarian Academy of Sciences

Dynamics of pH changes in corn (*Zea mays* L.) haploid cell suspension cultures
--Kovács, G

The loss of embryogenic competence of long term cell suspension cultures is one of the major problems faced in the development of in vitro regeneration systems for propagation as well as genetic manipulation. Currently, the causes for the loss of embryogenic competence, which is associated with the time of culture and the number of subcultures, are believed to be genetic or physiological. On the contrary, according to our hypothesis these changes are generated by the repeated stress caused by the suboptimal physical culture conditions, such as pH, dissolved oxygen, etc. The pH of a cell culture medium is one of the most important factors, as growth promoting properties and the selectivity of the culture media are pH dependent. In a majority of cases the pH of a culture medium lies between 5.5 and 6.0. Murashige and Skoog (1962) reported that a pH value of 5.7-5.8 is suitable for maintaining all the salts in soluble form, even with relatively high phosphate levels, and is enough to permit rapid growth and differentiation of the tissue.

Several workers observed that the presence of plant tissue affects the pH of the medium and in most cases there is a fast pH drop from 5.8 to around 4.5 during the first two days of cultiva-

tion. The pH optimum of cell growth is not a constant pH value. It changes with the medium composition, and probably there is a different optimum for maximum growth (cell proliferation) and for differentiation.

Supposing that plant cells have an active role in changing the medium pH, we should choose the initial pH of the medium so that the cells spend less energy on changing their circumstances. The aim of the present work is to determine the effect of the initial pH in consecutive subcultures of corn haploid cell suspensions in order to minimize the stress conditions caused by the periodic pH changes.

An anther culture originated haploid corn cell line was used throughout. This cell line was proved to be able to regenerate green plants in a separate experiment even in large scale cultures.

BM medium supplemented with 1 mg/l 2,4-D and 3% sucrose was used in all experiments. The pH of the medium was adjusted after heat sterilization by sterile 1N HCl or 0.5N KOH. The initial pH of the medium was adjusted to 5.8 in experiment A) and to 4.6 in experiment B). Cell suspension culture was cultivated in 250 ml Erlenmeyer flasks containing 100 ml medium prior to culture inoculation, and was rotated on a shaker at 100 rpm for 7 days at 27 C. The initial dry cell concentration at the time of inoculation was around 0.2 g/l. After 7 days the cells were settled and media was removed. Cell mass was divided and fresh media with adjusted pH was added for the next 7 days subculture.

A New Brunswick 1500 ml CelliGen Cell Culture System fermenter was used for determination of the pH profiles during 7 days of cultivations. Mixing was accomplished using Cell-lift impeller at an agitation rate of 100 rpm. Temperature was controlled at 27 C. Cultures were continuously sparged with air at a rate of 500 ml/min. The fermenter was exposed to warm white fluorescent light (45 μmol/m^{2}s) for a 16 h photoperiod. pH was measured by sterilizable Ingold electrodes. The medium was the same as in shaking flask experiments, pH of the medium was adjusted after heat sterilization. The cells grown in 4 shaking flasks for 7 days were collected and used as inoculum for the fermentation process. pH electrode signal was recorded on-line by a computerized process monitoring system.

Cell suspension was poured into vacuum filter with a preweighed filter paper. Medium was removed by vacuum and fresh cell weight was measured.

The embryogenic competence of the cell cultures from pH 5.8 and 4.6 was tested in a hormone free liquid BM medium (pH: 5.8) at the end of the experiment.

Our first corn haploid cell suspension culture experiments were carried out in shaking flasks. The initial pH of the media was adjusted to the "traditional" pH 5.8. The pH of the medium at the end of one week of cultivation varied between 4.3 and 4.6. The cells formed 1-3 mm size aggregates and had a brownish color indicating pigment production.

A series of parallel shaking flask experiments were carried out with different initial pH values. The initial pH of the subculture media was 5.8 in experiment A) and 4.6 in experiment B). These initial pH values were used in all consecutive subcultures. Four subcultures were examined for each initial pH and the pH values at the end of the 7 days culture were recorded. (Fig. 1.)

Despite the fact that there was almost 1.5 pH unit difference between the initial adjusted pH of the subcultures there were no significant differences between the two experiments in final media pH values. However there were differences in the "behavior" of

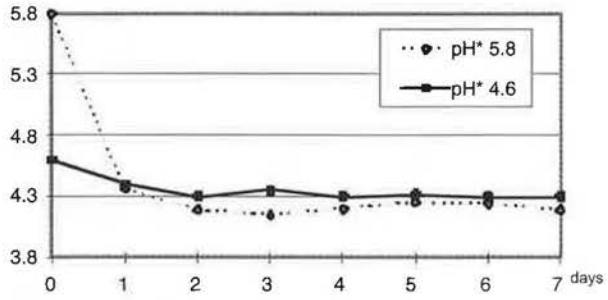


Figure 1. Final pH of the media after 7 days cultivation. Initial pH of the media was 5.8 for experiment A) and 4.6 for experiment B)

the cultures. Cultures on pH 4.6 have lost their bright-yellow color and formed lighter-color or white aggregates. Cell growth was also different. Fresh cell weight after 7 days was 4 times higher than the inoculum weight at pH 5.8 and 9 times higher at pH 4.6 .

The changes in medium pH were determined by taking sterile samples from both of the experiment series. Samples were taken and pH was measured once a day during the 7 days cultivation. Measured pH values are presented in Fig. 2.

The result of the pH measurements during 7 days cultivation reflects that pH dropped rapidly from 5.8 to around 4.3 during the first two days of cultivation. After that sudden change pH remains quite stable. On the contrary there was no such dramatic pH drop in the cultures initiated from pH 4.6 where pH slowly drifts to a pH value around 4.3. According to the results of embryoid induction, cell cultures originated from 5.8 media produced dramatically less embryoids than when the initial pH was 4.6. Under the given conditions used for embryo induction only globular embryos were observed in both cases.

More careful examination of the changes in medium pH were carried out in two experiments in a bioreactor. The initial pH of the media in the bioreactor was adjusted to 5.8 and to 4.6. Fig. 3. presents the pH changes during the cultivations.

The results of the bioreactor experiments show the same overall behavior as the shaking flask experiments. The pH changes during the first two days are even more striking. The pH drop at the beginning of the process seems to be a more complex process. After the inoculation at pH 5.8 the medium pH first drops to around pH 4.4 already in the first hours of the cultivation. This pH drop is followed by a short increase then pH monotonously de-

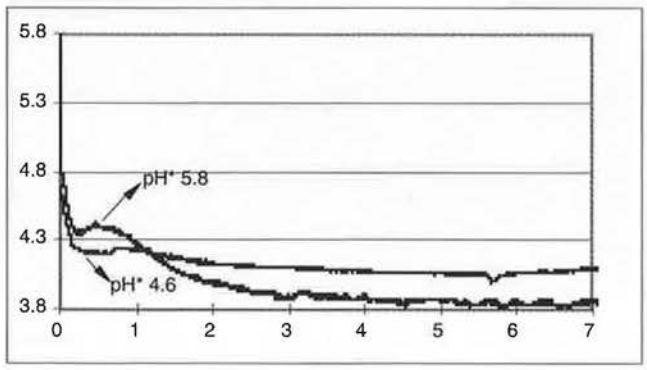


Figure 2. Changes in pH of the media during 7 days cultivation in shaking flasks. pH* in the legend indicates the initial pH of the culture media.

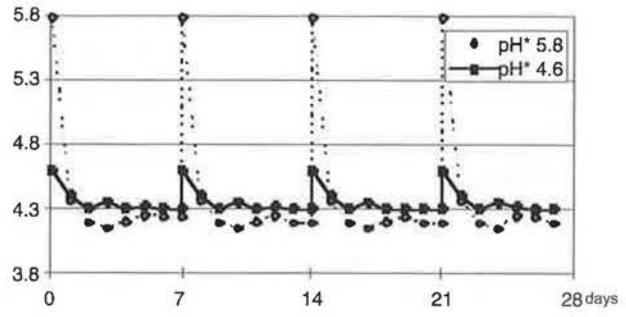


Figure 3. Changes in pH of the media during 7 days cultivation in bioreactor. pH* indicates the initial pH of the culture media.

creases during the first two days reaching a steady state for the remaining time of the cultivation. This pH fluctuation does not occur in the experiments started from pH 4.6. In this case there is only a small but rapid drop to a value around 4.3 where pH stabilizes already after 12 hours.

It has to be noted that the final pH values of the cultures in bioreactors are a little bit lower than in the shaking flask experiments probably due to the different aeration.

The results of monitoring the changes in the cell culture medium pH suggest that the traditional method of starting subcultures from pH 5.8 without any attention paid to the final pH of the medium leads to periodically changing environment. Each subculture inoculation to high pH causes dramatic stress condition for the cells (Fig. 4.). The rapid pH changes during the first 2 days of the culture indicate that cells must spend energy on adjusting the medium pH to a more favorable value after each subculture inoculation. This energy consumption for maintenance purposes results in a lower cell production yield, and decreased embryogenic capacity.

The adjustment of the initial pH of the subculture media to 4.6 eliminates the periodic pH changes during the first two days of the culture. This also means eliminating stress conditions which result in higher cell production efficiency and embryogenic competence.

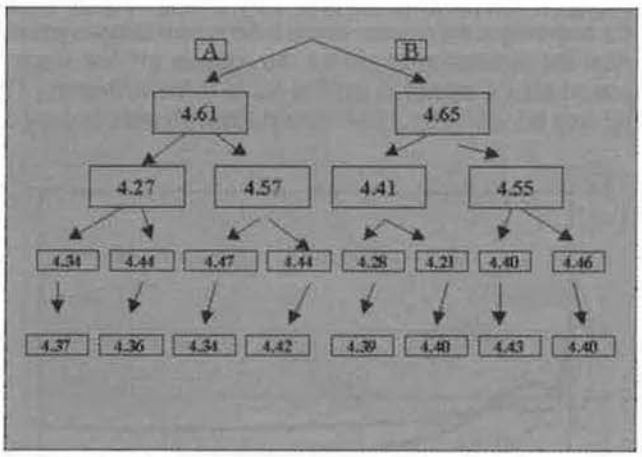


Figure 4. Periodic changes in pH of the media during consecutive subcultures. pH* indicates the initial pH of the subculture media.

MILAN, ITALY
Università degli Studi di Milano

Molecular analysis of *abs*-7065*, a mutant with severe impairment in seed development

--Giulini, A, Consonni, G, Aspesi, C, Gavazzi, G

The mutant we are going to describe was originally isolated in the selfed progeny of F1 obtained by outcrossing a *+/lil1* heterozygous plant to the W64A inbred line. Since *lil1* (*lil*iputian) was isolated by insertional mutagenesis with the mutator (Dolfini et al., 1999), the new mutant could have arisen by insertion of an element of the *Mu* family into a functional gene thus leading to an aborted seed phenotype. This mutant was accordingly named *abs*-7065* (*aborted seed*). Crosses with the A-B translocation stocks locate *abs*-7065* on chromosome 1L.

Phenotype of the mutant seed. Immature (16 DAP) mutant seeds obtained by selfing *+/abs7065* heterozygous plants, are easily distinguishable from normal sibs by a reduction in size and a pale, translucent appearance.

Their endosperm has a soft and fluid consistency while the embryo, not different in size from that of wild-type, appears retarded in its morphogenesis. Mature seeds are completely collapsed but retain a reduced amount of endosperm tissues.

Mutant embryo rescue. Immature embryos of mutant and normal sibs segregating on a selfed *+/abs7065* ear were cultured on minimal or enriched media to assay their germination and growth capacity.

Starting at 13 DAP, germination of excised wt embryos can be obtained, while mutant embryos do not germinate until 25 DAP (Table 1). This observation may indicate a delay in mutant development, that affects its germination capacity. Furthermore the percentage of germination is significantly lower (13.2%) in comparison to wt (100%) and seedlings obtained from homozygous *abs7065* embryos are retarded and impaired in their growth. No promoting effect on germination or growth is observed by culturing mutant embryos on enriched media.

A plausible interpretation of these observations that takes into account the close relationship between endosperm and embryo development is that the primary effect of the mutation is impairment of endosperm development leading to a retarded morphogenetic potential of the embryo as a secondary effect, likely due to lack of component(s) elaborated by the endosperm and supplied to the embryo to accomplish its regular development.

The failure to observe a complete phenotypic repair of mutant seedlings obtained by immature embryos would suggest impairment of an early effect of the endosperm upon the embryogenetic process.

Molecular analysis. Since these mutants have been isolated from a progeny derived from an active Robertson's *Mutator* maize stock, its origin can be ascribed to an insertional event. To verify the association between the mutant phenotype and a molecular

Table 1. Growth of mutant and normal sib embryos at two developmental stages on a minimal (MS) medium or on media supplemented with amino acids, vitamins or hormones.

Developmental stage	Cultured embryos		Germination %		Seedling growth: MM		MV		MA		MM(1)	
	±	m	±	m	±	m	±	m	±	m	±	m
13 DAP	146	42	100	0	8.3	-	10,7	-	3,7	-	ND	ND
25 DAP	218	83	100	13.2	15.6	1.7	6.6	1.0	ND	ND	6.0	0.5

(1) gibberellic acid and benzyladenine

polymorphism, cosegregation analysis was performed. Heterozygous plants were crossed to the W64A line and the genotype (+/- or +/+) of individuals from the progenies was determined by selfing. Genomic DNA was extracted from single plants (110 individuals) and analysed by Southern blot. A 12 Kb *Eco*RI fragment was found in association with all heterozygous plants tested using an internal fragment of *Mu3* as a probe. By digesting with *Pst*I, a restriction enzyme internal to *Mu3*, two polymorphic fragments have been detected of 9Kb and 2kb respectively (Fig. 1). The 2kb fragment was cloned; sequence data confirm the presence of the 5' portion of the *Mu3* element and of 1115 bp flanking genomic DNA. A 390 bp *Xho-Mlu*1 internal fragment was used as a probe on Southern analysis: this probe confirms the presence of the 12kb *Eco*RI and 2kb *Pst*I fragments in +/- plants, previously highlighted in the *Mu3* profile. Isolation of the genomic clones is in progress.

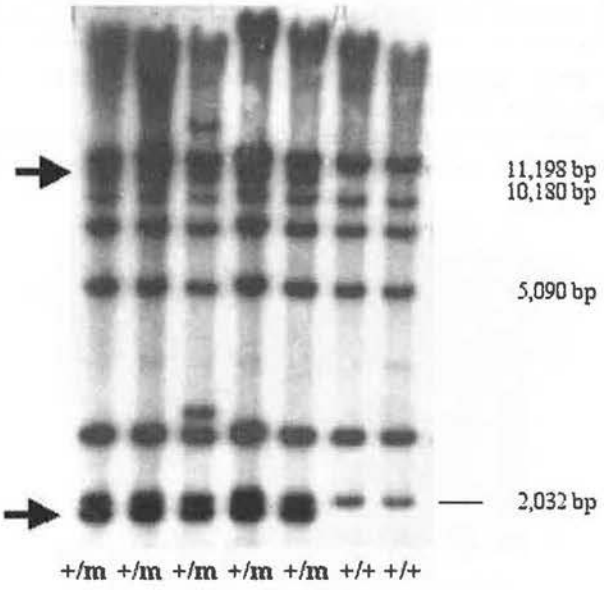


Figure 1. Identification of the *Mu3*-hybridizing *Pst*1 fragments that cosegregate with the *abs*-7065*/+ genotype. *Pst*1 digested DNA from sibling plants was analyzed by Southern blot, using an internal *Eco*RI/*Hin*dIII fragment of *Mu3*. The arrows indicate the *Mu3*-hybridizing fragments that cosegregate with the *abs*-7065*/+ genotype.

The twin trait in maize

--Pilu, R

The twin phenotype that I am going to describe was first observed in the selfed progeny of a plant heterozygous for *Ac* and *sml* (*s*hoot*m*eristem*l*ess : MNL73:69) with a frequency of 3% (Fig. 1A).

A further round of selfing yielded four ears, two without and two with twins (frequency: 2.5% and 5.7% respectively), and, occasionally, seedlings with a stem divided into two stems after the first node while the primary root remains undivided (Fig. 1B). When twin plants are outcrossed as male parents to a different line, no twins are recovered in the F1.

In the selfed progeny of a twin plant reproduced in the greenhouse, seeds with twin embryos (Fig. 1C) are recovered with a high frequency (20%) while seedlings obtained by germinating normal seeds exhibit abnormal leaves with two lobes and a stunted growth. Their karyotype shows a normal chromosome number excluding a chromosomal unbalance as the basis of this trait. Taken as a whole these preliminary observations seem to indicate that the twin character is due to a monogenic recessive mutant with low penetrance upsetting the genetic program subtending apical meristem organisation.

Since the character appeared in the presence of *Ac*; we will assay if its origin is related to *Ac* induced transpositional mutagenesis.

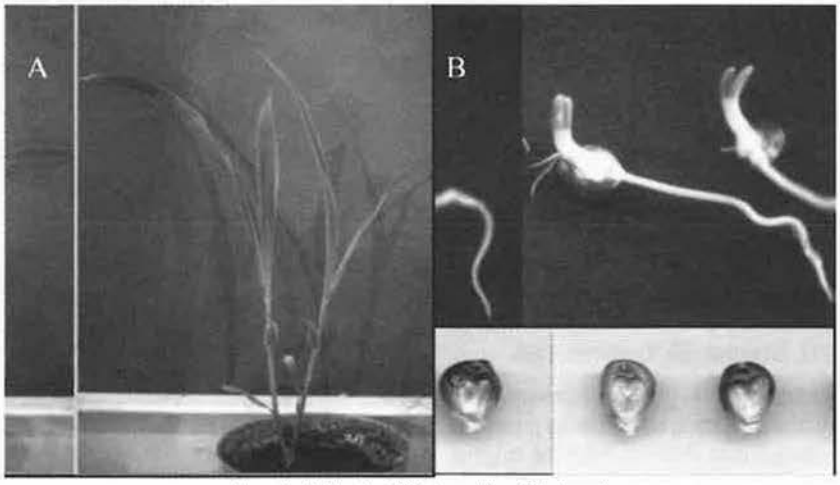


Figure 1. A) twin plant. B) twin seedlings. C) twin seeds

MINAS GERAIS, BRAZIL
EMBRAPA Maize and Sorghum

Mapping a novel opaque endosperm mutant using SSR markers

--Maki, CS, Carneiro, NP, Guimarães, CT, Lopes, MA, Paiva, E

To understand more about the relation among the opaque mutations, zein levels, endosperm hardness and nutritional quality of the maize endosperm we started to characterize native maize opaque endosperm mutants. The opaque mutants described in the literature have in general higher nutritional quality and lower zein levels compared to the wild type genotypes. These novel maize opaque endosperm mutants have soft endosperm, low nutritional quality and zein levels similar to the wild type endosperm (Guimarães et al., Rev. Bras. Genet. 18, 2, 259-264). One of the mutant genotypes (BOL II) was crossed to the wild type genotype (BR-201) and the F2 plants were self-pollinated to F4. DNA pools from homozygous vitreous and opaque plants were analyzed in bulked segregant analysis (Michelmore et al., PNAS 88:9829-9832) to map the locus related to endosperm hardness in this mutant. Four out of 276 primer pairs tested (Research Genetics) showed to be polymorphic when bulks of DNA from individuals of vitreous and opaque endosperm were compared: MAG.T01.D04, *bngl125*, *bngl382* and *phi083*, located at 2.01-2.02, 2.02, 2.03 and 2.04 respectively. To get more accurate map position, DNA from these individuals was tested separately (Figure 1). We are in the process of refining the data using RFLP markers surrounding this genomic region. The same F4 population will be used in a subtractive hybridization strategy to isolate genes involved in the definition of the soft endosperm trait.

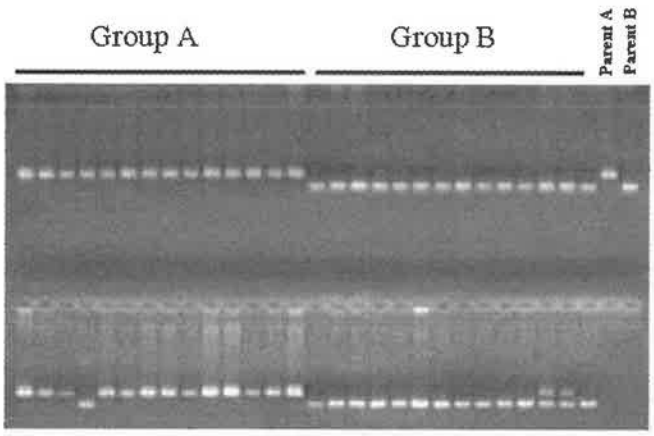


Figure 1. SSR marker analysis showing polymorphism among F4 individuals homozygous for vitreous (Group A) and opaque (Group B) endosperm. Parent A is the novel mutant and Parent B is the wild type.

MOSCOW, RUSSIA
Institute of Plant Physiology

RAPD markers variability in maize somaclones produced from inbred A188

--Osipova, ES, Dolgykh, YI, Shamina, ZB, Gostimsky, SA

Random amplified polymorphic DNA (RAPD) markers have been used to study genetic variation among somaclones produced from inbred A188. These somaclones differed from the original line in several inherited morphological and biochemical traits (Dolgykh et al., MNL 65:89, 1991; Khavkin et al., MNL 67:84, 1993; Dolgykh, MNL 73:70, 1999). The clones investigated represented two independently produced groups of plants: R11, R14, R27 and R54, which had been regenerated from scutellar callus after cultivation in vitro for two months; and R105, R106, R107 and R119 which had been regenerated after cultivation in vitro for eight months.

Sixteen 10-base primers were used to amplify genomic DNA extracted from leaves of three week old plantlets (Table 1).

Table 1. Sequences of 10-base primers used.

Primer	Sequence	Primer	Sequence
318	5'-CGG-AGA-GCG-A-3'	QR-5	5'-CGG-CCC-CGG-C-3'
340	5'-GAG-AGG-CAC-C-3'	B-1	5'-CGT-CGT-TAC-C-3'
450	5'-CGG-AGA-GCC-C-3'	B-2	5'-GTC-CTC-AGT-G-3'
QR-1	5'-CGG-TCA-CTG-T-3'	B-3	5'-GCA-GAC-TGA-G-3'
QR-2	5'-CGG-CCA-CTG-T-3'	B-4	5'-TCT-TAG-TGC-C-3'
QR-3	5'-CGG-CCC-CTG-T-3'	B-5	5'-GAC-AGT-AGC-A-3'
QR-4	5'-CGG-CCC-CGG-T-3'	B-6	5'-CTT-GGA-TGG-A-3'
10	5'-AGG-CGG-GTA-C-3'	11	5'-AGG-CGG-GAA-C-3'

All primers generated from 2 to 17 markers, which varied in size from 200 to 2000 bases.

RAPD analysis did not detect any DNA polymorphism among the individual plants of original inbred A188 (Fig. 1), confirming a

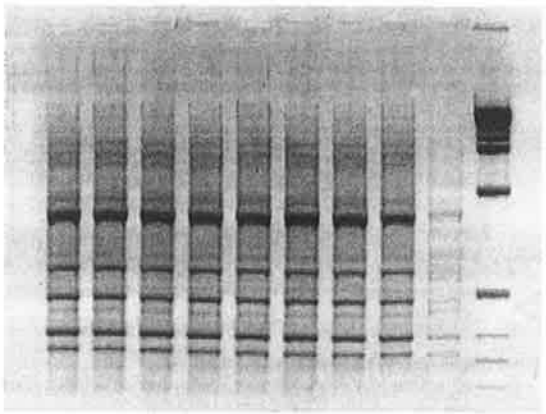


Figure 1. RAPD profiles of individual plants of inbred A188 generated by primer 11.

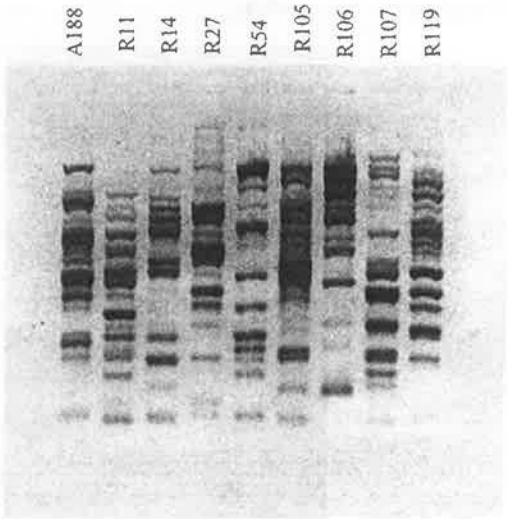


Figure 2. Individual spectrum of markers for each somaclone with primer QR-1.

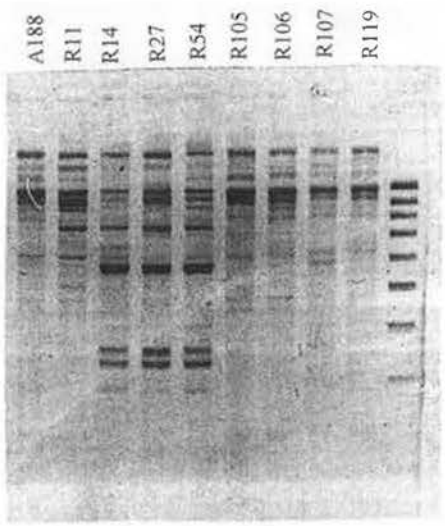


Figure 3. Specific RAPD bands for the somaclones R11, R14, R27 and R54 generated by primer QR-5.

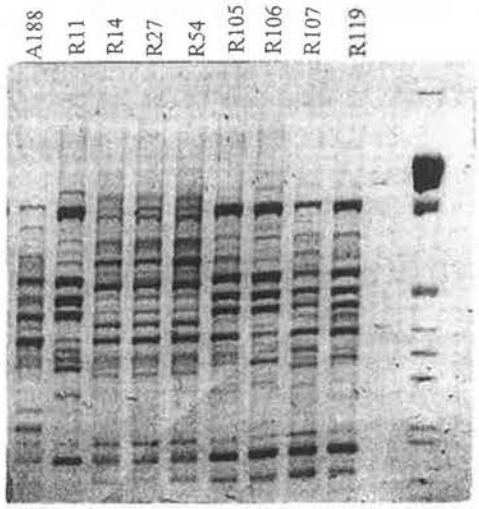


Figure 4. Group-specific RAPD profiles generated by primer B-5.

high level of inbreeding. At the same time multiplicity qualitative and quantitative differences were found between RAPD profiles A188 and somaclones. One type of the primers, for example QR-1, QR-3, B-2 and B-4, generated an individual spectrum of markers for each somaclone (Fig. 2). All genotypes examined, including A188, could be distinguished on the basis of their RAPD profiles with these primers. RAPD profiles produced by other primers (QR-2, QR-4, B-1, 10, 11, 318 and 340) included both polymorphic and monomorphic bands. The primers of the third type (QR-5, B-3, B-5, B-6 and 450) generated common markers for the somaclones of one group. For example, the RAPD spectrum of clones R11, R14, R27 and R54 produced using the primer QR-5 contained a band of about 870 bases, which was absent in RAPD profiles of A188 and other somaclones. The clones R14, R27 and R54 displayed three specific light amplification products with this primer (Fig. 3). Both groups of somaclones could be distinguished one from the other and from the line A188 with the primer B-5 (Fig. 4). The similarity of RAPD profiles of regenerated plants inside one group seems to be conditioned by common origin .

These results demonstrate that the RAPD technique can be applied for elucidation of genetic polymorphism of regenerated plants.

MOSCOW, RUSSIA
Institute of Agricultural Biotechnology

A putative transposed K-box homolog of *zmm1* and *zag2*

--Ermishev, VY, Karyagina, AS, Khavkin, EE, Naroditskii, BS, Zabrodina, MV

Previously we reported (MNL 73: 70, 1999) that the direct amplification of genomic DNA with degenerate primers recognizing the K-box sequence of the MADS-box genes produced DNA bands similar in size to the product of cDNA amplification. A 190-bp amplicon belonging to the *agamous* class was cloned from the A188 inbred, sequenced, and compared to maize *agamous* genes using the program BLAST 2.0.9 (Altschul et al., Nucl. Acids Res. 25: 3389, 1997). The nucleotide sequence of this K-box fragment was 90-94% identical to that of *zmm1* (nucleotides 3693-3758 and 4213-4332) and *zag2* (nucleotides 4760-4825 and 5269-5321).

Several sequences corresponding to the MADS-box motif (exon 1) were previously related to mobile elements (Fisher et al., Nucl. Acids Res. 23: 1901, 1995; Mena et al., Plant J. 8: 845–854, 1995; Montag et al., Nucl. Acids Res. 23: 2168, 1995). Following Fischer et al., 1995, we tentatively define the reported amplicon as a transposed K-box element of *Zea mays* (*tkz1*).

The putative transposed element comprises most of exons 3 and 5, a very short stretch of intron 3, and a longer fragment of intron 4 (Figure 1). The nucleotide and deduced amino acid sequences of exon 3 in *tkz1* are 90 and 95% identical to those of *zmm1* and *zag2*. Exon 5 contains a two-nucleotide insert; as a result, the corresponding identities of the nucleotide and amino acid sequences are 90 and 50%. A 6-bp stretch of intron 3 adjoining exon 3 is completely identical to the corresponding sequences in *zmm1* and *zag2*. The sequence downstream is 87% identical to intron 4 of *zmm1*, with a 17-bp stretch completely identical to nucleotides 4213-4229 of *zmm1* and a 12–bp stretch bounding exon 5, which is completely identical to nucleotides 4281-4292 in *zmm1* and to nucleotides 5269-5280 in *zag2*.

```
tkz1   1 ctaccagcaggaatcaccaaagctgcgcaaccagatccagatgctgcaaa-caactaacag gtagag  66
zmm1 3693 *********a*****cgt***a****************************a**-************
3758
zag2 4760 *********a******g****a****************************a**-************
4825

tkz1   67 ttctgatgtaagtaacttagagttcgtgtaggctaggttgtttatttgtcagactctaattgattgaa  136
zmm1 4213 ****************at*****t***at*************************g****c*****
4280

tkz1  137 ttcttgtttcagagtgagctgctgtctgctgagattgc-ttacatggcaaaaag  190
zmm1 4281 ***********************g****c*****--*a*********_*****  4332
zag2 5269 ***********************g****g*****--*g*********_*****  5321
```

Figure 1. Alignment of *tkz1* to the corresponding sequences of *zmm1* and *zag2* (GenBank accession numbers X81199 and X80206). Exons 3 and 5 are in bold. Identical residues are indicated by asterisks.

NEWARK, DELAWARE
DuPont Ag products - Genomics
JOHNSTON, IOWA
Pioneer Hi-Bred International, Inc

Single nucleotide polymorphisms (SNPs) in corn: early lessons

--Bhattramakki D, Ching A, Dolan M, Register J, Tingey S, Rafalski A

Single nucleotide polymorphisms (SNPs, pronounced as SNIPs) are the most common form of DNA polymorphisms that can be found in any genome. In crop plants like maize, they can be put to various uses like germplasm finger printing, marker assisted back cross conversion, and marker assisted breeding. SNPs are highly amenable to automation and can potentially be used to create a very high-density genetic map. Some of the SNPs in the coding region (cSNPs) may have functional significance in its correlation with altered phenotype. SNPs are viewed as the next generation of molecular markers that would either complement or replace the existing markers that are routinely used in many laboratories.

Previously we conducted a pilot study and demonstrated the high frequency of single nucleotide polymorphisms and their possible uses as genetic markers for corn. In an effort to isolate a large number of SNPs from maize, eight genotypes representing more than 90% of allelic diversity within the test population that were considered for pilot study, were further selected. A 'resequencing' strategy of isolation of SNPs was adopted and the sequences in the DuPont/Pioneer EST database served as initial templates for primer design. The primer pairs designed from 3'untranslated regions of the genes of interest were amplified from the genomic DNA extracted from the 8 preselected genotypes. The PCR products (an average length of 350 bases) were sequenced using dideoxy terminator chemistry and their sequences aligned to identify the polymorphic sites. The Phred/Phrap/Consed suite of software was custom modified so that the SNPs and small insertion/deletions (indels) are tagged and a file of polymorphic sites is generated. At this context, it needs to be borne in mind that the term "SNP" is being used very loosely here; polymorphisms involving more than just single nucleotides are identified during the process, this included indels as stated above, and also some novel 'Miniature Inverted Repeat Transposable Elements (MITES)'.

To date we have designed primers from more than 700 genes/ESTs of interest. PCR, sequencing, scoring of SNPs and cataloguing was finished for 530 loci, from which we identified variants in 311 loci. The remaining 219 loci were either monomorphic, primers failed to amplify, or the majority of the genotypes did not sequence well. A total of 1655 polymorphic sites were identified within the 311 loci, spanning 107,606 base regions of the maize genome, and catalogued. Indels accounted for 27 percent of all the total variations observed and the rest were comprised of transitions and transversions (SNPs), transitions being 25 percent more than expected. An average of 5.3 variants was detected per locus, although there were hot spots of mutations in many loci. Our results from this large-scale study confirmed the high rate of SNP polymorphism (1/80 bp) and the high rate of indels (1/240 bp). Out of 311 loci for which we have SNP information, 164 of them could be easily mapped due to the fact that they are also polymorphic between the parents (B73 and MO17) of a recombinant inbred mapping population. 41 percent of 168 loci also result in length difference that could be visualized on a polyacrylamide gel to generate mapping data. An example of haplotypes found in one of the loci is depicted in Table1.

Table 1: The major haplotypes found in the region spanning intronIV of Globulin1-S locus among the 8 genotypes analyzed for SNP.

Haplotype	Genotype Name/No.	Polymorphic base position 57	130 Exon IV	165	232	236 Intron IV	274	374 Exon V
H1	3	T	T	A	T	T	ND	C
	4	T	T	A	T	T	ND	C
	6	T	T	A	T	T	ND	C
	8	T	T	A	T	T	ND	C
	B73	T	T	A	T	T	ND	C
H2	MO17	C	T	A	C	C	D	C
	7	C	T	A	C	C	D	C
H3	5	C	C	T	T	T	D	T

Notes: 1. Sequence length covered: 403 bp. 2. Only the polymorphic positions are shown. 3. ND = No deletion

Apart from adding SNPs from additional loci, we are also evaluating high-density high throughput SNP genotyping using different approaches available commercially. SNPs will allow higher throughput; low cost multiplexed genotyping for molecular breeding, genetic diagnostics and research applications.

NORMAL, ILLINOIS
Illinois State University

A survey of *ig* containing materials

--Schneerman, MC, Charbonneau, M, Weber, DF

The indeterminant gametophyte (*ig*) mutation was recognized by Kermicle (1969, Science 166, 1422-1424) to produce haploids with a frequency of nearly 3%. This mutation can be used to produce paternal haploids whose chromosomes then can be doubled. These plants are diploid and homozygous at all loci and contain the cytoplasm of the female parent. This mutation therefore has been recognized as potentially useful for placing a given nuclear germplasm in a different cytoplasm. This is of interest to the seed industry because it would make it possible to place a nuclear genome in a different cytoplasm in far fewer generations than by using conventional backcrossing.

In an effort to identify the efficacy of this mutation, several stocks containing *ig* in different genetic backgrounds were grown and crossed as female parents by an unrelated glossy (*gl*) tester with normal cytoplasm (Table 1). Progeny were planted in the sandbench and individual *gl* plants were identified to determine the frequency of androgenic plants produced by each genotype. To verify that the *gl* plants were indeed haploids, root tips were harvested and the ploidy level of each plant determined cytologically by counting the metaphase chromosomes of at least 3-5 cells. Several androgenic diploids (10.8%) were also recovered in this study, a result consistent with Kermicle's observations (1974, p. 137 in Proc. First Intnl. Symp. on Haploids in Higher Plants, ed. K. Kasha, Guelph, Canada).

The materials from the Stock Center that were originally provided by B. Kindiger which should have possessed two normal chromosome 3's with *ig* and a B3-Ld with Ig did not produce the expected plant types in our hands. Every one of these stocks produced large numbers of small plants that had a distinctive abnormal phenotype that was different from the phenotype of haploids.

Table 1.

Source/Stock	Total # grown	Total *gl* identified	Total # Androgenic Haploids	Total # Paternal Diploids	% Androgenic Haploids/ Diploids produced
B. Kindiger (several cytoplasms) provided by the Stock center	5331	24	20	4	0.45%
J. Laughnan (various lines & cytoplasms), provided by the Stock center	6503	6	6	0	0.09%
Originally from Bob Brawn (Funks) N cytoplasm; D. Weber's collection	3482	13	12	1	0.09%
Originally from Bob Brawn (Funks) C cytoplasm; D. Weber's collection	2234	8	6	1	0.37%
Originally from David Alvey (Indiana); D. Weber's collection	1553	26	26	0	1.67%
Originally from Jerry Kermicle, U of Wis.;D. Weber's collection	936	5	3	2	0.53%
Originally from Bob Brawn (Funks) C cytoplasm; D. Weber's collection	2120	25	24	1	1.18%
Jerry Kermicle; U of Wisconsin	1739	8	8	0	0.46%
Jerry Kermicle; U of Wisconsin	1456	1	1	0	0.06%
Originally from Bob Brawn (Funks) N cytoplasm; D. Weber's collection	1013	4	2	2	0.39%

This abnormal phenotype was the same as plants that had lost much of the long arm of chromosome 3 or all of this chromosome (hypoploid for 3L or monosomic for chromosome 3). Furthermore, these exceptional plants were examined cytologically and were found to contain 20 chromosomes. From these and other observations, it appears that the complete B-A translocation was present in these stocks and that the chromosome constitution was not as described by Kindiger and Hamann (1993, Crop Sci. 33:342-344). Also, the frequency of haploids produced by these stocks was much lower than reported by Kindiger and Hamann (1993). The materials provided by J. Laughnan (Stock Center) were also difficult to discern. The fact that the plants were not definitely male sterile and did not produce haploids in the expected frequency suggested that *ig* was segregating, or a restorer gene was segregating. Certain plants of the two stocks originally provided by R. Brawn and D. Alvey that had been maintained for over 25 years in our culture collection did produce higher frequencies of paternal haploids than the other stocks, 1.67% and 1.18% respectively. The reason for this is unknown. The materials provided by Jerry Kermicle produced paternal haploids but at a lower frequency than expected. This survey has allowed us to determine which *ig* containing materials produce paternal haploids with the highest frequency and also suggests that genetic background is important.

NOVOSIBIRSK, RUSSIA
Institute of Cytology and Genetics, Russian Academy of Sciences
WOODWARD, OKLAHOMA
Southern Plains Range Research Station, USDA-ARS

The genetic programs of nonreduction and parthenogenesis in corn-gamagrass hybrids are inherited and expressed in an independent manner

--Sokolov, VA, Dewald, CL, Khatypova, IV

About 40 years ago Prof. D. F. Petrov proposed the hypothesis of the digenic control of apomixis in gamagrass: one gene is needed for the control of nonreduction and formation of the diploid egg-cell; the other for its parthenogenetic development to be realized (Petrov et al., In: Apomixis and its role in evolution and breeding, New Delhi, India, 9-73, 1984). The discussion about these constituents of reproduction through asexual seeds - Petrov called them elements - continues up to now (it is excellently set forth in a report by Andrea Mazzucato, Apomixis News Letter 9, 1997, http: // www. cimmyt. mx). Attempts to find segregants for nonreduction and parthenogenesis in backcrosses of apomicts on sexual forms are also in progress, which may give evidence for their genetic control being independent. We won't consider here all that was done in this direction since these results were discussed repeatedly and deeply (Asker and Jerling, Apomixis in plants, 1992; Nogler, In: Embryology of Angiosperms, 1984; Mogie, The evolution of asexual reproduction in plants, 1992). We'll note however, that proceeding from what is known now about the biology of reproduction and kernel development, two genes are an unacceptable simplification (Carman, Biol. J. Linnean Soc., 1997; Sokolov et al., Proc. Acad. Sci. (Russia), 347(5) : 714-717, 1996; Blakey et al., Genome, in press). We would remind you that besides apomeiosis and parthenogenesis their actions are strongly modified: 1) epigenetically (imprinting, paramutations); 2) by telomeres. As our knowledge is being accumulated other factors influencing apomictic development will undoubtedly be discovered.

It is quite obvious that in species with different types of reproduction through asexual seeds contributions of these factors are not the same. For that reason apparently it's more correct to discuss experimental results only in application to the object on which they were obtained in order that these might not be exaggerated more than the method used allows.

The present report is the result of studying the offspring of corn-gamagrass hybrids with different ratios of parental genomes (the pedigrees were published earlier in Sokolov et al., Russian Genetics 34 : 499-506, 1998), produced from backcrossing F1 (2n=56; 20Zm + 36Td) by corn and very rarely spontaneously obtained hybrids with doubled genomes: 1) 2n = 2 x 39 (30Zm + 9Td) = 78; 2) 2n = 2 x 38 (20Zm + 18Td) = 76.

Also, new crosses of corn with gamagrass were made and F1 hybrids were obtained that were backcrossed by corn with the purpose of analysing for segregation between nonreduction and parthenogenesis in gamagrass by family. Twenty three BC1 families were studied in all.

In Table 1 the results of segregation among the offspring of the corn-gamagrass hybrids are cited. From the data presented it's quite evident that the number of sexual offspring (B_{III} + B_{II}) increases with an increase in the corn portion of the genome of the hybrids (the sexual parent) relative to the number of genomes introduced into them by gamagrass (the apomictic parent). It need

be noted that the effect of the increase in the number of corn chromosomes on suppression of nonreduction (B_{II}-hybrids) and parthenogenesis (B_{III} + B_{II}- hybrids) is not the same. As we can see, parthenogenetic development may not be realized even with a 1:1 ratio of the genomes (F_1 hybrids).

Table 1. Segregation in the offspring of corn-gamagrass hybrids with different ploidy levels for apomeiosis and parthenogenesis traits.

Hybrids	Offspring type			
	Number of apomicts	Number of B_{III}-hybrids	Number of B_{II}-hybrids	Total
F_1 2n=56 (20Zm + 36Td)	98	7	0	105
BC 2n=38 (20Zm + 18Td)	177	4	0	181
BC 2n=39 (30Zm + 9Td)	132	13	0	145
BC 2n=40*	105	6	0	111
BC 2n=59 (50Zm + 9Td)	21	13	2	36
BC 2n=60**	102	54	5	161

* The genome of this line has one unusual chromosome 6 from corn carrying an extra NOR on the long arm in addition to the regular NOR on the short arm. It has been previously reported as a Mz -Tr translocation but as the analysis of spacer regions was not made such an affirmation is unproved (Kindiger, B et al., Genome 39 : 1133-1141, 1996). Besides, the line carries two different size telocentric chromosomes. The line is derived from a 39-chromosome line (30Zm + 9Td), so phenotypically and by its hybridological behavior it is close to the hybrids having this genome.

**B_{III}-hybrid produced from pollination of the 40-chromosome line with tetraploid corn.

The development without fertilization of egg-cells may not be realized even in lines with a 2 : 2 ratio of the genomes (F_1 hybrids) and we observed this in 7 cases among 105 plants. Such a proportion (about 10%) of B_{III} offspring holds in the hybrid lines up to a 5Zm : 0.5Td ratio of the genomes when their number sharply increases to 35%. For a significant increase in a proportion of egg - cells with a reduced chromosome complement (B_{II}-hybrids) a tenfold difference in a ratio of the parental genomes is needed but even in that case their number is by an order less than that of fertilized unreduced egg- celles(B_{III}-hybrids).

These results suggest the independent penetrance of the two constituents of apomixis as well as a difference in the number and quality of genes involved in their control. The apomeiotic constituent presented in Table 1 is programmed sufficiently rigidly and realized as dominant even with a multifold difference in the number of the genomes in favour of the sexual parent. At the same time parthenogenesis exhibits incomplete penetrance even in F_1 hybrids and further is highly labile and decreases inversely to increases in the ratio of corn genomes to gamagrass genomes.

A small sampling of results is presented in Table 2. The ability to obtain offspring from these hybrids is complicated enough by reason of their very high female sterility. High-productive tillering is characteristic of both, so we pollinated about 700 flowers of the 78 chromosome plants with tetraploid and diploid maize pollen and obtained 34 very shrunken kernels. All these were from pollination with the commercial hybrid ICI (2n=20). In total only 15 of them gave us plants. The results concerning the second plant were taken from the work of our laboratory published earlier (Yudin and Lukina, Proc. Acad. Sci. (Russia), : 273,#5, 1246-1248,1983). These hybrids were also actively pollinated and set 7 kernels from pollination with hexaploid corn (2n=6x=60).

It's noticeable that 14 plants from the 15 obtained from the 78-chromosome form, turned out to be dihaploids and the other was a B_{II}-hybrid., Unlike the 39-chromosome apomicts, which were always sufficiently homogeneous morphologically, the plants in question markedly differed from one another both in tillering degree (1 to 8) and in number of ears, character of their placement and development. In the given family we observed many off-types noticed earlier as being rare autosegregants in the 39-chromosome lines. It is possible that this is a consequence of epigenetic marking realized under the meiotic development of egg- cells.

Table 2. Segregation in the offspring of the doubled corn-gamagrass hybrids.

Hybrids	Offspring type			
	Apomicts	B_{III}-hybrids	B_{II}-hybrids	Total
BC 2n=78; 2 (30Zm + 9Td)	0	14	1	15
BC 2n=76; 2 (20Zm + 18Td)	7	0	0	7

We'll especially stress that isogeneity under the doubling of the small gamagrass complement in the 78-chromosome plants leads to the normal proceeding of meiosis and formation of reduced egg- cells which develop in the main parthenogenetically and we observe "dihaploid" offspring. This is affirmation of independent penetrance of hereditary structures responsible for apomeiosis and parthenogenesis as in the given case the latter is realized not after nonreduction but in an inverse variant after meiosis.

The second plant in Table 2 (the 76-chromosomes) produced only apomictic offspring. When comparing its hybridological behaviour with the preceding case one may suppose that the action of two haploid complements of gamagrass chromosomes differs from that in gamagrass with twice the complement of 9. Perhaps the effect of 60 corn chromosomes is also stronger in the 78-chromosome plants than the 40 in the 76-chromosome plants.

And finally the data presented in Table 3 generalize the results from backcrossing a 46-chromosome F1 hybrid by diploid corn. Most BC1 families (15 of 23) proved to be apomicts, that is, like the mother plant, they had 46 chromosomes. Another group (7 families) were represented mainly by apomictic offspring but in addition they had 1 to 3 B_{III}-hybrids. In that case, as well as in those considered before, parthenogenesis does not have 100% penetrance, though the ratio of the genomes 1 : 2 is in favour of the apomictic parent.

Table 3. Segregation in gamagrass (2n=72) for parthenogenesis trait.

Family	BC_1 plant ploidy		
	Apomicts	B_{III}-hybrids	Total
57	13	0	13
92	11	0	11
112	12	0	12
188	8	0	8
190	11	0	11
236	11	0	11
251	9	0	9
289	9	0	9
300	13	0	13
334	14	0	14
355	14	0	14
365	13	0	13
392	8	0	8
415	15	0	15
484	8	0	8
Total: 15	169	0	169
45	15	1	16
46	17	1	18
77	12	1	13
79	12	3	15
175	9	1	10
302	11	1	12
383	12	1	13
Total: 7	88	9	97
363	0	15	15
Total: 1	0	15	15

One family (363) was represented by B_{III}-hybrids only, i. e. parthenogenesis is absent from this form.

All the results present evidence for the independence of the control of apomeiosis and parthenogenesis and for the possibility of segregation in gamagrass. Besides, based on these results, one may suppose that the number of genes controlling development without fertilization exceeds the number of genes controlling nonreduction and their penetrance depends on many genotypical circumstances and external factors.

The authors express deep appreciation to Dr. J. G. Carman for fruitful discussion. The research was supported by the Netherlands Organization for Scientific Research Grant No. 047.007.019 and Russian Foundation of Basic Research Grant No. 97-04-49301.

PASCANI, REPUBLIC OF MOLDOVA
Maize and Sorghum Research Institute

Differences in sensitivity to herbicide of maize lines obtained under disruptive selection for reversion frequency of the mutable allele *o2-m(r)*

--Koterniak VV

Earlier (MNL, 73: 76-79) we reported about the lines obtained under disruptive selection (started in F5 progeny of one ear) for low and high content of whole endosperm revertants (WER). These lines were designated as LFWER and HFWER respectively. The frequency of WER formation is conditioned by the frequency of reversion of the mutable allele *o2-m(r)* (as a result of excision of the receptor element *rbg* from the *opaque2* (*o2*) locus in the presence of the regulatory element *Bg*) which takes place before the first meiotic division of the primary endosperm nucleus. We established that differences in WER content in lines obtained are determined by changes in state of the initial receptive allele *o2-m(r):3449* and the regulatory element *Bg-3449*. Changed forms of the receptive alleles and the regulatory elements presented in LFWER and HFWER lines were respectively designated as *o2-lf, Bg-lf* and *o2-hf, Bg-hf*. Lines obtained differed not only in WER content but also in some quantitative traits (MNL, 73: 76-79).

In 1999 we observed significant differences between LFWER and HFWER lines in their sensitivity to herbicide "Buctril D", Rhone Poulenc Inc., (22.5% of bromoxinil + 22.5% of 2,4-DMA) applied for weed control in the concentration of 0.56 kg/ha of the active substance at the plant stage of 3-5 leaves. Lines obtained under selection for low WER content (LFWER lines) did not show visible symptoms of sensitivity to the herbicide. In contrast with this, HFWER lines were characterized by high sensitivity to the herbicide expressed in strong leaf twisting (Fig. 1).

Stronger sensitivity of the HFWER lines to the herbicide was quite unexpected since the plants of these lines in comparison with the plants of LFWER lines are more vigorous, have higher kernel weight, kernel volume and number of leaves on the main stalk (MNL, 73: 76-79).

Sensitivity to herbicide of HFWER lines is not connected directly with the frequency of reversion of the mutable allele *o2-hf*. Either the "standard" HFWER lines or the *o2-hf; Bg-hf* genotype and their *o2-hf*; $+^{Bg}$ derivatives lacking the regulatory element *Bg* with the typical *o2* endosperm appearance and the absence of WER both were sensitive to herbicide. The same sensitivity to herbicide was observed in HFWER sublines with WER frequency close to that of LFWER lines obtained as a result of reverse selection for WER content. (The reverse selection, i. e. selection for low WER content at HFWER lines and high WER content at LFWER lines, was effective for the former and ineffective for the latter, suggesting deletion changes in the *o2-lf* allele and indicating that the change in state which led to formation of this allele was caused most likely by internal deletion of the *rbg* element (Maydica, 1999, in press)).

Figure 1. Herbicide sensitivity (expressed in leaf twisting) of the lines obtained under selection for high frequency ("HF", left row) and low frequency ("LF", right row) of whole endosperm revertants.

It seems that differences in sensitivity to herbicide between LFWER and HFWER lines are not linked with the possible differences in their developmental stages. Though LFWER lines are earlier in comparison with HFWER lines by 1-3 days (MNL, 73: 76-79). The big majority of the plants of these lines were in the same developmental stage as the plants of HFWER lines. Thus 79% (34 out of 43) of the plants of LFWER families had the same date of flowering of male inflorescences as the plants of HFWER families (indicated data were obtained on the families of LFWER and HFWER lines used in the reverse selection program). Notwithstanding this, the plants of LFWER lines did not show visible reaction to herbicide.

Since LFWER and HFWER lines were obtained under disruptive selection for WER content and are characterized by the different states of the *Bg-rbg* system components (which are responsible for WER content) we can suggest that differences between these lines in herbicide sensitivity (as well as differences in quantitative traits) are also connected either with disruptive selection for WER content or with the states of the *Bg* and *rbg* elements. In case of insertions of the *rbg* or *Bg* in the genes determining these traits (e. g. insertion in the gene(s) responsible for herbicide resistance) the activity of such genes can be changed and be dependent on the activity of the components of the *Bg-rbg* system of transposable elements. If the insertions of *rbg* or *Bg* elements in the mentioned genes were presented in the initial source of instability before the disruptive selection was started we can infer that later these elements could undergo spontaneous changes in state (as a result of internal deletions, intragenic transposition etc.) which could be picked up by the following disruptive selection. Such changes in state could have not only spontaneous character but be induced by the *rbg* or *Bg* elements responsible for low and high WER content in these lines (i. e. by the

rbg elements presented in *o2-lf* and *o2-hf* alleles and by the *Bg-lf* and *Bg-hf* elements). This kind of interaction can be assumed taking into account the paper of Cuypers et al. (EMBO J., 1988, 7: 2953-2960) in which it was established that a defective *En-I102* element reduced the excision frequency of both the autonomous *En-1* element and the inhibitor element *Spm-I5719A*.

Bg and *rbg* elements could affect the activity of the genes responsible for the herbicide resistance and quantitative traits also through their insertions in such genes arising as a result of transpositions of the receptor elements from the *o2-lf* and o2-*hf* alleles and transpositions of the regulatory elements *Bg-lf* and *Bg-hf*. Because of differences of these *Bg*s and *rbg*s (due to their changes in state) we can expect the differences in activity of the genes in which they are inserted.

RALEIGH, NORTH CAROLINA
North Carolina State University

A remarkable new teosinte from Nicaragua: Growth and treatment of progeny

--Bird, RMcK

On 7 November 1989, Allan J. Hruska, working for CARE (the relief agency), collected a sample of teosinte seed in northwest coastal Nicaragua near Honduras. Two years later Hugh Iltis, Bruce Benz and Alfredo Grijalva found and collected the teosinte at a ranch, Apacunca, in the department of Chinandega. The site is peculiar for teosinte, only 10 m elevation and frequently flooded during a 6-month rainy season. On a return visit, Iltis and two Nicaraguan associates collected large seed samples (Iltis 30919) at the Rancho Apacunca locality. This seed is available at the Maize Germplasm Bank of the International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico, accessioned as CIMMYT No. 11083 and at the United States Department of Agriculture's North Central Regional Plant Introduction Station (NC7) at Ames, Iowa (accession Ames 21893).

John Doebley planted seed of the Hruska collection in a growth chamber, observing:
"Plants produced mature seed four months after planting, this much more rapidly than is typical of this species [*Zea luxurians*]. This collection, clearly differentiated from the Guatemalan form of this species by its small stature and early maturity, may represent a new subspecies." (label on specimen Doebley No. 648).

I saw plants of the CIMMYT accession being grown for seed replication in the field station at Tlaltizapán, Morelos (940 masl), in early 1995 (planted in July, 1994). They were profusely tillered, standing about 1.6 m tall, and were flowering. I asked Dr. Suketoshi Taba to bring a plant to Texcoco (near Mexico City, 2255 m) after the seed were harvested, on the suspicion that it would continue growing. This done, the plant was cut into three parts, each of which grew well, one flowering twice more. The 1994 clones at El Batán were preparing to flower at the end of 1996 when killed by frost.

Shoot morphology: After soaking the fruit cases (rachis segments) in diluted pharmacy H_2O_2 (20%) for over an hour, I planted original and self-pollinated seed of CIM 11083 in a screenhouse at CIMMYT's El Batán station in March, 1996, and, on 25 Sep 98, in a North Carolina State University greenhouse in Raleigh, NC. For months the two stands grew similarly, but there was segregation of some interesting traits (Table 1). In Raleigh, the four plants started growing tillers four weeks after planting, at the lowest nodes of the main stem, and then at the lower nodes of larger primary tillers. Tillers eventually totaled 22-31, up to 15 being primary (on the main stem).

A week later, starting a new phase of juvenile Stage 1, about half the plants were prostrating themselves on the ground (for Plant 1, main stem at 20° from the ground, tillers at 0°), growing like maize with the *lazy* gene, while the rest relaxed to 50°-70°. By this time there were 5 leaves with auricles showing. At this point, I commenced numbering every fifth leaf on the main stem and the largest tiller.

At the start of Stage 2, 15 weeks after planting (8 Jan 99), the stems of all plants were growing more upright, including the 15-28 tillers. Main stems had 17-21 leaves. The main stems were inclined at 30°-80°, tillers at 40°-60°. A few more tillers were produced before 14 Feb, none thereafter. Perhaps the pots were too small, although fertilizer was added occasionally. Stage 2 continued into the autumn for many plants -- lights in nearby greenhouses and then long days prevented flowering. However, in cuttings transplanted to a garden, flowering was induced by artificially lengthening nights with black plastic covers (below).

As plants have matured, removal of old leaf sheaths has revealed very short internodes and masses of secondary prop roots. The approximate distances from node 1 to node 20 of the main stems of the original four plants are 3.5 cm, 7.5 cm, 11.5 cm and 10.2 cm. I recommend measuring internodes as soon as possible, before the secondary roots grow. Average internode lengths in the interval between nodes 20 and 25 of the four plants are 1.0 cm, 1.4 cm, 1.2 cm and 1.7 cm (the two shorter are the two more prostrate plants in Stage 1). Higher internodes are longer.

It has been easy to cut tillers and root them. Three were taken from Plant 1 on 29 Dec 98, and three were cut from each of the 4 original plants on 18 Jan 99. Those that were about 1 cm in diameter did well, better if a bit of root had emerged. The propagated plants grew much like the originals, but those cut after December did not recline as much, UNLESS they were transplanted outdoors (16 Apr) where angles were 40° to 60° less than the equivalents indoors (Table 1). The three cut in December from Plant 1 were lying flat or nearly so (0°-20°) by 9 Feb, and remained nearly prostrate through 21 Apr, even though they were treated in varying ways (below)! Those of Plant 3 reclined at an intermediate angle. Two cuttings made on 3 June have not tillered. Perhaps cuttings were imprinted by the stage they were in when cut, but crowding of plants, root binding and temperature seem to have had major effects. One January cutting of each original was transplanted to my backyard on 16 Apr, where those of Plants 1, 3 and 4 eventually produced 28-34 tillers and enormous plants; the Plant 2 cutting has remained smaller, eventually becoming shaded by the others.

Leaf counts show that cuttings reflect their source plants in relative numbers of leaves grown per month; Plants 2 and 4 had fewer leaves by 22 June than Plants 1 and 3. New leaves emerged in intervals as short as four days.

Leaves of this teosinte bear small (0.4 mm long) hairs visible with a loupe, but only on the upper surface of the blade, not the sheath or auricle. Leaf blade margins have sharp, apically directed barbs 0.06 mm long which can cut skin. When sheaths are rubbed upward, one feels (especially with the lips) a slight, downwardly directed roughness. Shapes of blades will be discussed.

A flooding experiment: Because of this ability to replicate

Table 1. Measurements of four original and 11 propagated plants of Nicaraguan teosinte. The seed source is CIMMYT's accession CIM 11083 (=Ilis 30919). The numbers of leaves are for those with auricles showing. Identifiers for cuttings (Cut.) include the number of the source plant, one unique letter if cut from the source plant, and two letters if cut from a cutting. Trt.: treatment; IN: internode; GH: greenhouse; FL: flooded in greenhouse; BY: backyard; NL: nights lengthened in backyard.

Identification	Trt.	Date When Cut	Tiller Angles June	No. of Tillers 16 Aug	No. of Leaves 22 Jun[1]	No. of Leaves 20 Sep	Length Leaf (mm) No. 24-26	Width Leaf (mm) No. 24-26
Plant 1	GH	---	80	25	39	49	86	45
Plant 2	GH	---	80	23	35	41	74	50
Plant 3	GH	---	55	31	34	42	83	47
Plant 4	GH	---	80	22	32	39	98	58
Cut. 1a	GH	29 Dec	60	16	31	42	79	43
Cut. 2b	GH	18 Jan	60	6	20	31		
Cut. 3b	GH	18 Jan	40	4	22	35		
Cut. 4b	GH	18 Jan	65	2	20	32		
Cut. 1c	GH-FL	29 Dec	65	5	31	46	80	45
Cut. 1d	BY	18 Jan	-5	34	26	45	92	47
Cut. 2a	BY	18 Jan	25	8	19	31		
Cut. 3a	BY	18 Jan	0	28	23	42		
Cut. 4a	BY	18 Jan	25	29	20	35		
Cut. 1f	BY-NL	18 Jan	60	12	25	45	87	46
Cut. 3aa	BY-NL	3 Jun	---	0	ca. 3	25*		

1 Node and leaf numbers for cuttings are counted as the number which have emerged since cutting.
* For Cut. 3aa, the final leaf number - started tasseling on 31 Aug.

plants of Nicaraguan teosinte, it has been possible to run several experiments on the "same" plant. Cuttings of Plant 1 ended up: (a) in a tank of water, (b) in 15 cm and 25 cm pots in the greenhouse, and in my backyard, (c) with and (d) without night-lengthening.

Plants in Chinandega, Nicaragua had been observed growing up to 5 m tall, yet none of the progeny grown in Mexico grew over 2 m tall, so I have been testing a hypothesis that extended flooding of the plants greatly stimulates growth. On 16 Feb one of the December cuttings (Plant 1, Cut. 1c) was placed in a tank of water so that the roots, but not the shoots, were constantly underwater (another plant was submerged 5 cm deeper but suffered badly so after 16 days it was removed). Cut. 1c did not recline as much as twin cuttings. It has grown well in a 25 cm pot. By 5 June it had grown only 7 tillers, while an unflooded twin had 12. On 23 Sep it was growing taller than the controls and other plants, about 1.8 m to the tallest auricles, versus 1.0-1.3 m. It, as the others experiencing short nights, remains in vegetative Stage 2. It continues to grow ever taller than the others.

Cuttings of Plants 2 and 3 (Cut. 2c & 3c), in 15 cm pots, were placed in a separate water tank on 22 Apr, with the roots just under water. These two were lowered 6 cm on 5 June, and even more about a month later. One soon rotted at the base and the other later, but nodal roots have grown and the plants still live, hydroponically. All three plants have grown masses of secondary roots in the water, with small tertiary roots 0.2-0.4 mm in diameter extending upward, either 1/2 cm above the soil surface, or 1.5-2.5 cm towards the water surface. The small roots are dense, about 40 per 3 cm of secondary root. A bluish green alga has been found growing over the roots of the cutting from Plant 1, possibly affecting its growth.

Photoperiod adjustment: In North Carolina, short summer nights and extraneous greenhouse light in the winter has prevented flowering, so artificial night-lengthening was applied to cuttings of Plants 1 (cut on 18 Jan) and 3 (cut on 3 June). Three of an additional four plants grown from seed from Nicaraguan teosinte plants pollinated in Mexico (planted 16 Apr 99) were also treated, as were plants of *Z. luxurians* and of *Z. mays* ssp. *huehuetenangensis*. Frames 4 ft. high were covered in black plastic, 4 mil thick. These were placed over the plants for 21 nights (17 July-7 Aug), 13 hours each night. By 13 Aug it was obvious that changes had occurred -- emerging leaves had a different shape (below).

A tassel appeared on Cutting 1f on 19 Aug, silking started on 29 Aug and pollen shed started on 31 Aug. Tassels on Cutting 3aa appeared 6 to 16 days later, but shed much later, slowed by cool weather after 16 Sep or by crowding. Perhaps the older the plant or cutting, the more quickly it can be induced to flower, or being a cutting from a cutting may have affected the later one. Seedlings may be ready to flower later than cuttings made on the planting date. None of the untreated plants showed a sign of flowering by 22 Sep, but by 25 Oct branches were growing from upper nodes and leaves were broadening at the base.

Flowering stage: The third, reproductive stage of shoot development is quite different. The forms of the tassels, ear branches and earlets will be described in a later report. As reproductive structures developed, upright stems, leaves and upper branches changed. An indicator of Stage 3 was the shape of the leaves, broadened at the base and shortened. Broadening started at nodes roughly where the lowest ear branches occurred. Widths at 5 cm from the ligule, for leaves in the middle of the zone of ear branches, ranged 45-59 mm, lengths ranged 54-70 cm, and maximum widths ranged 49-63 mm at 30-45% of the distance from ligule to tip. Grown outdoors, leaves on untreated plants, still in Stage 2, reached 1.4 m long. Their widths at 5 cm from the ligule were only 15-30 mm. Their maximum width was at more than 1/2 of the ligule-tip distance. On plants at Stage 2 in the greenhouse, leaves were smaller in all dimensions but had the same shape as the outdoor vegetative cuttings.

Discussion: Several trends and much variation have been noted. While taking measurements and notes, many questions occurred to me. Maybe the flooding noted at the Apacunca site ebbs and flows with the size of storms. Would the plants grow taller and better if flooding alternately covered stems for up to 30 cm and uncovered roots to a depth of 5 cm? Might the 5 m height of some plants at the collection site be due to introgression from maize? F1 hybrids of maize with *Z. luxurians* and this teosinte reach that height. What are the youngest age, shortest days and fewest days of treatment which will give consistently good flowering? Does the "quality" of a cutting change over time or over generations of cutting? Do cool temperatures increase the "lazy" effect? In many ways this is a most unusual teosinte.

SAINT PAUL, MINNESOTA
University of Minnesota

Zea diploperennis backcross adapted to U. S. corn belt

--Carlson, LA

Zea mays backcrossed twice to *Zea diploperennis* and selected for blooming in a long day environment creates a perennial strain closer to *Zea diploperennis*.

Pollen was collected from adapted descendants of *Zea diploperennis* x Maize plants (MNL #63, Pages 106 and 107) and backcrossed to *Zea diploperennis* P.I. No. 441931. About 100 plants from the above cross were grown in the greenhouse during the winter of 1995-1996. Two fans were directed on these plants and the few Maize plants for crossing purposes were detasseled.

About 2000 seeds were harvested from these plants. One isolation plot and one breeding plot were planted in the spring of 1996. The isolation plot plants that produced seed were transplanted to the greenhouse in the fall with the seed from each plant identified. Only seed that came from plants that survived the transplantation and grew was planted in 1997.

Another isolation plot was planted in 1998. Seed from these plants was identified and saved and the plants were transplanted into the greenhouse. Again in 1999 an isolation plot was planted U.of M. Experiment Station at Rosemount with seed that came from the perennial plants. The perennial plants from the greenhouse were planted in a separate isolation plot and any that didn't grow in 1999 were identified. Rows of seedlings that descended from plants that didn't grow when replanted in the spring were chopped off when they were about three feet high.

Most of this population bears seed partially extruded from the cupule but some seed is completely enclosed in the fruit case. All plants bear many ears and have numerous tillers. Seed can be obtained from Mark Millard at the Plant Introduction Station at Ames, Iowa or from the Author.

SAINT PAUL, MINNESOTA
University of Minnesota and USDA-ARS

Towards a radiation hybrid map for maize chromosomes

--Kynast, RG, Okagaki, RJ, Odland, WE, Russell, CD, Livingston, SM, Rines, HW, Phillips, RL

Radiation hybrid mapping is one alternative to traditional genetic chromosome mapping. Genetic mapping allocates markers to linkage groups and provides information on the probable sequential order along the chromosomes. Distances between the marker loci are based on the frequency of their meiotic recombination and usually expressed in centiMorgans. Genetic maps are commonly derived by intercrossing genotypes possessing distinct characters of interest and analyzing trait segregations in the offspring. On the other hand, a detailed radiation hybrid map of the human genome is accomplished by using hamster-human somatic cell fusion lines. Human cells are first irradiated to fragment the chromosomes. These cells are then fused with hamster cells. The generated cybrids are propagated under selective conditions to create cybrid lines carrying different fragments of human chromosomes in addition to the entire hamster genome. Cybrid lines with human chromosome fragments are screened for the presence versus absence of markers. Maps may then be created by using the set of cybrid lines as a collection of overlapping deletions and/or translocations. The deletion and translocation breakpoints define the physical segments. Because radiation rather than genetic recombination breaks the linkage between loci, physical segments are generated, which do not exclude recombination-poor areas along the whole chromosome. The distances between markers are estimated by calculating the probability of any pair of markers being separated. The distances are expressed in centiRays. We are developing a radiation hybrid mapping system for maize chromosomes based on oat-maize radiation hybrids generated from a set of oat-maize monosomic chromosome addition lines.

The principle for the production of oat-maize chromosome addition lines has been described previously (Riera-Lizarazu et al., TAG 93: 123-135, 1996). From more than 25,000 crosses of maize cv. Seneca 60 to the oat cvs. Starter, Gaf Park, Preakness, Kanota, Sun II, Stout, and an experimental hybrid (MN97201-1 x MN841801-1), we have rescued about 2000 embryos by in vitro culture on 1/2 MS medium. About 12 % of the F1 embryos germinated, and 175 of them grew to vigorous plantlets which were analyzed by molecular and cytogenetic means. The majority of these F1s were haploid oat plants without maize chromosomes. Approximately 36 % of these F1s were partial hybrids, however, retaining one or more maize chromosomes (Figure 1). We observed a maximum of six retained maize chromosomes in addition to the haploid oat complement in one plant. Most plants had lower numbers of added maize chromosomes (Figure 1). The generally low numbers of retained maize chromosomes among the recovered F1 hybrids indicate a competitive advantage for cells having eliminated maize chromosomes. The specific chromosomes and their allelic constitutions lead to distinct phenotypes.

Figure 2 summarizes the identity of the added maize chromosomes and their frequencies in the F1 hybrid population. A total of 87 maize chromosome additions have been identified to date. In 37 F1 plants a single maize chromosome was retained along with the haploid oat complement. All ten maize chromosomes have been recovered as single monosomic additions. Maize chromosome 9 was most frequent among the single monosomic additions. Chromosome number 5 was slightly less frequent as a single addition, but most frequent in combination with other chromosomes. Apart from maize chromosome number 3, all of the other chromosomes also were found together in combination with other chromosomes added to the haploid oat complement. There was no obvious preferential combination of the maize chromosomes among the multiple

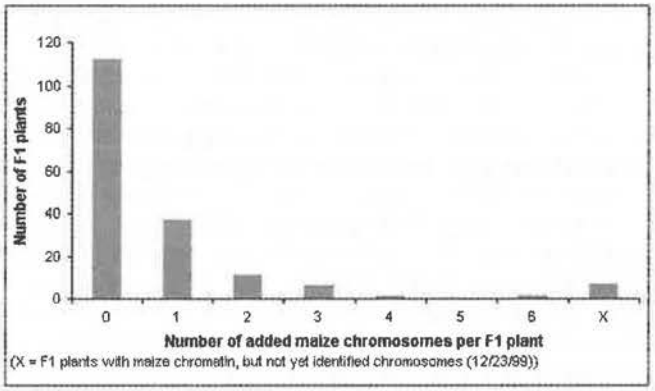


Figure 1: Frequencies of the number of maize chromosomes retained in 175 oat- maize F1 plants.

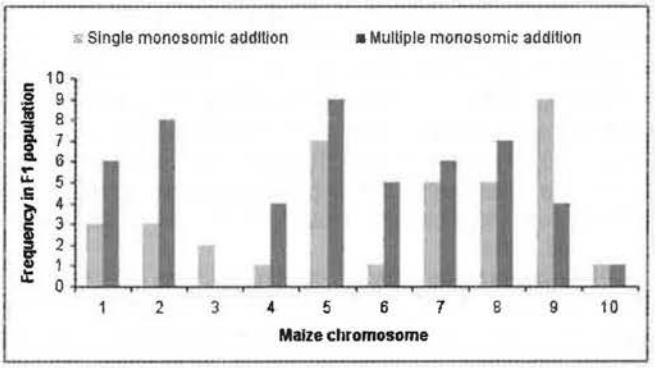


Figure 2: Frequencies of 87 identified maize chromosomes as single and multiple monosomic additions in 56 oat-maize F1 plants.

additions. The double monosomic additions involved the combinations of the maize chromosomes 5 + 9, 5 + 8, 1 + 5, 2 + 6, 1 + 8, 2 + 5, 4 + 7, 5 + 6, 4 + 6, and twice 2 + 7. Triple monosomic additions included the chromosome combinations 6 + 7 + 9, 2 + 7 + 8, 2 + 6 + 9, 2 + 5 + 8, 1 + 4 + 5, and 1 + 5 + 8. In one F1 plant we identified the added maize chromosomes 1 + 2 + 8 + 9. And one F1 plant had the maize chromosomes 1 + 4 + 5 + 7 + 8 + 10. The most important result is that we detected for the first time an entire set of oat-maize chromosome addition plants, which carry singly each of the ten maize chromosomes.

Haploid oats are known to produce up to 40 % seed set (Davis, MS Thesis, University of Minnesota, 1992). As it occasionally occurs with haploids of other allopolyploid and amphidiploid cereals, e.g. wheat and *triticale*, haploid oats can produce doubled haploid or even aneuploid (e.g. monosomic or nullisomic) offspring by completely or partially unreduced gametes. The high seedset frequency in oats, however, is mainly caused by restitution of meiosis I. Meiotic restitution results in unreduced spores, which eventually generate fertile gametes. Our haploid F1 hybrids with or without maize chromosome additions occasionally underwent the same or very similar processes. As a result, hexaploid oat plants with nullisomic, monosomic, or disomic additions of maize chromosomes were formed among the F2 genotypes. We recovered disomic additions for maize chromosomes 2, 3, 4, 6, 7 and 9 and a monosomic addition for maize chromosome 8. Details on this material and its availability are presented in Table 1.

To produce radiation hybrid lines a disomic chromosome 9 addition line was backcrossed to oat to generate a line monosomic for maize chromosome 9. These seed were irradiated, grown to maturity, and allowed to self pollinate. Seed from individual panicles were planted and tested for the presence of maize DNA. Approximately 10% of the progeny carried maize DNA. Lines carrying maize DNA were characterized cytologically and with molecular markers. We have made over 40 radiation hybrid lines for chromosome 9, and are developing radiation hybrid lines for chromosomes 2 and 4.

At present we are working with 38 radiation hybrid lines for chromosome 9. Our first step has been to map markers previously mapped to either the consensus SSR map or the 1998 UMC map.

Table 1. Oat-maize single monosomic and disomic addition lines.

		F1 hybrids			Availability	
Added maize chromosome	Oat background	Before 1999	In 1999	Offspring F2, F3, F4, ...	Seed	DNA
1	Preakness	1		0	None	Plenty
	Starter		1		None	Limited
	Gaf Park		2		None	Limited
2	Starter	4	3	> 100	Plenty	Plenty
3	Sun II	1		> 100	Plenty	Plenty
	Preakness		1	29	None	Limited
	Starter		1	0	None	Limited
4	Starter	5	1	> 100	Plenty	Plenty
5	Starter	1?	3		None	Limited
	Gaf Park		2	0	None	Limited
	Hybrid		2		None	Limited
6	Starter	1	1	> 100	Plenty	Plenty
	Dumont			> 100	Plenty	Plenty
7	Gaf Park	1		> 100	Plenty	Plenty
	Starter		5	> 100	Plenty	Plenty
8	Gaf Park	1	2	> 100*	None	Limited
	Starter	1?	3		None	Limited
9	Starter	2	6	> 100	Plenty	Plenty
	Kanota		1	1	None	Limited
	Gaf Park		2	30	None	Limited
10	Gaf Park		1		None	Limited

* monosomic offspring only

This allows us to look at the distribution of breaks along chromosome 9 and provides a basis for future comparisons between the radiation hybrid map and other chromosome 9 maps. The first 56 markers placed on the map defined 41 segments. Many more markers need to be mapped before we can be confident that the chromosome breaks are adequately defined.

A major effort this year has been to develop new markers. Using EST sequences from ZmDB (http://www.zmdb.iastate.edu/), primers have been designed and tested. To date 385 primers were developed that amplified maize DNA and distinguished maize from oat sequences. These primers have been mapped to chromosome. Using our new oat-maize addition lines a total of 178 EST sequences have been allocated to chromosome 2 (27 ESTs), chromosome 3 (34 ESTs), chromosome 4 (46 ESTs), chromosome 6 (28 ESTs), and chromosome 9 (23 ESTs). Thirty-four EST markers were located to chromosome 9; 11 of these markers were also located on one or more of the other chromosomes tested. These markers are being placed onto the chromosome 9 radiation hybrid panel. 20 ESTs showed loci on more than one chromosome tested. One EST sequence, AI737657, mapped at the *Sh1* locus. A BLAST search revealed that this EST was a *Sh1* sequence.

The data for 385 EST primer sequences, their chromosome allocation and the description of the radiation hybrid lines will soon be available on our website: http://www.agro.agri.umn.edu/rp/genome/

This material is based upon work supported by the National Science Foundation under Grant No. 9872650.

SAN DIEGO, CALIFORNIA
University of California

barren stalk1 is epistatic to teosinte branched1

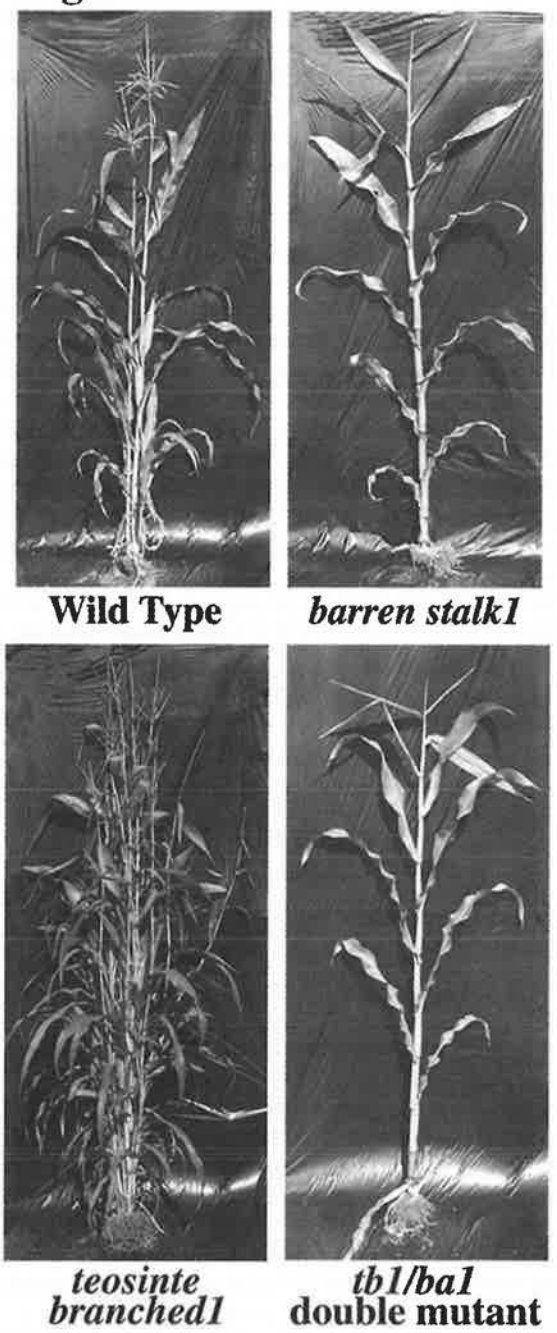
--Ritter, MK, Padilla, CM, Schmidt, RJ

Plants homozygous for the recessive *barren stalk1* (*ba1*) mutation, first described by Hofmeyr in 1930, are characterized by having a tassel which lacks branches and spikelets. These plants also have no ears or tillers. To establish whether the lack of tillers in *ba1* mutants is due to true suppression of vegetative branching or to background effects, a double mutant was generated with *teosinte branched1* (*tb1*), a mutation which causes excess tillering.

Plants heterozygous for the *ba1* mutation were crossed to homozygous *tb1* mutants and the resulting F1 progeny were then selfed. F2 populations were screened to determine which ones segregated for the *ba1* and *tb1* phenotypes. Two hundred and thirty seven F2 progeny were genotyped at the *tb1* locus by Southern blots with a genomic *tb1* probe. (Probe kindly provided by John Doebley). Of these 237 F2 plants, 47 were *tb1* homozygous mutants. Eleven of those 47 *tb1* mutants also showed the *ba1* phenotype. Due to the fact that these *ba1/tb1* double mutant plants shed a small amount of pollen, one of these 11 plants was crossed to a heterozygous *ba1* tester to confirm the presence of the *ba1* allele. Ten progeny were planted from this cross, of which 6 showed the *ba1* phenotype.

The *tb1/ba1* double mutant is indistinguishable from the *ba1* single mutant (Figure 1, page 62). Thus it appears that *ba1* is epistatic to *tb1* and completely suppresses lateral branching in both the vegetative and inflorescence meristems.

Figure 1



SHANGHAI, CHINA
Chinese Academy of Sciences

Genetic diversity and its relationship to hybrid performance and heterosis in maize as revealed by AFLPs and RAPDs

--Wu, M

The recent advances in genome research have generated considerable interest in predicting hybrid performance using molecular markers in crop breeding programs. PCR-based techniques, which include AFLPs and RAPDs, have been proven useful in genetic diversity studies. In the study presented here, AFLPs and RAPDs were employed to study the genetic diversity among seventeen elite inbred lines widely used in hybrid maize breeding programs in China. The relationship between genetic distance and hybrid performance in a diallel set of crosses between them was assessed.

Of the 90 RAPD primers used to amplify DNA from the seventeen lines, 73 (81%) revealed polymorphisms among the seventeen parents. These 73 primers generated a total of 453 non-redundant polymorphic bands with an average of 6.2 and a range of 3 RAPD profiles. Among the 453 RAPD variants, 10 (2.2%) were present in only one of the seventeen parents. For AFLP analysis, sixteen primer combinations were used to assay the seventeen inbreds. These primer combinations revealed approximately 1038 selectively amplified DNA fragments ranging in size from 80 to 900 bp nucleotides. Among 1038 AFLP variants, 621 (59.8%) were polymorphic bands with an average of 38.8 and a range of 30 to 59 per AFLP primer combination, 75 (7.2%) were detected in only one of the seventeen parents. These results suggest that AFLPs can reveal a large number of polymorphisms in a more efficient way compared with RAPDs.

Nei's genetic distance (GDs) were computed for all 136 combinations of the seventeen parents based on AFLP and RAPD markers. GDs based on AFLP data among the seventeen parents ranged from 0.19 to 0.56, with an average of 0.43 across all 136 pairs. GDs based on RAPD data among the seventeen parents ranged from 0.09 to 0.67, with a mean of 0.59 across all 136 pairs. These results indicate that GDs based on AFLP data were significantly different from GDs based on RAPD data used in this study.

Cluster analysis based on the AFLP data and RAPD data resolved the seventeen parental lines into five major groups that were consistent with pedigree information, GD values among groups were significantly greater than that within group. Dendrograms were also constructed on the basis of either 21 AFLP or 453 RAPD variants and compared with the dendrogram generated from the entire data set of AFLPs and RAPDs. The AFLP-based dendrogram had quite similar clustering structure as the dendrogram developed from the RAPD data. The only discrepancy was that 1301 and P167 (1301 and P167 were synthetical-bred inbreds) were in different groups. These results suggest that AFLP and RAPD markers used in this study can assign genotype to different heterotic or subspecific groups.

The correlations of genetic distance with the F1 yield (F1Y)/mid-parent heterosis (MPH)/specific combining ability (SCA) of the 136 hybrids are presented in Table 1.

Table 1. Correlations of genetic distance (GD) based on AFLP and RAPD data respectively, with F1 yield (F1Y)/mid-parent heterosis (MPH)/specific combining ability (SCA) of grain yield for 136 crosses.

Variables	F1Y	MPH	SCA
GD-AFLP	0.4352**	0.3453**	0.4732**
GD-RAPD	0.4018**	0.3247**	0.4217**

**Significant at 0.01 probability level

As shown in Table 1, genetic distances based on AFLP and RAPD data were significantly correlated with F1 yield. The correlation coefficient(r) was 0.4352 for AFLPs and 0.4018 for RAPDs. The correlation coefficients of GDs calculated for AFLP and RAPD data with mid-parent heterosis for 136 hybrids were highly significant. Genetic distance based on AFLP and RAPD data were also correlated with specific combining ability (SCA)($P<0.01$). Finally, it was worth noting that correlation be-

tween GD computed from AFLP data and F1Y/MPH/SCA were higher than those based on RAPD data.

In summary, the results from the current study indicate that AFLP and RAPD offer a reliable and effective means of assessing genetic variation and of assigning maize inbred lines into different heterotic groups and thus reduce the field work associated with making cross and hybrid field testing. In particular, AFLP technique may allow maize breeders to predict combinations of lines that result in high-yielding, single-cross hybrids.

SOFIA, BULGARIA
Institute of Genetics, Bulgarian Academy of Sciences

Studying the possibilities for transfer of regeneration capability in vitro via classical crosses

--Nedev, T, Kruleva, M, Krapchev, B

We have previously reported (MNL 72:76) about good regeneration capability of in vitro culture of inbred maize line A619. The protocol of embryo isolation, media used, and the in vitro tissue culture procedures were published there too. The aim of the present work was to verify the possibility of transferring the trait "good capability" from line A619 to hybrid combinations A619 x M320 or M320 x A619 (A619 as a female and male partner). The results obtained for the two combinations are presented in Table 1.

Table 1. Regeneration frequencies of A619 x M320 or M320 x A619 hybrids.

Genotype	Number of embryos with morphogenesis	Number of regenerated plants
A619 x M320	109	71
M320 x A619	62	43

The results differed in dependence on the line used as a female parent. It is obvious that maternal genotype A619 had a valuable effect on the frequency of plants produced in vitro.

We speculate that a factor determining these results may be the importance of combination between nucleus and cytoplasm. It is true for the case where A619 is the female partner, which is not the situation with A619 being the male partner, where there is only a nucleus.

The viability of regenerated plants was not high. During development part of the plants did not elongate and their appearance was like that established by Pilu et al. (MNL 73:69). Future experiments will elucidate differentiation in other traits of hybrids obtained in a "classical crosses" way.

This work was supported by grant B-602 from the National Fund of Scientific Investigations of the Bulgarian Ministry of Education and Science.

Genetic relationship among cytoplasm analogs and morphogenic potential of maize inbreds

--Nedev, T, Kruleva, M, Krapchev, B

Three maize inbred lines, A654, B37, Wf9 and their analogs, possessing cytoplasm of S and C type (A654 S, A654 C; B37 S, B37 C; Wf9 S, Wf9 C) were used as experimental materials. We set up this survey to answer the question whether there is any relationship between different cytoplasm and morphogenic potential of maize inbreds. Maize inbreds used in this experiment have different potential for callus formation and plant regeneration (Nedev et al. MNL 72:76). Data for media and procedures for cultivation are available in the same paper. A summary of the obtained results is given in the Table 1.

Table 1. The plant regeneration of maize inbreeds with cytoplasm analogies. Summary. (%).

A654	A654S	A654C	B37	B37S	B37C	Wf9	W9S	Wf9C
33.1	41.4	18.5	-	1.3	2.5	4.8	6.6	1.5

As is shown in the table, the frequency of regeneration was the highest in A654 S. The reason for differences in regeneration potentials among inbreds is clear - different frequencies of genes controlling regeneration. The question about relations between A 654 (normal cytoplasm) and their male sterile analogs A654 S, A654 C in respect to their regeneration potential is more complicated. Maybe the explanation should be searched for in the connections between nucleus and cytoplasm at the physiological and genetic levels. A genetic aspect may be very attractive if one speculates about the relationship (or lack of relationship) between nucleus genes and genes located in cytoplasm - mitochondria, chloroplasts.

This work was supported by grant B-602 from the National Fund of Scientific Investigations of the Bulgarian Ministry of Education and Science.

TAIPEI, TAIWAN, REP. OF CHINA
Academia Sinica, National Taiwan University
KAOHSIUNG, TAIWAN, REP. OF CHINA
National Sun Yat-sen University
BUFFALO, NEW YORK
State University of New York

Multi-photon excited fluorescence and absorption properties of maize tissues

--Lin, B-L, Kao, F-J, Sun, C-K, Cheng, P-c

In order to interpret the images obtained with confocal and multi-photon fluorescence microscopy, it is important to obtain the basic absorption and autofluorescence properties of maize tissues, in particular, the multi-photon excited fluorescence spectra. It has been demonstrated that *Arabidopsis* mesophyll protoplasts and leaves exhibit significantly different autofluorescence between single- and two-photon excitation (Cheng et al., Proc. Optics and Photonics Taiwan, 1099-1101, 1999; Cheng et al., SPIE Proceedings 3919, 2000; Cheng et al., Micron, 2000). A 495nm two-photon excited fluorescence emission peak was detected from *Arabidopsis* protoplasts when illuminated with high intensity 760nm IR pulse. This green emission is very close to the emission peak of GFPs, hence, may interfere with the detection of GFP in plant cells under multi-photon fluorescence microscopy.

Leaves from Ohio43 inbred were used in this study. Figure 1 shows the absorption properties of normal leaf and waterlogged leaf. Note the normal leaf shows a significantly higher optical density as a result of light scattering from air chambers within the leaf. The lower optical density in the longer wavelength region favors the use of multi-photon fluorescence microscopy for the study of thicker tissues.

Figures 2 and 3 show two-photon excited fluorescence spectra of both whole leaf and the acetone extract of the leaf. When 780nm excitation was used, two major fluorescence peaks (678nm and 512nm) were observed in whole leaf. In contrast, fluorescence emission at 512nm and 668nm was observed in leaf acetone

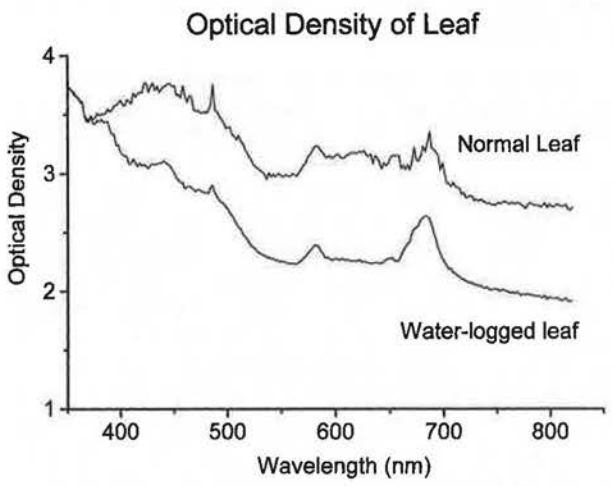
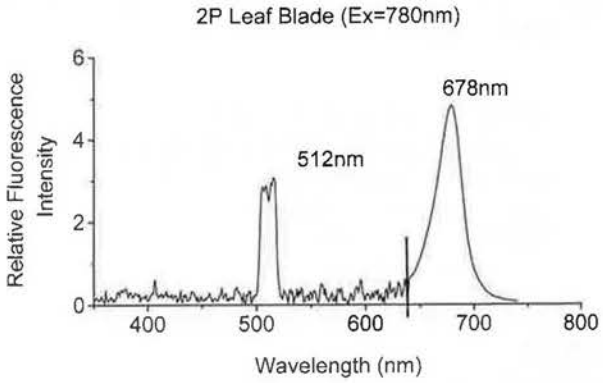


Figure 1.



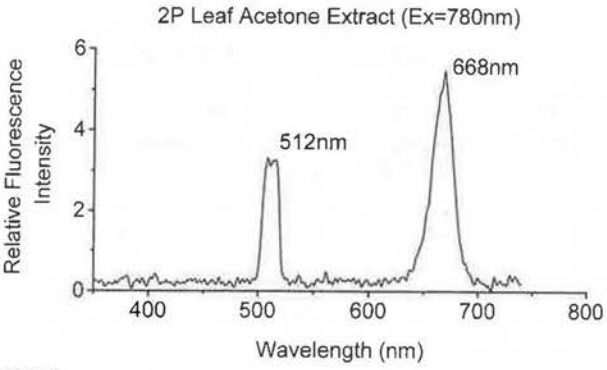
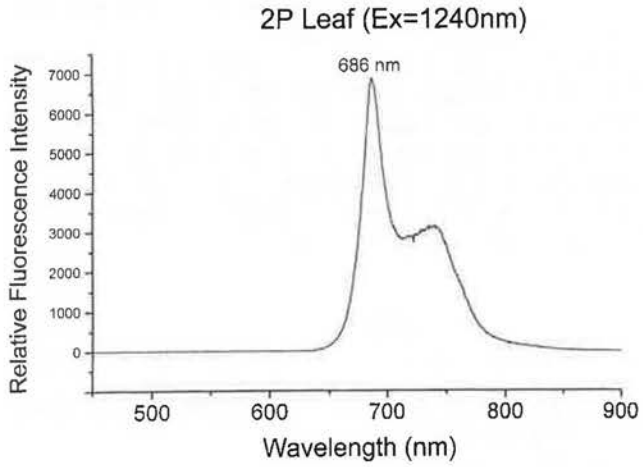


Figure 2.

extract. Two-photon fluorescence emission at 686nm was detected from leaf blade under 1240nm illumination. A second red fluorescence shoulder was observed around 740nm. Leaf acetone extract shows similar fluorescence peaked at 671nm and a longer shoulder around 730nm. The green fluorescence (512nm) observed in the 800nm excited spectra was absent due to the longer wavelength of the illumination (1240nm) which is not capable of producing 2-photon excitation at this wavelength. Although three-photon excitation is possible, no green fluorescence was observed in our current set-up.



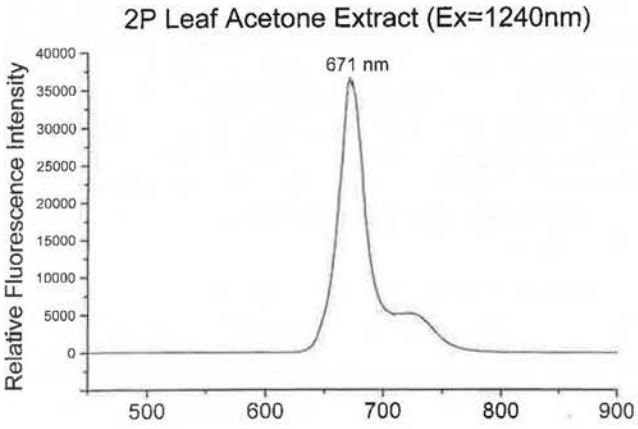


Figure 3.

Two-photon fluorescence spectra were obtained by using a Coherent Verdi pumped Spectra-Physics Tsunami mode-locked Ti-sapphire laser operated at 780nm with 100fs IR pulse. The 1240nm IR excitation was obtained from a Spectra-Physics Millennium IR (1064nm) pumped Chromium-doped Forsterite (home-built) laser operated at 1240nm with a pulse width of 130fs.

TIFTON, GEORGIA
USDA-ARS and University of Georgia
COLUMBIA, MISSOURI
USDA-ARS

Identification of a gene at the syntenic *sh2-a1* region in maize

--Guo, BZ, Butron, A, Li, RG, Zhang, ZJ, Widstrom, NW, McMullen, MD, Lynch, RE

Using common DNA probes in comparative genetic mapping of cereal crops, a number of research groups have demonstrated an impressive degree of synteny, genes in related species tending to be collinear in order on chromosomes, at the genetic level (Gale and Devos, PNAS 95:1971-1974; Feuillet and Keller, PNAS 96:8265-8270, 1999). Genetic mapping of sorghum, maize, rice and other species has revealed the existence of conserved gene content, gene

order, and gene sequence in the family *Poaceae* (Devos and Gale, Plant Mol. Biol. 35:3-15, 1997; Bennetzen et al., PNAS 95:1975-1978, 1998). In the family *Poaceae*, rice has the smallest genome. The rice genetic map can be divided into sets of linked genes known as linkage blocks, and these blocks can be rearranged to form the maps of other cereals (Moore et al., Curr. Biol. 5:737-739, 1995; Devos and Gale, Plant Mol. Biol. 35:3-15, 1997). The chromosomes of other species in this family can be arrayed or aligned in concentric circles such that a radial line from the central species, rice, passes through regions with similar genic content in other species. This integrated grass genome map allows genetic information from different species to be combined, thus providing a large potential marker/gene pool. The collinearity of genes in the family *Poaceae* provides a unique tool for gene discovery and gene isolation.

Dr. Bennetzen's group has demonstrated microcolinearity in the *sh2-a1* homologous regions of rice, sorghum, and maize (Chen et al., PNAS 94:3431-3435, 1997) and has completely sequenced this homologous region in rice (Chen and Bennetzen, Plant Mol. Biol. 32:999-1001, 1996) and sorghum (Chen et al., Genetics 148:435-443, 1998). A putative transcription factor gene X has been identified in this *sh2-a1* homologous region in rice and sorghum in Bennetzen's lab. At the nucleotide level, gene X of rice and gene X of sorghum share 82% identity of exons and 57% identity of introns. The order and orientation of the genes are conserved among rice and sorghum as demonstrated by Bennetzen's group. The questions are whether gene X exists in maize and if so, where is its position in the maize genome.

To answer these questions, we used maize inbred SC102 and inbred B31857 in this study. The SC102 is a dent corn with *A1/A1* genotype and high silk maysin concentration. Inbred B31857 with *shrunken-2* (*sh2*) endosperm is an *a1/a1* genotype and has no silk maysin (Guo et al., J. Econ. Entomol. 92:746-753, 1999). A pair of primers were synthesized based on the homologous region of gene X in rice and sorghum (Chen et al., Genetics 148:435-443,1998, GenBank accession number AF010283; Chen and Bennetzen 1996, Plant Mol. Biol. 32:999-1001, GenBank accession number U70541) and used to amplify maize genomic DNA. PCR was performed on 50 ng of template DNA, in a final volume of 50 µg containing 0.2 mM of each dNTP (Pharmacia), 0.3 µM of each primer, and 2.5 U of *AmpliTaq* Gold DNA polymerase (PE Applied Biosystems) in the buffer supplied by the enzyme manufacturer. The DNA amplification reaction was carried out using a GeneAmp PCR System 9700 Thermocycler (PE Applied Biosystems) under the following conditions: pre-PCR held 10 min at 94 C; PCR, 40 cycles of 45 s at 94 C, 45 s at 60 C, and 2 min at 72 C; post-PCR held 7 min at 72 C and kept at 4 C. We cloned the Taq-amplified PCR product using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). DNA sequencing was done using an ABI Prism-377 automatic sequencer. The search for homologous sequences at the GenBank databases was carried out using the BLAST program.

RT-PCR was done by the same primers using the Access RT-PCR system (Promega). Northern and Southern analyses were done by using the PCR product as probe to detect the gene X transcript and gene copy number. Probes were prepared by random-primer labeling of the template DNA with [α-^{32}P]dCTP using the High Prime labeling kit according to the manufacturer's instructions (Ambion). After hybridization, blots were exposed to a phosphor screen which was monitored using a PhosphorImager (Cyclone Storage Phosphor System) from Packard Instrument Company. The software package ImageQuant Version 3.3 from Molecular Dynamics was used to analyze and quantify the hybridization signals. To map this gene X in relationship with location of *sh2* and *a1*, we used a F2 population (300 plants) derived

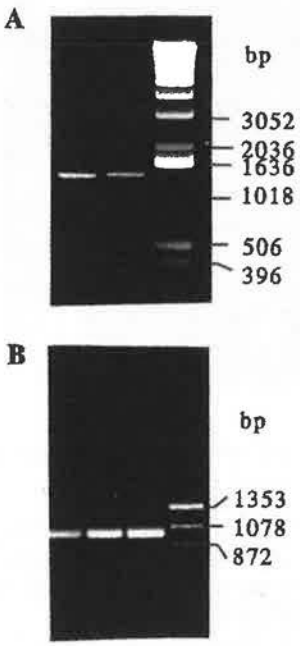


Figure 1. PCR (A) and RT-PCR (B) analyses, using the primers designed based on the sequences (GenBank accession number AF010283 and U70541).

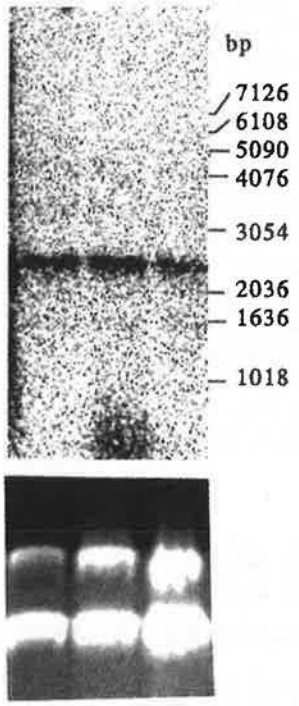


Figure 2. Northern blot analysis of gene expression in leaf, cob, and silk. The blot was hybridized with the cloned PCR product (Fig. 1A). Equal loading was verified by visualizing rRNA in the gel stained with ethidium bromide (bottom).

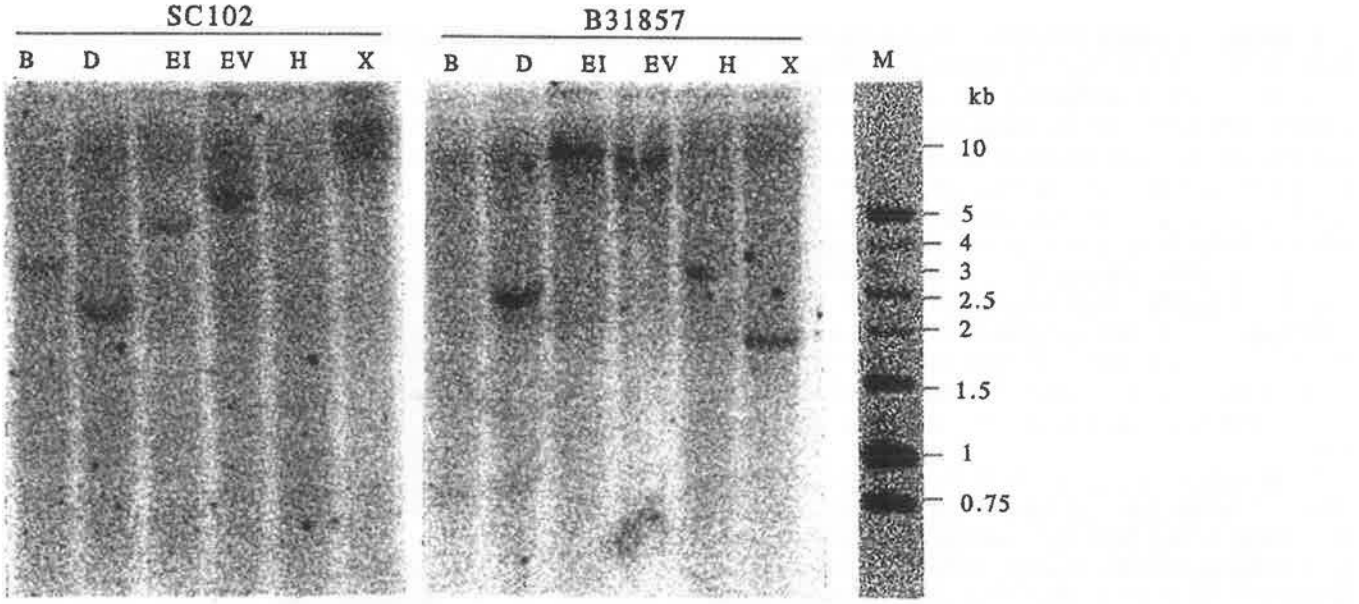


Figure 3. Southern blot analysis of PCR clone with *Bam*HI (B), *Dra*I (D), *Eco*RI (EI), *Eco*RV (EV), *Hind*III (H), or *Xba*I (X) digested genomic DNA of maize.

from inbreds SC102 and B31857. Restriction fragment length polymorphism (RFLP) analyses were performed with probes specific for *a1*, *sh2*, and the cloned X-gene. The linkage analysis was done using the MAPMAKER program.

Using primers designed from gene X of rice and sorghum, we amplified and cloned a PCR product with molecular weight of 1.4 kb from maize leaves of SC102 and B31857 (Fig. 1A). The DNA sequence of the cloned PCR product has been deposited in GenBank database under accession number AF136530. This sequence has more than 90% and 80% homology with the 5'-end sequence of the putative X gene in sorghum and rice, respectively. Transcripts of the gene X in maize were detected by reverse transcriptase-PCR (Fig. 1B) with primers that yield a 1.4 kb PCR product from genomic DNA (Fig. 1A). We detected a gene transcript (1 kb) in leaves, cobs, and silks using RT-PCR (Fig. 1B). Northern analysis also revealed the X-gene transcript of about 2.3 kb in leaves, cobs, and silks (Fig. 2). This X-gene identified in maize is a single copy gene (Fig. 3). Mapping and linkage analysis showed this X gene is closely linked to loci of *sh2* and *a1*, and placed in the same linkage group with *sh2* and *a1*. Therefore, the X gene homologous to rice and sorghum exists in the maize genome. The position of the X gene in maize is closely linked with *sh2* and *a1* on chromosome 3. The functional feature of this X-like gene identified in *sh2-a1* region in maize should be investigated in the future.

TUCSON, ARIZONA
University of Arizona

Npi402 and *ncsu1* are identical ; *inra1* (*tmp*) maps upstream of the *b* promoter

--Stam, M, Lavin, T, Chandler, VL

The analysis of intragenic recombinants showed that *b* is transcribed, 5'→ 3', from the telomere towards the centromere and that the sequences required for paramutation of the *b* gene map upstream of the *b* coding region (Patterson et al., Genetics 140:1389, 1995). To further define the sequences involved in paramutation we wanted to identify recombination events between the *B'* (paramutagenic) and *B-P* (non-paramutagenic) alleles further upstream of the *b* gene. To simplify the screen for recombinants we needed to convert RFLP markers tightly linked and upstream of *b* into a PCR assay to detect sequence length polymorphisms (SLPs) linked to the different *b* alleles. The RFLP markers *npi287A*, *umc61*, *ncsu1* and *npi402*, which map 1.1 cM upstream of *b* on chromosome 2S (www.agron.missouri.edu/images/bnlc2.jpg), were sequenced. Two markers, *ncsu1* and *npi402*, were found to have exactly the same sequence and therefore are the same RFLP marker. Primers designed to amplify the genomic sequences corresponding to *npi287A*, *umc61*, *ncsu1/npi402* did not reveal any SLP between *B'* and *B-P*. No further experiments were performed with these markers.

The map UMC 98 2 showed *inra1* (*tmp*) mapped to 1.2 cM 3' of *umc61*; *inra1* was not mapped relative to *b*, however. Southern analysis using DNA from *B'*, *B-P* and three *B-P//B'* recombinant alleles (recombinations verified by PFGE analysis) showed that all three *B-P//B'* alleles had the *B-P inra1* RFLP, while *B'* had another sized fragment (not shown). This suggested that *inra1* is located upstream of *b* and could be used to look for a PCR SLP between *B'* and *B-P*. *Tmp* is an mRNA for a transmembrane protein that is highly conserved. We hypothesized that SLPs might be present within the introns of the *tmp* alleles linked to *B'* and *B-P*. To predict the intron positions in the *tmp* gene, the protein sequence was used to search the database for homologous genes. The genomic DNA sequence of the homologous *Arabidopsis thaliana* water channel-like protein (EMBL accession nr. CAA20461) contained three introns. Protein sequence alignment between the translated maize *tmp* cDNA and the *Arabidopsis* protein predicted the intron positions in the maize gene. Primers surrounding the predicted third intron gave rise to different sized PCR products for the *tmp* alleles linked to *B'* and *B-P*.

Sequence analysis of both PCR products identified multiple deletions and insertions in the *tmp* intron 3 between the alleles linked to *B'* and *B-P*. Primers were designed that only amplified *tmp* sequences linked to *B-P*. This enabled us to screen for recombination events upstream of the *b* promoter. The *B'* allele (colorless seeds due to *B'* promoter proximal region), flanked with the homozygous recessive phenotypic markers *gl2* (19 cM 5' of *b*) and *wt* (11 cM 3' of *b*), was combined with the *B-P* allele (purple seeds), flanked with the wild-type alleles of *gl2* and *wt*. The F1 was crossed to *gl2 B-I wt* plants (colorless seeds); colorless seeds were planted and screened for a recombination event upstream of *b* using the *gl2* marker. 1861 seedlings wild-type for the marker *gl2*, were tested for a recombination event near to *b* using the *B-P* specific primers on pooled DNA samples. 18 recombinant alleles (0.97% of 1861 seedlings) were identified using PCR and verified by Southern blot analysis. All 18 alleles have *wt* as the 3' marker, consistent with a single recombination event between *tmp* and *b*. These results show that the *tmp* allele maps 0.18 cM upstream of *b*. This was calculated by multiplying the 19 cM between *gl2* and *b* by the % of recombinants obtained within this region.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center

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

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

Approximately 1 acre each year is devoted to the propagation of the large collection of A-A translocation stocks. In this collection is a series of *Waxy1*-linked translocations that are used for mapping unplaced mutants. Each translocation is maintained in separate M14 and W23 inbred backgrounds which are crossed together to produce vigorous F1's to fill seed requests. Over the years, pedigree and classification problems arose during the propagation of these stocks. We have been able to sort through the problem ones, and can now supply good sources proven by linkage tests to include the correct translocated chromosomes.

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

Table 1. *Wx1* T1-9(4995) (1L.19; 9S.20)

A) The M14 source showed linkage of *wx1* with *bz2*.

2 point linkage data for ***bz2*-*Wx1*** T1-9(4995)
Testcross: [*Bz2 Wx1* 1-9(4995) x *bz2 wx1* N] x *bz2 wx1* N

source:94-1863-2^M14

Region	Phenotype	No.	Totals
0	+ Wx	581	
	bz wx	537	1118
1	bz Wx	138	
	+ wx	176	314

% recombination *bz2-Wx1* = 21.9±1.1

B) The W23 source showed linkage of *wx1* with *f1* & *bz2*.

1) 2 point linkage data for ***bz2*-*Wx1*** T1-9(4995)
Testcross: [*Bz2 Wx1* 1-9(4995) x *bz2 wx1* N] x *bz2 wx1* N

source:94-1865-3^W23

Region	Phenotype	No.	Totals
0	+ Wx	485	
	bz wx	425	910
1	bz Wx	148	
	+ wx	115	263

% recombination *bz2-Wx1* = 22.4±1.2

2) 2 point linkage data for ***f1*-*Wx1*** T1-9(4995)
Testcross: [*F1 Wx1* 1-9(4995) x *f1 wx1* N] x *f1 wx1* N

source:94-1865-3^W23

Region	Phenotype	No.	Totals
0	+ Wx	1919	
	f wx	1726	3645
1	f Wx	327	
	+ wx	251	578

% recombination *f1-Wx1*= 13.7±0.5

Table 2. *Wx1* T4-9e (4S.53; 9L.26)

A) The M14 source showed linkage of *wx1* with *su1*:

2 point linkage data for *su1-Wx1* T4-9e
Testcross: [*Su1 Wx1* T4-9e x *su1 wx1* N] x *su1 wx1* N

source:87-987 / 986 Bulk ^M14

Region	Phenotype	No.	Totals
0	+ Wx	788	
	su wx	684	1472
1	su Wx	81	
	+ wx	22	103

% recombination su1-*Wx1*= 6.5±0.6

B) The W23 source showed linkage of *wx1* with *su1*.

2 point linkage data for *su1-Wx1* T4-9e
Testcross: [*Su1 Wx1* T4-9e x *su1 wx1* N] x *su1 wx1* N

source:87-985 x sib ^W23

Region	Phenotype	No.	Totals
0	+ Wx	840	
	su wx	782	1622
1	su Wx	194	
	+ wx	34	228

% recombination *su1-Wx1*= 12.3±0.8

Table 3. *Wx1* T9-10b (9S.13; 10S.40)

A) The F1 source showed linkage of *wx1* with *bf2*:

2 point linkage data for *bf2-Wx1* T9-10b
Testcross: *bf2 wx1* N x [*Bf2 Wx1* T9-10b x *bf2 wx1* N]

source:87-1050 / 1049 bulk (1)

Region	Phenotype	No.	Totals
0	+ Wx	729	
	bf wx	610	1339
1	bf Wx	39	
	+ wx	41	80

% recombination *bf2-Wx1* =5.6±0.6

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

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

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

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

Table 1. *wx1* T1-9c (1S.48; 9L.22)

A) The W23 source showed linkage of *wx1* with *P1-ww:*

2 point linkage data for *P1-ww-wx1* T1-9c
Testcross: [*P1-wr Wx1* N x *P1-ww wx1* T1-9c] x *P1-ww wx1* N wx1

source:94-1891-1^W23

Region	Phenotype	No.	Totals
0	P1-wr Wx	62	
	P1-ww wx	72	134
1	P1-ww Wx	10	
	P1-wr wx	3	13

% recombination *P1-ww-Wx1* =8.8±2.3

Table 2. *wx1* T1-9(4995) (1L.19; 9S.20) COOP source

A) The new M14 c/o sources showed linkage of *wx1* with *bz2* & *f1*.

1) 2 point linkage data for ***bz2*-*wx1*** T1-9(4995)
Testcross: [*Bz2 wx1* 1-9(4995) x *bz2 wx1* N] x *bz2 wx1* N

source:94-1845-1c/o^M14

Region	Phenotype	No.	Totals
0	bz Wx	595	
	+ wx	693	1288
1	+ Wx	222	
	bz wx	191	413

% recombination *bz2-Wx1* = 24.3±1.0

Testcross: *bz2 wx1* N x [*bz2 wx1* N x *Bz2 wx1* 1-9(4995)]
source:94-1845-2c/o^M14

Region	Phenotype	No.	Totals
0	bz Wx	71	
	+ wx	82	153
1	+ Wx	19	
	bz wx	37	56

% recombination *bz2-Wx1* = 26.8±3.1

source:94-1851-1c/o^M14

Region	Phenotype	No.	Totals
0	bz Wx	177	
	+ wx	133	310
1	+ Wx	71	
	bz wx	30	101

% recombination *bz2-Wx1* = 24.6±2.1

2) 2 point linkage data for ***f1*-*wx1*** T1-9(4995)
Testcross: [*F1 wx1* 1-9(4995) x *f1 wx1* N] x *f1 wx1* N

source:94-1845-1c/o^M14

Region	Phenotype	No.	Totals
0	f Wx	830	
	+ wx	724	1554
1	+ Wx	79	
	f wx	48	127

% recombination *f1-Wx1*= 7.6 ±0.6

Testcross: [*f1 wx1* N x *F1 wx1* 1-9(4995)] x *f1 wx1* N
source:94-1845-2c/o^M14

Region	Phenotype	No.	Totals
0	f Wx	760	
	+ wx	704	1464
1	+ Wx	87	
	f wx	60	147

% recombination *f1-Wx1*= 9.1±0.7

Testcross: [*F1 wx1* 1-9(4995) x *f1 wx1* N] x *f1 wx1* N
source:94-1851-1c/o^M14

Region	Phenotype	No.	Totals
0	f Wx	975	
	+ wx	970	1945
1	+ Wx	95	
	f wx	66	161

% recombination *f1-Wx1*= 7.6 ±0.6

Testcross: [*f1 wx1* N x *F1 wx1* 1-9(4995)] x *f1 wx1* N
source:94-1851-2c/o^M14

Region	Phenotype	No.	Totals
0	f Wx	727	
	+ wx	662	1389
1	+ Wx	82	
	f wx	35	117

% recombination *f1-Wx1*= 7.8 ±0.7

B) The W23 source showed linkage of *wx1* with *bz2* & *f1*.

1) 2 point linkage data for ***bz2*-*wx1*** T1-9(4995)
Testcross: *bz2 wx1* N x [*bz2 wx1* N x *Bz2 wx1* 1-9(4995)]

source:84H-2410-4^W23

Region	Phenotype	No.	Totals
0	bz Wx	329	
	+ wx	233	562
1	+ Wx	108	
	bz wx	93	201

% recombination *bz2-Wx1* = 26.3±1.6

2) 2 point linkage data for ***f1*-*wx1*** T1-9(4995)
Testcross: [*f1 wx1* N x *F1 wx1* 1-9(4995)] x *f1 wx1* N

source:84H-2410-4^W23

Region	Phenotype	No.	Totals
0	f Wx	1111	
	+ wx	975	2086
1	+ Wx	85	
	f wx	52	137

% recombination *f1-Wx1*= 6.2 ±0.5

C) The new Sisco c/o source showed linkage of *wx1* with *bz2*.

1) 2 point linkage data for ***bz2*-*wx1*** T1-9(4995)
Testcross: [*bz2 wx1* N x *Bz2 wx1* 1-9(4995)] x *bz2 wx1* N

source:94-1841-9c/o^Sisco

Region	Phenotype	No.	Totals
0	bz Wx	714	
	+ wx	530	1244
1	+ Wx	434	
	bz wx	247	681

% recombination *bz2-Wx1* = 35.4±1.1

Table 3. *wx1* T2-9c (2S.49; 9S.33)

A) TheF1 source showed linkage of *wx1* with *lg1gl2*.

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

source:93-432-2 x 433 ^F1

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

% recombination *lg1-g12* =17.2±0.7
% recombination *gl2-Wx1* =8.1±0.5
% recombination *lg1-Wx1*=24.9 ±0.8

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

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

source:87-838 x 839 ^W23

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

% recombination *lg1-g12* =17.9±1.0
% recombination *gl2-Wx1* =8.6±0.7
% recombination *lg1-Wx1*=25.9 ±1.1

Table 4. *wx1* T6-9(4505) (6L.13; 9ctr.)

A) The M14 sources showed linkage of *wx1* with *y1:*

1) 2 point linkage data for *y1-wx1* T6-9(4505)
Testcross: [*Y1 wx1* T6-9(4505) x *y1 Wx1* N] x *y1 wx1* N

source:93W-1411-3^M14

Region	Phenotype	No.	Totals
0ZZ	y Wx	177	
	+ wx	194	371
1	+ Wx	13	
	y wx	6	19

% recombination *y1-Wx1*=4.9±1.1

source:94-1965-9c/o^M14

Region	Phenotype	No.	Totals
0	y Wx	955	
	+ wx	846	1801
1	+ Wx	47	
	y wx	63	110

% recombination *y1-Wx1*=5.8±0.5

source:95-1034-4 fr. 94-1965-9c/o^M14

Region	Phenotype	No.	Totals
0	y Wx	736	
	+ wx	678	1414
1	+ Wx	84	
	y wx	44	128

% recombination *y1-Wx1*=8.3±0.7

source:94-1965-10c/o^M14

Region	Phenotype	No.	Totals
0	y Wx	675	
	+ wx	571	1246
1	+ Wx	31	
	y wx	33	64

% recombination *y1-Wx1*=4.9±0.6

source:94-1965-11c/o^M14

Region	Phenotype	No.	Totals
0	y Wx	817	
	+ wx	832	1649
1	+ Wx	48	
	y wx	40	88

% recombination *y1-Wx1*=5.1 ±0.5

source:94-1965-12c/o^M14

Region	Phenotype	No.	Totals
0	y Wx	684	
	+ wx	640	1324
1	+ Wx	38	
	y wx	32	70

% recombination *y1-Wx1*=5.0±0.6

B) The W23 sources showed linkage of *wx1* with *y1:*

1) 2 point linkage data for *y1-wx1* T6-9(4505)
Testcross: [*Y1 Wx1* N x *y1 wx1* T6-9(45045)] x *y1 wx1* N

source: 87-910 x sib bulk 1^W23

Region	Phenotype	No.	Totals
0	+ Wx	237	
	y wx	209	446
1	y Wx	34	
	+ wx	31	65

% recombination *y1-Wx1*=12.7±1.5

source:87-911-2 x sib^W23

Region	Phenotype	No.	Totals
0	+ Wx	593	
	y wx	523	1116
1	y Wx	136	
	+ wx	134	270

% recombination *y1-Wx1*=19.5±1.1

Testcross: *y1 wx1* N x [*Y1 Wx1* N x *y1 wx1* T6-9(45045)]
source:87-911-2 x sib^W23

Region	Phenotype	No.	Totals
0	+ Wx	641	
	y wx	566	1207
1	y Wx	47	
	+ wx	60	107

% recombination *y1-Wx1*=8.1 ±0.8

Table 5. *wx1* T8-9(6921) (8L.85; 9L.15)

A) The Robertson Accession source showed linkage of *wx1* with *j1:*

2 point linkage data for *j1-wx1* T8-9(6921)
Testcross: [*J1 wx1* T8-9(6921) x *j1 Wx1* N] x *j1 wx1* N

source:90-563-1^Rob.

Region	Phenotype	No.	Totals
0	j Wx	39	
	+ wx	51	90
1	+ Wx	1	
	j wx	1	2

% recombination *j1-Wx1*=2.2±1.5

Allelism testing of miscellaneous stocks in Maize COOP phenotype only collection

--Jackson, JD

This report summarizes allele testing of miscellaneous stocks characterized by phenotype only in the Maize Genetics COOP Stock Center collection. Some of these stocks have been found in other COOP stocks and some have been sent in by cooperators over the years. In most cases crosses were made between known heterozygotes and homozygous plants. Plants were scored at the seedling stage and again at maturity. Proposed new designations have been assigned to these alleles. These stocks have been increased and placed on the 2000 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.

previous designation	allelism test with *ra1*	allelism test with *ra2*	new designation
ra-PI262495*	positive (ra*/ra1= 24/24 plts.) (+/ra* // ra1=10/24)	negative	*ra1-PI262495*
ra-4889*	negative (+/ra* // ra1=0/90)	negative (+/ra* // +/ra2=0/96)	

note: *ra1-PI262495* tassel has good ra1 phenotype. Ear has weak or no ramosa phenotype. gs1-PI262495 may segregate in this stock.

note: *ra*-4889* has ramosa-like tassel; ear has no ramosa phenotype.

previous designation	allelism test with *ps1-Sprague*		new designation
ps-8205*	positive (3 out of 3)		*ps1-8205*

previous designation	allelism test with *gl1*	allelism test with *gl8*	new designation
gl-dy*	positive (2 out of 2)	negative	*gl1-dy*

note: *gl1-dy* traces back to Coop1953-159: Nelson 9947-4. This is also source of *mn1* & *dy1*.

Three-point linkage data for *Og*-0376 Wx1* T9-10b on 10S

--Jackson, JD

A new dominant yellow stripe stock maps to chromosome 10S near *Og1*. This new mutation was isolated from a *ms2* stock of E. B. Patterson. Its phenotype is very similar to *Og1*. Crosses were done with the *waxy1*-marked translocations: T9-10b and T8-9(043-6) to genetically determine its chromosomal loction.

The results of a three-point linkage test for *Wx1*, T9-10b and *Og*-0376* are presented in Table 1. The linkage test was set up as a modified backcross. *Wx* and *wx* kernels from the backcross ears were planted in the field and the resulting plants were scored for yellow stripes and for the presence of the translocation by pollen sterility. The following linkage relationship was established: *Wx1* – 9.7 – T9-10b – 3.2 – *Og*-0376*. Crosses are underway with *Og1* to determine if *Og*-0376* is an allele of *Og1*.

The results of a two-point linkage test for *Wx*1, T8-9(043-6) and *Og*-0376* are presented in Table 2. The linkage test was also set up as a modified backcross. Wx and wx kernels from the backcross ears were planted in the field and the resulting plants were scored for yellow stripes. Results indicate no linkage of *Og*-0376* with either chromosome 9 or chromosome 8.

Table 1. Three point linkage data for *Og*-0376- Wx1*-T9-10b.

Testcross: *og* wx1* N x [*Og*-0376 Wx1* N x *og* wx1* T9-10b]

Region	Phenotype	No.	Totals
0	Wx N str	44	
	wx T gr	38	82
1	Wx T gr	4	
	wx N str.	4	8
2	Wx N gr	2	
	wx T str	0	2
1+2	Wx T str	0	
	wx N gr	1	1

% recombination *Wx1*-T =9.7±2.9
% recombination T -*Og**=3.2±1.4
% recombination *Wx1-*Og*=11.8±3.3

Table 2. Two point linkage data for *Og*-0376- Wx1*-T8-9(043-6).

Testcross: *og* wx1* N x [*Og*-0376 Wx1* N x *og* wx1* T8-9(043-6)]

Region	Phenotype	No.	Totals
0	Wx str	15	
	wx gr	28	43
1	Wx gr	32	
	wx str	13	45

% recombination *Wx1*-Og*= 51.1±5.3

The brown kernel mutant of maize consists of a duplicate factor pair *brn1 brn2*

--Stinard, PS

The mutant *brown kernel1* (*brn1*) was previously described by this author as being a simple Mendelian recessive mutation (Stinard. 1994. Maydica 39:273-278). Several mutant alleles have been isolated, and in order to study and compare them in a uniform genetic background, backcrosses of the different alleles were made to the inbred B73. Self-pollination of the F1 ears yielded considerably fewer than the expected 1/4 brown mutant kernels, approximating instead a 15:1 ratio of nonmutant to mutant kernels suggestive of a duplicate factor relationship. Further generations of backcrossing to B73 were done, and self-pollinations made after each generation of backcrossing also gave 15:1 ratios. At the fifth backcross generation, nonmutant kernels from 15:1 segregating ears of *brn1-R* were planted, and the resulting plants were self-pollinated and scored for *brn1* mutant kernels. Four ears segregating 3:1 for nonmutant to brown kernels were identified; these ears would be presumably homozygous mutant for one of the two duplicate factors and segregating for the other. Nonmutant kernels from each of these 3:1 ears were planted and the resulting plants self-pollinated. Ears segregating 3:1 as well as ears with only nonmutant kernels were obtained. The latter ears would be expected to be homozygous mutant for one duplicate factor and homozygous nonmutant for the other duplicate factor; such ears will be referred to as single factor lines.

Crosses were made between all possible combinations of the single factor lines, and the F1 ears were self-pollinated to produce F2 ears. Intercrosses among three of the single factor lines yielded only nonmutant ears in the F2, but crosses of these three single factor lines with the fourth single factor line gave all F2 ears segregating 15:1. Thus, we succeeded in isolating the two duplicate factors as single factor lines. I name these two factors *brn1* and *brn2*. *brn1* is the factor that was located to the short arm of chromosome 3; *brn2* remains unmapped.

The question remains as to why when *brn1* was originally isolated, it was thought to be only a single factor. *brn1* originally arose in a Robertson's *Mutator* population that had been propagated by crossing an active *Mutator* line alternate generations to the two non-*Mutator* hybrids Standard B70 (B77 X B79) and Standard Q60 (Q66 X Q67). Apparently, these two hybrid lines are already homozygous mutant for the other factor, *brn2*, so when the *brn1* mutation occurred, it segregated 3:1 on a self-pollinated ear. Outcrosses of *brn1* to Standard B70, Standard Q60, and M14/W22 all give F2's segregating 3:1, proving that they are homozygous for *brn2*. Similarly, the TB-3Sb line used in the arm-locating cross as well as the linkage stocks used for mapping *brn1* must also have been homozgyous for *brn2*. Crosses to inbred B73 gave F2's segregating 15:1; therefore B73 is homozygous nonmutant for both factors. Crosses are being planned to confirm the *brn2* status of the Robertson hybrid lines.

brn1 brn2 is not the first duplicate factor pair discovered upon crossing a mutant into B73. Two other mutants previously considered to be single factor traits, *su3* (now *su3 su4*; Stinard. 1997. MNL 71:83) and *ns1* (now *ns1 ns2*; Scanlon and Freeling. 1995. MNL 69:23), were also shown to have duplicate factor inheritance upon crossing into B73.

John Deere green aleurone corn carries two *R1*-specific dominant inhibitors of aleurone color

--Stinard, PS

John Deere corn is a variety of novelty corn with green kernels (Sprague. 1994. MNL 68:105). In order to isolate the cause of the green pigmentation, we crossed the John Deere line to various colored aleurone stocks. We found that in crosses to stocks carrying specific alleles of *R1* (members of the *R1-d* class and *R1-*

ch), dominant inhibition of aleurone color occurred in a pattern similar to that found for *Inhibitor of R1* (*Inr1*; MNL 73:89-90). After backcrossing the John Deere line to our W23 ACR conversion, which carries an *R1* allele susceptible to inhibition, for several generations, a pattern emerged. Plants were obtained that produced ears giving a 15:1 ratio of colorless and pale to colored aleurone kernels, and whose outcrosses to our W23 ACR gave a 3:1 ratio of colorless and pale to colored aleurone kernels. These crosses are indicative of segregation for two dominant aleurone color inhibitors. Through several generations of self-pollination, we were able to obtain lines homozygous for each dominant inhibitor separately.

Allelism tests of these factors with *Inr1* were done by crossing these separate lines to a homozygous *Inr1-R* line, and then crossing the resulting F1 by our W23 ACR line. One of the factors produced only colorless kernels in a population of thousands of kernels, indicating probable allelism. (There is always the remote possibility that the factor is not allelic to *Inr1*, but is instead extremely tightly linked to *Inr1*--these tests cannot rule out that possibility). The other factor produced ears segregating 3:1 for colorless to colored kernels in these crosses, indicating non-allelism with *Inr1*. We have given the second factor the temporary designation *Inr*-JD* until allelism with other known aleurone color factors can be ruled out. We are currently in the process of conducting linkage tests of *Inr*-JD* with a comprehensive set of *wx1*-marked A-A translocations.

We still do not have a precise explanation as to why John Deere corn is green. The *R1* allele that is present in the John Deere line is apparently only weakly suppressed by the dominant color inhibitors. Perhaps the weak suppression of aleurone color occurring over the surface of yellow endosperm (John Deere is *Y1 Y1*) gives a green appearance through the combination of the light purple and yellow. Another possibility is the production of a unique anthocyanin pigment or some other chemical alteration in the aleurone cells that gives rise to a green color. Further analysis will need to be done in order to resolve this question.

Three-point linkage data for *fl2 bm3 su1* on 4S

--Stinard, PS

The results of a three-point linkage test for *fl2*, *bm3*, and *su1* on chromosome 4 are presented in Table 1. The linkage test was set up as a modified backcross as indicated in Table 1. Kernels from the backcross ears were planted in the field and the resulting plants were self-pollinated and scored for *bm3*, and the self-pollinated ears were scored for the presence of *fl2* and *su1*. The following linkage relationship was established: *fl2* - 5.0 - *bm3* - 2.1 - *su1*. These data are consistent with the *fl2* - *su1* distance (8 cM) given on the most recent genetic map of chromosome 4, and clearly place *bm3* distal to the TB-4Sa breakpoint.

Table 1. Three-point linkage data for *fl2* - *bm3* - *su1*.
Testcross: (*fl2 Bm3 Su1* / *Fl2 bm3 su1*) X *Fl2 bm3 Su1*.

Reg.	Phenotype	No.	Totals
0	fl2 + +	208	
	+ bm3 su1	197	405
1	fl2 bm3 su1	8	
	+ + +	14	22
2	fl2 + su1	3	
	+ bm3 +	6	9
1+2	fl2 bm3 +	0	
	+ + su1	0	0

% recombination *fl2--bm3* = 5.0 +/- 1.0
% recombination *bm3--su1* = 2.1 +/- 0.7
% recombination *fl2--su1* = 7.1 +/- 1.2

Miscellaneous allelism tests

--Stinard, PS

Over the past few years, we have conducted allelism tests between mutants with similar phenotype, especially if they have been found to have the same chromosome location. We report here the results of four such positive allelism tests.

We obtained preliminary TB mapping data last year placing the pale yellow endosperm mutant *y2* to the short arm of chromosome 7. Since *y8*, also on chromosome 7, has a similar phenotype, we conducted an allelism test and found them to be allelic. We did a pedigree search on the origin of our *y2* stock and found that it came from George Sprague. The lineage is not clean, and at one point, it had been reisolated by Sprague after being crossed to the *y8* reference allele and to other pale endosperm factors. *y2* was originally described as being a white endosperm mutant tightly linked to *vp2* on chromosome 5 (Eyster, 1931. Genetics 16:574-590). Since *vp2* mutant kernels have white endosperm, it is probable that Eyster was simply observing the white endosperm of *vp2* and that the crossovers he observed were either dormant *vp2* kernels or heterofertilization events. The *y2* stock that the COOP has is certainly not the original *y2* described by Eyster, and is probably identical to the *y8* reference allele, which became introgressed into the line by accident.

The virescent mutant *v*-JRL* that we mapped to 9L last year (MNL 73:90) was crossed to the 9L virescents *ar1*, *v1*, and *v30*. Only the crosses to *v1* produced virescent progeny. We have renamed *v*-JRL* as *v1-JRL*.

We conducted an allelism test of our uncharacterized terminal ear mutant *te*-Galinat* with *te1* and found them to be allelic. We now call the mutant *te1-Galinat*.

Last year, we located the mutant *y11* to the long arm of chromosome 2 using B-A translocations (MNL 73:90). Last winter, we crossed homozyous *y11* plants to heterozygous *w3-R* plants and noted 1:1 segregation for yellow and pale yellow kernels on the progeny ears. This summer, we planted the pale yellow kernels and self-pollinated the resulting plants. All ears segregated in a 3:1 ratio for pale yellow dormant to white endosperm viviparous kernels. The most logical explanation is that the pale yellow dormant kernels are homozygous *y11* or heterozygous for *y11* and *w3-R*, and the white endosperm viviparous kernels are homozygous for *w3-R*. We conclude that *w3* and *y11* are allelic, and propose that *y11* be renamed *w3-y11*. It should be noted that *w3-y11* kernels are dormant and produce green seedlings, whereas *w3-R* kernels are viviparous and produce albino seedlings. This appears to be an example of allelic diversity similar to that which exists at other carotenoid loci in maize.

Results of TB tests of unplaced mutants

--Stinard, PS, and Jackson, JD

The past two years, we have reported the results of TB mapping crosses made to symbolized unplaced mutants in the Coop's collection (MNL 72:79 and MNL 73:90). This year, we observed grow-outs of the TB crosses made last year to mature plant mutants. The mutants for which we obtained positive results are

summarized in Table 1. Additional crosses with linkage markers will be made to confirm chromosome arm placement, and allelism tests will be conducted with mutants with similar phenotype located on the same chromosome arm.

Table 1. Results of TB tests of symbolized unplaced mutants.

Mutant	Arm-locating TB Cross	Number of Positive Tests/Total Number of Crosses with this TB	Mutants on Same Chromosome Arm with Similar Phenotype	Note
les-3F-3330*	TB-5Sc	2/2		1
les-PI262474*	TB-4Lf	2/2		
zb3	TB-1Sb	2/2	*zb4*	

1. A repeat and confirmation of tests reported in MNL 73.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center
BERKELEY, CALIFORNIA
University of California at Berkeley

The *reverse germ orientation2-Vl* mutation maps to chromosome 1L near *Ts6* and shows allelism to *ids1*

--Jackson, JD, Kaplinsky, N

As previously reported (MNL 70:66), *rgo*-Vl* arose in a *Rf*-Vl* strain of John Laughnan. This mutant was shown not to be allelic to *rgo1* and was therefore called *rgo2-Vl.* The *rgo2* mutant sometimes produces extra florets within the tassel spikelets and in the female spikelets as well, giving a reverse germ orientation phenotype. Floret number seems to vary between two and three in the *rgo2-Vl/rgo2-Vl* tassels, but kernels on *rgo2-Vl/rgo2-Vl* ears are all rgo in straight rows. Another *rgo** from Frances Burr was shown to be allelic to *rgo2-Vl* and was therefore referred to as *rgo2-Burr.* Mutations with similar phenotypes have been reported by Brieger (MNL 22:55,1948), Joachim (MNL 29:53,1955; MNL 30:84-85,1956; Proc. Minn. Acad. Sci. 24:37-43,1956) and Sachan and Sarkar MNL 52:119-120, 1978).

One of these is *reversed germ orientation1* (*rgo1*). This is a recessive mutation, which causes the spikelet meristem to produce three instead of the normal two floret meristems. Development of the third floret causes reversed kernels due to the distichous pattern of floret initiation in the spikelet. *rgo1* also prevents the abortion of lower florets in some ear spikelets. *rgo1* maps to chromosome 9 near *wx1* (Nick Kaplinsky et al., Maize Genetics Conference Poster Abstracts 41: 1999).

The *rgo2* phenotype is similar to the *rgo1* phenotype in the tassel and the ear. Ears from plants heterozygous for *rgo1* and *rgo2* have a reversed germ phenotype, suggesting that *rgo1* and *rgo2* are in the same developmental pathway and are sensitive to each other's dosage.

Crosses were undertaken with the comprehensive set of B-A translocations (TB's) to determine the chromosomal location of the *rgo2-Vl* mutation. Plants homozygous for *rgo2* were crossed by the respective TB's and seeds from the resulting F1 ears showing the rgo phenotype were planted in the field and scored for hypoploid status by plant height and pollen sterility. The tassels on hypoploid plants were checked for floret number and ears were self-pollinated and at harvest scored for rgo phenotype. The results of crosses with TB-1Sb, TB-1La, TB-7Sc and TB-9Lc are summarized in Table 1. Results placed *rgo2-Vl* to chromosome 1.

Table 1. Summary of TB crosses with *rgo2*.

Mutant	Arm-locating TB Cross	Summary of hypoploid ears	floret # / spikelet in hypoploid tassels	Mutants on Same Chromosome with Similar Phenotype
rgo2-Vl	TB-1Sb	3 ears all rgo: 2 ears some rgo: 7 ears some rgo	3 3 2	
rgo2-Vl	TB-1La	10 ears all rgo: 11 ears all rgo: 1 ear some rgo: 5 ears some rgo	3 2 3 2	*Ts6, ids1*
rgo2-Vl	TB-7Sc	16 ears all +rgo: 5 ears some rgo	2 2	
rgo2-Vl	TB-9Lc	26 ears all +rgo: 8 ears some rgo	- -	*rgo1* maps to 9L

Crosses were then made of *rgo2-Vl* with *Vg1*, *Kn1* and *Ts6* on chromosome 1 to confirm chromosomal arm placement. These results are summarized in Tables 2-4. The linkage test was set up as a backcross as indicated. Kernels from the backcross ears were planted in the field and the resulting plants were scored for the chromosome 1 markers and self-pollinated. The resulting ears were scored for reversed germ orientation. Tight linkage was obtained with *Ts6* placing *rgo2-Vl* on chromosome 1L very close to *Ts6*.

Table 2:
2 point linkage data for *rgo2-Ts6*

Testcross: [*Rgo2 Ts6* x *rgo2 ts6*] x *rgo2*

Region	Phenotype	No.	Totals
0	Ts +	84	
	+ rgo	97	181
1	Ts rgo	5	
	++	2	7

% recombination *rgo2-Ts6* =3.7±1.4

Table 3:
2 point linkage data for *rgo2-Kn1*

Testcross: [*Rgo2 Kn1* x *rgo2 kn1*] x *rgo2*

Region	Phenotype	No.	Totals
0	Kn +	60	
	+ rgo	62	122
1	Kn rgo	23	
	++	28	51

% recombination *rgo2-Kn1*=29.5±3.5

Table 4:
2 point linkage data for *rgo2-Vg1*

Testcross: [*Rgo2 Vg1* x *rgo2 vg1*] x *rgo2*

Region	Phenotype	No.	Totals
0	Vg +	25	
	+ rgo	46	71
1	Vg rgo	22	
	++	13	35

% recombination *rgo2-Vg1* =33.0±4.6

Another mutation with a similar phenotype (*indeterminate spikelet1=ids1*) has been mapped to 1L with recombinant inbred lines (George Chuck et al., Genes & Development 12:1145-1154, 1998). According to Ben Burr, *ids1* maps to the long arm of chromosome one and showed zero recombination with RFLP marker *chi1*, which is out on 1L, 15 cM distal to *Ts6* (George Chuck, personal communication).

The *rgo2* phenotype is similar to the *ids1* phenotype in the tassel and they both give reversed kernels. Southern blots using

ids1 as a probe suggested that *rgo2* is an allele of *ids1* (Nick Kaplinsky et al., Maize Genetics Conference Poster Abstracts 41: 1999).

Crosses were then undertaken to test for allelism between *ids1* and the two *rgo2* alleles. Results are presented in Table 5. The allelism crosses were between plants homozygous for *rgo2* and heterozygous for *ids1*. In both cases crosses gave a good 1:1 segregation indicating a positive allelism test. In further crosses the mutant segregated out in good 3:1 ratios as would be expected. We suggest the proposed new designations for these two new alleles of *ids1*.

Table 5:

Allelism tests of *ids1* and *rgo2*

previous designation	allelism test with +/*ids1*	self of + ear from Allelism Test	self of rgo ear from Allelism Test	proposed new designation
rgo2-VI	38 + : 32 rgo (1:1)	5 + : 2 rgo (3:1)		*ids1-rgo2-VI*
rgo2-Burr	32 + : 36 rgo (1:1)	11 + : 4 rgo (3:1)	0 + : 27 rgo (all mut)	*ids1-rgo2-Burr*

URBANA, ILLINOIS
University of Illinois
GAINESVILLE, FLORIDA
University of Florida

Transposon tagging of nuclear genes that control mitochondrial gene expression

--Gabay-Laughnan, S, Chase, CD

The S system of cytoplasmic male sterility (CMS-S) in maize presents an unprecedented opportunity for the identification, cloning and functional characterization of nuclear genes regulating mitochondrial gene expression in a higher eukaryote. In this system, expression of a novel chimeric gene in the mitochondria results in the collapse of starch-filling pollen and, consequently, a male-sterile phenotype. Loss-of-function mutations in nuclear genes required for mitochondrial gene expression behave as restorer-of-fertility (*Rf*) alleles, blocking expression of the chimeric mitochondrial gene and the male sterility trait. Such mutations are visible in pollen because it is haploid. *Rf* alleles also block expression of essential mitochondrial genes These mutations are tolerated in pollen because late-stage pollen development and pollen germination do not require high levels of mitochondrial gene expression or function.

Based upon these observations, we propose that *Rf* alleles for CMS-S maize can be generated by transposon mutagenesis and cloned via transposon tagging. These mutations should be recovered at high frequency due to the large number of nuclear genes involved in mitochondrial gene expression. The recovery of new *Rf* alleles by transposon mutagenesis is being approached by screening populations of male-sterile, S-cytoplasm plants carrying active *Ac-Ds* or *I-En* (*Spm*) transposons. Pollen from plants with male-fertile tassels or tassel sectors will be used to fertilize the ears of CMS-S tester plants for recovery of the new *Rf* alleles.

To demonstrate the feasibility of this transposon mutagenesis, we conducted a preliminary screen of 1,241 CMS-S plants carrying an active *Ac* element in chromosome 9 and a *Ds* reporter at the *a1* locus (*a1-m4*). This screen was carried out by Chase and Gabay-Laughnan in Gabay-Laughnan's 1999 summer nursery at the University of Illinois, Champaign-Urbana. The screening population was developed in the Mo17 inbred background, and the plants were derived from spotted kernels indicative of *Ac* activity. Twenty-four plants had visible sectors of male fertility on otherwise male-sterile tassels. Pollen samples from each sector were examined through a field microscope and observed to consist of starch-filled and collapsed grains in roughly equal proportions. This demonstrated that each sector resulted from a nuclear mutation. (Sectors resulting from cytoplasmic mutants consist entirely of starch-filled pollen). Of the 24 sectors, 12 were large enough to be used for testcrosses. Testcrosses from 10 of the 12 plants resulted in seed set. In CMS-S maize, only *Rf* pollen is functional. All of the testcross progeny are therefore expected to carry an *Rf* allele. This will be confirmed in our 2000 summer nursery.

Our preliminary observations indicate that we have recovered 10 independent *Rf* alleles from 1,241 plants. This high rate of mutation is consistent with the large number of nuclear genes known to regulate mitochondrial gene expression in yeast. Most independent *Rf* alleles are expected to result from mutations at different nuclear loci. This will be confirmed through allelism tests. The high rate of mutation in the Mo17-S *a1-m4* plants contrasts with the mutation rate in Mo17-S plants lacking an active *Ac*. Over the past five years, we have examined over 2,000 Mo17-S plants and recovered only two sectors of male fertility. The *Ac-Ds* system is almost certainly responsible for the *Rf* alleles recovered in the Mo17-S *a1-m4* materials. This will enable us to recover molecular clones of these alleles.

In summary, observations made in Gabay-Laughnan's summer nursery provide a strong indication that we will efficiently recover new *Rf* alleles from CMS-S plants carrying active transposable element systems. If, as expected, the majority of mutations are transmitted to the next generation and result from mutations at different loci, the recovery of mutants will not be a limiting factor in our study. We are hoping to clone on the order of 10 different *rf* loci. Given the high-throughput methods that are now being applied to clone transposon-tagged loci, our target is very reasonable. Indeed it may be possible to clone many more loci.

The value of the molecular clones we will recover is significantly enhanced by our ability to examine the effects of mutations at *rf* loci on the expression of mitochondrial genes in developing maize pollen. The mutants and gene sequences derived from this project will provide an invaluable resource for the future genetic and molecular dissection of mitochondrial function in higher organisms.

Chromosome location of two Oh51A CMS-S restoring alleles and their potential usefulness in the molecular cloning of *rf* loci

--Gabay-Laughnan, S, Chase, CD

Gabay-Laughnan has been searching for exceptional male-fertile plants in a number of CMS-S inbred line backgrounds for three decades. The inbred background exhibiting the highest frequency of nuclear reversion is Oh51A. Twenty-four restorer-of-fertility (*Rf*) alleles have been identified from 2,635 Oh51A-S plants, a rate just under 1%. Six new *Rf* cases arose in the 476 Oh51A-S male-sterile plants grown in the summer of 1999. Heritability and transmissibility of these *Rf* cases will be determined in our 2000 summer nursery.

We are using the *wx*-marked translocation series to map new *Rf* alleles to chromosome (Laughnan and Gabay-Laughnan, The Maize Handbook pp. 255-257, 1994). We have previously mapped three of the newly arisen Oh51A *Rf* alleles to chromosomes 3, 6 and 8 by these means (Gabay-Laughnan, Maydica 42:163-172,

1997) and will be using RFLP marker analysis to more accurately estimate their relative positions on their respective chromosomes. We report here that an additional two Oh51A *Rf* alleles have been located to chromosome. *Rf* 81-94-5 is on chromosome 2 according to our crosses with *wx* T2-9c. *Rf* 91-1066-3 has also been mapped to chromosome 2, but through use of *wx* T2-9d. These two newly mapped restorers are of special interest to us since the standard CMS-S restorer, *Rf3*, maps to the long arm of chromosome 2 (Laughnan and Gabay, Maize Breeding and Genetics pp. 427-447, 1978; Kamps and Chase, Theor. Appl. Genet. 95:525-531, 1997).

We hypothesize that the high rate of nuclear reversion in the Oh51A nuclear background reflects the presence of an active transposable element system. If that is the case, at least some of the new Oh51A *Rf* alleles may be tagged with a transposable element. Since molecular probes are available for the maize transposable element systems *Ac-Ds*, *I-En* (*Spm*) and *Mu*, and tester stocks are available to determine the presence of active *Ac*, *En* (*Spm*) and *Mu* elements, we have begun screening Oh51A for the presence of these three elements by genetic means. The Oh51A inbred line has been crossed with *Ac*, *En* (*Spm*) and *MuDR* tester stocks. The resulting F1s will be back-crossed by the appropriate tester stocks in Gabay-Laughnan's winter nursery. When mature ears are harvested, they will be analyzed for the kernel phenotypes indicative of active transposable elements. If Oh51A carries active *Ac*, *En* (*Spm*) or *Mu* transposons, we will generate segregating populations of CMS-S *Rf/rf* and CMS-S *rf/rf* plants for each independent *Rf* allele recovered in this background. These segregating populations will be screened for transposable element sequences that co-segregate with *Rf* alleles. Those populations will be used for the molecular cloning of *rf* loci.

VARANASI, INDIA
Indian Institute of Vegetable Research

Attributes of maize genotype for baby corn production

--Kumar, S, Kalloo, G

Baby corn is a diversified product of the maize plant, where baby ears are harvested before fertilization and consumed as a fresh or canned vegetable. In fact, young dehusked ears of maize have been eaten as a vegetable by the farmers for a long time in Thailand, Taiwan and China. In the recent past, it is a modern food habit, which accepted tender-dehusked ears as a cooking ingredient for use in salad, soup, pickle and several Chinese preparations. The canning industry has made baby corn even more important as an industrial crop in countries like Thailand, where baby corn production technology is a success story. The success can be visualized by observing the export figures of canned baby corn, which increased dramatically from 67t, worth US $38,059 in 1974 to 36,761 t, worth US $33 million in 1992 (Paroda & Chamnan. "Baby corn production technology in Thailand. A success story". APARI publication, 1994). Thailand is the world leader in the export of both fresh and canned baby corn and the world market for fresh and canned baby corn is expanding very rapidly (Jason. "World market for fresh and canned baby corn". RAP market information Bulletin No. 5, 1995). In many other countries like Taiwan, Sri Lanka, Indonesia, India, Zambia, Zimbabwe, South Africa, Guatemala, Nicaragua, etc., baby corn production technology is gaining ground and baby corn is emerging as an export oriented vegetable. Baby corn production technology consists of several components like specific maize variety/hybrid, specific production practices, post harvest practices etc. Among these, the most important one is specific maize variety/hybrid suitable for baby corn production. Albeit any kind of maize genotype can be grown for baby corn production in the kitchen garden, for commercial purpose a variety should be characterized by certain following genetic traits.

Early maturity: Among the hierarchy of baby corn varietal attributes, earliness is one which attracts vegetable growers habituated to harvesting short duration crops. Earliness of the variety not only provides the opportunity to take baby corn as a catch crop, but also helps the crop escape many of those biotic and abiotic stresses which appear after the flowering stage. Plants of an ideal baby corn variety should produce all the ears (of silk emergence stage) between 45 to 55 days of duration.

Prolificacy (more number of cobs/plant): Ideal plant ideotype for dent corn/field corn (corn for grain purpose) is two ears per plant with maximum number of bold kernels. But for baby corn production, an ideal plant should bear at least three ears per plant without losing quality, size and shape of young ears. In corn, prolificacy is highly influenced and negatively correlated with the planting density. Therefore, with respect to prolificacy, the variety should not only bear more cobs but also it should be tolerant to high density planting. Selection of small tassel may be the one criterion to select genotypes tolerant to high density planting. Furthermore, the advice of Dr. Galinat for yield improvement of field corn through increased plant density (Galinat. "Canopy and yield enhancement per acre with dense populations". MNL 70:67,1996; Galinat. "Reverse maize breeding for high density populations". MNL 73:91, 1999), can also be taken into account by the baby corn breeders.

Synchronized ear emergence: Synchronized earing reduces the harvesting and storage cost of young ears drastically. Therefore, for commercial production purposes, the variety should be preferably a single cross hybrid. Even in certain single cross hybrids, synchronization can not be achieved, especially during second or third ear emergence. This problem can be solved either by developing baby corn hybrids having an additional attribute in the form of sugary (*su1*)/shrunken (*sh2*) gene(s) or over-sized ears can be harvested for grain production. In the former case, over sized ears can be harvested 15-18 days after fertilization (at milking stage)as sweet corn. However, this alternative is less attractive because there is no taste advantage in using a sweet corn variety over a dent corn variety for baby corn production, as immature ears are harvested and at this stage sugar is not accumulated in kernels. Further, crop and seeds of sweet corn varieties are more prone to certain insects and storage pests.

Yellow kernel: Yellow immature kernel with uniform row arrangement is the prescribed standard for the international market. Hence, a yellow seeded corn variety fulfills one of the criteria for a baby corn cultivar. Plant height: Plant height and ear height on the plant affect ease of picking. Optimum plant height varies from 2 to 2.5 m with the preferable height of lower and upper ears at least 1.5 ft and not more then 6 ft, respectively. Proper ear height on the plant reduces the cost of picking, which requires hand labor.

VIÇOSA, BRAZIL
Universidade Federal de Viçosa

Cytogenetic mapping of sister chromatid exchange points in maize
--Borges, CMO , Carvalho, CR

In order to map the sister chromatid exchange (SCE) points in maize, image analysis resources by computational methods (Rasband. NIH-image 1.60 anonymous ftp from zippy.nimh.ninh.gov) were used in association with cytogenetic techniques. Maize seeds from the test line from Universidade Federal de Viçosa were germinated and the roots treated with a 100 µM 5-bromo-2`-deoxyuridine solution for 22 hours and then with a 0.02% colchicine solution for 2 to 3 hours, both done in the dark at 28 C. The roots were fixed in a methanol:acetic acid (3:1) solution and slides were prepared by the air-drying technique after enzymatic maceration (Carvalho, Saraiva. Heredity, 70:515-519,1993). The preparations were stained with Hoechst 33258 for 10 minutes, irradiated with a UV-254 nm light for 5 to 15 minutes and stained with a 2% Giemsa solution, for 10 minutes (Perry, Wolff. Nature, 251,156,1974). Images of chromosome figures were captured directly from the microscope by means of a video camera coupled to a computer and digitized with an image analysis software. To generate 256 gray value density profile plots, chromosome images were calibrated to the range of 0 (white) to 255 (black) and spatial calibration from chromosome length was performed in micrometers. This methodology permitted the identification of the SCE points with enough resolution to measure the relative and absolute positions of the exchanged segments (Fig. 1). These results were used to elaborate the maps of the positive and negative regions of the SCEs.

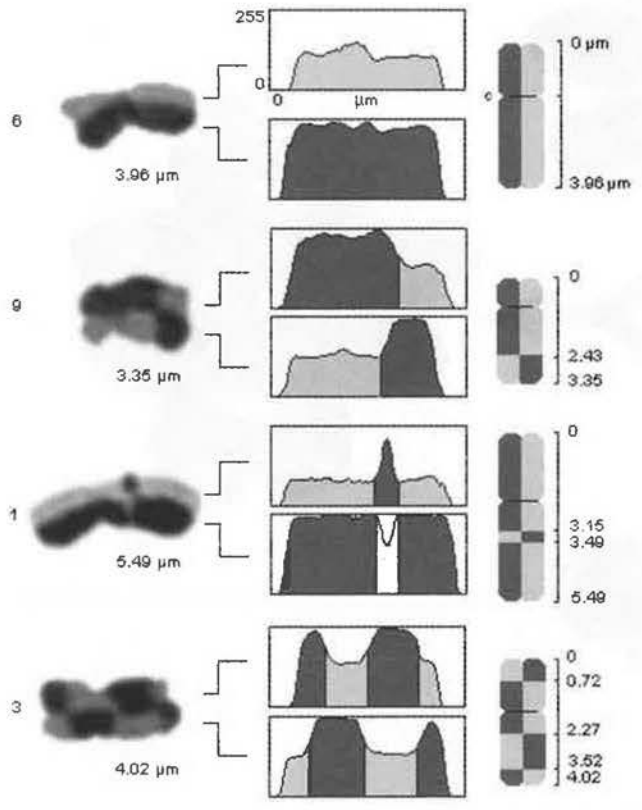


Figure 1. The plot pixel value of 256 gray scale of sister chromatid exchanges in maize chromosomes (numbers: 6, 9, 1 and 3). The background picture was set to a 0 gray value and chromosome length in micrometers. C = centromere.

WALTHAM, MASSACHUSETTS
University of Massachusetts

Is the recombination of genes during natural domestication an act of genetic engineering?
--Galinat, WC

Has Fink (1999, Web Access Excellence, About Biotech [http://www.accessexcellence.org/AB/WYW/fink/fink_3.html]) incorrectly redefined the use of the term recombination (or recombinant) in order to serve an artificial agenda? The term recombination was first used by Darwin to describe the power of the breeder during the domestication of plants and animals and by Mendel to describe genetic recombination during segregation of various traits in the Garden Pea.

According to Fink, recombination means genetically engineered and, since most food has undergone human-directed recombination of mutant genes during domestication, we would starve to death without Fink's genetic engineering. He states, "Of course they (the restaurants) will serve recombinant corn because it is the only corn we have."

I may add the only sexual-based life we have on Earth is recombinant life. In an appeal to those who have a fear reaction to the unknown breeding created or modified by genetic engineering, I quote from Franklin Roosevelt: "The only thing we have to fear is fear itself." To those who respond to logic, I ask questions. If it is natural evolution for flu viruses to pick up human genes that trigger sneezing for the sake of virus survival, isn't it also natural evolution for humans to use transgenic genes for the sake of human survival? Are we just another living thing or are we a unique life form capable of amazing grace, of cultural evolution and of travel beyond our planet of origin? Are we destined to understand and populate the Universe while we have a chance before we become extinct and our "Pale Blue Dot" non-existent?

Does a good goal justify bad means? The botanical terminology Fink uses is incorrect, such as his misuse of the term spike to refer to spikelet, each morphologically different and genetic-pathway different. He incorrectly plagiarizes (and allows recopyrighting without permission) my drawing showing the role of recombination in the domestication of teosinte leading to the origin of corn, already copyrighted by the University of Chicago Press in my article "Corn, Columbus and Culture," Perspectives in Biology and Medicine 36:1-12. 1992. My drawing in Fig. 2 on page 5 of the article is described in correct botanical terminology.

Fink's confusion between spike and spikelet may be corrected in the dictionaries and glossaries of grasses as follows:

> A spike is a head (ear) of grain (grass seed) bearing many spikelets sometimes in many rows (ranks) borne around the main axis (rachis) of a compound inflorescence. In the case of corn, the entire ear (spike), sometimes bearing 1000 kernels, may be bracted (enclosed) by husks borne below on the shank.
>
> A spikelet is bracted by two glumes. The axis (rachilla) of the spikelet may carry several florets, with each bracted by a lemma on the outer side and a palea next to the rachilla. The spikelets may be paired as in both the sep-

arate male and female inflorescences of corn, or they may be single in just the female spikelets of teosinte (corn's wild ancestor).

The recombinant of two different double rowing pathways quadruples the kernel row number per ear (spike) during the domestication of maize from teosinte

--Galinat, WC

The importance of my 1992 cross-sectional drawing of spikelets and spikes during the first recombinant jump of teosinte domestication toward maize has been recognized by others (Fig. 2 below).

Although the selection that transformed teosinte into maize was probably deliberate domestication for increased food supply, some of the selection may have been unconscious because the more productive seed ears would contribute more progeny (Galinat. 1995:The origin of maize: Grain of humanity. Economic Botany 49:3-12).

The diagrams represent: (a) the ancestral teosinte; (b)a four rowed mutant teosinte from two ranks of paired spikelets; (c) a four rowed mutant teosinte from four ranks of single spikelets; (d)The double mutant teosinte derived from the hybrid is eight-rowed corn.

The cupules with spikelets are from two successive nodes, with the lower ones in surface view. The upper ones are shown in solid black from cross sections revealing some details of rachillae, spikelets, and kernels. The single spikelets are illustrated in a more reflexed position than normal in order to reveal their sessile condition, in contrast with the pedicelate spikelet activated as a key corn trait.

The art work shown above on the two morphological pathways of teosinte transformation leading to maize is from my original balanced representation while the caption is directly from the published paper (Galinat, W.C.1992 Corn, Columbus, and Culture. Perspectives in Biology and Medicine 36:1-12). The art work as published became unbalanced because the truly great editor, Richard L. Landau, had to condense the spacing between items to obtain more room for my poem, sent in late after the page proofs were already printed.

Epilog to figure 2, Corn, Columbus and Culture.

Here you see how art and poetry
became the communication key
to render information on recombination
between mutants of teosinte
in the amazing creation of maize.

It is tragic that editors of most scientific journals and their reviewers deplore and often refuse combinations of art and poetry in their publications. The general public knows that art and poetry are the best tools for communication.

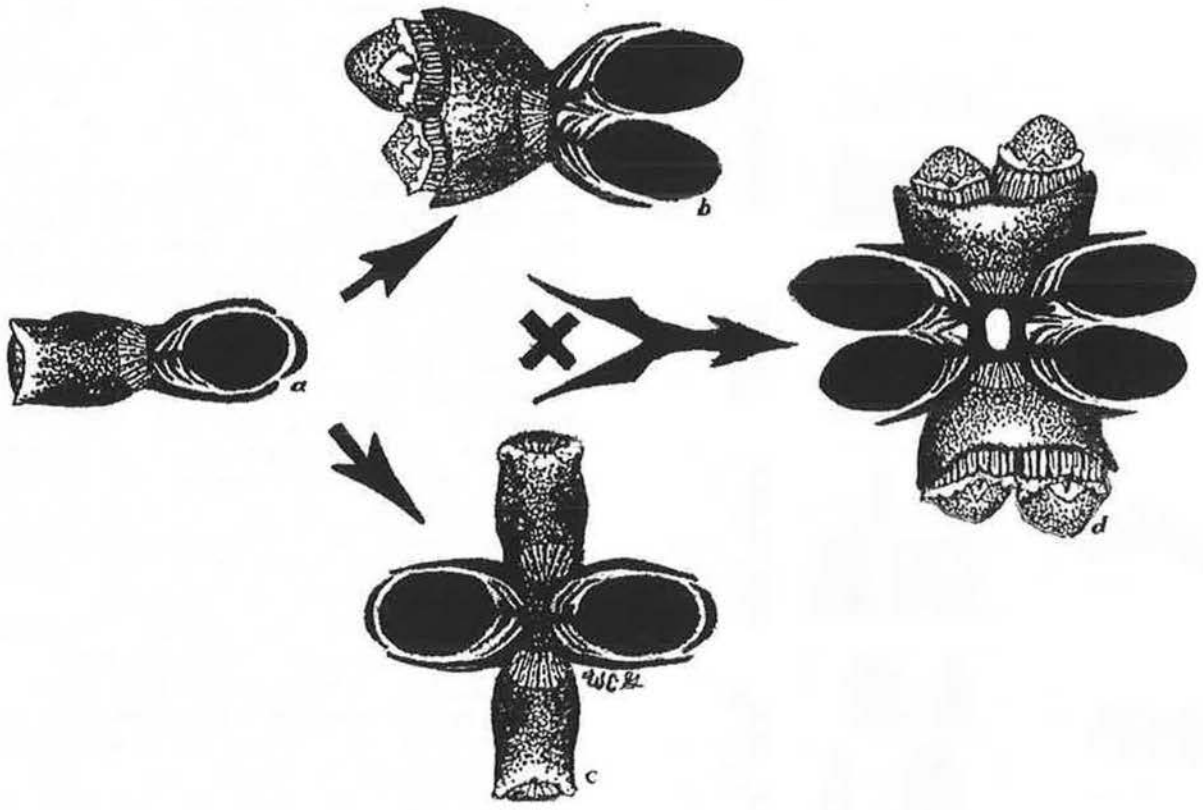


Figure 2—The transformation of teosinte into corn:
Here you see, how two kinds of four-rowed teosinte,
became the key, to corn's pedigree
from their hybrid one morn, the first corn was born,
as an amazing creation from recombination.

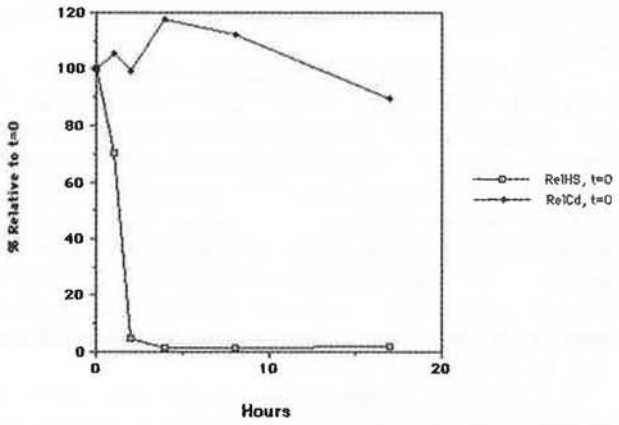
WOOSTER, OHIO
The College of Wooster and Ohio Agricultural Research & Development Center
LONDON, ONTARIO
The University of Western Ontario

Persistence of 18kDa HSP mRNA accumulation in metal-ion insulted maize seedling radicles relative to heat shock

--Bouchard, RA, Yang, Z, Walden, DB

We reported previously that maize root tips insulted with the heavy metals Cd and Zn accumulate mRNAs encoding 18kDa HSPs (shsp18s) in the epidermal and cortex regions of the root tip (Yang and Walden, MNL 71: 55, 1997), and more recently relative quantification of the mRNA levels (Bouchard, Yang and Walden, MNL 72: 85, 1998). We have now examined the relative levels of shsp18 mRNAs during recovery from cadmium stress versus heat shock using quantitative RNA-Dot hybridization.

Seedling growth, heat shock (HS), and heavy metal-ion insults were performed as reported in the notes cited above, while RNA extraction, filter-binding, preparation of labeled DNA probes, hybridization conditions, scanning, and quantification were as described in Bouchard et al., 1993. Maydica 38: 135-144). Seedlings subjected to heat shock at 40C were shifted to 25C at the end of the 2 hour period and held on damp filter paper until harvest. Seedlings incubated in $CdCl_2$ solution were rinsed in sterile distilled water for 15 minutes with gentle agitation, then shifted to damp filter paper at 25C until harvest. The RNA Dots were probed with the insert fragment from plasmid pscMHSP18-9-2, a sub-clone containing the ORF of clone pMHSP18-9 (map designation *uwo11*), which is a common probe for mRNAs from all members of the maize shsp gene family. The results, relative to the signals observed after each treatment in radicles harvested immediately after the period of stress set to 100%, are shown in the accompanying graph.



These results reveal an interesting difference in the persistence of relative RNA levels after the two types of stress treatment. Once heat shock ends, shsp18 mRNA levels decline quickly, and are already close to baseline after only two hours. By contrast, the overall levels of shsp18 mRNA remain essentially unchanged for many hours after cadmium insult.

It is possible that substantial amounts of cadmium remain in the tissues of seedlings even after the 15-minute rinse that follows treatment with the metal. However, the perdurance of shsp18 mRNA following cadmium insult would still contrast with what is seen with prolonged heat stress. As reported in Greyson et al. (1996, Developmental Genetics 18: 244-253), under continuous heat shock shsp18 mRNAs essentially decline to baseline levels after eight hours. It is thus likely that the control mechanisms modulating shsp-gene response to metal stress are at least partially distinct from those governing their response to heat stress.

WUHAN, CHINA
Wuhan University
BEIJING, CHINA
Chinese Academy of Sciences

Identification of *Zea diploperennis* chromatins introgressed to maize via genomic in situ hybridization

--Wang, L, Song, YC, Ning, SB, Liu, LH, Gu, MG, Guo, LQ

Zea diploperennis (DP) is a new teosinte found by Iltis et al. (1979) from Mexico. It shows many important traits, such as disease and pest resistance, and adaptation to extreme, stressful environmental conditions. DP and maize are close relatives. They not only have the same chromosome number but also show similarities in size and morphology for most of the chromosomes (Pasupuleti and Walton, 1982). DP is interfertile with maize (Iltis et al. 1979). Therefore, it provides geneticists and maize breeders with a potentially valuable source of germplasm (Iltis et al. 1979).

Guo et al.(1997 and 1998) crossed maize inbred line Zi 330 with DP, and made backcrosses of the hybrids with Zi330. Parthenogenesis was successfully induced in the BC1 by chemicals and the alloplasmic pure line 540 was obtained at the second generation of chemical-induced parthenocarpy (Pa2)(Guo et al. 1998). The pure line 540 was proliferated by inbreeding and crossed with maize inbred line Zi 50003. The F1 (the first hybrid generation) named Yi Dan 6 has already been applied to maize production in China. Both 540 and the F1 hybrids, Yi Dan 6, showed much stronger *Helminthosporium turcicum* and *Helminthosporium maydis* resistance and wider adaptation to stressful conditions, compared with the maize parents and the F1 intraspecific hybrids of maize (Guo et al.1997). It could be deduced that the DP chromatin dictating these characters had been introgressed into the parthenocarpic progenies (Pa2), the 540 should be an alloplasmic pure line and it had transferred the introgressed chromatin to its F1 hybrids, Yi Dan 6.

The karyotypes and chromosome C-banding patterns among maize Zi330, DP and the alloplasmic pure line 540 have already been analyzed comparatively (Yang et al., 1995; Yan et al., 1997). Although some chromosome variations of 540 were found in their studies, it was still unknown whether the DP chromatin was introgressed into the parthenocarpic progenies or not and if it was, where it was located. GISH using total genomic DNA as a probe to identify alien chromosomes or chromosome segments was well-established in many other plant species (Le et al. 1989; Heslop-Harrison et al 1990; Leitch et al. 1990; Mukai and Gill 1991; Schwarzacher, et al. 1992; Anamthawat-Jonsson and Heslop-Harrison 1993.; Kenton, et al. 1993; Mukai and. Nakahara 1993; Yasuhiko and Yumiko 1993; Chen and Armstrong 1994; Jellen et al. 1994; Jiang and Gill 1994; King et al. 1994; Fernandez-Calvin et al. 1995; Humphreys et al. 1995; Jacobsen, et al. 1995; Leggett

and Markhand 1995; Keller et al. 1996; Zhong et al. 1996; Garriga-Caldere et al. 1997; Pickeing et al. 1997; Tang et al. 1997; Belyayev and Raskina 1998; Chen et al. 1998; Mikhailova et al. 1998; Stevenson and Armstrong 1998; Kamstra et al. 1999), while, as we know, no report about this respect has been published yet in the distant hybrids of crosses of maize with DP. In this study, the introgressed DP chromatins were detected and located in both the pure line 540 and Yi Dan 6 by genomic in situ hybridization.

Zea diploperennis (DP) was supplied by Mr. Li Dongyu, Guangxi Zang Autonomous Region of China. Maize Inbred lines Zi330, alloplasmic pure line 540 and the F1 hybrids of 540 X Zi 50003, Yi Dan 6, were supplied by Institute of Genetics, Chinese Academy of Sciences, Beijing. Their pedigree is given in Fig. 1.

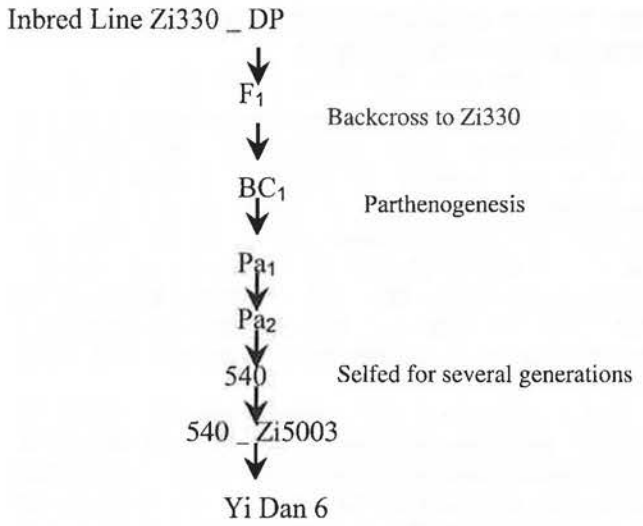


Figure 1. Brief illustration of the relationships between the tested materials.

Genomic DNA was extracted from DP and inbred line 330 following the procedure described by Doyle and Doyle (1990). The total genomic DNA from DP for use as labeled probe was mechanically broken to 10-12kb fragments prior to labeling, and the total genomic DNA from 330 was fragmented to pieces about 100-250bp by autoclaving for 5min and used as blocking DNA according to the procedures reported by Heslop-Harrison et al. (1990), Schwarzacher et al. (1992), and Jacobsen et al. (1995).

The preparation technique was that described by Song et al. (1994) with some modification. The harvested root tips were pretreated in saturated α-bromonaphthalene solution for 2hr at room temperature (RT). After rinsing in tap water, the root tips were fixed in fresh fixative (3 methanol: 1 glacial acetic acid) for 2-3hr at RT and then, digested in 1% cellulase and 1% pectinase (SERVA) at 28 C for about 2.5hr. The softened tips were subsequently rinsed gently with distilled water. Following removal of the water, 3-4 root tips were smeared with fixative onto a clean slide. The slide was flame-dried.

Nick translation of DP genomic DNA was adopted with the kit offered by Sino-American Biotechnology Company, China. 15μl of dNTP (mixture of average amount of dATP, dCTP and dGTP), 5μl of 10X buffer, 4μl of DP genomic DNA, 5μl of Bio-11-dUTP and 5μl of DNAse1 and DNA polymerase1 mixture, and ddH_2O were added to total volume 50μl. It was incubated at 15 C for 2hr, and the reaction was stopped by adding 5μl of 0.2M EDTA. The labeled probe was purified by passing the reaction solution through a Sepharose CL-6B (Sigma) column.

The procedures of in situ hybridization and signal detection followed the modified methods published by Leitch and Heslop-Harrison (1993), Schwarzacher et al. (1992), Schwarzacher (1994) and Jacobsen et al. (1995). The hybridization mix was 42μl in the total volume and it contained 1.0μg of probe (0.2μg/μl), 50% deionized formamide (Sigma), 10% sodium dextran sulphate (Sigma), 4.2μg ssDNA and 2XSSC, then mixed with excess blocking DNA of inbred line 330 which was 10-50μg respectively based on different tested ratios. Subsequently the hybridization mixture was denatured at 100 C for 10min, immediately chilled on ice for 10min. Hybridization was performed overnight at 37 C. The slides were then washed in formamide (20%), 2XSSC, 0.1XSSC, at 42 C for 15min each step, and subsequently washed in 0.1% TritonX-100, PBS at RT, for 5min each step.

DAB detection was performed according to the procedure outlined by Gustafson et al. (1990), Gustafson and Dille (1992).

The procedure included the following steps: 1) after the washes, the slide was covered with 30 μl (10 ng/μl) anti biotin FITC conjugate developed in goat (Sigma) and incubated in a humid chamber at 37 C for 45 min and the slides were washed 3 times, each for 5 min in PBS at RT; 2) subsequently covered with 30μl (11ng/μl) anti-goat-IgG conjugated with biotin (Sigma) instead, and incubated in a humid chamber at 37 C for 45 min and the slides were washed 3 times, each for 5 min in PBS at RT; 3)repeated step 1; 4) the slide was mounted in 20μl p-phenylenediamine dihydrochloride(10μg/ml) containing 3μg/ml propidium iodide (PI). Slides were analyzed using an Olympus fluorescence microscope.

An average of the hybridization site measurements was taken by calculating the distance from the centromere to the detection site and using that as a percentage of the arm on which the site was located. The arm ratio of the chromosome showing a detection site was also measured in order to determine on which chromosome the site was located. The standard deviations for both the percentage distance of the hybridization site and the arm ratio were calculated.

Different blocking ratios of the labeled DP genomic DNA: unlabeled maize Zi330 genomic DNA including 1:10, 1:20, 1:30, 1:40, and 1:50 were tested. Many scattered signal spots were distributed irregularly on different chromosomes and interphase nuclei of the tested pure line 540, Yi Dan 6 and the control, maize Zi330 with the lower blocking ratio including 1:10 and 1:20. The signals were not easy to detect even on chromosomes of the tested 540 and Yi Dan 6 with the higher blocking ratios 1:40 and 1:50. The optimum blocking ratio is 1:30. The following regular and stable results were observed through the eight repeated experiments with the blocking ratio of 1:30.

The genomic in situ hybridization signals of the pure line 540 appeared on the long arm of chromosomes 1 (Fig. 2A and C) and 8 (Fig. 2A), and chromosomes 2 (Fig. 2B) and 5 (Fig. 2A, 2B and C). Their detection rates were 10.10%, 11.15%, 8.01%, and 13.24% respectively and the percentage distances from centromeres to the hybridization sites were 86.3±4.5, 68.3±4.4, 12.2±2.5, 73.7±3.0 for the long arms of chromosomes 1, 2, 5, and 8 respectively (Table 1 and Fig. 2). In some mitotic cells, the signals showed simultaneously on the two members of the detected homologous chromosome pairs 1, 2, 5, and 8, each of the two mem-

Table 1. Introgressed chromatin location and arm ratio average of the chromosomes detected by GISH with the blocking ratio of 1 probe: 30 Zi330 DNA

Materials	arm	DAB detection			FISH detection		Average percent distances of DAB and FISH detection (%)	Ratio of long to short arm
		No. of detection	Detection rate (%)	Percent distances (%)	No. of detection	Percent Distances (%)		
Alloplasmic pure line 540	1L**	29	10.10	90.23±8.54*	5	82.42±4.45	89.08±8.03	1.18±0.03*
	2L	32	11.15	73.77±3.48	9	62.85±7.56	71.37±4.32	1.48±0.05
	5L	23	8.01	14.25±1.75	6	10.23±3.32	13.42±2.04	1.04±0.02
	8L	38	13.24	75.02±5.01	8	72.41±1.12	74.57±4.39	3.05±0.04
Yidan6	1L	13	6.07	81.18±6.72	4	78.24±7.78	80.49±6.93	1.27±0.04
	2L	12	5.61	64.29±7.26	6	56.56±8.44	61.71±7.62	1.46±0.07
	8L	15	7.01	48.85±5.52	6	41.61±3.49	46.78±4.99	2.98±0.02

* Standard deviation, ** Long arm

bers of chromosome 8 showing the signals at the same region can be visualized in Fig. 2A.

The hybridization signals of Yi Dan 6 showed on the long arm of only one of two members for each of chromosome 1 (Fig. 2D), chromosome 2 (Fig. 2E), and chromosome 8 (Fig. 2F). No chromosome pair showed the signals simultaneously on their two homologues in all the mitotic cells observed. The detection rates of the signals were 6.07%, 5.61%, and 7.01%, and the percentage distances of the hybridization sites were 80.7±3.3, 60.4±2.9, and 45.2±4.4 for chromosomes 1, 2, and 8 respectively (Table 1). The control, maize, Zi330 did not show any hybridization signals on its chromosomes.

The signals observed by our fluorescence detection procedure showed yellow-green, while the chromosomes were red (Fig. 3). The blocking ratio was 1 labeled DP genomic DNA : 30 unlabeled maize Zi330 genomic DNA, just like that of DAB detection. The hybridization signals of the alloplasmic pure line 540 appeared on the long arm of chromosome 2 (Fig. 3A), chromosomes 1 and 5 (Fig. 3B and C), chromosome 8 (Fig. 3C). In some mitotic cells, the signals showed simultaneously on the two homologues of the detected chromosomes 1, 2, 5, and 8. Each of the two members of chromosome 2 showing the signals at the same region can be seen in Fig. 3A. The percentage distances from centromeres to the hybridization sites were 82.42±4.45, 62.85±7.56, 10.23±3.32, and 72.41±1.12 for the long arms of chromosomes 1, 2, 5, and 8 correspondingly (Table 1).

Compared with the results obtained by DAB and FISH detection systems, the chromosomes introgressed by DP chromatins were the same and the signal positions showed on the detected chromosomes, the percentage distances were very close to each other and had no significant differences statistically in both the pure line 540 and Yi Dan 6. Therefore, we have constructed the cytogenetic maps of the introgressed segments in both pure line 540 and Yi Dan 6 with the average value of the data obtained by two kinds of detection methods (Table 1 and Fig. 4).

The detection rates observed by FISH were about 30% for each hybridization site of the pure line 540, and about 20% for Yi Dan 6 with the blocking ratio 1:30. There are much higher than those by DAB detection.

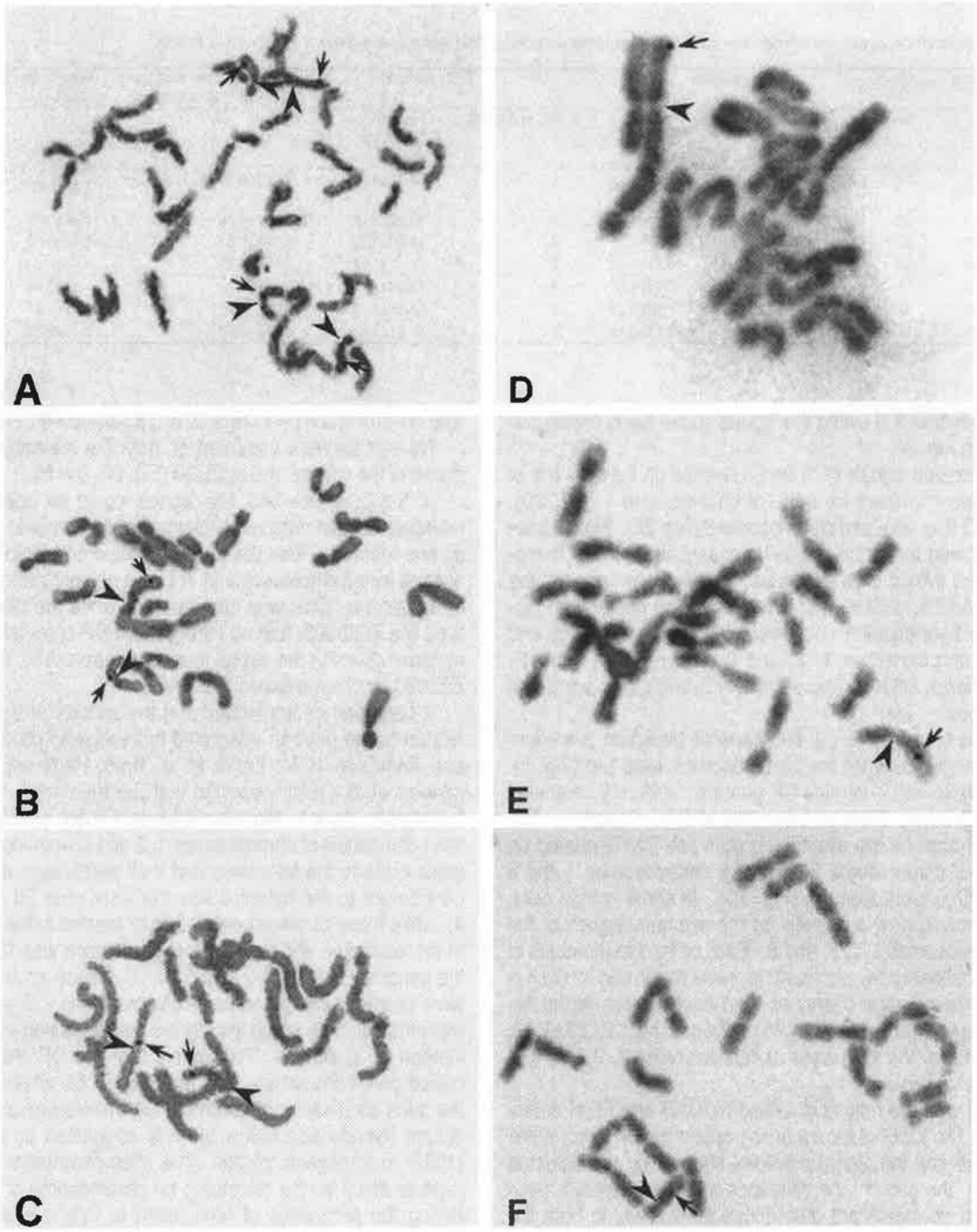
The hybridization signals of Yi Dan 6 showed on the long arm of chromosome 2 (Fig. 3D), chromosome 1 (Fig. 3E), and chromosome 8 (Fig. 3F). The signals only appeared on one member of each detected chromosome pair and no signals on the two members of a chromosome pair were observed simultaneously in all the mitotic cells observed. The percentage distances of the hybridization sites were 78.24±7.78, 56.56±8.44 and 41.61±3.48 for the long arms of chromosomes 1, 2, and 8 respectively (Table 1).

No signals were observed on both the metaphase and interphase of the control, maize Zi330 (Fig. 3G and H).

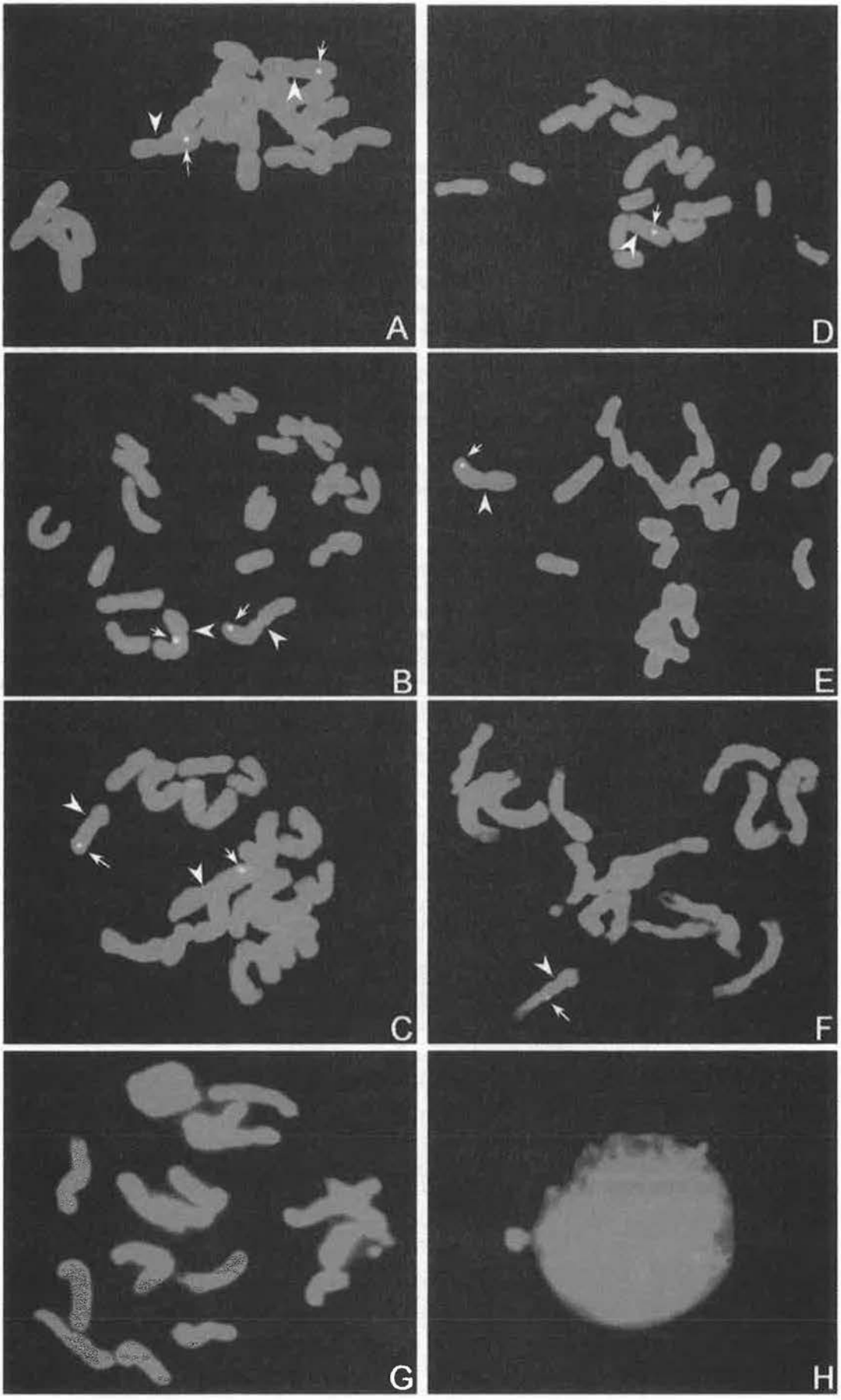
In the pure line 540, the signals could be observed on two members of each detected chromosome pair, while in Yi Dan 6, only on one member. That the signals showed only on one member of each detected chromosome in Yi Dan 6 demonstrated that only one of its parents, 540, was introgressed, while the other, maize inbred line Zi 50003, had no introgressed DP chromatin at all. One member showing the signal must come from 540, the other from Zi50030 for chromosomes 1, 2, and 8.

It has been demonstrated that the stability of transgenes was related to the position integrated in transgenic plants (Jorgensen and Andersen 1994; Frello et al. 1995; Pedersen et al. 1997). Iglesias et al. (1997) reported that the transgenes integrated on the regions close to the telomeres were more stable. In 540, the alien chromatins of chromosomes 1, 2 and 8 were located in the regions close to the telomeres and their percentage distances from centromere to the hybridization site were over 70 (Table 1, Fig. 4), while those of chromosome 5 were located in the regions distal to the telomeres and the percentage distance was 13.42±2.07. In the genomes of both 540 and Yi Dan 6, the introgressed segments were located in the long arms on chromosomes 1, 2, and 8 except of chromosome 5 on which the signals only appeared in pure line 540 instead of Yi Dan 6. This meant that the DP chromatins integrated on chromosomes 1, 2, and 8 could be inherited stably, but the alien chromatins of chromosome 5 were unstable. Probably distant hybrids also follow the rule suggested by Iglesias et al. (1997) in transgenic plants. The alien chromatins integrated in regions distal to the telomeres on chromosome 5 might be lost during the processes of fertilization or hybrid seed production. Therefore, Yi Dan 6 had no signal on chromosome 5 at all.

In addition, the percentage distances of the signals showed smaller differences on chromosomes 1 (89.08 and 80.49) and 2 (71.37and 61.71), but they were more distinct on chromosome 8 (72.41 and 41.61) between 540 and Yi Dan 6 (Table 1). Because different regions of the chromosomes have varying states of condensation during the mitotic process, it is possible that the percentage distance of a giving hybridization site from the centromere will show some variation in different mitotic stages. For the differences of percentage distances on chromosomes 1 and 2 it could be explained by the fact that the observed samples could not be kept in the same mitotic stages between 540 and Yi Dan 6. Whether it was induced by chromosome rearrangement (translocation or inversion) or not in Yi Dan 6 for the larger position change on chromosome 8 remains to be studied.



In our laboratory, several important disease resistance genes of maize including *Helminthosporium turcicum* Pass, *Helminthosporium maydis* Nisik, and *Helminthosporium carbonum* Ullstrup resistance genes *ht*, *rhm* and *hm* with RFLP markers flanking each of them were mapped by in situ hybridization. The results showed that the hybridization site of *ht1* was between the percentage distances 61.78 and 63.01 on the long arm of chromosome 2 (Li et al. 1998a); the hybridization site of homologous sequence of *hm1* was located at 58.23 on the long arm of chromosome 5 (Li et al. 1998b); and that of the homologous sequences of *rhm* was positioned at percentage distances 65.06 and 72.40 on chromosomes 1 and 8 correspondingly (unpublished data). This demonstrated that all of the chromosome arms integrated by the alien chromatins had the distributions of the different disease resistance genes mentioned above in 540. Moreover, the sites of the disease resistance genes or their homologous sequences were very close to those of the alien chromatins on chromosomes 1, 2, and 8 except chromosome 5 on which the homologous sequence of *hm1* was located at percentage distance 58.23, while the alien chromatin was at 13.42 (Table 1). As mentioned above, both 540 and the F1 hybrids, Yi Dan 6, showed much stronger *Helminthosporium turcicum* and *Helninthosporium maydis* resistances and wider adaptation to stressful conditions. The regions integrated by DP chromatins basically corresponded to those located by


A
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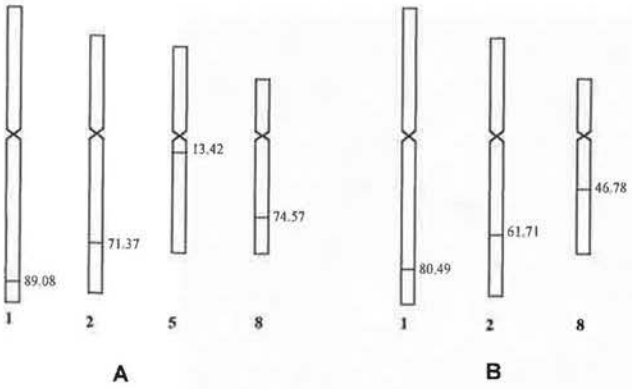

Fig. 4. Ideograms of the chromosomal locations of the introgressed chromatins in the tested materials. The integrated sites were indicated with percentage distances on the right of each chromosome. The percentage distances and arm ratios were averaged for DAB and fluorescence detection systems. The number at bottom is chromosome number. A. Inbred line 540 and B. Yidan 6.

Helminthosporium turcicum and *Helminthosporium maydis* resistance genes *ht* and *rhm* on chromosomes 1, 2, and 8. Therefore, we think genes *ht* and *rhm* between maize and DP should be homologous or homoeologous, and the DP chromatins were integrated by homoeologous or homologous recombination from crossing over during meiosis instead of random process in the hybrids of maize X DP. Because the unexpressed introns should have different sequences in the genes between DP and maize, the introgressed DP chromatins could not be blocked easily with the blocking ratio 1 : 30. Because the differences of the introns between DP and maize were not absolute, the integrated DP chromatins were still blocked to a certain degree by maize genomic DNA with a high blocking ratio. Therefore, the signals of DP chromatins could not be detected completely with 1:40 and 1:50 blocking ratios. DP is interfertile with maize (Iltis et al. 1979), during meiosis the synapsis of their hybrids should be normal basically. Thus, many DP chromosome fragments or genes besides genes *ht* and *rhm* should be able to be integrated in the maize genome by crossing over. The alloplasmic pure line 540 was obtained by selection through several generations. As a result, it only showed the integration sites which were mainly corresponding to the loci of disease resistance genes in maize.

The breeding of alloplasmic pure lines, which showed disease resistance traits, offered us a new opportunity for establishing the specific gene library and isolating genes or chromatins specifying important agricultural characters, for example, resistance to disease and pests from wild germplasm by microdissection of the special regions on chromosomes. Based on our results with GISH, the establishment of the specific library containing genes *ht* and *rhm* of DP and their isolation could probably be performed by the microdissection of the DP integrated sites on the chromosomes of 540 or Yi Dan 6.

Acknowledgments: This research was supported by the National Natural Science Foundation in China and the Doctorate Vesting Point Foundation of the Education Ministry of the People's Republic of China

ZARIA, NIGERIA
Ahmadu Bello University

Induced multiple ear mutants in popcorn (*Z. mays* var. praecox Sturt.)

--Adamu, AK

The study was carried out with the objective of inducing beneficial mutants. Air dried seed of popcorn (*Z. mays* var. *praecox* Sturt.) was irradiated with gamma-rays cobalt 60 at the following doses 10, 20, 30, 40, and 400Gy and Thermal Neutrons (5 curie 241AM/Be neutron source flux 1.5 x 10^4n.cm^{-2}S^{-1}) at 25, 40, 50, 60 minutes and 12 hours. Result obtained indicated an increase in the number of ear induced per plant in both M1 and M2 generations and the increase was in direct proportion to the dose for both gamma-rays and thermal neutrons. The number of ears induced in the M1 generation ranges from zero (none) to five and in some, up to 6 ears per plant. The frequency of occurrence in the M2 generation of these mutants was relatively low when compared with the M1 generation.

Gamma-ray (^{60}CO) and thermal neutron induced tassel seed and male sterile mutants in popcorn (*Z. mays* var. praecox Sturt.)

--Adamu, AK

Male sterility is an important tool, which may effectively eliminate hand emasculation in genetic improvement. Air dried seed were irradiated with different doses of gamma-rays and thermal neutrons, with the aim of inducing male sterile mutants. The different types of mutants induced were plants developing seeds on tassels (tassel seed). "Highly Sterile" are plants whose tassels were completely turned into female ears (unisexual mutants) and are usually seed sterile. Partially "Sterile" mutants are plants with ears on the normal part of the stem and an additional ear in place of tassels. The appearance of the different categories of male sterile mutants tends to increase with dose in both gamma-ray and thermal neutron treatment. However, only about 4% of the M1 male sterile mutants induced were transmitted to the M2 generation.

III. ADDRESS LIST

(Most-current addresses are available on the web (http://www.agron.missouri.edu/))

Abedon, Bruce; Rhodes College; 2000 North Parkway; Memphis TN 38112; 901-843-3431; 901-843-3565 (fax); abedon@rhodes.edu
Adams, Karen R.; 2837 E. Beverly Dr.; Tucson AZ 85716-5716; 520-326-8994
Adamu, A. Kasim; Department of Biological Sciences; Ahmadu Bello University; Zaria; NIGERIA; 069-50581 ext. 108; 234 69 50891 (fax)
Agrama, Hesham A; Agronomy Department; 2004 Throckmorton; Kansas State University; Manhattan KS 66506-5501; 785-532-6796; 785-532-6094 (fax); hagrama@ksu.edu
Aguiar-Perecin, Margarida; Dept Genetica - ESALQ; Universidade de S. Paulo; 13400-970 Piracicaba, SP; BRAZIL; 55 (0) 19 429 4125; 55 (0) 19 433 6706 (fax); mlrapere@carpa.ciagri.usp.br
Akamatsu, Toyokazu; Sakata Seed Corp; Plant Biotechnology Center; 358 Uchikoshi Sodegaura City Chiba; JAPAN; 0438-75-2369; 0438-75-2594 (fax)
Albertsen, Marc C.; Pioneer Hi-Bred International; 7300 N.W. 62nd Ave., Box 1004; Johnston IA 50131-1004; 515-270-3648; 515-253-2149 (fax); ALBERTSNM@PHIBRED.COM
Alexander, Danny; Novartis Corp; 3054 Cornwallis Rd.; Research Triangle Park NC 27709-2257; 919-541-8687; 919-597-3036 (fax); danny.alexander@nabri.novartis.com
Alfenito, Mark; 61 Avondale Ave; Redwood City CA 94062; 650-368-1206; alfenito@targetquest.com
Alleman, Mary; 253 Mellon Hall; Duquesne University; Dept. Biol. Sci.; Pittsburgh PA 15282; 412-396-1660; 412-396-5907 (fax); ALLEMAN@DUQ.EDU
Allen, Jim; Department of Biological Sciences; Florida Internatl Univ; Miami FL 33199; 305-348-6632; 305-348-1986 (fax); allenjo@fiu.edu
Altosaar, Illimar; Department of Biochemistry; University of Ottawa; 40 Marie Curie Private; Ottawa, Ontario K1N 6N5; CANADA; 1-613-562-5800 Ext. 6374; 1-613-562-5180 (fax); altosaar@oreo.chem.uottawa.ca
Alvey, David; 237 Myrtle Drive; W. Lafayette IN 47906; 765-567-2115; 765-567-4046 (fax)
Amano, Etsuo; Biol Resources Res & Dev Centre; Fukui Prefectural Univ; 88-1, Futaomote, Awara-cho, Sakai-gun; Fukui-ken, 910-41; JAPAN; 81-776-77-1443; 81-776-77-1448 (fax); amano@fpu.ac.jp
Ananiev, Evgueni; Pioneer Hi-Bred Int'l Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004; 515-253-2477/5740; 515-270-3367 (fax); ananievev@phibred.com
Andersen, Tim; 7300 NW 62nd Ave; Box 129; Johnston IA 50131-0129; 515-276-6541; 515-270-4312 (fax); andersent@phibred.com
Arbuckle, John; Genome Research; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave Box 1004; Johnston IA 50131-1004; 515-270-4350; 515-270-3376 (fax)
Armstrong, Charles L.; Monsanto, GG4C; 700 Chesterfield Parkway North; St. Louis MO 63198; 314-737-7229; 314-737-6567 (fax); charles.l.armstrong@monsanto.com
Arpat, Hacimurat; Ohio State University; OARDC, Dept of Agronomy; 1680 Madison Avenue; Wooster OH 44691-4096; 330-263-2882; 330-263-3658 (fax)
Arruda, Paulo; Univ. Estadual de Campinas; Cidade Univ. Zeferino Vaz; Distrito de Barao Geraldo; Campinas - SP; BRAZIL; (192)398351; (192)394717 (fax)
Arthur, Lane; Life Cycle Information Management; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004; arthurwl@phibred.com
Artim-Moore, Lori; Ciba-Ag Biotechn; PO Box 12257; Research Triangle Park NC 27709
Atanassov, A.; Institute of Genetic Engineering; 2232 Kostinbrod 2; BULGARIA; 359.0721.2552; 359.721.4985 (fax); atanos@icgeb.trieste.it
Auger, Donald L.; Biol Sci; Tucker Hall; Univ Missouri; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); dauger@biosci.mbp.missouri.edu
Ayala-Osuna, Juan; Rua Dos Trabalhadores 906; 14870.000 Jaboticabal-SP; BRAZIL; 016-3220260; 016-3224275 (fax)
Aycock, Harold S; Cornnuts Genetic Research; P.O. Box 215; Greenfield CA 93927; 831-674-3131; 831-674-5139 (fax); aycockh@nabisco.com
Ayers, John E; Dept Plant Pathology; 308 Buckhout Lab; Pennsylvania State Univ; University Park PA 16802; 814-865-7776; 814-863-7217 (fax); jea@psu.edu
Azanza, Fermin; Novartis Seeds; Field Crops Europe Biotech Dept; 12 Chemin de l'Hobit; B.P. 27 - 31790 Saint-Sauveur; FRANCE; (33)5.62.79.98.09; (33)5.62.79.99.96 (fax); fermin.azanza@seeds.novartis.com
Bai, Yongyan; Inst. of Plant Physiol.; 300 Fengling Road; Shanghai 200032; CHINA
Bailey II, J. Clinton; Biol Sci; 324 Tucker Hall; Univ Missouri; Columbia MO 65211; 573-882-8033; 573-882-0123 (fax); cbailey@mail.biosci.missouri.edu
Bailey-Serres, Julia; Botany & Plant Sciences; Batchelor Hall; Univ. Calif. Riverside; Riverside CA 92521; 909-787-3738; 909-787-4437 (fax); SERRES@UCRAC1.UCR.EDU
Bairoch, Amos; Centre Medicale Universitaire; 1211 Geneva 4; Geneva; SWITZERLAND; 41-22-784-40-82; BAIROCH@cmu.unige.ch
Baker, Barbara; USDA; Plant Gene Expression Center; 800 Buchanan Street; Albany CA 94710; 510-559-5912; 510-559-5678 (fax); bbaker@garnet.berkeley.edu
Ball, Dale W.; Wilson Seeds, Inc.; P.O. Box 391; Harlan IA 51537; 712-755-3841; 712-755-5261 (fax); dballmaize@aol.com
Bar-Zur, Avri; Moshava; P.O. Box 32; Yokneam 20600; ISRAEL; 972-4-989-4026; 972-4-985-3291 (fax)
Barbour, Eric; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004
Barkan, Alice; Institute of Molecular Biology; University of Oregon; Eugene OR 97403; 541-346-5145; 541-346-5891 (fax); abarkan@molbio.uoregon.edu
Barros, MEC; PO Box 395; Pretoria, 0001; SOUTH AFRICA; 27-12-841-3221
Bass, Hank W.; Dept Biol Sci; Biology Unit 1, Chieftan Way; Florida State Univ; Tallahassee FL 32306-4370; 850-644-9711; 850-644-0481 (fax); bass@bio.fsu.edu
Baszczynski, C. L.; Pioneer Hi-Bred International, Inc.; 7250 N.W. 62nd Avenue; P.O. Box 552; Johnston IA 50131-0552; (515)270-3693; (515)334-4729 (fax); BASZCZYNSKI@PHIBRED.COM
Bates, Lynn S.; Alteca Ltd.; 731 McCall Road; Manhattan KS 66502; 913-537-9773; 913-537-1800 (fax); alteca@kansas.net
Baysdorfer, Chris; Biological Sciences; California State U; Hayward CA 94542; 510-885-3459; 510-888-4747 (fax); 72652.662@CompuServe.COM
Beach, Larry; Pioneer Hi-Bred Internatl; PO Box 1004; Johnston IA 50131-9410; 515-270-3798; 515-270-3367 (fax); beachl@phibred.com
Beavis, Bill; Nat Center for Genome Resources; 1800-A Old Pecos Trail; Santa Fe NM 87505; 800-450-4854x4412; 505-995-4432 (fax); wdb@ncgr.org
Beckert, M.; INRA Station D'Amelioration des Plantes; 63039 Clermont Ferrand; FRANCE; (33)73 624319; (33)73 624453 (fax); beckert@clermont.inra.fr
Beckett, Jack; 607 Longfellow Lane; Columbia MO 65203-1630; 573-445-3472; 573-884-7850 (fax)
Becraft, Philip; Zoology & Genetics/Agronomy Depts; 2116 Molecular Biology Bldg; Iowa State University; Ames IA 50011; 515-294-2903; 515-294-0345 (fax); becraft@iastate.edu
Bedinger, Patricia; Biology Dept; Colorado State Univ.; Fort Collins CO 80523-1878; 970-491-2879; 970-491-0649 (fax); bedinger@lamar.colostate.edu
Belanger, Faith; Dept of Crop Science; Cook College, Lipman Hall; Rutgers University; New Brunswick NJ 08816; 908-932-8165; belanger@aesop.rutgers.edu
Bell, Duane; 304 Pearl St.; Blissfield MI 49228; (517)486-3520; (517)486-2631 (fax)
Benner, Michael; Science and Technology Center; Rider University; 2083 Lawrenceville Rd; Lawrenceville NJ 08648-3099; 609-896-5097; 609-895-5782 (fax); benner@rider.edu
Bennett, Michael; Jodrell Laboratory; Royal Botanic Gardens; Kew, Richmond; Surrey, TW9 3AB; UNITED KINGDOM; 44-1181-332-5000; 44-1181-332-5310 (fax); M.Bennett@rbgkew.org.uk
Bennetzen, Jeff; Dept of Biological Sciences; Purdue University; W. Lafayette IN 47907-1392; 765-494-4763; 765-496-1496 (fax); Maize@bilbo.bio.purdue.edu
Bensen, Robert; Pioneer Hi-Bred Int'l Inc.; 7300 NW 62nd Ave., P.O. Box 1004; Johnston IA 50131; 515-270-3645; 515-253-2149 (fax)
Benzion, Gary; 303 W. Lanvale St.; Baltimore MD 21217; 703-308-1119; benzion@USPTO.GOV
Bergquist, Richard R.; 401 East Sixth Street; El Paso IL 61738; 309-527-6000
Bergvinson, David; CIMMYT; Apdo. Postal 6-641; 06600 Mexico, DF; MEXICO; 525-726-9091; 525-726-7558 (fax); DBERGVINSON@ALPHAC.CIMMYT.MX
Berke, Terry; Box 42; Shanhua; Tainan, Taiwan 741; REPUBLIC OF CHINA; 8866 583 7801; 8866 583 0009 (fax); TERRY@NETRA.AVRDC.ORG.TW
Berlyn, Mary; Dept. of Biology; Yale University; New Haven CT 06520; 203-432-9997; 203-432-3854 (fax); mary@fetalpig.biology.yale.edu
Bernardo, Rex; Dept of Agronomy; Purdue University; 1150 Lilly Hall Life Sci; West Lafayette IN 47907-1150; 765-494-8088; 765-496-2926 (fax); bernardo@purdue.edu
Bernot, John; Dept. of Biological Sciences; Duquesne University; Pittsburg PA 15282
Berthaud, Julien; UR 31 Centre ORSTOM; 911, Avenue Agropolis; BP 5045; 34032 Montpellier cedex; FRANCE; 33(0)467 41 6165; 33(0)467 54 7800 (fax); julien.berthaud@mpl.orstom.fr
Bertrand, Ralph L.; 14 E Cache La Poudre; Dept. of Biology; Colorado College; Colorado Springs CO 80903; (719)389-6402; (719)389-6940 (fax);

rbertrand@cc.colorado.edu
Berville, A.; INRA; Station d'Amelior des Plantes; 2 Place Viala; 34060 Montpellier Cedex; FRANCE
Bhattramakki, Dinakar; DuPont Ag Products; Delaware Technical Park, Suite 200; PO Box 6104; Newark DE 19714-6104; 302-733-8815; 302-631-2607 (fax); bhatt2d@cdcln05.lvs.dupont.com
Bianchi, Angelo; Ist Sper Cerealicoltura; Via Cassia 176; 00191 Rome; ITALY; 06-3295705; 06-3630-6022 (fax)
Biradar, Madhuri; 320 ERML, Crop Sci; Univ. of Illinois; 1201 W. Gregory; Urbana IL 61801
Birchler, James; Biological Sciences; Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-4905; 573-882-0123 (fax); birchler@biosci.mbp.missouri.edu
Bird, Robert; 1211 Dogwood Lane; Raleigh NC 27607; 919-787-8452; rmckbird@earthlink.net
Bjarnason, Magni; IM Aufeld 5; D-77815 Buehl; GERMANY; 49-7227-5691; 49-7227-5691 (fax); bjarnason@t-online.de
Blakey, Cynthia Ann; Cooper Science Bldg; Dept. of Biology; Ball State University; Muncie IN 47306; 765-285-8820; 765-285-2351 (fax); ablakey@gw.bsu.edu
Blewitt, Michael; Brookhaven National Lab; Bldg. 463; Upton NY 11973
Bocanski, Jan; Faculty of Agriculture; Univ of Novi Said; 21000 Novi Sad; YUGOSLAVIA; bocanski@polj.ns.ac.yu
Bogorad, Lawrence; Biol Labs; Harvard Univ; 16 Divinity Ave; Cambridge MA 02138; 617-495-4292; 617-495-4292 (fax); bogorad@biosun.harvard.edu
Bokde, S.; 599 Laurel Ave. #3; St. Paul MN 55102-2047
Bollman, Krista; Dept. of Biology; Plant Science Institute; University of Pennsylvania; Philadelphia PA 19104-6018
Bommineni, Venkat R; Agritope, Inc; 16160 SW Upper Boones Ferry Rd; Building C; Portland OR 97224-7744; 503-670-7702; 503-670-7703 (fax); venkat@agritop.com
Borovskii, Genadii; Siberian Inst Plant Physiol Biochem; P.O. Box 1243; Irkutsk-33, 664033; RUSSIA; root@sifibr.irkutsk.su
Bosch, Lluis; Escola Superior d'Agricultura; Comte d'Urgell, 187; 08036 Barcelona; SPAIN; 3-4304207; 3-4192601 (fax)
Boston, Rebecca S.; Box 7612; Dept Botany; North Carolina State Univ; Raleigh NC 27695-7612; (919)515-2727; (919)515-3436 (fax); boston@unity.ncsu.edu
Bouchard, Robert A.; College of Wooster; Biology Dept.; Wooster OH 44691; 330-263-2433; 330-263-2378 (fax); rbouchard@acs.wooster.edu
Bowen, Ben; Lynx Therapeutics, Inc; 25861 Industrial Boulevard; Hayward CA 94545; 510-670-9441; 510-670-9302 (fax); ben@lynxgen.com
Boyer, Charles D.; Dept. of Horticulture; Oregon State University; Ag & Life Sciences 4017; Corvallis OR 97331-7304; 503-737-5474
Brakke, Myron; Box 57, Route 1; Crete NE 68333-9606; 402-826-5569; PATH010@UNLVM.UNL.edu
Brar, Gurdip; Monsanto Co.; 8520 University Green; Middleton WI 53562; 608-821-3483; 608-836-9710 (fax); gsbrar@monsanto.com
Braun, David; Dept Plant Biology; 111Koshland; Univ California; Berkeley CA 94720; 510-642-8058; 510-642-4995 (fax); dbraun@nature.berkeley.edu
Breto, Paz; Pioneer Hi-Bred Int. Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004
Brettell, Richard; CSIRO Div Plant Industry; P.O. Box 1600; Canberra City ACT 2601; AUSTRALIA; 062-46-5581; dick@pican.pi.csiro.au
Bretting, Peter K.; USDA/ARS, NPS; 5601 Sunnyside Ave.; Bldg. 4, Room 2212; Beltsville MD 20705-5139; 301-504-5541; 301-504-6191 (fax); pkb@ars.usda.gov
Brewbaker, James; Horticulture; Univ of Hawaii; 3190 Maile Way; Honolulu HI 96822; 808-956-7985; 808-956-3894 (fax); brewbake@Hawaii.Edu
Briggs, Kristen; Pioneer Hi-Bred Internat Inc; Trait and Technol Development; 7300 NW 62nd Ave PO Box 1004; Johnston IA 50131-1004; 515-254-2623; 515-254-2619 (fax)
Briggs, Robert W.; Crow's Hybrid Corn Company; Box 306; Milford IL 60953; 815-889-4151; 815-889-5253 (fax)
Briggs, Steven; Novartis Agrib Disc Inst Inc; 3050 Science Park Rd Suite 102; San Diego CA 92121-1102; 616-784-8670; steven.briggs@nabri.novartis.com
Brinkman, Mark; Mycogen Seeds; 809 4th Street; Gothenburg NE 69138; 308-537-2838; 308-537-2960 (fax); brinkmanm@mycogen.com
Britt, Anne Bagg; Section of Plant Biology; U of CA; Davis CA 95616; 916-752-0699; 916-752-5410 (fax); abbritt@ucdavis.edu
Brockman, Laura L.; Cargill Hybrid Seeds; 2600 W. Galena Blvd; Aurora IL 60506; 630-801-2352
Brouwer, Cory; Pioneer Hi-Bred Intl; 7300 NW 62d Ave; PO Box 1004; Johnston IA 50131-1004; 515-254-2633; 515-270-4312 (fax); brouwercr@phibred.com
Brown, Douglas; Dept of Biology; Bishops University; Lennoxville J1M 1Z7 Quebec; CANADA; 819-822-9632; 819-822-9661 (fax)
Browne, Chris; Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-7616; cbrowne@socketis.net
Bruce, Wes; Pioneer Hi-Bred International, Inc.; 7300 NW 62nd Ave.; P.O. Box 1004; Johnston IA 50131-1004; (515)270-3647; (515)270-3367 (fax); Brucewb@phibred.com
Bruggemann, E; Pioneer Hi Bred Intl; 7250 NW 62nd Ave; PO Box 1000; Johnston IA 50131-1000; 515-270-4143; 515-334-4788 (fax); bruggeep@phibred.com
Brutnell, Thomas P.; Dept Plant Sci; University of Oxford; South Parks Road; Oxford, OX1 3RB; UNITED KINGDOM; 011-44-1865-275030; 011-44-1865-275147 (fax); tom.brutnell@plant-sciences.oxford.ac.uk
Bubeck, David; Northrup King Co.; 317 330th St.; Stanton MN 55018-4308; (507)663-7666
Buckler, Carlyn S. Keith; Dept Statistics; North Carolina State Univ; Box 8203; Raleigh NC 27695-8203
Buckler, Edward; Dept Statistics; North Carolina State Univ; Box 7614; Raleigh NC 27695-7614; 919-513-1475; 919-515-3355 (fax); buckler@statgen.ncsu.edu
Buckner, Brent; Div. Science, Science Hall; Truman State Univ; 100 East Normal; Kirksville MO 63501-4221; 816-785-4083; 816-785-4045 (fax); bbuckner@truman.edu
Bullock, W. Paul; Garst Seeds Company; 2369 330th St; P.O. Box 500; Slater IA 50244; 515-685-5116; 515-685-5080 (fax); paul.bullock@garstseedco.com
Bureau, Thomas; Dept. of Genetics; University of Georgia; Athens GA 30602
Burr, Benjamin; Biology Dept; Brookhaven National Lab; Bldg 463; Upton NY 11973; 631-282-3396; 631-282-3407 (fax); burr@bnl.gov
Burr, Frances; Brookhaven National Lab; Biol Dept; Bldg 463; Upton NY 11973; 631-344-3396; 631-344-3407 (fax); burr@bnl.gov
Bushman, B. Shaun; 302 Curtis Hall; Univ Missouri; Columbia MO 65211; 573-882-2033; shaun@teosinte.agron.missouri.edu
Butler, Lou; Dept of Agronomy; Curtis Hall; Univ of Missouri; Columbia MO 65211; 573-882-2674; ButlerL@missouri.edu
Butnaru, Gallia; Univ Stiinte Agricole A Banatului; Disciplina de Genetica; C. Post 136, O.P. 1; Timisoara 1900; ROMANIA; 40.56.141424; 40.56.200296 (fax)
Byrne, Mary; Cold Spring Harbor Laboratory; PO Box 100; 1 Bungtown Rd; Cold Spring Harbor NY 11724; 516-367-8836; 516-367-8369 (fax); byrne@cshl.org
Byrne, Patrick; Dept Soil and Crop Sci; Colorado State Univ; Ft Collins CO 80523; 970-491-6985; 970-491-0564 (fax); pbyrne@lamar.colostate.edu
Cabulea Iancu, I.; Library; Agric Res Station; Str. Agriculturii 27; Turda R03350; ROMANIA; 040-064-311680; 040-064-311134 (fax)
Caldwell, Elizabeth E. Oberthur; 4505 Regal Ave NE; Cedar Rapids IA 52402-2143; 319-335-8333
Callis, Judy; Section of Molecular and Cellular Biol.; Univ of CA; Davis CA 95616; (916)752-1015; (916)752-3085 (fax); JCallis@ucdavis.edu
Camara-Hernandez, J.; Altolaguirre 1295; Buenos Aires 1427; ARGENTINA; 54-1-521-6464; 54-1-522-1687 (fax)
Camargo, Luiz; Depto. Fitopatologia -ESALQ/USP; C.P. 09; 13418-900 Piracicaba-SP; BRAZIL; 55-194-294124; 55-194-344839 (fax); leacamar@carpa.ciagri.usp.br
Campbell, Wilbur H.; Dept. of Biological Sciences; Michigan Technological Univ.; 1400 Townsend Drive; Houghton MI 49931-1295; 906-487-2214; 906-487-3355 (fax); WCAMPBEL@MTU.EDU
Camussi, Alessandro; Genetic Unit - Fac of Agriculture; Via S. Bonaventura 13; I-50129 Firenze; ITALY
Cande, Zac; Dept of Molec & Cell Biology; Box 341 LSA; Univ of California; Berkeley CA 94720-3200; 510-642-1669; 510-643-6791 (fax); zcande@uclink4.berkeley.edu
Cardinal, Andrea J.; Agronomy Hall 1571; Iowa State Univ; Ames IA 50011; 515-294-3635; 515-294-3163 (fax); cardinal@iastate.edu
Carlson, Lawrence A.; 7 North Winthrop Street; St. Paul MN 55119-4674; 612-738-8812; carls245@tc.umn.edu
Carlson, Wayne; Dept. of Biol. Sci.; University of Iowa; Iowa City IA 52242; (319)335-1316; (319)335-3620 (fax); wayne-carlson@uiowa.edu
Carneiro, Newton; University of Arizona; 3401 N. Columbus Blvd, 16F; Tucson AZ 85721; 520-325-9186
Carson, Chris; Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-0832; 573-884-7850 (fax); carson@teosinte.agron.missouri.edu
Carson, Martin L.; Dept. Plant Pathology; Box 7616; NCSU; Raleigh NC 27695-7616; (919)515-3516; (919)515-7716 (fax)
Carvalho, Carlos Roberto de; Universidade Federal de Vicosa; Depto Biologia Geral; 36571.000 Vicosa - MG; BRAZIL; 31-899-2568; 31-899-2203 (fax); ccarvalh@mail.ufv.br
Causse, Mathilde; Station de Genetique Vegetale; Ferme du Moulon; 91190 Gif/Yvette; FRANCE; 33 1 6941 6727; 33 1 6941 2790 (fax); causse@moulon.inra.fr
Chalyk, Sergey; M. Spataru 1, Box 134; Kishinev 2075; MOLDOVA; 37-32-78-83-67; 37-32-55-61-80 (fax); schalyk@hotmail.com
Chan, Annette; Dept. of Plant Biology; 345 LSA; University of California; Berkeley CA 94720-3102; 510-643-8277; 510-643-6791 (fax); ACHAN@UCLINK3.BERKELEY.EDU
Chandler, Vicki; Dept Plant Sciences; 303 Forbes Hall; University of Arizona; Tucson AZ 85721; 520-626-8725; 520-621-7186 (fax); chandler@ag.arizona.edu
Chandrakanth, E.; Crop Biotechnology Center; Texas A&M Univ; College Station TX 77843; 409-260-4563
Chang, Ming-Tang; ExSeeds Genetics L.L.C.; 1567 Food Sciences Bldg.; Iowa State Univ.; Ames IA 50011-1061; 515-294-2993; 515-294-2644 (fax);

mingchang@ames.net
Chang, S. C.; 13, LN36, Chung Shan Rd.; Chiayi, ROC; TAIWAN; 05-278-4603
Chao, Shiaoman; Centre Plant Conserv Genetics; Southern Cross Univ; PO Box 157; Lismore NSW 2480; AUSTRALIA; 61 2-6620-3485; 61 2-6620-2080 (fax)
Charlton, Wayne; Rodney Porter Building; Dept. of Plant Sciences; University of Oxford, South Parks Road; Oxford, OX1 3RB; UNITED KINGDOM; 44-1865-275814; 44-1865-275805 (fax)
Chase, Christine D.; Horticultural Sciences Dept.; 1143 Fifield Hall; Univ of Florida; Gainesville FL 32611-0690; 352-392-1928x316; 352-392-6479 (fax); ctdc@gnv.ifas.ufl.edu
Chase, Sherret S.; Chase Road; P.O. Box 193; Shokan NY 12481; 914-657-2392
Chen, Fure-Chyi; Dept. Plant Industry; National Pingtung Polytechnic Inst; Neipu, Pingtung 91207; TAIWAN; 886-8-774-0267; 886-8-774-0264 (fax)
Chen, Jychian; Institute of Molec Biol; Academia Sinica; Taipei 11529; TAIWAN; 011-8862-2789-9208; 011-8862-2782-6085 (fax); mbjchen@ccvax.sinica.edu.tw
Chen, Shouyi; Academia Sinica; Institute of Genetics; Datun Road, Andingmen Wai; Beijing,100101; CHINA
Chen, Weicheng; Dept. of Agronomy; Henan Agric. University; ZhengZhou 450002; CHINA
Cheng, Kan-Sheng; Yunnan Academy of Agricultural Sciences; Kunming; Yunnan 650205; CHINA
Cheng, Ping-chin; Advanced Microscopy and Imaging Lab; Dept Elect Comp Eng, 201 Bell Hall; State University of New York at Buffalo; Buffalo NY 14260; (716)645-3868; (716)645-3868 (fax); elepcc@corn.eng.buffalo.edu
Cheng, Ruiyang; Nankai University; Biology Division; Plant Molecular Biology Dept.; Tianjin 300071; CHINA
Chernov, Alex; Institute of Genetics; Acad Sci Mold Repub; Kishinev; MOLDOVA; a_chernov43@hotmail.com
Chilton, Scott; Dept of Botany; Box 7612; N. C. State University; Raleigh NC 27695-7612; (919)515-3792; (919)515-3436 (fax); schilton@unity.ncsu.edu
Choe, Bong-Ho; Agronomy Dept; College of Agriculture; Chungnam National University; Dae-Jon 305-764; KOREA; KOREA-042-821-5723; 82-42-823-8050 (fax)
Choi, Keun-Jin; Dept. of Corn Breeding; Upland Crop Div.; Crop Experiment Station; Suwon; KOREA; (0331)292-3823; (0331)292-4560 (fax)
Chojecki, Jan; Plant Bioscience Limited; Norwich Research Park; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44-(0)1603-456500; 44-(0)1603-456552 (fax); ajsc@plantbioscience.com
Chomet, Paul; DeKalb Plant Genetics/Monsanto; 62 Maritime Drive; Mystic CT 06355-1958; 860-572-5224; 860-572-5282 (fax); PCHOMET@DEKALB.COM
Chongkid, Boonhong; Dept. of Agricultural Technology; Fac. of Science & Technology; Thammasat Univ., Rangsit Campus; Pathum Thani 12121; THAILAND; 5160020-39 ext. 1712, 1713; 5160963 (fax)
Chopra, Surinder; 2204 Molecular Biology Building; Iowa State University; Ames IA 50011; (515)294-5054; (515)294-0345 (fax); chopra@iastate.edu
Chourey, Prem; USDA-ARS; Plant Pathology Department; University of Florida; Gainesville FL 32611-0680; 352-392-2806; 352-392-6532 (fax); PSCH@GNV.IFAS.UFL.EDU
Chuck, George; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5922; 510-559-5678 (fax); gchuck@nature.berkeley.edu
Chughtai, Sajjad R.; Maize Program; NARC, P.O. NIH; Islamabad; PAKISTAN; (051)241534; 51-812968 (fax)
Clark, Janice K.; Dept. of Biology; University of North Dakota; Grand Forks ND 58202-9019; (701)777-2621; (701)777-2623 (fax); janclark@plains.nodak.edu
Close, Timothy J.; Dept. Bot. & Plant Sci.; Univ. of California Riverside; Riverside CA 92521; 909-787-3318; 909-787-4437 (fax); TIMCLOSE@UCRAC1.UCR.EDU
Clutter, Mary; BIO/BIO; National Science Foundation Room 605N; 4201 Wilson Blvd.; Arlington VA 22230; 703-306-1400; mclutter@note.nsf.gov
Cocciolone, Suzy; 2288 Molecular Biology Building; Iowa State University; Ames IA 50011; 515-294-5054; 515-294-0345 (fax); scocciol@iastate.edu
Coe, Ed; Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-2768; 573-884-7850 (fax); ed@teosinte.agron.missouri.edu
Colasanti, Joseph J.; Univ California-Berkeley; PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5908; 510-559-5678 (fax); colasant@uclink4.berkeley.edu
Colbert, Terry; Pioneer Hi-Bred Int., Inc; RR1 P.O. Box 90-I; Princeton IN 47670; colberttr@phibred.com
Coleman, Craig; Dept Botany & Range Sci; Brigham Young Univ; Provo UT 84602; 801-378-3784; 801-378-7499 (fax); cec3@email.byu.edu
Colless, J. M.; Agric Research Sta; Grafton, NSW 2460; AUSTRALIA; 066-420420; 066-447251 (fax)
Cone, Karen C.; Biological Sciences; University of Missouri; Columbia MO 65211; 573-882-2118; 573-882-0123 (fax); Cone@biosci.mbp.missouri.edu
Cook, Bill; Biology Department; Midwestern State University; 3410 Taft Blvd.; Wichita Falls TX 76308; 940-397-4192; 940-397-4442 (fax); fcookb@nexus.mwsu.edu
Cooper, Jennifer; Univ of Missouri; 105 Tucker Hall; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); C635704@showme.missouri.edu
Cooper, Pamela S.; Univ. of Missouri; Div. of Biological Science; Columbia MO 65211; 573-882-1168; 573-882-0123 (fax); cooper@biosci.mbp.missouri.edu
Coors, James G.; Department of Agronomy; University of Wisconsin; 1575 Linden Drive; Madison WI 53706; 608-262-7959; jgcoors@facstaff.wisc.edu
Corke, Harold; 45 Highwest; 142 Pokfulam Road; Hong Kong; HONG KONG; 011 852 2859 2820; 011 852 2858 3477 (fax); harold@hkuxa.hku.hk
Cormack, Jean; DeKalb Genetics Corp.; 3100 Sycamore Rd; DeKalb IL 60115; 815-758-9682; 815-758-4106 (fax); jcormack@dekalb.com
Cornu, A.; Sta d'Amelior des Plantes; B V 1540; 21034 Dijon; FRANCE; 80-63-3159; 80-63-3263 (fax)
Cowen, Neil; DowElanco Discovery Research; 9410 Zionsville Rd., CI/#245; Indianapolis IN 46268-1053; 317-337-3684; 317-337-3228 (fax)
Crane, Virginia; Trait and Technology Development; Pioneer Hi-Bred International, Inc.; 7300 N. W. 62nd Ave, Box 1004; Johnston IA 50131-1004; (515)270-3645; (515)253-2149 (fax); CRANEVC@PHIBRED.COM
Creech, Roy G.; Plant & Soil Sciences; Box 9555; Mississippi State University; Mississippi State MS 39762; 601-325-2699; 601-325-8742 (fax); rgcreech@ra.msstate.edu
Culley, David; 24106 N. Bunn Rd; Prosser WA 99350; 509-786-9236/9241; dculley@tricity.wsu.edu
Cummings, D. P.; Dekalb Genetics Corporation; P.O. Box 367; 908 North Independence; Windfall IN 46076-0367; 317-945-7125; 317-945-7152 (fax)
Currie, Randall; SWREC; 4500 E. Mary; Garden City KS 67842; 316-276-8286; 316-276-6028 (fax); RCURRIE@OZNET.KSU.EDU
D'Halluin, Kathleen; Plant Genetic Systems N.V.; Jozef Plateaustraat 22-B 9000; Gent; BELGIUM; (32) (9)2358486; (32) (9)2240694 (fax); pgs@pgsgent.be
Damerval, Catherine; Station de Genetique Vegetale; Ferme du Moulon; 91190 Gif Sur Yvette; FRANCE; (1)01 69 33 23 66; 33(1)01 69 33 23 40 (fax)
Dankov, Toma; Ivan Assen Str.-93; Sofia 1124; BULGARIA; 43-82-73; nedeva@biofac.uni-sofia.bg
Daohong, Xie; Maize Research Institute; Jilin Acad.; 5 W. Xing Hua Street; Gongzhuling, Jilin, P.R. 136100; CHINA; (86)-04441-215179; (86)-04441-214884 (fax)
Darrah, Larry; 110A Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-2349; 573-884-7850 (fax); agrolld1@showme.missouri.edu
Davis, Georgia; Biol Sci - 403A Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-884-4620/3715; gdavis@teosinte.agron.missouri.edu
Dawe, R. Kelly; Department of Botany; University of Georgia; Athens GA 30602; 706-542-1658; 706-542-1805 (fax); kelly@dogwood.botany.uga.edu
Day, Peter R.; Center for Ag Molec Biology; Cook College, Rutgers Univ; Foran Hall, Dudley Road; New Brunswick NJ 08903-0231; 908-932-8165; 908-932-6535 (fax); day@aesop.rutgers.edu
De Leon, Carlos; Maize Program; c/o CIAT; Apdo. Aereo 67-13; Cali; COLOMBIA
De Oliveira, Antonio Costa; Centro de Biotecnologia, Predio 19; Campus UFPel, P.O. Box 354; 96001-970, Pelotas, RS; BRAZIL; 055532757158; 055532759031 (fax); acosta@ufpel.tche.br
de Wolff, F; Advanta Seeds B.V.; PO Box 1; 4410 AA Rilland; NEDERLAND; 31-1135-2151; 31-1135-2237 (fax)
Dean, Caroline; BBSRC; John Innes Centre; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 011-44-1603-452571; 011-44-1603-505725 (fax); caroline.dean@bbsrc.ac.uk
Dean, Ralph; Dept Plant Path & Physiol; Clemson Univ; Clemson SC 29634; 864-656-5737; 864-656-4293 (fax); rdean@clemson.edu
DeBroux, Steve; 700 E. Butler Ave.; Delaware Valley College; Doylestown PA 18901; 215-345-1500; 215-345-5277 (fax)
Delannay, Xavier; Monsanto - N3SB; 800 N. Lindbergh Blvd.; St. Louis MO 63167; 314-537-6611; 314-694-3644 (fax); x.delannay@monsanto.com
Dellaporta, Steve; Yale University; Molec Cell Devel Biol; New Haven CT 06520-8104; 203-432-3895; 203-432-3854 (fax); stephen.dellaporta@yale.edu
Delzer, Brent; Novartis Seeds Inc; 4133 E County Road "O"; Janesville WI 53546; 608-757-1102; 608-757-0080 (fax); brent.delzer@seeds.novartis.com
DeMason, Darleen; Botany & Plant Sciences; Univ of California; Riverside CA 92521; 909-787-3580; 909-787-4437 (fax); demason@ucrac1.ucr.edu
Dempsey, Ellen; 7 Prospect St; Cornwall-on-Hudson NY 12520; 914-534-5285
Deutsch, James A.; ICI Seeds, Inc.; RR2; Box 16; Marshall MO 65340; 816-886-6363; 816-886-9877 (fax)
Dewald, Chester L.; ARS-USDA; 2000 18th St.; Woodward OK 73801; 580-256-7449; 580-256-1322 (fax); sdewald@ag.gov
Dhillon, B. S.; CIMMYT, Maize Program; Apartado Postal 6-641; C.P. 06600 Mexico, D.F.; MEXICO; (5)726-90-91; (595)410-69 (fax)
Dietrich, Chuck; B420 Agronomy Hall; Iowa State University; Ames IA 50011; 515-294-1659; 515-294-2299 (fax); bones@iastate.edu
Dijkhuizen, Arian; W-203 Turner Hall; 1102 South Goodwin Ave.; Urbana IL 61801; 217-244-3388; 217-333-9817 (fax)
Dille, John E.; Winthrop College Biology Dept; Rock Hill SC 29733; 803-323-2111; 803-323-3448 (fax); dillej@winthrop.edu

Dilworth, Machi; Plant Sci Initiatives; National Science Foundation; 4201 Wilson Blvd.; Arlington VA 22230; 703-306-1439; mdilwort@nsf.gov
Ding, Qun-Xing; 1435, S. Limeston St., Apt 285; Lexington KY 40503
Dinges, Jason; 2182 Molec Biol Bldg; Iowa State Univ; Ames IA 50011; 515-294-8202; 515-294-0453 (fax); jdinges@iastate.edu
Dodd, J. L.; Professional Seed Research, Inc; 7 South 437 Dugan Road; Sugar Grove IL 60554; 630-466-1060; 630-466-1068 (fax)
Doebley, John F.; Genetics Department; University of Wisconsin; Madison WI 53706; 608-265-5803/5804; 608-262-2976 (fax); jdoebley@facstaff.wisc.edu
Dogra, Anjali; 105 Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); c651666@showme.missouri.edu
Dolfini, Silvana Faccio; Dipartimento di Genetica; University of Milano; Via Celoria 26; 20133 Milano; ITALY; 39 2 266051; 39 2 2664551 (fax)
Dolgykh, Yulia; Inst of Plant Physiology; ul. Botanicheskaya, 35; Moscow 127276; RUSSIA; 7-095-9039392; 7-095-4821685 (fax); ifr@ippras.ru
Dombrink-Kurtzman, Mary Ann; Natl. Ctr. for Agric. Utilization Research; USDA, ARS; 1815 N. University St.; Peoria IL 61604-3902; (309)681-6254; (309)681-6686 (fax); dombrink@mail.ncaur.usda.gov
Dooner, Hugo K.; The Waksman Institute; Rutgers University; P.O. Box 0759; Piscataway NJ 08855; 732-445-4684; 732-445-5735 (fax); dooner@waksman.rutgers.edu
Doring, Hans-Peter; Deutz-Kalker-St. 136; 50679 Koln; GERMANY
Dorweiler, Jane; Dept Plant Sciences; 303 Forbes Hall; University of Arizona; Tucson AZ 85721; 520-621-8964; 520-621-7186 (fax); jdorweil@ag.arizona.edu
Dowd, Pat; USDA-ARS; 1815 N. University St.; Peoria IL 61604; 309-681-6242; 309-681-6686 (fax); dowdpf@mail.ncaur.usda.gov
Doyle, Greg; Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-2674
Dresselhaus, Thomas; Univ Hamburg, AMP II; Ohnhorststr 18; Hamburg D-22609; GERMANY; 49 40 82282 360; 49 40 82282 229 (fax); dresselh@botanik.uni-hamburg.de
Drummond, Bruce; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004
Dudley, John W.; Crop Sciences, S112 Turner Hall; University of Illinois; 1102 S Goodwin Ave; Urbana IL 61801; 217-333-9640; 217-333-9817 (fax); jdudley@uiuc.edu
Duncan, David; Monsanto Agricultural Group GG4H; 700 Chesterfield Parkway N.; Chesterfield MO 63198; 314-537-6923; 314-537-6567 (fax); drdunc1@ccmail.monsanto.com
Durkovicova, Viera; Prachaticka 43; SK-96001 Zvolen; SLOVAK REPUBLIC
Duvick, Donald N.; P.O. Box 446; 6837 N.W. Beaver Drive; Johnston IA 50131; 515-278-0861; 515-253-2125 (fax); dnd307@AOL.com
Duvick, Jonathan P.; Pioneer Hi-Bred Internatl; Dept of Biotechnology Res; 7300 N.W. 62nd Ave; Johnston IA 50131-1004; 515-270-3176; 515-253-2147 (fax); duvickj@phibred.com
Duvick, Susan; 1501 Agronomy; Iowa State University; Ames IA 50011; 515-294-5545
Earle, Elizabeth D.; Dept Plant Breed & Biom; Cornell University; 252 Emerson Hall; Ithaca NY 14853-1902; 607-255-3102; 607-255-6683 (fax); ede3@cornell.edu
Eathington, Samuel R.; 910 Gaskill; Ames IA 50014; 515-956-3073; 515-232-7170 (fax); Samuel.R.Eathington@Monsanto.com
Eaton, Dana; Asgrow Seed Co; 32545 Galena Sassafras Rd; Galena MD 21635; 410-827-9240; 410-827-9763 (fax)
Edgerton, Mike; Dekalb Plant Genetics/Monsanto; 62 Maritime Dr; Mystic CT 06355-1958; 860-572-5269; 860-572-5282 (fax); medgerto@dekalb.com
Edwards, Keith; Crop Genetics; IACR-Long Ashton Res Stn; Univ Bristol, Long Ashton; Bristol BS18 9AF; UNITED KINGDOM; 44 1275 549431; 44 1275 394281 (fax); KEITH.EDWARDS@BBSRC.AC.UK
Edwards, Marlin; Northrup King Co.; 317 330th Street; Stanton MN 55018-4308; 507-663-7623; 507-645-7519 (fax)
Efremov, Alexander; Max-Planck-Institute; Carl-von-Linne-Weg 10; D-50829 Koln (Vogelsang); GERMANY
Eggleston, Bill; Department of Biology; Virginia Commonwealth University; 816 Park Ave; Richmond VA 23284; 804-828-1562; 804-828-0503 (fax); Weggles@saturn.vcu.edu
Egli, Margaret A.; Dept Agron & Plant Genetics; Univ of Minnesota, 411 Borlaug Hall; 1991 Buford Circle; St. Paul MN 55108; (612)625-5215/5793; (612)625-1268 (fax); peggy@biosci.cbs.umn.edu
Eichholtz, David A.; Monsanto, BB4D; 700 Chesterfield Village Pkwy; St. Louis MO 63198; 314-537-6227; 314-537-6047 (fax); DAEICH@CCMAIL.MONSANTO.COM
Eisses, John; Dept. of Biological Sciences; University of Idaho; Moscow ID 83844-30521; 208-885-6370; 208-885-7805 (fax); eiss4477@novell.uidaho.edu
Elsing, Evan; Pioneer Hi-Bred Intl Inc.; Kekaha Research Sta; PO Box 596; Kekaha, Kauai HI 96752-0596
England, Dan; Curtis Hall; Univ of Missouri; Columbia MO 65211-7020; 573-882-7818; 573-884-7850 (fax); england@teosinte.agron.missouri.edu
Esen, Asim; Dept Biology; Va Polytech Inst & State Univ; Blacksburg VA 24061; 540-231-5894; 540-231-9307 (fax); esen@vt.edu
Eubanks, Mary W.; Dept Botany; 139 Biological Sci Bldg; Duke Univ; Durham NC 27708-0338; 919-660-7301; 919-660-7293 (fax); eubanks@duke.edu
Evans, Matthew; Laboratory of Genetics; 445 Henry Mall; University of Wisconsin; Madison WI 53706; 608-262-3286; 608-262-2976 (fax); mmevans@facstaff.wisc.edu
Evola, S. V.; Novartis Crops; 410 Swing Rd; Greensboro NC 27419-8300; 910-547-1038; 910-547-1030 (fax); evolas@a1.abru.cg.com
Falbel, Tanya; Department of Horticulture; University of Wisconsin; 1575 Linden Dr.; Madison WI 53705; 608-262-1622; 608-262-4743 (fax); falbel@macc.wisc.edu
Farish, Guy; Biology Dept; Adams State College; Alamosa CO 81102; 719-587-7969; 719-587-7242 (fax); gefarish@adams.edu
Fauron, Christiane; University of Utah; 2100 Eccles Genetics Bldg; Salt Lake City UT 84112; 435-581-4435; 435-585-7177 (fax); christiane.fauron@genetics.utah.edu
Fedoroff, Nina; Biology; 0519 Wartik Laboratory; University Park PA 16802; 814-863-5717; 814-863-1357 (fax); nvf1@psu.edu
Feix, Gunter; Institut Fur Biologie III; Universitat Freiburg; 79104 Freiburg; GERMANY; 497-6120-32724; 497-6120-32745 (fax); FEIX@biologie.UNI-FREIBURG.DE
Feng, Jia Shi; Maize Research Institute Shandong; Academy of Agricultural Sciences; 11 Sang Yuan Road; Jinan, Shandong 250100, P.R.; CHINA; (0531)860329
Fergason, Virgil; Custom Farm Seed; 100 East Shafer, P.O. Box 380; Forsyth IL 62535; 217-875-2826; 217-875-9437 (fax)
Ferl, Robert; Horticultural Sciences; Univ Florida; Gainesville FL 32611; 352-392-4711 x313; 352-392-4072 (fax); Robferl@NERVM.NERDC.UFL.EDU
Fincher, Robert R.; Pioneer Hi-Bred Intl; 7300 NW 62nd Avenue; P.O. Box 1004; Johnston IA 50131-9410; 515-270-3797; 515-253-2288 (fax); fincher@phibred.com
Flament, Pascal; Biocem; Campus Universitaire des Cezeaux; 24 Avenue des Landais; 63170 Aubiere; FRANCE; (33)73 42 79 70; (33)73 27 57 36 (fax)
Flint-Garcia, Sherry; Univ Missouri; 110 Curtis Hall; Columbia MO 65211; 573-882-2349; 573-884-7850 (fax); saflint@teosinte.agron.missouri.edu
Fluminhan, Antonio; RIKEN-Inst Phys Chem Res; Div Biosciences, Hirosawa 2-1; Wako-shi,; Saitama-ken 351-0198; JAPAN; 81-48-467-9518; 81-48-462-4676 (fax); antonio@postman.riken.go.jp
Foley, Terry; Holden's Foundation Seeds L.L.C.; P.O. Box 839; Williamsburg IA 52361; 319-668-1100; 319-668-2453 (fax); terry.foley@holdens.com
Foster, Toshi; Plant Gene Expression Center; USDA; 800 Buchanan St.; Albany CA 94710
Fowler, John; Botany and Plant Path Dept; 2082 Cordley Hall; Oregon State Univ; Corvallis OR 97331-2902; 541-737-5295; 541-737-3573 (fax); fowlerj@bcc.orst.edu
Fox, Tim; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave. NW; P.O. Box 1004; Johnston IA 50131
Frances, Shannon; Lab Photopercep Sign Transd; FRP/RIKEN, Hirosawa 2-1; Wako-shi; Saitama 351-01; JAPAN; TOM80WMN@POSTMAN.RIKEN.GO.JP
Francis, T. R.; Novartis Seeds Canada Inc; R. R. 1; Arva, Ont N0M 1C0; CANADA; 519-461-0072; 519-461-0275 (fax)
Frank, Mary; Dept. of Biology; MC0116; Univ. of California - San Diego; La Jolla CA 92093-7108
Franklin, Amie; Dept Molec Cell Biol; 345 LSA; Univ of California; Berkeley CA 94720-0001; 510-643-8277; 510-643-6791 (fax); franklin@candelab.berkeley.edu
Freeling, Michael; Dept of Plant Biology; 111 Genetics & Plant Biology Bldg; Univ of California; Berkeley CA 94720; 510-642-8058; 510-642-4995 (fax); freeling@nature.berkeley.edu
Frei, Mark; LG Seeds; Eggenkamp 1; Greven 48268; GERMANY; 49-2571-55939; 49-2571-53808 (fax); lggrev@aol.com
Frey, Monika; Lehrstuhl fur Gentechnik Hoherer Pflanzen; TUM; Lichtenbergstrabe 4; 85747 Garching; GERMANY; 49-89-2891-3532; 49-89-2891-2892 (fax); frey@gen.chemie.tu-muenchen.de
Freymark, Peter J.; 16 Cameron Road; P.O. Borrowdale; Harare; ZIMBABWE; 011-263-4-726061; 011-263-4-726061 (fax); FREYMARKP@PHIBRED.COM
Friedberg, Jeremy; 24 Silvergrove Rd; North York, Ont M2L 2N6; CANADA; 416-449-2098; jfriedb@plant.uoguelph.ca
Friedman, Robert B.; Cerestar USA; 1100 Indianapolis Blvd; Hammond IN 46320-1094; 219-659-2000 ext 390; 219-473-6607 (fax)
Friedrich, James W.; Maize Genetic Resources, Inc; 10570 Hwy. 50 North; Angier NC 27501; 919-894-5594; 919-894-5660 (fax)
Frova, Carla; Dept of Genetics & Microbiology; University of Milano; Via Celoria 26; 20133 Milano; ITALY; 39 2 26605244; 39 2 2664551 (fax); carla.frova@unimi.it
Fu, Xi-Qin; Hunan Academy of Agricultural Sciences; Hybrid Rice Research Center; Mapoling, Dong Jiao, Changsha; Hunan 410125; CHINA; 86-731-469-1443; 86-731-469-1877 (fax)
Fuerstenberg, Susan; 1206 Orkney Drive; Ann Arbor MI 48103; 313-647-5718; sifuerst@umich.edu
Gabay-Laughnan, Susan; Plant Biology/265 Morrill Hall; University of Illinois; 505 S. Goodwin Avenue; Urbana IL 61801; 217-333-2919; 217-244-7246 (fax); gabaylau@life.uiuc.edu

Gaillard, Antoine; Maisadour Semences; Unite Biotechnologie; BP 27; 40001 Mont-de-Marsan; FRANCE; 58 05 84 54; 58 05 84 87 (fax); gaillard@maisadour.com
Gale, Michael; John Innes Centre; Norwich Research Park; Colney; Norwich NR4 7UH; UNITED KINGDOM; 44 1603 450 599; 44 1603 450 024 (fax); mike.gale@bbsrc.ac.uk
Galinat, Walton C.; Eastern Agric. Center, U. Mass.; 240 Beaver Street; Waltham MA 02154-8096; 617-891-0650; 617-899-6054 (fax)
Gallie, Daniel; Dept. of Biochemistry; University of California; Riverside CA 92521; (909)787-7298; (909)787-3590 (fax); DRGALLIE@UCRAC1.UCR.EDU
Gao, Min-Wei; Zhejiang Agricultural University; Institute of Nuclear-Agric. Science; Hangzhou; Zhejiang 310029; CHINA
Garcia-Olmedo, F.; Lab Biochemistry and Molecular Biology; Dept. Biotechnology; E T S Ingenieros Agronomos; 28040-Madrid; SPAIN; 34-1-3365707; 34-1-3365757 (fax)
Gardiner, Jack; 214 Curtis Hall; University of Missouri; Columbia MO 65211-7202; 573-884-3134; 573-884-7850 (fax); GardinerJ@Missouri.edu
Gardiner, Michele; Rogers NK Seed Co; 6338 Highway 20-26; Nampa ID 83687; (208)466-0319; (208)467-4559 (fax); michele.gardiner@seeds.novartis.com
Gardner, Candice; USDA-ARS Research Leader; NCR Plant Introduction Station; G214 Agronomy Hall; Iowa State University; Ames IA 50011-1170; 515-294-7967; 515-294-4880 (fax); gardnerc@iastate.edu
Garnaat, Carl W.; Pioneer Hi-Bred Int'l; Department of Biotechnology Research; 7300 N. W. 62nd Ave-P.O. Box 1004; Johnston IA 50131-1004; (515)253-2251; (515)270-3367 (fax); GARNAATC@PHIBRED.COM
Garwood, D. L.; Garwood Seed Company; 1929 N. 2050 East Rd.; Stonington IL 62567-5306; 217-325-3715; 217-325-3578 (fax)
Gaut, Brandon; 321 Steinhaus Hall; Dept Eco & Evol; UC Irvine; Irvine CA 92697-2525; 949-824-2564; 949-824-2181 (fax); bgaut@uci.edu
Gavazzi, Giuseppe; Universita de Milano; Dip Fisiol Pianta Coltivate Chim Agrar; Via Celoria 2; 20133 Milano; ITALY; 02-26607221; 02-2663057 (fax); gavazzi@imiucca.csi.unimi.it
Geadelmann, Jon L.; Holden's Foundation Seeds, Inc; 2440 Highway 19 Blvd; Stanton MN 55018-7220; 507-263-3476; 507-263-4839 (fax); jon@holdens.com
Gebauer, Juan E.; Corn Breeding; Casilla 190; Buin; CHILE; 011(56-2)821-1552; 011(56-2)821-3564 (fax)
Geiger, Hartwig H.; Univ Hohenheim; 350 Inst Pflanzenzucht; Seed Sci and Pop Genetics; D-70593 Stuttgart; GERMANY; 49-711-459-2644; 49-711-459-2343 (fax); geigerhh@uni-hohenheim.de
Gengenbach, Burle G.; Agron & Plant Genetics; Univ of Minnesota; 1991 Upper Buford Cir.; St Paul MN 55108; 612-625-6282; 612-625-1268 (fax); burle@biosci.cbs.umn.edu
Gierl, Alfons; Lehrstuhl fur Genetik; Technische Universitat Munchen; Lichtenbergstrasse 4; 85747 Garching; GERMANY; 49-89-289-12890; 49-89-289-12892 (fax); gierl@bio.tum.de
Gilliam, Jacob; Pioneer Hi-Bred Internat Inc; Crop Protection; 7300 NW 62nd Ave., PO Box 1004; Johnston IA 50131-1004
Gillies, Christopher; Macleay Bldg A12; Univ of Sydney; Sydney NSW 2006; AUSTRALIA; 61-2-351-2688; 61-2-351-4771 (fax); cgillies@extro.ucc.su.oz.au
Giorio, Giovanni; c/o Metapontum Agrobios; SS. Ionica Km 448.2; I-75010 Metaponto (MT); ITALY; 39-835-740276; 39-835-745306 (fax)
Girard, Lisa; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102
Glover, David V.; Dept of Agronomy; Purdue University; W. Lafayette IN 47907; 765-494-8067; 765-494-6508 (fax); DGLOVER@dept.agry.purdue.edu
Godshalk, E. Brent; Dekalb Genetics Corp.; 423 S. Colebrook Rd.; Manheim PA 17545-9144; 815-758-9372
Goff, Steve; CIBA-GEIGY Biotechnology; 3054 Cornwallis Road; Research Triangle Park NC 27709
Goldman, Irwin; Department of Horticulture; Univ. of Wisconsin; 1575 Linden Drive; Madison WI 53706; (608)262-7781; (608)262-4743 (fax)
Goldman, Stephen; Dept of Biology; University of Toledo; Toledo OH 43606; 419-530-1540; 419-530-7737 (fax); stephen.goldman@utoledo.edu
Golubovskaya, Inna; Cande Lab; Dept of Molec & Cell Biology; Box 341 LSA; Univ of California; 190 000 St Petersburg; RUSSIA
Gong, Zuxun; Academia Sinica; Shanghai Inst. of Biochem.; 320 Yue-Yang Road; Shanghai 200031; CHINA
Gonzalez de Leon, Diego; Paseo del Atardecer 360; Villas de Irapuato; Irapuato 36650 Guanajuato; MEXICO; 52-462-31137 (phone/fax); dgdeleon@prodigy.net.mx
Goodman, Major M.; Department of Crop Sciences; North Carolina State Univ; P.O. Box 7620; Raleigh NC 27695; 919-515-7039; 919-515-7959 (fax)
Gordon, P. N.; CT Forest & Park Assoc.; 16 Meriden Rd.; Rockfall CT 06481-2961; (203)346-2372; 203-347-7463 (fax)
Gordon-Kamm, William; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave., PO Box 1004; Johnston IA 50131-1004
Gouesnard, B; INRA Centre de Montpellier; Sta Genet Amel Plantes; domaine de Melgueil; 34130 Mauguio; FRANCE; 67 29 39 90 (fax); gouesnar@montpellier.inra.fr
Gracen, Vernon; Cargill Worldwide Seed; Box 5645; Minneapolis MN 55440; 612-742-7244; 612-742-7235 (fax)
Graham, Geoffrey; Asgrow Seed Co.; 9866 Highway 66; Newburgh IN 47630; 812-853-2425; 812-853-5688 (fax); geoffrey.graham@monsanto.com
Grant, David; G304 Agronomy Hall; Iowa State Univ; Ames IA 50011-1010; 515-294-1205; 515-294-2299 (fax); dgrant@iastate.edu
Gray, John; Dept of Biology; Univ of Toledo; 2801 West Bancroft St; Toledo OH 43606; 419-530-1537; 419-530-7737 (fax); jgray5@uoft02.utoledo.edu
Greene, Thomas W.; Univ Florida; Dept Hort Sci; P.O. Box 110690; Gainesville FL 32611; 352-392-1928 x314; 352-392-5653 (fax); twg@gnv.ifas.ufl.edu
Grier, Stephen L.; Novartis Seeds; 317 330th St.; Stanton MN 55018-4308; 507-663-7662; 507-645-7519 (fax); steve.grier@seeds.novartis.com
Griffin, Daniel; 745 Beach Ave; Beachwood NJ 08722; 908-244-6721; grriffin@rider.edu
Griffor, Matthew; DeKalb Genetics; 62 Maritime Road; Mystic CT 06355; 860-572-5229; 860-572-5240 (fax)
Grimanelli, Daniel; ORSTOM - CIMMYT; Apdo Postal 6-641; 06600 Mexico D.F.; MEXICO; (52) 5 726-9091; (52) 5 726-7558 (fax); dgrimanelli@cgiar.org
Grobman, Alexander; Semillas Penta del Peru S.A.; Apartado 270227; Lima 27; PERU; (5114)426375; (5114)425465 (fax)
Grossniklaus, Ueli; Cold Spring Harbor Laboratory; PO Box 100; Cold Spring Harbor NY 11724; 516-367-8825; 516-367-8369 (fax); grossnik@cshl.org
Grotewold, Erich; Ohio State Univ Biotech Ctr; 232 Rightmire Hall; 1060 Carmack Rd; Columbus OH 43210; 614-292-2483; 614-292-5379 (fax); grotewold.1@osu.edu
Gu, Ming-Hong; Jiangsu Agricultural College; Dept. of Agronomy; Yangzhou; Jiangsu 225001; CHINA
Gu, Mingguang; Institute of Genetics; Chinese Academy of Sciences; Beijing; CHINA
Guiltinan, Mark; Penn State Biotechnology Institute; 306 Wartik Lab; Dept of Horticulture; Univ. Park PA 16802-5807; 814-863-7958; 814-863-6139 (fax); mjg9@psu.edu
Guimaraes, Marco A; Caixa Postal 9; Via Dionisio Bortoloti Km 0.5; Santa Cruz das Palmeiras; SP 13650-970; BRAZIL; 55196-72-1917; 55196-72-2424 (fax)
Guo, Baozhu; USDA/ARS/IBPMRL; PO Box 748; Tifton GA 31793-0748; 912-387-2326; 912-387-2321 (fax); bguo@tifton.cpes.peachnet.edu
Guo, Jun-Yuan; Academia Sinica; South China Institute of Botany; Guangzhou 510650; CHINA
Guo, Mei; Pioneer Hi-Bred Intl, Inc; 7250 NW 62nd Ave; PO Box 552; Johnston IA 50131-552; 515-253-2146; 515-334-4788 (fax); guom@phibred.com
Gupta, Manju; Mycogen; 9330 Zionsville Rd; Building 306/C-1; Indianapolis IN 46268-1054; 317-337-5980; 317-337-5989 (fax); mgupta@dowelanco.com
Haag, Wayne L.; Sasakawa Global 2000; C. P. 4247; Maputo; MOZAMBIQUE; 258-1-490004; 258-1-491417 (fax)
Hake, Sarah; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5907; 510-559-5678 (fax); maizesh@nature.berkeley.edu
Hall, Lisa Naomi; Oxford Univ; Dept Plant Sci; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; 865-275030; 865-275147 (fax)
Hallauer, Arnel R.; Agronomy Building; 1505 Agronomy Hall; Iowa State University; Ames IA 50011-1010; 515-294-3052; 515-294-3163 (fax)
Hamilton, RI; 3199 Klondike Road; North Gower; Ontario K0A2T0; CANADA; 613-489-3166; 613-489-3166 (fax); rih@cyberus.ca
Han, Changdeok; Gyeongsang National University; Gazwa Dong; Chinju 660-701; KOREA; 082-591-751-6029; 82-591-759-9363 (fax); cdhan@nongae.gsnu.ac.kr
Hancock, Denis C.; 213 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-1722; 573-884-7850 (fax); dhancock@teosinte.agron.missouri.edu
Hannah, Curtis; Veg Crops Dept; Univ of Florida, IFAS; 1143 Fifield Hall, P.O. Box 110690; Gainesville FL 32611-0690; 352-392-1928x315; 352-392-5653 (fax); Hannah@GNV.IFAS.UFL.EDU
Hansel, W. C.; Hansel Cons & Mgmt; Box 283; Carrollton MO 64633; 816-542-1616
Hansen, Joel; 2254 Molecular Biology Bldg.; Iowa State University; Ames IA 50011; 515-294-0347; 515-294-0453 (fax); jdhansen@iastate.edu
Hansen, Leon A.; Novartis Seeds, Inc.; 6338 Highway 20-26; Nampa ID 83687; 208-465-8554; 208-467-4559 (fax)
Hanson, Maureen R.; Section Genetics/Develop.; Cornell Univ.; Biotech Bldg; Ithaca NY 14853; 607-254-4833; 607-255-2428 (fax)
Hantke, Sabine; Pioneer Hi-Bred Intl, Inc; 7300 NW 62nd Ave; P.O. Box 1004; Johnston IA 50131; 515-253-2493; 515-270-3367 (fax); hantkess@phibred.com
Hardeman, Kristine; Dekalb Plant Genetics/Monsanto; 62 Maritime Dr; Mystic CT 06355; 860-572-5282 (fax); lbrooks@dekalb.com
Harper, Lisa; Dept Molec & Cell Biol; 345 LSA; University of CA; Berkeley CA 94720; 510-643-8277; 510-643-6791 (fax); ligule@nature.berkeley.edu
Harris, John W.; Dept of Biology; Tennessee Tech Univ; Cookeville TN 38505; 615-372-3143; 615-528-4097 (fax)
Harris, Linda J.; Eastern Cereal & Oilseed Res Centre; Agriculture & Agri-food Canada; Bldg. #21, Central Exp. Farm; Ottawa, Ontario K1A 0C6; CANADA; (613)759-1314; (613)759-6566 (fax); harrislj@em.agr.ca
Hartman, Carl; 155 South St. Rd. 2; Valparaiso IN 46383; 219-462-1927

Havukkala, Ilkka; Genesis R & D Corp. Ltd.; P.O. Box 50; Auckland; NEW ZEALAND; 64-9-373-5600; 64-9-373-5601 (fax); i.havukkala@genesis.co.nz
Hawk, James A.; Dept Plant & Soil Sciences; University of Delaware; Newark DE 19717-1303; 302-831-1379; 302-831-3651 (fax); jhawk@strauss.udel.edu
He, Zuhua; Zhejiang Agricultural University; Biotechnology Institute; Hangzhou; Zhejiang 310029; CHINA
Heinlein, Manfred; The Scripps Research Institute; Dept. of Plant Cell Biology, MRC 7; 10666 North Torrey Pines Rd; La Jolla CA 92037; 619-554-2854; 619-554-6330 (fax)
Helentjaris, Tim; Pioneer Hi-Bred Int., Inc.; Agronomic Traits/TTD; 7300 N.W. 62nd Ave. P.O. Box 1004; Johnston IA 50131-1004; 515-270-3691; 515-254-2619 (fax); helentjartg@phibred.com
Heredia-Diaz, Oscar; Monsanto; 700 Chesterfield Pkway North GG6A; St. Louis MO 63198; 314-537-6902; 314-537-6950 (fax); oohere@monsanto.com
Heslop-Harrison, J. S.; Karyobiology Group; JI Centre for Plant Science Research; Colney Lane; Norwich NR4 7UH; ENGLAND; 44-1603-452571; 44-1603-456844 (fax); HHARRISON@bbsrc.AC.UK
Hetz, Winfried; Institue of Biology; University of Freiburg; Schlanzlestr, 1; 79104 Freiburg; GERMANY
Heun, Manfred; Population Genetics; Dept Chem & Biotechnol; PO Box 5040, Agric Univ Norway; N-1432 AAs - NLH; NORWAY; 47-64947679; 47-64947691 (fax); manfred.heun@ikb.nlh.no
Hiatt, Evelyn N.; Dept of Genetics; Life Sciences Bldg; Univ Georgia; Athens GA 30602; 706-542-1010; 706-542-3910 (fax); hiatt@dogwood.botany.uga.edu
Hile, Glenn C.; Northrup King Co; 7113 Alt 49 East; P.O. Box 249; Arcanum OH 45304; 513-692-5164; 513-692-8256 (fax)
Hill, Martha; CIBA-GEIGY; PO Box 12257; 3054 Cornwallis Rd; Research Triangle Park NC 27709-2257; 919-541-8580; 919-541-8585 (fax); HILLM@ABRU.CG.COM
Ho, David; Department of Biology; Washington University; St. Louis MO 63130; 314-935-4632; 314-935-4432 (fax); HO@WUSTLB.wustl.edu
Hodges, Tom; Botany & Plant Pathology; Agricultural research Building; Purdue University; W. Lafayette IN 47907; 765-494-4657; 765-494-5896 (fax); hodges@btny.purdue.edu
Hoegemeyer, Thomas C.; Hoegemeyer Hybrids Inc; 1755 Hoegemeyer Rd; Hooper NE 68031; 402-654-3399; 402-654-3342 (fax)
Hoekenga, Owen; Boyce Thompson Inst Plant Res; Tower Rd; Ithaca NY 14853-1801; 607-254-1310; 607-254-1242 (fax); oah1@cornell.edu
Hoisington, David; CIMMYT; Lisboa 27, Aptdo. Postal 6-641; 06600 Mexico, D. F.; MEXICO; 525-726-7575; 525-726-7558 (fax); DHOISINGTON@CIMMYT.MX
Hole, David; Plant Soils & Biometeorology Dept.; Utah State Univ.; Logan UT 84322-4820; 435-750-2235; 435-750-3376 (fax); dhole@mendel.usu.edu
Holland, Greg; Asgrow Seed Co; Box 460 Hwy 20 W; Parkersburg IA 50665; 319-347-6633; 319-347-2720 (fax)
Holley, Randall; 340 Southside Dr.; Henderson KY 42420
Hollick, Jay; Institute of Molecular Biology; University of Oregon; Eugene OR 97403-1229; 541-346-5123; hollick@darkwing.uoregon.edu
Hong, Guo-Fan; National Center for Gene Research; Chinese Academy of Science; 500 Cao Bao Road; Shanghai 200233; CHINA; 86-21-482-2885; 86-21-482-5775 (fax)
Hong, Meng-Min; Chinese Academy of Sciences; Shanghai Inst Plant Physiol; 300 Fenglin Road; Shanghai 200032; CHINA; 86-21-64042090-4429; 86-21-64042385 (fax); hongmm@iris.sipp.ac.cn
Houchins, Kate; 302 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2033; 573-884-7850 (fax); Kate@teosinte.agron.missouri.edu
Howard, John; Prodigene; 1500 Research Pkwy Suite 220; College Station TX 77845; 409-862-8537; 409-862-8538 (fax)
Hsia, An-Ping; B420 Agronomy Hall; Iowa State University; Ames IA 50011; 515-294-1659; 515-294-2299 (fax); hsia@iastate.edu
Hu, Gongshe; Dept Plant & Microbial Biol; Plant Gene Expression Ctr; 800 Buchanan St; Albany CA 94710; 510-559-5919; 510-559-5678 (fax); gongshe@uclink4.berkeley.edu
Hu, Jianping; Dept of Botany; University of Georgia; Athens GA 30602; 706-542-1857; 706-542-1805 (fax)
Huang, Anthony; Dept. Bot. & Plant Sci.; Univ. of California; Riverside CA 92521-0124; 909-787-4783; 909-787-4437 (fax); ahuang@ucrac1.ucr.edu
Huang, Bing-Quan; Biology Dept; Univ of North Dakota; Grand Forks ND 58202; 701-777-4479; 701-772-5041 (fax)
Huang, Danian; China National Rice Research Institute; Hangzhou; Zhejiang 310006; CHINA
Huang, Wei-Da; Fudan University; Dept. of Biochem.; Handan Road 220; Shanghai 200433; CHINA
Huang, Yih-Ching; Department of Agronomy; National Taiwan University; Taipei; TAIWAN; (02)363-0231 ext. 2727; (02)362-0879 (fax)
Hubbard, Lauren; USDA-ARS-PGEC; 800 Buchanan St.; Albany CA 94710; 510-559-5922; 510-559-5648 (fax); LHUBBARD@Nature.Berkeley.EDU
Huffman, Gary A.; Pioneer Hi-Bred International; 7300 NW 62nd Ave.; P.O. Box 1004; Johnston IA 50131-1004; 515-270-3502; 515-270-3367 (fax); HUFFMANG@PHIBRED.COM
Hulbert, Scot; Kansas State University; Dept. of Plant Pathology; Throckmorton Hall; Manhattan KS 66506-5502; 913-532-1392; 913-532-5692 (fax); shulbert@plantpath.ksu.edu
Hunsperger, John P.; P.O. Box 2217; Gilroy CA 95021-2217; (408)848-1161; jhunsp@ix.netcom.com
Hunter, Brenda; Dept Plant Sci; Forbes Hall, Room 303; Univ of Arizona; Tucson AZ 85721
Hunter, Clifford; Hybritech; 5912 N. Meridian; Wichita KS 67204-1699
Hussey, Patrick J.; University of London; Royal Holloway New College; Dept. Biochem., Egham Hill; Egham, Surrey TW20 OEX; UNITED KINGDOM
Iida, Shigeru; Div Gene Expr & Regulation I; Nat Inst for Basic Biology; Okazaki 444; JAPAN; 0564-55-7680; 0564-55-7685 (fax); shigiida@nibb.ac.jp
Im, Kyung Hoan; Dept Plant Path; Univ of Florida; Gainesville FL 32611
Innes, R. L.; DeKalb Canada Inc.; R.R. 2; Glanworth, Ont. N0L 1L0; CANADA
Inoue, Yasuaki; National Grassland Res Inst; 768 Nishinasuno; Tochigi 329-27; JAPAN; 0287-36-0111
Irish, Erin; Department of Biological Sciences; 312 Chemistry Bldg; University of Iowa; Iowa City IA 52242; 319-335-2582; 319-335-3620 (fax); erin-irish@uiowa.edu
Isaac, Peter; Agrogene S.A.; 620, rue Blaise Pascal; Z.I.-Immeuble Alligator; 77555 Moissy Cramayel; FRANCE; 33-1-64 13 31 80; 33-1-64 13 31 81 (fax); pete.i@agrogene.com
Ishige, Teruo; Biol Resources Div; JIRCAS; 1-2 Ohwashi; Tsukuba 305-8686; JAPAN; 81-298-38-6305; 81-298-38-6650 (fax)
Ishikawa, Ryuji; Faculty of Agriculture; Hirosaki Univ; Hirosaki Aomori 036; JAPAN; 011-81-172-39-3778; 011-81-172-39-3750 (fax); ishikawa@cc.hirosaki-u.ac.jp
Islam-Faridi, M. Nurul; Dept Soil and Crop Sci; Texas A&M Univ; College Station TX 77843-2474
Jackson, David; Cold Spring Harbor Lab; 1 Bungtown Rd; P.O. Box 100; Cold Spring Harbor NY 11724-2212; 516-367-8467; 516-367-8369 (fax); jacksond@cshl.org
Jackson, Janet Day; Maize Coop Stock Center/AW-101 Turner; University of Illinois; 1102 S. Goodwin Ave; Urbana IL 61801; 217-333-6331; 217-333-6064 (fax); j-day@uiuc.edu
James, Martha G.; Dept. of Biochemistry & Biophysics; Molecular Biology Building, Room 2152; Iowa State University; Ames IA 50011; 515-294-3818; 515-294-0453 (fax); mgjames@iastate.edu
Jampatong, Chaba; Natl Corn & Sorghum Res Ctr; Kasetsart Univ; Klangdong, Pakchong; Nakhonratchasima, 30320; THAILAND; 66-44-361771-4; 66-44-361108 (fax); raisuwan@korat.loxinfo.co.th
Jampatong, Sansern; Natl Corn & Sorghum Res Ctr; Kasetsart Univ; Klangdong, Pakchong; Nakhonratchasima,30320; THAILAND; 66-44-361771-4; 66-44-361108 (fax); raisuwan@korat.loxinfo.co.th
Jankovsky, Julia; Biology Dept c/o Tim Nelson; PO Box 208104; Yale University; New Haven CT 06520-8104; 203-432-3862; julia.Paxson@Yale.edu
Jeffries-Griffor, Joanne; DeKalb Genetics; 62 Maritime Rd; Mystic CT 06355
Jegla, Dorothy; Dept Biol Sci; 316 CB; University of Iowa; Iowa City IA 52242
Jenkins, Bethany; Institute of Molecular Biology; University of Oregon; Eugene OR 97403
Jesaitis, Lynn; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102; 510-642-8058; 510-642-4995 (fax); ljesaiti@nature.berkeley.edu
Jewell, David C.; CIMMYT Maize Research Station; P.O. Box MP 163; Mount Pleasant; Harare; ZIMBABWE; (263)(4)301807; (263)(4)301327 (fax); D.Jewell@CGNET.com
Jobling, Steve; Unilever Research; Colworth House; Sharnbrook; Bedford MK44 1LQ; UNITED KINGDOM; 44 1234 222575; 44 1234 222552 (fax); Steve.Jobling@unilever.com
Johal, Guri; Pioneer Hi-Bred Internatl; Dept Biotechnol Res; PO Box 552; Johnston IA 50131-0552; 515-253-2154; 515-253-2147 (fax); johalgu@phibred.com
Johns, Mitrick A.; Dept Biological Sciences; Northern Illinois University; DeKalb IL 60115; 815-753-7836; 815-753-0461 (fax); majohns@niu.edu
Johnson, Elizabeth; CIBA-GEIGY Corporation; P.O. Box 1830; Kaunakakai HI 96748; 808-567-6146; 808-567-6753 (fax)
Johnson, Elmer C.; 1525 Vine St.; Belmont CA 94002; 650-593-1525
Johnson, MW; Dept of Agronomy; Pennsylvania State Univ; University Park PA 16802; 814-865-0324; 825-863-7043 (fax)
Johnson, Scott; Mycogen Plant Sciences; 301 Campus Dr; P.O. Box 280; Huxley IA 50124; 515-597-3284; 515-597-2875 (fax)

Johri, M. M.; Molecular Biology Unit; Tata Inst Fundamental Res; Homi Bhabha Road; Mumbai 400 005; INDIA; 215-2971; 091-22-215-2110 (fax); MMJOHRI@tifrvax.tifr.res.in
Jondle, Doug; Cargill Hybrid Seeds; 1502 N Gault; St. Peter MN 56082; 507-931-2940; 507-931-9691 (fax)
Jones, John Edward; 8429 Meadow Green Way; Gaithersburg MD 20877
Jones, John K.; Dept Agric Bot; University of Reading; Whiteknights; Reading RG6 2AS; UNITED KINGDOM; 44-1734-875123 EXT 7950; 44-1734-750630 (fax)
Jones, Jonathan; Sainsbury Laboratory; John Innes Centre; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44-1603-452571; 250024 (fax); JONESJ@bbsrc.AC.UK
Jones, Mark; 031 Selby Hall; 1680 Madison Ave; Wooster OH 44691; 330-263-3838x2837; 330-263-3841 (fax); jones.390@osu.edu
Jones, Todd; E. I. DuPont de Nemours & Co; Stine Research Center 614/106; Newark DE 19714; 302-283-2404; 302-283-2449 (fax); todd.jones@usa.dupont.com
Julstrom, Paul; c/o CIMMYT; Lisboa 27, Apdo. Postal 6-641; 06600 Mexico, D.F.; MEXICO; 52 5 726 9091; 52 5 726 7558 (fax)
Jun, Wei; Box 8118; Beijing 100081; CHINA
Juvik, J. A.; Dept. Nat. Res. Environ. Sci.; University of Illinois; 307 ERML; Urbana IL 61801; 217-333-1966; 217-333-4777 (fax); j-juvik@uiuc.edu
Kaeppler, Shawn; Dept of Agronomy; University of Wisconsin; 1575 Linden Drive; Madison WI 53706; 608-262-9571; 608-262-5217 (fax); smkaeppl@facstaff.wisc.edu
Kahler, Alex; Biogenetic Services, Inc; 2308 6th Street E.; P.O. Box 710; Brookings SD 57006; 605-697-8500; 605-697-8507 (fax); biogene@brookings.net
Kaleikau, Ed; NRI Plant Genome Program; Stop 2241; 1400 Independence Avenue SW; Washington DC 20250-2241; 202-401-1931; 202-401-6488 (fax); EKALEIKAU@REEUSDA.GOV
Kalia, V.; Regional Research Station; Dhaulakuan-173001; Distt Sirmur (H. P.); INDIA
Kang, Chun-Lin; Hunan Agricultural College; Dong Jiao; Changsa; Hunan 410128; CHINA
Kang, Manjit; Department of Agronomy; Louisiana State University; Baton Rouge LA 70803-2110; 504-388-2110; 504-388-1403 (fax); mkang@agctr.lsu.edu
Kannenberg, L. W.; Dept of Crop Science; University of Guelph; Guelph, Ontario N1G 2W1; CANADA; 519-824-4120 EXT 2506; 519-763-8933 (fax); LKANNENB@CROP.UOGUELPH.CA
Karpoff, Arnold; Dept of Biology; Univ of Louisville; Louisville KY 40292; 502-852-5934; 502-852-0725 (fax); AJKARP01@homer.Louisville.edu
Kasha, Ken; Dept of Crop Science; Univ of Guelph; Guelph, Ontario N1G 2W1; CANADA; 519-824-4120EXT 2507; 519-763-8933 (fax)
Kass, Lee; L. H. Bailey Hortorium; 462 Mann Library; Cornell Univ; Ithaca NY 14853; 607-255-2131; 607-255-7979 (fax); LBKBHWON@aol.com
Kaszas, Etienne; Univ California-Berkeley; 345 LSA; Berkeley CA 94533; 510-643-8277; 510-643-6197 (fax); ekaszas@lsa.berkeley.edu
Kato, Akio; Univ Missouri; 117 Tucker Hall; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); kato@mail.biosci.missouri.edu
Kato Y., T. Angel; Colegio de Postgraduados; Inst Rec Genet Productividad; Km 35.5 Carr Mexico-Texcoco; 56230 Montecillo, Texcoco; MEXICO; 595 1 02 30; 595 1 02 30 (fax)
Keeling, Peter; ExSeeds Genetics L.L.C.; 1573 Food Sci Bldg; Iowa State Univ; Ames IA 50011-1061; 515-294-3259; 515-294-2644 (fax); pkeeling@iastate.edu
Kellogg, Elizabeth A.; Dept of Biology; Univ of Missouri - St Louis; 8001 Natural Bridge Rd; St. Louis MO 63121; 314-516-6217; 314-516-6233 (fax); tkellogg@umsl.edu
Kendall, Tim; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave., PO Box 1004; Johnston IA 50131-1004; 515-270-5952; 515-270-3367 (fax); KENDALLTL@PHIBRED.COM
Kendra, David F.; Northrup King Company; 317 330th Street; Stanton MN 55018; 507/663-7636; 507/645-7519 (fax); dkendra@MR.Net
Kermicle, Jerry; 218 Genetics Dept; 445 Henry Mall; University of Wisconsin; Madison WI 53706; 608-262-1253; 608-262-2976 (fax); kermicle@facstaff.wisc.edu
Kerstetter, Randall; Plant Gene Expression Center, USDA; 800 Buchanan Street; Albany CA 94710; (510)559-5922; (510)559-5648 (fax); RAND@NATURE.BERKELEY.EDU
Khairallah, Mireille; CIMMYT; Apdo. Postal 6-641; Mexico, D.F. 06600; MEXICO; 415-833-6655; 415-833-6656 (fax); mkhairallah@cimmyt.mx
Khavkin, Emil E.; Inst Agric Biotech; 42 Timiryazevskaya ul.; Moscow, 127550; RUSSIA; (7-095)976-6544; (7-095)977-0947 (fax); emil@agrobio.msk.su
Kiefer, Mike; Northrup King Co.; 317 330th St.; Stanton MN 55018-4308
Kim, Insoon; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102
Kim, Soon Kwon; Internat Agric Research Inst; College of Agriculture; Kyungpook National Univ; Taegu 702-701; SOUTH KOREA; kimsk@bh.kyungpook.ac.kr
Kindiger, Bryan; USDA-ARS; Grazinglands Research Laboratory; 7207 West Cheyenne St; El Reno OK 73036; 405-262-5291; BKindiger@GRL.ARS.USDA.GOV
Kiss, Charles; 18 Avenue Gallieni; 49130 Les Ponts de Ce; FRANCE; 33-2-41-44-97-97; 33-2-41-44-98-69 (fax)
Kleese, Roger; 6700 80th Ave. N.; Brooklyn Park MN 55445; 612-566-3561; 515-254-2744 (fax)
Klein, Anita S.; Department of Biochemistry; Spaulding Life Science Bldg; University of New Hampshire; Durham NH 03824; 603-862-2455; 603-862-4013 (fax); anita.klein@unh.edu
Kloeckener, Barbara; Univ. of CA - Berkeley; Dept. of Plant Biology; 111 Koshland Hall; Berkeley CA 94720; 510-642-7085; 510-642-4995 (fax); bkg@nature.berkeley.edu
Knapp, Steve; Department of Crop & Soil Science; Oregon State University; Corvalis OR 97331-2902
Koch, Karen E.; 2147 Fifield Hall; Horticulture Dept, Plant Molec Cell Biol; University of Florida; Gainesville FL 32611; 352-392-4711 ext 309; 352-392-6479 (fax); kek@gnv.ifas.ufl.edu
Koester, Ruth; Genetic Design, Inc.; 7017 Albert Pick Rd.; Greensboro NC 27409; 919-668-1432; 919-665-3966 (fax)
Kohashi, Josue; Dept of Botany; Colegio de Postgrad; Chapingo, Edo de Mex; MEXICO; (595)4-22-00 ext.5294; (595)428-73 (fax)
Koinuma, Keiichi; Hokkaido Natl Agric Exp Stn; Hitsujigaoka, Toyohira-ku; Sapporo; Hokkaido 0628555; JAPAN; koinuma@cryo.affrc.go.jp
Konstantinov, Kosana; Maize Research Institute; S. Bajica 1; Belgrade 11080; YUGOSLAVIA; (381)11-617-434; (381)11-197-890 (fax)
Konstantinov, Yuri; Siberian Inst Plant Phys Bioch; P.O. Box 1243; Irkutsk 664033; RUSSIA; 39-52-46-09-03; 39-52-51-07-54 (fax); yukon@sifibr.irk.ru
Konstantinov, Yuri; Siberian Inst Plant Phys Bioch; P.O. Box 1243; Irkutsk 664033; RUSSIA; 39-52-46-09-03; 39-52-51-07-54 (fax); plantgene@sifibr.irk.ru
Kowalewski, Shirley; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2674; 573-884-7850 (fax); shirley@teosinte.agron.missouri.edu
Kowles, Richard V.; Biology Department - Box 10; 700 Terrace Heights; St. Mary's University of Minnesota; Winona MN 55987; 507-457-1554; 507-457-1633 (fax); dkowles@smumn.edu
Krebbers, Enno; DuPont de Nemours & Co.; Agricultural Biotechnology; Experimental Station 402/2253; Wilmington DE 19880-0402; (302)695-8577; (302)695-7361 (fax); enno.krebbers@usa.dupont.com
Krivov, Nikolai V.; Institute of Genetics; 20 Padurii St.; Kishinau 2002; MOLDOVA; 0422-52-20-68; 3732 556180 (fax); n.krivov@usa.net
Kriz, Al; DeKalb Plant Genetics/Monsanto; 62 Maritime Dr.; Mystic CT 06355-1958; 860-572-5217; 860-572-5280 (fax); akriz@dekalb.com
Krone, Todd; Asgrow Seed Co; 634 E. Lincoln Way; Ames IA 50010-6598; 515-232-6955; 515-232-6905 (fax)
Kross, Heike; Univ Missouri; 209 Curtis Hall; Columbia MO 65211-7020; 573-884-2343; 573-884-7850 (fax); heike@teosinte.agron.missouri.edu
Krueger, Roger W.; Monsanto; 800 N. Lindbergh Blvd. C2NA; St. Louis MO 63167; 314-694-3677; 314-694-5926 (fax); RWKRUE@ccmail.monsanto.com
Kuhn, William E; U.S. Corn Research Director; Pioneer Hi-Bred Internatl., Inc.; 7300 NW 62d Ave., PO Box 1004; Johnston IA 50131-1004; 515-270-3362; 515-253-2288 (fax); kuhnw@phibred.com
Kumar, M.; Department of Genetics; Rajendra Agric. Univ., Bihar; Pusa (Samastipur)-848125; INDIA
Kumar, Sanjeet; Project Directorate Veg Res; Post Box # 5002; Varanasi-221005; INDIA; sanjeetk1@mailcity.com
Kumar, Sushil; Central Inst Medicinal & Arom Plants; PO CIMAP; Lucknow 226015; INDIA; 91-0522-342683; 91-0522-342666 (fax); root@cimap.sirnetd.ernet.in
Kunze, Reinhard; Institut fuer Botanik II; Universitaet zu Koeln; Gyrhofstr. 15; 51931 Koeln; GERMANY; Tel. +49-221-470-6596; Fax+49-221-470-5039; Reinhard.Kunze@uni-koeln.de
Kuzmin, Eugene; Dept Biol Sci; Univ of Missouri; Columbia MO 65211; 573-882-8033; 573-882-0123 (fax); ekuzmin@biosci.mbp.missouri.edu
Labate, Joanne; Institute for Genomic Diversity; Biotechnology Bldg; Cornell Univ; Ames IA 50011; (607)254-4849; (607)255-6249 (fax); jl265@cornell.edu
Laccetti, Lucille B.; DeKalb Plant Genetics; 62 Maritime Park; Mystic CT 06355; 860-572-5247; 860-572-5240 (fax)
Lai, Jinsheng; Waksman Institute; State Univ of NJ; 190 Frelinghuysen Rd; Piscataway NJ 08854-8020; 732-445-3801; 732-445-5735 (fax); lai@mbcl.rutgers.edu
Lambert, Antoine; Verneuil Recherche; 33, rue de la Croix Blanche; 49630 Corne; FRANCE; 41-66-17-00; 41-45-02-61 (fax)
Lamkey, Kendall; Agronomy Building; Iowa State University; Ames IA 50011; 515-294-7826; 515-294-9359 (fax); KRLAMKEY@IASTATE.EDU
Lampoh, E.; Crops Res Inst; PO Box 3785 Kumasi; Ashanti Region; GHANA; 6221
Lane, Barbara; College of Natural Resources; Dean's Office; University of California; Berkeley CA 94720-3100; 510-643-2203; 510-642-4612 (fax); babs@nature.berkeley.edu

Lang, Timothy; USAID/KATHMANDU/6190; Washington DC 20523
Langdale, Jane; Department of Plant Sciences; University of Oxford; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; (44)1865275099; (44)1865275147 (fax); jane.langdale@plants.ox.ac.uk
Larkins, Brian; Dept Plant Sciences; University of Arizona; Building #36; Tucson AZ 85721; (520)621-9958; (520)621-3692 (fax); LARKINS@ag.arizona.edu
Laudencia-Chingcuanco, Debbie; Plant Gene Expression Center; USDA-ARS; 800 Buchanan St.; Albany CA 94710; 510-559-5968; 510-559-5678 (fax); dchingcu@nature.berkeley.edu
Laurie, David; JI Centre for Plant Science Res.; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44-01603-452571 x2610; 44-1603-502241 (fax); LAURIED@BBSRC.AC.UK
Lawson, Emily; Avitech Diagnostics Inc; 30 Spring Mill Dr; Malvern PA 19355
Leaver, Chris; Dept Plant Sciences; University of Oxford; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; 01865 275143; 01865 275144 (fax); chris.leaver@plants.ox.ac.uk
Ledger, Elizabeth; NMSU; Biology Dept; Box3AF Dept 30001; Las Cruces NM 88003; 505-646-7963; 505-646-5665 (fax); eledger@nmsu.edu
Lee, Danny; Section of Plant Biology; Robbins Hall c/o Neelima Sinha; UC Davis; Davis CA 95616
Lee, Elizabeth A.; OAC Plant Agric Dept; Crop Science Div; Rm 223 Crop Sci Bldg; Guelph, Ont N1G 2W1; CANADA; 519-824-4120x3360; 519-763-8933 (fax); elee@plant.uoguelph.ca
Lee, Hee Bong; Dept Agronomy, Coll Agriculture; Chungnam Natl Univ; 220 Gung-Dong, Yusung-Gu; Taejon 305-764; KOREA; 82-042-821-5721; 82-042-823-8050 (fax)
Lee, Hsueh-Sheng; Zoology Dept; University of Texas; Austin TX 78712-1064
Lee, In-sup; Department of Biology; College of Science; Kyungsung University; Pusan, 608-736; SOUTH KOREA; 051-620-4647; 051-627-4115 (fax)
Lee, Michael; Department of Agronomy; Iowa State University; Ames IA 50011-1010; 515-294-7951; 515-294-3163 (fax); mlee@iastate.edu
Leland, Tim; Monsanto BB3K; 700 Chesterfield Parkway; Chesterfield MO 63198; 314-737-7208; 314-737-5223 (fax)
Lemaux, Peggy; Department of Plant Biology; 111 Koshland Hall; University of California, Berkeley; Berkeley CA 94720; 510-642-1589; 510-642-7356 (fax); lemauxpg@nature.berkeley.edu
Leroy, Philippe; INRA; Domaine De Crouelle; 63039 Clermont-Ferrand; Cedex 2; FRANCE; 33 73 624337; 33 73 624453 (fax); leroy@valmont.clermont.inra.fr
Lesnick, Marc; Institute of Molecular Biology; University of Oregon; Eugene OR 97403; lesnick@molbio.uoregon.edu
Letovsky, Stan; Cereon Genomics, LLC; One Kendall Sq.; Baltimore MD 21210; 410-614-1061; letovsky@gdb.org
Levings, C. S.; Department of Genetics; North Carolina State Univ; Box 7614; Raleigh NC 27695-7614; 919-515-7115; 919-515-3355 (fax)
Levites, E. V.; Inst Cytol Genetics; Novosibirsk; RUSSIA
Levy, Avraham; Plant Genetics Department; Weizmann Institute of Science; Rehovot, 76100; ISRAEL; 972-8-342421; 972-8-466966 (fax); LPLEVY@WEIZMANN.WEIZMANN.AC.IL
Li, De-Bao; Zhejiang Agricultural University; Biotechnology Institute; Hangzhou; Zhejiang 310029; CHINA
Li, Jiansheng; Maize Research Lab; Department of Agronomy; Huazhong Agricultural University; Wuhan, Hubei 430070; CHINA; 86-27-7815681-8545; 86-27-7815057 (fax)
Li, Ping; Sichuan Agricultural University; Rice Research Institute; Yaan; Sichuan 625014; CHINA
Li, Qing; China Natl. Cente Biotech. Development; P.O. Box 8118; Beijing 10008; CHINA
Li, Xiao-Fang; DuPont Company; DuPont Experiment Station; E402-2247; Wilmington DE 19880-0402; CHINA; 302-695-8357; 302-695-4296 (fax)
Li, Yongzhong; S Plains Range Res Stn; 2000 18th St; Jilin, P. R., 136100 OK 73801; CHINA; 04441-215179; 04441-214884 (fax); yli@ag.gov
Li, Yongzhong; S Plains Range Res Stn; 2000 18th St; Jilin, P. R., 136100 OK 73801; CHINA; 04441-215179; 405-256-1322 (fax); yli@ag.gov
Li, Yongzhong; S Plains Range Res Stn; 2000 18th St; Jilin, P. R., 136100 OK 73801; CHINA; 405-256-7449; 04441-214884 (fax); yli@ag.gov
Li, Yongzhong; S Plains Range Res Stn; 2000 18th St; Jilin, P. R., 136100 OK 73801; CHINA; 405-256-7449; 405-256-1322 (fax); yli@ag.gov
Li, Yuxing; 2312 Food Sciences; Iowa State Univ; Ames IA 50011
Li, Zhaohui; Lehman College, CUNY; Biology Dept; 250 Bedford Park Blvd. West; Bronx NY 10468; 718-960-8643; 718-960-8236 (fax); ZHLLC@CUNYVM.CUNY.EDU
Library; Univ Philippines Los Banos; 4031 College; Laguna; PHILIPPINES
Lightfoot, David; Southern Illinois Univ; P.O. #23086-0006Z; Agriculture 174; Carbondale IL 62901-4415; 618-453-1797; 618-453-7457 (fax); GA4082@siu.edu
Liljegren, Sarah; Dept of Biology 0116; UC San Diego; La Jolla CA 92093-7108
Lim, Jun; New York Univ; Biology Dept Room 1009; Washington Square East; New York NY 10003; 212-998-3962; 212-995-4204 (fax); limj03@mcrcr6.med.nyu.edu
Lin, Bor-yaw; Institute of Molecular Biology; National Chung Hsing University; Taichung 402; TAIWAN; (886-4)285-1885; (886-4)287-4879 (fax); bylin@dragon.nchu.edu.tw
Lin, Liang-Shiou; USDA/CSREES/NRICGP; STOP 2241; 1400 Independence Ave. SW; Washington DC 20250-2241; 202-401-5042; 202-401-6488 (fax); llin@reeusda.gov
Liu, Aimin; Jiangsu Academy of Agric. Science; Institute of Agrobiol. Genet. & Physiol.; Nanjing 210014; CHINA
Liu, Liang-Shi; Zhongshan University; Biotechnology Research Center; Guangzhou 510275; CHINA
Liu, QinQin; Dept of Biology; Univ of Minnesota; Duluth MN 55812-2496; 218-726-7271; 218-726-8142 (fax); qliu1@d.umn.edu
Liu, Xiangdong; South China Agricultural University; Agronomy Dept.; Wushan, Tianhe, Guangzhou; Guangdong 510642; CHINA
Liu, Xiaochuan; China National Rice Research Institute; Genetics & Breeding Dept.; Hangzhou 310006; CHINA
Liu, Zhixian; 11 Sangyuan Road; Maize Research Inst; Shandong Academy of Agri. Science; Jinan, 250100; CHINA; (0531)8963721-2313; (0531)8962303 (fax)
Lonsdale, David M.; Cambridge Laboratory; IPSR - John Innes Centre; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44 1603 452571; 44 1603 456844 (fax); LONSDALE@bbsrc.AC.UK
Lopes, Mauricio A.; Natl. Maize & Sorghum Res. Ctr.; CNPMS/EMBRAPA; Caixa Postal 151, CEP 35 701-970; Sete Lagoas MG; BRAZIL; 011-55-31-773-2866; 011-55-31-773-9252 (fax); mauricio@cnpms.embrapa.br
Lorenzen Dahl, Lisa; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004
Lorenzoni, C.; Istituto di Genetica Vegetale; Universita Cattolica; Sede di Piacenza; 29100 Piacenza; ITALY; (523)599210; (523)599283 (fax); genetica@ipcucsc.bitnet
Lorz, Horst; Institut Allgemeine Botanik; Universitat Hamburg; Ohnhorststrasse 18; 22609 Hamburg; GERMANY; 49-40-82282-420; 49-40-82282-229 (fax)
Lowe, Brenda; DeKalb Genetics/Monsanto; 62 Maritime Dr; Mystic CT 06355-1958; 860-572-5216; 860-572-5280 (fax); blowe@dekalb.com
Lu, Guihua; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004
Lu, Mei-Kuang; 305 Manter Hall; Univ. Nebraska; Lincoln NE 68503; (402)472-6084
Lu, Yong-Gen; South China Agricultural University; Guangzhou 510642; CHINA
Ludwig, Steven; 709 E. Capitol St., S.E.; Washington DC 20003
Luebberstedt, Thomas; Inst fur Pflanzenzuchtung; Universitat Hohenheim; 70593 Stuttgart; GERMANY; ++711-4593483; Ge-711-4592343 (fax); LUEBBIT@UNI-HOHENHEIM.DE
Luo, Ming; Sichuan Agricultural University; Rice Research Institute; Yaan; Sichuan 62500; CHINA
Luo, Rui Bai; Biology Dept, Lehman College; 250 Bedford Park Blvd West; CUNY; Bronx NY 10468; RBLLC@CUNYVM.CUNY.EDU
Luthe, Dawn S; Dept Biochemistry; Miss State Univ; Mississippi State MS 39762; 601-325-7733; 601 -325-8664 (fax); dsluthe@ra.msstate.edu
Lutz, Joseph; Pillsbury/Green Giant; 1201 N 45th St; LeSueur MN 56058; 507-665-4457; 507-665-2682 (fax); jlutz@pillsbury.com
Lutz, Sheila; Dept Agron Plant Genetics; 411 Borlaug Hall, 1991 Buford Circle; Univ of Minnesota; St. Paul MN 55108; 612-625-1728; smlutz@puccini.crl.umn.edu
Lynch, Margaret; 1883 21st Ave; San Francisco CA 94122
Lysikov, Valery; Inst. of Genet. of AS RM.; st. Paudurie 20; Kishinev-277002; MOLDOVA; (0422)622068; 3732-556180 (fax); Lysikov@gen.as.md
Ma, Hong; Dept Biol, Life Sci Consortium; 519 Wartik Laboratory; Penn State Univ; University Park PA 16802; 814-863-6144/8082; 814-863-1357 (fax); hxm16@psu.edu
MacDonald, M. V.; Plant Breeding International; Maris Lane; Trumpington; Cambridge CB2 2LQ; UNITED KINGDOM; 01223-840411; 01223-844425 (fax)
MacRae, Amy F.; Dept of Biology, Campus Box 1229; McDonnell Hall; Washington University; St. Louis MO 63130; 314-935-7509; 314-935-5125 (fax); macrae@biology.wustl.edu
Maddock, Sheila E; Pioneer Hi-Bred Int Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004; 515-270-4047; 515-270-3444 (fax); maddocks@phibred.com

Maddox, Joyce; Pioneer Hi-Bred Internat Inc; Crop Protection, PO Box 1004; 7300 NW 62nd Ave.; Johnston IA 50131-1004; 515-270-3636; 515-253-2149 (fax); maddoxj@phibred.com
Magill, Clint; Texas A & M University; Dept. of Plant Pathology; College Station TX 77843; 409-845-8250; 409-845-6483 (fax); c-magill@tamu.edu
Maguire, Marjorie; Zoology Department; University of Texas; Austin TX 78712-1064; 512-471-7451; 512-471-9651 (fax); marjm@mail.utexas.edu
Maheshwari, J. K.; National Botanical Res Inst; H.I.G.-130, Sector 'E'; Aliganj Extension, Aliganj; 226 020, U.P. - Lucknow; INDIA; 72655; 244330 (fax)
Maillet, Dan; Dept Plant Sciences; Univ of Western Ontario; 1151 Richmond St; London, Ontario N6A 5B7; CANADA; 519-679-2111 ext 6467; dmaillet@julian.uwo.ca
Maluf, Mirian; Crop Science; Turner Hall, 1102 S. Goodwin Ave.; University of Illinois; Urbana IL 61801
Mangano, MaryLou; DeKalb Genetics Corp.; 62 Maritime Dr.; Mystic CT 06355
Manzocchi, Lucia; Consiglio Nazionale delle Ricerche; Istituto Biosintesi Vegetali; Via Bassini 15; 20133 Milano; ITALY; (39) 02.23699.408; (39) 02.23699.411 (fax); luman@icm.mi.cnr.it
Marillonnet, Sylvestre; Dept of Botany; 2502 Plant Sciences; Univ of Georgia; Athens GA 30602-7271; 706-542-1857; 706-542-1805 (fax); MARILLONNET@BSCR.UGA.EDU
Marocco, Adriano; Universita Cattolica S. Cuore; Istituto Di Genetica Vegetale; Via E. Parmense 84; 29100 Piacenza; ITALY; 39-523-599207; 39-523-599283 (fax); amarocco@pc.unicatt.it
Marshall, Lori; Holden's Foundation Seeds, L.L.C.; P.O. Box 839; 503 S. Maplewood Ave; Williamsburg IA 52361; 319-668-1100; 319-668-2453 (fax); lori.marshall@monsanto.com
Martienssen, Rob; Cold Spring Harbor Labs; P.O. Box 100; 1 Bungtown Rd; Cold Spring Harbor NY 11724-2212; 516-367-8322; 516-367-8369 (fax); MARTIENS@CSHL.ORG
Martin, Michael J.; 10454 NW 114th St; Granger IA 50109; 515-999-2548; 515-685-5204 (fax)
Martin Sanchez, J. A.; UdL-IRTA Centro R S D; Alcalde Rovira Roure 177; 25006 Lerida; SPAIN; 34-73-702569; 34-73-238301 (fax)
Mascarenhas, Joseph P.; Department of Biol Sci; State University of New York; Albany NY 12222; 518/442-4388; 518/442-4354 (fax); jm558@cnsunix.albany.edu
Mascia, Peter N.; Ceres, Inc.; 3007 Malibu Canyon Road; Malibu CA 90265; 310-317-8911; 310-317-9978 (fax); pmascia@ceres-inc.com
Mather, Diane E.; Plant Sci Dept - Macdonald Col; McGill Univ - 21111 Lakeshore; Ste-Anne-de-Bellevue; Quebec H9X 3V9; CANADA; (514) 398-7854; (514)398-7897 (fax); mather@agradm.lan.mcgill.ca
Mathur, D. S.; Div. of Genetics; Indian Agr Res Inst; New Delhi-110012; INDIA; 581481
Matthews, Paul; Lehman College, CUNY; 250 Bedford Park Blvd West; Bronx NY 10468; 718-960-4984; 718-960-8236 (fax)
Matz, Eileen C.; Dept of Biology; Building 463; Brookhaven National Laboratory; Upton NY 11973; 516-344-3396; 516-344-3407 (fax); matz@bnl.gov
Mawgood, Ahmed L. Abdel; Univ Wisconsin; Plant Pathology Dept; Madison WI 53706; amawgood@plantpath.wisc.edu
Mazoti, Luis B.; Carlos Croce 145; 1832 Lomas de Zamora; ARGENTINA
Mazur, Barbara J.; DuPont Agr Products; Biotechnol Res & Devel; Experimental Sta E402/3226; Wilmington DE 19880-0402; 302-695-3700; 302-695-7361 (fax); Barbara.J.Mazur@usa.dupont.com
McCarthy, Susan A.; National Agricultural Library; 10301 Baltimore Blvd; Beltsville MD 20705; 301-504-5510; 301-504-5022 (fax); smccarth@nal.usda.gov
McCarty, Donald R.; Vegetable Crops Department; 1255 Fifield Hall; University of Florida; Gainesville FL 32611; 352-392-1928 ext 322; 352-392-6479 (fax); drm@gnv.ifas.ufl.edu
McConnell, R. L.; Research & Product Development; Pioneer Hi-Bred International; Box 1004; Johnston IA 50131-1004; (515)270-3363; (515)253-2478 (fax); McConnelR@phibred.com
McCormick, Sheila; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5906; 510-559-5678 (fax); sheilamc@nature.berkeley.edu
McCullough, Andrew; Dept. of Biochemistry; Baylor College of Medicine; Houston TX 77030; 713-798-4622
McCurdy, Leroy; P.O. Box 77; McCurdy Seed Co; Fremont IA 52561; 515-933-4291
McFerson, John; AsgrowSeed Co.; 634 E. Lincoln Way; Ames IA 50010; (515)232-7170; (515)232-6905 (fax)
McLaren, James S.; Inverizon Int Inc; 2214 Stoneridge Terrace Court; St. Louis MO 63017-7120; 636-530-6943; 636-530-6945 (fax); mclaren@inverizon.com
McMullen, Michael; USDA ARS MWA; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-7606; 573-884-7850 (fax); mcmullen@teosinte.agron.missouri.edu
McWhirter, Ken; 127 Victoria Road; West Pennant Hills; NSW 2125; AUSTRALIA; 02 9484 7417; 02 9484 7417 (fax); kmcwhirt@ozemail.com.au
Mead, Doug; Northrup King Co.; 317 330th St.; Stanton MN 55018-4308; (507)663-7623; (507)645-7519 (fax)
Meeley, Robert; Trait and Technology Dev.; Pioneer Hi-Bred Int'l; 7300 NW 62nd Ave. - Box 1004; Johnston IA 50131-1004; 515-270-3770; 515-253-2149 (fax); MeeleyRB@phibred.Com
Meghji, Moez; Novartis Seeds; RR13/Box166; Bloomington IL 61704; 309-823-8578; 309-823-9468 (fax); moez.meghji@seeds.novartis.com
Mei, Mantong; South China Agricultural University; Genetic Engineering Laboratory; Guangzhou 510642; CHINA
Melchinger, Albrecht E.; Universitat Hohenheim; Institut fur Pflanzenzuchtung; Postfach 70 05 62 (350/1); D-70593 Stuttgart; GERMANY; 0711-459-2334; 0711-459-2343 (fax); melchinger@uni-hohenheim.de
Melia-Hancock, Susan; Curtis Hall - Agronomy; University of Missouri; Columbia MO 65211; 573-882-6566; 573-884-7850 (fax); susan@teosinte.agron.missouri.edu
Mello-Sampayo, Tristao; R. Padre Francisco 16, 5.F.; 1300 Lisboa; PORTUGAL
Messing, Jo; Rutgers, The State Univ; Waksman Institute; 190 Frelinghuysen Rd; Piscataway NJ 08854-8020; 732-445-4256; 732-445-0072 (fax); messing@mbcl.rutgers.edu
Mettler, I. J.; Northrup King Co.; 317 330th Street; Stanton MN 55018-4300; 507-663-7643; 507-645-7519 (fax)
Meyer, Terry; Pioneer Hi-Bred International; 7300 N.W. 62nd Avenue; P. O. Box 1004; Johnston IA 50131-1004; 515-270-3962; 515-270-3367 (fax); MEYERTE@PHIBRED.COM
Meyerowitz, Elliot; Biology Dept 156-29; California Inst Tech; Pasadena CA 91125; 818-395-6889; 818-449-0756 (fax)
Michelini, Luiz Antonio; R. Ayrton Playsant, 21; Ponta Grossa; Parana 84100-550; BRAZIL; 55-42-223-2774; 55-42-223-2774 (fax)
Michiels, Frank; Plant Genetic Systems; Jozef Plateaustraat 22; Gent B-9000; BELGIUM; 32-9-2358475; 32-9-2240694 (fax); FRAL@PGSGENT.BE
Micu, V. E.; Scientific Res Inst Maize & Sorghum; Pashcani; Criuleni; 278336 Moldova; MOLDOVA; (3732)-22-24-78; (3732)-22-73-02 (fax)
Mies, David; Northrup King; 306 Meadow Drive - Box 710; St. Joseph IL 61873; 217-469-2746; 217-469-2407 (fax)
Mihm, John A.; French Agricultural Research; RR2, Box 294; Lamberton MN 56152; 507-752-7274; 507-752-6132 (fax); JMihm@Prairie.lakes.com
Mikula, Bernard C.; Defiance College; 901 College Drive; Defiance OH 43512; 419-784-4010 EXT 426; 419-784-0426 (fax)
Milach, Sandra; 1991 Buford Circle; Room 411 Borlang Hall; St. Paul MN 55108; (612)625-6223; (612)625-1268 (fax)
Miles, Donald; Tucker Hall; Div. Biological Sciences; University of Missouri; Columbia MO 65211; 573-882-7933; miles@biosci.mbp.missouri.edu
Millard, Mark; 117 Plant Intro; Ames IA 50011-1170; 515-294-3715; 515-294-1903 (fax); mjmillar@iastate.edu
Min, Hwang Kee; Hongcheon Maize Exper Sta; 814 Jangnam; Doochon; Hongcheongun; KOREA; 82-366-435-3757; 82-366-435-6876 (fax)
Min, Shao-Kai; China National Rice Research Institute; Genetics and Breeding; 171 Ti Yu Chang Road, Hangzhou; Zhejiang 310006; CHINA
Ming, Ray Reiguang; Hawaii Agric Research Center; 99-193 Aiea Heights Dr; Aiea HI 96701-3911; 808-486-5374; 808-486-5020 (fax); rming@harc-hspa.com
Miranda, Luiz Eugenio C de; Av Barao de Itapura 1481; Cx Postal 28; Campinas SP 13001-970; BRAZIL; 019-23154222150
Miura, Y; Maize Breeding Station; Hokkaido Natl. Agr. Exp. Stn.; Hitsujigaoka; Sapporo 062; JAPAN
Miyoshi, K.; Sakata Seed Corp.; Kakegawa Breed. STN; Yoshioka 1743-2; Kakegawa City, Shizuoka City 436-01; JAPAN
Modena, Stephen; Dept. of Crop Science; North Carolina State Univ.; Box 7620; Raleigh NC 27695-7620; 919-515-2246; 919-515-7959 (fax)
Mogensen, Lloyd; Northern Arizona Univ; Dept of Biology, Box 5640; Flagstaff AZ 86011; 520-523-7328; 520-523-7500 (fax); Hans.Mogensen@nau.edu
Molina, M. C.; Del Valle Iberlucea 3711; 1826 Remedios de Escalada; Buenos Aires; ARGENTINA
Montagu, Jonathan; Cold Spring Harbor Lab; PO Box 100; Cold Spring Harbor NY 11724
Mooney, Mark; UC Berkeley; Plant Biology Dept; 111 Koshland Hall; Berkeley CA 94720-3102
Moore, Kathy; Molec & Cell Biol Dept; LSS 444; University of Arizona; Tucson AZ 85721; (520)621-3381; (520)621-3709 (fax)
Moore, Paul H.; USDA ARS PWA; Experiment Station HSPA; P.O. Box 1057; Aiea HI 96701; 808-487-5561; 808-486-5020 (fax)
Moose, Stephen P.; Dept of Crop Sciences; Univ of Illinois; 389 ERML; 1201 W. Gregory Drive; Urbana IL 61801; 217-244-6308; 217-333-4582 (fax); smoose@uiuc.edu
Moreira-Filho, Carlos A.; Dept. de Imunologia do ICP-USP; Av. Prof. Lineu Prestes, 2415; 05508-900 Sao Paulo S. P.; BRAZIL
Morgante, Michele; DuPont Ag Biotech; Delaware Technol Park; Suite 200 PO Box 6104; Newark DE 19714-6104; 302-631-2638; 302-631-2607 (fax);

michele.morgante@usa.dupont.com
Mori, Naoki; Dept Agron Plant Genetics; Univ of Minnesota, 411 Borlaug Hall; 1991 Buford Circle; St. Paul MN 55108; 612-625-9261; 612-625-1268 (fax)
Moro, Gloverson Lamego; Novartis Seeds; Caixa Postal 585; 38406-270 Uberlandia MG; BRAZIL; 034-216-6005; moro@nanet.com.br
Morrow, Sasha; Cargill Hybrid Seeds; 2600 W. Galena Blvd; Aurora IL 60506; 630-801-2326; 630-801-2345 (fax)
Mottinger, John; Dept Bioch Microb Mol Gen; University of Rhode Island; Kingston RI 02881; 401-874-2625; 401-874-2202 (fax); john.mottinger@uri.edu
Motto, Mario; Ist Sper Cereal - Sez Bergamo; Via Stezzano 24; 24100 Bergamo; ITALY; 39-035-313132; 39-035-316054 (fax); motto@tin.it
Mozoub, Daniel; Lehman College, CUNY; Biology Dept, c/o Elli Wurtzel; 250 Bedford Park Blvd West; Bronx NY 10468
Mueller, Suzanne; Univ of Wisconsin; 1575 Linden Drive; 430 Moore Hall; Madison WI 53706-1597; 608-262-3660; 608-262-5217 (fax); smmuell1@students.wisc.edu
Muenchrath, Deborah A.; Agronomy; Iowa State Univ; Ames IA 50011-1010; 515/294-1360; 515/294-8146 (fax); mnchrath@iastate.edu
Mulcahy, David; Botany Department; Univ of Massachusetts; Amherst MA 01003; 413-545-2238; 413-545-3243 (fax); dmulcahy@bio.umass.edu
Mulligan, Timothy P.; P. O. Box 523; Cold Spring Harbor NY 11724; 516-367-8829; 516-367-4031 (fax); MULLIGAN@CSHL.ORG
Mungoma, Catherine; Mt. Makulu Research Station; P. B. 7; P.O. Box 30563; Chilanga; ZAMBIA; 260-1-278008; 260-1-249127 (fax)
Murigneux, Alain; BIOCEM-Groupe Limagrain; Lab Biol Cell Molec, Campus Univ Cezeaux; 24 av des Landais; 63170 Aubiere; FRANCE; 33-4-73-42-79-70; 33-4-73-27-57-36 (fax); alain.murigneux@limagrain.com
Murray, Michael G.; Dow AgroSciences; 9330 Zionsville Rd; Indianpolis IN 46268; 317-337-3982; 317-337-4266 (fax); mmurray@dowagro.com
Musket, Theresa; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2033; 573-884-7850 (fax); musket@teosinte.agron.missouri.edu
Mustell, Robert A.; National Corn Growers Assoc.; 1000 Executive Parkway, Suite 105; St. Louis MO 63141; 314-275-9915x113; 314-275-7061 (fax); mustell@NCGA.com
Muszynski, Michael G.; Pioneer Hi-Bred Intl.; Agronomic Traits/TTD; 7300 NW 62nd Ave, POB 1004; Johnston IA 50131-1004; 515-254-2637; 515-254-2608 (fax); muszynskimg@phibred.com
Nagle, Barry; National Starch & Chemical Co.; PO Box 380; Forsyth IL 62535; 217-875-2826; Barry.Nagle@nstarch.com
Nakagawa, Yoichi; Takii & Company LTD; P.O. Box 7; Kyoto C. P. O.; JAPAN; (075)365-0123; (075)365-0110 (fax)
Naranjo, Carlos; Inst Fitotecn Santa Catalina (UNLP); C.C.4 (1836) Llavallol; Buenos Aires; ARGENTINA
Nedev, Trenfafil; Dept Tissue & Cell Cult; Inst of Genetics; Bulgarian Acad Sci; Sofia; BULGARIA; (+359 2) 75 40 41x233; (+359 2) 75 70 87 (fax); nedeva@biofac.uni-sofia.bg
Negrotto, David; Novartis; 3054 Cornwallis Rd; Research Triangle Park NC 27709-2257; 919-541-8686; 919-541-8557 (fax); negrottod@am.abru.cg.com
Nel, P. M.; Dept of Genetics; Univ of The Witwatersrand; P.O. WITS; Transvaal 2050; SOUTH AFRICA; 011-716-2154; 011-716-8030 (fax)
Nelson, Jennifer; UC Berkeley; Plant Biology Dept; 111 Koshland Hall; Berkeley CA 94720-3102; 510-642-7085; 510-642-4995 (fax); jnelson@nature.berkeley.edu
Nelson, Oliver E.; Department of Genetics; 445 Henry Hall; University of Wisconsin; Madison WI 53706; 608-265-4636; 608-262-2976 (fax); oenelson@facstaff.wisc.edu
Nelson, Timothy; Department of Biology; PO Box 208104; Yale University; New Haven CT 06520-8104; 203-432-3860; 203-432-5632 (fax); TIMOTHY.NELSON@YALE.edu
Nemeth, Janos; Cereal Research Non-Profit Co.; Also Kikoto sor 9; Szeged; HUNGARY; 62-54-555; 62-54-588 (fax)
Neuffer, M. G.; 202 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-7735; 573-884-7850 (fax); Gneuffer@aol.com
Newman, T. S.; Wyffels Hybrids, Inc; P.O. Box 246; Atkinson IL 61235; 309-936-7833; 309-936-7930 (fax)
Newman, Thomas C; MSU-DOE Plant Res Lab; Plant Biology Bldg; East Lansing MI 48824-1312; 517-353-0854; 517-353-9168 (fax); newmant@pilot.msu.edu
Newton, Kathleen; Dept Biol Sci; University of Missouri; Columbia MO 65211; 573-882-4049; Newton@biosci.mbp.missouri.edu
Nguyen, Henry T.; Dept of Plant & Soil Science & Inst for Biotech; Texas Tech University; Lubbock TX 79409-2122; 806-742-1622; 806-742-2888 (fax); henry.nguyen@ttu.edu
Noble Jr., Stephen W.; Dept of Corn Breeding; Pioneer Hi-Bred Int'l Inc; P.O. Box 385; Johnston IA 50131-0385; 515-270-3318; 515-270-4314 (fax); NOBLES@PHIBRED.COM
Norton, Robert A.; USDA, ARS, NCAUR Mycotoxin R.U.; 1815 N. University; Peoria IL 61604; 309-681-6251; 309-671-7814 (fax)
Nowell, David C; Pannar (Pty) Ltd; PO Box 19; Greytown 3500; SOUTH AFRICA; 27-334-31131; 27-334-71208 (fax)
Nutter, Robert; Pioneer Hi-Bred; 7300 NW 62nd Street; Johnston IA 50131-1004; (515)270-3349; (515)270-3367 (fax)
Ober, Eric; 1-87 Agriculture Bldg.; University of Missouri; Columbia MO 65211; 573-882-6832; 573-882-1469 (fax)
Ogle, Charles W.; P.O. Box 484; Sugar Grove IL 60554; 312-466-4742
Ohta, Y.; Kasuga 2-5-1-301; Tsukuba 305; JAPAN
Oishi, Karen; Molec & Cell Biol Dept; University of Arizona; Tucson AZ 85721; 520-621-3381
Okagaki, Ronald; Univ Minnesota; Dept Agron Plant Genet; 411 Borlaug Hall, 1991 Buford Circle; St. Paul MN 55108; 612-625-8756; 612-625-1268 (fax); okaga002@tc.umn.edu
Okuno, Kazutoshi; National Inst of Agrobiol Resources; 2-1-2 Kannondai; Tsukuba; Ibaraki 305; JAPAN; 81-298-38-7458; 81-298-38-7408 (fax); okusan@abr.affrc.go.jp
Oldroyd, Giles; UC Berkeley; Plant Biology Dept; 111 Koshland Hall; Berkeley CA 94720-3102
Olhoft, Paula; Dept Agron & Plant Genetics; 1991 Buford Circle; 411 Borlaug Hall, Univ of Minnesota; St. Paul MN 55113; 612-625-9258; 612-625-1268 (fax)
Openshaw, Steve; Novartis Seeds; 317 330th St; Stanton MN 55018-4308; 507-663-7696; 507-645-7519 (fax); steve.openshaw@seeds.novartis.com
Orr, Alan R.; Department of Biology; University of Northern Iowa; Cedar Falls IA 50614; 319/277-4381; 319/273-2893 (fax); ORR@UNI.EDU
Osterman, John C.; School of Life Sciences; University of Nebraska-Lincoln; Lincoln NE 68588; 402-472-5129; 402-472-2083 (fax); josterman@unl.edu
Ostrander, Brad; Plant Genetics Group; National Starch and Chemical, Plant Genetics Group; 5648 W. 73rd Street; Indianapolis IN 46278; 317-295-4124; 317-295-4121 (fax); brad.ostrander@nstarch.com
Ottoboni, Laura; CBMEG Inst. Biol.; UNICAMP/Caixa Postal 6109; CEP 13083-970; Campinas SP; BRAZIL; 55-192-397030; 55-192-39-4717 (fax); OTTOBONI@TURING.UNICAMP.BR
Padilla, Chris; UC San Diego; Dept of Biology, 0116; La Jolla CA 92093-7108
Paez, Alix V.; Genetic Enterprises Int'l; 6165 Crabapple Lane; Johnston IA 50131; 515-278-1170; 515-276-9360 (fax); paezgei@worldnet.att.net
Page, Brent; Univ of Missouri; 117 Tucker Hall; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); c670769@showme.missouri.edu
Palmer, K. E.; Dept. of Microbiology; University of Cape Town; Private Bag; Rondebosch 7700; SOUTH AFRICA; 21-650-3269; 21-650-4320 (fax)
Palmer, Reid; USDA-ARS-CICGR; Iowa State University; Agronomy Department, Room G301; Ames IA 50011; 515-294-7378; 515-294-2299 (fax); rpalmer@iastate.edu
Pan, Caixian; Chinese Academy of Agrucultural Sciences; 30 Baishiqiao Road; West Suburb Beijing; Beijing; CHINA
Pareddy, Dayakar R.; DowElanco; R&D Building, 306/B-1; 9330 Zionsville Road; Indianapolis IN 46268; (317)337-3646; 317-337-3228 (fax)
Parlov, Dragomir; Inst Breeding & Prod Field Crops; Marulicev trg 5; 41000 Zagreb; YUGOSLAVIA; 041-750-311; 41-750-523 (fax)
Partas, E. C.; Maize and Sorghum Res. Inst.; Pashcani; Criuleni; 278336 Moldova; MOLDOVA
Pataky, Jerald K.; Crop Science, Turner Hall; 1102 S. Goodwin Ave; University of Illinois; Urbana IL 61801; 217-333-6606; 217-244-1230 (fax); j-pataky@uiuc.edu
Paterson, Andrew H.; University of Georgia; Rm 162, Riverbend Research Center; 110 Riverbend Road; Athens GA 30602; 706-583-0162/0161; 706-583-0160 (fax); paterson@uga.edu
Patil, M. S.; Agric. Research Station; Gulburua 585101; INDIA; 21120; 091-08472-21120 (fax)
Paul, Anna-Lisa; Dept Horticultural Sciences; 1255 Fifield Hall; Univ Florida; Gainesville FL 32611; 352-392-4711x313; 352-392-6479 (fax); ALP@NERVM.NERDC.UFL.EDU
Pawar, S. E.; Nuclear Agriculture Division; Bhabha Atomic Research Center; Trombay Bombay 400085; INDIA; 5563060 ext 2638; 9122-556-0750 (fax)
Peacock, Jim; Division of Plant Industry; CSIRO; Canberra ACT 2601; AUSTRALIA; 062-465250
Peng, Jinzhi; State Science & Tech. Commission; China Natl. Ctr. Biotech. Development; 54 Sanlihe Road; Beijing; CHINA
Penzes, Eva A.; Dept Molec Cell Biol; 345 LSA; UC Berkeley; Berkeley CA 94720-0001; 510-643-8277; 510-643-6791 (fax)
Pereira de Souza, Anete; CBMEG/UNICAMP; Caixa Postal 6109; CEP 13083-970; Campinas SP; BRAZIL; 55-19-239-8351; 55-19-239-7030 (fax); anete@turing.unicamp.br

Perez, Pascual; Biocem - Groupe Limagrain; 24 Av. Des Landais; 63130 Aubiere; FRANCE; (33) 73427977; (33) 73275736 (fax)
Perotti, Enrico; Lisboa 27; Apdo. Postal 6-641; CIMMYT; 06600 Mexico; MEXICO; 5-726-9091; 525-726-7567 (fax); eperotti@cimmyt.mx
Peschke, Virginia M.; Plant Sciences, GG6A, Monsanto Co.; 700 Chesterfield Village Parkway; St. Louis MO 63198; (636)737-6939; (636)737-6189 (fax); virginia.m.peschke@monsanto.com
Peterson, Peter A.; Dept of Agronomy; Iowa State University; Ames IA 50011; 515-294-9652; 515-294-2299 (fax); pap@iastate.edu
Peterson, Thomas A.; Dept. of Zoology & Genetics; 2206 Molecular Biology; Iowa State Univ.; Ames IA 50011; 515-294-6345; 515-294-0345 (fax); thomasp@iastate.edu
Pham, Hiep; Cargill Seed Research; P.O. Box 774; Grinnell IA 50112; (515)236-4911; (515)236-3607 (fax)
Phelps-Durr, Tara; Tucker Hall; University of Missouri; Columbia MO 65211; 573-449-4871; 573-882-0123 (fax); c675971@showme.missouri.edu
Phillips, Ronald; Agronomy & PI Genetics; 411 Borlaug Hall; University of Minnesota; St. Paul MN 55108; 612-625-1213; 612-625-1268 (fax); Phill005@maroon.tc.umn.edu
Phinney, Bernard O.; Dept. of Biology; 405 Hilgard Avenue; UCLA; Los Angeles CA 90024-1606; (310)825-3177; (310)825-3177 (fax); bop@ucla.edu
Pixley, Kevin V; PO Box MP 163; Mount Pleasant; Harare; ZIMBABWE; 263-4-301807; 263-4-301327 (fax); k.pixley@cgnet.com
Plehn, Steve J.; Cargill Hybrid Seeds; P.O. Box 762; Mt. Vernon IN 47620; (812)838-5218; (812)838-8864 (fax)
Plesset, Judith; Plant and Microbial Development; National Science Foundation; 4201 Wilson Boulevard; Arlington VA 22230; (703) 306-1417; jplesset@nsf.gov
Plunkett, Dave; Green Giant Company, M.S. 9921; 330 University Ave. S.E.; Minneapolis MN 55408; 612-330-8007; 612-330-8064 (fax)
Podolskaya, Anna P.; N. I. Vavilov All Union Inst Plant Industry; 44 Herzen Street; 190000, St. Petersburg; RUSSIA; 311-99-45; 311-8762 (fax)
Poethig, R. Scott; Biology Department; Leidy Labs, Univ Penn; Philadelphia PA 19104-6018; 215-898-8915; 215-898-8780 (fax); SPOETHIG@MAIL.SAS.UPENN.EDU
Poggio, Lidia; Inst Fitotecnico de Santa Catalina (UNLP); C.C.4 (1836) Llavallol; Buenos Aires; ARGENTINA
Polacco, Mary; 203 Curtis Hall; University of Missouri; Columbia MO 65211; 573-884-7873; 573-884-7850 (fax); maryp@teosinte.agron.missouri.edu
Pollacsek, M.; Station Amelior PI-INRA; 63039 Clermont Ferrand; FRANCE; 73-62-43-01; 73-62-44-53 (fax)
Pollak, Linda; USDA-ARS; Dept. Agronomy; Iowa State Univ.; Ames IA 50011; 515-294-7831; 515-294-9359 (fax); lmpollak@iastate.edu
Pollmer, W. G.; Universitat Hohenheim; Egilolfstr. 25; D-70599 Stuttgart; GERMANY; 49-711-4586315; 49-711-4569008 (fax)
Poneleit, Charles G.; Agronomy; University of Kentucky; Lexington KY 40546-0091; 606-257-4934; 606-258-1952 (fax); AGR021@UKCC.UKY.EDU
Porter, Hedera L.; PO Box 516; Lamberton MN 56152; 507-752-7012; (fax)
Prasanna, B.M.; Division of Genetics; Indian Agricultural Research Institue; New Delhi-110 012; INDIA; bmp@bic-iari.ren.nic.in
Pratt, Richard C.; Dept. Hort. & Crop Science; OSU/OARDC; 1680 Madison Avenue; Wooster OH 44691; 330-263-3972; 330-263-3887 (fax); pratt.3@osu.edu
Price, Carl; Waksman Inst; Rutgers University; Piscataway NJ 08855-0759; 732-445-2920; 732-445-5735 (fax); Price@mbcl.rutgers.edu
Prigge, Michael; Institute Molec Biol; Univ of Oregon; Eugene OR 97403
Pring, Daryl R.; Dept of Plant Pathology; 1453 Fifield Hall; University of Florida; Gainesville FL 32611; 352-392-3638; 352-392-6532 (fax); drpg@gnv.ifas.ufl.edu
Prioli, Laudenir M.; Depto. Genetica; IB/CBMEG, Univ Estad Campinas; CP 6109; Campinas 13083-970; BRAZIL; 55-192-397030; 55-192-394717 (fax); LAUDENIR@CCVAX.UNICAMP.BR
Prioul, Jean-Louis; IBP Bat 630; Universite de Paris-Sud; 91405 Orsay Cedex; FRANCE; 33169336373; 33169336424 (fax); prioul@ibp.u-psud.fr
Prosen, Dennis; Harris Moran Seed Co.; 100 Breen Rd.; San Jun Bautista CA 94045; (408)623-4323; (408)623-2260 (fax)
Pryor, Tony; Plant Industry CSIRO; PO Box 1600; Canberra ACT 2601; AUSTRALIA; 61-02-6246 5494; 61-02-6246 5000 (fax); tony.pryor@pi.csiro.au
Puigdomenech, Pedro; Centro de Investigacion y Desarrollo; c/ Girona Salgado, 18-26; 08034 Barcelona; SPAIN; 34-3-400-61-29; 34-3-204-59-04 (fax); pprgmp@cid.csic.es
Purugganan, Michael; Dept of Genetics; Box 7614; NC State University; Raleigh NC 27695-7614; michaelp@unity.ncsu.edu
Qin, Lu; Institute of Genetics and Crop Breeding; Fuzhou 0591; CHINA
Qiu, Lijuan; G403 Agronomy Hall; Iowa State Univ; Ames IA 50011
Qu, F; Institute of Genetics; Lab No. 801; Beijing 100101; CHINA
Qualset, Calvin O.; Genetic Resources Cons Program; Univ of California; 1 Shields Ave; Davis CA 95616-8602; 530-754-8502; 530-754-8505 (fax); coqualset@ucdavis.edu
Quarrie, Steve; John Innes Centre; Norwich Research Park; Colney; Norwich NR4 7UH; UNITED KINGDOM; 44-1603-452571; 44-1603-502241 (fax); QUARRIE@BBSRC.AC.UK
Quayle, Tom; The American University; 113 Sharia Kasr El-Aini; 11511 Cairo; EGYPT; quayle@aucegypt.edu
Quebedeaux, Bruno; Dept Nat Res Sci & Landsc Arch; Plant Sci Bldg 2130; University of Maryland; College Park MD 20742; 301-405-4336; 301-314-9308 (fax); BQ1@umail.umd.edu
Qun Hui, Lin; Fujian Agricultural College; Dept. of Agronomy; Jingshian, Fuzhou; Fujian 350002; CHINA
Raboy, Victor; USDA-ARS-NSGGRF; PO Box 307; Aberdeen ID 83210; 208-397-4162; 208-397-4165 (fax); vraboy@uidaho.edu
Rabson, Robert; Div. of Energy Bioscience; Office of Basic Energy Sci; U.S. Dept of Energy, ER-17 GTN; Washington DC 20545; 301-903-2873; 301-903-1003 (fax)
Racchi, Milvia L.; Genetics Unit; Univ of Florence; Via San Bonaventura 13; 50145 Firenze; ITALY; 55-573201; 55-580341 (fax); gene_agr@cesit1.unifi.it
Rafalski, Antoni; DuPont Agric Biotechnol; Delaware Technol Park, Suite 200; 1 Innovation Way, PO Box 6104; Newark DE 19714-6104; 302-631-2612; 302-631-2607 (fax); J-Antoni.Rafalski@usa.dupont.com
Ragot, Michel; Novartis Seeds; 12, Chemin de l'Hobit; F-31790 Saint-Sauveur; FRANCE; 33(0)562799902; 33(0)562799996 (fax); michel.ragot@seeds.novartis.com
Raizada, Manish; Dept Biol Sci; Stanford Univ; Stanford CA 94305-5020; 650-723-2609; 650-725-8221 (fax); raizada@leland.stanford.edu
Ralston, Ed; DNA Plant Technology Corp; 6701 San Pablo Avenue; Oakland CA 94608; 510-547-2395; 510-547-2817 (fax); ralston@dnap.com
Ramsey, Ann; P.O. Box 3311; Urbana IL 61801; 217-384-5699; amramsey@uiuc.edu
Rao, P. N.; Dept of Botany; Andhra University; Visakhapatnam 530003; INDIA; 54871 ext 390
Rapp, William; Department of Biology; Univ. of Missouri-St. Louis; 8001 Natural Bridge Rd.; St. Louis MO 63121-4499; 314-553-6225; 314-553-6233 (fax); swdrapp@umslvma.umsl.edu
Rayburn, A. Lane; Crop Sci, 320 ERML; 1201 W. Gregory; Univ of Illinois; Urbana IL 61801; (217)333-4777; (217)333-9817 (fax); arayburn@uiuc.edu
Reddy, Arjula; School of Life Sci; Univ of Hyderabad; Hyderabad-500 046; INDIA; 0091-40-3010265,3033123; 0091-40-3010120 (fax); arjulsl@uohyd.ernet.in
Reddy, Gurjal; Department of Genetics; Osmania University; Hyderabad-500007; INDIA; 868951 Ext. 375; 91-0842-868087 (fax)
Redinbaugh, Margaret; USDA-ARS; OARDC; Dept Plant Pathology; Wooster OH 44691; 330-263-3965; 330-263-3841 (fax); redinbaugh.2@osu.edu
Reid, L. M.; Eastern Cereal & Oilseed Res Centre; Agriculture and Agri-Food Canada; Bldg 121 Central Experimental Farm; Ottawa, Ontario K1A 0C6; CANADA; 613-759-1619; 613-952-9295 (fax); reidl@em.agr.ca
Rhee, Seung; Carnegie Institution of Washington; 290 Panama St.; Stanford CA 94305
Rhodes, Carol; 219 Bay Tree Rd.; San Carlos CA 94070-3816; 650-598-9469; 650-598-9469 (fax); carolrhodes@hotmail.com
Ribaut, Jean-Marcel; CIMMYT; Apdo. Postal 6-641; 06600 Mexico D.F.; MEXICO; 595-54400/54410; 595-54425 (fax); JRIBAUT@ALPHAC.CIMMYT.MX
Rice, Doug; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 22; Johnston IA 50131-1004
Richards, Eric; Biology Dept.; Washington University; St. Louis MO 63130; 314-935-7196; richards@biodec.wustl.edu
Richter, Todd E.; Plant Path. Dept.; Throckmorton Hall; Manhattan KS 66506; (913)532-6176; (913)532-5692 (fax); trichter@ksuvm.ksu.edu
Riera-Lizarazu, Oscar; Dept. Agronomy & Plant Genetics; Univ. Minnesota; 411 Borlaug Hall, 1991 Buford Circle; St. Paul MN 55108-6026; (612)625-6223; (612)625-1268 (fax)
Rinehart, Karl; Box 187; Marshalltown IA 50158; 515-752-2170
Rinehart, Tim; Univ Idaho; Biological Sciences; Moscow ID 83844-3051; 208-885-2550; 208-885-7905 (fax); rine9558@uidaho.edu
Rivin, Carol; Dept. of Botany; Cordley 2082; Oregon State University; Corvallis OR 97331-2902; 503-737-5281; rivinc@cgrb.orst.edu
Robbins Jr., W. A.; P.O. Box 158; Ag. Alumni Seed Imp. Assn.; Romney IN 47981; 317-538-3145; 317-538-3600 (fax)
Roberts, Jean L.; Dow Agrosciences Discovery Research; 9330 Zionsville Road; Indianapolis IN 46268; 317-337-3126; 317-337-3228 (fax); jlroberts@dowagro.com
Robertson, Donald S.; 1707 Woodhaven Circle; Ames IA 50010-4198; 515-232-2892; donnrrr@aol.com
Robison, Glenn; DeKalb Plant Genetics; 3100 Sycamore Road; DeKalb IL 60115; 815-758-9531; 815-758-4106 (fax)
Rocheford, Torbert R.; Crop Sci, W221 Turner Hall; University of Illinois; 1102 S. Goodwin Av.; Urbana IL 61801; 217-244-3388; 217-333-9817 (fax); trochefo@uiuc.edu

Rocky, Sally; USDA, NRICGP; 901 D. Street, SW, Room 323; Washington DC 20250
Rodermel, S.; Department of Botany; Bessey Hall; Iowa State University; Ames IA 50011; 515-294-8890; 515-294-1337 (fax); S1SRR@ISUVAX.BITNET
Rogowsky, Peter; RCAP; ENS-Lyon; 46 Allee d'Italie; F-69364 Lyon cedex 07; FRANCE; 33-472-72-86-07; 33-4-72-72-86-00 (fax); progowsk@ens-lyon.fr
Romero-Severson, Jeanne; Purdue University; Department of Forestry and Natural Resources and; Department of Agronomy; Room 206C WSLR; 1159 Forestry Bldg.; West Lafayette IN 47907-1159; 765-496-6801; 765-496-2422 (fax); romeros@fnr.purdue.edu
Rood, Tracy; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004
Rosato, S. Caprice; 1220 Oak Villa Road; Dallas OR 97338; 541-750-8750 (fax); rosatoc@ucs.orst.edu
Rosichan, Jeffrey; Novartis Seeds, Inc.; Research Center; 317 330th St.; Stanton MN 55018-4308; 507-663-7642; 507-645-7519 (fax); jeff.rosichan@seeds.novartis.com
Rosielle, A.; Monsanto-International Assignment; Mail Stop 5045; 800 N Lindbergh; St. Louis MO 63167
Roth, Brad; Pioneer Hi-Bred Internat, Inc.; 7300 NW 62nd Ave.; Box 1004; Johnston IA 50131-1004; (515)270-3789; (515)253-2478 (fax); rothba@phibred.com
Rubenstein, Irwin; 1838 Parliament Rd; Leucadia CA 92024-1030
Ruff, Thomas G.; Monsanto-Ceregen; 700 Chesterfield Pkwy North; AA3E; St. Louis MO 63198; 314-737-7023; 314-737-6047 (fax); thomas.g.ruff@monsanto.com
Russell, Doug; Monsanto-Agrocetus, Inc.; 8520 University Green; Middleton WI 53562; 608-821-3443; 608-836-9710 (fax); douglas.a.russell@monsanto.com
Russell, Kelly; Institute of Molec Biol; University of Oregon; Eugene OR 97403
Russell, Ken; Univ of Nebraska; Dept of Agronomy; PO Box 830915; Lincoln NE 68583; 402-472-1562; 402-472-7904 (fax); KRUSSELL3@unl.edu
Saab, Imad; Crop Sci, Turner Hall; 1102 S. Goodwin Ave.; Univ of Illinois; Urbana IL 61801; 217-333-7585; 217-333-6064 (fax); i-saab@uiuc.edu
Sachan, JKS; Division of Genetics; I.A.R.I.; New Delhi-110012; INDIA; 91-011-5783077; 91-011-5752006 (fax)
Sacher, Robert; Hunt-Wesson; Research and Development; 1645 W. Valencia Drive; Fullerton CA 92833-3899; 714-680-2822; 714-449-5166 (fax); RFSACHER@CLASS.ORG
Sachs, Marty; USDA/ARS; S108 Turner Hall; 1102 S. Goodwin Ave; Urbana IL 61801; (217)244-0864/333-9743lab; (217)333-6064 (fax); msachs@uiuc.edu
Saedler, Heinz; Max-Planck Inst Zuchtungsf; Carl-von Linne-Weg 10; D 50829 Koln; GERMANY; 221-5062-100; 221-5062-113 (fax)
Saghai Maroof, M. A.; CSES Department; VPI & SU; Blacksburg VA 24061; 540-231-9791; 540-231-3431 (fax); SMAROOF@VT.EDU
Salamini, Francesco; Max Planck Inst Zuchtungsf; Abt Pflanzenz Ertragsphysiol; D-50829 Koln; GERMANY; 49-221-5062400; 0049-221-5062413 (fax)
Salerno, Juan C.; PJE. San Sebastian 439; 1405 Buenos Aires; ARGENTINA; 54-1-15-949-3685; 54-1-450-0805/1876 (fax); jsalerno@inta.gov.ar
Salimath, S. S.; Plant Biology Div; PO Box 2180; The S.R. Noble Foundation; Ardmore OK 73402; 405-221-7349; 405-221-7380 (fax); sssalimath@noble.org
Salvador, Ricardo J.; Iowa State University; Dept. of Agronomy; 1126 Agronomy Hall; Ames IA 50011-1010; 515-294-9595; 515-294-8146 (fax); RJSALVAD@IASTATE.EDU
San Miguel, Phillip; HANS 339; Dept. of Biological Sciences; Purdue University; West Lafayette IN 47907; 765-494-4919; 765-496-1496 (fax); pmiguel@bilbo.bio.purdue.edu
Santos, MA; Dept Genetica Molecular; Centro Invest Desarrollo; Gorge Girona 18-24; 08034 Barcelona; SPAIN; 34-3-4006100; 34-3-2045904 (fax)
Sarkar, Kumud R.; 77B, Ekta Apartments; Block A-2/B; Paschim Vihar; New Delhi 110 063; INDIA; kurasar@del3.vsnl.net.in
Sasaki, Takuji; Rice Genome Research Program; STAFF Institute, 446-1, Ippaizuka; Kamiyokoba, Tsukuba; Ibaraki 305-0854; JAPAN; 81-248-38-7441; 81-248-38-7468 (fax); tsasaki@abr.affrc.go.jp
Saura, Fulgencio; Dpto Tecnologia; Univ. Nacional Lujan; CC 221-6700 Lujan(Bs As); ARGENTINA; pantuso@mail.unlu.edu.ar
Savidan, Yves H.; APONET/CIMMYT; Aptdo. Postal 6-641; 06600 Mexico D.F.; MEXICO; (52)5-726.90.91; (52)5-7267558 (fax); y.savidan@cgiar.org
Savidan, Yves H.; APONET/CIMMYT; Aptdo. Postal 6-641; 06600 Mexico D.F.; MEXICO; (52)5-726.90.91; (52)5-7267558 (fax); Yves.Savidan@mpl.orstom.fr
Scandalios, John; Dept. of Genetics; North Carolina State Univ.; Box 7614; Raleigh NC 27695-7614; (919)515-7079; (919)515-3355 (fax); jgs@unity.ncsu.edu
Schichnes, Denise E.; Dept of Plant Biology; 111 Koshland Hall; Univ California Berkeley; Berkeley CA 94720; (510)642-8058; (415)642-4995 (fax); Schichne@nature.berkeley.edu
Schiefelbein, John W.; 4085 Natural Science Bldg.; University of Michigan; Ann Arbor MI 48109; 313-764-3579; 313-747-0884 (fax); schiefel@umich.edu
Schillinger, John; Asgrow Seed Company; 9672-190-16; 7000 Portage Rd.; Kalamazoo MI 49001
Schmidt, Robert J.; Univ of California-San Diego; Dept of Biology 0116; 9500 Gilman Dr; San Diego CA 92093-0116; 619-534-1636; 619-534-7108 (fax); rschmidt@ucsd.edu
Schnable, Patrick; Dept of Agronomy; G405 Agronomy Hall; Iowa State Univ; Ames IA 50011; 515-294-0975; 515-292-2299 (fax); Schnable@iastate.edu
Schneeberger, Richard G.; CERES, INC; 3007 Malibu Canyon Road; Malibu CA 90265; 310-317-8924; 310-317-8998 (fax); rschnee@ceres-inc.com
Schneerman, Martha C; Illinois State University; 4120 Biological Sciences; Normal IL 61790-4120; 309-438-3088; 309-438-3722 (fax); MCSCHNEE@ilstu.edu
Schnicker, Bruce; Cornnuts; 1000 S. Edgewood Ave.; P.O. Box 830; Urbana OH 43078; 513-652-1321; 513-653-3675 (fax)
Scholl, Randy; Arabidopsis Biol Resource Center; Ohio State; 1735 Neil Ave.; Columbus OH 43210; 614-292-0603; 614-292-0603 (fax); scholl.1@osu.edu
Schramke, Mary; Bio-Rad Laboratories, Life Sciences Group; 2000 Alfred Nobel Drive; Hercules CA 94547; 510-741-6717; 510-741-1051 (fax)
Schulman, Alan H.; Univ Helsinki; Institute of Biotechnology; P.O. Box 56, Viikinkaari 9; 00014 Helsinki; FINLAND; 358-0-708-59366; 358-0-708-59422 (fax); aschulman@operoni.helsinki.fi
Schultes, Neil; Dept Biochem & Genetics; Conn Agric Exper Sta; 123 Huntington St; New Haven CT 06511; 203-789-6912; 203-789-7232 (fax)
Schwall, Michael; Suedwestdeutsche Saatzucht; Im Rheinfeld 1-13; 76437 Rastatt; GERMANY; 49-7222-7707-0; 49-7222-770777 (fax); schwall@sun1.ruf.uni-freiburg.de
Schwartz, Drew; Biology Dept; Indiana University; Bloomington IN 47405; 812-855-6060; 812-855-6705 (fax); Schwartz@bio.indiana.edu
Scott, Lu Ann; Dept Biol Sci; University of Idaho; Moscow ID 83844-3051
Selzer, Gerald; Research Resources; National Science Foundation Room 615; 4201 Wilson Blvd.; Arlington VA 22230; (703)306-1469; gselzer@nsf.gov
Senior, Lynn; Novartis Agribusiness Biotech Res, Inc; 3054 Cornwallis Rd; Research Triangle Park NC 27709; 919-597-3041; 919-541-8585 (fax); lynn.senior@nabri.novartis.com
Seo, Beom-Seok; 2154 Molec Biol Bldg; Iowa State Univ; Dept Biochem, Biophys & Mol Biol; Ames IA 50011; 515-294-8202; 515-294-0453 (fax); beoseok@iastate.edu
Setter, Tim L.; Department of Soil Crop and Atm. Sci.; 519 Bradfield Hall; Cornell University; Ithaca NY 14853; 607-255-1701; 607-255-2644 (fax); tls1@Cornell.edu
Settles, Mark; Univ Florida; 2235 Fifield Hall, PO Box 110690; Horticultural Sci; Gainesville FL 32611; 352-392-1928 ext 308; 352-392-6479 (fax)
Sevilla P., Ricardo; Programa de Maiz; Univ Nacional Agraria; Aptdo 456, La Molina; Lima; PERU; 51-14 495647; 51-14 495670 (fax); rsevilla@lamolina.edu.pe
Shadley, Jeff and Gwen Krill-; 7018 Chestnut St.; Milwaukee WI 53213-2742
Shamina, Zlata; Academy of Sciences; K. A. Timiryazev Inst. Plant Phys.; Botanicheskaya 35; 127276 Moscow; RUSSIA
Shands, Henry L.; USDA-ARS-OA, Rm 319-A; Jamie L. Whitten Federal Bldg; 14th & Independence Ave, SW; Washington DC 20250-0300; 202-205-7835; 202-690-1434 (fax); shands@sun.ars-grin.gov
Shao, Qi-Quan; Academia Sinica; Genetics Institute 917 Bldg.; Datun Road, Andingmen Wai; Beijing 100101; CHINA
Sharp, Peter; Plant Breeding Institute; University of Sydney; Cobbitty Road; Cobbitty NSW 2570; AUSTRALIA; 61-46-512-600; 61-46-512-578 (fax); pbic00@angis.su.oz.au
Shaver, Donald L; Western Corn Genetics Co.; 20250 Palou Dr.; Salinas CA 93908; 831-455-1492; 831-455-0467 (fax)
Shcherbak, Victor; Krasnodar Res Inst Agric; c/o Zeneca Moscow; Bolshoi Strochenovski Pereulok; Moscow 113054; RUSSIA; 7-503-2306111; 7-503-2306119 (fax)
Sheen, Jen; Dept. of Molecular Biology; Wellman 11, MGH; Boston MA 02114; 617-726-5916; 617-726-6893 (fax); sheen@frodo.mgh.harvard.edu
Shen, Daleng; Fudan University; Inst. of Genetics; Shanghai 200433; CHINA
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; (302)695-4296 (fax); jennie.b.shen@usa.dupont.com
Shen, Yu-Wei; Zhejiang Agricultural University; Institute of Nuclear-Agric. Science; Hangzhou; Zhejiang 310029; CHINA
Shen, Zong-Tan; Zhejiang Agricultural University; Dept. of Agronomy; Hangzhou; Zejiang 310029; CHINA
Sheridan, William; Biology Department; Univ of North Dakota; PO Box 9019 Univ Station; Grand Forks ND 58202-9109; 701-777-4479 or -4705; 701-777-2623 (fax)
Shiga, Toshio; Sakata Seed Corp; Plant Bio Center, SAKATA SEED Corp; 358 Uchikoshi Sodegaura; Chiba 299-02; JAPAN; 438-75-2369
Shigemori, I; Chusin Agr. Exp. Stn.; Sooga Shiojiri; Nagano 399-64; JAPAN; shige@chushin-exp.pref.nagano.jp
Short, Kent E.; Carnia Seed (Pty) Ltd.; P.O. Box 7424; Petit 1512; SOUTH AFRICA; (011)965-1905; (011)965-1906 (fax)
Shotwell, Mark A; Dept of Biology; 123 Vincent Science Hall; Slippery Rock Univ; Slippery Rock PA 16057-1326; 724-738-2476; 724-738-2188 (fax);

mark.shotwell@sru.edu
Siddiqui, Khushnood A.; Int Assoc for Promotion of New; Genetical Approaches to Crop Imp; 387 Talpur Colony; Tandojam Sind; PAKISTAN; 92 2233-5759; 92 2233-5728 (fax)
Sidorenko, Lyuda; 2292-1 Molec Biol; Iowa State Univ; Ames IA 50011; 515-294-3277; luda@iastate.edu
Simcox, Kevin; Pioneer Hi-Bred Intl Inc.; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004; 515-270-4178; 515-270-3444 (fax); simcoxkd@phibred.com
Sims, Lynne E.; Trait & Technology Development; Pioneer Hi-Bred Internatl; 7300 NW 62nd Ave/P.O. Box 1004; Johnston IA 50131-1732; 515-270-3652; 515-270-3367 (fax); SIMSL@PHIBRED.com
Singh, Karam; Dept Mol, Cell & Devel Biol; 405 Hilgard Avenue; UCLA; Los Angeles CA 90095-1606; 310-206-8259; 310-206-3987 (fax); Ksingh@ucla.edu
Sinha, Neelima; Section of Plant Biology; Division of Biological Sciences; University of California; Davis CA 95616; 530-754-8441; 530-752-5410 (fax); NRSINHA@UCDAVIS.EDU
Sinibaldi, Ralph; 1780 Acacio Ct.; Fremont CA 94536; 510-794-6410
Sisco, Paul; The American Chestnut Foundation; 14005 Glenbrook Ave.; Meadowview VA 24361; 540-944-4631; paul@acf.org
Sleper, David A.; Agronomy Dept; 210 Waters Hall; University of Missouri; Columbia MO 65211; 573-882-7320; 573-882-1467 (fax); dsleper@psu.missouri.edu
Smith, Alan G.; Dept Horticultural Science; 356 Alderman Hall, Univ. of MN; 1970 Folwell Av.; St Paul MN 55108; 612-624-9290; alan@molbio.cbs.umn.edu
Smith, Howie; Pioneer Hi-Bred International; 7300 NW 62nd Ave.; P.O. Box 1004; Johnston IA 50131-1004; 515-270-3539; 515-270-4312 (fax); SMITHO@Phibred.com
Smith, James D.; PO Box 2132; Department Soil & Crop Sci; Texas A & M University; College Station TX 77843; 409-845-8276
Smith, Laurie G.; Biology Dept 0116; U. C. San Diego; 9500 Gilman Drive; La Jolla CA 92093-0116; 858-822-2531/2558; 858-534-7108 (fax); lsmith@biomail.ucsd.edu
Smith, Margaret E.; Cornell Univ; 252 Emerson Hall; Dept of Plant Breeding; Ithaca NY 14853; 607-255-1654; 607-255-6683 (fax); mes25@cornell.edu
Smith, Steve; Pioneer Hi-Bred Internatl; P.O. Box 1004; Johnston IA 50131-9410; 515-270-3353; 515-270-4312 (fax); SMITHS@PHIBRED.COM
Snape, John W.; Cambridge Laboratory; JI Centre for Plant Science Research; Colney Lane; Norwich NR4 7UJ; UNITED KINGDOM; 44-1603-452571; 44-1603-502270 (fax)
Sokolov, Victor A.; Institute of Cytology and Genetics; Russian Academy of Sciences; Lavrentjev str., 10; Novosibirsk 630090; RUSSIA; 383-2-33-34-71; 383-2-33-12-78 (fax); sokolov@ghost.bionet.nsc.ru
Somers, David A.; Dept Agron & Plant Genet; University of Minnesota; 1991 Upper Buford Cir.; St. Paul MN 55108; 612-625-5769; 612-625-1268 (fax); somer001@maroon.tc.umn.edu
Somerville, Chris; Plant Biology Dept; Carnegie Institution; 290 Panama St; Stanford CA 94305; 650-325-1521x203; 650-325-6857 (fax); crs@andrew.stanford.edu
Song, Xiangfu; China National Rice Research Institute; 171 Tiyuchang Road; Hangzhou 310006; CHINA
Song, Yunchun; Life Science College; Wuhan University; Wuhan 430072 P.R.O.; CHINA; (027)7822712-4505; 7813833 (fax); ycsong@usa.net
Sowinski, Steve; Illinois State Univ; 4120 Dept Biological Sci; Normal IL 61790; 309-438-3088; 309-438-3722 (fax); sgsowin@ilstu.edu
Spray, Clive R.; Dept. of Biology; 405 Hilgard Av; UCLA; Los Angeles CA 90024-1606; 310-825-3177; 310-825-3177 (fax)
Springer, Patricia; Univ of California; Dept Bot and Plant Sci; Riverside CA 92521; 909-787-5785/4549; 909-787-4437 (fax); patricia.springer@ucr.edu
Srinivasan, Ganesan; CIMMYT Maize Program; Lisboa 27, Aptdo Postal 6-641; 06600 Mexico, D. F.; MEXICO; 52-5-726-9091 Ext. 1116; 52-5-726-7558/59 (fax); GSRINIVASAN@CIMMYT.MX
St. Clair, Grace; Pioneer Hi-Bred Internat Inc; Trait & Technology Dept; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004
Stack, Stephen; Department of Biology; Colorado State University; Fort Collins CO 80523-1878; 970-491-6802; 970-491-0649 (fax); sstack@lamar.colostate.edu
Staiger, Chris; Dept. of Biological Sciences; Purdue University; 321A Hansen Bldg.; West Lafayette IN 47907; 765-496-1769; 765-496-1496 (fax); CSTAIGER@BILBO.BIO.PURDUE.EDU
Start, William; DeKalb Genetics; 62 Maritime Drive; Mystic CT 06355; 860-572-5223; 860-572-5240 (fax)
Steffensen, D. M.; 506 Morrill Hall, Cell Biol; 505 S. Goodwin Ave; University of Illinois; Urbana IL 61801; 217-333-3087; 217-244-1648 (fax)
Stein, Nils; Inst Plant Biology; Univ of Zurich; Zollikerstr 107; Zurich 8008; SWITZERLAND; 41-1-634-8223; 41-1-634-8257 (fax); nstein@botinst.unizh.ch
Stern, David B.; Boyce Thompson Inst. Plant Res. Inc.; Tower Road; Ithaca NY 14853-1801; (607)254-1306; (607)254-1242 (fax); ds28@cornell.edu
Stiles, J. I.; Dept. of PI & Molec Physiology; Univ. Hawaii at Manoa; Honolulu HI 96822; 808-956-7354; 808-956-3542 (fax); stiles@hawaii.edu
Stinard, Philip; USDA/ARS; S123 Turner Hall; 1102 S. Goodwin Ave.; Urbana IL 61801-4798; (217)333-6631; (217)333-6064 (fax); pstinard@uiuc.edu
Stuber, Charles W.; Dept of Genetics; North Carolina State UnivBox 7614; 3513 Gardner Hall; Raleigh NC 27695-7614; 919-515-5834; 919-515-3355 (fax); cstuber@ncsu.edu
Styles, E. D.; Biology; Univ of Victoria, PO Box 3020; Victoria BC V8W 3N5; CANADA; 250-477-4337; styles@uvic.ca
Subbaiah, Chalivendra; University of Illinois; Crop Sci, S-27 Turner Hall; 1102 S. Goodwin Ave.; Urbana IL 61801-4730; 217-333-9743; 217-333-6064 (fax); subbaiah@uiuc.edu
Sullivan, Sue; Garst Seed Co.; PO Box 8; Kunia HI 96759; 808-688-1477; 808-688-1479 (fax)
Sullivan, Tom; Laboratory of Genetics; 445 Henry Mall; University of Wisconsin; Madison WI 53706; 608-262-4934; 608-262-2976 (fax); tdsulliv@facstaff.wisc.edu
Sun, Chongrong; Fudan University; Dept. of Biochem.; Handan Road 220; Shanghai 200433; CHINA
Sundaresan, V.; Institute of Molecular Agrobiology; National University of Singapore; 59A The Fleming, 1 Science Park Dr; Singapore 118240; INDIA; 65-8723339; 65-8725349 (fax)
Sundberg, Marshall D.; Div Biol Sciences; Emporia State Univ; 1200 Commercial St; Emporia KS 66801; 316-341-5605; 316-341-6055 (fax); sundberm@esumail.emporia.edu
Sung, Tong Ming; Dept of Agronomy; Beijing Agric Univ; 912 Research Building; Beijing 100094; CHINA; 86-010-62891851; 010-62891055 (fax); gyymz@public.cau.edu.cn
Suprasanna, P.; Plant Biotechnology Division; Bhabha Atomic Research Centre; Trombay, Bombay 400 085; INDIA; 91-22-556-3060x2571/3276; 91-22-556-0750 (fax)
Suresh, Jayanti; Dept. of Agronomy; 513 Borlaug Hall; 1991 Buford Cr.; St. Paul MN 55108; (612)625-1208
Suttie, Janet; CIBA; PO Box 12257; 3054 Cornwallis Rd; Research Triangle Park NC 27709-2257
Swiecicki, W.K.; Polish Academy of Sciences; Institute of Plant Genetics; ul. Strzeszynska 34; 60-479 Poznan; POLAND; 48-61-8233-511; 48-61-8233-671 (fax); wswi@igr.poznan.pl
Sylvester, Anne W.; Botany Dept; PO Box 3165; Univ of Wyoming; Laramie WY 82071-3165; 307-766-6378; 307-766-2851 (fax); annesyl@uwyo.edu
Szalma, Stephen; 302 Curtis Hall; Univ Missouri; Columbia MO 65211; 573-882-2033; szalma@bigfoot.com
Szick, Kathy; Bot Plant Sci; UC Riverside; Riverside CA 92521
Tadmor, Yaakov; Dept of Horticulture; 1201 Gregory; University of Illinois; Urbana IL 61801-4798; 217-244-3388; 217-333-9817 (fax)
Tagliani, Laura; Pioneer Hi-Bred Internat Inc; PO Box 1004; Johnston IA 50131-1004; 515-270-4188; 515-270-3367 (fax); TAGLIANILA@PHIBRED.COM
Tan, C. C. (Jia Zheng); Fudan University; Inst. of Genet.; Shanghai 20043; CHINA
Tanksley, Steven D.; Dept. Plant Breeding; Cornell University; 252 Emerson Hall; Ithaca NY 14853
Tao, Quanzhou; 320 Yue-Yang Road; Shanghai 200031; CHINA; 86-21-4374430; 86-21-4378357 (fax)
Tausta, Susan; Dept of MB&B; 205 Hall-Atwater Labs/Lawn Ave; Wesleyan Univ; Middletown CT 06459; 860-685-3373
Taylor, Brian H.; Dept of Biology; Texas A&M Univ; College Station TX 77843-3258; 409-845-7754; 409-845-2891 (fax)
Taylor, Loverine P.; Genetics & Cell Biology; 301 Science Hall; Washington State University; Pullman WA 99164-4234; 509-335-3612; 509-335-8690 (fax); ltaylor@wsu.edu
Taylor, W. C.; CSIRO; Division of Plant Industry; G.P.O. Box 1600; Canberra ACT 2601; AUSTRALIA; (61-6)246-5223; (61-6)246-5000 (fax); bt@pican.pi.csiro.au
Tenborg, Robin; Pioneer Hi-Bred Intl, Inc; 733 NW 62nd Ave; Johnston IA 50131-1004; 515-270-5951; 515-253-2149 (fax)
Thompson, Richard; Max Planck Inst Zuchtungsf; Carl-von-Linne Weg 10; D-50829 Koeln; GERMANY; 49-221-5062-440/441; 49-221-5062-413 (fax); thompson@mpiz-koeln.mpg.de
Thornsberry, Jeffry; Biol Sci; 324 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-8033; 573-882-0123 (fax); jmt@biosci.mbp.missouri.edu
Tierney, Mary L.; Marsh Life Sciences Bldg.; University of Vermont; Burlington VT 05405-0086; 802-656-0434; mtierney@moose.uvm.edu
Tiffany, Doug; Corn Research; Pioneer Hi-Bred, Int.; Rt. 8, Box 113A; Mankato MN 56001; (507)625-3252; (507)625-6446 (fax); TIFFANYD@PHIBRED.COM
Till, Bradley; Inst Molec Biol; Univ of Oregon; Eugene OR 97403
Timmermans, Marja; Cold Spring Harbor Lab; 1 Bungtown Rd; Cold Spring Harbor NY 11724; 516-367-8835/6818; 516-367-8369 (fax); timmerma@cshl.org

Ting, Yu-Chen; Biology, Boston College; Chestnut Hill 67; Boston MA 02167; 617-552-2736; 617-552-2011 (fax); tingy@bc.edu
Tingey, Scott V.; Du Pont Company; DTP Suite 200, PO Box 6104; 1 Innovation Way; Newark DE 19714-6104; 302-631-2602; Scott.V.Tingey@usa.dupont.com
Tomas, Adriana; Pioneer Hi-Bred International; 7300 NW 62d Ave; PO Box 1004; Johnston IA 50131-1004; 515-253-2116; 515-253-2149 (fax); tomasa@phibred.com
Tomes, Dwight T.; Pioneer Hi-Bred Intl; 7300 NW 62nd Ave; P.O. Box 1004; Johnston IA 50131-1004; 515-270-3646; 515-270-3444 (fax); tomesd@phibred.com
Tonelli, Chiara; University of Milan; Dept. of Genetics & Microbiology; Via Celoria 26; Milano 20133; ITALY; 39-02-26605210; 39-02-2664551 (fax); chiara.tonelli@unimi.it
Tossberg, John; Pioneer Hi-Bred Internat Inc; Reid Research Center; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004; 515-270-3645; 515-253-2149 (fax)
Tracy, William; Department of Agronomy; 1575 Linden Drive; University of Wisconsin; Madison WI 53706; 608-262-2587; 608-262-5217 (fax); wftracy@facstaff.wisc.edu
Trimnell, Mary; 7301 NW 62nd Ave; Pioneer Hi-Bred International; PO Box 85; Johnston IA 50131-0085; 515-270-3297; 515-270-3667 (fax); trimnellm@phibred.com
Troxell, Cynthia; Dept. MCD Biology; Campus Box 347; University of Colorado; Boulder CO 80309-0347; 303-492-8534; 303-492-7744 (fax); troxell@beagle.colorado.edu
Troyer, A. Forrest; Corn Breeder; 611 Joanne Ln; DeKalb IL 60115-1862; 815-758-4375; 630-801-2345 (fax)
Tsai, Charles; Dept Bot; Natl Taiwan Univ; Taipei 10764; TAIWAN
Tu, Zeng-Ping; Guangdong Acad. of Agric. Sciences; Rice Research Institute; Wushan, Guangzhou; Guangdong 510640; CHINA
Tuerck, Jutta; Advanced Technol (cambridge) Ltd; 210 Science Park; Cambridge CB4 0WA; UNITED KINGDOM; 44-1223-420 284; 44-1223-423 448 (fax); atc.biotech@dial.pipex.com
Tuttle, Ann Marie; CIBA-Geigy Corp; PO Box 12257; Research Triangle Park NC 27709-2257
Tyers, Randall; Plant Biol Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102; 510-642-7948; 510-642-4995 (fax); tyersome@nature.berkeley.edu
Tyrnov, Valery; Genetics Dept; 83 Astrakhanskaya Str; Saratov State University; Saratov 410026; RUSSIA; (845-2)240446 (fax); tyrnov.bi.sgu@oda.ssu.runnet.ru
Uhr, David V.; Northrup King Co.; 340 Southside Drive; Henderson KY 42420; (502)827-5787; (502)827-5703 (fax)
Ujiie, Katsuhiro; HOKUREN Naganuma Res. Stn.; Minami-2, Higashi-9; Naganuma-Cho, Yuubari-Gun; Hokkaido 069-1316; JAPAN; 01238-8-3330; 01238-8-3200 (fax)
Ulrich, Valentin; P.O. Box 451; Morgantown WV 26506; 304-292-5262
Valdez, Gregorio; Lehman College CUNY; Biology Dept c/o Elli Wurtzel; 250 Bedford Park Blvd West; Bronx NY 10468
Vallejos, Ruben H.; CEFOBI; Univ Nac de Rosario; Suipacha 531; 2000 Rosario; ARGENTINA; 54-41-371955; 54-41-370044 (fax); RNCEFOBI@ARCRIDE.EDU.AR
Van Heeckeren, Wim; Inst Molec Biol; Univ of Oregon; Eugene OR 97403-1229; wim@morel.uoregon.edu
Van Montagu, M.; Lab Genetics; K L Ledeganckstr 35; B-9000 Gent; BELGIUM; 32-9-264.51.70; 32-9-264.53.49 (fax); mamon@gengenp.rug.ac.be
Van Schaik, N.; Dept Genetics/U Witwatersrand; PO Wits 2050; SOUTH AFRICA; (011) 716-2125; 27-11-403-1733 (fax)
Vanderslice, Olin L.; Vanderslice Enterprises; Lake Viking, 106 Mooney Drive; Gallatin MO 64640-6340; 660-663-2946
Vantoai, Tara T.; USDA Agronomy; 590 Woody Hayes Dr; Columbus OH 43210; 614-292-9806; 614-292-9448 (fax); tvantoai@magnus.acs.ohio-state.edu
Varagona, Rita; Monsanto Co.; O3C; 800 N. Lindbergh Blvd.; St. Louis MO 63167; (314) 694-2007; RITA.J.VARAGONA@stl.Monsanto.com
Vasal, SK; CIMMYT; PO Box 9-188; Bangkok 10900; THAILAND; 5794858,5790577,9405789; 662-5614057 (fax); BANGKOK@CIMMYT.MX
Vasil, Indra K.; Laboratory of Plant Cell & Molecular Biol; 1143 Fifield Hall; University of Florida; Gainesville FL 32611; 352-392-1193; 352-392-9366 (fax); ikv@gnv.ifas.ufl.edu
Vega, Shifra; Dept Biol; Plant Sci Inst; Univ of Pennsylvania; Philadelphia PA 19104-6018
Veit, Bruce; Dept Biol and Biotech; Massey Univ; Private Bag 11222; Palmerston North; NEW ZEALAND; B.Veit@massey.ac.nz
Veldboom, Lance; PO Box 839; Holden's Foundation Seeds, L.L.C.; Williamsburg IA 52361; 319-668-1100; 319-668-2453 (fax); lance@holdens.com
Vergne, Phillippe; RCAP; ENS-Lyon; 46 Allee d'Italie; 69364 Lyon cedex 07; FRANCE; 33/72-72-86-08; 33/72 72 86 00 (fax); Philippe.Vergne@ens-lyon.fr
Vermerris, Wilfred; Forest Biotechnology Group; 6113 Jordan Hall; North Carolina State Univ; Raleigh NC 27695-8008; 919-515-7799; 919-515-7801 (fax); weverme1@unity.ncsu.edu
Vidakovic, Mirceta; Maize Research Institute; Slobodana Bajica 1; 11080 Zemun; Belgrade; YUGOSLAVIA; 381-11-617434; 381-11-197890 (fax); djelovac@eunet.yu
Vierling, Richard; Indiana Crop Improvement; 3510 U.S. 52 South; Lafayette IN 47905; 317-474-3494; 317-474-8959 (fax); Vierling@purdue.edu
Vincent, Leszek; Curtis Hall; University of Missouri; Columbia MO 65211-7020; 573-882-2674; 573-884-7850 (fax); leszek@missouri.edu
Violic, Alejandro D.; Vital Apoquindo 180; Santiago (Las Condes); CHILE; (562)229-0685; (562)735-5892 (fax)
Viotti, Angelo; Ist Biosintesi Vegetali; Via Bassini 15; 20133 Milano; ITALY; 39 2 706.001.70/309.85; 39 2 2362946 (fax); aviotti@icm.mi.cnr.it
Vodkin, Lila Ott; 384 ERML, Dept Crop Sci; Univ Illinois; 1201 W. Gregory; Urbana IL 61801; 217-244-6147; 217-333-9817 (fax); l-vodkin@uiuc.edu
Voelker, Rodger; Inst Molec Biol; Univ of Oregon; Eugene OR 97403
Vogel, Julie; DuPont Agric Biotechnol; DTP Suite 200; 1 Innovation Way, PO Box 6104; Newark DE 19714-6104; 302-631-2630; 302-631-2607 (fax); julie.m.vogel@usa.dupont.com
Vollbrecht, Erik; Cold Spring Harbor Lab; 1 Bungtown Rd; PO Box 100; Cold Spring Harbor NY 11724; 516-367-8836; 516-367-8369 (fax); vollbrec@cshl.org
Von Wettstein, D.; Dept Crop Soil Sci, Genet Cell Biol; Washington State Univ; Pullman WA 99164-6420; 509-335-3635; 509-335-8674 (fax); diter@wsu.edu
Waines, J. Giles; Dept of Botany/Plant Sciences; University of California; Riverside CA 92521; 909-787-3706; 909-787-4437 (fax)
Walbot, Virginia; Dept Biol Sci; 385 Serra Mall; Stanford Univ; Stanford CA 94305-5020; 650-723-2227; 650-725-8221 (fax); walbot@stanford.edu
Walden, D. B.; Dept of Plant Sciences; Univ of Western Ontario; London N6A 5B7; CANADA; 519-661-3103; 519-661-3935 (fax)
Walker, Elsbeth L.; Biology Dept; Morrill Science Center; Univ of Mass, Amherst; Amherst MA 01003; 413-545-0861; 413-545-3243 (fax); ewalker@bio.umass.edu
Walker, John; Biol Sci; 308 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-3583; 573-882-0123 (fax); jcw@biosci.mbp.missouri.edu
Walker, Nigel; Plant Biol Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102; 510-642-8058; 510-642-4995 (fax); nigel@nature.berkeley.edu
Walko, Richard; Pharmacia LKBBiotechnology; Alameda CA 94501
Walsh, Justine; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102; 510-642-8058; 510-642-4995 (fax); justice@nature.berkeley.edu
Walton, Jonathan; DOE Plant Research Lab; Michigan State University; East Lansing MI 48824; 517-353-4885; 517-353-9168 (fax); walton@pilot.msu.edu
Walton, Mark; LInkage Genetics; 2411 South 1070 West; Suite B; Salt Lake City UT 84119; 435-975-1188; 435-975-1244 (fax); waltonm@linkgene.com
Wan, Yuechun; Monsanto Agricultural Group; 700 N Chesterfield Pkwy - GG4H; St. Louis MO 63198; 314-537-6734; yuechun.wan@monsanto.com
Wang, Andrew S.; Novartis Seeds Inc; 317 330th Street; Stanton MN 55018-4308; 507-663-7658; 507-645-7519 (fax)
Wang, Bin; Academia Sinica; Institute of Genetics; Beijing 100101; CHINA; 8610-64870491; 8610-64873428 (fax); bwang@ss10.igtp.ac.cn
Wang, Guo-Ying; China Agric Univ Dept Biotech; Yuanmingyuan West Road No. 2; Beijing 100094; CHINA; (010)62892577; (010)62582332 (fax); wanggy@cpy.cjfh.ac.cn
Wang, Qinnan; National Natural Science Foundation; Dept. of Life Science; Beijing 100083; CHINA
Wang, Ya-hui; Academia Sinica; Inst. of Cell Biol.; 320 Yo-Yang Road; Shanghai 200031; CHINA
Warner, Todd; Northrup King Co; 317 330th St; Stanton MN 55018-4308
Warren, Jana; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004; 515-270-4390; 515-270-4312 (fax); warrenjl@phibred.com
Weaver, Sam; Quaker Oats Company; 617 West Main St.; Barrington IL 60010-4113; 708-304-2135; 708-304-2166 (fax)
Weaver, Sam; Quaker Oats Company; 617 West Main St.; Barrington IL 60010-4113; 708-304-2135; 847-304-2149 (fax)
Weaver, Sam; Quaker Oats Company; 617 West Main St.; Barrington IL 60010-4113; 847-304-2135; 708-304-2166 (fax)
Weaver, Sam; Quaker Oats Company; 617 West Main St.; Barrington IL 60010-4113; 847-304-2135; 847-304-2149 (fax)
Webb, Craig; Dept Plant Pathol; 4024 Throckmorton Plant Sci Ctr; Kansas State Univ; Manhattan KS 66506-5502; 913-532-2328; 913-532-5692 (fax); webbcr@plantpath.pp.ksu.edu
Weber, David; Illinois State Univ; 4120 Biological Sciences; Normal IL 61790-4120; 309-438-2685; 309-438-3722 (fax); dfweber@ilstu.edu
Weber, Gerd; Institut fur Pflanzenzuchtung; Universitat Hohenheim; Fruwirthshr. 21; D 70599 Stuttgart; GERMANY; 49 (711)459 2341; 49 (711)459 2343 (fax); weberg@uni-hohenheim.de
Weck, Edward A.; 901 College; Northfield MN 55057; 507-663-1244; eweck@microassist.com
Weil, Cliff; Dept. of Biological Sciences; University of Idaho, 229 Gibb; Moscow ID 83844-3051; (208)885-6370; (208)885-7905 (fax); cweil@uidaho.edu
Wen, Tsui-Jung; B422 Agronomy Hall; Iowa State University; Ames IA 50011; 515-294-1659; 515-294-2299 (fax); TJWEN@IASTATE.EDU
Wendel, Jonathan F.; Department of Botany; Bessey Hall; Iowa State University; Ames IA 50011; 515-294-7172; 515-294-1337 (fax); jfw@iastate.edu

Wenxiong, Lin; Fujian Agricultural College; Dept. of Agronomy; Jingshan, Fuzhou; Fujian 350002; CHINA
Werr, Wolfgang; Institut Fur Entwicklungsbiologie; Universitat zu Koln; Gyrhofstr 17; 50931 Koln 41; GERMANY; 49 221 470 2619; 49 221 470 5164 (fax); wwerr@biolan.uni-koeln.de
Wessler, Sue; University of Georgia; Dept of Genetics; Life Sciences Bldg; Athens GA 30602; 706-542-1870; 706-542-3910 (fax); sue@dogwood.botany.uga.edu
West, Dennis R.; Dept Plant and Soil Sci; Univ of Tennessee; Knoxville TN 37901-1071; 423-974-8826; 423-974-7997 (fax); DWEST3@UTK.EDU
Westhoff, Peter; Ins. Ent. Mol. Bio. Pflanzen; Heinrich-Heine-Univ Dusseldorf; D-40225 Dusseldorf; GERMANY; 49-211-81-12338; 49-211-81-14871 (fax); West@Uni-Duesseldorf.de
Wetzel, Carolyn; Dept of Botany; Bessey Hall; Ames IA 50011; 515-294-7724; 515-294-1337 (fax); cmwetzel@iastate.edu
Whalen, Richard; Dept of Biology; South Dakota State Univ; Brookings SD 57007; 605-688-4553; 605-688-6677 (fax); Richard_Whalen@sdstate.edu
Whitwood, W.; Seneca hybrids/SVS; 5271 Flat Street; Hall NY 14463; 716-526-5879; 716-526-5350 (fax)
Widholm, Jack M.; Crop Sciences; Univ of Illinois; ERML, 1201 W. Gregory; Urbana IL 61801; 217-333-9462; 217-333-4777 (fax); Widholm@UIUC.EDU
Widstrom, Neil W.; Coastal Plain Exp Sta; PO Box 748; Tifton GA 31793; 912-387-2341; 912-387-2321 (fax); nwidstro@tifton.cpes.peachnet.edu
Wienand, Udo; Inst. Allge. Bot.; Univ Hamburg; Pflanzen, AMP I, Ohnhorststrasse 18; D-22609 Hamburg; GERMANY; (49)40 822 82 501; (49)40 882 82 503 (fax); wienand@nw01.uni-hamburg.de
Wilkes, H. Garrison; Biology-Dept.; Univ of Mass/Boston; 100 Morrissey Blvd; Boston MA 02125-3393; 617-287-6600; 617-287-6650 (fax); WILKES@UMBSKY.CC.UMB.EDU
Willcox, Martha; CIMMYT; Apartado Postal 6-641; Mexico, D.F. 06600; MEXICO; 52(5)726-9091 ext.1128; 52(5)726-7559 (fax)
Williams, Claire G.; Texas A&M Univ; Dept of Soil & Crop Sci MS-2135; College Station TX 77843-2135; 409-862-3745; 409-845-6049 (fax); claire_williams@tamu.edu
Williams, Mark; DuPont de Nemours & Co Agric Biotech; Stine-Haskell Res Cent 210N/253; 1090 Elkton Rd; Newark DE 19714-0030; 302-366-5102; 302-451-4832 (fax); mark.e.williams@usa.dupont.com
Williams, Robert E.; PO Box 294; Pittsfield IL 62363; 217-285-2530
Williams, Rosalind; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720
Williams, W. Paul; USDA-ARS-CHPRRU; Box 9555; Miss. State MS 39762; 601-325-2735; 601-325-8441 (fax); pwilliams@dorman.msstate.edu
Willman, Mark R.; Hunt-Wesson Inc; 463 U.S. Hwy. 30 East; Valparaiso IN 46383; 219-464-9602x233; 219-462-6293 (fax); mwillman@hwfoods.com
Wing, Rod; Clemson Univ. Genomics Inst.; 100 Jordan Hall; Clemson University; Clemson SC 29634-5727; 864-656-7288; 864-656-4293 (fax); rwing@clemson.edu
Winkler, Rodney G.; Genomica Corporation; 4001 Discovery Drive; Suite 130; Boulder CO 80303; 303-544-4025; 303-402-9877 (fax); winkler@genomica.com
Winter-Vann, Ann Marie; CIBA Ag Biotech; PO Box 12257; Research Triangle Park NC 27709-2257
Wise, Roger; USDA-ARS, 351 Bessey Hall; Dept. Plant Pathology; Iowa State Univ.; Ames IA 50011-1020; 515-294-9756; 515-294-9420 (fax); rpwise@iastate.edu
Wolfe, Kenneth; Dept. of Genetics; University of Dublin; Trinity College; Dublin 2; IRELAND; 353-1-702-1253; 353-1-679-8558 (fax); KHWOLFE@VAX1.TCD.IE
Woo, Claudine; 2667 Parker St; Berkeley CA 94704
Woodman, Wendy; Dept. of Agronomy; Iowa State Univ.; Ames IA 50011; 515-294-3635; 515-294-3163 (fax); wlwoodma@iastate.edu
Woodruff, Dorde; 6366 Cobblerock Lane; Salt Lake City UT 84121-2304; 435-277-5526
Woodward, Wendy; Agronomy Dept; Waters Hall; Univ of Missouri; Columbia MO 65211; 573-882-1168; woodward@psu.missouri.edu
Woody, Laura; Biol Sci; 110 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-4871; lwoody@biosci.mbp.missouri.edu
Wright, James; Pioneer Hi-Bred Intl; PO Box 245; Wilson NC 27894-0245
Wright, Scott; Linkage Genetics; 1515 West 2200 South, Suite C; Salt Lake City UT 84119; 435-975-1188; 435-975-1244 (fax)
Wrobel, Russell; Dept Veg Crops; University of California-Davis; Davis CA 95616; rlwrobel@ucdavis.edu
Wurtzel, Eleanore; Dept Biol Sci; Davis Hall, Lehman College; City Univ New York; Bronx NY 10468; 718-960-4994, -8643; 718-960-8236 (fax); etwlc@cunyvm.cuny.edu
Xia, Yiji; B420 Agronomy Hall; Iowa State Univ; Ames IA 50011
Xia, Zhen-Ao; Academia Sinica; Shanghai Inst. of Plant Physiol.; 300 Fonglin Road; Shanghai 200433; CHINA
Xie, You-Ju; College of Biology; China Agricultural University; Beijing 100094; CHINA; 86 (10) 62631895; 0086-1-2582332 (fax); daimin@public3.bta.net.cn
Xiong, Chenmin; China National Rice Research Institute; Ti Yu Chang Road 171; Hangzhou; Zhejiang 310006; CHINA
Xu, Wenwei; Texas A&M Univ; Agric Res and Ext Center; Route 3, Box 219; Lubbock TX 79401; 806-746-6101; 806-746-6528 (fax); we-xu@tamu.edu
Xu, Xiaojie; B420 Agronomy Hall; Iowa State Univ; Ames IA 50011
Xu, Yun-Bi; Zhejiang Agricultural University; Dept. of Agronomy; Hangzhou; Zhejiang 310029; CHINA
Xu, Zeng-Fu; Zhongsan Univ.; Biotechnology Res. Center; 135 West Xingang Road, Guangzhou; Guangdong 510275; CHINA
Xu, Zhi-Hong; Shanghai Inst. of Plant Physiol.; 300 Fenglin road; Shanghai 200032; CHINA
Yaklin, Paul; Pioneer Hi-Bred Internat Inc; Trait & Technol Devel; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004
Yamada, Minoru; N.I. Vavilov Res Inst Plant Ind; Bolshaya Morskaya St 42; 190000 St. Petersburg; RUSSIA; 7-812-314-4848; 7-812-311-8762 (fax); viv@glasnet.ru
Yamaguchi, Judy; Plant Gene Expression Center; USDA-ARS; 800 Buchanan St; Albany CA 94710
Yang, Hong; Chinese Academy of Agric. Sciences; Biotech. Research Centre; Beijing 100081; CHINA
Yang, Jin Shui; Fudan University; Institute of Genetics; Shanghai 200433; CHINA
Yang, Ren-Cui; Fujian Agricultural College; Heterosis Utilization Lab.; Chinmen, Fuzhou; Fujian 350002; CHINA
Yang, Yuesheng; South China Agricultural University; Experimental Center; Guangzhou; Guangdong 510642; CHINA
Yano, Masahiro; Rice Genome Research Program; Nat. Inst. Agrobiol. Resources; 2-1-2, Kannondai; Tsukuba, Ibaraki 305; JAPAN; 81-298-38-7441,2199; 81-298-38-7468,2302 (fax); myano@abr.affrc.go.jp
Yatou, Osamu; Lab Rice Genet Resources; Dept Rice Research; Hokuriku Agric Exp Stn; 1-2-1 Inada, Joetsu-shi, Niigata-ken 943-01; JAPAN; 81-255-26-3304; 81-255-24-8578 (fax); yatou@inada.affrc.go.jp
Ye, Ke-Nan; Zhongsan University; Biotechnology Research Centre; Guangzhou 510642; CHINA
Ye, Sheng-Yu; Shanghai Inst. of Biochem.; 320 Yue Yang Road; Shanghai 200031; CHINA
Yi, Bu-Young; Nat Agric Sci & Technol Inst; RDA; SEO DUN DONG 249; Suwon 441-707; KOREA; 82-331-296-9436; 82-331-294-1072 (fax)
Yoganathan, Arulmolee; Department of Biology; Lehman College/CUNY; Bedford Park Blvd. West; Bronx NY 10468; 212-960-8235; 212-960-8227 (fax); ARYLC@CUNYVM.CUNY.EDU
Yong, Gao; Liaoning Academy of Agric. Sciences; Rice Research Institute; Sujiatun; Shenyang 110101; CHINA
Yoon, Elizabeth; Dept Bot Plant Path; Oregon State Univ; Cordley 2082; Corvallis OR 97331-2902; 541-737-3308; 541-737-3573 (fax); yoone@bcc.orst.edu
You, Chong-biao; Chinese Academy of Agric. Sciences; IAAE, Dept. of Biotechnology; P.O. Box 5109; Beijing 100094; CHINA
Young, Todd; Biochemistry Dept; UC Riverside; Riverside CA 92521-0129; 909-787-3580; 909-787-4437 (fax); teyoung@citrus.ucr.edu
Yu, Di-qiu; Zhongsan(Sun Yat-Sen)University; Biotechnology Research Center; Guangzhou; Guangdong 510275; CHINA
Yu, Jia; Dept. Biological Science; Lehman College; 250 Bedford Park Blvd. West; Bronx NY 10468; 212-960-4994; 212-960-8236 (fax)
Yu, Li; China National Rice Research Institute; Library; Tiyuchang Road No. 171, Hangzhou; Zhejiang 310006; CHINA
Yue, Yong Gang; Dow Agrosciences; Building 306 / C2; 9330 Zionsville Rd; Indianapolis IN 46268; 317-337-3861; ygyue@dowagro.com
Zabala, Gracia; Plant Biol/265 Morrill Hall; Univ Illinois; 505 S. Goodwin Avenue; Urbana IL 61801; 217-333-3736; 217-244-7246 (fax); Gracia.Zabala@Qms1.Life.uiuc.edu
Zavalishina, Alexandra; Genetics Dept; Saratov State University; 83, Astrakhanskaya St.; 410026, Saratov; RUSSIA; 845-2-240446 (fax); zavalish.bi.sgu@oda.ssu.runnet.ru
Zehr, Brent; Maharashtra Hyb Seeds Co; Rehsam Bhavan, 4th Floor; 78, Veer Nariman Road; Mumbai 400020; INDIA; mahycord@bom2.vsnl.net.in
Zeng, Mengqian; Institute of Genetics; Academia Sinica; 3 Datun Rd.; 100101 Beijing; CHINA; 64857495; 64854896 (fax); mengzeng@public.fhnet.cn.net
Zhang, Deyu; Jiangsu Academy of Agricultural Sci.; Inst. of Genet. and Physiol.; Nanjing 210014; CHINA
Zhang, Fan; Monsanto U4C; 800 North Lindbergh; St. Louis MO 63167; 314-694-8415; 314-694-8275 (fax); fan.zhang@monsanto.com
Zhang, Gui-Quang; South China Agricultural University; Dept. of Agronomy; Guangzhou 510642; CHINA
Zhang, Jianbo; 2288 Molecular Biology Building; Iowa State University; Ames IA 50011; (515)294-2922; (515)294-0345 (fax); jzhang@iastate.edu
Zhang, Peifen; 2288 Molecular Biology Bldg; Iowa State University; Ames IA 50011; 515-294-2922; 515-294-0345 (fax); zpf@iastate.edu

Zhang, Qifa; Huazhong Agricultural University; Nat Key Lab Crop Genet Imp; Wuhan 430070; CHINA; 86-27-7393392; 86-27-7393392 (fax); qifazh@public.wh.hb.cn
Zhao, Qiquan; Zhejiang Agricultural University; Dept. of Tea Science; Hangzhou; Zhejiang 32100; CHINA
Zhao, Zuo-Yu; Biotechnology Research; Pioneer Hi-Bred Int'l; 7300 NW 62nd Ave. P.O. Box 1004; Johnston IA 50131-1004; 515-270-3644; 515-270-3444 (fax); zhaoz@phibred.com
Zhen, Zhu; Academia Sinica; Institute of Genetics; Beijing 100101; CHINA
Zheng, Kangle; China National Rice Research Institute; 171 Ti Yu Chang Road; Hangzhou 310006; CHINA
Zheng, Yin-Zhou; Div Biol Sci; 117 Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-4871; 573-882-0123 (fax); yzheng@biosci.mbp.missouri.edu
Zhong, Zhen-Ping; Fujian Agricultural College; Dept. of Agronomy; Fuzhou; Fujian 350002; CHINA
Zhou, Hongsheng; Institute of Crop Breeding and Cultiv; Chinese Acad Agric Sci; 30, Bai Shi Qiao Lu; Beijing 100081; CHINA; 86-1-8351731; 86-1-8316545 (fax); dyhzhs@public3.bta.net.cn
Zhou, Kaida; Sichuan Agricultural University; Rice Research Institute; Yaan; Sichuan 625014; CHINA
Zhou, Zhaolan; Chinese Academy of Sciences; Institute of Genetics; Group 601; Beijing 100101; CHINA
Zhu, Li-Hong; Nanjing Agric University; Dept Agronomy; Nanjing; Jiangsu 210095; CHINA
Zhu, Li-huang; Academia Sinica; Institute of Genetics; Datun Road, Andingmen Wai; Beijing 100101; CHINA; 86-10-62033491; 86-10-64913428 (fax); lhzhu@igtp.ac.cn
Zhu, Xiaoyang; Inst Crop Germplasm Resources; Chinese Academy of Agric Sci; 30 Bai Shi Qiao Road; Beijing, 100081; CHINA; 86-10-62186647; 86-10-62174142 (fax)
Zhu, Ying-Guo; Wuhan University; Genetics Dept; Wuchang; Hubei 430072; CHINA; 27-7822712-4560; 27-7812661 (fax)
Zhu, Z. P.; Shanghai Inst. of Plant Physiol.; 300 Fengling Road; Shanghai 200032; CHINA
Zimmer, Elizabeth; Lab of Molecular Systematics MRC 534; Support Ctr. Nat'l Museum Nat. History; Smithsonian Inst; Washington DC 20560; 301-238-3444x106; 301-238-3059 (fax); zimmer@onyx.si.edu

BIZARRO **By Dan Piraro**

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

S-123 Turner Hall
1102 South Goodwin Avenue
Urbana, IL 61801-4798

(217) 333-6631 [*phone*]
(217) 333-6064 [*fax*]
maize@uiuc.edu [*internet*]
http://www.uiuc.edu/ph/www/maize [*URL*]

2365 seed samples have been supplied in response to 271 requests, for 1999. Of these, a total of 74 requests were received from 21 foreign countries. Approximately 85% of our requests were received by electronic mail or through our order form on the World-Wide Web.

Working backwards, we have been continuing to computerize data from the Stock Center's planting pedigrees. To date, we have entered all planting notes back through 1966, which facilitate pedigree information searches. Also completed are indexes for several reprint collections we hold. Among these are the complete collections of Earl Patterson, George Sprague and Marcus Rhoades.

We have added many new 'Phenotype Only' stocks. These are stocks that have been donated to the COOP over the years, and have been classified according to their mutant phenotype only. For the most part, these stocks have not as yet been allele tested, nor has their gene been located to a chromosome arm. While we expect that most of these will represent new alleles of known loci, some will represent unique, as yet undescribed loci. Over the past few years, some mutants in this class have been mapped and/or allele tested and where appropriate, the now characterized mutant stock was added to our main catalog. We are now listing all of these mutants to give cooperators that are interested in specific traits, easier access to these mutants.

Approximately 5 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Optimal spring growing conditions were followed by a hot summer, but with additional water supplied by irrigation, we obtained good increases of most stocks grown this year

Special plantings were made of several categories of stocks:

1. Approximately 1.5 acres was devoted to the vast mutant collection of Gerry Neuffer with special attention also given to the collection of mutants that we have obtained from Donald Robertson. We have made good progress in increasing the Neuffer collection, and have increased most of the Robertson collection. These stocks will be placed in the 'Phenotype Only' category of stocks created last year.
2. Plantings were also made from donated stocks from the collections of Don Auger (translocated *Ac* lines), Ed Coe (various genetic stocks), Jerry Kermicle (*R1* alleles), Michael McMullen (Brink pericarp color collection), Donald Miles (high chlorophyll fluorescence mutants), Robert Brawn (dwarf mutants), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.
3. We conducted allelism tests of several categories of mutants with similar phenotype or chromosome location. We found additional alleles of *glossy1*, *pink scutellem1*, *ramosa1*, *terminal ear1*, *virescent1*, *white3*, *yellow endosperm1*, and *yellow endosperm8*. In this manner, we hope to move stocks from our vast collection of unplaced uncharacterized mutants and integrate them into the main collection.
4. We conducted linkage tests of several mutants that had been placed to chromosome arm using B-A translocations. More precise locations were determined for *brown midrib3*, *inhibitor of r1*, and *reverse germ orientation2*.
5. Approximately 1 acre each year is devoted to the propagation of the large collection of A-A translocation stocks. Additional linkage tests were conducted on the *Wx1* and *waxy1*-linked A-A translocations with results presented elsewhere in this edition. We can provide good sources and complete pedigree information on stocks that were previously found not to carry the correct translocated chromosomes. Additional translocation stocks will be tested as time allows.

We continue to grow a winter nursery of 0.5 acres at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico. We had an excellent winter crop last year, and despite the passage of Hurricane Lenny over Puerto Rico, our crop this year looks as good if not better. We plan to continue growing our winter nurseries at this location.

We hired an additional Research Specialist who is responsible for the new stocks generated by the NSF project "Maize Gene Discovery, Sequencing and Phenotypic Analysis", in which we are involved along with Virginia Walbot and others in the maize community. We anticipate that this and other projects recently funded by the NSF Plant Genome Program will greatly enhance our collection along with greatly increasing our understanding of maize as a biological organism.

Marty Sachs	Philip Stinard	Janet Day Jackson	Shane Zimmerman
Director	Curator	Senior Research Specialist	Research Specialist

CATALOG OF STOCKS

CHROMOSOME 1 MARKER

101A sr1 zb4 p1-ww
101B sr1 P1-wr
101C sr1 p1-ww
101D sr1 P1-rr
101F sr1 ts2 P1-rr
102A Ws4-N1589
102D Blh1-N1593
102F ms28
103D vp5
103DA vp5-DR3076
103DB vp5-86GN4
103DC vp5-86GN3
103DD vp5-86GN6
103DE vp5-86GN11
103DF vp5-Mumm#1
103DG vp5-N81
103E zb4 ms17 p1-ww
104A Ts3
104F ms*-6034
104G ms*-6044
105A zb4 p1-ww
105B zb4 P1-wr
105C zb4 p1-ww br1
105E ms17 P1-wr
105F ms17 p1-ww
106B ts2 P1-rr
107A P1-cr
107B P1-rr
107C P1-rw
107D P1-cw
107E P1-mm
107F P1-vv::Ac
107G P1-or
107H p1-ww
109A gs1-PI228173
109B gs1-PI262495
109D P1-rr ad1 bm2
109E P1-wr br1 f1
110A P1-wr an1 Kn1 bm2
110D P1-wr an1 bm2
110E P1-wr ad1 bm2
110F P1-wr br1 Vg1
110H P1-wr br1 f1 bm2
110K P1-wr br1
111B hcf3-N846B
111C hcf3-N1242B
111D hcf44-N1278B
111F Les20-N2457
111G rs2
111H Les5-N1449
112B p1-ww br1 f1 bm2
112E as1
112H p1-ww br1
112I p1-ww br1 gs1 bm2
113B rd1
113BA rd1-Wasnok
113C br1 f1
113E br1 f1 Kn1
113K hm1; hm2
113L Hm1; hm2
114C br1 bm2
114D Vg1
114F br2 hm1; Hm2
114G br2 hm1; hm2
115C v22-8983
115CA v22-055-4
115J bz2-m::Ds; A1 A2 C1 C2 Pr1 R1
116A bz2-m::Ds; A1 A2 Ac C1 C2 Pr1 R1
116C an1 bm2
116D def(an1..bz2)-6923; A1 A2 Bz1 C1 C2 Pr1 R1
116G an1
116GA an1-93W1189
116I bz2 gs1 bm2 Ts6; A1 A2 Bz1 C1 C2 R1
117A br2
117D tb1
117DA tb1-8963
117E Kn1
118B Kn1 bm2
118C lw1
118CA lw1-3108
118CB lw1-6474
118J Adh1-3F1124r53
118K Adh1-1S5657; Adh2-33
118L Adh1-3F1124::Mu3
118M Adh1-3F1124r17
118N Adh1-IL14H; su1
118O Adh1-Cm
118P Adh1-FCm
118Q Adh1-Ct
119A Adh1-1S; Adh2-1P
119B vp8
119C gs1
119D gs1 bm2
119E Ts6
119F bm2
119H Adh1-FkF(gamma)25; Adh2-N
119J Adh1-Fm335::Ds1
119K Adh1-Fm335RV1
119L Adh1-2F11::Ds2
119M Adh1-1F725
120A id1
120B nec2-8147
120C ms9
120CA ms9-6032
120CB ms9-6037
120CC ms9-6042
120D ms12
120E v22-055-4 bm2
120F Mpl1-Sisco
120G Mpl1-Freeling
121A ms14
121AA ms14-6005
121B br2-mi8043
121C D8
121D lls1
121DA lls1-N501B
121E ty*-8446
121G ct2
121GA ct2-rd3
124A v*-5688
124B j*-5828
124C w*-8345
124CA w*-013-3
124CB w*-8245
124D v*-5588
124E w*-018-3
124F w*-4791
124G w*-6577
124H w*-8054
124I v*-032-3
124J v*-8943
125A Les2-N845A
125B Mpl1-Jenkins
125C hcf13-N1097B
125D hcf41-N1275C
125E hcf50-N1481
125F hcf2-N506C
125G hcf31-N1268B
126A bz2 gs1 bm2; A1 A2 Bz1 C1 C2 R1
126B id1-N2286A
126C dek1-N928A
126D dek1-N971
126E dek32-N1322A
126F o13
126H P1-vv::Ac bz2-m::Ds
126I P1-vv::Ac
126J P1-ww-1112
126K P1-ovov-1114
126L P1-rr-4B2
126M P1-vv-5145
126N dek1-N1348
126O dek1-N1394
126P dek1-N1401
127A bz2 zb7-N101 bm2
127B dek1-N792
127C dek2-N1315A
127D dek22-N1113A
127E f1
127F Msc1-N791A
127G Tlr1-N1590
127I gt1
128A ij2-N8
128B I16-N515
128C I17-N544
128D pg15-N340B
128E pg16-N219
128F v25-N17
128G py2-N521A
128H spc2-N262A
129A w18-N495A
129AA w18-571C
129B wlu5-N266A
129C zb7-N101
129D emp1-R
129E ptd1-MS1568
129F dek*-MS2115
129G dek*-MS6214
130A o10-N1356
130B cp3-N888A
130BA cp3-N888A; mn4-N888C
130C id1-NA972
130D dek1-PB388
6502A P1-ww-4Co63
6502C P1-ovov-CFS-29
6502D P1-rr(11)-CFS-33
6502E P1-rr(10)-CFS-36
6502F P1-rr(4-5)-CFS-47
6502G P1-rr(9)-CFS-53
6502I P1-rr(8-9)-CFS-75
6502K P1-vv-CFS-96
6502L P1-vv-CFS-110
6502M P1-vv-CFS-116
6502N P1-ovov-CFS-124
6502O P1-vv-CFS-138
6502P P1-rr(7)-CFS-140
6502Q P1-vv-CFS-155
6502R P1-o-grained-red-CFS-167
6502S P1-r pale(8)-CFS-181
6502T P1-rr(9)-CFS-186
6502V P1-vv-CFS-245
6502W P1-vv-CFS-246
6502X P1-vv-CFS-249
6502Y P1-vv-CFS-252
6502Z P1-vv-CFS-255
6502ZA P1-vv-CFS-256
6502ZB P1-vv-CFS-259
6503A P1-rr(11)-CFS-272
6503B P1-vv-CFS-273
6503C P1-vv-CFS-278
6503D P1-vv-CFS-279
6503E P1-vv-CFS-281
6503F P1-vv-CFS-282
6503G P1-vv-CFS-283
6503H P1-vv-CFS-284
6503I P1-r pale(5)-CFS-285
6503J P1-vv-CFS-286 (Brazil)
6503K P1-mm-CFS-286
6503L P1-mm-CFS-287
6503M P1-mm-CFS-289
6503N P1-mm-CFS-290
6503O P1-mm-CFS-291
6503P P1-mm-CFS-292
6503R P1-mm-CFS-294
6503S P1-mm-CFS-297
6503T P1-mm-CFS-301
6503U P1-rw(9)-CFS-302
6503V P1-rr(11)-CFS-303
6503W P1-rr(10)-CFS-305
6503X P1-rr(10)-CFS-315
6503ZA P1-rr(2)-CFS-319
6503ZB P1-rr(8)-CFS-320
6503ZC P1-rr(7)-CFS-321
6504A P1-rw(8)-CFS-324
6504B P1-rw(6-7)-CFS-325
6504C P1-rr(9)-CFS-327
6504D P1-rw(7)-CFS-330
6504E P1-rw(9)-CFS-332
6504F P1-rw(8)-CFS-334
6504G P1-o-grained-red-CFS-335
6504H P1-rw(5-6)-CFS-336
6504I P1-rw(7-9)-CFS-342
6504J P1-rr(5)-CFS-345
6504K P1-rw(7)-CFS-350
6504L P1-rr-CFS-360
6504M P1-rw(5)-CFS-369
6504N P1-ww(1)-CFS-376
6504O P1-vv-CFS-497
6504Q P1-rr(11)-CFS-548

CHROMOSOME 2 MARKER

201A mrl1-IHO
201B hcf106-Mum1::Mu1; hcf106c
201C hcf106-Mum2::Mu1; hcf106c
201D hcf106-Mum3::Mu1; hcf106c
201F ws3 lg1 gl2 b1
201G sm2-Brawn180
201H sm2-Brawn189
201I sm2-Brawn190
201J sm2-Brawn191
202A lg1-PI200299
202B lg1-PI262493
202C lg1-32TaiTaiTaSarga
202D lg1-ZCXGRB
202E lg1-64-4
203B al1
203BA al1-Brawn
203BB al1-y3
203D al1 lg1
203G al1-y3 gl2
204A al1-lty3
205A al1 lg1 gl2
205B lg1
205C lg1 gl2
205G al1 gl2 B1
206A lg1 gl2 B1
206C D10-N2428
206D Wrp1-NA1163
206E oro2
207A y11
208B lg1 gl2 B1 sk1
208C lg1 gl2 B1 sk1 v4
208D lg1 gl2 B1 v4
208E lg1 gl2 b1
208H gl2-Salamini
209E lg1 gl2 b1 sk1
209I gl2-Parker's Flint
210E gl2-3050-3
210F gl2-PI200291
210G gl2-PI239114
210H gl2-PI251009
210i gl2-PI251885
210J gl2-PI251930
210K gl2-PI262474
210L gl2-PI262493
210M gl2-PI267186
210N gl2-N718
210O gl2-N239
211A lg1 gl2 b1 fl1
211H gl2 wt1
212B lg1 gl2 b1 fl1 v4
212D lg1 gl2 b1 v4
213B lg1 gl2 wt1
213F lg1 B1-v::Bg Ch1
213H lg1 gl2 B1-v::Bg
214A wt1-PI251939
214B lg1 b1 gs2
214C d5
214D gl11 B1
214E B1 ts1
214J sk1
214L lg1 gl2 mn1
215A gl14

215B gl11
215C wt1
215CA wt1-N472A
215CB wt1-N666B
215CC wt1-N178C
215D mn1
215E fl1
215EA fl1-o4
215G fl1 v4
215H wt1 gl14
216A fl1 v4 Ch1
216D fl1 w3
216E fl1 v4 w3
216G fl1 v4 w3 Ch1
217A ts1
217B v4
217G v4 Ch1
217H ba2 v4
217I Les10-NA607
217J Les11-N1438
217K Les15-N2007
217L Les18-N2441
217M Les19-N2450
217N cpc1-N2284B
218A w3
218C w3 Ch1
218D Ht1-GE440
218DA Ht1-Ladyfinger
218DB Ht1
218E ba2
218G B1-Peru; A1 A2 C1 C2 r1-r
218GA B1-Peru; A1 A2 C1 C2 R1-r
218H w3-8686
218I w3-86GN12
219A B1-Peru; A1 A2 C1 C2 r1-g
219B b1; A1 A2 C1 C2 r1-g
219C Ch1
219D Ht1 Ch1
219F B1-Peru; A1 A2 bz2 C1 C2 r1-g
219G B1-Bolivia-706B; A1 A2 C1 C2 r1-g
219H B1-Bolivia; A1 A2 C1 C2 Pl1-Rhoades Pr1 r1-g
219I B1-I; A1 A2 C1 C2 Pl1-Rhoades r1-r
219J B1-I; A1 A2 C1 C2 Pl1-Rhoades r1-g
219K B1-S; pl1-McClintock R1-g
219L B1-S; pl1-McClintock R1-r
220A Les1-N843
220B ws3 lg1 gl2; Alien Addition T2-Tripsacum
220D hcf15-N1253A
220F os1
221A gs2
221AA gs2-0229
221C wlv1-N1860 Ch1
221G wlv1-N1860
224B v*-5537
224H whp1; A1 A2 C1 c2 gl1 in1 R1
224I ws3-7752
224J ijmos*-7335
224K glnec*-8495
224L ws3-8949
224M ws3-8991
224N ws3-8945
226A ws3-N2357
226B b1-m1::Ds1; A1 A2 C1 C2 r1-g
226C b1-md2::Ds1; A1 A2 C1 C2 r1-g
226D b1-Pm5; A1 A2 C1 C2 r1-g
226E b1-Perum216; A1 A2 C1 C2 r1-g
227A dek3-N1289
227B dek4-N1024A
227C dek16-N1414
227D dek23-N1428
227E Les4-N1375
227I nec4-N516B
227K et2-2352
227L et2-91g6290-26
228A I18-N1940
228B spt1-N464
228C ws3-N453A
228CA ws3-N605A
228E B1-Bh
228F ms33-6019
228G ms33-6024
228H ms33-6029
228I ms33-6038
228J ms33-6041
229A rf3 Ch1
229B v24-N424
229BA v24-N576A
229BB v24-N588A
229BC v24-N350
229C w3 rf3 Ch1
229E emp2-MS1047
229F dek*-MS1365
229G dek*-MS4160
229H dek*-MS2159
229J dek*-PIE

CHROMOSOME 3 MARKER

301A cr1
301B bif2-N2354
301C spc3-N553C
301D Wi2-N1540
302A d1-6016
302AA d1-N446
302AB d1-N339
302B d1 rt1
302E d1-tall
303A d1 rt1 Lg3-O
303F g2
303FA g2-pg14::I
303FB g2-v19
303FD g2-56-3040-14
303FE g2-59-2097
303FF g2-94-1478
303G g2 d1
304A d1 ys3
304F d1 Lg3-O ys3
304G Lg3-O Rg1
304I d1 h1
305A d1 Lg3-O
305D d1 Rg1
305K d1 cl1; Clm1-4
306D d1 Rg1 ts4
306F ref1-MS1185
307A Sdw2-N1991
307C pm1
308B d1 ts4
308E ra2
308F ra2 Rg1
309A a1-m3::Ds Sh2
309B a1-m1-5718::dSpm
309C a1-m1-5719A1::dSpm
309D a1-m1-5719A1::dSpm; Mod Pr1
309E a1 Sh2; Spm-w
309F a1-m2-8417::dSpm
309G a1-m2(os)-o1
309H a1-m2-7991A-o2
309I a1-m2-7995::dSpm
309J a1-m2-7977B::dSpm
309K a1-m2-8012A-p1
309L a1 Sh2; Spm-s
309M a1-m1-5719A1::dSpm sh2
309N a1-m2-7995B
309O a1-m1-5996-4::dSpm
309P a1-m1-5719A1::dSpm; Spm-i
309Q a1-m5::Spm-w; Spm-s
309S a1-m2-8411A::Spm-w Sh2
309T a1-m2-7981B6::Spm-w
309U a1-m2-8409::Spm-i
309V a1-m5::Spm-w Sh2
309W a1-m2-8011::Spm-w Sh2
309X a1 Sh2; Spm-w-8745
309Y a1 Sh2; Spm-i
309Z a1-m1-5720-o2
310C ra2 lg2
310D Cg1
311A cl1
311AA cl1-N2
311B cl1; Clm1-2
311BA cl1-7716; Clm1-2
311C cl1; Clm1-3
311D cl1-p; Clm1-4
311E rt1
311F ys3
311G Lg3-O ys3
312B Les17-N2345
312D Lg3-O
312G brn1-R
312H g2 brn1-R
312I brn1-R cr1
312J brn1-R ra2 lg2
312K brn1-Nelson
312L brn1-3071
312M ms23
313A gl6
313AA gl6-gl7
313AB gl6-N672B
313D ms3
313DA ms3-6008
313DB ms3-6009
313DC ms3-6043
313DD ms3-6020
314A gl6 lg2 A1; A2 C1 C2 R1
314C gl6 lg2 a1-m et1; A2 C1 C2 Dt1 R1
314F Rg1 gl6 lg2
314G gl6 lg2
315B Rg1 gl6
315C Rg1
315D A1-b(P415); A2 C1 C2 R1
315I A1-m2(os)-p1
315J A1-m2(os)-r2
315K a1-m2-7991A-o1
315L a1-m2-7991A-p2
315M a1-m2-7991A-p3
315N a1-m2-7991A-p4
315O a1-m2-7991A-p4b
315P a1-m2-7991A-p5
315Q a1-m2-8010A-o2
315R A1-m3-r1a sh2-m1::Ds
315S a1-m5-o1
315T a1-m5-o2
315U A1-m5-r1
315V A1-m5-r4
315W A1-m5-r5
316A ts4
316B a1-N796
316C dek5-N1339A
317F gl6 ts4 lg2
317I a1-m1-5996-4m::dSpm; Spm
317J a1-m2::Spm-s; Spm-w
317K a1-m2-7991A::Spm-s
317L a1-m2-8004::dSpm
317M a1-m2-8010A::Spm-s
317N a1-m2-8011::Spm-w
317O a1-m2-8012A
317P a1-m2-8147
317Q a1-m2-8167::dSpm
317R a1-m2-8414C
317S a1-m2-8549C
317T a1-m5::Spm-w Sh2
317U a1-m5::Spm-w sh2-1
317V a1-m1-OS::Spm
317W a1-m1-5720::Spm
317X a1-m1-6078::dSpm
317Y a1-m2-8409-2
317Z A1 def-1260
318A ig1
318B ba1
318C y10-7748
318D hcf19-N1257A
318E sh2-N391B
318EA sh2-N2307
318F sh2-N2340
318G na1
318H vp1-Mc
318I y10-8624
319A lg2 A1-b(P415) et1; A2 C1 C2 Dt1 R1
319C lg2 a1-m et1; A2 C1 C2 dt1 R1
319D lg2 a1-m et1; A2 C1 C2 Dt1 R1
319F lg2 a1-st et1; A2 C1 C2 Dt1 R1
319G lg2 a1-st et1; dt1
320A lg2
320B lg2-PI184281
320C lg2 na1
320E et1
320F A1 sh2; A2 b1 C1 C2 pl1 R1
320K sh2-94-1001-11
320L sh2-94-1001-58
320M sh2-94-1001-1003
320N a3-Styles; B1-b Pl1-Rhoades r1-g
320O a3-Styles; B1-b Pl1-Rhoades R1-nj
321A A1-d31; A2 C1 C2 R1
321B lg2 a1; A2 C1 C2 dt1 R1
321C lg2 A1-b(P415) et1; A2 C1 C2 dt1 R1
321D a1-m4::Ds; A2 C1 C2 R1
321E a1-rUq; A2 C1 C2 R1
321F a1-Mum1; A2 C1 C2 R1
321H a1-Mum3; A2 C1 C2 R1
321I a1-Mum4; A2 C1 C2 R1
321J a1-Mum5; A2 C1 C2 R1
322A A1-d31 sh2; A2 C1 C2 dt1 R1
322B A1-d31 sh2; A2 C1 C2 Dt1 R1
322C A1-Mum3-Rev; A2 C1 C2 R1
322F a1-m; A2 b1 C1 dt1 pl1 R1
322I et1-24
322J et1-27
322K et1-34
322L et1-2162
322M et1-2320
322N et1-2424
322O et1-2457
322P et1-3191
322Q et1-3328
322R et1-5079
322S et1-84-6013
322T et1-88g-9733
322U et1-43
323A a1-m; A2 C1 C2 Dt1 R1
323D a1-m sh2; A2 C1 C2 Dt1 R1
323E a1-m et1; A2 C1 C2 Dt1 R1
323G a1-m1::rDt (Neuffer); A2 C1 C2 Dt1 R1
323H a1-st; A2 C1 C2 dt1 Mrh R1
323I a1-m1::rDt (Neuffer); A2 C1 C2 dt1 R1
324A a1-st; A2 C1 C2 Dt1 R1
324B a1-st sh2; A2 C1 C2 Dt1 R1
324E a1-st et1; A2 C1 C2 Dt1 R1
324G a1-st; A2 C1 C2 dt1 R1
324H a1 et1; A2 C1 C2 dt1 R1
324I a1-st et1; A2 C1 C2 dt1 R1
324J a1-sh2-del-Robertson; A2 C1 C2 R1
324K a1-Mus1; A2 C1 C2 R1
324L a1-Mus2; A2 C1 C2 R1
324M a1-Mus3
324N a1-Mus4
325A a1-p et1; A2 C1 C2 dt1 R1
325B a1-p et1; A2 B1 C1 C2 Dt1 Pl1 R1
325C a1-x1; A2 C1 C2 R1
325D a1-x3; A2 C1 C2 R1
325E A1 ga7; A2 C1 C2 R1
325G a3
325I a1-p; A2 C1 C2 Dt1 R1
325J a1-p; A2 C1 C2 Pr1 R1
325K a1-m3::Ds sh2-m1::Ds; A2 Ac C1 C2 R1
326A sh2-Elmore
326AA sh2-Garwood
326AB sh2-60-156
326B vp1
326BA vp1-Mum3
326BC vp1-86N6
326BD vp1-86GN14
326BE vp1-86GN18
326BF vp1-86GN19
326BG vp1-Mum2
326BH vp1-Mum1::Mu
326C Rp3
326D te1-1

326DA te1-Forester
326DB te1-Grogan
329A v*-9003
329B v*-8623
329C w*-022-15
329D yd2
329E w*-8336
329F yg*-W23
329G w*-062-3
329H v*-8609
329HA v*-8959
329I pg2
329K yel*-8630
329L yel*-5787
330A h1
330G a1-mrh; A2 C1 C2 Mrh R1
330H A1-b(P415) Ring 3; A2 C1 C2 R1
330I a1-Mum2; A2 C1 C2 MuDR R1
330J a1-Mum2; A2 C1 C2 R1
330K a1 sh2; A2 C1 C2 dt1 R1
330L a1-mrh; A2 C1 C2 R1
332B dek5-N874A
332C dek24-N1283
332D Wrk1-N1020
332F gl19-N169
332G dek6-N627D
332H dek17-N330D
332I Lxm1-N1600
332M Spc1-N1376
332N wlu1-N28
332S Mv1
333A dek5-25
333AA dek5-MS33
333B te1-Galinat

CHROMOSOME 4 MARKER

401A Rp4-a
401C Ga1 su1
401D Ga1-S
401E Ga1-S; y1
401I ga1 su1
401J Ga1-M
401K Ga1-S su1
402A st1
402D Ts5
403A Ts5 fl2
403B Ts5 su1
404A su3-5081; su4-5081
404B su3-89-1303-18; su4-89-1303-18
404C su3-94-4079-6; su4-94-4079-6
405B la1-PI239110
405BA la1-Funk:1087
405BB la1-Funk:2232
405BC la1-N2020
405BD la1-N2276B
405BE la1-PI184284
405D la1-R su1 gl3
405G la1-R su1 gl4
406C fl2
406CA fl2-DR9234
406D fl2 su1
407D su1
407DA su1-N86
407DB su1-N2316
407DC su1-BKG489-13
407DD su1-PI
407DE su1-R2412
407DF su1-N896A
407DG su1-N1161A
407DH su1-N2313
407DI su1-N2314
407DJ su1-N959
407DK su1-N1968
407DL su1-N1994
407E su1-am
407F su1-am; du1
408B bm3-Burnham su1
408C su1 zb6
408E bm3-91598-3
408J su1 ra3
408K su1; se1
408L su1 zb6 Tu1
409A su1-st
409B su1-66
409C su1-P
409D su1-5051
409F su1-28510
409G su1-28511
409H su1-28512
409I su1-28513
409J su1-28515
409K su1-28516
409L su1-28517
409M su1-28518
409N su1-28519
409O su1-28520
409P su1-30394
409Q su1-30397
409R su1-30398
409S su1-30399
409T su1-30400
409U su1-30401
409V su1-Bn2
410D su1 zb6 gl3
410E su1-A3
410F su1-4582::Mu1
410G su1-8064
410H su1-2401
410I su1-3837
410J su1-7110
410K su1-2857
410L su1-2859
411B su1 gl4 o1
411F gl7 su1 v17
412C su1 gl3
412E su1 j2 gl3
412G su1 gl4 Tu1
413A su1 o1
413B su1 gl4
413D su1 C2-Idf1(Active-1); A1 A2 C1 R1
413F su1 de*-414E
413G v23 Su1 gl3; bm*-COOP
414A bt2
414AA bt2-Williams
414AB bt2-60-158
414AC bt2-9626
414AD bt2-5288
414B gl4
414BA gl4-Stadler
414BB gl4-gl16
414BD gl4-N525A
414C gl4 o1
414E de*-414E
415A j2
415B o1-N1243
415C o1-N1478A
416A Tu1-A158
416B Tu1-I(1st)
416C Tu1-I(2nd)
416D Tu1-d
416E Tu1-md
416F Tu1 gl3
417A j2 gl3
417B v8
417C gl3
417D o1 gl3
418A gl3 dp1
418B c2; A1 A2 C1 R1
418D C2-Idf1(Active-1); A1 A2 C1 R1
418E dp1
418F o1
418G v17
419A v23-8914
419E gl7
419F Dt6 gl3 C2; a1-m A2 C1 R1
419G Dt6 C2; a1-m A2 C1 R1
419H c2-m1::Spm; A1 A2 C1 R1
419I c2-m2::dSpm c2-m3::Mpi1
419J c2-Mum1
419K c2-m2::dSpm; Spm-s
419L c2-m881058Y::IRMA; En Mod wx1-m8::Spm-I8
420A su1 Dt4 C2; a1-m A2 C1 R1
420C nec*-rd
420CA nec*-016-15
420D yel*-8957
420F dp*-4301-43
420G w*-9005
420H Dt4 C2; a1-m A2 C1 R1
424C gl3-64-4
424D gl3-56-3120-2
424E gl3-56-3129-27
424F gl3-60-2555
424G gl3-PI183683
424H gl3-PI251928
424I gl3-PI251938
424J gl3-PI254858
424K gl3-PI267180
424L gl3-PI267219
424M gl3-PI-311517
424N gl3-15
426A Gl5 Su1; gl20
426B gl3-PI251941
427A cp2-o12
427AA cp2-N211C
427AB cp2-N1875A
427AC cp2-MS2608
427AD cp2-N912
427B dek25-N1167A
427C Ysk1-N844
427D orp1-N1186A; orp2-N1186B
427E dek8-N1156
427F dek10-N1176A
427G Ms41-N1995
427H dek31-N1130
427I Sos1-ref
428A gl5 Su1; gl20
428C nec5-N642
428D spt2-N1269A
428E wt2-N10
428F lw4; Lw3
428G bx1
428H gl5 su1; gl20
428L dsc1-MS2058

CHROMOSOME 5 MARKER

501A am1 a2; A1 C1 C2 R1
501B lu1
501D ms13
501E gl17
501F gl17-N260B
501G gl17 a2; A1 C1 C2 R1
501I am1
502B A2 ps1-Sprague pr1; A1 C1 C2 R1
502C D9-N2319
502D A2 bm1 pr1; A1 C1 C2 R1
502E Ms42-N2082
502F Nl2-N1445
502G A2 ga10; Bt1
503A A2 bm1 pr1 ys1; A1 C1 C2 R1
503B hcf43-N1277B
504A A2 bt1 pr1; A1 C1 C2 R1
504C A2 bm1 pr1 zb1; A1 C1 C2 R1
504E A2 bt1; A1 C1 C2 R1
505B A2 pr1 ys1; A1 C1 C2 R1
505C A2 bt1 pr1 ga*-Rhoades; A1 C1 C2 R1
505D pr1-N1515A
505E pr1-N1527A
506A A2 v3 pr1; A1 C1 C2 R1
506B A2 pr1; A1 C1 C2 R1
506C A2 pr1 v2; A1 C1 C2 R1
506D na2 A2 pr1; A1 C1 C2 R1
506F A2 pr1 v12; A1 C1 C2 R1
506L A2 br3 pr1; A1 C1 C2 R1
507A a2; A1 C1 C2 R1
507AA a2-Mus2; A1 C1 C2 R1
507AB a2-Mus3; A1 C1 C2 R1
507AC a2-Mus1; A1 C1 C2 R1
507F a2 bm1 bt1 ga*-Rhoades; A1 C1 C2 R1
507G a2 bm1 bt1; A1 C1 C2 R1
507H A2 bv1 pr1; A1 C1 C2 R1
507I a2-m4::Ds; wx1-m7::Ac7
508A a2 bm1 bt1 pr1; A1 C1 C2 R1
508C a2 bm1 bt1 bv1 pr1; A1 C1 C2 R1
508F a2 bm1 pr1 ys1; A1 C1 C2 R1
508H a2-Mum1
508I a2-Mum2
508J a2-Mum3
508K a2-Mum4
508L bv1 pr1
509G a2-m1::dSpm; Bt1
509H a2-m1(II)::dSpm(class II)
509I pr1-m1
509J a2-m1::dSpm pr1-m2
509K a2-m1(ps)
509L a2-m1::dSpm; Spm-s
509M a2-m5::dSpm
509N A2-m1(os)-r1
510A a2 bm1 pr1 v2; A1 C1 C2 R1
510D a2 pr1 gl8; A1 C1 C2 R1
510E a2 ae1 pr1 gl8; A1 C1 C2 R1
510G a2 bm1 pr1 eg1; A1 C1 C2 R1
511C a2 bt1 pr1; A1 C1 C2 R1
511F a2 bt1 Pr1 ga*-Rhoades; A1 C1 C2 R1
511H a2 bt1; A1 C1 C2 R1
512C a2 bt1 pr1 ga*-Rhoades; A1 C1 C2 R1
512D vp2-N1136B
512F pb4
512G gl8-N166A
512H v13
512I lw2-vp12
513A a2 pr1; A1 C1 C2 R1
513C a2 pr1 v2; A1 C1 C2 R1
513D A2 pr1 sh4; A1 C1 C2 R1
513E a2 pr1 v12; A1 C1 C2 R1
514A a2 bm1 pr1; A1 C1 C2 R1
514B ae1-PS1
514C ae1-PS2
514D ae1-PS3
514E ae1-PS4
514F ae1-PS5
514G ae1-PS6
514H ae1-PS7
514I ae1-PS8
514J ae1-PS9
514K ae1-PS10
514L ae1-PS11
514M Ae1-5180-r4
514N bt1-m1::dSpm
514O bt1-m2
514P bt1-m3::dSpm
514Q bt1-m4::Ds
514R Bt1-m1-r1
515A vp2
515AA vp2-DR5180
515AB a2 vp2-green mosaic; A1 C1 C2 R1
515C ps1-Sprague
515CA ps1-8776
515CB ps1-881565-2M
515CC ps1-N80
515CD ps1-8205
515D bm1
515E bt1-N1992
515F bt1-N2308
515G bt1-N2309
516B bt1-R
516BA bt1-Elmore
516BB bt1-C103
516BC bt1-Singleton
516BD bt1-sh3
516BE bt1-sh5
516BF bt1-Eldridge
516BH bt1-6-783-7
516BI bt1-Vineyard
516BJ bt1-T
516BK bt1-W187R
516BL bt1-3040

516BM bt1-N797A
516C ms5
516D td1 ae1
516DA td1-Nickerson
516G A2 bm1 pr1 yg1; A1 C1 C2 R1
517A v3
517AB v3-8982
517B ae1
517BA ae1-EMS
517BB ae1-PS12
517BC ae1-PS13
517BD ae1-PS14
517BE ae1-PS15
517BF ae1-PS16
517BH ae1-Elmore
517E ae1 pr1 gl8
518A sh4
518AA sh4-Rhoades
518AB sh4-o9
518B gl8-Salamini
518BA gl8-R
518BB gl8-6:COOP
518BC gl8-6:Salamini
518BD gl8-10:COOP
518BE gl8-PI180167
518C na2
518D lw2
519AA ys1-W23
519AB ys1-5344
519AC ys1-N755A
519AD ys1-74-1924-1
519B eg1
519C v2
519D yg1
519E A2 pr1 yg1; A1 C1 C2 R1
519F A2 pr1 gl8; A1 C1 C2 R1
519H zb1
519I zb1-2
520A hcf38-N1273
520B v12
520C br3
520F A2 Dap1; A1 C1 C2 R1
520G A2 pr1 Dap1; A1 C1 C2 R1
520H Dap1-2
521A nec3-N409
521B Nec*-3-9c
521C nec*-8624
521D nec*-5-9(5614)
521E nec*-7476
521F nec*-6853
521G nec*-7281
521H nec*-8376
521I v*-6373
521J yg*-8951
521K lw3; lw4
521L w*-021-7
521N lnec*-5931
521NA lnec*-8549
521P lw3; Lw4
524A v*-PI267226
524B les*-3F-3330
527A dek18-N931A
527B dek9-N1365
527C dek26-N1331
527D dek27-N1380A
527E grt1-N1308B
527F nec7-N756B
527G dek33-N1299
527H Msc2-N1124B
527I ppg1-N199
527J nec6-N493
528A Hsf1-N1595
528B wgs1-N206B
528C anl1-N1634
528CA anl1-330C
528E prg1-MS8186
528F ren1-MS807
528H dek*-MS2146
528I dek*-MS1182
529A anl1-N1643
529B anl1-N1645
529C anl1-N1671
529D anl1-N1685
529E anl1-N1691
529F anl1-N1673

CHROMOSOME 6 MARKER

601C rgd1 y1
601F po1-ms6 y1 pl1
601H rhm1 rgd1 y1
601I rhm1 y1 l11
601J Wsm1 Mdm1; Wsm2 Wsm3
601K wsm1 mdm1; wsm2 wsm3
601L Mdm1 y1
602A po1-ms6 wi1 y1
602C y1
602D rhm1 Y1
602J y1-w-mut
602K y1-gbl
602L y1-pb1
602M y1-8549
602N y1-Caspar
602O y1-0317
602P y1-129E
603A y1 l10
603AA y1 l10-1359
603B y1 l11-4120
603C y1 l12-4920
603D w15-8896 y1
603H mn3-1184 y1
604D y1 l15-Brawn1
604F y1 si1-mssi
604FA y1 si1-ts8
604FB y1 si1-Sam
604H y1 ms1
604HA y1 ms1-Robertson
604I Y1 ms1
604IA ms1-6050
605A wi1 y1
605C y1 pg11; pg12 Wx1
605E wi1 Y1 Pl1
605F wi1 Y1 pl1
605G l3
606A Y1 pg11-4484; pg12-4484 Wx1
606AA pg11-8925; pg12-8925
606AB pg11-48-040-8; pg12-48-040-8
606AC pg11-8563; pg12-8563
606AD pg11-8322; pg12-8322
606B y1 pg11; pg12 wx1
606C Y1 pg11; pg12 wx1
606E y1 pl1
606F y1 Pl1
606I y1 pg11 su2; pg12 Wx1
607A y1 Pl1-Bh1; A1 A2 c1 C2 R1 sh1 wx1
607C y1 su2
607E y1 pl1 su2 v7
607H y1 Pl1-Bh1; A1 A2 c1 C2 R1 sh1 Wx1
607I y1 Pl1-Bh1; A1 A2 c1 C2 R1 sh1 skb1 wx1
607J sm1-Brawn168
607K sm1-Brawn178
607L sm1-Brawn184
608A gs3-N268
608B Y1 l12
608C sbd1-N2292
608D Les13-N2003
608F y1 pl1 w1
608G Y1 l11
609D Y1 su2
609DA Y1 su2-89-1273
609DB su2-PS1
609DC su2-PS2
609F ms1-Albertsen
610B Dt2 Pl1; a1-m A2 C1 C2 R1
610F Y1 pl1 su2 v7
610G hcf34-N1269C
610H Y1 Dt2 pl1; a1-m A2 C1 C2 R1
610I hcf36-N1271B
610J hcf48-N1282C
610K hcf26-N1263C
610M hcf5-N510C
611A Pl1 sm1; P1-rr
611D Pt1
611E Y1 pl1 w1
611EA w1-7366
611I sm1 tan1-py1; P1-rr
611K Y1 Pl1 w1
611L w1; l1
611M afd1
611N sr4-N65A
611O o14-N924
612A w14
612B po1
612BA po1-ms6
612C l*-4923
612D oro1
612DA oro1-6474
612I tan1-py1
612J w14-8657
612K w14-8050
612L w14-6853
612M w14-025-12
612N w14-1-7(4302-31)
613A 2NOR y1; A1 a2 bm1 C1 C2 pr1 R1 v2 wx1
613D vms*-8522
613F w14-8613
613I tus*-5267
613J gm*-6372
613L w*-8954
613M yel*-039-13
613N yel*-7285
613O l*-4-6(4447)
613P yel*-8631
613T pg11-6656; pg12-6656
627A dek28-N1307A
627B dek19-N1296A
627C vp*-5111
627G dek*-MS1104; l*-1104

CHROMOSOME 7 MARKER

701B In1-D
701D o2
701E o2-Mum1
701F Hs1
702A o2 v5
702B o2 v5 ra1 gl1
702I In1-Brawn
703A o2 v5 gl1
703D o2 ra1 gl1
703J Rs1-O
703JA Rs1-1025::Mu6/7
703K Rs1-Z
704B o2 ra1 gl1 sl1
704C o2-NA696
704D o2-NA697
705A o2 gl1
705B o2 gl1 sl1
705D o2 bd1
706A o2 sl1
706B vp9-Bot100
707A y8 v5 gl1
707B in1; A1 A2 C1 C2 pr1 R1
707C in1 gl1; A1 A2 C1 C2 pr1 R1
707D v5
707E vp9-R
707EA vp9-3111
707EB vp9-86GN9
707EC vp9-86GN15
707F y8 gl1
707G in1 gl1; A1 A2 C1 C2 Pr1 R1
708A ra1
708AA ra1-PI262495
708B bd1-N2355
708C o15-N1117
708G y8
709A gl1
709AA gl1-56-3013-20
709AB gl1-56-3122-7
709AC gl1-PI183644
709AD gl1-PI218043
709AE gl1-PI251652
709AF gl1-PI257507
709AG gl1-Istra
709AH gl1-BMS
709AI gl1-7L
709AJ gl1-9:COOP
709AK gl1-N212
709AL gl1-N269
709AM gl1-N345B
709C gl1-m
710A gl1 Tp1
710B gl1 mn2
710E o5 gl1
710I gl1 Bn1
710J gl1-N271
710K gl1-dy
711A Tp1
711B ij1-ref::Ds
711G ts*-br
712A ms7
712AA ms7-6007
712B ms7 gl1
713A Bn1
713E Bn1 bd1
713H Bn1 ij1
713I bd1 Pn1
714A Pn1
714B o5
714BA o5-PS3038
714BC o5-N874B
714C o5-N1241
714D va1
715A Dt3; a1-m A2 C1 C2 R1
715C gl1 Dt3; a1-m A2 C1 C2 R1
716A v*-8647
716B yel*-7748
716C dlf1-N2389A
716D dlf1-N2461
716F Les9-N2008
727A dek11-N788
727B wlu2-N543A
727D v27-N590A
727DA v27-N53B
727DB v27-N413C
727E gl1-cgl
727F Rs4-N1606
727G Rs1-O o2 v5 ra1 gl1
727H ms34-6004
727I ms34-6010
727J ms34-6013
727K ms34-6014
728A Px3-6
728B ptd2-MS3193
728C mn2-cp1
728D sh6-8601
728E sh6-N1295
728F ren2-NS326
728G dek*-MS2082
728H dek*-MS5153

CHROMOSOME 8 MARKER

801A gl18-g
801B v16
801I yel*-024-5
801K v16 ms8
802A rgh1-N1285
802B emp3-N1386A
802C Ht2
802G ms43
802H gl18-PI262473
802I gl18-PI262490
803A ms8
803B nec1-025-4
803D gl18-g ms8
803F nec1-7748
803G nec1-6697
804A v21-A552
804B dp*-8925
804C tb*-poey1013
805A fl3
805C gl18-g v21-A552

805E el1
805G ms8 j1
808A ct1
808B Lg4-O
808C Htn1
810A v16 j1; l1
810B j1
827A dek20-N1392A
827B dek29-N1387A
827C Bif1-N1440
827CA Bif1-N2001
827D Sdw1-N1592
827E Clt1-N985
827F pro1-N1058
827G pro1-N1121A
827H pro1-N1528
827I pro1-N1533
827J wlu3-N203A
827K pro1
827L pro1-Tracy
828A ats1
828C pro1-N1154A
828D pro1-NA342

CHROMOSOME 9 MARKER

901B yg2 C1 sh1 bz1; A1 A2 C2 R1
901C yg2 C1 sh1 bz1 wx1; A1 A2 C2 R1
901E yg2 C1 bz1 wx1; A1 A2 C2 R1
901H yg2 C1 Bz1; A1 A2 C2 R1
901I yg2 C1 sh1 Bz1 wx1 K9S-l; A1 A2 C2 R1
902A yg2 c1 sh1 bz1 wx1; A1 A2 C2 R1
902B yg2 c1 sh1 wx1; A1 A2 C2 R1
902C yg2 c1 sh1 wx1 gl15-Hayes; A1 A2 C2 R1
902D yg2 c1 sh1 Bz1 wx1 gl15 K9S-s; A1 A2 C2 R1
903A C1 sh1 bz1; A1 A2 C2 R1
903B C1 sh1 bz1 wx1; A1 A2 C2 R1
903D C1-I sh1 bz1 wx1; A1 A2 C2 R1
904B C1 sh1; A1 A2 C2 R1
904C C1 sh1 wx1; A1 A2 C2 R1
904D C1 wx1 ar1; A1 A2 C2 R1
904F C1 sh1 bz1 gl15 bm4; A1 A2 C2 R1
905A C1 sh1 wx1 K9S-l; A1 A2 C2 R1
905C C1 bz1 Wx1; A1 A2 C2 R1
905D C1 sh1 wx1 K9S-l; A1 A2 C2 K10-l R1
905E C1 sh1 wx1 v1; A1 A2 C2 R1
905G C1 bz1 wx1; A1 A2 C2 R1
905H c1 sh1 wx1; A1 A2 b1 C2 R1-scm2
906A C1 wx1; A1 A2 C2 Dsl Pr1 R1 y1
906B C1 wx1; A1 A2 C2 Dsl pr1 R1 Y1
906C C1-I Wx1; A1 A2 C2 Dsl R1
906D C1-I; A1 A2 C2 R1
906G C1-I Sh1 Bz1 Wx1; Dsl
906H C1 Sh1 bz1 wx1; Ac
907A C1 wx1; A1 A2 C2 R1
907E C1-I wx1; A1 A2 C2 R1 y1
907G c1-p; A1 A2 B1-b C2 pl1 R1
907H c1-n; A1 A2 b1 C2 pl1 R1
907I C1-S wx1; A1 A2 C2 R1
908A C1 wx1 da1 ar1; A1 A2 C2 R1
908B C1 wx1 v1; A1 A2 C2 R1
908D C1 wx1 gl15; A1 A2 C2 R1
908F C1 wx1 da1; A1 A2 C2 R1
909A C1 wx1 Bf1-ref; A1 A2 C2 R1
909B c1 bz1 wx1; A1 A2 C2 R1
909C c1 sh1 bz1 wx1; A1 A2 C2 R1
909D c1 sh1 wx1; A1 A2 C2 R1
909E c1 sh1 wx1 v1; A1 A2 C2 R1
909F c1 sh1 wx1 gl15; A1 A2 C2 R1
910B c1 sh1 wx1 gl15 Bf1-ref; A1 A2 C2 R1
910D c1; A1 A2 C2 R1
910G C1 sh1-bz1-x2 Wx1; A1 A2 C2 R1
910H C1 sh1-bz1-x3; A1 A2 C2 R1
910I sh1-bb1981 bz1-m4::Ds
910IA sh1-bb1981 bz1-m4::Ds; Ac
910L yg2-str
911A c1 wx1; A1 A2 C2 R1
911B c1 wx1 v1; A1 A2 C2 R1
911C c1 wx1 gl15-Hayes; A1 A2 C2 R1
911D Fas1
911E sem1-1364
912A sh1
912AA sh1-1746
912AB sh1-9026-11
912AC sh1-3-6(6349)
912AD sh1-60-155
912AE sh1-EMS
912AF sh1-4020
912AG sh1-9552
912AH sh1-9626
912AI sh1-3017
912AJ sh1-6
912B sh1 wx1 v1
912E lo2
912H lo2 wx1
913C sh1 l7
913D sh1 l6
913E baf1
913F yg2-Mum1
913G yg2-Mum2
913H yg2-Mum3
913I yg2-Mum4
913J yg2-Mum5
913K yg2-Mum6
913L yg2-Mum7
913M yg2-Mum8
913N yg2-Mum9
913O yg2-DR83-106-3
913P yg2-DR83-106-5
914A wx1 d3-COOP
914B dek12-N1054
914K Wc1-ly; Y1
914L bz1-Mus1
914M bz1-Mus2
914N bz1-Mus3
914O bz1-Mus5
914P bz1-Mus6
914Q bz1-Mus7
914R bz1-Mus10
915A wx1
915B wx1-a
915C w11
915D wx1-N1050A
915E wx1-Alexander
915F wx1-N1240A
916A wx1 v1
916B wx1 v1-JRL
916C wx1 bk2
916E wx1 v1 gl15
916G Trn1-N1597
916H v31-N828
916I d3-8201
917A wx1 Bf1-ref
917C v1
917D ms2
917DA ms2-6002
917DB ms2-6012
917E gl15-Sprague
917EA gl15-Lambert
917EB gl15-KEW
917F d3-COOP
917FA d3-d2
917FB d3-015-12
917FC d3-072-7
917FD d3-8054
917FF d3-d2-Harberd
917FG d3-d2-Phillips
917FH d3-N660B
918A gl15 Bf1-ref
918B gl15 bm4
918C bk2 Wc1
918D Wc1
918F Wx1 Bf1-ref
918G Wc1 Bf1-ref bm4
918GA Wc1-Wh Bf1-ref bm4
918K bk2 v30
918L wx1 Wc1
919A bm4
919B Bf1-ref bm4
919C l6
919D l7
919G l6; l1
919I Bf1-DR-046-1
919J bz1-Mum9; MuDR
919K bz1-Mum4::Mu1
919L bz1-Mum1
919M bz1-Mum2
919N bz1-Mum3
919O bz1-Mum5
919P bz1-Mum6
919Q bz1-Mum7
919R bz1-Mum8
919S bz1-Mum9
919T bz1-Mum10
919U bz1-Mum11
919V bz1-Mum12
919W bz1-Mum15
919X bz1-Mum16
919Y bz1-Mum18
920A yel*-034-16
920B w*-4889
920C w*-8889
920E w*-8950
920F w*-9000
920G Tp3L-9SRhoades
920L ygzb*-5588
920M wnl*-034-5
920N pyd1
923A wx1-a
923B wx1-B
923C wx1-B1
923D wx1-B2::TouristA
923E wx1-B3::Ac
923F wx1-B4::Ds2
923G wx1-B6
923H wx1-B7
923I wx1-B8
923J wx1-BL2
923K wx1-BL3
923L wx1-C
923M wx1-C1
923N wx1-C2
923O wx1-C3
923P wx1-C4
923Q wx1-C31
923R wx1-C34
923S wx1-F
923T wx1-90
923U wx1-H
923V wx1-H21
923W wx1-I
923X wx1-J
923Y wx1-M
923Z wx1-m1::Ds
923ZA wx1-m6R
923ZB wx1-m6NR
923ZC wx1-m8::Spm-l8
923ZD wx1-P60
923ZE wx1-R
923ZF wx1-Stonor
924A Wd1 wd1 C1 C1-I Ring 9S; A1 A2 C2 R1
924B C1-I Ring 9S; A1 A2 C2 R1
924C yg2
924D wd1
924E wd1 C1 sh1 bz1
924F C1 Sh1 sh1 Bz1 bz1 wx1 tiny fragment 9
924G C1-I Bz1; Ac Dsl
924H c1 sh1 bz1 wx1; Ac
925A bz1-m1::Ds wx1-m9::Ac
925C bz1-m2::Ac
925D Wx1-m9r1
925E bz1-m2(Dll)::Ds wx1-m6::Ds
925F C1 sh1 bz1 wx1-m8::Spm-l8
925H bz1-m2(Dl)::Ds wx1; R1-sc
925I c1-m2::Ds Wx1; Ac
925J c1-m858::dSpm wx1
925K c1-m1::Ds
926A sh1-m5933::Ds
926B Sh1-r3(5933)
926C Sh1-r6(5933)
926D Sh1-r7(5933)
926E Sh1-r8(5933)
926F Sh1-r9(5933)
926G Sh1-r10(5933)
926H Sh1-r11(5933)
926I sh1-m6233::Ds
926J Sh1-r1(6233)
926K Sh1-r2(6233)
926L C1-I sh1-m6258::Ds
926M Sh1-m6258-r1
926N Sh1-r6795-1
926O bz1-m5::Ac
926P Bz1-wm::Ds1
926Q Bz1-m1-p
926R Bz1-m2-r1
926S Bz1-m2(Dll)-r1
926T Bz1-m2(Dll)-r2
926U Bz1-m2(Dll)-r3
926V sh1-bb1981 Bz1-m4-p1
926W sh1-bb1981 Bz1-m4-r6851
926X sh1-bb1981 Bz1-m4-r7840B
926Y sh1-bb1981 Bz1-m4-r8332
926Z Bz1-m5-p1
926ZA Bz1-m5-r1
926ZB Bz1-m5-r2
927A dek12-N873
927B dek13-N744
927C dek30-N1391
927D Les8-N2005
927E Zb8-N1443
927H C1 Dt7; a1-r A2 C2 R1
927I G6-N1585
927K Rld1-N1990
927L Rld1-N1441
928A yg2-N27
928AA yg2-N585
928AB yg2-N697
928AC yg2-N610
928B wlu4-N41A
928C ms20
928G c1-m5::Spm wx1-m8::Spm-l8; A1 A2 C2 R1
928H wx1-m7::Ac7
928I C1 bz1-mut::rMut; A1 A2 Bz2 C2 Mut R1
928J C1 bz1-(r)d; A1 A2 C2 R1
928K C1 Sh1 bz1-s; A1 A2 C2 Mut R1
928L ms45-6006
928M ms35-6011
928N ms35-6018
928O ms*-6021
928P ms*-6022
928Q ms35-6027
928R ms35-6031
928S ms*-6046
928T ms*-6047
929E Dp9
930A wx1-Mum1
930B wx1-Mum2
930C wx1-Mum3
930D wx1-Mum4
930E wx1-Mum5::Mu
930F wx1-Mum6
930G wx1-Mum7
930H wx1-Mum8
930I wx1-Mum9
930J wx1-Mum10
930K wx1-Mum11
930L wx1-Mus16
930M wx1-Mus181
930N wx1-Mus215
931A Wx1-m5::Ds
931B wx1-m6::Ds
931C wx1-m6-o1
931D Wx1-m7-i1
931E Wx1-m8-r10
931F Wx1-m9-r3
931G Wx1-m9-r4
931H wd1-Mus1
931I wd1-Mus2

931J wd1-Mus3
931K wd1-Mus4
931L wd1-Mus5
931M wd1-Mus6

CHROMOSOME 10 MARKER

X01A oy1-Anderson
X01AA oy1-yg
X01AB oy1-8923
X01B oy1 R1; A1 A2 C1 C2
X01C oy1 bf2
X01E oy1 bf2 R1; A1 A2 C1 C2
X02C oy1 zn1 R1; A1 A2 C1 C2
X02E oy1 du1 r1; A1 A2 C1 C2
X02G oy1 zn1
X02H Oy1-N1459
X02I Oy1-N1538
X02J Oy1-N1583
X02K Oy1-N1588
X02L Oy1-N1989
X03A sr3
X03B Og1
X03D Og1 R1; A1 A2 C1 C2
X03E oy1 y9
X03F Inr1-Ref
X04A Og1 du1 R1; A1 A2 C1 C2
X04B ms11
X04BA ms11-6051
X04D bf2
X04DA bf2-N185A
X05A Og*-0376
X05B Gs4-N1439
X05E bf2 sr2
X05G bf2 g1 R1-r; A1 A2 C1 C2
X06A bf2 r1 sr2; A1 A2 C1 C2
X06C nl1 g1 R1; A1 A2 C1 C2
X06F bf2 R1 sr2; A1 A2 C1 C2
X07A nl1 g1 r1; A1 A2 C1 C2
X07C y9
X07CA y9-y12
X07D nl1
X08A vp10
X08B vp10-86GN5
X08C vp10-TX8552
X08F li1
X08FA li1-IL90-243Tco
X09B li1 g1 R1; A1 A2 C1 C2
X09EA g1-g4
X09EB g1-56-3005-24
X09EC g1-1-7(X-55-16)
X09ED g1-68-609-13
X09EE g1-ws2
X09EF g1-PI262473
X09F ms10
X09FA ms10-6001
X09FB ms10-6035
X09G li1 g1 r1; A1 A2 C1 C2
X10A du1
X10AA du1-PS1
X10AB du1-PS2
X10AC du1-PS3
X10AD du1-PS6
X10AE du1-PS4
X10AF du1-PS5
X10D du1 g1 r1; A1 A2 C1 C2
X10F zn1
X10FA zn1-N25
X10G du1 v18
X11A zn1 g1
X11D Tp2 g1 r1; A1 A2 C1 C2
X11E g1 R1 sr2; A1 A2 C1 C2
X11F g1 r1; A1 A2 C1 C2
X11H zn1 R1-r; A1 A2 C1 C2
X11I Tp2 g1 sr2
X12A g1 r1 sr2; A1 A2 C1 C2
X12C g1 R1-g sr2; A1 A2 C1 C2
X12E g1 R1; A1 A2 C1 C2
X13D g1 r1-r sr2; A1 A2 C1 C2
X13E g1 r1-ch; A1 A2 C1 C2 wx1
X14A r1-r lsr1-Ej; A1 A2 C1 C2
X14E r1; A1 A2 C1 C2 wx1
X14F v18 r1; A1 A2 C1 C2
X14I r1-sc:m3::Ds
X14J R1-nj::Ac
X14K r1-Del902
X14L r1-g; A1 A2 C1 C2
X15B l1 r1 sr2; A1 A2 C1 C2
X15C R1-g; A1 A2 C1 C2
X15D r1-ch; A1 A2 C1 C2
X15F lsr1 R1-g sr2
X15G isr1 r1-g sr2
X15H isr1 R1-r:PI302369
X15HA isr1 R1-r:PI302369 sr2
X15I isr1 R1-nj Mst1
X16B r1 K10-I; A1 A2 C1 C2
X16C R1-ch; A1 A2 C1 C2 Pl1
X16CA R1-ch
X16D r1 sr2; A1 A2 C1 C2
X16E r1 K10-II; A1 A2 C1 C2
X16F R1 K10-II; A1 A2 C1 C2
X17A r1-g; A1 A2 C1 C2
X17B r1-r; A1 A2 C1 C2
X17C R1-mb; A1 A2 C1 C2
X17D R1-nj; A1 A2 C1 C2
X17E R1-r; A1 A2 C1 C2
X18A R1-lsk; A1 A2 C1 C2
X18B R1-sk:nc-2; A1 A2 C1 C2
X18C R1-st; A1 A2 C1 C2
X18D R1-sk; A1 A2 C1 C2
X18E R1-st Mst1
X18G R1-scm2; A1 A2 bz2 C1 C2
X18H R1-nj; A1 A2 bz2 C1 C2
X18I r1; A1 A2 C1 C2
X19A R1-sc:124
X19B w2
X19BA w2-Burnham
X19BB w2-2221
X19C l1 w2
X19D o7
X19E R1-r Lc1-Ecuador; b1
X19F r1 w2
X19G r1-n19 Lc1; b1
X19H r1-g:e Lc1; b1
X20B l1
X20C v18
X20I R1-d:Arapaho
X20J R1-d:Catspaw
X24A cm1
X24B lep*-8691
X24C v*-8574
X25A R1-scm2; a1-st A2 C1 C2
X25B R1-scm2; A1 A2 C1 c2
X25C R1-sc:122; A1 A2 C1 C2 pr1
X25D R1-scm2; A1 a2 C1 C2
X25E R1-scm2; A1 A2 c1 C2
X26A r1-X1 / R1; A1 A2 C1 C2
X26B R1-scm2; A1 A2 C1 C2
X26C R1-sc:122; A1 A2 C1 C2
X26D R1-sc:5691; A1 A2 C1 C2
X26E R1-scm2; A1 A2 C1 C2 pr1 wx1
X26F R1-scm2; A1 A2 C1 C2 In1-D
X26G R1-scm2; A1 A2 C1 c2-m2::dSpm
X26H R1-scm2; A1 A2 C1 C2 wx1
X27A dek14-N1435
X27B dek15-N1427A
X27C w2-N1330
X27D Les6-N1451
X27E gl21-N478B; gl22-N478C
X27F Vsr1-N1446
X27G Oy1-N700
X27H orp2-N1186B; orp1-N1186A
X27I l19-N425
X27J l13-N59A
X27K v29-N418
X27L Les12-N1453
X28B R1-scm2; a1-m1::rDt (Neuffer)
X28C R1-nj:Cudu; A1 A2 C1 C2
X28D Vsr*-N716
X28E Les3
X28F cr4-6143
X28G R1-nj:Chase; A1 A2 C1 C2
X28I R1-scm2; a1-m1-5719::dSpm A2 C1 C2
X28J R1-scm2; A1 A2 bz1 C1 C2
X29A ren3-MS1339
X29B dek*-MS2181

UNPLACED GENES

U140C l4
U140G ms22
U140H ms24
U240A Les7-N1461
U240D o11
U240E zn2
U240F zn2-PI251887
U240G zn2-PI236997
U240H zn2-PI239110
U240I zn2-56-3012-10
U340D ws1-COOP ws2-COOP
U340DA ws1-Pawnee ws2-Pawnee
U340H oro4
U440B gl13
U440C hcf49-N1480
U440D ub1-76C
U440E frz1
U440F mg1-Sprague
U540A dv1
U540B dy1
U640A dsy1-Doyle
U640B dsy1-Russian
U640C pam1
U640D pam2
U640E ada1
U640F atn1 Adh1-1S5657
U740A abs1-PI254851
U740B y2
U740C lty1
U740D lty2
U740F pi1 pi2
U740G Fbr1-N1602
U740H ad2-N2356A
U840A csp1-NA1173
U840B rli1-N2302A
U840C rli1-N2276
U840D Les21-N1442
U840E zb3
U840F agt1
U840G Wi3-N1614
U840H nld1-N2346
U840I Mc1
U940A Ht3

MULTIPLE GENES

M141A A1 A2 B1 C1 C2 Pl1 Pr1 R1-g
M141AA A1 A2 B1 C1 C2 Pl1-Rhoades Pr1 R1-g
M141B A1 A2 B1 C1 C2 pl1 Pr1 R1-g
M142A A1 a2 b1 C1 C2 pl1 R1-r
M142B a1 A2 b1 C1 C2 pl1 R1-r
M142C A1 a2 b1 C1 C2 pl1 R1-r
M142D A1 A2 b1 bz1 C1 C2 pl1 R1-r
M142E A1 A2 b1 bz2 C1 C2 pl1 R1-r
M142F A1 A2 b1 c1-p C2 pl1 R1-r
M142G A1 A2 b1 C1-l C2 pl1 R1-r
M142H A1 A2 b1 C1 c2 pl1 R1-r
M142I A1 A2 b1 C1 C2-ldfm pl1 R1-r
M142J A1 A2 b1 C1 C2-ldf1(Active-1) pl1 R1-r
M142K A1 A2 b1 C1 C2 pl1 pr1 R1-r
M142L A1 A2 b1 C1 C2 gl1 in1 pl1 R1-r
M142M A1 A2 b1 C1 C2 In1-D pl1 R1-r
M142O C1 sh1 bz1 wx1; A1 A2 C2 R1-r
M142P c1 sh1 wx1; A1 A2 C2 R1-r
M142Q yg2 c1 sh1 wx1; A1 A2 C2 R1-g
M142R A1 A2 C1-l C2 R1-r wx1
M142S su1 c2; A1 A2 C1 R1-r
M142T A1 A2 b1 C1 C2 pl1 r1-g
M142U A1 A2 b1 C1 C2 pl1 r1-r
M142V A1 A2 C1 C2 R1-nj
M142W A1 A2 C1 C2 R1-st
M142X A1 A2 b1 C1 C2 Pl1 r1-g
M142Y A1 A2 B1 C1 C2 Pl1 r1-g
M142Z a1-st A2 b1 C1 C2 pl1 R1-scm2
M142ZA A1 a2 b1 C1 C2 pl1 R1-scm2
M142ZC A1 A2 b1 bz2 C1 C2 pl1 R1-scm2
M142ZD A1 A2 b1 c1-n C2 pl1 R1-scm2
M142ZE A1 A2 b1 c1-p C2 pl1 R1-scm2
M241A A1 A2 B1 C1 C2 Pl1 Pr1 r1-g
M241C A1 A2 B1 C1 C2 Pl1 Pr1 R1-r
M241D A1 A2 b1 C1 C2 Pl1-Rhoades r1-g
M242A A1 A2 b1 C1 c2 pl1 R1-scm2
M242B A1 A2 b1 C1 C2 pl1 pr1 R1-scm2
M242C in1 gl1; A1 A2 b1 C1 C2 pl1 R1-scm2
M242D a1 sh2; A2 b1 C1 C2 pl1 R1-scm2
M242E c1 sh1 wx1; A1 A2 b1 C2 pl1 R1-scm2
M242F su1 c2; A1 A2 b1 C1 pl1 R1-scm2
M242G A1 A2 b1 C1 C2 pl1 R1-scm2
M242H A1 A2 b1 C1 C2 pl1 r1-g
M242I A1 A2 b1 C1 C2 pl1 r1-r
M340A A1 A2 B1 c1 C2 pl1 Pr1 R1-g
M340B A1 A2 B1 c1 C2 Pl1 Pr1 R1-g
M340C A1 A2 b1 c1 C2 pl1 Pr1 R1-g
M341B A1 A2 B1 C1 C2 pl1 Pr1 R1-r
M341C A1 A2 b1 C1 C2 Pl1 Pr1 R1-r
M341CA A1 A2 b1 C1 C2 Pl1-Rhoades Pr1 R1-r
M341D A1 A2 B1 c1 C2 Pl1 Pr1 R1-r
M341F A1 A2 b1 C1 C2 pl1 Pr1 R1-r
M441B A1 A2 B1 C1 C2 pl1 Pr1 R1-r wx1
M441D A1 A2 B1 C1 C2 Pl1 Pr1 r1-r
M441F A1 A2 b1 C1 C2 pl1 Pr1 R1-g wx1
M541B A1 A2 b1 C1 C2 pl1 Pr1 R1-g
M541F a1 A2 C1 C2 R1-nj
M541G A1 a2 C1 C2 R1-nj
M541H A1 A2 c1 C2 R1-nj
M541I A1 A2 C1-l C2 R1-nj
M541J A1 A2 C1 c2 R1-nj
M541K A1 A2 C1 C2-ldf1(Active-1) R1-nj
M541L A1 A2 bz1 C1 C2 Pr1 R1-nj
M541M A1 A2 Bz1 C1 C2 pr1 R1-nj
M541N A1 A2 C1 C2 gl1 in1 R1-nj
M541O A1 A2 C1 C2 In1-D R1-nj
M541P ae1 wx1
M641C A1 A2 b1 C1 C2 pl1 Pr1 R1-r wx1
M641D A1 A2 C1 C2 Pr1 r1 wx1 y1
M641E A1 A2 C1 C2 r1-g wx1 y1
M741A A1 A2 b1 C1 C2 pl1 Pr1 r1-g wx1
M741B Stock 6; A1 A2 B1 C1 C2 Pl1 R1-r
M741C Stock 6; A1 A2 B1 C1 C2 pl1 R1-r
M741F Stock 6; A1 A2 C1 C2 pl1 R1-g y1
M741G Stock 6; A1 A2 C1-l C2 pl1 R1-g wx1 y1
M741H Stock 6; A1 A2 B1 C1 C2 Pl1 R1-nj
M741I Stock 6; A1 A2 C1 C2 R1
M841A A1 A2 C1 C2 pr1 R1 su1
M841B f1 wx1
M841C v4 wx1
M841D v2 wx1
M841F A1 A2 bz2 C1 C2 R1-scm2 wx1
M841G A1 A2 C1 c2 R1-scm2 wx1
M841H gl6 wx1
M841I su1 wx1
M841J v16 wx1
M841K gl4 wx1
M841L gl2 lg1 wx1

M941A A1 A2 c1 C2 Pr1 R1 wx1 y1
M941B Mangelsdorf's tester; a1 bm2 g1 gl1 j1 lg1 pr1 su1 wx1 y1
M941C a1 Dt1 gl2 lg1 wt1
M941D gl1 wx1 y1
M941E gl8-R wx1 y1
MX40A A1 A2 C1 C2 P1-vv::Ac r1-sc:m3::Ds
MX40B A1 A2 Ac2 bz2-m::Ds C1 C2 R1
MX40C A1 A2 C1 C2 r1-sc:m3::Ds trAc8168
MX40D P1-vv::Ac r1
MX41A A1 A2 C1 C2 gl1 pr1 R1 wx1 y1
MX41B A1 A2 C1 C2 gl1 pr1 R1 su1 wx1 y1
MX41C a1 a2 bz1 bz2 c1 c2 pr1 r1 wx1 y1
MX41D a1 A2 C1 C2 gl1 pr1 R1 su1 wx1 y1
MX41E a1-m1-n::dSpm A2 C1 C2 R1 wx1-m8::Spm-I8

B-CHROMOSOME

B542A_Black Mexican Sweet; B chromosomes present
B542B_Black Mexican Sweet; B chromosomes absent

TRISOMIC

123A trisomic 1.
223A trisomic 2
328A trisomic 3
422A trisomic 4
523A trisomic 5
615A trisomic 6
718A trisomic 7
922A trisomic 9
X23A trisomic 10

TETRAPLOID

N102A Autotetraploid; A1 A2 B1 C1 C2 Pl1 Pr1 R1
N102D Autotetraploid; A1 A2 C1 C2 R1
N102E Autotetraploid; B chromosomes present
N102EA Autotetraploid; B chromosomes present
N102F Autotetraploid; A1 a2 C1 C2 R1
N103A Autotetraploid; P1-rr
N103B Autotetraploid; P1-vv::Ac
N103C Autotetraploid; P1-ww
N103D Autotetraploid; P1-wr
N103E Autotetraploid; P1-mm
N104A Autotetraploid; su1
N104B Autotetraploid; A1 A2 C1 C2 pr1 R1
N105B Autotetraploid; wx1 y1
N105D Autotetraploid; A1 a2 bt1 C1 C2 R1
N105E Autotetraploid; bt1
N106C Autotetraploid; wx1
N107B Autotetraploid; W23
N107C Autotetraploid; Synthetic B
N107D Autotetraploid; N6

CYTOPLASMIC STERILE/RESTORER

C736A R213 (N); mito-N Rf1 rf2
C736AB R213 (T) Sterile; cms-T Rf1 rf2
C736B Ky21 (N); mito-N Rf1 Rf2 Rf3 RfC
C736C B37 (N); mito-N rf1 Rf2 rf3 rfC
C736CA B37 (T) Sterile; cms-T rf1 Rf2
C736CB B37 (T) Restored; cms-T Rf1 Rf2
C736E Tr (N); mito-N Rf3 rfC rfT
C736EA Tr (S) Restored; cms-S Rf3 rfC rfT
C736F W23 (N); mito-N rf1 Rf2 rf3 RfC
C736FA W23 (N); mito-N rf1 Rf2 rf3 RfC
C736G B73 (N); mito-N rf1 Rf2 rf3 rfC
C736H L317 (N); mito-N rf3 RfC rfT
C836A Wf9 (T) Sterile; cms-T rf1 rf2
C836B Wf9 (N); mito-N rf1 rf2 rf3 rfC
C836C Wf9 (T) Restored; cms-T Rf1 Rf2 rf3 rfC
C836D Wf9 (S) Sterile; cms-S rf1 rf2 rf3 rfC
C836F Mo17 (N); mito-N rf1 Rf2 rf3 rfC
C836G Mo17 (C) Sterile; cms-C rf1 Rf2 rf3 rfC
C836H Mo17 (S) Sterile; cms-S rf1 Rf2 rf3 rfC
C936D K55 (N); mito-N Rf1 Rf2 rf3 RfC
C936DA K55 (N); mito-N Rf1 Rf2 rf3 RfC
C936F N6 (N); mito-N rf1 Rf2 rf3 RfC
C936FA N6 (N); mito-N rf1 Rf2 rf3 RfC
C936G N6 (T) Sterile; cms-T rf1 Rf2
C936H N6 (T) Restored; cms-T Rf1 Rf2
C936I SK2 (N); mito-N rf1 Rf2 rf3 rfC
C936J SK2 (T) Sterile; cms-T rf1 Rf2
C936K SK2 (T) Restored; cms-T Rf1 Rf2
C936M 38-11 (N); mito-N rf1 Rf2 rf3 rfC
CX36A N6 (C) Restored; cms-C rf1 Rf2 rf3 RfC
CX36B N6 (S) Sterile; cms-S rf1 Rf2 rf3 RfC
CX36C B37 (C) Sterile; cms-C rf1 Rf2 rf3 rfC
CX36D B37 (S) Sterile; cms-S rf1 Rf2 rf3 rfC

CYTOPLASMIC TRAIT

C337A NCS2
C337B NCS3

TOOLKIT

T0318AA TB-3Ld lg1; ig1R1-nj
T0318AB cms-L; ig1 R1-nj
T0318AC cms-MY; ig1 R1-nj
T0318AD cms-ME; ig1 R1-nj
T0318AE cms-S; ig1 R1-nj
T0318AF cms-SD; ig1 R1-nj
T0318AG cms-VG; ig1 R1-nj
T0318AH cms-CA; ig1 R1-nj
T0318AI cms-C; ig1 R1-nj
T0318AJ cms-Q; ig1 R1-nj
T0940A Hi-II Parent A (for producing embryogenic callus cultures)
T0940B Hi-II Parent B (for producing embryogenic callus cultures)
T0940C Hi-II A x B (for producing embryogenic callus cultures)
T0940D KYS (for chromosome observations in pachytene microsporocytes)
T0940E Mu off; a1-Mum2 A2 C1 C2 R1
T3302A Inv1m; P1-vv::Ac bz2-m::Ds
T3302C T1-2b; P1-vv::Ac bz2-m::Ds
T3302D T1-2(036-7); P1-vv::Ac bz2-m::Ds
T3302E T1-2c; P1-vv::Ac bz2-m::Ds
T3302G T1-3k; P1-vv::Ac bz2-m::Ds
T3302H T1-3(5597); P1-vv::Ac bz2-m::Ds
T3302I T1-3(5982); P1-vv::Ac bz2-m::Ds
T3302J T1-4i; P1-vv::Ac bz2-m::Ds
T3302K T1-4(064-20); P1-vv::Ac bz2-m::Ds
T3303B T1-5b; P1-vv::Ac bz2-m::Ds
T3303C T1-5(4613); P1-vv::Ac bz2-m::Ds
T3303D T1-5(5045); P1-vv::Ac bz2-m::Ds
T3304I bz2-m::Ds
T3304J Inv1m; P1-vv::Ac r1-sc:m3::Ds
T3304K Inv1a; P1-vv::Ac r1-sc:m3::Ds
T3304M T1-2c; P1-vv::Ac r1-sc:m3::Ds
T3305A T1-3(5597); P1-vv::Ac r1-sc:m3::Ds
T3305B T1-4i; P1-vv::Ac r1-sc:m3::Ds
T3305C T1-4(064-20); P1-vv::Ac r1-sc:m3::Ds
T3305F T1-4b; P1-vv::Ac r1-sc:m3::Ds
T3305H T1-5(6899); P1-vv::Ac r1-sc:m3::Ds
T3305J T1-5(4613); P1-vv::Ac r1-sc:m3::Ds
T3305M T1-6(5495); P1-vv::Ac r1-sc:m3::Ds
T3305N T1-6e; P1-vv::Ac r1-sc:m3::Ds
T3305O T1-6(028-13); P1-vv::Ac r1-sc:m3::Ds
T3306C T1-7(4444); P1-vv::Ac r1-sc:m3::Ds
T3306D T1-7(4405); P1-vv::Ac r1-sc:m3::Ds
T3306H T1-8(6591); P1-vv::Ac r1-sc:m3::Ds
T3306L T1-9(8302); P1-vv::Ac r1-sc:m3::Ds
T3306M T1-9(6762); P1-vv::Ac r1-sc:m3::Ds
T3306N T1-10g; P1-vv::Ac r1-sc:m3::Ds
T3307A trAc8178; T2-9b (2S.18; 9L.22) wx1
T3307B trAc8178; T2-9c (2S.49; 9S.33) wx1
T3307C trAc8178; T2-9d (2L.83; 9L.27) wx1
T3307D trAc8163; T3-9(8447) (3S.44; 9L.14) wx1
T3307E trAc8163; T3-9c (3L.09; 9L.12) wx1
T3307F trAc8183; T3-9(8447) (3S.44; 9L.14) wx1
T3307G trAc8183; T3-9c (3L.09; 9L.12) wx1
T3308A trAc8200; T4-9g (4S.27; 9L.27) wx1
T3308D trAc8175; T5-9c (5S.07; 9L.10) wx1
T3308E trAc8193; T5-9c (5S.07; 9L.10) wx1
T3308F trAc8179; T5-9a (5L.69; 9S.17) wx1
T3308G trAc8181; T5-9a (5L.69; 9S.17) wx1
T3308H trAc8186; T5-9a (5L.69; 9S.17) wx1
T3309A trAc8196; T5-9a (5L.69; 9S.17) wx1
T3309B trAc6062; T6-9b (6L.10; 9S.37) wx1
T3309C trAc6063; T6-9b (6L.10; 9S.37) wx1
T3309D trAc8172; T6-9b (6L.10; 9S.37) wx1
T3309E trAc8184; T6-9b (6L.10; 9S.37) wx1
T3310A trAc8161; T7-9(4363) (7ctr; 9ctr) wx1
T3310B trAc8173; T7-9(4363) (7ctr; 9ctr) wx1
T3310C trAc8173; T7-9a (7L.63; 9S.07) wx1
T3310D trAc8190; T7-9(4363) (7ctr; 9ctr) wx1
T3310E trAc8194; T7-9(4363) (7ctr; 9ctr) wx1
T3310F trAc8185; T7-9a (7L.63; 9S.07) wx1
T3311A trAc8162; T8-9d (8L.09; 9S.16) wx1
T3311B trAc8182; T8-9d (8L.09; 9S.16) wx1
T3311C trAc8182; T8-9(6673) (8L.35; 9S.31) wx1
T3311D trAc6059; T9-10b (9S.13; 10S.40) wx1
T3311E trAc6059; T9-10(8630) (9S.28; 10L.37) wx1
T3311F trAc8180; T9-10b (9S.13; 10S.40) wx1
T3311G trAc8180; T9-10(8630) (9S.28; 10L.37) wx1
T3312A Ds-1S1 P1-vv::Ac Dek1
T3312B Ds-1S2 P1-vv::Ac Dek1
T3312C Ds-1S3 P1-vv::Ac Dek1
T3312D Ds-1S4 P1-vv::Ac Dek1
T3312E Ds-1L1 P1-vv::Ac Bz2
T3312F Ds-1L3 Bz2; Ac
T3312G Ds-2S1 B1-Peru; P1-vv::Ac
T3312I Ds-2S3 B1-Peru; P1-vv::Ac
T3312J Ds-2S4; P1-vv::Ac
T3312L Ds-3L1 A1 Sh2; P1-vv::Ac
T3312M Ds-3L2 A1 Sh2; P1-vv::Ac
T3312O Ds-4L1 C2; P1-vv::Ac
T3312P Ds-4L3 C2; P1-vv::Ac
T3312Q Ds-4L4 C2; P1-vv::Ac
T3312S Ds-4L6 C2; P1-vv::Ac
T3312T Ds-4L7 C2; P1-vv::Ac
T3312U Ds-5L1 A2 Pr1 Bt1; P1-vv::Ac
T3312V Ds-5S1 A2 Pr1 Bt1; P1-vv::Ac
T3312W Ds-5S2 A2 Pr1 Bt1; P1-vv::Ac
T3312Y Ds-9S1 C1-I wx1; Ac
T3312Z Ds-10L2 R1-sc; P1-vv::Ac

B-A TRANSLOCATIONS (BASIC SET)

122A TB-1La
122B TB-1Sb
222A TB-1Sb-2L4464
222B TB-3La-2S6270
327A TB-3La
327B TB-3Sb
421A TB-4Sa
423E TB-4Lf
522A TB-5La
522C TB-5Sc
614B TB-6Sa
614C TB-6Lc
717A TB-7Lb
719A TB-7Sc
809A TB-8Lc
922B TB-9Lc Wc1
922D TB-9Sd

X21B TB-10L19
X22A TB-10Sc

B-A TRANSLOCATIONS (OTHERS)

122C TB1-Lc
126G TB-1Sb P1-vv::Ac bz2-m::Ds A1 A2 Bz1 C1 C2 R1
221I TB-2Sa B1-Peru
221J TB-2Sb
225A TB-3La-2L7285
225B TB-1Sb-2Lc
320P TB-1La-3Le
320Q TB-5La-3L(1)
320R TB-5La-3L(2)
320S TB-5La-3L(3)
327C TB-3Lc
327D TB-3Ld
329Z T3-B(La); T3-B(Sb)
331A TB-1La-3L5267
331B TB-1La-3L4759-3
331C TB-1La-3L5242
331E TB-3Lf
331F TB-3Lg
331G TB-3Lh
331H TB-3Li
331I TB-3Lj
331J TB-3Lk
331K TB-3Ll
331L TB-3Lm
420B TB-9Sb-4L6504
420I TB-9Sb-4L6222
421B TB-1La-4L4692
421C TB-7Lb-4L4698
423A TB-4Lb
423B TB-4Lc
423C TB-4Ld
423D TB-4Le
423F TB-1Sb-2L4464-4f
425A TB-4Sg
425B TB-4Lh
425C TB-4Li
428I Dt6 TB-4Sa
522B TB-5Lb
522D TB-5Ld
528D TB-1La-5S8041
614A TB-6Lb
627E TB-6Lc Dt2; a1-m A2 C1 C2 R1
720A TB-7Lb Dt3; a1-m1::rDt (Neuffer)
806A TB-8La
806B TB-8Lb
921A TB-9La
921B TB-9Sb
921C TB-9Lc
922C TB-9Sb C1-I
929A IsoB9-9 isochromosome Type 1
929B IsoB9-9 isochromosome Type 2
929C T9-B(La); T9-B(Sb)
929D IsoB9-9 isochromosome (original)
929F T9-B (La + Sb)
929G TB-9Sb; T9-8(4453)
929H TB-9Sb; T9-3(6722)
929I TB-9Sb-1866
929J TB-9Sb-1852
929K TB-9Sb-2150
929L TB-9Sb-14
929M TB-9Sb-2010
TX40D TB-1Sb P1-vv::Ac r1-sc:m3::Ds
TX40E TB-3La a1-m Dt1
TX40F TB-8Lc Ac2 bz2-m::Ds
TX40G TB-9Sd a1-m Dt1
TX40H TB-9Lc trAc81 r1-sc:m3::Ds
X21A TB-10La
X21C TB-10Ld
X22B T1La-B-10L18
X22C TB-10Lb
X30A TB-10L1
X30B TB-10L2
X30C TB-10L3
X30D TB-10L4
X30E TB-10L5
X30F TB-10L6
X30G TB-10L7
X31A TB-10L8
X31B TB-10L9
X31C TB-10L10
X31D TB-10L11
X31E TB-10L12
X31G TB-10L14
X31H TB-10L15
X31I TB-10L16
X31J TB-10L17
X32A TB-10L18
X32C TB-10L20
X32D TB-10L21
X32E TB-10L22
X32F TB-10L23
X32G TB-10L24
X32H TB-10L25
X32I TB-10L26
X32J TB-10L27
X32K TB-10L28
X33A TB-10L29
X33B TB-10L30
X33C TB-10L31
X33D TB-10L32
X33E TB-10L33
X33F TB-10L34
X33G TB-10L35
X33H TB-10L36
X34A TB-10L37
X34B TB-10L38

INVERSION

I143A Inv1a (1.S.30; 1.L.50)
I143B Inv1c (1.S.30; 1.L.01)
I143C Inv1d (1.L.55; 1.L.92)
I143D Inv1k (1.L.46; 1.L.82)
I243A Inv2b (2S.06; 2L.05)
I243B Inv2h (2L.13; 2L.51)
I343A Inv3a (3L.38; 3L.95)
I343B Inv3b (3L.21; 3L.70)
I343C Inv3c (3L.05; 3L.95)
I343D Inv3(8582) (3S.55; 3L.82)
I344A Inv9a (9S.70; 9L.90)
I443A Inv4b (4S.10; 4L.12)
I443B Inv4c (4S.89; 4L.62)
I443D Inv4d (4L.40; 4L.96)
I443E Inv4f (4L.17; 4L.63)
I444A Inv2a (2S.70; 2L.80)
I543A Inv4e (4L.16; 4L.81)
I743A Inv5(8623) (5S.67; 5L.69)
I743B Inv6d (6S.70; 6L.33)
I743C Inv6(3712) (6S.76; 6L.63)
I743D Inv6a (6S.76; 6L.63)
I843A Inv6e (6S.80; 6L.32)
I943A Inv7f (7L.17; 7L.61)
I943B Inv7(8540) (7L.12; 7L.92)
I943C Inv7(3717) (7S.32; 7L.30)
I943E Inv7a (7L.05; 7L.95)
IX43A Inv8a (8S.30; 8L.15)
IX43B Inv9b (9S.05; 9L.87)

RECIPROCAL TRANSLOCATIONS (wx1 AND Wx1 MARKED)

wx01A T1-9c (9L.22; 1.S.48); wx1
wx01B T1-9(5622) (9L.12; 1.L.10); wx1
wx02A T1-9(4995) (9S.20; 1.L.19); wx1
wx02AA T1-9(4995) (9S.20; 1.L.19); wx1
wx03A T1-9(8389) (9L.13; 1.L.74); wx1
wx04A T2-9c (9S.33; 2S.49); wx1
wx05A T2-9b (9L.22; 2S.18); wx1
wx06A T2-9d (9L.27; 2L.83); wx1
wx07A T3-9(8447) (9L.14; 3S.44); wx1
wx08A T3-9c (9L.12; 3L.09); wx1
wx09A T3-9(8562) (9L.22; 3L.65); wx1
wx10A T4-9e (9L.26; 4S.53); wx1
wx11A T4-9g (9L.27; 4S.27); wx1
wx12A T4-9(5657) (9S.25; 4L.33); wx1
wx13A T4-9b (9L.29; 4L.90); wx1
wx14A T5-9c (9L.10; 5S.07); wx1
wx14B T5-9(022-11) (9L.27; 5S.30); wx1
wx15A T5-9(4817) (9S.07; 5L.06); wx1
wx16A T5-9d (9L.10; 5L.14); wx1
wx17A T5-9a (9S.17; 5L.69); wx1
wx18A T6-9(4778) (9L.30; 6S.80); wx1
wx19A T6-9a (9L.40; 6S.79); wx1
wx19B T6-9e (9L.24; 6L.18); wx1
wx20A T6-9b (9S.37; 6L.10); wx1 y1
wx21A T6-9(4505) (9ctr.00; 6L.13); wx1
wx22A T7-9(4363) (9ctr.00; 7ctr.00); wx1
wx23A T7-9a (9S.07; 7L.63); wx1
wx24A T8-9d (9S.16; 8L.09); wx1
wx25A T8-9(6673) (9S.31; 8L.35); wx1
wx26B T9-10(059-10) (9S.31; 10L.53); wx1
wx27A T9-10b (9S.13; 10S.40); wx1
Wx30A T1-9c (9L.22; 1.S.48); Wx1
Wx30B T1-9(4995) (9S.20; 1.L.19); Wx1
Wx30C T1-9(8389) (9L.13; 1.L.74); Wx1
Wx31A T2-9c (9S.33; 2S.49); Wx1
Wx31B T2-9b (9L.22; 2S.18); Wx1
Wx31C T2-9d (9L.27; 2L.83); Wx1
Wx32A T3-9(8447) (9L.14; 3S.44); Wx1
Wx32B T3-9(8562) (9L.22; 3L.65); Wx1
Wx32C T3-9c (9L.12; 3L.09); Wx1
Wx33A T4-9e (9L.26; 4S.53); Wx1
Wx33B T4-9(5657) (9S.25; 4L.33); Wx1
Wx33C T4-9g (9L.27; 4S.27); Wx1
Wx34A T5-9c (9L.10; 5S.07); Wx1
Wx34B T5-9(4817) (9S.07; 5L.06); Wx1
Wx34C T4-9b (9L.29; 4L.90); Wx1
Wx35A T5-9(8386) (9S.13; 5L.87); Wx1
Wx35B T5-9a (9S.17; 5L.69); Wx1
Wx35C T5-9d (9L.10; 5L.14); Wx1
Wx36A T6-9(4778) (9L.30; 6S.80); Wx1
Wx37A T6-9(8768) (9S.61; 6L.89); Wx1
Wx37B T7-9(4363) (9ctr.00; 7ctr.00); Wx1
Wx37C T6-9(4505) (9ctr.00; 6L.13); Wx1
Wx38A T7-9a (9S.07; 7L.63); Wx1
Wx38B T8-9d (9S.16; 8L.09); Wx1
Wx38C T8-9(6673) (9S.31; 8L.35); Wx1
Wx39A T9-10(8630) (9S.28; 10L.37); Wx1
Wx39B T9-10b (9S.13; 10S.40); Wx1

PHENOTYPE ONLY

Kernel Mutants

blotched aleurone
Bh*-86-1381-1
Bh-Tu*-Mumm

brittle endosperm
bt*-011-11
bt*-0601-Alexander
bt*-1979-14
bt*-1979-16
bt*-1982
bt*-4380
bt*-4539
bt*-4973
bt*-60-151
bt*-8101
bt*-8102
bt*-83-84-3541-1
bt*-84-4
bt*-84-5
bt*-84-5091-9
bt*-84-5257-1
bt*-84-6
bt*-85-3096-6
bt*-85-3098-15
bt*-85-3099-16
bt*-85-3372-27
bt*-87-2132-39
bt*-87-2297-1
bt*-87-88-2630-28
bt*-88-3177-14
bt*-88-3177-2
bt*-88-3177-7
bt*-8804
bt*-8805
bt*-89-1265-18
bt*-90286
bt*-A4109
bt*-Alexander
bt*-Briggs-1998-1
bt*-F-15
bt*-F-23
bt*-F-31
bt*-F-34
bt*-F-36
bt*-F-8
bt*-F10
bt*-Panzio
bt*-PetersonResHy
bt*-PI200197
bt*-PI251887
bt-gm*-84-5045-39
bt-gm*-85-3017-24
bt-sh*-PI251930

brown endosperm
brn*-1981-1
brn*-1981-2
brn*-1981-3
brn*-1981-4
brn*-84-23
brn-bt*-81-F-24

brown kernel
lt-brn-sm*-86-1302-37

brown pericarp
bp*-PI183639

colored plumule
Pu*-1976-RYDCO

colorless aleurone
cl*-85-86-3559-1
cl*-86-1478-16
cl-crown-pale-base*-85-86-3558-23
r*-86-1590-6

defective kernel
de*-1276
de*-17
De*-1976-RYDCO
de*-2080
de*-2192
de*-2424
de*-2915
de*-2919
de*-3188
de*-4309

de*-5044Hagie
de*-85-86-3567-35
de*-8505
de*-8507
de*-8508
de*-86-1472-6
de*-8808
de*-8809
de*-8810
de*-8811
de*-8818
de-small*-8813
de-small*-8814
de-small*-8815
de-small*-8816
de-small*-8817
def*-8101
def*-8102
def*-8103
def*-8104
def*-8105
def*-8106
def*-8107
def*-8108
def*-8109
def*-8110
def*-8111
def*-8112
def*-8113
def*-8114
def*-8116
def*-8118
def*-8119
def*-8120
def*-8121
def*-8122
def*-8123
def*-8125
def*-8126
def*-8127
def*-8128
def*-8130
def*-8131
def*-8132
def*-8134
def*-8136
def*-8137
def*-8138
def*-8201
def*-84-22
def*-84-28
def*-84-29
def*-84-30
def*-84-31
def*-84-37
def*-84-40
def*-84-41
def*-84-45
def*-84-48
def*-84-49
def*-84-53
def*-84-54
def*-84-58
def*-84-60
dek*-1979-32
dek*-1981-1
dek*-74-0060-4
dek*-84-14
dek*-86-1496-35
dek*-8902
dek*-8903
dek*-8904
dek*-99-6273-1
dek*-F-16
dek*-PS602
wrinkled-de*-86-1473-5
wrinkled-gm*-86-1582-32

discolored kernel
pig*-84-5080-18
pig*-86-1178-6
pig-gm*-1979-51
pig-gm*-1979-52
pig-gm*-1979-9
pig-gm*-1981-A
pig-gm*-1981-B
pig-gm*-1982-3
pig-gm*-5020-14
pig-gm*-84-5078-10
pig-gm*-86-1200-3
pig-gm*-87-2275-15
pig-gm*-87-2305-22
pig-gm*-Briggs 1998-1
pig-gm*-Briggs 1998-2
pig-gm*-PI251930
ptd-dek*-1976-RYDCO
ptd-dek*-1981
ptd-dsc*-87-2490-22
small-pig-gm*-88-89-3554-44

dull endosperm
du*-0203

etched endosperm
et*-3130
et*-3576
et*-5191
et*-6-9321-1
et*-73-766-1
et*-8-M-4
et*-84-5266-26
et*-84-5270-40
et*-85-86-3518-21
et*-86-1493-6
et*-8616
et*-87-2349-13
et*-88-89-3525-22
et*-88-89-3554-33
et*-89-90-1547-19
et*-89-90-1548-13
et*-Mu1767
et*-Mu2349
et-mutable*-87-2519-31
et*-Osturana
et-de*-88-89-3526-8
et-gm*-86-1475-34
et-gm*-86-87-1742-38
et-gm*-87-2502-19
granular-o*-84-5274-30
sm-et*-85-3522-29
su-sh-et*-98-1887-1

flint kernel
flint*-87-2126-22

floury endosperm
fl*-67-412
fl*-83-3386-19
fl*-84-44
fl*-8515
fl*-Mojo
fl*-N7B-65-1294
fl*-shoepeg
fl*-sucaxo
fl-cap*-1981
fl-cap*-66-519-1
fl-de*-8905
small-fl-cap*-1981

germless
brn-gm*-85-3315-6
brn-gm*-85-86-3587-46
brn-gm*-85-86-3595-3
brn-gm*-86-1161-5
emb*-85-3100-32
emb*-85-3378-8
gm*-1387
gm*-1979-11
gm*-1979-53
gm*-5234
gm*-6372
gm*-8510
gm*-86-1011-2
gm*-86-1013-4
gm*-86-1097-3
gm*-86-1335-1
gm*-86-1591-7
gm*-86-87-1742-18
gm*-87-2456-9
o-gm*-84-44
o-gm*-98-5733-1
pr-gm*-86-1109-1
sh-gm*-84-5045-32
sh-gm*-88-3082-4
sm-o-gm*-86-1323-4
small-dsc-gm*-95W-240
w-o-gm*-85-3135-4
w-o-gm*-86-1349-1
w-o-gm*-88-3270-10
y-gm*-85-3288-28

glassy endosperm
ae*-1979-1
ae*-1979-7
ae*-1981-MuT
ae*-84-7
ae*-92-1365-3
ae*-96-1449-1
ae*-Briggs 1998-1
ae*-Mu32

lemon white
lw*-1979-45
lw*-1979-46
lw*-1981
lw*-1998-1
lw*-1998-2
lw*-1998-4
lw*-73-2548
lw*-82-1
lw*-85-3076-28
lw*-85-3252-5
lw*-8509
lw*-8513
lw*-8514
lw*-89-90-3609-5
lw*-PI200203
pale-y*-83-84-3549-13
pale-y*-84-5082-33
pale-y*-84-5167-48
pale-y*-84-5288-19
pale-y*-85-3005-22
pale-y*-85-3006-30
pale-y*-85-3007-40
pale-y*-85-3010-40
pale-y*-85-3016-15
pale-y*-85-3017-31
pale-y*-85-3065-25
pale-y*-85-3069-6
pale-y*-85-3087-29
pale-y*-88-89-3551-35
pale-y*-89-1313-3
pale-y*-89-90-1525-23
pale-y*-90-3220-1
pale-y*-90-3220-26

marbled aleurone
Dap*-3
dap*-86-8126-2
Dap*-89-3177.0
Dap*-89-3177.5
Dap*-89-3178.3
Marbled*-Sprague

miniature kernel
mn*-1981-51
mn*-87-2215-17
mn*-87-2346-20
mn*-87-2347-36
mn*-87-2422-14
mn*-88-2177-2
mn*-88-89-3509-40
mn*-88-89-3564-25
mn*-PI239110
mn*-PI245132
small-k*-97-4784-1

mottled aleurone
Mt*-2313
Mt*-65-2238
Mt*-Sprague

multiple aleurone layer
Mal*-Galinat
Mal*-Nelson
Mal*-PI515052

opaque endosperm
lg-o-crown*-89-1275-17
o*-1979-54
o*-1981-11-Fox-19
o*-1981-3-Fox-7
o*-1981-5-Fox-9
o*-1981-6-Fox-10
o*-1981-8-Fox-15
o*-1982
o*-1982-2-Fox-13
o*-2-Fox-6
o*-3015
o*-73-798-1
o*-76GH-76
o*-8129
o*-82:288-1
o*-83-84-3549-39
o*-84-5025-15
o*-84-5025-17
o*-84-5025-8
o*-84-5044-35
o*-84-5091-13
o*-84-5094-4
o*-84-5095-23
o*-84-5117-16
o*-84-5261-37
o*-84-5270-40
o*-84-5282-27
o*-84-5295-13
o*-84-5321-28
o*-84-5324-29
o*-84-8a
o*-85-3084-8
o*-85-3088-3
o*-85-3335-35
o*-86-87-1767-10
o*-87-2285-33
o*-87-2350-2
o*-88-89-3550-27
o*-97-4784-6
o*-Briggs-1998-1
o*-BS20-Fox-3
o-de*-1981-9-Fox-18
o-dek*-87-2279-12
o*-Fox-12
o*-PI195245
o*-PI200285
o-dek*-6
o-gm*-83-3398-6
o-gm*-84-33
o-sh*-86-1297-2
o-sh*-F1979-19
os*-2162
pro*-Mu1
sh-o*-87-2455-7
small-o*-87-88-2692-5
small-o*-PI195243

pale aleurone
pale-Cl*-86-1476-14
pale-Cl*-LGC65
pale-Cl-gm*-84-5251-1

pale aleurone, with pigmented sectors
pa-Cl*-m-86-1474-39
pa-Cl*-m-86-1478-4
pa-Cl*-m-87-2224-33

pale crown
pa-crown*-85-86-3558-23

pale yellow endosperm
al*-84-5020-32
pale-endo*-73-3
pale-endo*-73-4004

pale-y*-83-3382-16
pale-y*-83-3382-18
pale-y*-83-84-3548-25
pale-y*-84-5027-22
pale-y*-84-5103-16
pale-y*-84-5275-14
pale-y*-85-3016-30
pale-y*-85-3036-38
pale-y*-85-3042-7
pale-y*-85-3044-34
pale-y*-85-3134-46
pale-y*-85-3374-13
pale-y*-85-3377-2
pale-y*-85-3511-18
pale-y*-85-3562-31
pale-y*-85-86-3533-9
pale-y*-86-1151-7
pale-y*-86-1155-2
pale-y*-86-1155-3
pale-y*-86-87-1723-27
pale-y*-87-2160-16
pale-y*-87-2339-10
pale-y*-87-2350-2
pale-y*-87-2350-25
pale-Y*-87-2422-14
pale-y*-87-88-2679-1
pale-y*-88-2177-2
pale-y-gm*-Rsssc-77-110
pale-y-o*-84-5288-2
pale-y-o*-86-1296-27
small-y*-95-1930-2
y*-84-5272-12
y*-84-5288-1
y*-85-3041-2
y*-85-3078-41
y*-85-3087-12
y*-85-3125-7

purple pericarp
PI*-CFS-69

red pericarp
r*-ch-Burbank-CFS-80
r*-ch-PI213730

red silk scar
red-silk-scar*-MTC

shrunken kernel
pale-y-su-sh*-88-3133-28
sh*-1979-10
sh*-1982-2
sh*-2927-Mumm
sh*-2928-Mumm
sh*-83-3328-24
sh*-84-3
sh*-84-5248-20
sh*-84-5317-44
sh*-85-3045-7
sh*-85-3104-27
sh*-85-3112-20
sh*-85-3375-38
sh*-8502
sh*-8503
sh*-8506
sh*-8511
sh*-8517
sh*-86-1565-17
sh*-87-2045-25
sh*-87-2045-6
sh*-87-2050-1
sh*-87-2050-3
sh*-87-2213-19
sh*-87-2215-12
sh*-87-2355-29
sh*-87-2406-3
sh*-87-2496-21
sh*-88-89-3540-1
sh*-8806
sh*-8807
sh*-8906
sh*-8907
sh*-97P-29-5
sh*-Alexo1968
sh*-F-11
sh*-F-2
sh*-F-25
sh*-KERR
sh*-RJL
sh-bt*-85-3392-31
sh-crown*-Briggs-1998-1
sh-de*-6607
sh-de*-RSSSC-117
sh-fl*-9180
sh-fl*-9392
sh-o*-87-2410-24
sh-wx*-F-18
su-sh*-F-5

spotted aleurone
cl-mut*-85-86-3564-1
cl-mut*-99-2170
coarse-mutable*-86-1417-7

sugary kernel
su*-1979-5
su*-1979-8
su*-1981
su*-1982
su*-83-3383-21
su*-83-3383-4
su*-84-5167-6
su*-84-5267-18
su*-84-5350-2
su*-84-5350-31
su*-85-3113-11
su*-85-3133-32
su*-85-3217-10
su*-85-3436-29
su*-8501
su*-8504
su*-87-2046-27
su*-87-2279-12
su*-8801
su*-8802
su*-8803
su*-89-1279-14
su*-8908
su*-F-37
su*-MOEWS
su*-PI193430
su*-PI228183
su-sh*-Briggs-1998-1
su-sh*-F-22

viviparous kernel
pale-vp*-87-2286-1
pale-vp*-87-2286-18
pale-vp*-87-2286-2
pale-vp*-87-2286-25
pale-vp*-87-2286-3
pale-y*-84-5032-21
pale-y-vp*-83-3100-31
pale-y-vp*-83-3124-33
pale-y-vp*-84-5266-5
pale-y-vp*-85-3140-15
pale-y-vp*-85-3240-5
pale-y-vp*-85-3267-6
pale-y-vp*-85-3267-9
pale-y-vp*-85-3385-34
pale-y-vp*-86-1316-27
pale-y-vp*-88-3177-14
ps*-85-3288-28
ps*-85-3492-36
ps*-85-86-3567-1
ps*-86-1105-2
ps*-86-1352-4
ps*-86-1499-3
ps*-86-87-1742-18
ps*-89-90-1588-37
ps*-90-3222-27
ps*-90-91-8549-7
ps*-96-5032-6
ps*-98-5691-5
ps*-99-2157-1
ps*-Mu85-3061-21
ps*-Mu86-1105-1
vp(ps)*-86-1449-3
vp(ps)*-86-1565-17
vp*-0118
vp*-0315
vp*-2-8c
vp*-71-1367
vp*-73-30173
vp*-8101
vp*-8104
vp*-8106
vp*-8107
vp*-8108
vp*-8109
vp*-8110
vp*-8111
vp*-8112
vp*-8113
vp*-8114
vp*-8115
vp*-8116
vp*-8117
vp*-8201
vp*-8203
vp*-8204
vp*-8208
vp*-8209
vp*-8210
vp*-8211
vp*-84-5079-29
vp*-84-5279-29
vp*-84-5315-29
vp*-8418
vp*-8420
vp*-85-3011-11
vp*-85-3017-9
vp*-85-3040-29
vp*-85-3042-7
vp*-85-3099-16
vp*-85-3135-4
vp*-85-3182-6
vp*-85-3250-1
vp*-85-3339-25
vp*-85-3422-13
vp*-85-86-3567-20
vp*-86-1109-1
vp*-86-1407-15
vp*-86-1573-27
vp*-87-2146-18
vp*-87-2213-19
vp*-87-2224-3
vp*-87-2274-37
vp*-87-2299-1
vp*-87-2339-1
vp*-88-89-3555-1
vp*-88-89-8625-5
vp*-89-1181-8
vp*-89-1279-14
vp*-89-90-1561-18
vp*-92-1401-8
vp*-93-1017-2
vp*-95-2086-1
vp*-PI183642
vp*-PI185847
vp*-PI200204
vp*-PI254854
vp*-PI430482
vp-de*-87-2406-23
vp-dek*-99-2197-1
vp-Y*-86-1267-31
vp-Y*-86-1361-7
w-vp*-84-5020-4
w-vp*-85-3014-6
w-vp*-85-3304-13
w-vp*-91-1859-8
w-vp*-91-2544-7
w-vp*-92-1408-1
y-vp*-0730
y-vp*-1982-1
y-vp*-1982-2
y-vp*-2062-Coop
y-vp*-60-153
y-vp*-65-792
y-vp*-6961
y-vp*-73-2656
y-vp*-80-6118
y-vp*-8102
y-vp*-8103
y-vp*-8105
y-vp*-8206
y-vp*-8207
y-vp*-83-1A
y-vp*-83-3101-36
y-vp*-8336
y-vp*-84-13
y-vp*-8419
y-vp*-85-3572-30
y-vp*-8512
Y-vp*-87-2339-10
y-vp*-87-2340-36
y-vp*-8701
y-vp*-88-89-3563-33
y-vp*-88-89-3613-25
y-vp*-Alexho68-195

waxy endosperm
wx*-0208
wx*-98-1406-6

white cap kernel
Wc*-1982-1
Wc*-DC

white endosperm
y*-1981
y*-1982-3
y*-73-2
y*-73-2262-1
y*-73-2262-2
y*-73-2394
y*-73-324-1
y*-73-4035
y*-73-426
y*-84-8b
y*-87-2201-3
y*-syn-DOCI
y*-Williams-60-154

Seedling Mutants

adherent leaf
ad*-87-2285-18

albino seedling
nlw*-85-3357-17
peach-albino-mutable*-87-2209-30
w*-002-12
w*-005-19
w*-009-6
w*-010-4
w*-011-11
w*-017-14-A
w*-017-14-B
w*-034-16
w*-037-14
w*-039-15
w*-2065
w*-2246
w*-3858
w*-4670
w*-4873
w*-5201
w*-5255
w*-5267
w*-5602
w*-5863
w*-6293
w*-6504
w*-6575
w*-7165
w*-7219
w*-7281
w*-78-297-3
w*-8105W
w*-8129

w*-8147
w*-8201
w*-84-5205-46
w*-84-5222-30
w*-85-3359-11
w*-85-3552-25
w*-85-3559-30
w*-8549
w*-8569
w*-86-1078-6
w*-86-1265-30
w*-86-2222-5
w*-8630
w*-8635
w*-8637
w*-8670
w*-87-2215-8
w*-8963
w*-8977
w*-8992
w*-9235
w*-B-75
w*-Canario Hembrilla Enano
w*-MontenegrinFlint
w*-PI184276
w*-PI201543
w*-PI228176
w*-PI228179
w*-PI232965
w*-PI232968
w*-PI232972
w*-PI239103
w*-PI239110
w*-PI251009
w*-PI251885
w*-PI251930
w*-PI251932
w*-PI254851
w*-PI267162
w*-PI267179
w*-PI267204
w*-Singleton-16
w*-Singleton-24
w*-Singleton-25
w*-Singleton-31
w*-Tama
wh*-053-4
wh*-2083
wh*-89-578-6
wh*-BMS-Rhoades

clasping leaf
clsp*-87-2320-9
clsp*-88-89-3522-1

glossy leaf
gl*-218-1
gl*-32TaiTaiTaSarga
gl*-4339
gl*-5249
gl*-56-3023-6
gl*-56-3023-9
gl*-56-3036-7
gl*-6
gl*-60-2484-8
gl*-63-2440-8
gl*-85-3095-12
gl*-8654
gl*-87-2215-8
gl*-87-2215-30
gl*-88-3142-4
gl*-97P-261-5
gl*-Bizika
gl*-gl12
gl*-LGC-117
gl*-LGC-27
gl*-Manglesdorf
gl*-Moritsa
gl*-PI184286
gl*-PI200203
gl*-PI228177
gl*-PI239101
gl*-PI239110
gl*-PI251885
gl*-PI251933
gl*-PI262474
gl*-PI262476
gl*-PI262494
gl*-PI262500
gl*-PI267203
gl*-PI267209
gl*-PI267212

high chlorophyll fluorescence
hcf*-88-3005-3

luteus yellow seedling
l*-009-6
l*-017-3
l*-025-4
l*-062-3
l*-2215
l*-2673
l*-4356
l*-4871
l*-549-1 Derived Flint
l*-570-2 Cincantin
l*-5783-straw
l*-6474
l*-6923
l*-6973
l*-7165
l*-73-563
l*-7748
l*-8321
l*-8376
l*-84-5225-33
l*-85-3215-2
l*-85-3225-4
l*-85-3457-40
l*-85-3513-1
l*-85-3541-20
l*-86-1112-1
l*-86-1354-9
l*-88-89-3555-13
l*-89-90-1552-10
l*-d-8694
l*-LGC-43
l*-PI183642
l*-PI183643
l*-PI193433
l*-PI213737
l*-PI218038
l*-PI239110
l*-PI239114
l*-PI251884
l*-PI254854
l*-PI262495
l*-PI267215
l*-PI267226
l*-Tama
l*-y wx 6-9b
y-l*-8910 Briggs
yd*-87-2278-34
yel*-5344
yel*-8721
yel*-8793
yg*-8962

orobanche
oro*-6577
oro*-69-9291-8
oro*-84-5080-15
oro*-85-3087-3
oro*-85-3106-41
oro*-85-3113-11
oro*-88-3237-31
oro*-88-89-3550-32

pale green seedling
pas*-90-3222-13
pg*-2142
pg*-6372
pg*-69-5079-2
pg*-6923
pg*-7122
pg*-8129
pg*-84-5234-29
pg*-8412
pg*-8911
pg*-8959
pg*-Caspar
pg*-PI183648
pg*-PI193424
pg*-PI262473
pg*-PI262495
pg*-PI267162
pg*-PI267215

piebald leaf
pb*-2-7-4400
pb*-87-2442-5

red seedling leaf
red-leaf*-86-1569-7

translucent leaf
trans-leaf*-56-3122-7
trans-leaf*-68F-958
trans-leaf*-79-6533
trans-leaf*-PI228176

tube leaf
fused-leaves*-PI228170
tbl*-6504

virescent seedling
v*-002-17
v*-007-18
v*-022-17
v*-025-4
v*-037-5
v*-1-2(5376)
v*-1-9(5622)
v*-2-9(5257)
v*-388-Sprague
v*-4308
v*-4698
v*-5-10(5355)
v*-5287
v*-5413
v*-5575
v*-56-3012-10
v*-5828
v*-60-151
v*-60-2397-15
v*-65-1433
v*-7230
v*-7281
v*-7312
v*-74-1690-1
v*-74-1873-1
v*-74-1948-1
v*-8070
v*-8129
v*-8201
v*-8339
v*-8522
v*-8654
v*-8743
v*-8806
v*-8957
v*-8958
v*-9026
v*-Funk-84-13
v*-leng
v*-LGC-111
v*-LGC-142
v*-LGC-98
v*-pb-3019-16
v*-PI180165
v*-PI180231
v*-PI183640
v*-PI185851
v*-PI195244
v*-PI195245
v*-PI200197
v*-PI200201
v*-PI218042
v*-PI228174
v*-PI228176
v*-PI236996
v*-PI239105
v*-PI239114
v*-PI239116
v*-PI251883
v*-PI251891
v*-PI251930
v*-PI254856
v*-PI262476
v*-PI262487
v*-PI262489
v*-PI267184
v*-PI267209
v*-PI267212
v*-PI270293
v*-Pollacsek
v*-RumanianFlint
v*-Singleton-22
v*-Singleton-34

white striped seedling
str*-84-5222-7
str*-86-1494-27

yellow green leaf
yg*-0130
yg*-4369
yg*-4484
yg*-4889
yg*-5-8(5575)
yg*-56-3021-18
yg*-6697
yg*-68-1429
yg*-74-1827-1
yg*-77-585
yg*-8105
yg*-8631
yg*-8682
yg*-8946
yg*-910J
yg*-B73
yg*-Caspar
yg*-PI239114
yg*-PI267224
yg*-Singleton -23
yg*-Singleton-30
yg-nec*-95-5320-7
Yg-str*-Mu

zebra striped seedling
zb*-89-3137-5

Plant Mutants

absence of leaf blade
bladeless*-87-2406-23

albescent
al*-1479

barren stalk
ba*-1447
ba*-68-679-8
ba*-74-304-12
ba*-74-369-2
ba*-PI200290
ba*-PI218135
ba*-PI239105
ba*-PI251885
ba-ub*-94-4712

brachytic plant
br*-2180
br*-78-136KEW
br*-OSIJEK-Yugoslavia
br*-PI228171
br*-PI239105
br*-Singleton-8
br*-Singleton1969-252
td*-PI262476

brown midrib
bm*-PI228174
bm*-PI251009
bm*-PI251893
bm*-PI251930
bm*-PI262480
bm*-PI262485
bm*-PI267186

chromosome breaking
Chrom-breaking*-Mu

colored leaf
lc*-PI239110

crinkled leaf
cr*-97P-111
cr*-98-1698

defective tassel
Tp*-54-55-Jos
Tp*-PI213734
Tp*-Pk41-Jos
Tp*-T8-Jos
Tp*-Tenn61

dwarf plant
d*-018-3
d*-119
d*-136-220
d*-1821
d*-2108
d*-2201
d*-2447-8
d*-3-eared-JC
d*-3047
d*-5312
d*-56-3037-23
d*-60-2428
d*-64-4156-1
d*-74-1701-5
d*-75-6071-1
d*-76-1304-9
d*-76-2186
d*-78-282-3
d*-78-286-1
d*-78-286-5
d*-85-3081-33
d*-87-2198-36
d*-gl11
d*-MarovacWhiteDent
d*-PI180231
d*-PI183644
d*-PI184286
d*-PI200303
d*-PI213769
d*-PI228169
d*-PI228171
d*-PI239110
d*-PI245132
d*-PI251652
d*-PI251656
d*-PI251885
d*-PI254854
d*-PI262495
d*-PI267219
d*-rosette
d*-shlf-9-436-1
d*-su
d*-su2
d*-Teo
d*-ts1

green striped leaf
gs*-98-5700-5

liguleless
lg*-56-3037-5
Lg*-64-36
lg*-PI228170
lg*-podcorn

male sterile
ms*-6015
ms*-6025
ms*-6026
ms*-6028
ms*-6033
ms*-6036
ms*-6039
ms*-6040
ms*-6045
ms*-6048
ms*-6049
ms*-6052
ms*-6053
ms*-6054
ms*-6055
ms*-6057
ms*-6058
ms*-6059
ms*-6060
ms*-6061
ms*-6062
ms*-6064
ms*-6065
ms*-6066
ms*-PI217219
ms-si*-355

multiple midrib
multiple-midrib*-87-2406-23

narrow leaf
nl*-5688
nl*-PI245132

necrotic leaf
nec*-011-7
nec*-017-3
nec*-4889
nec*-5588 early
nec*-5619
nec*-5876
nec*-77-574-1
nec*-8624
nec*-8737
nec*-fraz
nec*-PI228174
nec*-PI267184
nec*-Vasco
nec-pg*-PI239116

pale green plant
pg*-56-3012-10
pg*-8321
pg*-Hy2 Nob 7-5
pg*-LGC-61

ramosa
ra*-412E
ra*-4889
ra*-63-3359
ra*-D
ra*-PI184279
ra*-PI239103
ra*-PI267181
ra*-PI267184

tassel seed
ts*-0174
ts*-69-Alex-MO17
ts*-Anderson
ts*-PI200203
ts*-PI251881
ts*-PI267209
ts*-Sprague

tasselless
tls*-Funk
tls*-Va35

yellow stripe leaf
ys*-1479
ys*-5-8(5575)
ys*-67-2403
ys*-8912
ys*-PI-262172
ys*-PI-262475
ys*-PI228180
ys*-whorled

Ear Mutants

distichous ear
distichous*-68-1227

distorted segregation
off-ratio*-85-3255-6
off-ratio*-86-1155-1
wx-off-ratio*-86-1110-4

polytypic ear
pt*-McClintock
pt*-Mu

reduced pollen fertility
ga*-0188
ga*-0213
ga*-3615
ga*-94-764
Ga*-Yugoslavia

silky
si*-0443
si*-0503
si*-0648
si*-8104

tunicate
Tu*-5090B

unpaired rows
up*-Shirer

V. MAIZE GENOME DATABASE

MaizeDB-- www.agron.missouri.edu

New Data

In brief. MaizeDB has added over 84,000 new records this year, many with links to external databases. Of the new records, 50% are 'Probes', 16% references, 14% Gel Patterns and 7% Variations . Images of over 1000 agarose screening blots for SSR and RFLP markers are now available. MNL issues are now retroactive to 1988, and accessible to full-text searching as are abstracts for the Annual Maize Genetics Conference, retrospective to 1991. See also the Stock Center report; new stocks are entered into MaizeDB , largely by the staff at the Stock Center.

Data Sources

Data for SSR have been imported from the Missouri Maize Project, the Acemaz database , Pioneer, and Keith Edwards (UK). Clone data for ESTs are imported from dbEST, GenBank, and ZmDB. When SSR are found and mapped in an EST, the link from the SSR primer pair and map information is made to the EST clone, which also inherits the map information. In all cases, acknowledgment of sources, complete with links to the external database source, is entered into each record.

External Database Links

New databases added this year include ZmDB (www.zmdb.iastate.edu) and TIGR(www.tigr.org). EMBL records are now linked to MaizeDB. We continue to regularly update links to dbEST, dbSTS, GenBank; SwissProt; and Medline/PubMed. Some 12% of the non-MNL references have links to PubMed; PubMed provides abstracts, related articles and sequences. GRIN links for genetic stocks are kindly provided by Marty Sachs at the Stock Center. MaizeDB also links to the Plant Genome Database suite at Cornell for comparative map data in GrainGenes and RiceGenes. In response to community requests for files of mapped cDNA sequences, with map coordinates, a utility that creates files of map coordinates and/or accessions for retrieval from GenBank by formats provided at NCBI, has been installed on the database EST page.

Literature Annotation

The past 2-3 years MaizeDB has not had resources to keep up with the flow of literature; this year some additional funding to MaizeDB from the USDA-ARS is being used to enhance this effort. Literature citations are selected and entered automatically, with links to authors. Of 33,837 references, 9,614 are MNL or Maize Meeting Abstracts. Many are currently linked to database objects, such as loci, map data, PCR primers and agronomic traits.

Navigation aids

•A side-bar directory that is inherited on the main pages for the database. It is organized into 4 sections: (1) general information, (2) access styles and (3) category-specific access (Locus, Stocks, Trait, Person, etc.) listings, followed by (4) links to other resources.

•Category-specific navigation helps are available , linked to the sidebar categories and maintained by a staff member most expert in that area and listed on the page for contact . When multiple staff members are involved, users are encouraged to contact the staff, at db_request@teosinte.agron.missouri.edu.

•Enhanced 'table of contents' , called 'Browser' in the side-bar. Alphabetical lists of the individual items, organized by category, Locus, Probes, Stocks, Images, Persons, and References may be explored and are dynamically created from the underlying database tables. Many of the lists permit restriction of listings to items based on map location and, where applicable , provide information about map and sequence accessions. Lists are created dynamically . The lists may be saved to any desktop, using the browser utility to save as 'source' or 'html' and then imported into standard software, such as an Excel workbook, all-the-while maintaining hypertext links .

•Integrated EST and SSR compilations, with links to sequence and map data. These are accessible from the sidebar list of categories.

Works in Progress

The work described below is largely supported by NSF award # 9872655.

Interactive Maize Plant - 'a feel for the organism'

An interactive set of images of the maize plant, complete with glossary of terms for anatomical features, is being developed for different stages of the maize plant, complete with links to underlying MaizeDB records. One goal is to provide access to the underlying maize genome to any person interested in maize, including elementary-high school students or teachers, undergraduates and researchers in various disciplines. The sort of access to be provided may be viewed at Flybase, flybase.bio.indiana.edu, a Drosophila genome database that integrates community information similarly to MaizeDB. The glossary will be harmonize d with trait and phenotype terminology in the germplasm, and also in other related species, in particular the grasses.

Custom Table-Making

A copy of the database has been placed under OPM (Object Protocol Management), which provides a graphical display of data relationships, custom query access, as well as defaults. Access to the work-in-progress is provided on the 'about MaizeDB' page, accessible from the homepage sidebar. In progress, access to a glossary of all the fields in the database. The software is proprietary, and licensed to MaizeDB, free of charge, from Data Logic, www.genelogic.com. We thank Dr. I-Min Chen and her colleagues for providing support in this work.

Interaction with cereal genome databases

OPM (see above section) also permits interactive database query interfaces across platforms, such as Oracle and Sybase, and at different locations. This is distinct from the record-to-record linking typically employed. We are testing a prototype rice genes dataset in collaboration with our colleagues in Tsukuba , Japan , using distinct schema and database management softwares (Oracle, Sybase). We are in contact with the USDA-ARS database suite at Cornell regarding this utility for providing distributed queries across different databases. The OPM software also supports interactions with applications, such as BLAST.

Graphical Representation

Currently, maps are viewable by ACEDB software at the Cornell site and by postscript files, stored at MaizeDB . Viewers, such as that developed for the Japan Rice Genome Program , and also facile custom-map displays are works in progress.

User Statistics

Access to the database has approximately doubled over the past year, from 25,000-30,000 visitors/month to over 60,000. Some 17% of resolvable hits is US educational, 56 % US commercial or network. Data categories most accessed include References, Locus, Person, Maps, Variations, Stock, Probes, GelPatterns, Gene Products, Images; these, however, account for only 22% of hits. Other hits are to various entry pages; a major referral page is the Maize Genetics Stock Center, Urbana.

Mary Polacco, Curator MaizeDB

SYMBOL INDEX

Silencing
(or,
Anti-sense,
or,
Epistasis)

To every gene action
there is an evil and obstinate
gene action

Coe-suppressor

AUTHOR INDEX

(* identifies articles authored in this Newsletter)

Bees' wings swarm when harvest ends

By CATHY SALTER

There is a swarm of activity across the Corn Belt in these early weeks of autumn. Farmers across the region are busy harvesting 40 percent of the world's annual corn crop. Vigorous hybrids developed in the 20th century by American agronomists have increased yields while acreage planted has declined. Across the four-month growing season, armies of corn stalks supported by deeply dug in roots grow tall and straight, each stalk bearing a single ear of corn — the principal food plant of the Western world.

Hartsburg is my little window on the Corn Belt. This is farmland that breathes — its patterns a reflection of the planting seasons. Farmers who grow up on the land breathe in sync with its rhythms. By autumn, soybeans planted after the June wheat harvest turn golden and hundreds of acres of corn turn the color of ancient parchment. Such abundance makes the land feel closed in. When the farmers sense this, crops are harvested and the land breathes again.

The sweet corn I knew before moving to the heartland was a hybrid variety harvested when the meat of the kernel is in the sugar stage and the ears are wrapped in an unripened green husk. The ears of local field corn are twice as big. Their hard, dented, long-toothed kernels are a rich gold, the color sweet corn achieves only after being boiled. Husks ripen, too, turning brittle and brown while farmers wait for the corn to reach the right moment for harvesting.

Like the carefully planted fields they come from, corn kernels grow in long, straight rows. They are living fruit — over a thousand plant embryos on a single ear of corn, each in a state of arrested development. Dried to the right moisture level by blowers in the grain bin and cooled for long-term storage, the kernels maintain their nutritional food value. Harvesting corn is anything but routine work.

Recently, I drove to the Hartsburg bottoms to witness the corn harvest. At the Beckmeyer grain bins, Glen was dumping a load of corn driven up from their fields into an auger hole where the corn began its journey to the top of a grain bin. While the grain spilled forth from the truck bed, Glen opened the dryer bin for me to climb in. Inside, I stretched out on a sea of gold and looked up at the sky through the auger hole 40 feet above me. I was a kernel of corn — a tiny spot dwarfed by the scale of the ocean I had fallen into.

When the grain truck headed back to the fields for another load of corn, I rode along. Before long, Glen's brother Orion circled by in the family's old International combine and signaled for me to climb into the cab. As the combine began to move and the rotary below the cab floor began spinning, we looked down six rows of stalks as high as the cab itself.

As Orion guided the rocket-like picker headers between the rows of corn, dry stalks snapped down and disappeared under the cab. Ears fell onto a center-feed auger and soon had their cobs stripped clean. Finally, kernels of corn were spit up into the combine's grain bin as pieces of pink cob and chaff spewed out through the combine's monstrous teeth.

For the final two passes, I rode on top of the combine's cab. All around me, the golden fields were washed by the pink and lavender hues that follow the western sun as it slipped down into the Missouri River. After filling the combine's bin, we transferred some of the corn into a grain chariot and filled the grain truck for its final run up the hill for the day.

It was dark when Orion began to transfer the final load of corn. As we stood near the auger hole, the moon came up full. In its light, a swarm of bees appeared to have emerged from the grain as it spilled into the auger hole. A thousand tiny pieces of pink chaff hung in the night. "They're called bees' wings," Orion said. "One flake on each side of the corn kernel keeps it nested on the cob."

The evening ended at Barbara and Orion's farm where we shared a late meal. From their table, we looked out at the moon hanging like a lamp over the fat grain bins just across the road. I went home that night pleasantly tired, conscious of how farmers must feel at the end of a long day in the fields. Later, when I brushed my hair, pink bees' wings flew out, encircling my head — traces of a sweet corn harvest and friends I will treasure for seasons to come.

Cathy Salter is a geographer and columnist who lives with her husband, Kit, near Hartsburg at a place they call Breakfast Creek.

By permission of the author (originally appeared in the Boone County Journal, October 16, 1996).

March, 2000

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This newsletter shares current research on genetics, cytogenetics, molecular biology, and genomics of maize. Information is shared by Cooperators with the understanding that it will not be used in publications without their specific consent.

Send your notes for the 2001 Maize Genetics Cooperation Newsletter now, anytime before January 1. Your MNL Notes will go on the Web verbatim promptly, and will be prepared for printing in the annual issue. Be concise, not formal, but include specific data, tables, observations and methods. Check MaizeDB for the most current information on submission of notes. Send your notes as attachments or as the text of an email addressed to coee@missouri.edu (we will acknowledge receipt, and will contact you further if necessary). You may also send by FTP(see http://www.agron.missouri.edu/mnl/MNL74call.html), and alert us with an email. If email is not feasible, please mail a double-spaced, letter-quality copy of your note, preferably with a disk containing the electronic version. Please follow the simple style used in this issue (city /institution title /--authors; tab paragraphs; give citations with authors' initials --e.g., Maizer, BA et al., J Hered 35:35, 1995, or supply a bibliography). Figures, charts and tables should be compact and camera-ready, and supplied in electronic form (jpg or gif) if possible. To separate columns in tables, please tab instead of using spaces, to ensure quality tabulations on the web. Your MNL Notes will go on the Web verbatim promptly, and will be prepared for printing in the annual issue. Mailing address:

Ed Coe
202 Curtis Hall
University of Missouri
Columbia, MO 65211-7020

SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

MNL 63ff. on line	MaizeDB - http://www.agron.missouri.edu
Author and Name Indexes (and see **MaizeDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeDB**)	
Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue and MaizeDB
Rules of Nomenclature (1995)	MNL69:182 and **MaizeDB**
Cytogenetic Working Maps	MNL 52:129-145; 59:159; 60:149 and **MaizeDB**
Gene List	MNL69:191; 70:99 and **MaizeDB**
Clone List	MNL 65:106; 65:145; 69:232 and **MaizeDB**
Working Linkage Maps	MNL69:191; 70:118; 72:118 and **MaizeDB**
Plastid Genetic Map	MNL 69:268 and **MaizeDB**
Mitochondrial Genetic Maps	MNL 70:133 and **MaizeDB**

Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you report in this Newsletter or in publications, and stocks of new combinations, to the collection.

(2) Inform the Stock Center on your experience with materials received from the collection.

(3) Acknowledge the source, and advice or help you received, when you publish.

MaizeDB needs Cooperators (this means you) to:

(1) Look up "your favorite gene or expression" in **MaizeDB** (see section V in this Newsletter) and send refinements and updates to polaccom@missouri.edu, coee@missouri.edu, or db_request@teosinte.agron.missouri.edu.

(2) Compile and provide mapping data in full, including the ordered array of map scores for molecular markers or counts by phenotypic classes; recombination percentage and standard error.

(3) Provide probe or primer information per http://www.agron.missouri.edu/cgi-bin/sybgw_mdb/mdb3/Probe/query; fingerprint data and fragment sizes are significantly useful to colleagues.

May you find a Unique corn in MM!