MAIZE GENETICS COOPERATION

NEWSLETTER

71

April 15, 1997

The data presented here are not to be used in publications without the consent of the authors.

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

DONORS TO THE ENDOWMENT FUND FOR THE NEWSLETTER

Hectare Patron (\$10,000)
PIONEER HI-BRED INTERNATIONAL, INC. (Donald Duvick)

Nursery Patron (\$5,000)
CIBA-GEIGY SEEDS; FUNK SEEDS INTERNAT.; CIBA-GEIGY AGR. BIOTECH. RES. UNIT

Whole Plant Endowment (\$1,000)

AGRIGENETICS CORPORATION

BIOSEM - LIMAGRAIN GROUPE

CARGILL HYBRID SEED

CUSTOM FARM SEED RESEARCH (Virgil L. Fergason)

DEKALB GENETICS CORPORATION
DNA PLANT TECHNOLOGY CORP.
E. I. DU PONT DE NEMOURS & CO.

ICI SEEDS (Keith Edwards; Cliff Hunter)

David C. Jewell

MAX-PLANCK-INSTITUT f. ZUCHTUNGSFORSCHUNG (ERWIN-BAUR-INSTITUT) Abt. Saedler Barbara McClintock

NORTHRUP KING COMPANY
PIONEER HI-BRED INTERNATIONAL, INC. (Marc C. Albertsen)

SANDOZ CROP PROTECTION CORP. SEMENTES AGROCERES S. A., BRASIL

A. Forrest Troyer UNITED AGRISEEDS INC.

Kernel Endowment (\$150)
(Please see also the donors listed in MNL 63-70)

Byung-Dong Kim Andrew S. Wang

| I. FOREWORD | 1 |
|--|-----|
| II. REPORTS FROM COOPERATORS | 2 |
| ALDANIY OALIFODNIA AND DEDIVELEY OALIFODNIA | |
| ALBANY, CALIFORNIA AND BERKELEY, CALIFORNIA A gene required for floral and inflorescence meristem determinacy Laudencia-Chingcuanco, D, Hake, S | 0 |
| A gene required for floral and inflorescence mension determinacyLaudencia-Chingcuanco, D, Hake, S | 2 |
| Surfing for mutants: crinkly4 alleles in the Neuffer collectionBecraft, PW | 2 |
| Transposon (Ac)-induced homologous recombination at maize P locus and in transgenic ArabidopsisXiao, Y, Peterson, T | 3 |
| BEIJING, CHINA | |
| Ovary manipulation: transformation and in vitro maturationDing, Q, Xie, Y, Dai, J, Mi, J, Kuo, Z | 3 |
| Analysis for biochemical markers of multiplasmic lines in maize (Zea mays L.)Liu, X, Zeng, M, Liu, Y, Yang, T | 5 |
| Isozymic analysis of Chinese waxy maize (Zea mays sinensis)Zeng, M, Yang, T, Liu, Y, Li, S | 7 |
| BEIJING, CHINA Construction of genic male sterile system marked by yellow-green seedlingsZhou, H, Li, J, Deng, Y, Sun, R, Jiang, W | Ω |
| Study on a new male sterile gene and apoptosisZhou, H, Li, J | |
| BELGRADE, YUGOSLAVIA | |
| Complementary genes Rf4, Rf5 and Rf6 are not the unique genetic system for fertility restoration in cmsC of maize (Zea mays L.) | |
| Vidakovic, M, Vancetovic, J, Vidakovic, M | 10 |
| BERGAMO, ITALY | |
| Analysis of nitrogen partitioning in maizeBalconi, C, Bosio, D, Motto, M | |
| Do pedicel and placento-chalazal tissues play a role in the amino acid supply to the developing endosperm of IHP and ILP maize strains? | |
| Balconi, C, Bosio, D, Motto, M | 12 |
| Genetic and molecular analysis of the <i>gl3-m3</i> allele of maizeDonini, G, Maddaloni, M, Salamini, F, Motto, M | |
| The methylation pattern of the Opaque-2 promoter in maize endosperm and leafRossi, V, Motto, M, Pellegrini, L | |
| Effects of DNA methylation on DNA binding and transcriptional activity of the Opaque2 proteinRossi, V, Solinas, G, Motto, M, | 576 |
| Pellegrini, L | 14 |
| BOLOGNA, ITALY Magning O'll a far leaf absolute gold concentration in a major population. Landi B. Sagguinett MC. Salvi S. Tuberasa B. | |
| Mapping QTLs for leaf abscisic acid concentration in a maize populationLandi, P, Sanguineti, MC, Salvi, S, Tuberosa, R, Casarini, E, Conti, S | 15 |
| Molecular marker analysis of maize populations divergently selected for abscisic acid concentration in the leafSalvi, S, Tuberosa, R | |
| Sanguineti, MC, Lándi, P, Conti, S | 15 |
| BUFFALO, NEW YORK | |
| Two-photon fluorescence confocal microscopy of maize tissuePan, SJ, Shih, A, Liou, WS, Cheng, PC | 16 |
| BUFFALO, NEW YORK AND IOWA CITY, IOWA | |
| Visualize the three-dimensional arrangement of vascular bundles using cone-beam optical tomographyPan, SJ, Shih, A, Liou, WS, Park, MS, Wang, G, Cheng, PC | 18 |
| CEDAR FALLS, IOWA AND WOODWARD, OKLAHOMA AND BATON ROUGE, LOUISIANA | 10 |
| An SEM study of inflorescence development in <i>Tripsacum dactyloides</i> Orr, A, Kaparthi, R, Dewald, C, Sundberg, M | 19 |
| CHESTNUT HILL, MASSACHUSETTS | |
| Continued studies on the genic instability of maize inbred lines derived from anther cultureTing, YC, Nguyen, DQ | 20 |
| More studies on tassel plants of maizeTing, YC, Nguyen, DQ | 20 |
| CULD SPRING HARBOR, NEW YORK | 04 |
| Consequences of the ectopic expression of the Myb-domain protein PRabinowicz, P, Ma, H, Grotewold, E | 21 |
| Utilization of molecular probes to facilitate development of Quality Protein MaizeLin, KR, Bockholt, AJ, Smith, JD | 22 |
| COLORADO SPRINGS, COLORADO | |
| Analysis of a zebra stripe double mutantSurdam, D, Bertrand-Garcia, R | 23 |
| COLUMBIA, MISSOURI | |
| A ring chromosome on 2SNeuffer, MG | 24 |
| Location data for Les*-A7145Neuffer, MG, England, D | 24 |
| Designation of Les21Neuffer, MG, England, D | 24 |
| C1 is expressed at low levels in husksCooper, P, Cone, K | |
| COLUMBIA, MISSOURI AND ATHENS, GEORGIA AND TIFTON, GEORGIA | |
| Metabolic channeling suggested by QTL analysis of silk maysin and apimaysin concentration Byrne, PF, McMullen, MD, Snook, ME, | |
| Musket, TA, Xu, G, Lee, EA, Flint, S, Widstrom, NW, Wiseman, BR, Coe, EH | 26 |
| Major QTL on chromosome 9S affects silk maysin concentration in four populationsByrne, PF, McMullen, MD, Snook, ME, | 0- |
| Musket, TA, Lee, EA, Widstrom, NW, Wiseman, BR, Coe, EH. | 27 |
| COLUMBIA, MISSOURI AND NORWICH, ENGLAND Factors influencing efficiency of genetic mapping projectsWanous, MK, Snape, JW, Gustafson, JP | 25 |
| CORVALUS, OREGON | |
| The maize Tousled-Like Kinase gene familyYoon, E, Rivin, C | 29 |

| DAVIS, CALIFORNIA | |
|--|-----|
| Identity of extra cell-layers produced in the glossy* mutation in maizeKessler, S, Sinha, N | 30 |
| DEFIANCE OHIO | 0.4 |
| Initial conditions during tassel determination control the level of R-allele silencing (paramutation)Mikula, BC, Kappen, T | 31 |
| DHAULAKUAN, INDIA Screening for bacterial stalk rot resistance in cornKalia, V, Basandrai, AK, Singh, A | 22 |
| DNIEPROPETROVSK, UKRAINE | 02 |
| Fluorescence test for selection of high-tryptophan genotypes of maizeFedenko, VS, Struzhko, VS, Tikhomirov, AYe | 32 |
| DNIEPROPETROVSK, UKRAINE | |
| Relationship between cytological and cultural indices of corn anther culture ability Satarova, TN, Chernousova, NM | 33 |
| DULUTH, MINNESOTA | |
| Detect DNA contents in microsporogenesis of maize mutantLiu, Q, Splett, M | 34 |
| DURHAM, NORTH CAROLINA Further evidence for two progenitors in the origin of maizeEubanks, MW | 0.4 |
| Further evidence for two progenitors in the origin of maizeEubanks, MWFREIBURG, GERMANY AND AMES, USA | 34 |
| Isolation of the new recessive mutant Int1 deficient in lateral root developmentHochholdinger, F, Schnable, P, Feix, G | 35 |
| GAINESVILLE, FLORIDA | 00 |
| Corn Rab2 homologsLaughner, BH, Ferl, RJ | 36 |
| IBARAKI, JAPAN | |
| Nitrous oxide (N2O) is effective in chromosome doubling of maize seedlingsKato, A | 36 |
| IOWA CITY, IOWA | |
| Further construction of proximal duplication stocksZheng, Y-Z, Carlson, WR | 37 |
| IRKUTSK, RUSSIA Type I DNA-topoisomerase activity in mitochondria Tarasenko, VI, Konstantinov, YM | 20 |
| Mitochondrial translation under different redox states of the respiratory chainKonstantinov, YM, Subota, IY, Arziev, AS | |
| JINAN, CHINA | |
| Correlation analysis among kernel physical and quality characters in quality protein maize (QPM)Liu, Z | 41 |
| New maize self-lines bred by stock 6 inducing haploid techniqueLiu, Z | 41 |
| KISHINEV, MOLDOVA | 112 |
| Immature embryo culture of waxy maizeKravchenko, OA | |
| Effect of gamma radiation on callus formation and somatic embryogenesis of wax maizeKravchenko, OA, Lysikov, VN, Palii, AF The action of combined gamma-irradiation with stimulating and mutating doses on maize seedsRomanova, IM, Lysikov, VN | 42 |
| Studies on plant genetic variability induced by combining low and high doses of ionizing radiationsRomanova, IM | |
| Location of factors influencing grain yield by means of isoenzyme markers under different year conditions | |
| Chernov, AA, Mihailov, ME | 44 |
| Mutagenic effects of laser radiation and 6-mercaptopurine on maize seedlingsBurilkov, VK, Paschenko, VM, Lysikov, VN | 44 |
| KRASNODAR, RUSSIA The use of gene ig in sterile-counterpart production of corn lines Shatskaya, OA, Zabirova, ER, Shcherbak, VS | 15 |
| LLAVALLOL, ARGENTINA | 45 |
| B chromosomes in native races of maize from Argentina. I. Populational frequency and altitudeChiavarino, AM, Rosato, M, | |
| Cámara Hernández, J, Naranjo, CA, Poggio, L | 45 |
| B chromosomes in native races of maize from Argentina. II. Genome size and altitudeRosato, M, Chiavarino, AM, Naranjo, CA, | |
| Poggio, L | |
| B chromosomes in native races of maize from Argentina. III. Selection of high and low transmission genotypes and meiotic behavior of BsChiavarino, AM, Rosato, M, Rosi, P, Naranjo, CA, Poggio, L | |
| Recalibration of opaque-2 line of maize as a standard to estimate nuclear DNA amountRosato, M, Naranjo, CA, Poggio, L | 49 |
| Relationship among Zea luxurians, Z. diploperennis and Z. perennis (Sect. Luxuriantes) Poggio, L, Confalonieri, V, Comas, C, | |
| Gonzalez,G, Aulicino, M, Naranjo, CA | 49 |
| Waxy maizes from Argentina. I. Potential yield and endosperm protein contentCorcuera, VR, Naranjo, CA | |
| Waxy maizes from Argentina. II. Endosperm protein content of inbredsCorcuera, VR, Naranjo, CA | 51 |
| Description of commercial hybrids and mutant inbreds incorporated into a waxy maize breeding plan in ArgentinaCorcuera, VR, Naranjo, CA | 52 |
| Evaluation of combining ability of inbred maize lines for precocity and yieldAulicino, MB, Naranjo, CA | 53 |
| Preliminary studies to determine the degree of resistance to Ustilago maydis D.C. Corda in wild and cultivated species of the genus Zea | |
| Astiz Gassó, MM, Molina, MC | 54 |
| LOMAS DE ZAMORA, ARGENTINA | |
| Pathogenic behaviour of spontaneus populations of <i>U. maydis</i> (DC) Corda on maize commercial hybridsSandoval, MC | 54 |
| Different behavior of alloplasmic lines of Zea mays under tissue cultureGalian, LR, Hirschhorn, E, Caso, O | 55 |
| LONDON, ONTARIO | |
| Modifications to the Langdale protocol for in situ hybridization of antisense RNAGreyson, RI, Yang, Z, Walden, DB | 55 |
| The induction and localization of mRNA from the 18 kDA HSP genes in metal-ion insulted maize radiclesYang, Z, Walden, DB | 55 |
| Immunogold localization of the 18-kDa hsp in Zea mays L. radiclesDenison, SJ, Smith, RJ, Walden, DB | 56 |
| 18-kDa hsp localization to membraneous whorl structuresDenison, SJ, Smith, RJ, Walden, DB | 5/ |

| A method for preparing metaphase chromosomes from tapetal cells of maizeMaillet, DS, Walden, DB | 57 |
|---|-------|
| The threshold temperature for the thermal shift induction of mRNA from 18 kDA HSP genes in maize radicles Yang, Z, Greyson, RI, | |
| Walden, DB | 58 |
| LONDON, ONTARIO AND BUFFALO, NEW YORK | |
| A non-dehiscent mutant (ndh1)Walden, DB, Cheng, PC | 59 |
| MANHATTAN, KANSAS AND RALEIGH, NORTH CAROLINA | |
| A gene conferring a hypersensitive reaction to the rice bacterial streak pathogen in maizeHulbert, SH, Drake, J, Leach, JE, | |
| Senior, L, Stuber, CW | 60 |
| MEXICO CITY, MEXICO | |
| Development of PCR-based markers to facilitate large-scale screening in molecular maize breedingHu, X, Ribaut, JM, | • |
| González de León, D | 61 |
| SSR fingerprints of CIMMYT and other inbred linesBird, R McK, González de León, D, Hoisington, D. | |
| Designation of SSR morphsBird, RMcK, González de León, D, Hoisington, D | 03 |
| MUENCHEN, GERMANY Isolation and mapping of <i>mus1</i> , a putative maize MutS-homologHorwath, M, Kunze, R | 62 |
| MUENCHEN, GERMANY | 03 |
| Biosynthetic origin of oxygen atoms in DIMBOAGlawischnig, E, Eisenreich, W, Bacher, A, Frey, M, Gierl, A | 64 |
| MUMBAI, INDIA | 04 |
| Diversity of GTP-binding proteins (GPs) in corn and sorghumPanigrahi, KC, Johri, MM | 61 |
| NEW HAVEN, CONNECTICUT | 04 |
| The root and leaf isoforms of NADP-dependent malic enzyme are encoded by distinct genes Tausta, SL, Nelson, T | 65 |
| NEW HAVEN, CONNECTICUT AND COLD SPRING HARBOR, NEW YORK | 03 |
| Leafbladeless1 is allelic to raggedseedling1 and is required for dorsal cell identityTimmermans, M, Schultes, N, Martienssen, R, | |
| Nelson, T | 66 |
| NORMAL, ILLINOIS | 00 |
| Haploids and monosomics are produced by maize plants containing telocentrics for both arms of chromosome 3 and B chromosomes | |
| L'Heureux, TA, Muzumdar, DA, Schneerman, MC, Weber, DF | 66 |
| NORMAL, ILLINOIS AND COLUMBIA, MISSOURI | 10000 |
| Identification of an isochromosome for the long arm of chromosome 7 in maizeMuzumdar, DA, Schneerman, MC, Doyle, GG, | |
| Weber, DF | 67 |
| NORWICH, U.K. | |
| Isolation and characterization of the maize mitochondrial RNA polymeraseYoung, DA, Allen, R, Lonsdale, DM | 67 |
| Evidence that plant mitochondrial transcription requires promoter-specific factorsYoung, DA, Lonsdale, DM | 69 |
| NOTTINGHAM, UNITED KINGDOM | |
| Effect of silver nitrate and culture vessel on growth and quality of Type II callus of the A188 genotypeSouthgate, EM, Power, JB, | |
| Davey, MR | 71 |
| PUSA, BIHAR, INDIA | 0.000 |
| Comparative cytotoxicity of four herbicides on mitosis and meiosis in maizeKumar, M, Kumar, H, Nasar, S | 71 |
| RALEIGH, NORTH CAROLINA AND AMES, IOWA | -20 |
| Polymorphic microsatellites found in cDNA clonesSenior, L, Lee, M | 72 |
| ST. PAUL, MINNESOTA | |
| RFLP mapping of Semi-dwarf1Vladutu, CI, Phillips, RL | 73 |
| SARATOV, RUSSIA | |
| Producing of parthenogenetic forms of maizeTyrnov, VS | /3 |
| Development of seeds with haploid embryo on haploid plants of parthenogenetic lineTyrnov, VS | |
| Cytological manifestation of apomixis in AT-1 plants of cornEnaleeva, NCh, Tyrnov, VS | |
| SENDAI, JAPAN AND JOETSU, JAPAN | 7 3 |
| Visualization of methylated cytosine in maize somatic chromosomesFluminhan, A, Ohmido, N, Fukui, K, Mizugaki, M, Kameya, T | 75 |
| SIMNIC - CRAIOVA, ROMANIA | / 0 |
| The theoretical significance of a mutant in the phylogeny of the species <i>Zea mays</i> LRadu, A, Urechean, V, Naidin, C, Motorga, V | 77 |
| SOFIA, BULGARIA | |
| Seeds with two bilaterally situated embryos and endospermsDankov, T, Kruleva, M, Krapchev, B | 78 |
| Increase the percentage of maternal haploids by effect of herbicide basagran Dankov, T, Kruleva, M, Dimitrov, B, Krapchev, B | 78 |
| STANTON, MINNESOTA AND ST. PAUL, MINNESOTA | |
| Differential expression of chitinase in maize kernelsHanten, J, Kendra, D, Mettler, I, Harlander, S, Fulcher, RG | 79 |
| TOLEDO, OHIO AND COLUMBIA, MISSOURI AND MUNCIE, INDIANA | |
| Localization of autonomous pairing sites in maizeGoldman, SL, Doyle, GG, Blakey, CA | 79 |
| TUCSON, ARIZONA | |
| Allele-specific PCR in maizeNatividad, M, Winkler, RG | 81 |
| URBANA, ILLINOIS | |
| Bn1 status of selected inbred linesStinard, P | 83 |
| g/7 and v17 map to the short arm of chromosome 4Stinard, P | 83 |
| Isolation of a new su3 alleleStinard, P | 83 |

| The COOP's rd3 is allelic to ct2Stinard, P | 83 |
|---|-----|
| Pale aleurone-Brawn (Pa*-Brawn) is allelic to in1Stinard, P | 83 |
| ws1-Coop is allelic to ws1-Pawnee ws2-PawneeStinard, P | 84 |
| Clarification on ws1, ws2, and g1Stinard, P | 84 |
| da1 is on 10LStinard, P | 84 |
| Three-point linkage data for Ig1 gl2 et2 on 2SStinard, P | 84 |
| URBANA, ILLINOIS AND RASTATT, GERMANY | 0 |
| Effect of different carbohydrates in the corn anther culture mediumBeaumont, VH, Miao, SH, Widholm, JM | 0.5 |
| WALTHAM, MASSACHUSETTS | 00 |
| The CO science and the development of trait technology for yield appearance in regime University account discrete the consist. | |
| The O2-CO2 cycle and the development of trait technology for yield enhancement in maize: Umbrella canopy adjacent to the ear sink | 0.5 |
| Galinat, WC | 85 |
| Female spikelet architecture, outer glurne induration and rachilla elongationGalinat, WC | 86 |
| WOOSTER, OHIO AND LONDON, ONTARIO | |
| Localization of shsp mRNA synthesis in maize spikeletsBouchard, RA, Greyson, RI, Walden, DB | 87 |
| WUHAN, CHINA AND WUHAN UNIVERSITY | |
| Physical location of maize (Zea mays L.) cdc2 and prh1 genes by in situ hybridization Ren, N, Song, YC, Bi, XZ, Ding, Y, Liu, LH | 87 |
| WUHAN, CHINA AND XIAMEN, CHINA | |
| RFLP analysis of maize mtDNA using important function genes as probesJiang, YH, Zhang, FD, Xia, T, Zheng, YL | 89 |
| ZARIA, NIGERIA | |
| Induced chlorophyll deficient mutants in maizeAdamu, A. Kasim | 90 |
| Induced tiller formation in maizeAdamu, A. Kasim | 90 |
| | |
| III. USING MAIZE IN K-12 CLASSROOMS | 93 |
| III. OONG WAZE IN N-12 SEASO TOO WO. | |
| N ADDRESS LIST | 0.4 |
| IV. ADDRESS LIST | 94 |
| | |
| V. MAIZE GENETICS COOPERATION STOCK CENTER | |
| Cooperation in 1931 Emerson, RA | 119 |
| | |
| VI. MAIZE DATABASE | 120 |
| | |
| VII. MAIZE PROBE BANK | 123 |
| | |
| WIII CAMBOL INDEX | 10 |
| VIII. SYMBOL INDEX | 124 |
| IV AUTUOD AND MANE INDEV | |
| IX. AUTHOR AND NAME INDEX | 12 |
| | |

CLONE INFORMATION SHEET

SUBSCRIPTION FORM

An open letter to maize cooperators:

Will Provine and Lee Kass have recently been funded by the National Science Foundation to conduct research for an intellectual biography of Barbara McClintock. Over a decade ago Provine initiated this project when he interviewed McClintock, Rhoades, Beadle and others regarding their contributions to maize cytogenetics. More recently Kass has contacted and/or interviewed many of McClintock's friends, colleagues and former students to gain insights for this study. One of our goals is to discern the influence that McClintock had upon the development of individual scientists and their work. Most people have been more than generous with their time and many have given us correspondence to document the many stories that prevail regarding McClintock's life and scientific contributions. Kass has examined McClintock's papers at the American Philosophical Society Library. She plans to visit other archives where correspondence and other materials may be found that will document McClintock's career. In the spirit of the founding of this News Letter we invite you to help us by contacting Lee Kass or Will Provine with any insights, or information you have that may aid us in our research. We thank you in advance for any assistance you may offer. Please contact us at the following addresses:

Lee B. Kass
L. H. Bailey Hortorium
462 Mann Library
Cornell University
Ithaca New York 14853
607-255-2131; email LBKBHWON@aol.com

William Provine Corson Hall Cornell University Ithaca New York 14853 607-254-4264; wbp2@cornell.edu

I. FOREWORD

The Notes in this Newsletter are voluntarily shared information. 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. This is not a refereed journal, and should be treated as "Conversations among Cooperators". Cooperators present here brief technical notes, updates, mutants, segregation ratios, tables of mapping data, developmental and anatomical information and techniques, clones, biochemical functions, and the like. Comprehensive material and analyses are better directed to formal publication. Maize Cooperators have the tradition of sharing information with colleagues, in MNL and 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.

As announced in the fall, MNL is now a <u>Virtual Hotletter and Linkletter!</u> 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/71/). We progressively incorporate figures and tables as electronic images, 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 passed, the print version of this issue, simply containing the Notes received to that date, plus the Address List and the Stock Center Report, has been 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 List, Maps, Zealand, Recent Maize Publications) will be done regularly insofar as possible, but on a separate schedule from MNL. Syntheses will be present in MaizeDB, where they can be viewed or printed by MaizeDB users.

Prof. C. H. Li -- a correction: Marc Albertsen informs me that Prof. Li has been seriously ill for several years. The parenthetical statement about Prof. Li in MNL 70, page 30, was incorrect.

Please note the appeal, at the end of the Table of Contents, from Lee Kass and Will Provine, toward an intellectual biography of Barbara McClintock. One key item of new correspondence from 1931, found during their research, is reproduced on page 119 in this issue.

Gifts to the Endowment Fund for support of the Newsletter have grown to nearly \$110,000. 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 continue to be welcome, to assure that costs of production are met, and are very much appreciated.

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 Agricultural Research Service. MaizeDB project, Curator Dr. Mary Polacco. We urge you with strongest enthusiasm to use, assess, and contribute to the database.

Shirley Kowalewski skillfully refined and redacted the copy, twisted diverse electronic sources and exotic scripts to suit, structured indexes, and questioned quality or content, or gave creative advice, at key moments. Beth Bennett contributed with care and diligence to many tasks, including library and literature work, composition, and checking of accuracy and completeness. At University Printing Services, Yvonne Ball and the printshop staff again efficiently ensured the job was done promptly and well.

Details about the 1998 Maize Genetics Conference, at Grand Geneva Lodge, Lake Geneva, Wisconsin, March 19-22, 1998, will be available on the MaizeDB Web at the earliest date, and information will be mailed to former attendees in November 1997; others may request the mailing by providing their address to Coe. The program and abstracts are provided by Bill Sheridan. Electronic submission and "Webification" of abstracts for the 1996 and 1997 Maize Conference by Mary Polacco has been successful, and will be used for the Conference. The Steering Committee for the 1998 Maize Genetics Conference is:

Ben Bowen Barbara Kloeckener

Paul Sisco

Ex officio: Karen Cone, Treasurer

Kelly Dawe

Jane Langdale Julie Vogel

Marty Sachs, local coordinator

Curt Hannah

Mike McMullen, Chair

Cliff Weil

Preparing notes for the next issue (Number 72, 1998)? SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME. See details inside the back cover.

Your clone can be mapped, and deposited in the Maize Probe Bank. Please see the Clone Information Sheet in the back of this issue, or see http://www.agron.missouri.edu/Coop/clonesheet96.html.

If you would like to subscribe to this Newsletter please use the form in the back of this issue.

Editor Coe

ALBANY, CALIFORNIA USDA Plant Gene Expression Center BERKELEY, CALIFORNIA University of California

A gene required for floral and inflorescence meristem determinacy -- Laudencia-Chingcuanco, D; Hake, S

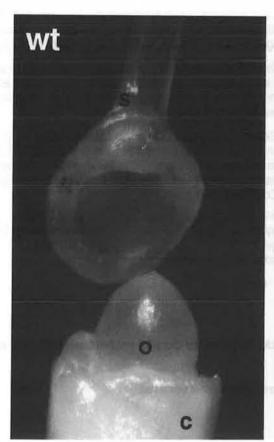
A recessive mutation, designated not under control (nuc), was identified from our Ac/Ds field in the summer of 1995. nuc plants are recognized in the field by the absence of protruding silks from otherwise normal looking ears. De-husked ears reveal florets with proliferous material at the center of the pistil. Upon closer inspection, the mutation appears to disrupt a gene required for the specification of a determinate floral meristem. In the female flowers, the floral apex, which is normally consumed in the formation of the gynoecium (see figure, wt) remains indeterminate. After formation of the carpels, an unorganized mass of pistillate tissue or an inflorescence-like, branching meristem is produced at the center of the flower (figure, nuc). The lower floret, which ultimately aborts, displays the same phenotype as the upper floret. Pollinated nuc ears failed to produce any kernels. In the tassel, additional male flowers form at the position of the aborted pistil. Occasionally, a nucellar-like mass is found at the center of these ectopic staminate flowers. nuc inflorescences also have extra spikelets and florets. In the ear, an extra spikelet forms between the spikelet pair. In the tassel, up to 6 extra spikelet pairs are produced after the pedicellate spikelet. Sometimes, an extra staminate flower is found between the two flowers in the spikelet.

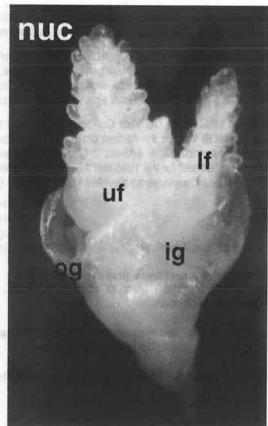
This transformation from flower to inflorescence shoot meristem has also been reported to occur in several species under particular growth conditions (Battey and Lyndon, Bot. Rev. 56:162, 1990; Okamuro et al., PNAS 93:13831, 1996), indicating that floral meristem determinacy is labile. Because *nuc* is a single gene that is required to prevent indeterminacy within the flower, we have a unique opportunity to explore the regulation of determinacy in flowers.

AMES, IOWA Iowa State University

Surfing for mutants: crinkly4 alleles in the Neuffer collection --Becraft, PW

Browsing through the maize genome database, I came upon images of 2 mutants from the Neuffer collection that were located on chromosome 10S and which bore striking resemblance to crinkly4, also on 10S (Stinard, MNL 65:17, 1991). The mutants were designated ad^* -N590C and ad^* -N647. Allelism tests between both these mutants and cr4 were positive.





s, silk; c, carpel; o, ovule; uf, upper floret; lf, lower floret; og, outer glume; ig, inner glume

Additionally, three mutants with similar phenotypes to *cr4* resulted from an EMS mutagenesis performed by Lisa Harper (Harper et al., MNL 69:22, 1995). The similarity to *cr4* was noted by Laurie Smith who sent them to me. The mutants were derived from the families LH624, LH651 and LH1231. Allelism tests between these three mutants and *cr4* were also positive.

The following designations for these mutants are proposed: cr4-N590, cr4-N647, cr4-H624, cr4-H651 and cr4-H1231.

Transposon (Ac)-induced homologous recombination at maize P locus and in transgenic Arabidopsis

--Xiao, Y; Peterson, T

The maize *P* gene encodes a Myb-homologous regulator of red phlobaphene pigment biosynthesis in the pericarp, cob and other floral tissues (Grotewold et al., Cell 76:543-553, 1994). The *P* locus has a unique structure with two 5.2kb direct repeats flanking the *P* gene coding region (Lechelt et al., Mol Gen Genet 219:225-234, 1989). When the transposon (*Ac*) inserts into one site between the two direct repeats in the *P-ovov-1114* allele, homologous recombination between the two 5.2kb repeats can occur and the whole *P* gene coding sequence is deleted (Athma and Peterson, Genetics 128:163-173, 1991).

To further study this transposon-induced homologous recombination, we examined six alleles which carry Ac insertions at different sites in the P locus, in both orientations. Each allele was tested as a heterozygote with P-wr, in the same hybrid (4Co63/W23) genetic background. After the cross with the rm3::Ds reporter, the mature ears were examined for the presence of colorless pericarp sectors. In a previous study, 80-90% of the colorless pericarp sectors were caused by deletions generated by recombination (Athma and Peterson, Genetics, 128:163-173, 1991). We found the three alleles with Ac inserted at different sites between the two direct repeats had twice the frequency of colorless sectors (average=4.1%) as did three alleles with Ac insertions either within or outside the 5' direct repeat (average=2.0%) (Figure 1). These results suggest that the transposon-induced homologous recombination is enhanced by the insertion of the element between the repeats. Ac orientation had no detectable effect.

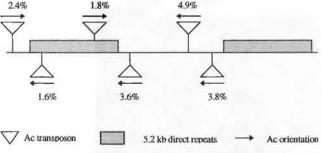


Figure 1. Frequency of kernels with colorless pericarp sectors observed with $\it Ac$ inserted at different sites in the $\it P$ locus.

To test if transposon-induced recombination is a general phenomenon in plants, we transformed *Arabidopsis* with a construct (GU.DS.US) containing a stable transposon (*Ds*) inserted between two deleted GUS parts with homologous direct repeats similar to *Ac* inserted in maize *P* locus. *Arabidopsis* transformants containing GU.DS.US will be crossed with an *Arabidopsis* strain containing stable *Ac*, which provides transposase to acti-

vate Ds. If transposon-induced homologous recombination occurs between the two deleted GUS parts, the GUS gene will be restored and result in blue sectors after staining with X-gluc reagent.

BEIJING, CHINA Academia Sinica

Ovary manipulation: transformation and in vitro maturation

--Ding, Q; Xie, Y; Dai, J; Mi, J; Kuo, Z

Ovary injection has been established as a transformation method (MNL 70:13-14) which has an obvious advantage: avoiding the limitation of genotypes on tissue culture and regeneration. On the other hand, this method has obvious shortcomings also: experience dependence and low efficiency. In order to improve this method some subjects were studied.

Carbon powders were used as tracers under the light microscope. Sampling at different times after injection, it was found that carbon powders could be injected into ovules and aggregate around the embryos along with their development. It was concluded that when the fertilized egg divided it could absorb nutritives from cells around itself and inert carbon particles dyed the embryo black (Figure 1e,f). For detailed information, tungsten particles were used as tracers under electron microscope according to the procedure for preparation of DNA for bombardment. The result showed that tungsten particles could be found in embryo cells four days after injection (Figure 1i). In this stage the embryo cells have a large nucleus (Figure 2a), after sampling and treatment the nucleus sometimes could stick to cell wall and tungsten particles could stick to the cell wall too (Figure 2b,c). Interestingly, one tungsten particle adhered to a nucleus that had stuck to the cell wall (Figure 1i, Figure 2b). These results could provide a demonstration for the pathway of donor DNA delivered into egg and embryo cells.

The main problem of ovary injection techniques has been that the injected ovaries were damaged by glass tubes and fungi, especially under the field condition. So I tried to culture isolated ovules and ovaries in order to increase the efficiency (Figure 1j,k,l; Figure 3). After two months culture the isolated ovules could just enlarge to 10 fold of their original size but with abnormal embryos. Although these ovules could be induced to calli they could not develop into embryos and plantlets. Isolated ovaries could develop into normal embryos but with very poor endosperm. Finally I cultured young fertilized ears. Normal seeds matured. After donor DNA injection, there are seeds matured but a lot of ovaries failed to produce seed (Figure 3f). At present about 180 injected seeds have been obtained and molecular identification is per-

formed.

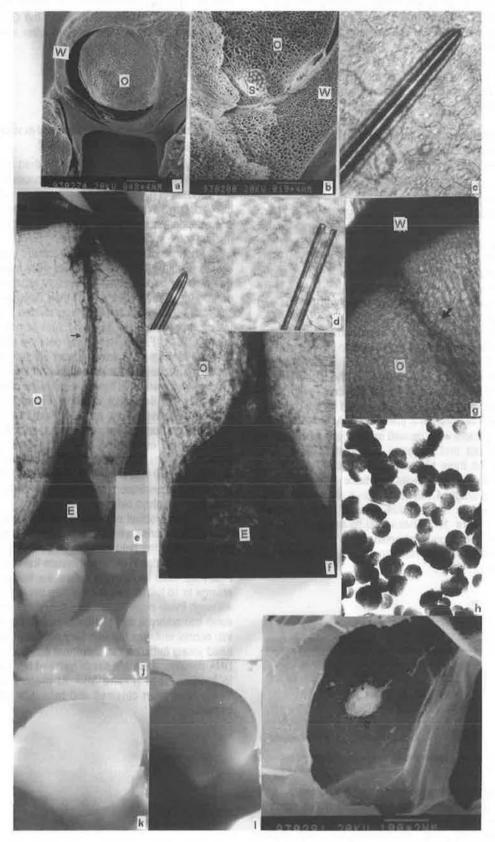


Figure 1. a: young ovary with ovule (O) and ovary wall (W); b: sac (S), ovule (O) and ovary wall (W) of ovary; c: tip of microcapillary (290X); d: comparison of tips of different microcapillaries (150X); e: carbon powder dyed embryo (E), normal ovule (O) and the trace (arrow) of injection (27X); f: carbon powder dyed embryo (E) (81X); g: trace of injection (arrow) (62X); h: tungsten particles (5000X); i: nucleus (double arrow) and tungsten particles (arrow) stuck to cell wall; j: isolated ovules (11X); k: growing ovule (13X) l: enlarged ovule (14X).

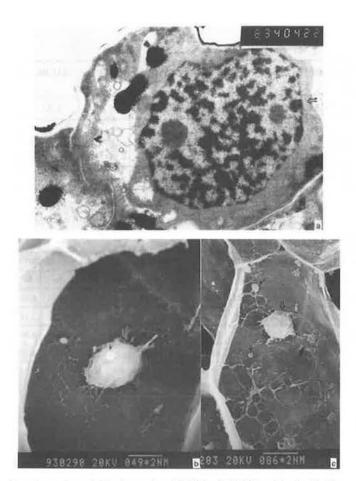


Figure 2. a: embryo cell with a large nucleus (4435X); b: detailed picture of nucleus (double arrow) and tungsten particles stuck to cell wall; c: nucleus (double arrow) and tungsten particles (arrow).

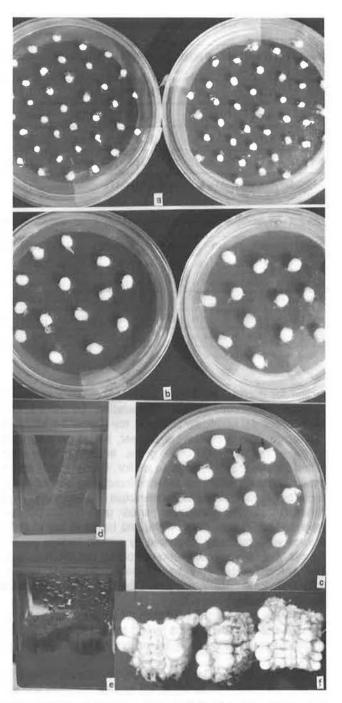


Figure 3. a: newly isolated ovaries; b: growing ovaries; c: some ovaries budding (arrow); d: cultured ears with vertical section; e: cultured ears with horizontal section; f: matured seeds after in jection.

Analysis for biochemical markers of multiplasmic lines in maize (Zea mays L.)

--Liu, X; Zeng, M; Liu, Y; Yang, T

Before this study, a group of multiplasmic lines with Mo17 nucleus, including (fli.)Mo17, (su1)Mo17, (sh2)Mo17, (bt1)Mo17, (pop)Mo17, (wx)Mo17, (teo.)Mo17, (cms-T)Mo17, (cms-S)Mo17, (cms-C)Mo17, (cms-21A)Mo17 etc., were successfully bred in our laboratory. The investigations of agricultural characters indicated the multiplasmic lines possess heterosis in the positive di-

Table 1. Protein content (%) of dry grain in multiplasmic lines.

| Sample Item | (fli)M017 | (sul)M017 | (sh1)M017 | (bt1)M017 | (teo)M017 | (wx)M017 | (pop)M017 | (T)M017 | (S)M017 | (C)M017 | (2IA)M017 |
|----------------|-----------|-----------|-----------|-----------|-----------|----------|-----------|---------|---------|---------|-----------|
| S.P | 1.02 | 1.00 | 1.33 | 1.24 | 1.21 | 1.09 | 1.07 | 1.16 | 1.05 | 1.01 | 1.20 |
| Zien | 4.87 | 4.74 | 5.19 | 5. 23 | 4.95 | 5.56 | 5.31 | 5.66 | 5.73 | 5.14 | 5.62 |
| Glu | 3.61 | 3.70 | 3.59 | 3.44 | 3.96 | 3.87 | 3.49 | 3.89 | 3.81 | 3.75 | 3.57 |
| Sum | 9.50 | 9.44 | 10.11 | 9.91 | 10.12 | 10.52 | 9.87 | 10.71 | 10.59 | 9.90 | 10.39 |

Notes: s. p-soluble protein Glu-Glutelin

Table 2. Protein content (%) for embryo and endosperm 34 days after pollination in multiplasmic lines.

| Sample | (III) | 1017 | (sul)Mi | 117 | (sh2)M | 017 | (bt1)M | 017 | (tea) | V017 | (wx) | 0M017 | (pop) | M017 | M(T) | 017 | (S)M | 017 | (C |)M017 | (21Å |)M017 |
|--------|-------|------|---------|------|--------|------|--------|------|-------|------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| Item | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En | Em | En |
| S.P | 9.71 | 0.56 | 10.67 | 0.66 | 11.01 | 0.64 | 10.24 | 0.51 | 9.14 | 0.51 | 11.24 | 0.73 | 11.12 | 0.76 | 10.08 | 0.53 | 10.70 | 0.58 | 0.90 | 0.51 | 10.34 | 0.50 |
| Zieh | 1.05 | 5.89 | 1.31 | 5.80 | 1.24 | 6.07 | 1.24 | 6.15 | 1.15 | 6.01 | 1.41 | 6.55 | 1.41 | 6.23 | 1.36 | 6.96 | 1.19 | 7.38 | 1.06 | 6.08 | 1.21 | 7.19 |
| Glu | 6.76 | 2.94 | 7.03 | 2.94 | 7.10 | 2.30 | 6.36 | 2.73 | 7.36 | 3.00 | 6.68 | 3.16 | 6.86 | 2.82 | 7.61 | 2.88 | 7.17 | 2.96 | 7.03 | 3.00 | 6.60 | 2.96 |
| Sun | 17.52 | 9.39 | 19.01 | 9.40 | 19.35 | 9.51 | 18.34 | 9.39 | 17.65 | 9,52 | 19.33 | 10.44 | 19.39 | 9.81 | 19.85 | 10.37 | 19.14 | 10.92 | 17.07 | 9.59 | 18.15 | 10.73 |

Nates: Em-Embryo En-Endospera

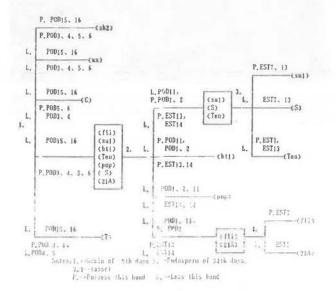
rection for nuclear-cytoplasmic interaction. The investigated agricultural characters involved 10 characters, i.e., plant height, height of ear, ear length, ear perimeter, row number per ear, grain number per row, grain number per ear, weight per ear, grain weight per ear, and weight of 100 grains. In addition, in the cross of multiplasmic line X Huangzhao shi line, the ear length, weight per ear and grain weight per ear were found to be markedly influenced by genetic factors and the interaction between heredity and circumstances. The row number per ear, grain number per row, weight of 100 grains, plant height, and height of ear of these crosses were markedly influenced by genetic factors, circumstances, and the interaction between heredity and circumstances. The ear perimeter may be influenced by other factors. (bt1)Mo17, (cms-T)Mo17, and (cms-C)Mo17 may be more useful for cross breeding.

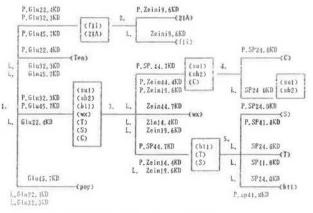
Heterosis in the positive direction for nuclear-cytoplasmic interaction was also found in the protein content, and variation for the soluble protein and zein were broader than for glutelin (Tables 1-2).

The leaf, tassel, pollen and grains from different developing stages were purified by centrifugation, and the isozymes and soluble protein, zein, and glutelin were analysed by isozymic electrophoresis and sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). The experimental results showed the multiplasmic lines possess some specific electrophoretic bands in the zymograms of peroxidase and esterase.

The experimental results also indicated the multiplasmic lines possess some specific zymogram bands in the soluble protein, zein, and glutelin.

The biochemical markers can be screened with these specific bands, and a plan was put forward to distinguish different multiplasmic lines.





Motestla—Embryo of 25th days 2.—Endosperm of 14th days 3.—Endosperm If 14th days 4.5—Dry grain p.—Possess this pand LaLess this band

Isozymic analysis of Chinese waxy maize (Zea mays sinensis)

--Zeng, M; Yang, T; Liu, Y; Li, S

Isozymes are one of the important biochemical indexes for examining relationships of plants. In our laboratory, we used 160 materials, including 119 of Chinese waxy maize, 21 of American dent waxy, 15 of *Coix* genus materials, 5 of teosinte and *Tripsacum*. Leaf and endosperm were examined. The experimental results proved that analysis of the zymograms of the peroxidases and malate dehydrogenases help to examine the relationship of waxy maize which originated from China.

The peroxidase isozymes of different tissues of different developmental stages in maize can be separated into 10-19 bands by polyacrylamide gel electrophoresis. But the fourth and fifth anodal bands (calculated from cathodal pole to anodal pole) were mainly a pair of specific bands of the green tissues (including leaf, sheath, shoot and husk, etc.). The relative activities of the fourth and fifth bands were similar to each other.

Results obtained from the detection of peroxidase isozymes showed that 86.6% of the waxy maize varieties (or inbred lines) from China show the fifth band, while 85.7% of dent waxy maize (including dent maize) from America show the fourth band (Table 1). In other words, the fifth band is the marker band of Chinese waxy maize, while the fourth band is the marker band of the dent waxy maize from America.

On the basis of phenotype, segregations of single cross combinations, F2, and BC1 can be deduced, so that the fifth and fourth bands of the peroxidase are genetically controlled by a locus with codominant alleles (Table 2 and Figure 1, 2).

Table 1. Comparison of peroxidase isozyme patterns of waxy maize from China and dent waxy maize (including dent) from America.

| Country or | Туре | Waxy or den | t maize |
|---------------------|---|-------------------------|----------------------------|
| Region | lszymer band | No. of materials | × |
| China | | Chinese waxy maize (| (Zea mays sinensis) |
| Yunnan Province | 4th band 5th band Mixed band Sum | 0 29 5 34 | 0 85.3 14.7 100.0 |
| Gungxi Province | 4th band 5th band Mixed band Sum | 0 17 2 19 | 0 89.5 10.5 100.0 |
| Sichuan Province | 4th bend 5th band Mixed band Sum | 0 18 2 20 | 0 90.0 10.0 100.0 |
| Gaishou Province | 4th band 5th band Mixed band Sum | 0 33 7 40 | 0 02.5 17.5 100.0 |
| Mubei Province | 4th band 5th band Mixed band Sum | 0 6 0 6 | 100.0 0 100.0 |
| America | Dent wax | y maize (including dent | maige)from America |
| .merica | 41h band 5th band Mixed band Sum | 18 0 3 21 | 85.7 0 14.3 100.0 |

Table 2. The fourth and fifth band segregations of the peroxidase isozymes.

| Crosses | | Phenot | Expected | X* | P | | |
|---|-------------|---------------------------|------------|-------|-------|-------|------|
| 4,5200,004 | Fourth band | Fourth and Fifth bands | Fifth band | Total | ralio | Α. | |
| Huangzao 4×Huangzao 4 | 38 | 0 | .6 | 30 | | | |
| Loxi×Loxi 1 | 0 | 0 | 3.0 | 38 | | | |
| Huungzao 4×Loxi 3 | 0 | 38 | 0 | 30 | | | |
| (Huangzao 4×Loxi 3) × (Huangzao 4 ×Loxi 3) | 9 | 14 | 9 | 32 | 1:2:1 | 0.500 | 0.75 |
| (Huangzao 4×loxi 3)× (G4046-5550×748788) | 12 | 26 | 12 | 50 | 1:2:1 | 0.000 | 0.95 |
| (Huangzao 4×Lexi 3)× Huangzao 4 | 23 | 21 | 0 | 50 | 1:1:0 | 0.320 | 0.95 |
| 748788×748788 | 0 | 0 | 30 | 30 | | | |
| G4048-5550×G4048-5550 | 30 | 0 | B | 38 | | | |
| 748788×G4040-5550 | 0 | 30 | .0 | 30 | | | |
| (749788×G4040-5550) ×(748788×G4040-5558) | 14 | 29 | 12 | 55 | 1,2,1 | 0.289 | 8.85 |
| (G4010-5550×740700) ×G4040-5550 | 25 | 25 | 0 | 50 | 1+1+0 | U | 1,00 |
| Ai- × Ai - | 0 | - 0 | 38 | 38 | | | |
| Tang+ × Tang + | 30 | - 5 | 0 | 38 | | | |
| Ai- × Tang + | 0 | 30 | 0 | 30 | | | |
| (Ai- × Tang+)× (Ai- × Tang +) | 10 | 20 | 16 | 40 | 1:2:1 | 0 | 1.00 |
| (Ai- × Tang+)×Tang+ | 15 | 15 | 0 | 30 | 1:1:5 | 0 | 1.00 |
| SIGDESIGD | 0 | 0 | 30 | 30 | | | |
| MO17 × MO17 | 30 | 0 | 0 | 38 | | | |
| Si(1) ×N017 | 0 | 100 | 0 | 100 | | | |
| [si(1)×N517]× [si(1)×N017] | 25 | 50 | 25 | 100 | le2c1 | 0 | 1.00 |
| (sl(1)×M017]× sl(1) | 0 | 50 | 50 | 100 | lelel | 0 | 1.00 |

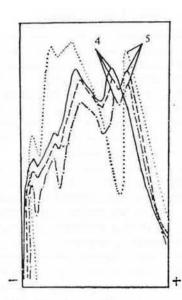


Figure 1. The segregations of the fourth, fourth+fifth, and fifth isozyme bands in the leaf tissue of (Ai- x Tang +) F2, showing 1:2:1 ratio.

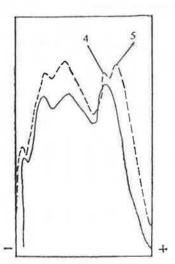


Figure 2. The segregations of the fourth, fourth+fifth, and fifth isozyme bands in the leaf tissue of [(Huangzao 4 x Loxi3) x Huangzao 4] BC1, showing 1:1:0 ratio.

BEIJING, CHINA Chinese Academy of Agricultural Sciences

Construction of genic male sterile system marked by yellow-green seedlings

-Zhou, H; Li, J; Deng, Y; Sun , R; Jiang, W

Nuclear male sterile gene ms2 and a yellow-green seedling gene (virescent) were introduced into an elite maize inbred line. Then a genic male sterile system marked by yellow-green seedling was constructed. The problem of segregation of genic male sterility was overcome by pulling out the normal green seedlings and leaving all the yellow-green seedlings (virescent) at three-four leaf stage. This new way of utilizing nuclear male sterility of maize is reported for the first time in the World and would find widespread use in developing countries.

Heterosis is usually described in terms of superiority of F1 performance over some measures of its parents. It's one of the modern achievements to utilize heterosis to produce more agricultural products. Artificial detasseling is often used in production of hybrid seeds, but it's more effective to utilize male sterile lines. Cytoplasmic male sterility is destroyed by specific diseases, e.g. Southern corn leaf blight. Maize nuclear male sterile genes were first found in 1930. Now more than 20 genes have been found, and most of them are recessive. They segregate in progenies and have no maintainers, so it is difficult to use them in breeding. Patterson (Proc. 6th Meeting Maize and Sorghum Section, Eucarpia, 1973) developed a duplication-deficiency system, but the seeds of the maintainer were small. It's difficult to use it in production.

From 1982, Jingxiong Li (pp. 4-5 in Recent Advances in Maize Breeding, China Science Press, 1992) used the linkage of ms1 and a white endosperm gene y in chromosome 6 to make backcrosses with some inbreds in order to select ms1 v/Ms1 Y linkage in China. The white seeds were male sterile and the yellow seeds were male fertile. Seeds produced can be separated into white and vellow seeds, i.e. male sterile and fertile seeds, by eyes or by machines. But the linkage between ms1 and y is not very close. They have a 5.72 cM recombination value. That means a seed producer must pull out 5-6% fertile plant tassels in the early flowering stage.

This also makes it difficult to be used in seed production.

We had been thinking of the problem. We found that the ms2 gene is very close to a yellow-green seedling gene virescent (v) in chromosome 9. So we designed a genic male sterile system marked by yellow-green seedlings in order to find a new way to utilize genic male sterility. This study is to construct the system.

The virescent gene (v) was sent from the maize stock center in Illinois in 1992. The phenotype of this gene is a yellow-green seedling in the 3-4 leaf stage, which then becomes a normal green seedling. Nine plants containing the ms2 gene were pollinated with v pollen in 1993. The hybrid seeds were planted and selfed and 10,000 of their progenies were planted. Normal green seedlings (V gene) were pulled out and all of the remaining seedlings were yellow-green (v gene). When most of the plants shed pollen, we looked for male sterile plants (v v ms ms). All the male sterile plants were pollinated with elite maize inbreds. We harvested the ears, planted 3/4 of the seeds and selfed. The selfed seeds and the remaining 1/4 seeds of the last generation were planted, and all of the normal green seedlings of the selfed seeds were pulled out. Male sterile plants were selected and pollinated with pollen of the remaining 1/4 plants. Their progenies segregate green-male sterile and yellow-male fertile seedlings 1:1.

Nuclear male sterile gene ms2 and the yellow-green seedling gene (virescent) were introduced into an elite maize inbred line after 5 generations of genetic manipulation and selection. The genic male sterility system marked by yellow-green seedlings was developed by us. The virescent gene has little effect on plant growth because it is yellow-green not white and becomes normal green after 3 leaves. The system employed seedling colour to distinguish male sterile and fertile plants, pulling out male fertile seedlings in one row and pulling out male sterile seedlings in another row. The male fertile row is the maintainer. The harvested seeds in sterile rows will be grown and manipulated in the same way next year. So, the system resolved the problem of maintainer in nuclear male sterility. To make hybrid seeds, the farmer can pull out green seedlings in the 4-5 female rows, the male row can be any normal inbred (Ms Ms V V), because they can all restore the male sterility. The hybrid seeds (Ms ms V v) will grow normally, with no yellowgreen male sterile plants. The farmer can grow them like other normal seeds. But the system needs the seed company to increase by 100% female seeds for pulling out. This means increasing the cost of hybrid seeds. But farmers have the custom of planting 3 times more seeds than should be planted and pulling out some weaker seedlings after 4-6 leaves, so this problem is not a problem in China. This system can be used in China or some developing countries. The design and manipulation is first reported by us. We think this is a new way of utilizing genic male sterility of maize.

Study on a new male sterile gene and apoptosis

-Zhou, H; Li, J

Dr. Jingxiong Li and his assistant once found a male sterile plant in their sweet breeding material several years ago and studied its genetics. He found the male sterility was controlled by one single recessive gene, temporally named msx. Using the B-A translocation system to locate the gene, he thought he had found a new male sterile gene. The gene was located on chromosome 4 with a recombination value of 15-20 with su1; msx was not the same gene as Ms41 and Ms44, also on chromosome 4, because these two genes were dominant and not at the same site according to crosses (unpublished data). The male sterility of msx was very stable, and

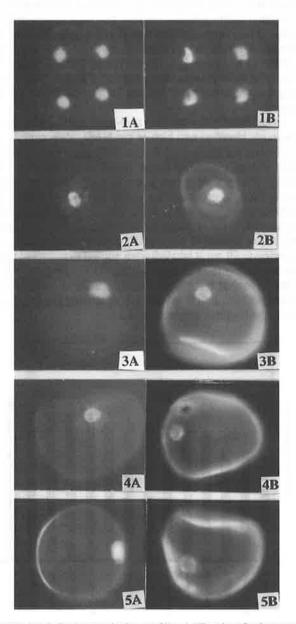
no fertile pollen grains were found in anthers in several years. Now we are mapping the gene on the maize RFLP map, and results will be published in our next paper.

We observed microspore development by employing a highly sensitive microfluorescent method this year. The msx msx and Msx msx genotypes were in the same inbred background. We took the anthers from two plants of msx msx and Msx msx each day after meiosis, fixed in Carnoy's solution, and marked nuclear DNA with DAPI overnight at 4C. The results were as follows:

- 1. Pollen in Msx msx anthers was normal (see Figure 1A-10A, male fertile plant) from quartet to binucleate stage.
- 2. Pollen in msx msx anthers was abnormal (see Figure 1B-10B), male sterile plant) from the late uninucleate stage (see 3B), when the chromatin began to condense (compare 3A to 3B).
- 3. The chromatin formed a death ring (4B and 5B) in msx while Msx was in first mitosis (4A and 5A).

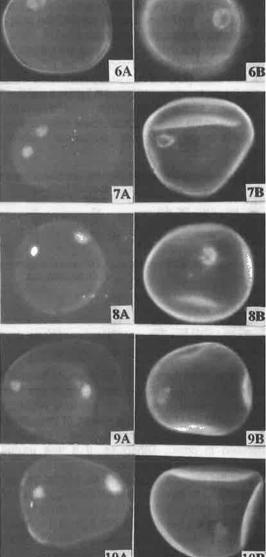
- 4. At the time of anaphase of first mitosis, the male sterile spore only showed a clear DNA digesting ring (6B), not a dividing nucleus (6A).
- 5. In the binucleate phase, msx showed DNA digesting (7B), but the spore was as big as the fertile one.
- 6. In 8B, the spore showed a split nucleus, as the normal spore (8A) was in second mitosis.
- 7. In 9B and 10B, the split nucleus was dissolving, with little DNA left, the fertile spore going into trinucleate phase. The sterile cell (cytoplasm, cell wall et al.) was not digested yet.

From the observations, we also found that DNA dissolution was earlier than cell dissolution. So we think that the microspore abortion of maize genic male sterility is a programmed cell death or apoptosis, a physiological cell death, not a cell necrosis.



9A

Figure 1-10A: Male fertile microspore development of Msx. 1-10B: male sterile microspore abortion of msx.



BELGRADE, YUGOSLAVIA Maize Research Institute

Complementary genes Rf4, Rf5 and Rf6 are not the unique genetic system for fertility restoration in cmsC of maize (Zea mays L.)

--Vidakovic, M; Vancetovic, J; Vidakovic, M

Using a group of five inbred lines in C-cytoplasm, converted to full restorers as female testers for the group of 24 inbreds known as restorers for cmsC, we found cases of segregation in F2 generations in the ratio of 15:1 male fertile to male sterile. Male sterile segregants did not appear in any of the two backcrosses, what was actually a crucial criterion for "absolute restorerness" of the crossed parents.

The above mentioned situation is possible only if fertility restoration of the two parents used in crosses was based on different genetic systems. It is obvious that the dihybrid segregation is in question. Therefore we assume that complementary genes *Rf4*, *Rf5* and *Rf6* represent the first system (System I), and as for the second one, it could be either a duplication, partial or complete of the first system or another parallel and independent genetic system with the same phenotypic effect.

The results of our screenings during two consecutive seasons (1995 and 1996) of all generations of the B73 cmsC RfC x V-312A RfC cross (RfC - a completely male fertile inbred containing cmsC cytoplasm) are presented in Tables 1 and 2.

The seeds for the 1996 screening were newly produced for all generations on the basis of consecutively selfed and pedigreed inbred plants in order to provide the maximal reliability of experimental results.

Table 1. Segregation of the B73 cmsC RfC x V312 RfC cross in Zemun Polje in 1995.

| | Plants per | ertility class | |
|---------------------------------|----------------|----------------|-----------------|
| Genotype | <u>Fertile</u> | Sterile | χ^2 (15:1) |
| B73 cmsC RfC x V312A RfC (F1) | 40 | 0 | |
| (B73 cmsC RfC x V312A RfC) (F2) | 272 | 13 | 1.39 |
| (B73 cmsC RfC) (BC1) | 80 | 0 | |
| (V312A RfC) (BC2) | 78 | 0 | |

Table 2. Segregation of the B73 cmsC RfC x V312A RfC cross In Zemun Polje in 1996.

| | Plants pe | r fertility class | |
|---------------------------------|-----------|-------------------|-----------------|
| Genotype | Fertile | Sterile | χ^2 (15:1) |
| B73 cmsC RfC (P1) | 27 | 0 | |
| V312A cmsC RfC (P2) | 23 | 0 | |
| B73 cmsC RfC x V312A RfC (F1) | 260 | 0 | |
| (B73 cmsC RfC x V312A RfC) (F2) | 2227 | 127 | 2,936 |
| (B73 cmsC RfC) (BC1) | 303 | 0 | |
| (V312A RfC) (BC2) | 1599 | 3 | |
| | | | |

As can be seen there are no discrepancies between the results obtained in 1995 and 1996 (Tables 1 and 2) and therefore they represent a direct proof of our previous report (Vidakovic, Maydica 33:51-64, 1988) that a system of three complementary genes might not be a unique genetic device for fertility restoration in cmsC. Otherwise, if it was unique, the F2 segregation would be impossible.

As for the dilemma duplications vs. parallel genetic system, it may be difficult to solve by classical genetic analysis of this type, and molecular geneticists might contribute much to it.

What is evident is the fact that both of the crossed parents studied here possess one recessive gene each, but in opposite genetic systems for fertility restoration in cmsC.

BERGAMO, ITALY Istituto Sperimentale per la Cerealicoltura

Analysis of nitrogen partitioning in maize

--Balconi, C; Bosio, D; Motto, M

Efficient use of nitrogen (N) fertilizer for maize production is important to maximize economic return to the grower, to minimize the potential impact on water quality, and to reduce the total energy required for manufacture of N. Current work indicates that the efficiency with which maize plants utilize N fertilizer is affected by several parameters including root morphology and extension, biochemical or physiological mechanisms regulating NO₃ uptake, redistribution and transport of N to different plant parts (Rizzi et al., Maydica 40:253-258, 1995). Therefore, the potential for developing superior N-efficient hybrids appears to exist. Evidence shows that maize can absorb substantial quantities of N following anthesis, and mobilization of vegetative N accumulated before anthesis provides the major source of N in the grain. It has been observed that N in the stalk of maize during vegetative growth can constitute as much as 40-50% the total-N content of the plant (Ta, Plant Physiol 97:1375-1380, 1991). Clearly, at least in some circumstances, the stalk is an important temporary reservoir for N that can be remobilized for ear development.

In this investigation we have analyzed 13 inbred lines of maize, previously identified with high or low N-NO₃ stalk content at anthesis, and the Illinois High Protein (IHP) and Illinois Low Protein (ILP) strains, previously tested (Bosio et al., MNL 69: 19-20, 1995). Plants were grown under field conditions at Bergamo, during 1995, to document the partitioning of ¹⁵N applied to the different genotypes, and to follow the pattern of subsequent remobilization of this N during ear development. For each genotype 340 mg of labeled N (as K¹⁵NO₃, 14.4 atom % excess, corresponding to 50 mg N labeled) were applied to individual plants in the two central rows of each subplot. In Figure 1 the ¹⁵N total plant content at black layer maturity (BLM) is reported for the genotypes tested. It is evident that substantial phenotypic variation in the N-uptake efficiency exists.

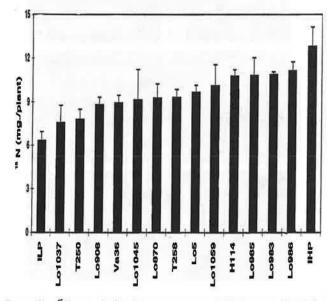


Figure 1. Plant ¹⁵N content (mg/plant) at BLM in the genotypes tested. Vertical bars indicate standard errors.

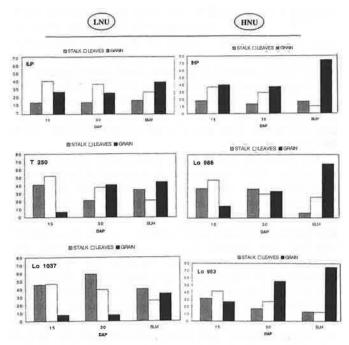


Figure 2. Distribution of ¹⁵N in plant parts in the genotypes identified for LNU (ILP, T250, L01037) and HNU (IHP, L0986, L0983).

For each subplot plant samples were harvested at 3 stages during grain filling: 15 and 30 days after pollination (DAP) and at BLM. The plants were divided into stalks, leaves and kernels for ¹⁵N determination. In Figure 2 the distribution of ¹⁵N in plant parts is reported for the genotypes identified for low (LNU: ILP,T250,L01037) and high (HNU: IHP,L0986,L0983) N-uptake. In the HNU plants we observed a higher relocation of ¹⁵N in the kernels than in the LNU plants; on the other hand, in the LNU plants ¹⁵N content in the stalk at BLM was higher than in HNU plants. These data suggest that the stalk plays an important role in storing and providing N for kernel development. It is proposed that this trait could be used as a selection criterion for developing more efficient plants in N utilization.

Do pedicel and placento-chalazal tissues play a role in the amino acid supply to the developing endosperm of IHP and ILP maize strains?

--Balconi, C; Bosio, D; Motto, M

The regulation of carbon (C) and nitrogen (N) supply to the developing maize kernel by the vegetative organs is a subject of great interest because it is responsible for the accumulation of starch, proteins and corresponding increases in dry weight of the kernel. Evidence has indicated that the relative proportion of starch and proteins in the endosperm may be determined by the nutrient supply, the sink demand and the interaction between them (Motto et al., Oxford Survey Plant Mol. Cell Biol. 6:87-114, 1989). The biochemical and physiological background of this relationship is complex and not fully understood.

Previous work in our laboratory, using field grown plants (Reggiani et al., Genet. Agr. 39:221-232, 1985) and endosperm cell cultures (Balconi et al., Plant Sci. 73:1-9, 1991) of the Illinois High Protein (IHP, a low kernel C/N ratio cultivar) and the Illinois Low Protein (ILP, a high kernel C/N ratio cultivar) also indicated that the composition of nutrients translocated from the plant to the developing grains may influence the relative concentration of

starch and proteins in the grains. Nutrient availability in the endosperm is also dependent on transfer layer, on the rate and efficiency of two different mechanisms:phloem unloading, and the uptake and utilization of assimilates for seed growth and storage protein formation (Gifford et al., Science 225:801-808, 1984). In maize, nutrients are unloaded from the phloem terminals located in the maternal tissue at the base of the seed, the pedicel. Susequently, the nutrients enter the apoplast of the placentochalazal region, which is the tissue that is directly in contact with the endosperm. Evidence shows that the processes of protein and starch biosynthesis are extremely dependent upon metabolic events taking place in these regions (Misra and Oaks, Plant Physiol. 77:520-523, 1985; Miller and Chourey, Plant Cell 4:297-305). The objective of this research was to evaluate the possible role of the cob and pedicel-placento-chalazal tissues in the amino acid (glutamine) supply in affecting zein and starch synthesis in the developing kernels.

Ears of the IHP and ILP maize strains were harvested at 9 days after pollination (DAP) and were cut into blocks containing 10 kernels per block as described by Gengenbach (Planta 134:91-93, 1977). The blocks were cultured on agar media containing salts as described in Nitsch and Nitsch (Science 163:85-87, 1969), 75 g/l sucrose and 4 g/l glutamine. After 7 days the blocks were transferred, until 25 DAP, to the same media supplemented with L-(U-14C) glutamine (12.5 uCi/ml; 4.56 uCi/mmol). The ¹⁴C content in endosperm, pericarp, pedicel and cob tissues was determined. The data indicated that the ¹⁴C content of cob and kernel tissues was lower in the ILP strain than in the IHP strain (Table 1). In Table 2 the concentrations, as percentage of dry matter, of various protein fractions (zeins, albumins plus globulins and glutelins) and carbohydrates (starch and sugars) are reported, in IHP and ILP endosperms harvested at 9 DAP (to) and grown in vitro until 25 DAP on culture media containing 4 g/l glutamine (t25). At 9 DAP (t0) low zeins and starch content was observed in all tissues of IHP and ILP kernels. At 25 DAP, both IHP and ILP kernels grown in vitro showed a phenotype similar to that observed in kernels grown to maturity in field conditions; in particular the ILP strain showed a reduced accumulation of the zein fraction and a higher starch content in comparison to the IHP strain; on the other hand, the sugar content was higher in IHP kernels than in ILP kernels.

Table 1. Distribution of ¹⁴C (cpm/20mg) in tissue extracts from IHP and ILP in vitro cultured caryopses until 25 DAP on culture medium containing 4 g/l glutamine.

| | Tissue | | | | | | | |
|---------|---------|----------------------|-----------|--|--|--|--|--|
| Strains | Cob | Pericarp and Pedicel | Endosperm | | | | | |
| IHP | 11780** | 11120** | 12595** | | | | | |
| ILP | 7260 | 5060 | 7740 | | | | | |

^{**} Significant at the 0.01 level of probability

Table 2. Protein fractions, starch and soluble sugars content as percent of the dry matter of the IHP and ILP endosperms at 9 DAP (t_0) and grown in vitro until 25 DAP on culture medium containing 4 g/l glutamine (t_{25}).

| | | Sample | time | | |
|-------------------------|-------|---------|-------|---------|--|
| | | 0 | t 25 | | |
| Endosperm components | ILP | IHP | ILP | IHP | |
| Zelns | 0.44 | 1.52** | 1.10 | 4.00** | |
| Albumins plus globutins | 1.16 | 1.88 | 1.00 | 1.70 | |
| Glutelins | 0.19 | 0.52 | 0.10 | 0.40 | |
| Starch | 14.70 | 16.90 | 60.30 | 35.20** | |
| Soluble sugars | 28.50 | 45.90** | 12.10 | 24.80** | |

^{**}Significant at the 0.01 level of probability

In summary, the results of this research suggest that the cob and pedicel-placento-chalazal tissues may be involved in determining the ILP phenotype, through a less efficient system of amino acid uptake, interconversion, and transport to the endosperm, in comparison with the IHP strain.

Biolistic-mediated transformation of maize endosperm cell cultures

--Balconi, C; Reali, A; Lupotto, E

Cell cultures derived from maize endosperms have been used to study endosperm physiology and biochemistry (Saravitz and Boyer, Theor. Appl. Genet. 73:489-495,1987). These cultures represent a useful tool for developing a homologous system for the study of endosperm-specific gene regulation. In order to establish a system for the routine application of GUS (beta-glucuronidase) transient gene expression assay, we investigated the response of the endosperm cells transfected with a series of plasmids in which the GUS coding region was driven by different constitutive promoters. Furthermore, the effect of osmotic treatments for enhancing the cell response was also evaluated.

For this research, long-term endosperm cell cultures (callus) and cell suspension cultures were established in the inbred line A69y, in the wild-type and *o2* mutant version, as described in Balconi et al. (Plant J. 3: 325-334, 1993). These endosperm cell cultures are not completely de-differentiated, and maintain in part the specific synthesis of starch and proteins characteristic of the genotypes. The analysis of zein and starch content indicates that the cell suspension cultures have a higher starch and a lower zein content in comparison with callus tissue. A more detailed analysis showed that the protein/starch ratio varies depending on the age of the cells, and along the four week time span of subculture.

Endosperm callus cultures, as described above, were subcultured to homogeneity, and utilized for the transformation experiments when the tissues were in the optimal stage of proliferation. This part of the work turned out to be essential for the success of transformation experiments, in order to guarantee constant operative conditions. Introduction of the plasmid vectors into endosperm-derived cells was performed via microbombardment using the Particle Gun device PDS He/1000 BioRad, with an operative pressure of 1100 PSI, and the target cells at about 7 cm from the stopping screen, in a partial vacuum atmosphere of 26 in Hg. The cells were bombarded with a series of plasmids all carrying the coding region of the GUS gene driven by: 35S CaMV, Ubiquitin-1 (maize), and Actin-1 (rice) promoter sequences in a similar plasmid backbone (pUC19). After bombardment, the cells were incubated 48h at 27 C in the dark, and subsequently histochemically stained for visualization and counting of the blue foci. Evaluations were performed calculating the number of blue foci per gram of fresh weight of tissue bombarded. Great attention was paid to reproducing the exact experimental conditions in each bombardment as far as size and amount of the cells used. A first set of experiments was indicative of good expression efficiency of the fusion p35S/GUS, with and without intron as enhancer. In addition, these results showed a dramatic positive effect of a short pre-treatment of the cells in the presence of osmotic pressure obtained by including 0.5M mannitol in the culture medium. The effect of the pretreatment enhanced up to 10 fold the number of blue foci recorded per bombardment (from 40 up to 900 on the average). The cells were also maintained in the same osmotic conditions 16h after transformation, and subsequently returned back to the original medium. Data gathered on the growth rate of the cells after bombardment including pre- and post-treatment in high osmotic medium, indicated that the whole procedure did not interfere with subsequent cell proliferation processes. These results are in good agreement with the general idea that a high osmotic pressure treatment of cells to be bombarded causes plasmolysis; this treatment which can reduce damage to cell membranes resulting in a better cell survival (Ye et al., Plant Mol. Biol. 15:809-819; Vain and McMullen, Plant Cell Rep. 12:84-88,1993).

The procedure described was subsequently used for further studies aimed at defining the rate of expression of the GUS gene in endosperm cells, when driven by different promoter sequences. To this purpose, the cells were bombarded with plasmids carrying the GUS coding region under the promoter sequences of *Ubi1* and Act1 genes, respectively, and the results obtained are compared with the data obtained in the case of p35SCaMV and p35SCaMVINT. For the osmotic treatment we chose a pretreatment of 4h followed by a post-treatment of 16h in 0.5M mannitol added to the culture medium. In each experiment, the data obtained from osmotically pretreated cells were compared with those obtained from non-treated cells. For both genotypes (wt and o2 versions), and for all plasmids used, the osmotic treatment enhanced particle bombardment-mediated expression of the GUS gene in the maize endosperm cells. By comparing the rate of expression obtained with the various promoters, we detected the highest values for pAct1 and pUbi1 promoters; these values were approximately twice the values obtained with p35SCaMV. In general, in the best conditions, we obtained with the fusion pUbi1/GUS an average value of 1200 blue foci/g fresh weight tissue. This value was also compared with fluorimetric determination of the GUS activity measured after enzymatic assays; the results obtained were in good agreement with the previous results. Interestingly, with respect to somatic cell systems, no enhancement in the GUS activity was detected when using p35SCaMV with the Adh1 intron respect to the p35SCaMV without intron. The data were compared to non treated cells during one year culture of the target tissues, and at different timing of growth during subculture. The results obtained allowed us to set up the technique for detailed investigations of promoter/GUS fusions in the maize endosperm system, by means of particle bombardment, in the study of regulatory mechanisms of genes involved in seed protein synthesis.

Genetic and molecular analysis of the gl3-m3 allele of maize

-- Donini, G; Maddaloni, M; Salamini, F; Motto, M

Epicuticular waxes from the outermost layer of aerial plant organs are thought to confer resistance to insect herbivore, fungal pathogens and drought (Martin and Juniper, The cuticle of plants, Edward Arnold, Ltd., 1970). The production of plant epicuticular wax is a biologically complex process involving a host of synthetic and transport mechanisms (von Wettstein-Knowles, In RJ Hamilton, ed, Waxes: Chemistry, Molecular Biology and Functions, The Oily Press, Dundee, UK, pp 91-129, 1995). In maize the biosynthesis and deposition of epicuticular waxes in the first five or six leaves of young plants is affected by at least 20 genetic loci (Coe, MNL 67:133-166, 1993). In this plant cuticular waxes are a complex mixture of very long chain fatty acids and their derivatives (Bianchi et al., Maydica 30:179-198, 1985). From genetic and biochemical analyses of maize plants carrying different muta-

tions affecting wax synthesis and depositions, it was hypothesised that two pathways can give rise to different kind of leaf waxes (Bianchi et al., Maydica 30:179-198, 1985). One pathway is responsible for wax synthesis in the first five or six juvenile leaves, whereas the second pathway produces waxes during the whole life cycle of the maize plant. The availability of mutants affecting cuticular wax accumulation and the isolation of the corresponding genes will assist in the elucidation of cuticular wax accumulation and the molecular mechanism by which its production is regulated. Therefore, a molecular genetic approach, based on transposable elements, is being used to isolate, clone and characterise the *Glossy3 (Gl3)* gene.

The *Gl3* locus is required for the biosynthesis of the epicuticular wax layer of the first five or six leaves of young plants. From genetic analyses and biochemical examination of the epicuticular wax composition of plants carrying a recessive mutation at the *Gl3* gene, it was concluded that the *Gl3* gene product may be involved in the elongation step from C28-C30 (Bianchi et al., Maydica 22:9-17, 1977). Mutated *gl3* plants produce approximately four times less wax than wild-type *Gl3* plants in the first five to six leaves and numerous variations in the distribution chain length within each class of wax compounds were found (Bianchi et al.,

Maydica 22:9-17, 1977).

A maize strain carrying the Ac transposable element inserted into the Waxy locus (wx-m7) was crossed as a male to the inbred line WF9 carrying the double mutation gl3-gl8. Out of 55,000 F1 seedlings scored, we have identified three unstable alleles at the GI3 locus; these alleles were named gl3-m1,-m2 and-m3. Despite the use of wx-m7 strain, we were not able to find Ac activity associated with the mutability at the G/3 locus. This finding was not surprising because, by using the wx-m7 strains, we generated several En/Spm unstable alleles at the Opaque-2 locus (Michel et al., Mol. Gen. Genet. 248:287-292, 1995) and at least one unstable allele at the GI2 locus (Tacke et al., The Plant J. 8:907-917, 1995). Therefore, a detailed molecular and genetic analysis was undertaken to characterise the transposable element inserted in each gl3 mutable allele. The gl3-mutable alleles were backcrossed twice to the WF9 gl3 strain and seeds of the second backcross generation were germinated; at the seedlings stage variegated and glossy stable shoots were bulked separately. The DNA extracted from each bulk was restricted with methylation-sensitive restriction enzymes and probed with an En/Spm specific probe. Results from Southern blot analyses were particularly encouraging with respect to the gl3-m3 allele, as fragments between 8 to 9 kb cosegregating with the mutable phenotype, were detected with each of the enzymes Sall, Pstl, and Pvul. In addition gl3-m3/gl3 plants were crossed to the c2-m1 En/Spm tester strain to establish an association between the mutability at the gl3-m3 locus and the appearance of coloured spots in the aleurone. The recorded data are in favour of the presence of an En/Spm element in the al3-m3 allele.

The methylation pattern of the *Opaque-2* promoter in maize endosperm and leaf

--Rossi, V; Motto, M; Pellegrini, L

In higher plants, 5-methylcytosine (m⁵C) can constitute as much as one-third of the cytosine residues of nuclear DNA. Moreover, DNA methylation has been found to control transposable element activity, genomic imprinting, gene silencing, inhibit gene transcription, and be implicated in the inheritance of a vari-

ety of epigenetic phenomena. A recent method based upon chemical treatment of genomic DNA (Frommer et al., PNAS 89:1827-1831, 1992) has allowed detailed analysis of the methylation state of several genes in different organisms. In transgenic plants, the application of this method allowed observation of the occurrence of m⁵C outside the canonical symmetrical CpG and CpNpG sites, and revealed association between the high level of m5C observed within a silenced 35S promoter and the transcriptional inactivation of the transgene (Meyer et al., EMBO J. 13: 2084-2088, 1994; Park et al., Plant J. 9:183-194, 1996). Furthermore, analysis of the methylation pattern of the maize Ac transposable element suggested a model to explain the association of Ac transposition with replication (Wang et al., Plant Cell 8:747-758, 1996). However, at present little is known about the methylation state of endogenous plant promoters (Ronchi et al., EMBO J. 14:5318-5328, 1995). To address this question, we determined the methylation state of a 390 bp region of the Opaque2 (O2) gene promoter (from -436 to -46) in endosperm and leaf cells by means of the bisulfite treatment of maize genomic DNA. This method is based upon a chemical treatment that converts unmethylated C residues into U residues that appear as T residues after PCR amplification of specific, single stranded DNA. Conversely, all m⁵C residues remain unmodified. For each tissue analyzed, separate PCR amplifications were performed for the upper and lower strands to produce DNA fragments that were subsequently cloned and sequenced.

In immature endosperm, where the O2 gene is expressed, 13 upper strand and 16 lower strand cloned PCR products were sequenced, respectively (Figure 1A). All clones displayed different methylation patterns and thus were derived from individual genomic O2 sequences. The O2 promoter has a considerably high level of C-methylation, 84%, present in this tissue. Particularly, almost all the C residues embedded within the O2-protein binding site appeared methylated. Interestingly, the methylation pattern of the lower strand differs markedly from that of the upper strand. Statistical analysis (student t test) of the mean methylation values obtained from the collected data indicated that, with p=1%, the lower strand is significantly less methylated than the upper strand (80% m⁵C vs. 89%). Moreover, an unequal m⁵C distribution is observed in the lower strand, less methylated at the most distal part (-436; -370) of the region analyzed. Student t analysis was also used to compare the methylation level of each C residue within their sequence context. Accordingly, we found that in endosperm, for both strands, CpT and CpG dinucleotides were significantly less methylated than CpA and CpC (p=1% and p=5%,

respectively).

The methylation pattern of the same *O2* promoter region was also analyzed in leaf by sequencing 10 upper strand and 10 lower strand clones (Figure 1B). Again, different methylation patterns were observed, thus indicating that they derive from individual genomic *O2* sequences. Student t analysis was also used to compare the mean methylation values obtained from leaf and endosperm. Data indicate that, with p=1%, the level of C-methylation in leaf is significantly higher than in endosperm (96% m⁵C vs. 84%). Similar to endosperm, in leaf an unequal m⁵C distribution is also observed in the lower strand of the most distal part of the region analyzed.

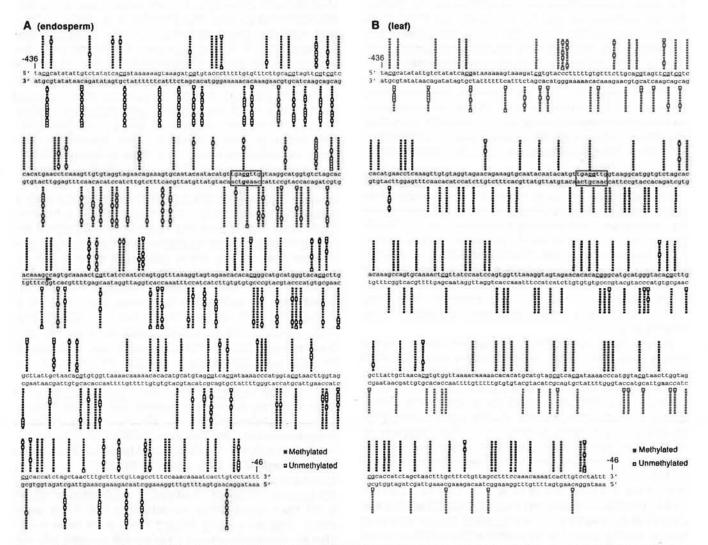


Figure 1. Methylation map of the -436, -46 O2 promoter region in endosperm and leaf. (A) Methylation map of the O2 promoter region in endosperm. Data from 13 and 16 clones have been compiled for the upper and lower strand, respectively. (B) Methylation map of the O2 promoter region in leaf. Data from 12 and 10 clones have been compiled for the upper and lower strand, respectively. Methylated C residues are indicated by black squares, whereas unmethylated C residues are indicated by white squares. CpG dinucleotides are underlined. The O2 consensus sequence is boxed.

Effects of DNA methylation on DNA binding and transcriptional activity of the *Opaque2* protein

--Rossi, V: Solinas, G: Motto, M: Pellegrini, L

In animals, DNA methylation appears to influence the tissue-specific expression of developmentally regulated genes by affecting the interactions of both chromatin proteins and transcription factors with DNA. Similarly, in plants methylation of the C residue embedded within the consensus sequence for mammalian CREB factors, TGACGTCA, was found to inhibit in vitro DNA binding activity of CREB-like factors present in nuclear extracts of pea, wheat, soybean and cauliflower (Inamdar et al., Plant Mol. Biol. 17:111-123, 1991). However, binding of plant nuclear proteins to hemimethylated target sequences has been reported for the tobacco CG-1 nuclear factor(s) (Staiger et al., PNAS 86:6930-6934, 1989) and the maize Ac-encoded transposase (Kunze and Starlinger, EMBO J. 8:3177-3185, 1989).

The maize Sn and O2 promoters have been shown to be highly methylated at the time and place of their expression (Ronchi et al.,

EMBO J. 14:5318-5328, 1995; Rossi et.al., unpublished). Particularly, the methylation pattern of the O2 promoter disclosed in immature endosperms indicates the occurrence of m⁵C within both DNA strands of the O2-protein binding site (TGACGTTG), thus suggesting the capability of this bZIP transcription factor to bind to a methylated consensus sequence and to activate the expression of a highly methylated promoter. To address these questions, gel-shift experiments were performed to investigate the DNA binding activity of a recombinant O2-protein to O2 promoter fragments (spanning from -399 to -211) whose methylation state mimics the O2 methylation patterns disclosed by genomic sequencing. We used O2 promoter fragments methylated at CpG sites, hemimethylated on either the upper or the lower strand, fully methylated, and containing different percentages of m5C. Results showed that O2 efficiently binds to a CpG-methylated O2 promoter. Band shifts were also observed with a 30%- and 50%-methylated promoter fragment. However, higher percentages of m⁵C were found to impair O2 in vitro binding activity. Interestingly, hemimethylated O2 promoter fragments can still be retarded, although a DNA fragment hemimethylated on the lower strand was bound with reduced efficiency compared to a promoter fragment hemimethylated on the upper strand.

An in vitro transcription initiation system consisting in a partially methylated *O2* promoter fragment (spanning from -762 to +60), HeLa cells nuclear extracts, and *E. coli* extracts expressing a recombinant O2-protein was developed to investigate the capability of the O2-protein to bind and activate transcription of a highly methylated promoter. Results showed that a 50%-methylated *O2* promoter allows formation of active preinitiation complexes and the synthesis of specific O2 transcripts.

BOLOGNA, ITALY University of Bologna

Mapping QTLs for leaf abscisic acid concentration in a maize population

--Landi, P; Sanguineti, MC; Salvi, S; Tuberosa, R; Casarini, E; Conti, S

Several studies have indicated that abscisic acid (ABA) has a pivotal role in the adaptive response of plants to drought-stress. In the present work, we have analyzed a maize population in order to identify QTLs affecting leaf-ABA (L-ABA) concentration and their overlap with QTLs for physiological traits involved in the response to drought. The population considered in this study included 80 random F4 families derived from the cross Os420 x IABO78. Previous experiments (Conti et al., Euphytica 78:81, 1994) have shown that under drought-stressed conditions Os420, as compared to IABO78, has a 2-3 fold higher L-ABA concentration. In 1994 and 1995, the F4 families were tested in replicated field trials conducted under conditions of partial water deficit. The following traits were investigated: L-ABA concentration measured, according to Hanway's classification, at stage 3 (L-ABA-3) and stage 4 (L-ABA-4), corresponding to rapid stem elongation and anthesis, respectively; drought stress index (DSI) visually scored; stomatal conductance (SC) at stage 4; leaf temperature (LT) at stage 4 in 1994 (mean of two measurements) and between stages 3 and 4 in 1995 (mean of seven measurements).

Southern analysis was carried out using 109 RFLP markers obtained with probes kindly provided by T. A. Musket (University of Missouri). The genetic map was computed using the program JOINMAP (Stam, Plant J. 5:503, 1993) and a LOD score of 2.5. QTL analysis was performed with the composite interval mapping procedure adopted in the program PLABQTL (Utz and Melchinger, JQTL 2:1, 1996.

The heritability values for L-ABA estimated on a family mean basis varied from 0.60 to 0.88. The genetic map spanned 1578 cM, which corresponds approximately to 75% of the length of the maize map. QTL analysis showed that the number of significant (LOD > 2.0) QTLs for L-ABA concentration, at each sampling, varied from two to eight (Table 1). In total, 16 QTLs were revealed in at least one sampling. The alleles increasing L-ABA were mainly contributed by Os420. The QTL with the strongest effect on L-ABA concentration was found on chr. 2, in the region between markers csu133 and csu4. In both years, this QTL was detected only at the second sampling, despite the fact that the mean value

Table 1. Additive effect (ng ABA g⁻¹ d.w.) and LOD score at the QTLs for leaf ABA (L-ABA) concentration investigated at two samplings (stage 3 and stage 4) in 1994 and 1995.

| | 1994 | | | | 1995 | | | | |
|--------------------|---------------|------|---------------|------|---------------|-------|---------------|------|--|
| | L-AB | A-3 | L-AE | 3A-4 | L-AE | 3A-3 | L-AE | A-4 | |
| Chrom. | Effect (1) | LOD | Effect (1) | LOD | Effect (1) | LOD | Effect (1) | LOD | |
| 1 | 47 | 2.97 | 15. | | *2 | | 16. | | |
| | | | 49 | 6.62 | *: | | | 24 | |
| 2 | 12 | 2 | - | 25 | 2. | - G-C | 34 | 2,54 | |
| 701 | -24 | | 41 | 5.56 | | - 1 | 72 | 8.88 | |
| | 14 | | 33 | 5.56 | 34 | 7.17 | 32 | 3.46 | |
| 3 | 2.1 | | | | 13 | 3.64 | | | |
| 341 | 24 | | 32 | 3.11 | - 50 | (40) | | 64 | |
| | -28 | 2.70 | | | | | 93 | 1.0 | |
| 4 | - 14 | 2 | 16 | 2.65 | 18 | 2.47 | 32 | 2.87 | |
| 5 | | | • | 7.5 | 18 | 2.83 | | 17 | |
| 6 | | * | * | | * | | 25 | 2.01 | |
| | 74 | * | | | | (4) | -25 | 2.22 | |
| 7 | | | | | -21 | 2.80 | | - | |
| | | | | | 25 | 4.85 | | | |
| | 12 | | -14 | 2.57 | -21 | 2.89 | 25 | | |
| 9 | 10 | * | -28 | 4.23 | -32 | 5.01 | - E | 196 | |
| QTL (no.) | 2 | | 7 | | 8 | | 6 | | |
| R ² (%) | 18 | 3 | 6 | 0 | 5 | 1 | 6 | 5 | |

(1) Computed as (Os420 - IABO78)/2.

of L-ABA concentration in 1994 was higher at the first sampling (587 vs. 366 ng ABA/g d.w.). Therefore the action of this QTL seems less influenced than others by the level of water stress experienced by the plant and its growth-stage.

The supporting intervals of the QTLs for L-ABA concentration did not overlap with the known location of ABA mutants, such as the *viviparous* mutants. It seems more likely that the effects of these QTLs on L-ABA relate to those of genes controlling morpho-physiological traits (e.g. root morphology, leaf area, etc.) which affect the water balance of the plant. The effects of genes modulating the intensity of the signal transduction associated with turgor loss, a major determinant for the concentration of ABA, could also be involved. Interestingly, the map position of the QTL on chr. 2 coincided with those of one QTL for L-ABA concentration and one QTL for root-pulling strength which have been found in a different maize population (Lebreton et al., J. Exp. Bot. 46:853, 1995).

Significant QTLs were also identified for the other investigated traits. Several of these QTLs overlapped with QTLs for L-ABA. As a general trend, an increase in ABA was associated to a higher DSI, lower SC, and higher LT. Only in a few cases, the reverse trend was noticed. Although it is not possible to ascertain if these associated effects are due to linkage and/or pleiotropy, our result indicate that in the population herein investigated an increase in L-ABA seems to have mainly represented an indicator of the degree of water-stress experienced by the plant at the time of sample collection, rather than having a causal role in the improvement of the level of drought-tolerance in the field.

Molecular marker analysis of maize populations divergently selected for abscisic acid concentration in the leaf

--Salvi, S; Tuberosa, R; Sanguineti, MC; Landi, P; Conti, S

Molecular markers represent an efficient tool for detecting the changes in allelic frequencies occurring at the loci controlling the response of a quantitative trait subjected to selection. We have investigated the effects of two cycles of divergent selection for leaf abscisic acid (L-ABA) concentration on the frequency of alleles at RFLP loci in a maize population derived from the cross between Os420 (high L-ABA) and IABO78 (low L-ABA).

In 1992, 480 random F2 plants were selfed and evaluated for L-ABA under conditions of partial drought stress at the stage of tassel emergence and pollen shed. In 1993, the F3 families derived from the 30 F2 plants with the highest L-ABA and the 30 F2 plants with the lowest L-ABA were tested in a replicated trial conducted under conditions of partial water deficit. The 8 F3 lines with the highest L-ABA and the 8 F3s with the lowest L-ABA were identified; selection was also conducted within these F3 families. The corresponding F4 families showing, respectively, the highest and the lowest L-ABA were then intercrossed according to the diallel scheme in order to obtain the high L-ABA and the low L-ABA populations. The two populations were evaluated in 1995 in a replicated field trial under conditions of partial water deficit. The high L-ABA population, as compared to the low L-ABA population, showed significantly higher L-ABA concentration (413 vs. 254 ng ABA/g d.w.), thus indicating the effectiveness of the divergent selection procedure.

The two groups of divergently-selected F4 lines were subjected to RFLP analysis with the same probes utilized in the preceding article. So far, the F4 families have been screened with 29 well-spaced probes localized on chromosomes 1, 2, 3, and 4. A significant departure (X2 test) of RFLP allele frequencies from the expected ones was observed in two chromosomal regions (csu133-csu4 on chr. 2 and umc31-umc193 on chr. 4). In both cases, the low L-ABA parental allele (from IABO78) was fixed in the eight low L-ABA families; among the eight high L-ABA families, there was only one family which was heterozygous at each region, while the remainder were homozygous for the high L-ABA parental allele (see Fig. 1 for csu4 on chr. 2). The QTL analysis carried out on 80 random F4 families derived from the same cross (see preceding article) also indicated that the two regions on chromosomes 2 and 4 contain two of the strongest and more stable QTLs for L-ABA. Therefore, the results of the divergent selection support the validity of the QTL analysis.

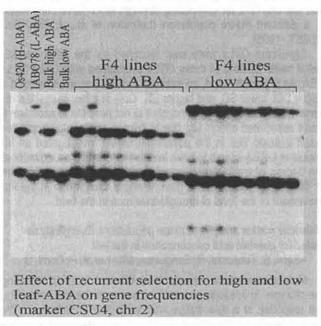


Figure 1.

In order to ascertain with more precision the effects associated with the QTLs for L-ABA on chromosomes 2 and 4, the heterozygosity at these regions will be maintained through three-four selfing cycles. Sets of nearly isogenic lines differing for the parental alleles at the chr. 2 and chr. 4 regions will then be derived and evaluated per se and in hybrid combination under different water regimes for L-ABA, yield, and other physiological traits.

BUFFALO, NEW YORK State University of New York at Buffalo

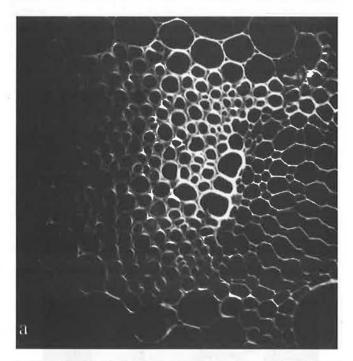
Two-photon fluorescence confocal microscopy of maize tissue --Pan, SJ; Shih, A; Liou, WS; Cheng, PC

Confocal laser scanning microscopy (LSM) has contributed significantly in the understanding of biological specimens in 3D. However, due to high attenuation (scattering and absorption) of light and strong autofluorescence, application of this technology to botanical specimens is generally more difficult than to animal specimens. Imaging maize tissue presents no exception. The light attenuation is particularly pronounced in the UV spectrum and becomes significantly lower in the IR (Cheng and Kriete, in: Handbook of Biological Confocal Microscopy, Plenum Press, 1995; Bhawalkar et al., Bioimaging, 1997).

Conventional confocal fluorescence microscopy uses shorter wavelength light to excite fluorophores to emit longer wavelength fluorescence. The high attenuation resulting from scattering and absorption (e.g. pigmented structures) in botanical specimens hinders the penetration of the excitation beam and the detection of fluorescence emitting from the specimen. Therefore, it frequently limits the imaging technique to the very surface of the specimen (Cheng et al., 1993, in Multidimensional Microscopy, Springer-Verlag). In addition, the presence of chlorophyll and other photoactive compounds in the plant cells exhibits strong autofluorescence, which hinders the feasibility of using conventional fluorophores such as FITC and Texas Red in multichannel fluorescence microscopy. It has been shown that botanical specimens (maize specifically) generally exhibit a much lower absorbance in deep red and near infrared light (Bhawalkar et al., Bioimaging, 1997), therefore, imaging the deeper part of the specimen by IR is desirable. However, the use of a longer wavelength results in lower transverse and axial resolution and prevents the use of most of the existing biological fluorescent dyes.

The use of two-photon induced fluorescence in conjunction with laser scanning microscopy (LSM) has provided an alternative method in the study of botanical specimens in three dimensions. The technique is based on simultaneous absorption of two photons, each having at least half the energy of the band gap, to excite a fluorophore and induce fluorescence. Because of the quadratic dependence of two-photon induced fluorescence intensity on the excitation intensity, it is possible to achieve depth discrimination even without a confocal aperture in front of the photo-detector (Denk et al., Science, 1990; 73-76). Since the two-photon absorption cross-section is much lower than the linear absorption cross-section, the two-photon process is very inefficient in comparison to the single-photon fluorescence process used in conventional fluorescence microscopy. As a result, the signal strength in two-photon fluorescence microscopy is frequently poor.

We report here the use of one of a group of newly developed highly efficient upconverters (fluorophores), 4-[N-(2-hydrox-



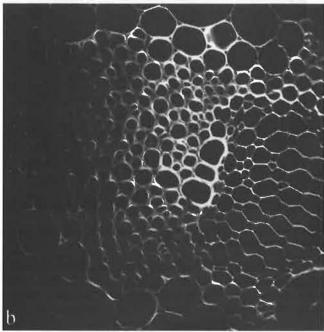


Figure 1 (a and b). Two-photon fluorescence image of maize vascular bundle stained by APSS.

yethyl)-N-methyl)amino pheny]-4'-(6-hydroxyhexyl sulfonyl)stilbene, abbreviated as APSS, for the imaging of maize stem. Two-photon technique, using this fluorophore, is capable of imaging structures over 250mm deep into the maize stem with a minimum degradation in image intensity and still capable of achieving submicron resolution. Maize stem fixed in 1:3 ethanol/acetic acid was used in this experiment. After fixation, the specimen was washed thoroughly in water and stained with 0.1% APSS in ace

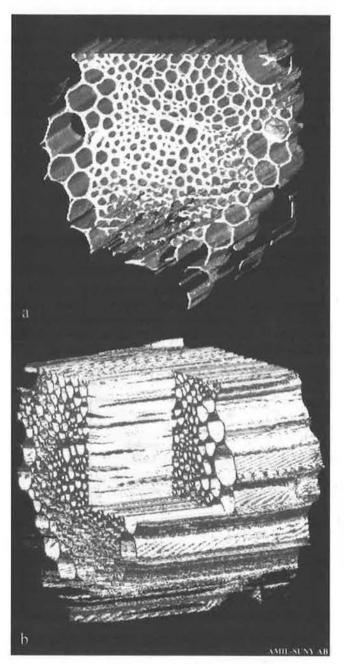


Figure 2 (a and b). Isosurface rendered views of a malze stem showing vascular bundles. The 3D rendering was generated from a stack of 250 optical sections.

tone. Following many washes in acetone, the tissue was cleared in methyl salicylate. The APSS has high affinity to cell wall.

For the two-photon confocal microscopy, an Olympus Fluorview laser scanning confocal microscope equipped with a Plan-apo 40X (NA=1.3) objective lens was used. The light source was a diode pumped mode-locked laser (850nm, Flare laser, Clark-MXR Inc.) producing a train of pulses of duration ~150fs each, at a frequency of ~120MHz. The average power in the laser beam was 30mW. A 800nm long-wavelength reflecting dichroic beam splitter (Chroma Technology Co., 725DCSP) was used to separate the illumination and detection paths and a 540nm short pass filter

was used in the detecting path. Figure 1a and 1b are two optical sections of a stem vascular bundle stained by APSS. Figure 2 (a and b) are isosurface renderings of a stack of 250 optical sec-

tions (at 1mm spacing).

This article is dedicated to Professor Dr. D. B. Walden on the occasion of his retirement after many years of continued inspiration through discussions with his students and colleagues in the filed of genetics. The APSS fluorophore was kindly provided by Dr. P. N. Parsad of the Department of Chemistry, SUNY. This project was supported in part by the Academic Development Fund of SUNY to PCC.

BUFFALO, NEW YORK State University of New York at Buffalo IOWA CITY, IOWA University of Iowa

Visualize the three-dimensional arrangement of vascular bundles using cone-beam optical tomography

-- Pan, SJ; Shih, A; Liou, WS; Park, MS; Wang, G; Cheng, PC

Adopting from the methodology and algorithm used in X-ray cone-beam tomography (Wang and Cheng, Zool. Studies, 34: Sup. 1. 159-161, 1995), a cone-beam optical tomographic imaging system (Figure 1) has been developed at our laboratory to visualize the three-dimensional arrangement of vascular bundles.

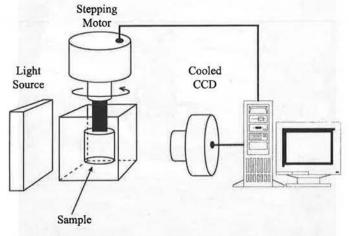


Figure 1. Schematic representation of a cone-beam optical tomo graphic imaging system.

Leaf sheaths (Oh43) were used to test the imaging system. Tissues were fixed in 3:1 ethanol/acetic acid mixture, washed in running water, stained in Schiff reagent, dehydrated in acetone, and cleared in methyl salicylate (MS) (Cheng, MNL 69:28-29, 1995). The procedure produced a highly cleared tissue with a relatively homogeneous refractive index and minimum scattering. The vascular bundle and cell wall stained in magenta. A Kodak Wratten #25 red filter was placed in front of the light source to reduce scattering and contrast. The specimen was rotated by a stepper motor in a rectangular glass container filled with MS. Projection images were captured with a cooled CCD camera (equipped with a Kodak 1400 CCD chip) at 12-bit dynamic range at 1277x1004 pixels. In order to maximize the depth-of-focus of the close-up lens (Olympus OM 50mm macro lens), aperture setting of f/22 was used. One hundred equiangular projections were obtained.

Figure 2 shows two views of a rolled-up leaf sheath (half of a leaf sheath) obtained by the CCD camera. The generalized Feldkamp cone-beam algorithm originally developed for X-ray microtomography (Wang et al., IEEE Trans. Med. Imag. 12:486-496, 1993) was used for the three-dimensional reconstruction. Figure 3 shows a three-dimensional isosurface rendering of the leaf sheath and its vascular bundles.

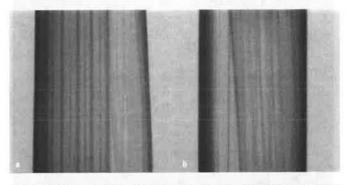


Figure 2 (a and b). Optical projection images of half a leaf sheath viewed at two different angles.

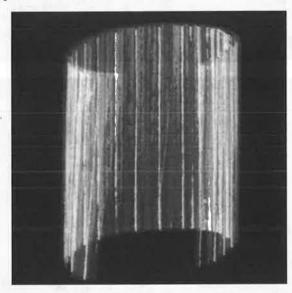


Figure 3. Isosurface view of the three dimensional data obtained from 100 optical projections. Note the vascular bundles within the leaf sheath.

The cone-beam optical tomographic technique demonstrated in this article can be used for visualizing plant structures in 3D. Since a volumetric data set is obtained from the tomographic reconstruction, viewing the specimen at any arbitrary angle can be easily accomplished by using a computer with appropriate software. It is important to note that the derivation of the original X-ray tomographic reconstruction algorithm was based on the X-ray absorption properties of the specimens. No consideration regarding scattering and refraction was given. Therefore, the reconstruction assumption in our optical tomography was also based on the condition that both scattering and refraction resulting from the MS-cleared tissue are negligible. If the specimen is not cleared entirely to a glass-like appearance, the optical tomography can not be performed reliably.

This article is dedicated to Professor Dr. D. B. Walden on the occasion of his retirement after many years of continued inspira-

tion through discussions with his students and colleagues in the fields of genetics. This project was supported in parts by the Academic Development Funds of SUNY to PCC.

CEDAR FALLS, IOWA
University of Northern Iowa
WOODWARD, OKLAHOMA
USDA-ARS, Southern Plains Range Research Station
BATON ROUGE, LOUISIANA
Louisiana State University

An SEM study of inflorescence development in Tripsacum dactyloides

--Orr, A; Kaparthi, R; Dewald, C; Sundberg, M

Although most current workers favor the hypothesis that maize arose from a teosinte-like ancestor, the debate gained renewed vigor with a recast of a Mangelsdorf hypothesis that *Tripsacum* played a role in the evolution of maize (Eubanks, Econ. Bot. 49:172-182, 1995). Fertile F1 *Tripsacum*-teosinte hybrid plants, that were derived when Eubanks crossed *Zea diploperennis* and *Tripsacum dactyloides*, produced hybrid inflorescences (ears) with fused, non-disarticulated cupules. According to Eubanks these first generation hybrid ears exhibit an intermediate morphological stage toward the evolution of the maize ear. If hybridization between *Tripsacum* and *Zea* played a role in the evolution of the maize inflorescence, it is likely that *Tripsacum* shares a similar pattern of inflorescence organogenesis with *Zea* (maize and the teosintes).

In previous studies by two of us (Orr and Sundberg) we described the development of inflorescences (ears and tassels) in the teosintes (see review Flowering Newsletter 18:48-53, 1994), and primitive maize (Am. J. Bot. 82:64-74, 1995; 83:1255-1265). The organogenic observations from these SEM studies were used to examine the prevailing ideas on the origin of the maize ear. These observations (Fig. 1) disclosed that: (1) ears and tassels in the genus Zea are derived on a common developmental background with bifurcation of spikelet pair primordia giving rise to paired sessile and pedicellate spikelet primordia; (2) the solitary spikelets of teosinte ears result from arrested growth and subsequent abortion of the pedicellate primordia; (3) the switch from 2-ranked to 4-ranked (distichy to polystichy) could be due to a change in the developmental timing program that controls the bifurcation of auxillary buds; and (4) a proposed switch from staminate to pistillate condition may have involved an intermediate bisexual (mixed) infloresence stage.

In light of Eubank's hypothesis indicating a possible role of *Tripsacum* in the origin of the maize ear, it became imperative that the early organogenesis of *T. dactyloides* bisexual inflorescences be investigated to test the developmental relationship among *Zea* and *Tripsacum*. Gamagrass plants (WW 1379) were grown at the USDA, ARS Southern Plains Research Station, Woodward, OK, and inflorescences were dissected from primary axes (A₁) and lateral (A₂) axes (Fig. 2). Material was prepared for scanning electron microscopy as described in previous work (Orr and Sundberg Am. J. Bot. 81:598-608, 1994).

Organogenesis along the infloresence proceeded acropetally and showed a dorsiventral morphology. Distichously produced bract primordia subtended axillary buds along the abaxial surface. The development of axillary bud (ab), spikelet pair primordia

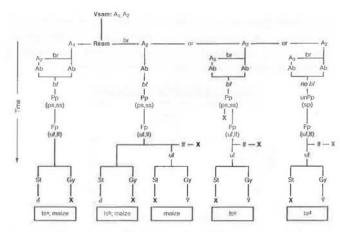


Figure Abbreviations: Ab, axillary bud; bf, bifurcation; br, branch; Fp, floret primordium; Gy, gynoecium; If, lower floret; Pp, spikelet pair primordia; ps, pedicellate spikelet; Rsam, reproductive shoot apical meristem; ss, sessile spikelet; st, stamen; uf, upper floret; unPp, unpaired spikelet primordium; Vsam, vegetative shoot apical meristem; letters indicate primordia initiated; X, subsequently aborted.

Figure 1. The genus Zea is represented by maize, and four teosinte taxa comprising two annuals and two perennials. Generalizations in the illustration may not reflect environmentally induced variability. A₁, main axis, A₂, first order lateral branch off A₁, A₃, second order lateral A₂.maize, Z. mays ssp. mays; ts, teosinte: (a, ssp. diploperennis, perennis, mexicana and parviglumis; b, ssp. diploperennis, mexicana and parviglumis; c, ssp. perennis, mexicana and parviglumis; d, ssp. diploperennis. Adapted from Orr and Sundberg, Am. J. Bot. 81:598-608, 1994.

(Pp), and lower floret (If) and upper floret (uf) primordia were similar to that described for maize (Cheng et al., Am. J. Bot. 70:450-462, 1983) and the teosintes (Fig. 1). Inflorescences developed two ranks (four rows) of staminate spikelet pair primordia (Pp) with two florets each on the distal portion of the rachis, and two ranks (two rows) of single female spikelets with one floret on the proximal portion of the rachis (Fig. 3). Gynoecial tissue aborted in both staminate florets. The single spikelet condition arose by arrest and abortion of the pedicellate spikelet (ps), and abortion of the lower floret in the sessile spikelet (Fig. 3). This ontogenetic derivation of the single, sessile spikelet is similar to

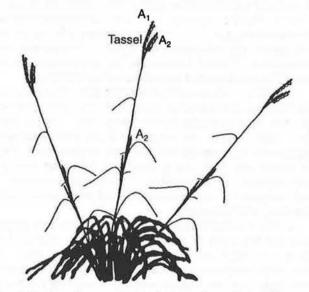


Figure 2. Morphology of gamagrass. The tassel and ear positions on the A_1 and A_2 axes are shown. A_1 represents main culm terminated by a sexual mixed tassel. A_2 represents a sexually mixed lateral branch of the tassel or a sexually mixed inflorescence lateral to an A_1 culm. Illustration modified from Jackson , 1990.

the pattern observed in the lateral (A₂) teosinte inflorescence (*Z. perennis*, *Z. mexicana* and *Z. parviglumis*) (Fig. 1). The sexually mixed condition is similar to the teosinte, *Zea diploperennis* (Sundberg and Orr, Am. J. Bot. 73:1699-1712, 1986).

ORGANOGENESIS OF GAMAGRASS

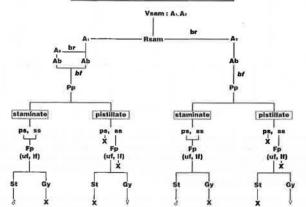


Figure 3. Comparative summary of early organogenic inflores cence development on A₁ and A₂ axis of *Tripsacum dactyloides*. A₁, main axis; A₂, secondary axis of A₁.

SEM analysis indicates that members of the genera *Zea* and *Tripsacum* share a common ontogenetic pattern of inflorescence ontogeny. We are continuing to characterize the development of *Tripsacum* inflorescences by investigating with SEM the organogenesis of a *T. dactyloides* gynomonoecious mutant, GSF-1.

CHESTNUT HILL, MASSACHUSETTS Boston College

Continued studies on the genic instability of maize inbred lines derived from anther culture

-- Ting, YC; Nguyen, DQ

In the summer of 1996, continued studies on the genic stability of maize inbreds derived from anther culture were carried out. In a total of 52 plants examined, 10 of them demonstrated dwarfism like that observed in 1995. These dwarf plants grew up to a little over one foot tall, with yellow green leaves. The stalks were completely barren. Three of them tasseled late in the season and only one of the tassels reached anthesis but without dehiscing.

In addition, it was found that among the above plants there was a curling plant and one robust green plant. This curling plant was the same as a previously reported (Abst. Proc. 17th Int. Cong. Gen., 1993) mutant. The robust and sturdy green plant never appeared in the progenies from the same ancestral plant. Since the number was small, one for each class, it does not seem that they originated by segregation of pre-existing mutations. It might suggest that the mutagenic effect of maize anther culture was active and transmitting.

In another consideration, polling last year's data from progeny tests and this year's, a total of 124 plants were examined; 21 plants were dwarf, approximately 17 percent of the total. The result does not follow any Mendelian expectations. Perhaps some other factor or factors may be involved. It might be that the tissue culture per se activated dormant transposable elements in the cells and tissues.

More studies on tassel plants of maize

-- Ting, YC; Nguyen, DQ

In the winter of 1996, seven plants propagated through cloning were transplanted to the greenhouse. Under natural light illumination, and within a temperature range of 60 F (night) to 80 F (day), all of them grew into adult plants. Tillers and ear shoots were as many as when they grew in the open field. Five months later, only one plant produced plantlets on its tassel. This plant had delayed senescence in growth, and the leaves were thicker and dark green. In other words, the whole plants appeared more vigorous than all the rest of its sibs. The pistillate inflorescences were well developed and fertile. Each ear bore more than 100 kernels upon backcrossing to the hybrid F1s.

One of five plants in the selfed progeny of the tassel plant also produced plantlets, but light green in plant and leaf color. The plant had reduced vigor and the plantlets sprouted out only on the

central spike of the tassel.

Since the same plantlets reappeared in the selfed progeny it is tenable to say that this characteristic is genetically controlled and under suitable conditions it would be transmitted to the next generation, even though the frequency of transmission was very low, 20 percent expression. None of the plants from the cross-fertilized progeny, a total of seven, initiated any plantlets on their tassels. These plants had on average three tillers, both staminate and pistillate florets were fertile, each ear bearing more than 50 kernels. The inflorescences of three plants in the crossed progeny were well developed and subsequently they were collected and fixed for meiotic observations. It was found that they were all tetraploid. Pachytene chromosomes were well-spread and stained intensely. Likewise, 12 plants of the backcrossed progeny, 95clone x 95 tp x 4n perennial maize, survived transplanting in the field. Six of the plants were of short stature and they had reduced vigor and barren stalks. The stalks had only a few antheses. No pollen sheds were observed. They seemed completely infertile. The other six plants of the same progeny grew into normal height. Both male and female flowers were highly fertile, but having a small number of ears per plant. None of the above plants regenerated any tassel plants in the tassels. The segregation ratio between short and tall plants was 6:6 or 1:1.

Seven plants (plantlets), propagated asexually (cloning) in the greenhouse, were also transplanted to the field. When they were about 3-inches in height, three of them converted into pistillate inflorescences with well developed silks. In consequence of this, all of them were pollinated with haploid pollen. None of them set any seeds. Then, these small plants (plantlets) soon senesced. The other four plantlets of the same pedigree grew normally into adult plants. Their male and female flowers were fertile. On average, each plant had three to four tillers. However, none of their tassels grew out plantlets.

In summary, the characteristic growth and their transmission of the tassel plants suggest the presence of a mutation. The expression of this mutation is regulated by, perhaps, a gene similar to det-2 in Arabidopsis. When this gene exists as a homozygote, it causes reduced fertility and aborted anthers. The senescence of chloroplasts, leaves and flowers is accordingly delayed. In addition, this mutation also blocks the biosynthesis of a hormone, brassinosteroid, which is required for development and light-regulated gene expression. In the last summer, among a total of 25 progeny plants of tp derived from cross-fertilization, backcrossed, as well as cloning propagated, none of them regenerated

into tassel plants (plantlets) on the tassels. It was postulated that in the summer, the natural light intensity was high. Thus, it might turn on the mutation (det-2 like), inhibiting the production of the hormone, brassinosteroid. The hormone-deficiency plants were unable to respond to light signals to call for tp expression. It is known that brassinosteroid may play an important role in regulation of gene expression of plants by responding to light signals. In the winter, the same progeny plants grown in the greenhouse without any artificial lights, synthesized adequate amounts of brassinosteroid and demonstrated the capacity to sprout out plantlets on the tassels. On average the daily light intensity during the winter in the greenhouse is much lower than that in the field during the summer. However, this explanation of light regulation of the expression of the mutant, tp, of perennial maize is tentative. The exact answer to the question of how to is expressed has yet to be elucidated in the future.

COLD SPRING HARBOR, NEW YORK Cold Spring Harbor Laboratory

Consequences of the ectopic expression of the Myb-domain protein P

--Rabinowicz, P; Ma, H; Grotewold, E

The P gene encodes a Myb-domain protein required for the transcriptional regulation of a subset of the maize anthocyanin biosynthetic genes regulated by C1/Pl + R/B. Whereas the accumulation of the P-regulated phlobaphene pigments seems to be restricted to the floral organs of some grasses including maize and sorghum, anthocyanin pigments can be expressed in all plant tissues and are widely found in the plant kingdom. The regulators of anthocyanin biosynthesis are functionally conserved, and the ectopic expression of R and C1 induces the accumulation of anthocyanins in a number of plant species.

As a way to determine whether phlobaphene accumulation could be induced in plant species normally lacking these pigments, we investigated the effect of expressing P in the dicot Arabidopsis thaliana. A functional P cDNA was ectopically expressed in Arabidopsis from the constitutive 35S promoter following an in planta Agrobacterium-mediated transformation. Kanamycin-resistant seedlings were selected, and to our surprise, we found a large number of very small plants present only in the material derived from 35S::P-transformed plants, but absent in plants transformed with unrelated plasmids (Fig. 1). Northern analysis of mRNA derived from total plant tissue demonstrated that the severity of the phenotype is dependent on high levels of P expression. P expression levels were 20-100 fold higher than in maize Prr pericarps. Every organ we looked for was present in the 35S::P plants, but was reduced in size. Microscopic investigation of these plants indicates that the reduced size is the product of the combination of smaller and fewer cells (Fig. 2A, B). As far as we can tell from comparing leaves of equal size from wild type and mutant plants, the density and shape of trichomes were not affected (Fig. 2C, D). This is in contrast to what has been determined for Arabidopsis plants ectopically expressing R, where an increased number of trichomes was observed (Larkin et al., The Plant Cell 5:1065-1076, 1994). 35S::P expressing plants often accumulate red pigments in the stem and the leaves. We have not yet carried out extensive biochemical characterization of these pigments, yet the color is very similar to the anthocyanin pigments



Figure 1. Effect of the ectopic expression of Pin Arabidopsis. About four week old plants containing the 35S::P transgene (two left pots) compared to wild type plants (right pot).

that accumulate in *R+C1*-expressing *Arabidopsis* plants (Lloyd et al., Science 258: 1773-1775, 1992). Provided that these pigments are anthocyanins, we believe that they could be induced by stress, from which the 35S::P plants clearly suffer. In maize, *P* does not activate anthocyanin biosynthesis, because it cannot bind to the promoters of anthocyanin-specific genes such as *bz1* (Grotewold et al., Cell 76:543-553, 1994).

What precedents are available for the small plant phenotype induced by *P* in *Arabidopsis*? A dominant genetic factor, called *Ufo* (for *Unstable Factor Orange*), has been identified by Dr. Charles Burnham that induces phlobaphene accumulation in maize vegetative tissues (Styles et al., MNL 61:100, 1987). Northern and RNase protection experiments indicate that *Ufo* induces the accumulation of *P*-homologous sequences in leaf sheath tissue at levels comparable to pericarp tissues (Grotewold, unpublished). Plants carrying the *Ufo* allele show a significant retardation in tissue growth, proportional to the amount of pigment present (Styles et al., 1987).

Ectopic expression of *P* from the strong 35S promoter in Black Mexican Sweet suspension cells does not appear to affect cell size, although the cultures grow more slowly. Yet, some unknown selection mechanisms allowed us to recover only transgenic lines with levels of expression of *P* comparable to the levels of *P* mRNA in *P-rr* pericarps (Grotewold et al., manuscript in preparation).

These observations are somehow consistent with what happens in the 35S::P transgenic *Arabidopsis* plants: P expression somehow interferes with cellular processes. Why does P cause such dramatic effects when expressed in plant tissues where it is not normally found?

- 1. \dot{P} ectopic expression could interfere with the normal expression of gene(s) with important cellular functions. This could be mediated by the conserved Myb domain present in a large number of other plant proteins, or by other regions of the P protein. It is remarkable, however, that ectopic expression of C1 (with an 80% identical Myb domain with P) does not show similar effects.
- P-regulated compounds, such as phenolics or isovitexin (a C-glycosyl flavone), could have an effect on cell growth/division.
 Although it is known that these compounds are regulated by P in

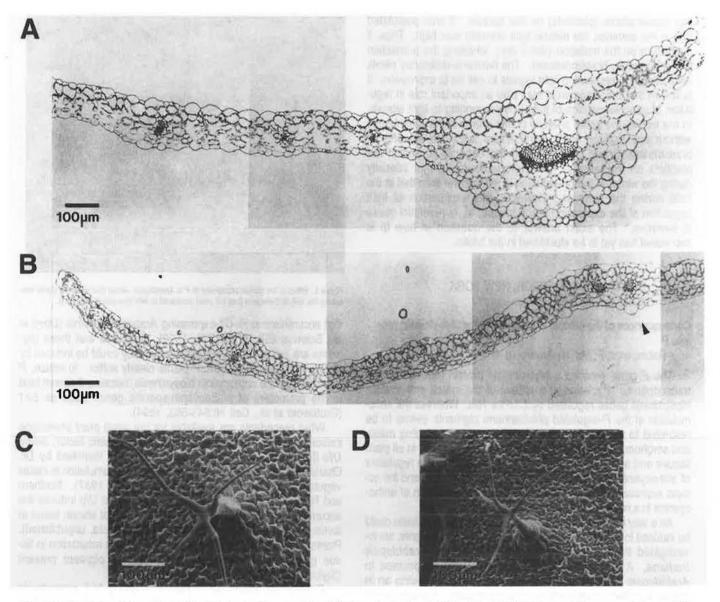


Figure 2. Leaf morphology of P-expressing Arabidopsis plants. A and B correspond to optical micrographs of sections of wild type (A) and 35S::P (B) leaves. Trichomes are not preserved in the fixation conditions used. The arrow in B indicates the central vasculature. C and D correspond to scanning electron micrographs of leaf trichomes of wild type (C) and 35S::P transgenic plants (D).

maize, the effect seen in *Arabidopsis* suggests that their accumulation in this dicot would also be induced by *P*. This remains to be determined.

3. *P* could have regulatory functions, in addition to controlling phlobaphene accumulation.

COLLEGE STATION, TEXAS Texas A&M University

Utilization of molecular probes to facilitate development of Quality Protein Maize

--Lin, KR; Bockholt, AJ; Smith, JD

The nutritional quality of maize can be dramatically improved by selecting for mutations in opaque-2 (o2), a recessive and endosperm specific gene that regulates expression of α -zein. However, o2 kernels are relatively soft, which results in problems with milling, disease and insect resistance. Quality protein maize

(QPM) is a name for o2 maize genetically modified for hard endosperm by selection (of modifier genes) in order to overcome the agronomic constraints. The QPM modifiers behave as a multigenic trait. Their introgression into elite inbreds is complicated because, in addition to maintaining the homozygous o2 gene, multiple modifiers must be selected. Although conventional breeding procedures have been used successfully to convert commercial lines to QPM forms, the procedure is highly inefficient and is not directly oriented toward improvement of grain quality. If genetic markers could be used to identify unknown modifiers that contributed to QPM phenotype, both the efficiency and the potential for grain quality improvement would be significantly increased. Therefore, the objective of this study was to find markers which could facilitate the identification of high quality maize before selection.

Triads of four inbreds, Mo17, B73, T224 and T232, were analyzed. Each triad consisted of 3 forms of the inbred, i.e., the normal inbred, an o2 form that was backcrossed at least 4 times to the normal inbred, and a QPM form that was backcrossed at least

twice to the o2 inbred with selection for hard endosperm and agronomic characteristics. These three lines of a triad are genetically similar, but not identical, and the genetic similarity of the original line is greater with the o2/o2 line than the QPM line. However, genetic similarity is much greater within than among triads. The QPM inbred from each triad may contain chromosomal segments introgressed from the original open-pollinated QPM varieties developed from CIMMYT. To the extent that introgressed regions exist in the same chromosomal locations in all four triads, they probably represent chromosomal regions that were retained by selection for seed characteristics during backcrossing. Thus, introgressed DNA that is present in QPM lines has the potential to carry modifier QPM genes.

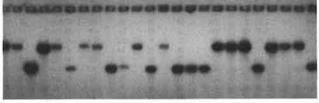
To detect these heterogeneous introgressed segments, we hybridized a collection of maize RFLP probes (obtained from Dr. David Hoisington, formerly of the University of Missouri, Columbia) to DNA from seedlings of the four triads. DNA isolation, digestion, electrophoresis, blot hybridization, probe labeling and autoradiography were described previously (Gardiner et al., Genetics 134: 917-930, 1993). About 100 UMC clones were selected for coverage of the maize genome at approximately 20 cM intervals based on the maize linkage map (Gardiner et al., 1993).

Fifty-four UMC probes were screened in the four triads with EcoRI and HindIII digests (Fig. 1). Of these probes, 9 detected

 ECoRI
 HindIII

 Mo17
 B73
 T224
 T232
 Mo17
 B73
 T224
 T232

 N o2 Q N o



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 1. Southern blot pattern using probe *umc64*. Genomic DNA of four triads was digested with *Eco*RI (lanes 1-12) and *Hind*III (lanes 13-24). Size was separated on 1% agarose gel, blotted to a nylon membrane (HybondTM-NC+) and hybridized to a radiolabelled probe. N: Normal maize. *o2*: opaque-2 maize. Q: Quality protein maize.

same size bands in all three forms of all four triads. Polymorphisms (12 out of 54) occurred between the o2 forms and the normal inbreds in one or more triads with either enzyme digest. These probes may reflect the different origins of O2 and o2 alleles. Ten probes (out of 54) were hybridized to different size bands in the normal, o2 and QPM forms in one or more triads with either enzyme digest. About half (26 out of 54) of the UMC clones detected identical bands in the normal and o2 lines, but bands were different in one or more QPM lines in one or two enzyme digests. Practically, a combination of molecular probes that would allow for selection of modifiers prior to selection for agronomic characteristics would be a powerful tool for QPM inbred conversion.

COLORADO SPRINGS, COLORADO Colorado College

Analysis of a zebra stripe double mutant

--Surdam, D; Bertrand-Garcia, R

Over the last several years we have been characterizing the zebra stripe mutants in maize. The recessive zebra stripe mutations, (zb1, zb2, zb3, zb4, and zb7), affect chloroplast development by reducing thylakoid membrane formation and the number of grana. Mutant plants typically express transverse yellow striping in the juvenile leaves of the plant and show wild type adult leaves.

Study of several zebra stripe double mutations in our lab has led to some very interesting results. We selfed heterozygous zebra stripe-3 (*zb3*)-zebra stripe-7 (*zb7*) mutant families, and recovered a double mutant phenotype.

Double mutants of zb7, zb3/zb7, zb3 segregated as 1/16 of the population; chi square analysis of 162 plants resulted in a x^2 =.107 giving us a p value =.975. The double mutants were phenotypically bleached-yellow, with little or no green pigment in the juvenile leaves (Fig. 1).



Figure 1. zb3-zb7 double mutant and wild type siblings.

Adult double mutants were stunted compared to wild type and single mutant siblings (mutant adults averaging about 23.7 cm in height). Expressivity of the bleached yellow phenotype was most severe in the first five (juvenile) leaves. Adult leaves were successively greener.

Analysis of the double mutants by Western analysis revealed some fascinating results. Antibodies to four chloroplast photosystem complexes (Sub 4, psaD, psbA, and ATPase) were used to assess wild-type, zb3, zb7 and the zb3-zb7 double mutants. Wild type, zb7, zb3 and zb3-zb7 double mutants were collected, chloroplast membrane proteins isolated and protein concentration determined by spectrophotometry. The proteins were separated by SDS-PAGE electrophoresis using a 12% gel. The proteins were blotted to nitrocellulose, the membrane was probed with specific antibodies and binding detected using an Amersham ECL-Western blotting analysis kit.

The blot in Figure 2 shows the results for juvenile leaves collected from all four genotypes. The most striking result is the lack of psaD in the *zb3-zb7* double mutant. psaD is a component of the photosystem I complex. All juvenile leaves of the double mutants revealed a lack of psaD. Overloading gels with 5X concentrations of chloroplast membrane proteins from the double mutant, and overexposure of films, still showed a lack of psaD. Analysis of

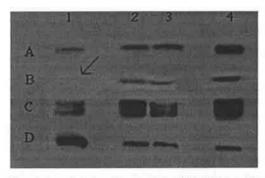


Figure 2. Lanes 1, 2, 3, and 4 represent the *zb3-zb7* double mutation, *zb7* mutation, and wild type chloroplast extracts, respectively. Rows A, B, C, and D represent the chloroplast photo-system complexes of Sub 4, psaD, psbA, and ATPase respectively. The arrow indicates the absence of psaD in the double mutant.

adult zb3-zb7 double mutant leaves revealed a small amount of the psaD protein, suggesting that the juvenile leaves lack PSI completely but recover small amounts of PSI in the adult leaves.

COLUMBIA, MISSOURI University of Missouri

A ring chromosome on 2S

--Neuffer, MG

I have been maintaining stock of a ring chromosome (Ring 2S) composed of a centromere and the segment of the short arm of chromosome 2 carrying the B1:Peru allele of the B1 locus. Transmission is through the male only. The stock may be maintained by crossing as a male on the mutant cp*-1122A (also called de*-N1122A), and selecting kernels that are sectored for the collapsed phenotype. These produce seedlings that have chimeras and pale green tassels (for photos, see http://www.agron.missouri.edu/db_images/variation/cd5133-2022-0660/83 and /84).

My supply of this stock is very small, so if anyone is interested in having a sample of this material please contact me soon.

Location data for Les*-A7145

-- Neuffer, MG; England, D

Location data for Les*-A7145 are given below:

| Marker | Breakpoint | # Pits | M.Wx | N.Wx | Mwx | N.wx | r± SE | chisq |
|-------------|------------|--------|------|------|-----|------|-----------------------|-------|
| T1-9c | S.48L.22 | 83 | 12 | 20 | 16 | 25 | 43.4±5.4 | 1.49 |
| T1-9(4995) | L.19S.20 | | | | | | | |
| T1-9(8389) | L.74L.13 | 81 | 17 | 24 | 21 | 19 | 44.4±5.5 | .99 |
| T2-9d | L83L.27 | 82 | 20 | 22 | 21 | 19 | 47. 615 .5 | .19 |
| T3-9c | L09L.12 | 94 | 29 | 19 | 20 | 26 | 41.515.0 | 270 |
| T3-9(8447) | S.44L.14 | 209 | 65 | 44 | 49 | 51 | 44.5±3.4 | 2.38 |
| T3-9(8562) | L.65L.22 | 92 | 24 | 23 | 21 | 24 | 47.8±5.2 | .18 |
| T4-9g | S.27L.27 | 90 | 28 | 19 | 18 | 25 | 41.1±52 | 281 |
| T4-9(5657) | L.33S.25 | 85 | 25 | 16 | 21 | 23 | 43.5±5.4 | 150 |
| T4-9b | L90L29 | 83 | 25 | 20 | 18 | 20 | 45.815.5 | .55 |
| T5-9c | S.07L.10 | 88 | 16 | 27 | 23 | 22 | 43.215.3 | 1.72 |
| T5-9a | L69S.17 | 96 | 31 | 19 | 24 | 22 | 44.B±5.0 | .94 |
| T6-9a | S.79L.40 | 86 | 18 | 23 | 28 | 17 | 40.7±5.2 | 289 |
| T6-9b | L.10S.37 | 64 | 18 | 13 | 11 | 22 | 45.3±6.2 | .59 |
| T7-9(4363) | .00.00 | 95 | 25 | 24 | 26 | 20 | 47.4±5.1 | .28 |
| T7-9a | L.63S.07 | 149 | 37 | 46 | 36 | 30 | 50.0±4.1 | 1.46 |
| T8-9d | L.09S.16 | 12 | 2 | 3 | 4 | 3 | 41.7±1.4 | .34 |
| T8-9(6673) | L.35S.31 | 98 | 35 | 14 | 36 | 13 | 49.0±5.1 | .05 |
| T9-10b | S.13S.40 | 310 | 121 | 47 | 38 | 104 | 27.4±2.5 | 63.11 |
| T9-10(8630) | \$.28L.37 | 410 | 107 | 93 | 100 | 110 | 47.0±2.5 | 1.42 |

The only statistically significant linkage is shown with T9-10b on 10S. This suggests that *Les*-A7145* is on the short arm of chromosome 10 left of the breakpoint of T9-10(8630).

This mutant originated as a spontaneous dominant necrotic mu-

tant found by Dr. Jack Beckett (his number was 9469C-3). It has been designated *Les*-A7145* in our cultures because the necrosis appears first as leaf lesions which enlarge and coalesce to form necrotic streaks.

Designation of Les21

--Neuffer, MG; England, D

Plants of Les*-1442 are yellowish green and grow slowly because of the many small chlorotic patches that appear below the leaf surface. Also fewer tiny round white necrotic lesions appear to arise from the chlorotic patches as the leaves mature. The necrotic lesions may enlarge and coalesce at senescence. Significant waxy translocation linkage data show no linkage to 22 of 23 translocation sites, covering the major portions of all 20 chromosome arms. This indicates that this mutant may be located in one of the regions not covered by these markers; namely, the distal end of 4S, 5S, 6L, 7S, 8L or 9L. The one missing test was for 7S. Since all the other designated lesion mutants have known locations, we have concluded that Les*-1442 could not be allelic to any of those; but an additional lesion site, which is designated Les21. The data are shown below:

Les*-1442:

| Marker | Breakpoint | # Plts | M.Wx | N Wx | Mwx | N.WX | I ± SE | chisq |
|-------------|------------|--------|------|------|--------|------|----------|-------|
| T1-9c | S.48L.22 | 198 | 53 | 46 | 49 | 50 | 48.0±3.5 | 32 |
| T1-9(4995) | L19S.20 | 136 | 33 | 36 | 38 | 29 | 45.6±4.3 | 1.08 |
| T1-9(8389) | L.74L.13 | 66 | 21 | 13 | 18 | 14 | 47.0±6.1 | 21 |
| T2-9b | S.18L.22 | 151 | 30 | 48 | 30 | 43 | 48.3±4.1 | .11 |
| T2-9c | S.49L.33 | 177 | 37 | 45 | 34 | 61 | 44.6±3.7 | 1.60 |
| T2-9d | L83L27 | 201 | 69 | 61 | 36 | 35 | 48.3±3.5 | .10 |
| T3-9(8447) | - S.44L.14 | 136 | - 29 | 28 | - 36 - | 43 | 47.1±4.3 | _ 37 |
| T3-9c | L.09L12 | 100 | 18 | 29 | 23 | 30 | 48.0±5.0 | 27 |
| T3-9(8562) | L65L22 | 81 | 22 | 18 | 19 | 22 | 45.7±5.5 | .61 |
| T4-9g | S27L27 | 124 | 32 | 32 | 29 | 31 | 49.2±4.5 | .03 |
| T4-9(5657) | L.33S.25 | 82 | 21 | 22 | 20 | 19 | 48.815.5 | .05 |
| T4-9b | L.90L29 | 270 | 69 | 69 | 65 | 67 | 49.6±3.0 | .02 |
| T5-9c | S.07L.10 | 114 | 29 | 30 | 26 | 29 | 49.1±4.7 | .04 |
| T5-9a | L69S.17 | 211 | 67 | 76 | 34 | 34 | 47.9±3.4 | .18 |
| T6-9a | S.79L.40 | 154 | 38 | 39 | 34 | 43 | 47.4±4.0 | .42 |
| T6-9b | L.10S.37 | 78 | 15 | 24 | 19 | 20 | 44.915.6 | .83 |
| T7-9(4363) | .00.00 | 150 | 23 | 52 | 32 | 43 | 44.0±4.1 | 2.33 |
| T7-9a | L.63S.07 | 148 | 16 | 65 | 16 | 51 | 45.3±4.1 | .37 |
| T8-9d | L09S.16 | 91 | 18 | 32 | 18 | 23 | 45.1±5.2 | .59 |
| T8-9(6673) | L.35S.31 | 121 | 35 | 41 | 23 | 22 | 47.1±4.5 | 29 |
| T9-10b | S.13S.40 | 255 | 53 | 75 | 57 | 70 | 48.2±3.1 | .31 |
| T9-10(8630) | S.28L.37 | 243 | 53 | 70 | 66 | 54 | 44.0±3.2 | 3.45 |

Mutant photographs on the World Wide Web

--Neuffer, MG

In the past few years I've been trying to get stocks of most of my maize mutant collection to the Stock Center. To make the collection more useful, we decided to make the information that had been accumulated about each mutant available on the World Wide Web. This was made possible, of course, by the fact that Ed Coe maintains MaizeDB (http://www.agron.missouri.edu), and I had access to the excellent services of the MaizeDB curator, Mary Polacco, and the database administrator, Denis Hancock. Our first order of business was to create forms for each of the mutants and enter as much information about each as possible.

By the time this was done, the technology had moved forward enough to make adding a picture of each mutant feasible. For many years I have been taking pictures (mostly slides) of maize mutants, accumulating over time about 8,000 slides. After a very laborious culling process, we had over 3,000 slides commercially transferred to Kodak Photo CDs (prices for this service vary widely, but we paid 88 cents per slide). We then cropped each image to reduce its size, and attached the image to the relevant MaizeDB variation

form. Other than cropping, the images were not edited or enhanced because there was no way to control the look of the image as it would appear on various browsers. (For those of you who are interested in the Photo CD technology, we have had very good success with it. Up to 100 images can be stored on each CD, and each image appears at 5 separate resolutions. If you have huge amounts of computer memory and a very good monitor, you can view the images at the highest resolution and zoom in to enlarge even the tiniest dots and sectors. Each PhotoCD comes with a "thumbnail" print, so a quick glance will show you what is on the disk. We have not done this, but the entire CD can be copied for about \$20.) Some of the images are not as good as I would have liked, but many of them are as good or better than the slide. If you go to the MaizeDB home page, you will see that the helpful curator has put a list of all these images under "Of Interest to Maize Cooperators" (http://www.agron.missouri.edu/NeufferImages.html).

The next project will be to improve the captions for the images and possibly to add the photos I have accumulated of other genes that are not in my mutant collection. We are still in the process of sending seed stocks to the Coop; they have now received stocks of

over 1300 mutants.

C1 is expressed at low levels in husks

-- Cooper, P; Cone, K

Genetic studies have established that members of the C1 / PI gene family act to induce anthocyanin production in a tissue-specific manner. The c1 gene controls pigmentation in the aleurone and embryo of the kernel, and pl controls pigmentation in the vegetative portions of the plant, such as husk, stem, leaves, anthers, and roots. Two indirect types of evidence have led to the notion that this tissue-specificity is due to tissue-specific transcription of these regulatory genes. First, sequence analysis reveals that although the coding regions of the two genes are very highly conserved, the 5' promoter regions are strikingly different (Cone et al., Plant Cell 5:1795-1805, 1993). Second, tissue-specificity is lost if c1 or pl expression is driven by a "constitutive" promoter such as the 35S promoter from cauliflower mosaic virus (P. Cooper, unpublished). These results, together with the genetic behavior of the regulatory genes, have led to the conclusion that c1 controls pigmentation in the kernel because c1 is transcribed only in the kernel; similarly, pl controls pigmentation in tissues other than the kernel because pl is transcribed only in those tissues. In this communication, we describe results that call for a more conservative interpretation of the mechanism for tissue-specific pigmentation.

We have been using reverse transcriptase-polymerase chain reaction (RT-PCR) to examine levels of transcript in husks from plants carrying different alleles of pl. For the amplification, we typically use a pair of primers that span the first intron of the gene. Because the sequences of pl and c1 are very similar in this part of the gene, the primers can amplify both pl and c1 sequences. Recently, we used these non-specific primers to quantitate mRNA in husks from plants carrying a putative null pl allele (pl-987). The results indicated a very low level of transcript relative to the progenitor allele, pl-W22. To ask if the transcript might arise from "ectopic" expression of the functional C1-W22 allele carried by these plants, we repeated the experiment with pl-and c1- specific primer pairs (Table 1). Not unexpectedly, the pl-specific pair detected transcripts in both the pl-987 and pl-W22 husks, although the levels were much lower in the pl-987 husks.

Table 1. Pigmentation patterns and expression of various pl and c1 alleles in husk and kernel tissue.

| | pigment | | pl n | RNA | c1 mRNA | |
|--------------------|---------|-------|------|-------|---------|-------|
| Genotype | husk | kemet | husk | kemel | husk | kemel |
| pl-987, C1-W22 | _ | + | + | nd | + | nd |
| pl-W22, C1-W22 | + | + | + | _ | + | + |
| PI-Rhoades, C1-McC | + | + | + | 2 | nd | + |

Surprisingly however, the c1-specific primer pair detected C1 transcripts in both samples. This result suggested that the C1-W22 allele in this line is normally expressed at low levels in husks, a tissue whose pigmentation is not genetically controlled by c1. We were also able to detect C1 transcripts in lines carrying a C1-McClintock (C1-McC) allele.

To test the possibility that pl might be expressed "ectopically" in kernels where pl, by genetic criteria, is not believed to act, we used gene-specific primer pairs to amplify transcripts from pigmented kernels from lines carrying pl-W22 or Pl-Rhoades alleles. In both cases, the c1-specific primer pair resulted in an amplification product but the pl-specific pair did not. These data indicate neither of these two pl alleles is expressed in kernels.

In an earlier publication (Cone et al., Plant Cell 5:1807-1816, 1993), we reported *pl* mRNA levels in husks from lines carrying different *pl* alleles--*Pl-Rhoades* and *pl-McClintock* (*pl-McC*). Because those experiments were carried out using primers that would amplify both *c1* and *pl* transcripts and both of the lines carried functional *C1*, the absolute levels of *pl* RNA reported earlier are probably overestimated. However, because the two lines carried the same *C1* allele, the relative levels of *pl* mRNA are still an accurate indicator of the differences in levels of *pl* activity.

The results presented here prompt a re-interpretation of the idea that the tissue-specificity of anthocyanin pigmentation is determined by on/off patterns of transcription of the c1 and p1 regulatory genes. Although we did not rigorously quantitate the level of c1 transcript in husks, it is roughly similar to the level of p1 transcript. Because the husks were unpigmented, the levels of both transcripts must be below the threshold required to activate the anthocyanin structural genes. Thus, even though c1 expression is technically "on" in these husks, the expression is not high enough to be physiologically significant in this tissue. This sub-threshold level of expression in husks therefore does not contradict the genetic interpretation of the c1 gene's lack of influence on anthocyanin production in vegetative tissues.

For RT-PCR, RNA was purified from kernels harvested 19-25 days after pollination or from inner husk leaves harvested from field-grown plants at the time of silk emergence. In most cases, poly(A)+ (approximately 1 µg) was used for the RT-PCR. RNA was converted to first-strand cDNA using a Gibco BRL Superscript Preamplification Kit. For PCR, samples were denatured for 5 min at 94 C and amplification was achieved by 35 cycles of denaturation at 94 C for 1 min, annealing at 54 or 55 C (depending on primer set), and extension at 72 C for 2 min. Final extension was at 72 C for 10 min.

Two sets of *pl*-specific primers were used. The first set was designed from sequences in the 5' region of the *Pl-Rhoades* gene. The upstream primer is located in the 5' untranslated region of the first exon (5'-ACCCTGCTGCTAGCTAGCTG-3') and the downstream primer is located near the 3' end of the second exon (5'-CTGTTGCCGAGGAGCTTGTG-3'). Amplification of properly spliced RNA with these primers yields a 316 bp product. The

second set of *pl*-specific primers was designed from sequences at the 3' end of the *pl-W22* gene. The upstream primer is located 53 bp 5' of the stop codon (5'-TCTCGAGTCCGACGAGG-3') and the downsteam primer is in the 3' untranslated region (5'-GTATACATACGCATGGCTA-3'). Amplification with these primers results in a 107 bp product.

One set of c1-specific primers was designed based on the sequence of a C1-W22 allele (Paz-Ares et al., EMBO J. 6:3553-3558, 1987). The upstream primer is located 53 bp 5' of the stop codon and is identical to the upstream primer from pl-W22 (5'-TCTCGAGTCCGACGAGG-3'). The downstream primer is located 20 bp 3' of the stop codon (5'-CCTCGTGCTTATTGGACA-3'). Amplification with this primer pair produces a 93 bp product from C1-W22 and a 369 bp product from pl-McC RNA. The larger size of the latter product is presumably due to a polymorphic insertion.

COLUMBIA, MISSOURI USDA-ARS and University of Missouri ATHENS, GEORGIA USDA-ARS TIFTON, GEORGIA USDA-ARS

Metabolic channeling suggested by QTL analysis of silk maysin and apimavsin concentration

--Byrne, PF; McMullen, MD; Snook, ME; Musket, TA; Xu, G; Lee, EA; Flint, S; Widstrom, NW; Wiseman, BR; Coe, EH

How does a cell maintain separate control over the synthesis of structurally similar compounds formed from common precursors? If enzymes and chemical precursors and intermediates were randomly diffused through the cytoplasm, such discrete control is difficult to envision. In contrast, the concept of metabolic channeling through membrane-bound multienzyme complexes (as presented by H. A. Stafford, Flavonoid Metabolism, CRC Press, Inc., Boca Raton, FL, 1990) accounts for the presence in a cell of an array of similar end products, exemplified by flavonoid compounds. In Stafford's model, flavonoid pathway enzymes are aligned on the endoplasmic reticulum, facilitating the transfer of metabolites from one enzyme to the next. The hypothesized final enzyme in the complex controls the transport of flavonoid glycosides into the vacuole or into vesicles that fuse with the vacuole, similar to the function of the glutathione-S-transferase encoded by bz2 (Marrs et al., Nature 375:397-400, 1995). Once sequestered in a specific complex, the flavonoid precursors and intermediates are unavailable to the action of enzymes in complexes with other end product specificities.

Results of our quantitative trait locus (QTL) study of silk maysin and apimaysin concentrations are consistent with the concept of metabolic channeling. Maysin and apimaysin are C-glycosylflavones that inhibit the growth of corn earworm larvae, Helicoverpa zea (Boddie). They differ by a single hydroxyl group at the 3'-position of the flavonoid B ring. Because the maize pr1 locus encodes a flavonoid-3'-hydroxylase that adds a hydroxyl group at this same position on anthocyanin molecules, we hypothesized that the pr1 locus also controls the 3'-hydroxylation of flavones, and therefore, the relative concentrations of maysin and apimaysin.

To test this hypothesis, we developed an F2 population from

the cross of inbred line GT114 (high maysin, low apimaysin) by line NC7A (high maysin, high apimaysin). Testcrosses had indicated functional *Pr1* in GT114 (purple aleurone in crosses to a recessive *pr1* tester), and a nonfunctional or reduced function *pr1* allele in NC7A (red aleurone in crosses with the *pr1* tester). In the summer of 1996 we grew 312 F2 plants, from which we collected leaves for RFLP analysis and silks for chemical evaluation via HPLC (see Byrne et al., Proc. Natl. Acad. Sci. USA 93:8820-8825 1996, for details of methodology). To date we have genotyped RFLPs at 53 loci distributed on 18 of 20 chromosome arms.

The F2 population displayed a broad distribution of values for both maysin and apimaysin concentrations (Table 1). Mean apimaysin concentration was about one-third that of maysin, but this ratio varied from near 0 to over 5. Surprisingly, maysin and apimaysin concentrations were not significantly correlated (r = 0.03).

Table 1. Mean and range of silk maysin and apimaysin concentrations of 312 (GT114 x NC7A) F2 plants and mean values for the parental lines and their F1 hybrid.

| | % fresh silk wt | | | | |
|-------------------------|-----------------------|-------------------|--|--|--|
| Entry | Maysin | Apimaysin | | | |
| F2 plants (mean) | 0.359 ± 0.020^{a} | 0.125 ± 0.010 | | | |
| F2 plants (range) | 0.003 - 1.376 | 0.000 - 1.272 | | | |
| GT114 | 0.274 ± 0.019 | 0.013 ± 0.002 | | | |
| NC7A | 0.487 ± 0.067 | 0.131 ± 0.029 | | | |
| (GT114 x NC7A) F1 | 0.326 ± 0.031 | 0.034 ± 0.017 | | | |
| a Moon + clandard orror | | | | | |

Single-factor analysis of variance detected a major QTL for apimaysin concentration in the pr1 region of chromosome 5L; the peak R² value (percent phenotypic variance explained) was 36.7% and occurred at locus bnl5.71 (Table 2). As predicted, plants homozygous for the NC7A allele at bnl5.71 had apimaysin concentrations much higher than GT114 homozygotes or heterozygotes (0.300% fresh silk weight vs. 0.054% and 0.061%, respectively). Gene action was dominant for low apimaysin, consistent with pr1 gene action in the anthocyanin pathway. Analysis of maysin concentration revealed only minor effects (maximum R2 = 3.2%) on chromosome 5 (Table 2).

Table 2. Significant (P < 0.01) chromosome regions in the single-factor analysis of variance of silk maysin and apimaysin concentration in the population (GT114 x NC7A) F2.

| | | % May | sin | % Apimay | sin |
|--------|--------------------|---------|-----------------|----------|----------------|
| Chrom. | Locus | P-value | B ^{2t} | P-value | B ₂ |
| 1L | umc128 | •• | 3.0 | | |
| 58 | umc90 | | | *** | 5.2 |
| 58 | umc107b | | | ***** | 8.1 |
| 58 | tub4 | | | ***** | 12.8 |
| 58 | bnl4.36 | •• | 3.2 | ***** | 17.6 |
| 5L | bnl5.71 (near pr1) | | | ***** | 36.7 |
| 5L | umc126 (near pr1) | | | | 34.9 |
| 5L | bnl5.24 | | | •• | 5.6 |
| 6L | npi393 | | | ** | 3.0 |
| 98 | umc109 | ***** | 10.9 | | |
| 98 | bz1 | ***** | 21.8 | | |
| 98 | wx1 | | 21.2 | | |
| 9L | umc95 | **** | 6.0 | | |

Significant at the 0.01, 0.001, 0.0001, and 0.00001 probability levels, respectively.

Percent phenotypic variance explained.

The only other region with major effects was on chromosome 9S, where results for the two traits were opposite those on 5L: a large effect on maysin concentration (peak $R^2 = 21.8\%$), but no significant effect on apimaysin level (Table 2). We have detected QTLs for maysin concentration in this same region of chromosome 9S, between bz1 and wx1, in three other populations (see following article).

Taken together, our results on chromosomes 5 and 9 suggest largely separate mechanisms for the synthesis and accumulation of maysin and apimaysin, such that one is not formed at the expense of the other. These results contrast with QTL analyses of compounds formed one from another along a linear pathway; Tanhuanpaa et al. (Theor. Appl. Genet. 91:477-480, 1995) found that the same chromosome region of spring turnip rape (*Brassica rapa* ssp. *oleifera*) increased seed oil content of palmitic acid and reduced oleic acid content, consistent with a single role for the detected QTL between the two compounds along the fatty acid biosynthetic pathway.

Some plants homozygous recessive for *pr1* had appreciable amounts of maysin, as did the parent NC7A (Table 1). This implies either that there is another 3'-hydroxylase gene (if the parental lines are not polymorphic at that locus, it would not be detected in our QTL analysis); or that the *pr1-NC7A* allele is capable of partial function. Specifications of that allele may affect 3'-hydroxylase abundance, conformation, or stability such that its function is reduced but not eliminated, and both maysin and apimaysin could be produced in individuals homozygous for that allele.

To interpret our results in terms of the multienzyme complex, one can envision the formation of two types of complex, one with and one without a 3'-hydroxylase. The relative frequency of the two complexes may be a function of 3'-hydroxylase concentration, physical characteristics that affect membrane binding, or variation in other enzymes of the complex that influences the rate at which the hydroxylase is recruited into the complex.

Stafford proposed that competition for precursors for different flavonoid end products occurs at the level of chalcone synthase, the first enzyme of flavonoid synthesis, or even earlier in the general phenylpropanoid pathway. Our unpublished results for another population, (GE37 x FF8) F2:3, show intriguing results for c2 and whp1, both of which encode chalcone synthase. The whp1 region had large effects on the concentration of maysin and on the sum of apimaysin and methoxymaysin (another maysin analog). However, the c2 region had a large effect on apimaysin + methoxymaysin, but no detectable effect on maysin. This suggests that the enzymes encoded by the two chalcone synthase loci have different affinities for flavonoid precursors with different Bring substitution patterns, or for the enzymes that catalyze those reactions.

Our data at first seemed at odds with previous observations on the relationship of maysin and apimaysin concentrations. Widstrom and Snook (unpublished) and Byrne et al. (unpublished) have found that over a broad range of germplasm, apimaysin concentrations are typically from 3 to 10% of maysin concentrations, and that the two compounds are highly correlated. This seems to be the standard situation, suggesting that even when functional *Pr1* is present, as in most inbred lines and populations, some of the time the hydroxylase enzyme does not become incorporated into the multienzyme complex. In contrast, the (GT114 x NC7A) F2 population has segregating *pr1* alleles, and apimaysin values much higher than normally encountered. It is in this situation that the inheritance of the two compounds appears largely independent.

Major QTL on chromosome 9S affects silk maysin concentration in four populations

--Byrne, PF; McMullen, MD; Snook, ME; Musket, TA; Lee, EA; Widstrom, NW; Wiseman, BR; Coe, EH

Our ongoing study of the genetic control of maysin synthesis in

maize silks has included QTL analysis of four populations to date (see Byrne et al., Proc. Natl. Acad. Sci. USA 93:8820-8825, 1996, for details of methodology). The most striking consistency is the detection in each population of a region on chromosome 9S significantly associated with maysin concentration (Table 1). The position of peak significance varied somewhat, but always fell in bin 9.02, between bz1 and wx1. In each case, the QTL has displayed dominance to partial dominance for low maysin concentration. The homozygous recessive genotype in this region has given a sizable boost in maysin level over the homozygous dominant genotype, ranging from a 49% increase in (GE37 x FF8) F2:3 grown at Columbia, MO to a 111% increase in (GE37 x Mp464) F2 (Table 1). The high-maysin allele was detected in three inbred lines (GT119, NC7A, and GE37), suggesting that it is fairly common in Southern U.S. germplasm.

Table 1. Genotype class means at chromosome 9S loci for silk maysin concentration in four populations. All trials were grown in Columbia, MO, except for population (GE37 x FF8) F2:3, which was grown both in Columbia, MO and Tifton, GA.

| Population/Genotype | Trait | (locus) |
|---------------------|----------------------|---------------------|
| (GT114 x GT119) F2 | % maysin (umc105a)* | (U.S. 0.500) |
| GT114 | 0.25 a¶ | |
| GT114/GT119 | 0.25 a | |
| GT119 | 0.46 b | |
| (GT114 x NC7A) F2 | % maysin (wx1) | |
| GT114 | 0.27 a | |
| GT114/NC7A | 0.31 a | |
| NC7A | 0.53 b | |
| (GE37 x Mp464) F2 | % maysln (bz1) | |
| Mp464 | 0.35 a | |
| GE37/Mp464 | 0.44 b | |
| GE37 | 0.74 c | |
| (GE37 x FF8) F2:3 | % maysin, MO (wx1) | % maysin, GA (wx1) |
| FF8 | 0.35 a | 0.24 a |
| GE37/FF8 | 0.39 a | 0.29 a |
| GE37 | 0.52 b | 0.42 b |

Loci in parentheses showed highest significance level. Indicated loci are on chromosome 9S in the order bz1 - umc105a - wx1. Distance between bz1 and wx1 ranges from 20 to 30 cM.

For a given population and trait, means followed by the same letter are not significantly different at P<0.05.

We currently have three main hypotheses to explain the nature of the gene on 9S:

1) We have previously proposed (Byrne et al., Proc. Natl. Acad. Sci. USA 93:8820-8825,1996), that the 9S locus is brown pericarp1, which we hypothesized to be a structural gene in the pathway leading to 3-deoxyanthocyanins. The homozygous recessive genotype blocks a step in that pathway, resulting in a shunting of intermediates to the flavone (maysin) pathway.

2) The gene on 9S is a negative regulator (inhibitor) of p1 expression. In the homozygous recessive class, this inhibition is released, resulting in enhanced p1 expression and hence, an increase in p1-controlled structural genes.

3) The 9S locus is a transcription factor that competes with or inhibits P1 protein from binding or activating the structural genes required for flavone synthesis. An analogy for this may be the IN1 protein which has sequence similarity to MYC-class transcription activators and down-regulates many of the structural genes of the anthocyanin pathway (Burr et al., Plant Cell 8:1249-1259, 1996). In addition, the MYB-homologous *zm38* gene has been shown to inhibit transcriptional activation of the *a1* promoter in the presence of functional C1 protein in transient expression assays (Franken et al., Plant J. 6:21-30, 1994). Experiments to differentiate among these hypotheses are currently underway.

Based on the consistent expression of an apparently single

QTL for enhanced maysin in all four populations examined, we wish to designate the corresponding genetic factor as *rem1* (recessive enhancer of maysin1), pending allelism tests with verified *brown pericarp1* stocks, if these become available.

COLUMBIA, MISSOURI USDA-ARS and University of Missouri NORWICH, ENGLAND John Innes Centre

Factors influencing efficiency of genetic mapping projects

--Wanous, MK; Snape, JW; Gustafson, JP

The development of restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) technologies have made it possible to construct highly saturated genetic maps as compared to maps utilizing classical morphological markers. Such maps have been constructed in human, as well as in model animal and plant species, and those of agricultural importance, including maize (Coe et al., MNL 69:247-267, 1995). Applications of molecular marker maps include gene tagging, analysis of synteny between species, analysis of quantitative trait loci, and map-based cloning. We have made some observations and calculations on strategies for map construction using different marker types and populations.

In constructing genetic maps, the amount of information generated depends on three factors: completeness of detection of recombinational events, linkage distance between loci, and number of individuals assayed. The first two factors are influenced by selection of parents for population construction and markers used. More polymorphism between parents and the utilization of more informative markers, increase the number of loci that can be mapped. Generally, the selection of parents for genetic map construction is optimized for maximum polymorphism between the parents. However, for specific applications such as gene tagging, where a specific population is used, the level of polymorphism may not be as high as for the initial mapping population. The recombination detected with different types of progeny populations varies.

F2 individuals completely classified with respect to linkage phase provide, on average, twice as much information as backcross individuals (Fig. 1; Mather, The Measurement of Linkage in Heredity. Methuen & Co., London, 1938, 1957). This is intuitively clear, given that an F2 individual possesses two meiotic products at a given locus rather than one as with a backcross progeny. However, the efficiency of mapping codominant markers (e.g., most RFLPs) in an F2 population ranges between that of a completely classified F2 and a backcross, depending on distance between markers (Fig. 1). This is because, with an F2 individual, two meiotic products are observed simultaneously, and some ambiguity occurs in that Ab/aB (two recombinant gametes) cannot be distinguished from AB/ab (two non-recombinant gametes) without progeny testing. The probability of a crossover having occurred in a given interval on both homologs of an F2 is the square of the probability of the event occurring once, which is proportional to the genetic length of the interval. Thus, the efficiency of mapping with codominant markers in an F2 population approaches that of a completely classified F2 population as the linkage distance between markers decreases (Fig. 1).

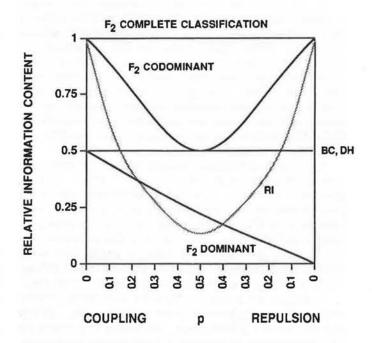


Figure 1. Relative information per individual (i) for different mapping population/marker type combinations. The population/marker type combinations are compared to the information content per individual of an F2 complete classification. Most RFLP markers are codominant Most RAPDs are dominant markers. BC = backcross; DH = doubled haploid; RI = recombinant inbred. p is the recombination frequency. For F2 complete classification, i = 2/p(1+p). F2 with codominant markers, $i = 2(1-3p+3p^2)/p(1-p)(1-2p+2p^2)$. BC and DH, i = 1/p(1-p). RI, $i = 2/p(1+2p)^2$. F2 with dominant markers, repulsion phase, $i = 2(1+2p^2)/(2+p^2)(1-p^2)$. F2 with dominant markers, coupling phase, replace (1-p) for p in above equation. After: Allard (Hilgardia 24:235-278, 1956); Mather (The Measurement of Linkage in Heredity, Methuen & Co., London, 1938, 1957); Reiter et al. (PNAS 89:1477-1481, 1992).

Random amplified polymorphic DNAs (RAPDs; Williams et al., NAR 18:6531-6535, 1990) are more cost- and time-efficient than RFLPs for small sample sizes (Ragot and Hoisington, TAG 86:975-984, 1993). However, when mapping dominant markers, such as RAPDs, in an F2 population, the efficiency is less than in a backcross population, but approaches this level as the linkage distance decreases if markers are in coupling phase. Markers in repulsion phase are not very informative (Fig. 1). In practice, when mapping many markers in an F2 or backcross population, mixed linkage phases will be encountered. This will affect overall mapping efficiency depending on the number of dominant markers used. Efficient approaches for mapping RAPDs include using backcrosses, doubled haploid (DH) populations and recombinant inbreds (RIs). The information content of these population types with dominant markers is unaffected by linkage phase. RIs are more informative when the distance between markers is ≤ 12.5 cM (Taylor, Recombinant inbred strains: use in gene mapping, in: Morse (ed.) Origins of Inbred Mice, pp. 423-438. Academic Press, New York, 1978).

A backcross population is more informative when greater genetic distances are involved (Fig. 1). A DH population is genetically equivalent to a backcross population derived from backcrosses to a completely recessive parent: one meiotic event is analyzed per individual. DH and RI mapping populations possess an additional advantage in that once constructed, they represent a practically inexhaustible 'immortal' population that is easily maintained and distributed to many collaborators. This facilitates interval mapping of clones of interest by merely mapping them on

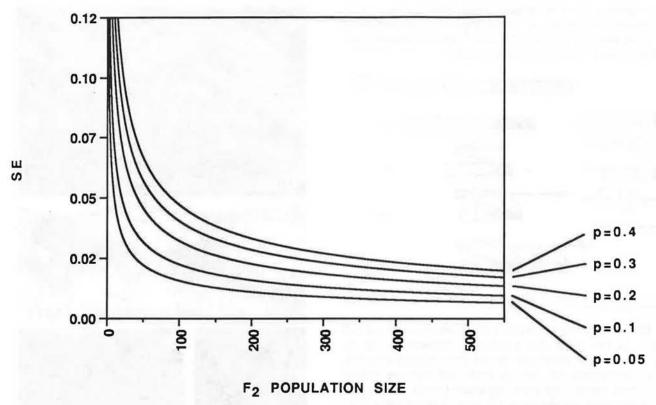


Figure 2. Standard error (SE) of recombination frequency (p) for codominant markers in an F2 mapping population. SE of p decreases as the number of individuals in the population is increased. SE of p decreases as p decreases, which, on average, is a function of the number of loci mapped. $i = 2(1-3p+3p^2)/p(1-p)(1-2p+2p^2)$. SE = $\sqrt{[1/n]}$ where n = number of individuals in the family. Derived from Allard (Hilgardia 24:235-278, 1956).

the immortal population. The genetic location can then be determined in relation to all other markers that have been previously mapped in the population.

For a given interval, two factors determine the standard error (SE) of the recombination fraction estimate (p): the magnitude of p itself and the number of individuals in the population (Fig. 2). The average p value is a function of the number of markers mapped. Obviously, increasing the number of individuals assayed will decrease the SE of p. The desired precision of the p estimate determines the number of progeny that should be included in a mapping project. In Figure 2, with p = 0.1 the slope of the curve is -1.0×10^{-4} at n = 108; with p = 0.05 the slope of the line is -1.0 x 10⁻⁴ at n = 86. Above these progeny numbers there is a diminishing return in precision gained by additional progeny. As population size moves below these thresholds the SE begins to increase (precision decreases) at a greater rate.

In planning a mapping project there are several factors to consider: i) types of populations available in the species being studied; ii) type and number of marker that will be used; and iii) number of individuals to be included in the mapping population. These factors determine the information content, precision, cost and time efficiency in generating genetic maps and should be considered in designing mapping projects.

CORVALLIS, OREGON Oregon State University

The maize Tousled-Like Kinase gene family

--Yoon, E; Rivin, C

The Tousled gene of Arabidopsis is essential for normal development of the plant (Roe et al., Cell 75:939, 1993). Mutants in tousled display mild vegetative abnormalities, but are severely impaired in floral development, having a variable, reduced number of floral organs and an unfused gynoecium. Tousled encodes a member of a novel class of serine-threonine kinases. The protein has an N' coiled-coil region, three nuclear localization signals, and a C' catalytic domain. TOUSLED kinase appears to be necessary for the proper maintenance and partitioning of the Arabidopsis shoot apical meristem.

Dr. Tim Helentjaris isolated a partial cDNA with very high homology to the catalytic domain of TOUSLED from developing maize seeds (MNL 69:232, 1995). This cDNA hybridizes to multiple bands on Southern blots, indicating that this gene is a member of a small gene family in maize. This gene and its homologues are the Tousled-Like Kinases, or TLK genes.

We used the maize cDNA from Helentiaris to screen an ear shoot cDNA library and a genomic library. We isolated a slightly longer cDNA of the same gene as Helentjaris' clone. Our cDNA encompasses the entire catalytic domain. In addition, we isolated genomic clones representing four distinct genes, including the one encoding the cDNAs. We have sequenced portions of the catalytic domains of each of these genes. They are strikingly similar, with over 92% identity to each other and over 85% identity to TOUSLED at the amino acid level. There is also conservation of

gene structure. Three of the genes contain introns in the same locations as each other and as *Tousled* (Figure 1). The sequence of the 3' untranslated region and the most 3' intron is also quite conserved in maize. The fourth gene lacks introns.

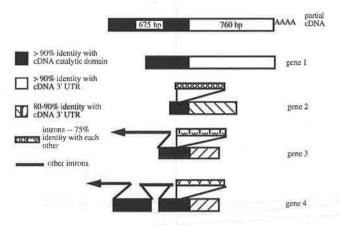


Figure 1. Sequence and structure of TLK genes. Sequenced portions of genes and a partial cDNA are shown,

We have detected very low level expression of at least one *TLK* gene. Northern blots show a transcript of between 2.5 and 3.0 kb. This size is consistent with the size of the *Tousled* transcript. Due to the extremely high homology of the maize genes, we have not determined whether this signal represents a single transcript, or the expression of more than one *TLK* gene. However, both cDNAs isolated correspond to the gene which lacks introns, indicating that this gene is expressed in both developing inflorescences and seeds.

Tousled is a single gene in Arabidopsis, but the TLK genes of maize form a small gene family. The sequenced portions of all four genes contain open reading frames encoding potentially functional kinases. We are interested in the function of these kinases in maize development and in whether the individual members of this gene family have redundant or distinctive roles. We are currently analyzing the non-catalytic domains of these genes. These regions are likely to be more divergent and may allow specific regulation of each of the TLK proteins. We have also initiated a TUSC reverse genetics search to obtain mutants in these loci.

DAVIS, CALIFORNIA University of California at Davis

Identity of extra cell-layers produced in the glossy* mutation in maize

-- Kessler, S; Sinha, N

The *glossy** mutant (Fig. 1A) arose in a mutator transposon background. It was identified by a spray test, but later determined to be a morphological mutant rather than a wax mutant (Schnable et al., Maydica (39) 1994: 279-287). The mutation is characterized by extra layers of cells (Fig. 1B) in leaves. We wished to ascertain the identity of these extra cell layers. Mutant leaves were examined under Scanning EM. Juvenile *glossy** leaves have both normal and abnormal regions of epidermal morphology and organization. The *glossy** regions have cells that are abnormally shaped (not regular rectangles) and are not as highly crenulated (Fig. 1D) as normal epidermal cells (Fig. 1C). The stomatal

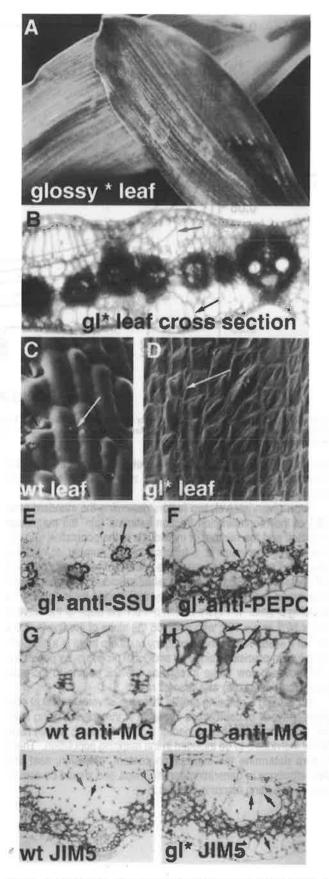


Figure 1. A: Juvenile leaf of glossy* plant showing regions of abnormal leaf surfaces. B: Transverse section of gl* leaf showing regions of extra cell layers (arrows). C: SEM of a normal

leaf surface showing cells with crenulated margins (arrows). D: SEM of gl^* leaf surface showing abnormal cells with no crenulated margins (arrow). E: Transverse section of gl^* leaf probed with anti-SSU antibody showing staining in the bundle sheath cells (arrow). F: Transverse section of gl^* leaf probed with anti-PEPC antibody showing staining in the mesophyll cells (arrow). G: Transverse section of wild-type leaf probed with anti-MG antibody showing staining in the epidermal cells (arrow). H: Transverse section of gl^* leaf probed with anti-MG antibody showing staining in the epidermal cells and extra cell (ayers (arrow). I: Transverse section of wild-type leaf probed with JIM5 antibody showing staining in the comers of epidermal and midrib cells (arrow). J: Transverse section of gl^* leaf probed with JIM5 antibody showing staining in the epidermal cells and extra cell layers. However not all cell junctions are stained (arrows).

organization also seems to be confused, stomates are found randomly throughout *glossy** regions instead of in definite, regularly spaced rows. The surface waxes of juvenile leaves in *glossy**

plants seem to be different from wild-type waxes.

We also performed immunolocalization experiments (immunogold with silver enhancement, followed by counterstaining with basic fuschin) to determine the identity of the extra cells found in glossy* regions of the leaf. We used two different kinds of antibodies, antibodies to photosynthetic proteins and antibodies to cell wall compounds. Antibodies to Small Subunit of RuBisCo (SSU) bound to chloroplasts in the bundle sheath cells in both the wild type and glossy* sections (Fig. 1E). The extra cell regions in the glossy* leaf did not stain, indicating that these cells probably do not have photosynthetic activity or bundle sheath identity. Antibodies to Phosphoenol Pyruvate Carboxylase (PEPC) bound to the mesophyll cells in both wild type and glossy* sections (Fig. 1F). The extra cell regions did not stain, indicating that the extra cells are not active in C₄ photosynthesis and do not have mesophyll identity. The anti-MG antibody recognizes mixedlinkage glycans in the cell wall of certain cells. Anti-MG stained the cell walls of the epidermal cells, midrib cells, and xylem in vascular bundles of both the wild type (Fig. 1G) and glossy* leaves (Fig. 1H). The bundle sheath and mesophyll cells did not show reactivity to the anti-MG antibody. The extra cells in glossy* leaves stained in their cell walls. This indicates that the cell walls of the extra cells are more like epidermal or midrib cells than bundle sheath or mesophyll cells, thus confirming the results from the photosynthetic antibodies. JIM5 is a monoclonal antibody against unesterified polygalacturonic acid and mostly decorates the corners where two epidermal cells join together. Therefore, we used this antibody to determine if the extra glossy* cells have epidermal identity. The corners of epidermal cells stained in both wild type (Fig. 11) and glossy* leaf sections (Fig. 1J). In addition, the midrib cells also stained in the corners. The extra glossy* cells, however, did not show this same pattern of staining. Therefore, we can conclude that the extra cells in the glossy * mutation do not have bundle sheath, mesophyll or pure midrib cell identity. They are also not identical to epidermal cells.

> DEFIANCE OHIO Defiance College

Initial conditions during tassel determination control the level of R-allele silencing (paramutation)

--Mikula, BC; Kappen, T

Paramutation, gene silencing for the *r* locus, has been reported, over the past 40 years, as pigment scores determined by sampling kernels from testcross ears. Kernels were matched against standard kernels representing varying grades of pigment from colorless to completely pigmented.

Score variations from year to year and experiment to experi-

ment have been attributed to environmental conditions or stochastic variation. In 1967 I showed a relationship betweeen pigment scores and environmental conditions which plants received during the third and fourth week of seedling development, the time of tassel determination. Experiments since 1967 have been directed toward exploring developmental intervals during which paramutation could be influenced, in seedlings, by temperature and light conditions.

It was found that maximum silencing, as judged by the lowest kernel pigment scores, was achieved for seedlings started in continuous light, days 1-10, and maintained at 32 C LL (continuous light), days 11-15. Seedlings started at 22 C LL, days 1-15, then switched to 32 C LL, days 16 to 21, showed similar reduced pigmentation.

The data of Table 1 explore the developmental interval affecting paramutation within days 11-15 in 32 C conditions and the days 16-21 at 22 C. Seeds of a heterozygote *R-g/r-m* (*r-m* weakly paramutagenic mutant from *R-st*) from a single ear were used in all the experiments. 50 kernels from six to eight testcross ears, representative of latest pollen samples, were scored by matching each seed against a set of standard kernels ranging from one, colorless, to 21, completely pigmented. Seedlings were held in continuous light except for the days given LD (12hr. light/12hr dark) conditions. At the end of each experiment at 15 days or at 21 days seedlings were transferred to field conditions for maturity to be testcrossed at anthesis.

Table 1. The role of temperature and light/dark cycles in the control of R-allele silencing under paramutagenic conditions following germination under 32 C and 22 C LL conditions.

| Days 1-10 | Days 11-15 | Score | Line |
|-----------|-------------------|-------|------|
| LL 32 C | 5LL 32 C | 8.1 | 1 |
| LL 32 C | 5LL 32 C 6LD 22 C | 7.6 | 2 |
| 10LL 32 C | 1LD 22 C 4LL 32 C | 7.2 | 3 |
| 10LL 32 C | 2LD 22 C 3LL 32 C | 11.5 | 4 |
| Days 1-15 | Days 16-21 | Score | Line |
| LL 22 C | 6LL 32 C | 8.8 | 5 |
| | 6LD 22 C | 13.4 | 6 |
| | 6LL 22 C | 11.8 | 7 |
| | 4LD 22 C 2LL 32 C | 13.5 | 8 |
| | 3LD 22 C 3LL 32 C | 13.4 | 9 |
| | 2LD 22 C 4LL 32 C | 11.2 | 10 |
| | 4LD 22 C 4LL 32 C | 13.8 | 11 |

Most silencing occurred in seedlings given 32 C LL conditions days 11-15, lines 1-3, Table 1. Similar scores were found following 15 days at 22 C if followed for six days, days 26-21, by 32 C LL conditions, line 5. In lines 2 and 3, LD conditions applied after 32 C LL had no effect on silencing. In line 3 a single LD cycle, day 11, had no effect on the pigment score, however, two LD cycles applied days 11-12 showed nearly four scoring units of reduced silencing, line 4, compared to line 2.

Plants started in 22 C LL conditions for days 1-15 showed least silencing (highest pigment scores) when LD cycles were applied days 16-21, line 6. If seedlings were given 6LL cycles at 22 C, days 16-21, a reduction of nearly two scoring units was found, line 7. The LL 32 C cycles applied within the 16-2l-day interval in line 5, showed no increased silencing when applied after 4 or 3 LD 22 C cycles, lines 8 and 9. When the number of LD cycles was reduced to two, days 16-17, and the number of 32 C LL cycles increased to four, increased silencing was found, line 10. Line 11 shows that 4 LL 32 C cycles following 4LD 22 C cycles produced the same pigment scores reported for lines 6, 8 and 9.

It is inferred from the table that developmental conditions fa-

voring increased *R*-allele silencing from a paramutagenic allele are available, following 32 C LL conditions, days 11-15 or a week later following 22 C LL conditions, from day 16-21. The table also shows that two LD 22 C cycles can influence silencing if applied days 11-12, line 4, or a week later, days 16-17, line 10. It is inferred the epigenetic conditions determining paramutation are temperature related and are separated by a week at the two temperatures of 32 C and 22 C. The source of variation in pigment scores from year to year becomes apparent when considering that much of the work in paramutation has depended on pollinations from field grown material. Given early spring growing conditions, variations in levels of gene silencing are to be expected from the data of the table since two-day intervals at the critical stage of tassel determination can bring about a change in silencing of four scoring units.

In MNL 70 we reported that seedlings from 22 C LD conditions, similar to line 6, showed nearly the same extremes of score variation as reported in the above table when pollen for testcrosses was sampled from a single plant. The earliest pollen collections from a single plant showed testcross scores significantly lighter than the last pollen collections made from the lowest tassel branches, seven days later. This means that under certain early spring temperature conditions considerable variation in pigmentation can be expected from the pollen samples of tassels of single plants over the seven days of anthesis.

Given this variation for the r-locus, under paramutagenic conditions, it is not surprising that geneticists in the middle of this century considered unstable genes "sick". The system shows a response to early temperature conditions resulting in pigment patterns in testcrosses that show gradients of R-allele expression when pollen is sampled and tested over the seven days of anthesis. The variation in pigment expression from single pollen samples was easily assumed to be stochastic rather than epigenetically determined. The stability of the Mendelian gene may undergo some revision as more unstable genes are explored in a developmental con-

The instability of the aleurone pigment expression in paramutation appears to be a product of developmental processes sensitive to the "initial conditions" of tassel determination (before tassel initiation). From the data above this period could involve an experimental window of two to three days. It would be interesting if the aleurone pigment expression controlled by *Spm*, *Mu* and *Ac*, reported by Walbot (Science 248:1534-1537) and Fedoroff (Genetics 121:591-608), might also be related to this specific period of tassel determination.

Because the *r*-allele can be silenced, incrementally, from generation to generation, genetic systems that show incremental silencing provide model systems for investigating how the environment can change Mendelian gene expressions. The role of the environment in genetics has been neglected throughout this century as pointed out by Jablonka and Lamb in their book, Epigenetic Inheritance and Evolution, 1995, Oxford Univ. Pr. Perhaps maize can provide systems where the role of the environment in gene stability can be examined. The longer developmental periods of maize provide larger "windows" for the dissection of developmental processes and their relationship to temperature and light. It may be considered that using a weakly paramutagenic allele, as well as the lower temperature of 22 C, attenuates the developmental process involved in paramutation making it possible to see results in pollen samples as recorded in Table 1.

DHAULAKUAN, INDIA Himachal Pradesh Agricultural University

Screening for bacterial stalk rot resistance in corn

--Kalia, V; Basandrai, AK; Singh, A

Bacterial stalk rot of maize caused by *Erwinia chrysanthemi* pv. *zeae* is a soft rot appearing at tasseling initiation stage leading to partial to complete death of the plant. This disease is a major problem in sub-tropical areas in low hills and valleys of the outer Himalayas where maize is predominantly grown during the wet season. The disease is reported to cause yield losses up to 85 percent (Lal et al., Ind. Phytol. 23:156-157, 1970). A temperature of 30-35 C and a relative humidity of 90 percent were found to be necessary for maximum disease development (Thind and Payak, Phytol. Medit. 17:59-63, 1978; Saxena, Ph.D. thesis, 1982). Thus, hot and humid conditions favour the disease incidence.

No definite sources of resistance against bacterial stalk rot are known within corn (*Zea mays* L.) although Sharma, Payak and Sachan (MNL69:111, 1995) reported resistance in allied genera of *Trilobachne*, *Chionachne* and wild Coix-25. The present studies were conducted to find out the sources of resistance within maize, and 111 inbred lines were screened under artificial inoculation during the wet season of 1995 at Regional Research Station, Dhaulakuan, Himachal Pradesh, India, which is a known hot spot for the disease. Inoculation was carried out using the syringe method, injecting 2 ml of freshly prepared bacterial cell suspension in the 2nd internode from the ground of each plant at pre-tasseling stage. Incidence was recorded after 25 days of inoculation. Of 111 inbreds, four (DK-3, DK-9, CM-119, 94067) were immune (Table 1) while one (DKI-9304) gave highly resistant reaction

Table 1. Incidence of bacterial stalk rot in different inbred lines of corn.

| Reaction | Percent incidence | Number of inbreds | Pedigree |
|--------------------|-------------------|-------------------|--|
| Immune | 0 | 4 | DK-3, DK-9, CM-119, 94067 |
| Highly resistant | <10% | 1 | DKI-9304 |
| Resistant | 10-30% | 14 | DKI-9418, DKI-9321, DKI- 9505, DKI-9536, DKI- 9412, DKI-9401, CM-209, CM-300, CM-210, L21, Bulb-13-2-1-1, Pool 27 (HGR), Pool 20 (Ht, R), GE- 440 (Ht1 Hiz), 94065 |
| Susceptible | 31-50% | 44 | 5.5 |
| Highly susceptible | 50% | 48 | |

(<10 percent). Fourteen inbreds were found to be resistant (10-30 percent), while the remaining were susceptible (44 inbreds) to highly susceptible (48 inbreds). Inbred lines showing immune and highly resistant reactions will be subjected to further genetic studies and used in breeding.

Contribution of inbred lines by various centres of All India Coordinated Maize Improvement Project is gratefully acknowledged.

DNIEPROPETROVSK, UKRAINE Dniepropetrovsk State University

Fluorescence test for selection of high-tryptophan genotypes of maize

--Fedenko, VS; Struzhko, VS; Tikhomirov, AYe

A distinctive feature of maize is that seeds are not balanced in protein composition and vital amino acids, such as tryptophan and lysine. To create maize with improved protein quality (QPM) a ge-

text.

netic method based on the endosperm *opaque-2* mutation is usually used. The problem of estimating the quality of the selection material is characterised by these methods. Presence of the *o2* gene is shown in the lowering of the portion of zein that does not have tryptophan, and increase of the relative portion of the tryptophan carrying fraction of protein. Thus, the index of tryptophan contained in grains may be used as an integral feature of protein balance improvement.

Accordingly, we seek an express-method for selection of hightryptophan maize genotypes based on fluorescence parameters of cut or milled grains. The suggested diagnostic criterion is based on optical features of QPM forms. It has long-wave displacement of ultraviolet fluorescence compared with ordinary analogs. As an object for investigation, lines based on o2 mutation and o2 double recessive have been used with forms that have endosperm modified type and high-lysine hybrids. It has been found that a fluorescence maximum of grain cut for ordinary hybrids is in the interval 312.7-313.3 nm; for high-lysine analogs this maximum is in the 327.7-329.0 nm range. As compared with other diagnostic tests that are well-known, it is possible to analyze an individual kernel without any biochemical reagents. One of the main important notes is that a grain is vital after analysis. This fluorescence test can carry out individual screening of high tryptophan grains and include them in another cycle of selection programmes.

DNIEPROPETROVSK, UKRAINE Institute of Grain Farm

Relationship between cytological and cultural indices of com anther culture ability

--Satarova, TN; Chernousova, NM

It is known that success in maize anther culture strongly depends on genotype with many genotypes being unresponsive. The early and rapid diagnosis of anther culture ability could help to find responsive genotypes among a great number. As the transition of a microspore to the sporophytic pathway of development is necessary for embryoid and callus formation, cytological determination of the amount of microspores having divided in an unusual manner, with embryoid and/or callus initiation, could characterize the androgenic ability of a genotype. Such characteristics, however, may be true only in case of strong correlation between the percentage of induced pollen grains in the initial period of cultivation (%IPG) on the one hand, and the percentage of responding anthers (%Resp.A), and the number of embryoids produced per 100 anthers plated (E/100A), on the other.

In this investigation the correlation between %Resp.A, E/100A, and %IPG was determined. Anthers for culture in vitro were taken from tassels of field donor plants in summer 1995 and after the cold pretreatment during 14 days at 8 C were planted on the medium UP (Genovesi and Collins, Crop Sci. 22:1137-1144, 1982). The first experiment was carried out for the responsive hybrid Wf9xLH148. Its anthers were plated at the stages of the uninucleate vacuolated pollen grain and the young binucleate pollen grain, before the generative cell migration. The second experiment included 9 genotypes, whose anthers were plated only at the young binucleate stage. %IPG was determined on the 8th and 15th days of culture by counting the number of promorphogenic pollen grains and different structures which could be the result of the induction of the sporophytic pathway. The following structures

were found in our material: bicellular pollen grains with abnormal position of cells (equal division in microspore), pollen grains in which one or several mitoses in the generative and/or vegetative cell occurred, multicellular pollen grains, coenocytic structures, young embryoids, young calli. The count of the number of the induced pollen grains was made for 6 anthers per tassel for Wf9xLH148. 1000 pollen grains for each anther were examined, then the average of six determinations was found. For the experiment with 9 genotypes, 6 anthers from 2-3 tassels for each genotype, 1000 pollen grains for each anther, were examined, then the average was found. %Resp.A and E/100A were determined on the 42nd day of culture.

The results of the first experiment for Wf9xLH148 are shown in Table 1. Correlation coefficients between %Resp.A and %IPG and between E/100A and %IPG (the 15th day of culture) for the uninucleate stage and for the young binucleate stage (the 8th day) were not significant.

For the young binucleate stage (the 15th day of culture) coefficients of correlation between the variables mentioned above were significant and high (0.824; 0.822), so the strong correlation is confirmed. Perhaps, it is very difficult to distinguish visually promorphogenic pollen grains on the 8th day of culture, some of them may not have started their development yet. For the stage of uninucleate vacuolated pollen grains, when the process of induction is unstable as a whole and is being realized with lower efficiency (Satarova, Izv. Akad. Nauk Ser. Biol. 5:771-778, 1994), visual estimate of induced pollen grains may be also difficult.

Taking into account all the reasons above it is necessary for future estimation of the androgenic ability of a genotype to determine the percentage of induced pollen grains on the 15th day of cultivation with the anthers having been plated at the stage of the young binucleate pollen grains.

Table 1. Correlation between the percentage of responding anthers (%Resp.A), the number of embryoids produced per 100 anthers plated (E/100A) and the percentage of induced pollen grains (% IPG) for the corn cross Wf9xLH148.

| Plant number | % Resp.A | E/100A | %IPG | Coefficients of correlation |
|--------------|-------------------|-----------------|---------------|-----------------------------|
| Th | e stage of the un | inucleate vacu | olated poller | grains, 15th day |
| 1 | 1.43 | 1.91 | 0.20 | 186 |
| 2 | 1.00 | 1.00 | 0.59 | |
| 3 | 0 | 0 | 0.13 | |
| 4 | 0 | 0 | 0.02 | r 12 = 0.988* |
| 5 | 0.40 | 0.40 | 0 | r 13 = 0.580 |
| 6 | 0 | 0 | 0.18 | r 23 = 0.483 |
| 7 | 0 | 0 | 0 | |
| Average | 0.40 | 0.47 | 0.16 | |
| | The stage of the | e vouna binucle | eate pollen a | rain. 8th day |
| 1 | 0 | 0 | 0.1 | |
| 2 | 7.39 | 9.85 | 0.15 | |
| 3 | 1.43 | 1.43 | 2.61 | r 12 = 0.985* |
| 4 | 7.23 | 12.05 | 0.70 | r 13 = - 0.014 |
| 5 | 1.23 | 1.23 | 0.03 | r 23 = -0.047 |
| 6 | 5.48 | 7.31 | 2.03 | |
| Average | 3.79 | 5.31 | 0.94 | |
| | The stage of the | young binucle | ate pollen qu | rain, 15th day |
| 1 | 7.23 | 12.05 | 4.38 | |
| 2 | 7.39 | 9.85 | 4.01 | |
| 3 | 1.54 | 1.54 | 2.92 | |
| 4 | 0.76 | 0.76 | 1.00 | r 12 = 0.990* |
| 5 | 3.33 | 3.33 | 1.20 | r 13 = 0.824* |
| 6 | 0.59 | 0.59 | 1.54 | r 23 = 0.822* |
| 7 | 1.23 | 1.23 | 2.78 | |
| 8 | 10.24 | 15.61 | 4.04 | |
| 9 | 0 | 0 | 0.02 | |
| 10 | 5.48 | 7.31 | 2.63 | |
| 11 | 0 | 0 | 0.17 | |
| Average | 3.44 | 4.75 | 2.25 | |

^{*}The coefficient of correlation is significant, P<0.05

In the second experiment we determined correlation between %Resp.A and %IPG and E/100A and %IPG for different corn genotypes (Table 2). Correlation coefficients were significant and high (0.827; 0.776) and this also proves the strong correlation between cytological (%IPG) and cultural (%Resp.A, E/100A) indices of androgenic ability. Genotypes which had %IPG being more than zero, were responsive in anther culture.

Table 2. Correlation between the percentage of responding anthers (%Resp.A), the number of embryoids produced per 100 anthers plated (E/100A) and the percentage of induced pollen grains (%IGP) for different com genotypes (the stage of the young binucleate pollen grains).

| Genotype | %Resp.A | E/100A | %IPG 15th day | Coefficient of correlation |
|-------------|---------|--------|------------------|----------------------------|
| WI9xLH148 | 2.46 | 3.39 | 2.35 | |
| And 44 | 12.14 | 29.57 | 2.71 | |
| H99xWf9 | 1.32 | 1.70 | 0.21 | |
| Wf9xH99 | 3.78 | 6.33 | 1.03 | |
| 3633xAnd44 | 0 | 0 | 0 | r 12 = 0.992* |
| LH59xAnd44 | 0 | 0 | 0 | r 13 = 0.827* |
| M377 | 0 | 0 | 0 | r 23 = 0.776* |
| 502xH99 | 0 | 0 | 0 | |
| And44xLH148 | 2.78 | 6.85 | 0.71 | |

*The coefficient of correlation is significant, P<0.05

Thus, the suggested approach allows taking in culture and analysis a minimum number of anthers during one vegetation to differentiate the large circle of corn genotypes and to pick out those with a potential for androgenesis.

The induced pollen grains in a great amount are contained in cultured anthers at the end of their cultivation, on the 42nd day of culture, when all the formed embryoids and calli are removed from an anther. Table 3 demonstrates that up to 5.42% of pollen grains in an anther (for B14xNf9, and in some lower rate for other genotypes) have entered the sporophytic pathway of development and formed the high unrealized androgenic potential of the anther.

Table 3. The percentage of induced pollen grains (%IPG) on the 42nd day of anther culture.

| Genotype | %IPG |
|-----------|------|
| WI9xLH148 | 3.70 |
| H99xWf9 | 0.65 |
| Wf9xH99 | 0.13 |
| B14xWf9 | 5.42 |

DULUTH, MINNESOTA University of Minnesota

Detect DNA contents in microsporogenesis of maize mutant --Liu, Q; Splett, M

The early work on cellular analysis of maize postmeiotic mutant po and alleles showed that premature chromosome condensation and fragmentation occurs at the end of meiosis II in microsporogenesis in po and alleles. Abnormal cell cycles occurred without chromosome replication in this mutant and its alleles (Liu et al., J. Cell Sci. 106:1169-1178). These chromosomal defects do not affect cell wall matrix formation during microspore development. Fluorescence cytometry has been used to detect DNA contents in microspores of po and wild type plants by using 4',6-diamidino-2phenylindole (DAPI, a fluorescent DNA label) and absorption cytophotometry with technical help from Dr. R. V. Kowles. Maize samples were collected and fixed with ethanol/acetic acid (3:1) at different stages of anther development. These microspore samples were isolated from anthers by using a dissecting microscope for fluorescence cytometric analysis. Results of the analysis indicate there is no DNA duplication before abnormal cell divisions and

DNA content was decreased during cell cycle progression in microspore development (Fig. 1 and 2). Correlated to such DNA content, cell size was also reduced during the formation of abnormal microspores (data not shown). This result will be confirmed by developing a new method using BrdU in vitro DNA labeling.

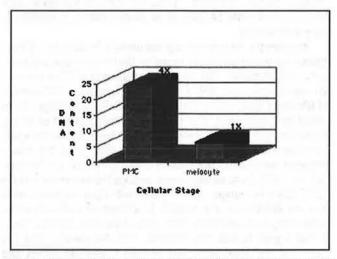


Figure 1. Fluorescence cytometry has been used to detect DNA content in pollen mother cells and individual melocytes during tetrad formation in the wild type plant as a control. Samples were then labeled with DAPI stain on the same slide and at least ten cells were detected and average measurements were used for their DNA contents. DNA content was about four times higher in maize pollen mother cells than that of individual melocytes in tetrads of the wild type plant.

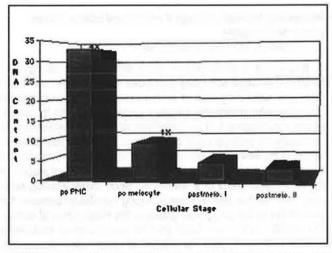


Figure 2. DNA content was measured for the samples on the same slide at different stages of abnormal cell cycles after meiosis in polmutants using the methods described in Figure 1. There was no DNA duplication in polmeiocytes before abnormal postmeiotic divisions. DNA contents were gradually decreased from polmeiocytes to cells in postmeiotic cell divisions I and II. Cell size was also reduced (data not shown).

DURHAM, NORTH CAROLINA Duke University

Further evidence for two progenitors in the origin of maize --Eubanks, MW

The most popular hypothesis for the origin of maize (*Zea mays* L.) today is that teosinte (*Zea mays* ssp. *mexicana*) is the wild progenitor of maize (Doebley, Econ. Bot. 44 (Suppl.):6-27, 1990; Iltis, Science 222:186-188, 1983; Galinat, Econ. Bot. 49:3-12,

1995). Fertile plants derived from crossing diploid perennial teosinte (*Zea diploperennis* Iltis, Doebley and Guzmán), hereafter referred to as diploperennis, and Eastern gamagrass (*Tripsacum dactyloides* L.), however, revived the possibility that the earlier hypothesis of Mangelsdorf and Reeves (TX Agr. Sta. Bull. 574,1939) proposing maize arose from intergeneric hybridization involving *Tripsacum* is the more likely scenario (Eubanks, Econ. Bot. 49:172-182, 1995).

Successful reciprocal crosses have been obtained using a tetraploid *T. dactyloides* and *Z. diploperennis* (MNL 67:39, 1993), and more recently with a diploid *T. dactyloides* and *Z. diploperennis*. Restriction fragment length polymorphism (RFLP) analysis of Southern blots hybridized with maize probes showed *Tripsacum* X diploperennis plants inherit unique restriction sites from both parents that are stably inherited in F2 progeny (Eubanks, Theor. Applied Genet. 1997, in press).

Findings reported here address two propositions that further test the hypothesis of a role for intergeneric hybridization in the origin of maize: (1) If *Tripsacum* was involved in maize evolution, unique restriction sites from *Tripsacum* should occur in maize but not in teosinte; (2) if hybrids between *Tripsacum* and diploperennis approximate a reconstructed prototype of early maize, restriction sites in the hybrids should also be present in maize.

A series of three molecular assays in which genomic DNA of all *Tripsacum* and diploperennis parent plants; F1, F2, and F3 *Tripsacum* X diploperennis progeny; maize lines B73 and Funk's G4522, and F1 and F2 progeny from crosses between B73 and G4522 with *Tripsacum*-diploperennis hybrids, was isolated, digested with *Eco*RI, *Bam*HI, *Eco*RV, and *Hin*dIII, transferred to Southern blots, then probed with 15 maize molecular markers (Eubanks, Econ. Bot. 49:172-182, 1995, Theor. Applied Genet. 1997, in press). Pairwise analysis of autoradiograph restriction sites was conducted to determine if any unique *Tripsacum* sites are found in maize but not in teosinte, and if new recombinant bands in *Tripsacum*-diploperennis hybrids are present in maize.

Eleven restriction sites revealed by nine of the 15 molecular markers are found in *Tripsacum* and maize but not in teosinte. Three of those sites are also present in *Tripsacum*-diploperennis hybrids.

Fourteen restriction sites revealed by ten of the 15 molecular markers appear in *Tripsacum*-diploperennis hybrids that are not present in either parent and evidently arose de novo through recombination as a result of intergeneric crossing. Molecular markers revealing these mutable sites correlate with phenotypic traits linked to developmental changes involved in the evolution of the maize ear. Four of the fourteen de novo recombinant sites are also observed in both maize lines.

Since some restriction sites are present in *Tripsacum* and maize but not teosinte, and since some recombinant sites in *Tripsacum*-diploperennis hybrids not present in either parent are found in maize, results of these small-scale molecular assays lend support to the hypothesis of a hybrid origin for maize involving *Tripsacum*. DNA fingerprinting with molecular markers spanning a greater segment of the maize genome and including other teosinte species will provide additional data for further elucidation of how maize originated.

FREIBURG, GERMANY Albert-Ludwigs-Universität Freiburg AMES, IOWA Iowa State University

Isolation of the new recessive mutant Irt1 deficient in lateral root development

-- Hochholdinger, F; Schnable, P; Feix, G

The root system of maize consists of embryogenic primaryand seminal lateral, and postembryonic crown- and brace roots. All of these root types form lateral roots from the pericycle cell layer. A mutant deficient in the formation of lateral roots (designated Irt1 for lateral-rootless1) has been identified in a seedling screen of segregating M2 families derived from an EMS mutagenized B73 population (established in 1994 in Ames). This recessive mutant is completely deficient in lateral root formation on early forming roots as evident from the comparison of 14-dayold wild-type and mutant seedlings shown in the figure. Mutant plants grow slower and are of smaller size than corresponding wild-type plants, but are, however, still fertile.



Figure. The root system of two 14 day old wt plants (left) in comparison to two Irt1 plants (right), lacking lateral roots.

Histological analysis of sections from 14-days-old primary roots of wild-type and mutant plants revealed that, in contrast to the large number of lateral roots already formed at that stage in wild-type plants, only a few lateral root primordia had formed in the primary root of mutant plants. Further, in mutant seedlings none of these primordia had grown out to form lateral roots. An equal number of such rudimentary primordia were also found in the primary root of 14-day-old wild-type plants. Hence, the relationship between these rudimentary primordia and the *Irt1* gene remains to be elucidated

Further characterization of this new mutant, including investigations at later stages of development and hormone action studies, are in progress.

GAINESVILLE, FLORIDA University of Florida

Com Rab2 homologs

--Laughner, BH; Ferl, RJ

We cloned three Rab2- homologous cDNAs, comprising two classes that share 83% nucleotide identity and 97% amino acid identity (GenBank Accession #s U22432 and U22433). RFLP analysis performed by Helentjaris's group at Arizona mapped only a single specific locus, near the centromere region of chromosome 10. These clones may then represent alleles of a single ZmRab2 gene. Indeed, other Rab subfamily members that are separate genes in other species typically do not share such identities. For example, human Rab5a and human Rab5b share 81% amino acid identity but are not able to cross hybridize since their nucleotide identity is 66% (Wilson and Wilson, J. Clin. Invest. 89:996-1005, 1992). Consequently, these corn clones are referred to as ZmRab2-A and ZmRab2-B. It is possible, however, that these clones represent distinct, but closely linked genes, as is apparently the case in two *Arabidopsis* Rab2 genes.

Alignments of Rab2 plant GTP binding proteins with animal homologs shows 82% identity with both ZmRab2s, with dissimilarities clustering primarily at the carboxyl ends. Since the first proteins identified in this subfamily were from yeast, some investigators prefer the designation "Ypt" rather than the "Rab" designation derived from the animal counterparts. Indeed, a Ypt designee from Zea mays (Ypt-Z3) has been reported to have approximately 80% amino acid identity with Rab2; however, the sequence information for this clone apparently is not deposited in any database (Fabry et al., Gene 118:153-162, 1992). Using available database matches and truncating the highly variable carboxyl ends, a PAUP analysis confirmed specific homology of our maize clones to the Rab2 group at a confidence level above 90% through bootstrap analysis with 100 replicates. Hence we retain the Rab2 designation.

The family of small GTP binding proteins, more correctly referred to as the "ras superfamily" (Kahn et al., FASEB J. 6:2512-2513, 1992), is characterized by a common mechanism for binding GTP which insures activation with subsequent inactivation rendered by GTP hydrolysis. This family is often divided into three major groups represented by Ras, Rho and Ypt/Rab proteins. Extensive biochemical analysis on the h-ras gene product p21ras has served as a model for other proteins in this family (Pai et al., Nature 341:209-214, 1989). When this model is used to more closely scrutinize the GTP binding motifs as delimited essentially by Valencia et al. (Biochemistry 30:4637-4648,1991), our ZmRab2 homologs exhibit extreme conservation in the GTP binding motifs. None of the substitutions that do exist affect the putative contact residues within the GTP binding motifs. The model predicts our ZmRab2s to have similar functions to the animal forms, and specifies the proteins to be in an active form when GTP is bound.

Further work is necessary, however, to demonstrate GTP binding and any purported role in vesicular trafficking. Even with the similarities to the animal Rab2s, important functional differences may exist. For example, animal Rab2s have been reported to be localized to a specific region of the cell between the endoplasmic reticulum and the Golgi apparatus, but differences in the carboxy-terminal modification signal could possibly change this intracellular targeting for these ZmRab2 homologs.

IBARAKI, JAPAN Institute of Radiation Breeding

Nitrous oxide (N₂O) is effective in chromosome doubling of maize seedlings

--Kato, A

Chromosome doubling of maize is important for subsequent haploid breeding and production of polyploid series. Recently successful chromosome doubling of maize calli has been reported using colchicine or other antimicrotubule herbicides in maize anther culture, though chromosome doubling of maize seedlings still remains difficult. The problems to address in the seedling treatment include the permeability of the leaf tissues of maize to the colchicine solution and/or prolonged effect of colchicine on maize growing tissues. The author proposes here the use of a gaseous substance, nitrous oxide (N₂ O), for chromosome doubling of maize seedlings.

A diploid inbred line Oh43 (2n=20) was used in this experiment. Ten seedlings with a shoot length of 2 cm were treated with a gaseous mixture (1 atm air and 8 atm N_2O) for one to four days in an iron container (15 cm diameter, 20 cm height) at 28 C. The shoot meristem tissues of the treated seedlings were cut and fixed in an ethanol acetic acid solution (3:1) just after the treatments, and the effect of nitrous oxide on cell mitosis and ploidy was examined microscopically for each of four preparations.

 N_2O gas affected significantly the mitosis of maize cells. Normal anaphase cells were completely replaced by N_2O -anaphase cells (Figure 1, Table 1). There was a reduction in the proportion of prophase cells and an increase in the number of metaphase cells. In the three- and four-day treatment, mitotic indexes decreased. Tetraploid cells were observed in all the treatments (1-4 days) (Figure 2, Table 2). In the four-day treatment, thirty-five percent of dividing cells were tetraploid and one octoploid cell was observed.

Table 1. Mitotic index and incidence of cells in prophase, metaphase and anaphase in the N₂ O-treated maize shoot maristem.

| Days of treatment | Mitotic in- dex (%) | Prophase (%) | Metaphase (%) | Anaphase (%) | N ₂ O-anaphase (%) | No. of dividing cells observed |
|-------------------|------------------------|-----------------|------------------|-----------------|----------------------------------|-----------------------------------|
| 1 | 32 | 4.8 | 88.1 | 0.0 | 7.1 | 378 |
| 2 | 4.1 | 116 | 80.7 | 0.0 | 7.7 | 259 |
| 3 | 0.7 | 15.3 | 77.6 | 0.0 | 7.1 | 98 |
| 4 | 1.4 | 7.1 | 85.0 | 0.0 | 7.9 | 140 |
| Control | 3.7 | 30.4 | 36.0 | 33.6 | 0.0 | 289 |

Table 2. Incidence of diploid, tetraploid and octoploid cells in N₂O-treated maize shoot meris-

| Days of treat- ment | Diploid cells (%) | Tetraploid cells (%) | Octoploid cells (%) | No. of dividing cells observed |
|------------------------|-------------------|-------------------------|------------------------|-----------------------------------|
| 1 | 99.4 | 0.6 | 0.0 | 317 |
| 2 | 83.9 | 16.1 | 0.0 | 242 |
| 3 | 73.2 | 26.8 | 0.0 | 82 |
| 4 | 64.0 | 35.4 | 0.6 | 178 |
| Control | 100.0 | 0.0 | 0.0 | 172 |

The seedlings subjected to the four-day treatment recovered from N₂O injury within two weeks. The ploidy level of three root tips and one shoot meristem of one of the seedlings (four-day treatment) was analyzed. The shoot and one root tip were diploid and one of the remaining two root tips was tetraploid, while the third root tip showed a mixture of diploid and octoploid cells. Further analysis on the remaining seedlings is currently underway.

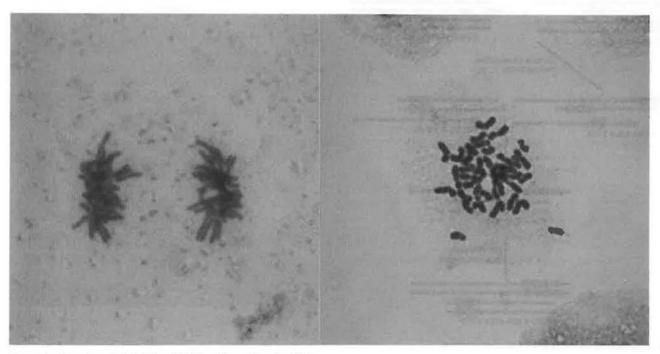


Figure 1. Anaphase of normal mitosis (left) and N2 O-induced ab normal anaphase (right).

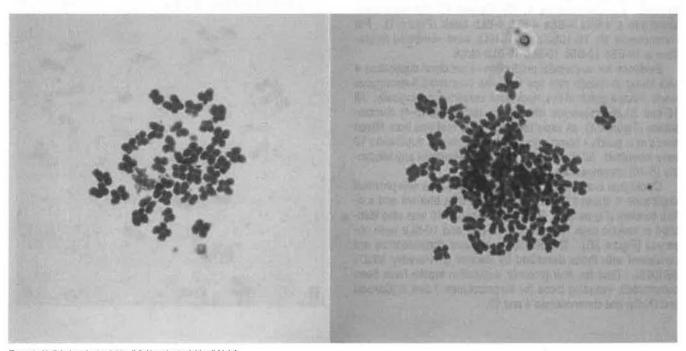


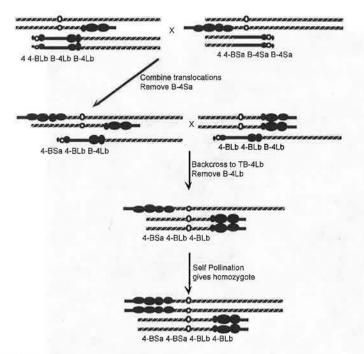
Figure 2. $\,\mathrm{N_2}\,\mathrm{O}\text{-induced}$ tetraploid cell (left) and octoploid cell (right).

IOWA CITY, IOWA University of Iowa

Further construction of proximal duplication stocks -- Zheng, Y-Z; Carlson, WR

B-A translocations can be used to construct unusual stocks, referred to as proximal duplication stocks. For example, 9-BSb 9-BSb 9-BLa 9-BLa is a proximal duplication stock. It was con-

structed by combining the 9-BSb from TB-9Sb with the 9-BLa from TB-9La. The B-9Sb and B-9La were discarded (Carlson and Curtis, Can. J. Genet. Cytol. 28:1034-1040, 1986). A pair of 9-BSb plus a pair of 9-BLa chromosomes is equal to a pair of standard chromosome 9's plus a duplication (4 doses) of a central region of chromosome 9. Proximal duplication stocks are useful in the construction of telocentric chromosomes (Zheng and Carlson, MNL, 70: 28) and may be useful in studies on gene duplication.



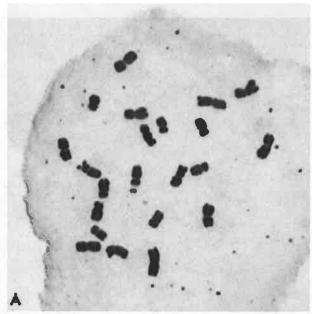
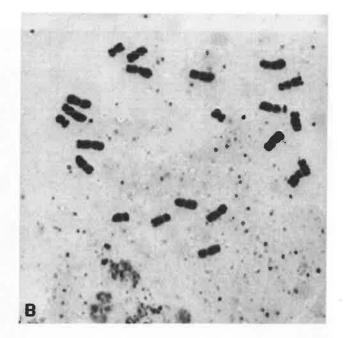


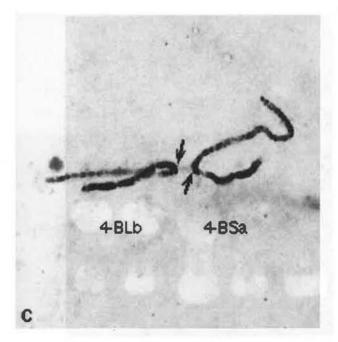
Figure 1. Construction of proximal duplication 4 by a series of crosses.

Following the same procedures as Carlson and Curtis, the proximal duplication stocks for chromosomes 4 and 10 were constructed. For chromosome 4, TB-4Sa and TB-4Lb were combined into a 4-BSa 4-BSa 4-BLb 4-BLb stock (Figure 1). For chromosome 10, TB-10Sc and TB-10Lb were combined to produce a 10-BSc 10-BSc 10-BLb 10-BLb stock.

Evidence for successful production of proximal duplication 4 was found in mitotic root tips from the presumed homozygous stock. Fifteen seeds of this stock were examined cytologically. All 15 had 22 chromosomes without any telocentric (B-4) chromosomes (Figure 2A), as expected. Similarly, root tips from fifteen seeds of a putative homozygous stock of proximal duplication 10 were examined. All showed 22 chromosomes without any telocentric (B-10) chromosomes (Figure 2B).

Cytological examination of meiotic cells of plants with proximal duplication 4 showed the presence of a 4-BSa bivalent and a 4-BLb bivalent (Figure 2C). Proximal duplication 10 was also identified in meiotic cells. Bivalents of 10-BSc and 10-BLb were observed (Figure 2D). The structures of these chromosomes are consistent with those described by Beckett (J. Heredity 69:27-36,1978). Thus far, four proximal duplication stocks have been constructed, including those for chromosomes 3 and 9 (Carlson and Curtis) and chromosomes 4 and 10.





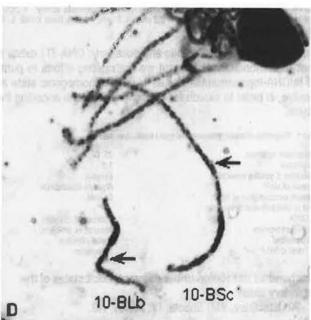


Figure 2. Proximal duplication stocks 4 and 10. A. 22 chromosomes In mitotic cell of proximal duplication 4. B. 22 chromosomes of proximal duplication 10. C. Pachytene stage of PD 10. Arrowheads indicate the centromeres of 4 and 10.

IRKUTSK, RUSSIA Institute of Plant Physiology and Biochemistry

Type I DNA-topoisomerase activity in mitochondria -- Tarasenko, VI; Konstantinov, YM

The type I DNA-topoisomerase (DNA-TI) plays a key role in control of the topological state of the DNA molecule to regulate its supercoiling, introducing a break into one of its chains. Such change is important for replication and transcription. Data have been obtained on involvement of the type I DNA-TI in regulation of genetic information expression by changing the topological state

of regulatory sites of particular genes (Caserta et al., J. Cell Biochem. 55:93-97, 1994). The present communication reports characteristics of the type I DNA-TI found in the genetic system of mitochondria from hybrid VIR42MV. The activity of the enzyme was mostly revealed in the solubilized fraction at 0.5% concentrations of Triton X-100. The DNA-topoisomerase activity was not found in intact mitochondria suspensions and in postmitochondrial supernatant, thus supporting intra-mitochondrial localization of the enzyme studied.

One of the characteristic properties of the type I DNA-topoisomerases is a dependence of the enzyme activity on Mg²⁺ ions. The presence of magnesium is necessary for topoisomerases of procaryotes to work, while the activity of eucaryotic nuclear topoisomerases is not absolutely dependent on Mg²⁺ ions though is greatly stimulated. However, the mechanism of the effect of Mg²⁺ ions on the mitochondrial enzymes is not clear. Figure 1 pre

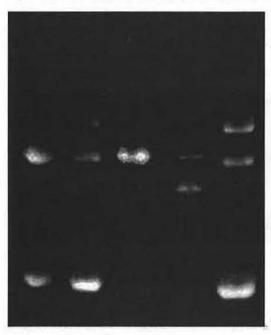


Figure 1. The influence of Mg $^{2+}$ and K $^+$ cations on type I mtDNA topoisomerase activity: 1, MgCl₂ + KCl; 2, KCl; 3, MgCl₂; 4, control (without cations); 5, pUC19 plasmid DNA (without enzyme).

sents data on dependence of the mitochondrial topoisomerase activity on the presence of magnesium and potassium ions in the reaction medium. The topoisomerase activity was found only in the presence of Mg²⁺ ions. Potassium ions had some inhibitory effect. The maximum activity was observed at 10-20 mM MgCl₂ (the data are not given). These results are in agreement with those of studies of type I TI from wheat mitochondria (Echeverria et al., Plant Mol. Biol. 6:417-427, 1986) and Chenopodium album (Meisner et al., Biochem. Intern. 27:1119-1125, 1992), also demonstrating an absolute dependence of the enzymes on Mg²⁺ ions. Since the interaction between the type I TI and the DNA molecule is actually electrostatic the ionic strength of the solution is important for the enzyme to exhibit its activity. Figure 2 shows the effect of KCI concentration on DNA relaxation by the topoisomerase. The maximum activity of the enzyme was observed at 50 mM KCl concentration. A further increase in the ionic strength of the solution had an inhibitory effect. Such dependence is typical of the type I plant topoisomerases. The effect of some inhibitors

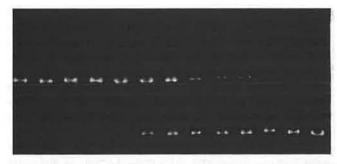


Figure 2. The dependence of type I mtDNA topoisomerase activity from ionic strength of KCI solution: 1, 0 mM; 2, 10 mM; 3, 30 mM; 4, 50 mM; 5, 70 mM; 6, 90 mM, 7, 120 mM; 8, 150 mM; 9, 180 mM; 10, 210 mM; 11, 250 mM; 12, 300 mM; 13, pUC19 plasmid DNA.

of the DNA-topoisomerase activity on mitochondrial DNA-TI has been studied in a particular set of experiments (Fig. 3). EDTA and ethidium bromide, which are characteristic inhibitors of procaryotic TI type I as well as type II TI, failed to affect the activity of the mitochondrial enzyme. Only very high concentrations of EDTA resulted in reduction of the activity (Fig. 3, lane 4). On the other hand, an addition of spermidine into the incubation medium caused certain decreases of the topoisomerase activity (Fig. 3,



Figure 3. The influence of potential inhibitors on topolsomerase activity: 1, 2 mM EDTA; 2, 10 mM EDTA; 3, 50 mM EDTA; 4, 0.5 mkg/ml ethidium bromide; 5, 5 mkg/ml ethidium bromide; 6, 10 mkg/ml ethidium bromide; 7, 2 mM spermidine; 8, 5 mM spermidine; 9, 20 mM spermidine; 10, pUC19 plasmid DNA.

lanes 8-10). Such effect of this compound is considered to be characteristic of DNA-topoisomerases of a procaryotic origin in contrast to eucaryotic enzymes which are shown to be stimulated by polyamines (Sitailo, Biopolymers and Cell (Russia) 7:97-103, 1991). The enzyme studied can use as a substrate the positively supercoiled DNA of the pUC19 plasmid (the data are not given), which is a typical property of eucaryotic TI. We have also attempted to study the effect of non-ionic detergent, Triton X-100, on the activity of mtDNA-TI (Fig. 4). It is shown that plasmid DNA is relaxed easier in the presence of nonsolubilizing (0.02%) concentrations of Triton X-100 (Fig. 4, lane 4,5). It is generally accepted that Triton X-100 affects the conformational state of the DNA to separate locally the chains of the double helix of nucleic acid. Such conformational variation is likely to be responsible for the enhanced activity of the enzyme.

The data on properties of the mtDNA-TI type I are summarized in Table 1. The majority of the properties described are similar to those of eucaryotic nuclear type I topoisomerases. However, such properties of the enzyme as Mg²⁺ ion dependence and partial inhibition by polyamines are characteristic of DNA-topoisomerases I of procaryotes. As similar data were obtained for mitochondrial TI of wheat and *Chenopodium album* it is assumed that an independent form of the enzyme with properties in-

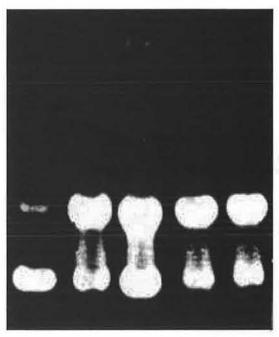


Figure 4. The effect of Triton X-100 on mitochondrial type I topoisomerase activity: 1, pUC19 plasmid DNA (without enzyme); 2, 2 units; 3, 0.2 units; 4, 2 units + 0.02% Triton X-100; 5, 0.2 units + 0.02% Triton X-100.

termediate between procaryotic and eucaryotic DNA-TI I exists in plant mitochondria. At present we are making efforts to purify the mtDNA-topoisomerase I to as high as a homogenic state as possible, in order to search for and isolate the gene encoding the enzyme.

Table 1. Properties of maize mitochondrial type I topoisomerase.

| Temperature optimum | 35 C |
|--------------------------------------|-----------------------|
| pH optimum | 7.5 |
| Relaxation of positive supercoils | Present |
| Influence of Mg ²⁺ | Absolute dependence |
| Optimum concentration of KCI | 50 mM |
| Effect of inhibitors and detergents: | |
| EDTA | Absence of Inhibition |
| Ethidium bromide | Absence of inhibition |
| Spermidine | Partial inhibition |
| Triton X-100 | Stimulation |
| | |

Mitochondrial translation under different redox states of the respiratory chain

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

We have previously reported (MNL 70:29-30) on the effect of different redox conditions on the protein synthesising activity in isolated mitochondria. It was found that under oxidising conditions created by the addition of potassium ferricyanide to mitochondria the protein synthesis was activated in organello, while under reducing conditions created by the addition of sodium dithionite it was strongly suppressed. We made an assumption of the existence of redox regulation of mitochondrial gene expression at the translational level. According to this hypothesis, the presence of a set of special redox sensors transmitting information on the changes in redox conditions to the genetic apparatus of the organelles seems to be a necessary element in the mechanism of this regulation.

Particular components of the respiratory chain complexes can, in our opinion, serve as such redox sensors of the mitochondrial system of redox regulation. To test this assumption, the present

work studied the effect of different states of the respiratory chain of mitochondria created by different respiratory inhibitors on the activity of the protein synthesising system of these organelles.

The mitochondria were isolated from 3-day-old etiolated maize seedlings of hybrid VIR42 MV by a standard method of differential centrifugation. Mitochondrial protein was determined by the Lowry method. Protein synthesis reactions were registered according to Bhat et al. (Biochemistry 21:2452-2460, 1982) with the use of [14C]-leucine (specific radioactivity was 1760 GBg/mol). In order to study the effect of oxidative phosphorylation uncoupler on mitochondrial translation carbonyl cyanide chlorophenylhydrazone (CCCP) at a final concentration of 1 uM was used. 1 ug/ml of antimycin A, 1 mM of potassium cyanide and 0.003 mM of rotenon were added to mitochondria in the inhibitory analysis of the respiratory chain. The kinetic data were obtained from at least 3 to 4 experiments.

Table. Activity of protein synthesis in isolated maize mitochondria under different redox states of respiratory chain. Incorporation of [14C]-leucine, % of control.

| Conditions | 5 min | 10 mln | 15 min | 20 min |
|-------------|-------|--------|--------|--------|
| Control | 100 | 100 | 100 | 100 |
| CCCP | 236 | 149 | 143 | |
| Rotenon | | 116 | 97 | 102 |
| Cyanide | 0 | 4 | 20 | 64 |
| Antimycin A | 17 | 58 | 88 | S(#6) |

The table shows that the addition of CCCP, an uncoupler of oxidative phosphorylation, caused an activation of protein synthesis as in the case of oxidising conditions following the addition of ferricyanide (MNL 70:29-30). Such an effect of CCCP presumably results from redox states of the carriers of the respiratory chain, which, as shown elsewhere (Muraoka and Slater, BBA 180:221-226, 1969), are converted to more oxidising states under uncoupling of mitochondria.

The addition of rotenon blocking the electron transport from NAD to cytochrome b failed to produce any effect on the translational activity of mitochondria, which is not surprising as sodium succinate was used as a respiratory substrate and oxidation is not inhibited by this agent. The protein synthesis in organello was shown to be drastically inhibited when the respiratory chain was blocked by potassium cyanide ceasing the final stage of the electron transport at the level of cytochrome a + a3, which can be accounted for by the conversion of the respiratory carriers into a reduced state. In contrast, the activity of the protein-synthesising system of mitochondria declined to a lesser degree under antimycin A inhibition blocking the electron transport from cytochrome b to cytochrome c than under cyanide inhibition. The different level of suppression of the protein synthesis can be accounted for by the location of the antimycin block in the respiratory chain when only carriers localized left of the "crossing point" proved to be in the reduced state. It should be emphasized that energy deficiency was not the reason for the protein synthesis suppression in our experiments with the inhibitors of respiratory chain, since otherwise cyanide and antimicin A could produce similar effects on the translational activity. In conclusion, the results obtained suggest the localization of the redox sensors in all multienzyme complexes of the respiratory chain of mitochondria.

JINAN, CHINA Maize Research Institute

Correlation analysis among kernel physical and quality characters in quality protein maize (QPM)

--Liu, Z

Five self-lines (QI205, QI208, ZhongXI042, Across 7741 and Population 69) and 3x2 crosses of QPM were used as experimental materials. The experiments were carried out to analyze correlation among kernel physical characters and quality characters in

The kernel quality characters included were lysine and protein contents of whole kernels. The lysine content was analyzed by an automatic and high speed amino acid analyzer (Model 835-50, Hitachi Ltd., Japan). The protein content was determined by the Micro-Kjeldahl method. The kernel physical characters included were endosperm hardness, grain density, 1000-kernel weight, and grain yield/plant. The endosperm hardness was divided into five classes (0, 1, 2, 3, 4) based on proportion of hard versus soft endosperm, in which 4 represents very hard and 0 represents very soft, floury kernel. The grain density was determined using the ratio between 100-kernel weight and 100-kernel volume. 1000kernel weight was determined with weight of 1000 whole, cleaned kernels at 13.5% moisture. Grain yield was measured by grain weight on a plant basis and adjusted to 13.5% moisture.

The main results of the experiment were as follows:

(1) There were positive correlations of endosperm hardness, grain density, 1000-kernel weight with grain yield/plant, R=0.853**, 0.804**, 0.976**, respectively.

(2) Lysine and protein contents of whole kernels have negative correlations with grain yield/plant, R=-0.845**, -0.736**, re-

spectively.

(3) Lysine content of whole kernel had a negative correlation with endosperm hardness, grain density and 1000-kernel weight, R=-0.854**, -0.813**, -0.674**, respectively

(4) Protein content of whole kernel had a negative correlation with endosperm hardness, grain density and 1000-kernel weight, but correlation coefficients were not significant, R=-0.125, -0.142, -0.163, respectively.

(5) Endosperm hardness had a positive correlation with grain density and 1000-kernel weight, R=0.882**, 0.925**, respectively.

New maize self-lines bred by stock 6 inducing haploid technique

--Liu, Z

Four self-lines have been bred from normal maize hybrids (female) crossed with stock 6 (male), which generates haploids and double haploids.

Stock 6, first identified by Edward H. Coe in 1956 (Chang, Sci. Agric. 40:53-80, 1992), carried the R-nj gene with full expression of the R-nj phenotype. This gene can induce haploids at a frequency of 1-5%. Seeds having homozygous R-nj are white flint with purple color on the crown and plumule. The haploids planted can be doubled into pure lines under natural conditions in a frequency of 10%.

Ql318xQl310 hybrid of normal maize was used as female, and was crossed with stock 6 (male). A total of 200 F1 ears or 17,600 F1 seeds were obtained. 493 haploids (2.8%) were identified among them by using kernel color screening technique. These 493 kernels were planted and 302 plants survived at seedling phase. 28 plants (9.3%) were doubled by pollen screening at maturity phase. 21 plants were self-pollinated and 15 self-ears were obtained. All of them were planted in ear-rows the next year and 4 good self-lines were selected. These lines have outstanding agronomic characters, good resistance to some diseases, and have been used in the production of hybrid maize.

KISHINEV, MOLDOVA Academy of Sciences of Moldova

Immature embryo culture of waxy maize

-- Kravchenko, OA

The behaviour of immature embryos in culture conditions in vitro was studied with respect to the following factors: genotype, age of embryos and 2,4-D concentration in nutrient medium. For callus tissue culture initiation the method of immature embryo cultivation (Green and Phillips, Crop Sci. 15:417-421, 1975) was used. The hybrid Pioneer 3978 and its parental forms, and the inbred lines 346 and 502 as well as their waxy counterparts, were used as initial material. Immature embryos at an age of 12, 13, 15 and 17 days were employed to produce callus. They were planted on the nutrient medium MS (Murashige and Skoog, Physiol. Plant. 15:473-497, 1962) supplemented with various concentrations of 2,4-D (0.5 mg/l, 1 mg/l, 2 mg/l). The data were processed by three factor analysis of variance.

The principal conclusions from the results obtained are as follows: (1) an influence of the waxy gene on callus formation, somatic embryogenesis and plant regeneration has been observed (Table 1). A similar conclusion was made by other scientists (Gulko,

Table 1. The influence of genotype on callus formation, somatic embryogenesis and plant regeneration.

| Genotype | Callusogenesis, % | Somatic embryogenesis, % | Plant regeneration, % |
|-----------------|----------------------|--------------------------|--------------------------|
| 346wx | 86.42*** | 65.10*** | 14.23* |
| 346 | 97.37*** | 84.59*** | 57.30* |
| 502wx | 52.36*** | 47.80*** | 36.15* |
| 502 | 77.33*** | 53.27*** | 35.23* |
| Pioneer 3978 | 53.28*** | 64.58*** | 45.14* |
| Pioneer 3978 wx | 81.19*** | 85.14*** | 25.31* |

*p < 0.05, *** p < 0.001

Kravchenko A., Palii, Bul. Acad. Stiin. Republ. Mold. 3:25-31, 1995). (2) The embryos at an age of 12 and 13 days proved to be the best explants for callus, somatic embryos and plantlet induction. (3) The processes of callusogenesis, embryogenesis and plant regeneration were influenced mostly by age of embryos and genotype. It should be noted that the composition of culture medium was the factor that affected these processes least. For reliable waxy maize regenerant production the best combinations of factors studied were also determined.

Effect of gamma radiation on callus formation and somatic embryogenesis of wax maize

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

For this study methods of immature embryo cultivation and induced mutagenesis were used. The application of these methods in combination leads to the increase of plant cell mutation frequency up to 10⁻³ (Sidorov and Maliga, Mol. Gen. Genet. 186:328-332, 1982). The experiment was conducted with the following inbred lines: 346, 346wx, 502 and 502wx. Immature embryos at age 15-

16 days were subjected to gamma rays applied at 0.16 Gr/second from the Co 60 radiation source. The treatments were 2, 4, 8 and 12 Gr. After irradiation embryos were planted on MS nutrient medium (Murashige and Skoog, Physiol. Plant. 15:473-497, 1962) supplemented with 2 mg/l 2,4-D. Analysis of variance has been carried out to determine the effect of genotype and gamma ray treatment for callus formation and somatic embryogenesis.

It should be noted that in the studied material different types of callus were observed. White colour callus of dense consistency was defined as type 1 and yellow colour callus of friable consistency (possessing rhizogenic ability) was defined as type 2. All variants of lines 346, 346wx and control variants of lines 502, 502wx proved to be type 1 callus-producing. In contrast, the irradiated embryos of lines 502 and 502wx produced type 2 callus formation. The results of the analysis of variance have shown that the type of callus formation was influenced by genotype, gamma rays and their interaction (Figures 1 and 2). During further cultivation the type 2 callus formation (for line 502wx) was suppressed in all variants of gamma ray treatment. A similar effect (for line 502) was induced by 8 and 12 Gr doses.

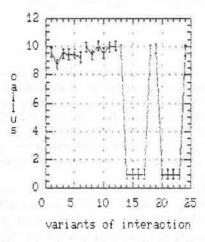


Figure 1. The influence of genotype and gamma rays on type 1 callus formation. 1-6 346wx; 7-12 346; 13-18 502wx; 19-24 502.

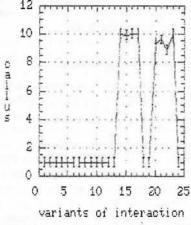


Figure 2. The influence of genotype and gamma rays on type 2 callus formation. 1-6 346wx; 7-12 346; 13-18 502wx; 19-24 502.

According to the results of the analysis of variance of somatic embryogenesis frequency significant differences have been determined between genotypes studied. The lines 346 and 502 have

demonstrated a higher somatic embryogenesis frequency than their waxy counterparts (Figure 3), however, inbred lines 502 and 502wx have been more radiosensitive in comparison to lines 346 and 346wx. It should be emphasized that the irradiated embryos of all genotypes studied had a lower somatic embryogenesis frequency than control variants (Figure 4).

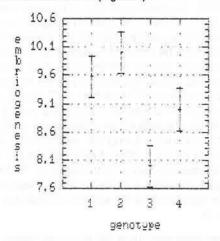


Figure 3. The influence of genotype on somatic embryogenesis. 1 346wx; 2 346; 3 502wx; 4 502.

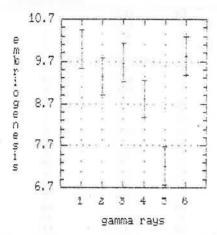


Figure 4. The influence of gamma rays on somatic embryogenesis. 1 control; 2 2Gr; 3 4Gr; 4 8Gr; 5 12Gr; 6 irradiated seeds.

In general, the results obtained indicate that the influence of gamma radiation on callus formation and somatic embryogenesis was reliable (on all significance levels) and resulted in decreasing of callusogenesis and somatic embryogenesis frequency.

The action of combined gamma-irradiation with stimulating and mutating doses on maize seeds

--Romanova, IM; Lysikov, VN

The goal of this work was to find out the influence of stimulating doses on the radiation reaction of maize seeds at later ontogenetic stages, using combined irradiation of maize dormant seeds with stimulating doses.

Significant influence of pre-irradiation of dormant seeds is observed at the earliest stage of plant development, following the initial cell division, expressed in a nearly double decrease in the number of damaged cells. The number of damaged anaphases in the range of 100-300 Gr is reduced if the seeds have been pre-ir-

radiated with a dose of 5 Gr at a 5 min or 24-hour interval between irradiations.

Observations over the growth and development of plants in a greenhouse experiment showed that air-dry kernels irradiated with a dose of 5 Gr initiated their germination two days earlier than the control. The seeds germinated at lower rates, and their emergence and plant height were nearly twice as low by the end of the vegetation period with irradiation at doses of 100 Gr, 200 Gr, 300 Gr in the treatments both with and without preliminary irradiation. This index is characterised by high variability of plants. In the treatments involving pre-irradiation with a 24-hour interval between irradiations (5+100 Gr, 5+200 Gr) the plants were on average 25% higher than in the controls (0+100 Gr, 0+200 Gr), as well as in the 5-min pre-treatment (5+100 Gr, 5+200 Gr), but the difference was not statistically significant.

The dose of 300 Gr, 5+300 Gr was lethal for the intervals of 5 min and 24 hr. The time interval between irradiations with stimulating and mutating doses was established to have significant importance. Thus, it was shown that the pre-irradiation of seeds with the stimulating dose of 5 Gr followed by the mutating dose after 5 min caused a sensibilizing effect, and in the case of additional irradiation after 24 hr with the same dose a distinct radio-protecting effect was induced. The combined irradiation of seeds with 5+100 Gr and 5+200 Gr gave rise to mutation forms with a changed growth pattern and even dwarf mutations.

Studies on plant genetic variability induced by combining low and high doses of ionizing radiations

--Romanova, IM

Pollination with pollen exposed to low and high doses of irradiation, the high doses being lethal, is a new technique to develop maize mutants. The present research was done with the aim of studying variability of maize qualitative and quantitative traits in M1-M4. Regularities of quantitative trait variability were studied involving statistically processed measurement data for 16 traits in 100 randomly drawn plants.

The inhibitory effect caused by the mutagenic action of gamma-irradiation of pollen, on quantitative traits, was expressed most in M2. When the pollen of K167 bm2 irradiated with low (5 Gr) and high (500, 800, 1000 Gr) doses was used as donor, followed by additional selfing, statistically significant decreases in all the plant traits studied were observed, except for leaf width and ear diameter. Changes in the traits plant height, leaf number, leaf length, tassel length, kernel number, ear length, kernel weight, and ear weight occurred with all the doses of gamma-irradiation. At the same time, the doses of 5+1000 Gr led to termination of changes in the traits node height of the first ear attachment, developed ears, number of branchlets on the tassel, and kernel height and diameter. The data obtained showed effects on 14 of the 16 quantitative traits studied. The traits of reproductive structures, i.e. the number of kernels per ear, kernel and ear weight, and the length of branchlets in the tassel, showed the greatest modification.

The DNA fragments of the pollen exposed to high doses of gamma-irradiation became mutagens and caused a high rate of mutations. Chlorophyll mutations were produced at doses of 5+500 Gr, 5+800 Gr, and 5+1000 Gr. Higher doses of gamma-irradiation resulted in a marked increase in the frequency of chlorophyll mutations, as well as in subsequent generations with a maximum in M3 and mutation decrease in M4. This was especially characteris-

tic of the percentage of modified plants - the frequency of chlorophyll mutations increased three-fold for the dose of 5+500 Gr, and four-fold for 5+500 Gr, 5+1000 Gr in M3. Chlorophyll mutations are a signal to detect mutations for other genes.

The highest increase in the total frequency of mutations was observed in cases of direct three-fold pollination of the recipient P346 with the donor pollen K167 *bm2*, irradiated with the dose of 5 Gr, followed by high doses and additional pollination with non-irradiated pollen as opposed to reverse irradiation with high doses, followed by a low one, and additional pollination with the recipient's own pollen.

Direct irradiation with the doses of 5+1000 Gr induced the highest genetic variability, i.e. for four groups of mutations, and reached 66.4% as well as the widest spectrum of variability, i.e. 26 traits, as opposed to reverse irradiation (1000+5 Gr), when the spectrum of variability included 16 traits, and variability reached 17.6%. Forms with increased number of ears, or huge ears, present a significant interest among the mutations produced. The lemon-yellow line P346 yielded forms with dark yellow, red, light brown, and dark brown kernels.

Pollen exposed to combined low (5 Gr) and high (100-1000 Gr) doses of gamma-irradiation induces peculiar mutagenic activity with various frequencies and with different doses of irradiation. The doses 5+200 Gr, 5+500 Gr produced red kernel mutations changing the kernel coloration due to the pericarp pigmentation.

Hence, combined irradiation of maize generative structures with low and high doses produces specific mutation types characteristic of chemical mutagenesis.

Location of factors influencing grain yield by means of isoenzyme markers under different year conditions

--Chernov, AA; Mihailov, ME

The objective of the present study was to establish the genotypic effects of the chromosome 1 segment marked by the Adh1 locus and the chromosome 7 segment marked by the Sod2 locus on a number of agronomic traits. The study was carried out on a widely cultivated high-yielding hybrid, Moldavsky 291. The parental lines, F1 and F2 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 internodes; 12) number of 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 ear 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) ear length; 27) ear diameter; 28) ratio of stem length to diameter; 29) weight of 100 kernels; 30) grain index and others.

The enzyme electrophoresis extracts from pollen were run in 14% starch gel (buffer system "G", pH=7.0) (Wendel and Stuber, 1984). The gel staining was performed using reaction

mixtures from Levites' (1986) list.

Isoenzyme analysis has shown the parental lines to differ with respect to the Adh1 and Sod2 loci. This allows 3 genotype classes to be distinguished in the F2 population for each locus: FF, FS and SS. For the quantitative traits studied, the following statistically significant differences between the above classes were observed for locus Adh1: 1) panicles and ears of SS enter flowering later than those of FS (for ears - FF and FS); 2) larger time lag of the onset of flowering between panicle and ear compared with FF; 3) top ear position on the stem of FF higher than of FS and SS; 4) number of ears with kernels of FF less than of SS; 5) lower weight of the top ear and cob of FS compared with FF; 6) number of kernel rows on the top ear of FF more than of FS and SS, of FS more than of SS; 7) number of kernels on the top ear of SS is more than of FS; 8) number of kernels in row on the top ear of SS more than of FS and FF, of FS more than of SS. Next year the results were reproduced only for the number of ears with kernels, which suggests the essential influence of year conditions. So for the Adh1 locus and year conditions a two-factor ANOVA was computed to evaluate the significance of the variation attributed to these factors (Table 1).

Table 1. Phenotypic variation explained by the Adh1 locus and year conditions.

| | | | tion (%) explained by | |
|--------------------------|------------|-----------------|---|------|
| Numbers of the traits | Adh1 locus | Year conditions | Adh1 locus-year conditions interaction | Rest |
| 1 | 0.6 | 18.8** | 2.8* | 77.8 |
| 2 | 0 | 14.4** | 0 | 85.6 |
| 4 | 0 | 4.2* | 0 | 95.8 |
| 5 7 | 0 | 0 | 0 | 100. |
| 7 | 0 | 9.0** | 0.6 | 90.4 |
| 8 | 0 | 2.2* | 0.2 | 97.6 |
| 9 | 0 | 24.7** | 0.6 | 74.7 |
| 10 | 0.4 | 40.8** | 0 | 58.7 |
| 11 | 0 | 0.4 | 0 | 99.6 |
| 12 | 0 | 0.3 | 0 | 99.7 |
| 13 | 2.3* | 0 | 0 | 97.7 |
| 14 | 0.1 | 11.0** | 0 | 88.8 |
| 15 | 0 | 0 | 0.2 | 99.8 |
| 16 | 0 | 4.2* | 0 | 95.8 |
| 17 | 0 | 0 | 1.6 | 98.4 |
| 18 | 0.5 | 6.5** | 0 | 93.0 |
| 19 | 0.7 | 0.1 | 0 | 99.2 |
| 20 | 0.1 | 8.8** | 0 | 91.1 |
| 21 | 0.6 | 3.3* | 0.5 | 95.6 |
| 22 | 0 | 5.9** | 1.1 | 93.0 |
| 23 | 1.3 | 9.9* | 4.6* | 84.3 |
| 24 | 0 | 0 | 2.5 | 97.5 |
| 25 | 0 | 3.6** | 3.5** | 92.9 |
| 26 | 0 | 0 | 0 | 100. |
| 27 | 0.3 | 4.0* | 0 | 95.7 |
| 28 | 0.2 | 0 | 0 | 99.8 |
| 29 | 0 | 15.5** | 0 | 84.5 |
| 30 | 0 | 5.6** | 0.2 | 94.3 |

For locus *Sod2* F2 segregation the only effect was observed for the time from emergence to the maturation of top ears.

The results suggest that the chromosome 1 segment marked by the *Adh1* locus and the chromosome 7 segment marked by the *Sod2* locus genetically affect the above traits in maize. The effect manifestation depends on year conditions. Future research is needed to ascertain the nature of the effects observed.

Mutagenic effects of laser radiation and 6-mercaptopurine on maize seedlings

--Burilkov, VK; Paschenko, VM; Lysikov, VN

Earlier, the mutagenic effects of acridine orange (AO) and ethidium bromide (EB) on prokaryotes and eukaryotes have been

studied (Burilkov and Krochik, Laser in the Life Sciences. v.2(4), pp. 253-274, 1988; Dragan, Khrapunov, Cytol. Genet. (USSR) 26:32-35, 1992). We have compared cytogenetic effects of the known sensitizers, AO and EB, and of previously unused ones, such as 6-mercaptopurine (6-MP) and Cloroxine (CX), each used in combination with laser radiation (LR).

Maize seedlings A-346 grown on media containing the above sensitizers at a concentration of 0.00001 M each, were exposed to LR for 1 min (=337.1 nm, I=70 MWt/m m sec). Counts of chromosome aberrations were made in temporary preparations during mitotic anaphase and telophase of maize (Gostimsky, Practical Guide on Cytogenetics, Moscow University Press, Moscow, 1974). The studies have shown that the highest rate of chromosome aberrations occurred when 6-MP was used as a sensitizer. This exceeded the rates of chromosome aberrations resulting from exposure to EB+LR, CX+LR, and AO+LR by factors of 1.5, 4, and 8 respectively.

One possible cause of chromosome aberrations induced by LR and sensitizers may be the formation of one- and two-strand breaks due to laser radiation energy which is transferred from the sensitizer molecule to certain DNA sites. The resultant one- and two-strand breaks may be repaired during mitosis, or they may eventually turn into chromosome aberrations. To test this hypothesis, we studied maize genomic DNA by the gel electrophoresis technique. Electrophoretic patterns and break counts from densitograms (Zhizina et al., Radiobiology (USSR) 23:783-786, 1983) have suggested that the hypothesis is not implausible (Table 1).

Table 1. The number of breaks in total DNA of maize resulting from exposure to sensitizers and LR.

| | C | LR | 6MP+LR | EB+LR | AO+LR | CX+LR |
|---|----|------|--------|-------|-------|-------|
| L | 67 | 65 | 45 | 56 | 61 | 60 |
| N | | 0.09 | 1.50 | 0.58 | 0.29 | 0.34 |

N-breaks number per molecule; L- average lengths of DNA fragments in the test and control DNA probes; C-control.

The induction of chromosome aberrations in maize plants with the aid of 6-MP and LR has a number of advantages over the conventional techniques:

-it shows relative selectivity: interaction is primarily with DNA;

-it enables the molecular mechanisms of mutation and recombination to be more precisely identified;

-it produces no cytotoxic effects.

P.S. A high level of mutations of the various types inherited in M2, M3 was discovered following the treatment of maize seedlings of the A-346 line. The analysis is in progress.

KRASNODAR, RUSSIA Krasnodar Research Institute of Agriculture

The use of gene *ig* in sterile-counterpart production of corn lines --Shatskaya, OA; Zabirova, ER; Shcherbak, VS

Gene *ig* (indeterminate gametophyte) was used as a source of high androgenetic haploid frequency for production of male-sterile counterparts of corn lines. Three types of stocks were included in the research work: stocks of *ig/+ R-nj* type from the genetic collection (USA), transferred into S and C cytoplasms (Chumak, 1977); stocks with different cytoplasms (Vg, CA, ME, SD, S, C) received from Kindiger, which have gene *ig* in a homozygous state due to B-A translocation, and hybrid stocks partly

adapted to Krasnodar climatic conditions. From published data ig/+ R-nj stocks increase the frequency of androgenetic haploids to 2% (Kermicle, 1969) and ig/ig TB-3Ld stocks to 9% (Kindiger, 1992).

The aim of this investigation was to show the possibility of practical use of these stocks for male-sterile counterpart production. Stocks with gene ig were used as females, and several Krasnodar inbreds as males. Haploid kernels were selected from hybrid ears for endosperm and embryo coloration. Supposed haploids were planted in the field, where supplementary selection was done for phenotype. Matroclinous haploids and hybrids were excluded from further investigation. The selected androgenetic haploids were pollinated by corresponding lines -- sterility maintainers. Natural female fertility of androgenetic haploids was high enough that there was no need for colchicine treatment for chromosome doubling. We obtained 67 androgenetic haploid seedlings in 1996 (Table 1). Ten of 67 haploid seedlings were identified among polyembryonic kernels: two (n:n), five (2n:n), one (2n:n:n), and two (2n:2n:n). In addition, in one (n:n) kernel one haploid proved androgenetic and the other maternal.

Table 1. Frequencies of androgenesis in crosses of several inbred lines as male to ig female stocks.

| | | | | | Comp. with cms ig+ R-nj | | |
|--|---------|---------|----------------------|----------------------|----------------------------|-------|--|
| Stocks | Ears Ke | Kernels | Haploid Seedlings | Freq. of haploidy, % | X2 | Р | |
| cms ig/+/R-nj | 626 | 142,399 | 17 | 0.012 | | | |
| cms iglig TB-3Ld R-nj Hybrid stocks | 127 | 29,111 | 12 | 0.041 | 10.58 | 0.001 | |
| cms iglig TB-3Ld R- nj/N igl+ R-nj | 361 | 86078 | 38 | 0.044 | 21.80 | 0.001 | |

Frequency of androgenetic haploids appeared to be significantly lower than described by Kermicle and Kindiger. It is necessary to note that we did not count the chromosome numbers of supposed haploids, and selection was done only on phenotypic traits. Some haploids, perhaps, were left in defective and aborted kernels. Only viable haploid plants have particular value for us.

Despite higher haploid frequency, cms *ig/ig* TB-3Ld *R-nj* stocks are less suitable for breeding practice against cms *ig/+ R-nj* stocks for some reasons. Their plants are not vigorous enough, the ears are small, endosperm and embryo coloration is not well enough developed, and their vegetation period is too long. Hybrid stocks appeared to be more suitable due to better plant phenotype.

All studied stocks will be convenient for practice after adaptation, and after increase of viable haploid frequency to at least 0.1%.

Sterile counterparts of Kr710Vg, Kr710CA, Kr726CA, Kr752ME, Kr752Vg, Kr10811S, RP-8C and Lu7C lines were obtained through these investigations.

LLAVALLOL, ARGENTINA Instituto Fitotéc. Santa Catalina (FCAF, UNLP), Centro Invest. Genét. (UNLP-CONICET-CIC)

B chromosomes in native races of maize from Argentina. I. Populational frequency and altitude

--Chiavarino, AM; Rosato, M; Cámara Hernández, J; Naranjo, CA; Poggio, L

In twenty one native populations, corresponding to 13 races of

maize cultivated at different altitudes (80-3620 m), the frequency of B-chromosome (Bs) was studied (Table 1). The sampling was made by the authors, from original indigenous populations, and was carried out in order to study the present correlations. The names and provenances of the races are the following:

ALTIPLANO -- Collection material: Salta Province, Santa Victoria Department, from El Puesto, at about 3000m above sea level, VAV-6167; Jujuy Province, Susques Department, from Susques, at about 3620m above sea level, VAV-6473; from Mal Paso of Susques, at about 3520 m above sea level, VAV-6474.

AMARILLO CHICO -- Collection material: Salta Province, Santa Victoria Department, , from El Condado, at about 2000m above sea level VAV-6451; Jujuy Province, Capital Department, from Termas de Reyes, at about 1690 m above sea level, VAV-6476; Jujuy Province, Tumbaya Department, from Tumbaya, VAV-6484.

AMARILLO GRANDE -- Collection material: Jujuy Province, Tumbaya Department, from La Ciénaga of Purmamarca, at about 2420 m above sea level, VAV-6480.

BLANCO POPULATION -- Grain collection: Jujuy Province, Tumbaya Department, from Purmamarca at about 2180 m above sea level, VAV-6479 and Tilcara Department, from Colonia San José, at about 2670m above sea level, VAV-6485.

CAPIA BLANCO -- Collection material: Salta Province, Santa Victoria Department, from Acoyte, at about 2600m above sea level, VAV-6418.

CAPIA ROSADO -- Collection material: Salta Province, San Victoria Department, from Rodeo Pampa, at about 2900m above sea level, VAV-6162.

CHIRIGUANO -- Collection material: Salta Province, Santa Victoria Department, from El Condado, at about 2000m above sea level, VAV-6218. Observations: Race Chiriguano is only distributed within Santa Victoria Department, province of Salta. It does not have the ample distribution of other races of the highlands in the provinces of Jujuy and Salta.

COLORADO -- Collection material: Formosa Province, Pilcomayo Department, from Frontera and Pilagás Department, from Misión Tacaaglé, both at less than 100m above sea level, VAV-6169 and

6223, respectively,.

HARINOSO -- Collection material: Salta Province, Iruya Department, from Iruya, at about 3400 m above sea level, VAV-6475.

PICHINGA -- Collection material: Formosa Province, Pirané Department, from Villa Dos Trece, at less than 100 m above sea level, VAV-6170.

PISINGALLO -- Collection material: Salta Province, Santa Victoria Department, from Acoyte, about 2600m above sea level and Catamarca Province, Ambato Department, from Piedras Blancas, Los Tordillos, at about 1600m above sea level, VAV-6416 and 6313, respectively.

OCHO RAYAS -- Collection material: Salta Province, Metán Department, from Metán Viejo, at about 750 m above sea level, VAV-6481; Tucuman Province, Trancas Department, Las Arcas, about 1250 m above sea level, VAV-6483.

ORGULLO CUARENTON -- Collection material: Salta Province, Candelaria Department, from La Candelaria, at about 910 m above sea level, VAV-6482.

The Bs were mitotically stable and present in at least one population of each race studied. A numerical polymorphism for Bs was found in 19 populations while it was absent in the remaining two (VAV 6473 and 6474) (Table 1). The number of Bs per plant ranged from 1 to 8, with 1, 2 and 3 being the more frequent. The variation in the percentage of plants with Bs among populations was very wide (0-94%) (1120 plants studied) (Table 1). A highly significant positive correlation between the adjusted mean number of Bs per plant per population and altitude of cultivation was demonstrated (r=0.6024, p=0.0063), when all populations with numerical polymorphism for Bs were considered (Fig. 1). Populations cultivated between 1600-3240m have, in general, the higher frequency of plants with Bs (19.6-94.2%) while those cultivated at altitudes lower than 1600m have the lower frequencies (2-26%) (Table 1). The populations without Bs (VAV 6474 and 6473), which grow at 3520 m and 3620 m, respectively, are placed in a marginal area in the Andean region of Jujuy Province under very extreme environmental conditions for this crop. When

Table 1. B chromosome frequencies and collection data.

| Race | Population (*) | Altitude (m) | | Number of plants with different dosis of Bs (0-8Bs) | | | | | Plants with Bs | Number of Bs | | | | |
|---------------------|-------------------|-----------------|----------|--|-----|----|----|---|-------------------|--------------|---|-------|------|-----------|
| | 17 | 17 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | total | (%) | per plant |
| Blanco | VAV 6479 | 2180 | 3 | 13 | 9 | 14 | 6 | 5 | 1 | | 1 | 52 | 94.2 | 2.615 |
| Blanco | VAV 6485 | 2670 | 10 | 10 | 11 | 9 | 2 | 1 | | 1 | | 44 | 77.3 | 1.795 |
| Amarillo grande | VAV 6480 | 2420 | 15 | 12 | 13 | 7 | 2 | 2 | 1 | | | 52 | 71.2 | 1.596 |
| Altiplano | VAV 6167 | 3000 | 15 | 4 | 9 | 2 | 4 | | | | | 34 | 55.9 | 1.294 |
| Harinoso | VAV 6475 | 3240 | 20 24 | 15 | 8 | 4 | 3 | | | | | 50 | 60.0 | 1.100 |
| Capia blanco | VAV 6418 | 2600 | 24 | 8 | 11 | 1 | 4 | | | | | 48 | 50.0 | 1.020 |
| Amarillo chico | VAV 6451 | 2000 | 45 | 44 | 9 | 11 | 1 | | | | | 110 | 59.1 | 0.900 |
| Pisingallo | VAV 6416 | 2600 | 20 | 13 | 3 | 2 | 2 | 1 | | | | 41 | 51.2 | 0.854 |
| Pisingallo | VAV 6313 | 1600 | 100 | 59 | 10 | 13 | 2 | | | | | 184 | 45.6 | 0.685 |
| Capia rosado | VAV 6162 | 2900 | 19 | 14 | 3 | | | | | 1 | | 37 | 49.6 | 0.649 |
| Colorado | VAV 6169 | 80 | 25 | 7 | 2 | | | | | | | 34 | 26.5 | 0.323 |
| Chiriguano | VAV 6218 | 2000 | 22 | 6 | 1 | | | | | | | 29 | 24.1 | 0.276 |
| Amarillo chico | VAV 6476 | 1690 | 41 | 7 | 3 | | | | | | | 51 | 19.6 | 0.255 |
| Blanco y ocho rayas | VAV 6481 | 750 | 48 | 1 | 5 | 1 | | | | | | 55 | 12.7 | 0.255 |
| Amarillo chico | VAV 6484 | 2010 | 45 | | 2 | 2 | | | | | | 49 | 8.2 | 0.204 |
| Orgullo cuarentón | VAV 6482 | 910 | 44 | 4 | 1 | | | | | | | 49 | 10.2 | 0.122 |
| Blanco y ocho rayas | VAV 6483 | 1250 | 34 | | 2 | | | | | | | 36 | 5.5 | 0.111 |
| Colorado | VAV 6223 | 80 | 37 | 2 | | | | | | | | 39 | 5.1 | 0.051 |
| Pichingá | VAV 6170 | 80 | 42 | 1 | | | | | | | | 43 | 2.3 | 0.023 |
| Altiplano | VAV 6474 | 3520 | 40 | | | | | | | | | 40 | 0.0 | 0.000 |
| Altiplano | VAV 6473 | 3620 | 43 | | | | | | | | | 43 | 0.0 | 0.000 |
| Total | | | 692 | 222 | 102 | 66 | 26 | 9 | 2 | 2 | 1 | 1120 | 38.2 | |

^{(*)-}The materials have been deposited in the seedbanks of Vavilov Laboratory (VAV, Fac. Agr., UBA) and of IFSC (UNLP).

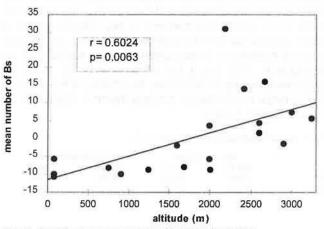


Figure 1. Correlation between mean number of Bs (adjusted) and altitude.

these populations were included in the statistical analysis the correlation was not significant (r=0.3498,p=0.1201).

The frequencies recorded in the present study would be the highest ones if compared with the previous few populational studies carried out on individuals with B frequencies in native races of maize.

B chromosomes in native races of maize from Argentina. II. Genome size and altitude

--Rosato, M; Chiavarino, AM; Naranjo, CA; Poggio, L

The DNA content of 17 populations (107 individuals) with varying mean frequencies of B-chromosomes (Bs) was determined (Table 1). The materials used are the same that were detailed in part I (Populational frequency and altitude) in this MNL. With

Table 1. Races, altitude of cultivation, mean number of Bs, and mean DNA content of plants without B chromosomes.

| Race | Population | Altitude (m) | Mean number of Bs | DNA content ±SE (pg) (no. plants) |
|---------------------|------------|-----------------|-------------------|--------------------------------------|
| Blanco | VAV 6479 | 2180 | 2.615 | 6.410 ±0.08 (3) |
| Blanco | VAV 6485 | 2670 | 1.795 | 5.800 ±0.09 (6) |
| Amarillo grande | VAV 6480 | 2420 | 1.596 | 6.105 ±0.10 (8) |
| Altiplano | VAV 6167 | 3000 | 1.294 | 5.007 ±0.25 (4) |
| Harinoso | VAV 6475 | 3240 | 1.100 | 6.450 ±0.13 (6) |
| Capla blanco | VAV 6418 | 2600 | 1.020 | 5.767 ±0.14 (6) |
| Amarillo chico | VAV 6451 | 2000 | 0.900 | 5.665 ±0.22(6) |
| Pisingallo | VAV 6313 | 1600 | 0.684 | 6.148 ±0.10 (8) |
| Capia rosado | VAV 6162 | 2900 | 0.649 | 5.741 ±0.22 (3) |
| Amarillo chico | VAV 6476 | 1690 | 0.255 | 6.280 ±0.18 (6) |
| Blanco y ocho rayas | VAV 6481 | 750 | 0.255 | 6.756 ±0.05 (7) |
| Amarillo chico | VAV 6484 | 2010 | 0.204 | 6.353 ±0.19 (7) |
| Orgulio cuarentón | VAV 6482 | 910 | 0.122 | 6.150 ±0.08 (6) |
| Blanco y ocho rayas | VAV 6483 | 1250 | 0.111 | 6.601 ±0.12 (4) |
| Pichingá | VAV 6170 | 80 | 0.023 | 6.172 ±0.10 (4) |
| Altiplano | VAV 6474 | 3520 | 0.000 | 6.514 ±0.05 (7) |
| Altiplano | VAV 6473 | 3620 | 0.000 | 6.493 ±0.20 (6) |

the aim of studying the variation in DNA content of A-chromosomes (A-DNA) independently from the variation supplied by the Bs, this estimation was performed in plants without Bs (2n=20) (5 to 6.756pg). In these populations a 36% variation in DNA content of the members of the regular complement was demonstrated. This range of variation is very high compared with the values reported for 32 populations of maize from the USA and Mexico (Laurie and Bennett, Heredity 55:307-313, 1985; Rayburn et al. Am. J. Bot. 72:1610-1617, 1985). It was found that A-DNA content is negatively correlated with altitude of cultivation (r=-0.3139, p=0.0022) (Fig. 1). However, when non-polymorphic

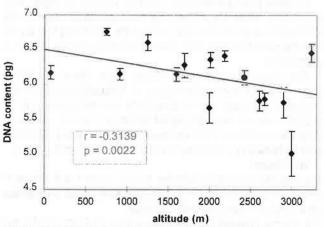


Figure 1. Correlation of A-DNA content (in plants without Bs) with altitude.

populations (VAV 6473 and 6474) were included in the analysis the correlation was not significant (r= -0.1012, p=0.3045) due in this case to their high A-DNA content.

Other authors also observed similar results. Rayburn (Evol. Trends Plant 4:53-57, 1990) found a negative correlation between total DNA content and altitude in 12 american populations of maize growing from 4900 to 7000 ft (1493 to 2134m).

The only exception was that reported by Rayburn and Auger (Theor. Appl. Genet. 79:470-474, 1990), who found an inverse relationship in 12 Indian maize populations collected from 100 to 5300 ft (31 to 1621m).

A significant negative correlation between mean DNA content (measured in 0B individuals) and the mean frequency of Bs per plant was found in the 17 populations (r=-0.3348, p<0.0001) (Figure 2) (Table 1). Interestingly, populations belonging to the same race also showed the same tendency. For instance, populations VAV 6484, 6476 and 6451, belonging to "Amarillo chico" race, with frequency of Bs of 0.20, 0.25 and 0.9 had decreasing values of A-DNA content (6.353, 6.280 and 5.665pg respectively). The same happened when populations VAV 6474, 6473 and 6167 (all from Altiplano race) were compared. The first two, cultivated at 3520m and 3620m, without Bs, had an A-DNA content of 6.5 pg and 6.49 pg whereas the VAV 6167, with a frequency of Bs of 1.29 showed a lower DNA content in the A genome (5.0pg) (Table 1).

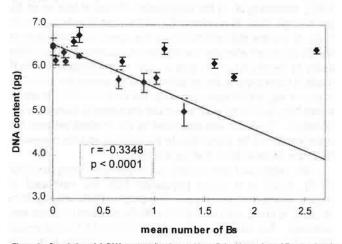


Figure 2. Correlation of A-DNA content (in plants without Bs) with number of Bs per plant in its population.

A similar phenomenon was found by Longley (J. Agric. Res. 56:177-195, 1938) when analyzing the association between the number of knobs and the occurrence of Bs. He found that Bs are more frequent in plants with fewer than seven knobs in their A chromosomes.

Tito, Poggio and Naranjo (Theor. Appl. Genet. 83:58-64, 1991) observed that higher values of heterochromatin content (equivalent to knobs) are correlated with increasing values of total DNA content in several varieties and lines of maize. As has already been mentioned, the decrease of DNA content in 0B plants could be related to a decrease in heterochromatic bands (knobs).

In conclusion:

-The negative correlation between A-DNA content and altitude of cultivation could be explained if genome size played an adaptive role in relation to environmental variables.

-The positive correlation between frequency of plants with Bs and altitude is closely related to the A-DNA amount.

-The negative correlation between frequency of plants with Bs and A-DNA amount indicates that Bs are better tolerated in populations with lower A-DNA values.

-The results obtained would indicate that there would be a maximum limit to the mass of nuclear DNA or nucleotype (Bennett, New Phytologist 106 (Suppl.):177-200, 1987) so that Bs would be tolerated as long as this maximum limit is not exceeded.

-The clinal variation of A-DNA content and the consequent inverse correlation of B frequencies over an altitudinal gradient could have an adaptive significance, since the populations analyzed were not distributed over a transect (Fig. 1). They were cultivated by aborigines and hardly interbreed with each other. Therefore, historical isolation of populations with high and low A-DNA content by genetic drift at both extremes of an altitudinal gradient, and subsequent gene flow between them, is not a very plausible explanation.

B chromosomes in native races of maize from Argentina. III. Selection of high and low transmission genotypes and meiotic behavior of Bs

-- Chiavarino, AM; Rosato, M; Rosi, P; Naranjo, CA; Poggio, L

In native populations from northern Argentina we found numerical polymorphism for accessory chromosomes (Bs). It is reported that Bs are maintained in populations due to various mechanisms of drive (Carlson, W. R. and Roseman, R. Genetics 131: 211-223, 1992), consisting of: 1) the suppression of meiotic loss when Bs are in single dose, 2) nondisjunction at the second pollen grain mitosis, 3) preferential fertilization by the sperm nucleus carrying the Bs produced after the nondisjunction process, 4) competitive ability of B-carrying pollen grains. One of the features of the B mode of inheritance is the variation in their transmission rate (TR) in such a way that in some progenies the Bs tend to be lost and in others they tend to increase in number compared to Mendelian expectation. The B TR was estimated by the quotient between the mean number of Bs transmitted to the progeny and the number of Bs of the parental plant that carried them.

This experiment was carried out in the race Pisingallo (VAV 6313), which is a native population from the northwest of Argentina collected on our own. The progeny (G0) obtained from 20 0B x 1B (male side) and 20 1B x 0B (female side) crosses was analysed. The B-TR variation ranged from 0.17 to 0.98 (mean TR= 0.52) and from 0.31 to 0.58 (mean TR= 0.48) for both male and female sides (Figures 1 and 2). Afterwards, the progenies

showing the highest and lowest male and female B-TR were selected through two generations (G1 and G2). The results obtained in G1 indicate the presence of two different groups of plants, high and low B-TR for male and female sides, and demonstrate the existence of polymorphism for genes controlling B-TR (Rosato et al. 1996, Am. J. Bot. 83(9): 1107-1112). In G2 the groups were kept up for both sides, nevertheless it was not possible to obtain more selection progress than that obtained in G1 (Table 1, Figures 1 and 2).

Table 1. Male and female transmission rate in G1 and G2.

| | Male tran | nsmission | Female transmission | | |
|----|-----------|-----------|---------------------|--------|--|
| | High TR | Low TR | High TR | Low TR | |
| G1 | 0.650 | 0.420 | 0.485 | 0.405 | |
| G2 | 0.670 | 0.485 | 0.532 | 0.411 | |

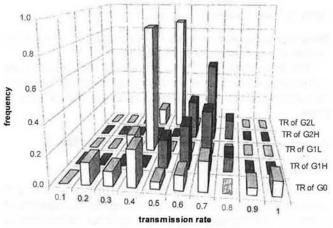


Figure 1. Male transmission rate of G0, G1 and G2.

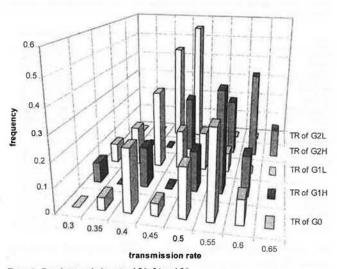


Figure 2. Female transmission rate of G0, G1 and G2.

The B univalent precociously migrates to a pole during anaphase I and integrates in one of the nuclei. The B univalent is lost when it lags or divides equationally during anaphase I. Rare meiotic loss of Bs was observed and no significant differences were found when high and low male B-TR plants from the parental population were compared (Table 2).

Generally, in 2B plants, Bs can be observed forming a bivalent and remaining in the metaphase I plate together with the A biva-

Table 2. Meiotic behavior of 1B in VAV 6313 population plants with different B TR.

| B TR of plants of VAV 6313 population (1B) | | agging in | | Bs split tically in Al (%) | 1000000 | onudei in vads (%) | 1424,4431 | ronuclei In etrads (%) |
|--|---|-----------|---|----------------------------------|---------|-----------------------|-----------|---------------------------|
| population (1.5) | | cell no. | | cell no. | | cell no. | | cell no. |
| 0.17 (low TR) | 0 | 108 | 2 | 108 | 3 | 133 | 0 | 152 |
| 0.17 (low TR) | 0 | 50 | 0 | 50 | 7 | 48 | 2 | 270 |
| 0.40 (low TR) | 1 | 322 | 2 | 322 | 8 | 339 | 0 | 155 |
| 0.66 (high TR) | 2 | 158 | 3 | 158 | 3 | 330 | 0 | 98 |
| 0.98 (high TR) | 1 | 150 | 1 | 150 | 4 | 130 | 1 | 284 |

lents; the two homologous Bs have regular meiotic behavior. High and low male B-TR plant groups have no significant differences forming two B univalents in G1 and G2. The low frequency of micronuclei in telophase I - dyad and telophase II - tetrad indicates the scarce meiotic loss of the two Bs (Table 3).

Table 3. Meiotic behavior of 2Bs in plants selected for high and low TR.

| Bs lagging or micronuclei in TI- | | ency of 2 diak and | | gging or nudei in | | gging or clei in TII - |
|-------------------------------------|------|-----------------------|-------|----------------------|-------|---------------------------|
| dyads (%) | M | (%) | TI-dy | ads (%) | tetra | ds (%) |
| 50 A33A | | cell no. | | cell no. | | cell no. |
| 0.27 (low TR) | 16.1 | 267 | 1.0 | 231 | 1.2 | 85 |
| 0.27 (low TR) | 2.7 | 263 | 9.6 | 124 | 4.6 | 109 |
| 0.44 (low TR) | 6.4 | 125 | 0.0 | 83 | 0.0 | 38 |
| 0.28 (low TR) | 11.8 | 136 | •••• | | 2.4 | 41 |
| | | | **** | | | |
| 0.28 (low TR) | 16.6 | 235 | 3.3 | 245 | 2.6 | 76 |
| 0.84 (high TR) | 2.1 | 192 | 5.7 | 56 | 1.4 | 69 |
| 0.84 (high TR) | 2.3 | 88 | 2.1 | 48 | 1.2 | 82 |
| 0.98 (high TR) | 8.4 | 333 | 4.0 | 99 | 1.6 | 64 |
| 0.92 (high TR) | 2.9 | 173 | 1.7 | 57 | 2.4 | 254 |
| 0.92 (high TR) | 1.0 | 386 | 1.7 | 121 | 2.6 | 190 |
| | | | | | | |

In conclusion, the variation found in male and female B-TR could be due to the presence of few genes controlling B-TR in this population. These genes do not affect the meiotic behavior of Bs in high and low B-TR plants. Therefore it seems that these genes could modify the frequency of differential fertilization by the sperm nuclei carrying 0B or 2Bs in the same pollen grain. So, the maintenance of the numerical polymorphism for Bs could be due to the balance of two opposite strengths in populations (pro B -high B-TR- and anti B genes -low B-TR-).

Recalibration of opaque-2 line of maize as a standard to estimate nuclear DNA amount

--Rosato, M; Naranjo, CA; Poggio, L

Measurements of DNA amount by microdensitometry usually are based on nuclei stained by the Feulgen method. It is generally accepted that Feulgen staining is specific for DNA (after removal of RNA by acid hydrolysis) and its proportionality between stain density and DNA amount can be assumed. Consequently the light absorption of a nucleus so stained is a quantitative measure of its DNA content (Bennett and Smith, Proc. Roy. Soc. London B:227-274, 1996).

When nuclear DNA content is measured by this method the results are obtained in arbitrary units (A.U.). The arbitrary units can be converted into absolute units in picograms (pg), through a standard species the DNA amount of which is already known.

Bennett and Smith (I.c.) gave the criteria to recalibrate several species against *Allium cepa* cv. Ailsa Craig (2C= 33.5 pg) in order to justify their use as known standards for calibrating other species. This procedure tends to minimize some technical errors inherent in Feulgen microdensitometry.

The standards available for species of the genus Zea in the literature are 2C= 5.155 pg for Va35 line (Rayburn et al., Am. J. Bot. 72:1610-1617, 1985; Rayburn et al., J. Exp. Bot. 40:1179-

1183, 1989), and 5.9 pg for c-tester line (Tito, Poggio and Naranjo, MNL 65:76, 1991). The latter is cultivated in the IFSC (FCAF, UNLP) and has reduced vigor and fertility as a result of many generations of inbreeding. Because in our laboratory we are working in intra- and interpopulation variation of DNA amount of Argentine races of maize, it was convenient to recalibrate a line available in our country and with good fertility.

The opaque-2 line (raised by M. Aulicino, IFSC) has all the characteristics suggested by Bennett and Smith to justify its use as standard; therefore, four experiments were done on different days to recalibrate carefully the 2C value of opaque-2 line against Allium cepa cv. Ailsa Craig. The technique of staining was performed as described by Tito, Poggio and Naranjo (Theor. Appl. Genet. 83:58-64,1991).

DNA content was measured in 20 telophase nuclei (2C) of the root tips of germinating seeds. Roots of 0.5-1 cm in length were fixed in 3:1 (ethanol:acetic acid). After fixation, the roots were rinsed for 30 min in distilled water. Hydrolysis was carried out with 5 N HCl at 20 C for 30 min The roots were then washed three times in distilled water for 15 min, and stained for 120 min in Schiff's reagents at pH 2.2. The material was then rinsed three times in SO2 water for 10 min each, kept in distilled water and squashed in 45% acetic acid. The coverslip was removed after freezing with CO2 and the slide was dehydrated in absolute alcohol and then mounted in Euparal. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm, using the scanning method with a Zeiss Universal Microspectrophotometer (UMSP 30). The DNA content per basic genome expressed in pg was calculated using A. cepa cv. Ailsa Craig as a standard. The differences in DNA content were tested through an analysis of variances and comparisons between means using Scheffe's method. The results obtained are shown in Table

Table 1. Recalibration of opaque-2 line as a standard to estimate nuclear DNA amount.

| Experiment | Nuclei mea experiment Individual (No.) | | DNA content ±SE (A.U.) | (2C) ±SE (pg) | | |
|------------|---|----|---------------------------|------------------|--|--|
| 1 | 1 | 20 | 13.34 ± 0.14 | 6.678 ± 0.07 | | |
| | 2 | 20 | 13.32 ± 0.17 | 6.662 ± 0.08 | | |
| | 3 | 20 | 13.29 ± 0.11 | 6.620 ± 0.06 | | |
| 2 | 4 | 18 | 13.60 ± 0.13 | 6.799 ± 0.06 | | |
| | 5 | 19 | 15.60 ± 0.16 | 6.736 ± 0.06 | | |
| | 6 | 10 | 15.81 ± 0.34 | 6.800 ± 0.15 | | |
| 3 | 7 | 20 | 15.82 ± 0.14 | 6.644 ± 0.06 | | |
| | 8 | 15 | 14.96 ±0.19 | 6.738 ± 0.08 | | |
| 4 | 9 | 20 | 13.17 ± 0.19 | 6.454 ± 0.09 | | |
| | 10 | 10 | 13.05 ± 0.33 | 6.450 ± 0.15 | | |

An anova test was made, and it was determined that the results do not show significant differences among themselves (F= 1.7203, p< 0.05). The results obtained point out a DNA average amount for opaque-2 line 2C= 6.658 ± 0.038 pg.

Relationship among Zea luxurians, Z. diploperennis and Z. perennis (Sect. Luxuriantes)

--Poggio, L; Confalonieri, V; Comas, C; Gonzalez,G; Aulicino, M; Naranjo, CA

The genus Zea (Tribe Maydeae) is composed, according to Doebley and Iltis (Am. J. Bot. 67:994-1004, 1980), of two sections: Sect. Zea and Sect. Luxuriantes. The last section includes the perennials Z. diploperennis Iltis, Doebley & Guzman (2n=20) and Z. perennis (Hitch.) Reeves & Mangelsdorf (2n=40) and the annual Z. luxurians (Durue & Archenon) Bird (2n=20).

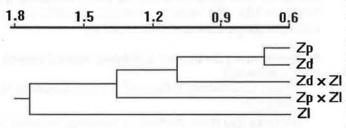
In the last few years, cytological evidence arose about the polyploid nature of all species of the genus Zea (Molina and Naranjo 1987, Theor. Appl. Genet. 73: 542-550; Poggio et al. 1990, Theor. Appl. Genet. 79:461-464; Naranjo et al. 1990, Acad. Nac. Cs. Ex. Fís. Nat., Buenos Aires, Monografía 5:43-53; Naranjo et al. 1994, Hereditas 120:241-244; Poggio and Naranjo 1995, Proceeding of III Latin American and XVI Andean Zone of Maize Researches Meeting. Bolivia, Tomo II:883-892). These authors postulated for the first time that five is the original basic number for the genus Zea. Some of the most suggestive cytological evidence which allowed postulating this hypothesis, stemmed from the analysis of meiotic behaviour of 2n=30 hybrids such as Z. perennis x Z. diploperennis and Z. perennis x Z. mays. The present contribution deals with the analysis of the meiotic behaviour of the intrasectional hybrid Z. luxurians x Z. perennis (2n=30). These hybrids are highly sterile (pollen stainability <10%). The most frequent meiotic configuration was 5III + 5II + 5I (X_{III} = 5.17, ranging from 8 to 3; $X_{II}=4.65$, ranging from 2 to 8; $X_{I}=4.52$, ranging from 2 to 7) (Table 1). The most parsimonious hypothesis to explain this result is to consider x=5 for the genus, as was postulated in previous reports from our laboratory.

Table 1. Meiotic configurations in the F1 Z. perennis x Z. luxurians hybrid.

| Hybrid | 2n | Meioti | c Configur | ations | No. of cells | |
|----------------------------|----|--------|------------|--------|--------------|--|
| | | III | 11 | 1 | | |
| Z. perennis x Z. luxurians | 30 | 8 | 2 | 2 | 4 | |
| | | 7 | 3 | 3 | 4 | |
| | | 6 | 4 | 4 | 2 | |
| | | 6 | 3 | 6 | 4 | |
| | | 5 | 6 | 3 | 2 | |
| | | 5 | 5 | 5 | 17 | |
| | | 5 | 4 | 7 | 1 | |
| | | 4 | 7 | 4 | 3 | |
| | | 4 | 6 | 6 | 6 | |
| | | 3 | 8 | 5 | 2 | |
| | X | 5.17 | 4.65 | 4.52 | 46 | |

The meiotic configuration of the *Z. luxurians* x *Z. diploperennis* (2n=20) hybrid was also analyzed. This hybrid is highly sterile and its pollen stainability lower than 10%. The most frequent configuration observed was 8II + 4I ($X_{II}=8.65$, $X_{I}=2.70$). At least three of these bivalents are very often heteromorphic.

The three species and both hybrids were also analysed by means of numerical techniques. With this purpose 13 vegetative morphological characters of plants and 10 reproductive ones (4 of prolificity, 4 from panicles and 2 from glumes) were used. The five OTU's (Operational Taxonomic Units) were grouped by means of UPGMA methods using the coefficient of mean taxonomic distance (DTM).



The phenogram constructed with these data shows that Z. diploperennis (Zd) and Z. perennis (Zp) are grouped, constituting the most proximal pairs of species. Then, both hybrids come out from this first group and finally, Z. luxurians (ZI) emerges at a

greater distance with respect to the other OTU's.

In order to estimate the molecular genomic affinities among all three species of the section, these were also analysed by means of "dot blot" hybridization. Total genomic DNA from *Z. perennis*, *Z. diploperennis* and *Z. luxurians* was labelled with digoxigenin dUTP and used as a probe over dot blots on nylon membranes of similar serial concentrations of DNA of the same species. The technique was carried out according to Ann Kenton et al. (Evolutionary Paterns and Processes, Chapter 12: 190-206, The Linnean Soc. Press, 1993) with some minor modifications.

Table 2. Relating genomic affinities analysed by means of "DOT blot".

| | Z. I. Z. p. ++++++++++++++++++++++++++++++++ | | | |
|------------------|--|-------|-------|--|
| | Z.1. | Z. p. | Z. d. | |
| Z. luxurians | ++++ | ++ | + | |
| Z. perennis | ++ | ++++ | +++ | |
| Z. diploperennis | + | +++ | ++++ | |

All probes were hybridized with all three species (Table 2), even with high stringency washes. However, *Z. perennis* and *Z. diploperennis* showed more molecular affinity between them than with *Z. luxurians*. This result is congruent with the morphological data.

Waxy maizes from Argentina. I. Potential yield and endosperm protein content

--Corcuera, VR; Naranjo, CA

During 1990 at the IFSC-CIGEN and CITIM, a maize breeding plan was initiated. The purpose is to obtain waxy endosperm commercial hybrids. Four foundational populations were used: SCV1, SCV2, WEM and FW. To obtain inbreds from these populations, the ear-to-row method proposed by Shull is being used. Simultaneously, a divergent selection procedure is being done. This is based on Illinois Selections, as individuals with high protein content in their endosperm are separated from those with a low value for the same trait in each generation of inbreeding. Selfings are practised to obtain new generations.

In this article, the results obtained through the characterization of the foundational materials, as well as from their S1 progenies, are presented. With this purpose some plant, prolificity, ear and evolutive cycle traits were measured according to maize descriptors recommended by IBPGR.

PLANT TRAITS: plant height (PH) in meters; upper ear insertion height (UEIH)in meters; stalk diameter (SD) in cm; stalk number (SN); total leaf number (TLN).

2. PROLIFICITY TRAITS: number of productive nodes per tiller (PN); ears in the uppermost node (EUN); ears per tiller (ET) and ears per plant (EP).

3. EAR TRAITS: ear length in cm (EL); ear diameter in cm (ED); number of rows (RN); number of kernels per row (NKR); ear weight in grams (EW); kernel weight per ear in grams (KWE) and cob percentage (%C).

4. EVOLUTIVE CYCLE TRAITS: days from plant emergence to silking (DS); heat units from plant emergence to silking (HUS) in degrees C and F.

Tables 1 to 3 show the average values found for plant, prolificity and ear traits in the foundational materials and their S1 progenies.

SCV1 and SCV2 are two similar populations from the IFSC. They segregate for the gene wx in a 3:1 ratio. Both populations

Table 1. Plant tralts measured in the populations (So) and their S1 progenies.

| Material | Gn | PH* (m) | UEIH 1 (m) | SD (cm) | SN | TLN |
|----------|-----|---------|------------|---------|-------|--------|
| SW1 | So | 1.23a | 0.37a | 1.67a | 1.13a | 9.75a |
| | S1 | 1.49b | 0.48a | 1.60a | 1.60b | 9.75a |
| SW2 | So | 1.40a | 0.43a | 1.58a | 1.07a | 10.00a |
| | S1 | 1.53a | 0.54a | 1.65a | 1.24a | 10.04a |
| WEM | So | 1.33a | 0.33a | 1.44a | 1.23a | 9.56a |
| | \$1 | 1.31a | 0.37a | 1.48a | 1.04a | 9.74a |
| FW | So | 1.86a | 0.68a | 1.93a | 1.12a | 13.03a |
| | S1 | 2.01a | 0.65a | 1.96a | 1.13a | 12.11b |

^{*} see abbreviations in the text.

Table 2. Prolificity traits measured in the populations (So) and their S1 progenies.

| Material | Gn | PN' | EUN 1 | ET | ₽ |
|----------|----|-------|-------|-------|-------|
| SCV1 | So | 1.50a | 1.10a | 1.50a | 1.50a |
| | S1 | 1.18 | 1.10a | 1.18a | 1.90a |
| SCV2 | So | 1.14a | 1.05a | 1.15a | 1.14a |
| | 51 | 1.46a | 1.12a | 1.58b | 1.84b |
| WEM | So | 1.40a | 1.07a | 1.37a | 1.62a |
| | S1 | 1.29a | 1.07a | 1.35a | 1.41a |
| PW | So | 1.14a | 1.07a | 1.04a | 1.11a |
| | S1 | 1.50a | 1.11a | 1.32a | 1.43b |

^{*}see abbreviations in the text

Table 3. Ear traits measured in the populations (So) and their S1 progenies.

| Material | Gn | EL* | ED ¹ (cm) | RN | NKR | EW g | WE g | % C |
|----------|----|--------|-------------------------|--------|--------|---------|--------|--------|
| SCV1 | So | 12.65a | 2.95a | 13.60a | 21.16a | 36.90a | 29.61a | 21.25a |
| | S1 | 12.30a | 3.12a | 15.00a | 22.25a | 54.50b | 44.00b | 19.24a |
| SCV2 | So | 13.96a | 3.62a | 14.40a | 27.00a | 70.80a | 75.40a | 18.87a |
| | S1 | 13.78a | 3.60a | 14.81a | 23.07a | 62.78a | 48.70a | 24.00b |
| WEM | So | 13.70a | 3.57a | 14.34a | 20.67a | 52.50a | 37.50a | 29.13a |
| | S1 | 14.85a | 3.77a | 14.61a | 25.52b | 79.13b | 60.43b | 25.00a |
| PW | So | 16.49a | 3.89a | 14.75a | 29.00a | 107.75a | 85.25a | 21.02a |
| | S1 | 18.62b | 4.17a | 15.34a | 29.34a | 102.50a | 80.00a | 24.64a |

^{*}see abbreviations in the text.

show short plants, with a low number of total leaves and slender stalks. Nevertheless, there are differences between them when prolificity traits are considered: SCV1 has high prolificity values whilst SCV2 shows low prolificity. SCV1 and SCV2 have small ears, but SCV1 has a high cob percentage.

WEM is another population from the IFSC. It carries the segment *c c sh sh wx wx* in the short arm of chromosome 9. The plants are short, with slender stalks, low number of total leaves, high prolificity, small and light ears with a high cob percentage.

FW was sent by CIMMYT in 1990. These are medium height plants, with approximately 13 leaves, low prolificity values, normal size ears but with high cob percentage.

Table 4 summarizes evolutive cycle trait values for the materials studied. All of them, present short evolutive cycle (measured from emergence to silking). Heat units in the period vary from 783.68 F to 988.16 F. Because of this, we recommend the sowing at a density of 70,000 to 80,000 plants/ha (35,000 to 40,000 plants/acre) in locations with a good water disposal. In Table 5, the potential yields are shown. In the estimations, prolificity values, yield/plant (grams of kernels/ear) and recommended sowing density were considered. The highest yielding inbred is FW followed in decreased order by SCV2, WEM and SCV1.

Table 4. Average days to silking and Heat Units measured in the populations (So) and their S1 properties.

| Material | Gn | Days | Heat | Units |
|----------|----|-------|-------|-------|
| 1-50100 | | | С | F |
| SCV1 | So | 54.50 | 459.6 | 859.3 |
| | S1 | 55.12 | 470.3 | 878.5 |
| SCV2 | So | 53.89 | 459.6 | 859.3 |
| | S1 | 48.77 | 417.6 | 783.7 |
| WEM | So | 54.79 | 470.3 | 878.5 |
| | S1 | 58.07 | 506.3 | 943.3 |
| PW | So | 58.82 | 518.2 | 964.8 |
| | S1 | 59.64 | 531.2 | 988.2 |

Table 5. Potential yield (kg/ha) of the populations (So) and their S1 progenies according to sowing density (70,000-80,000 plants/ha), EP and KWE.

| Material | Gn | EP | No. ears/ha | Potential yield (kg/ha) |
|----------|----|------|-----------------|-------------------------|
| SCV1 | So | 1.5 | 105,000-120,000 | 3,109-3,553 |
| | S1 | 1.9 | 133,000-152,000 | 5,852-6,688 |
| SCV2 | So | 1.14 | 79,800- 91,200 | 4,580-5,235 |
| | S1 | 1.84 | 128,800-147,200 | 6,273-7,169 |
| WEM | So | 1.62 | 113,400-129,600 | 4,252-4,860 |
| | S1 | 1.41 | 98,700-112,800 | 5,964-6,816 |
| PW | So | 1.11 | 77,700- 88,800 | 6,624-7,570 |
| | S1 | 1.43 | 100,100-114,400 | 8,008-9,152 |

Considering the potential yields and average endosperm protein content, the amount of protein per hectare produced by each population and their S1 progenies can be estimated. Taking into account the semola extraction percentage during the milling process (approximately 45% of the field yield), the amount of protein in semolas produced per hectare was also calculated (see Table 6). It must be pointed out that the S1 inbred derived from the population FW has 10.74% protein in its endosperm. This value is superior to the average shown by most commercial hybrids in Argentina.

Table 6. Protein yields per hectare considering semola production (kg/ha) in the populations (So) and their S1 progenies.

| Material | Gn | Endosperm protein content (%) | Semola yield (kg/ha) | Protein yield (kg protein/semola yield) |
|----------|----|----------------------------------|-------------------------|--|
| SCV1 | So | 10.80 | 1,399-1,599 | 151-173 |
| | S1 | 8.76 | 2,633-3,010 | 231-264 |
| SCV2 | So | 8.14 | 2,061-2,356 | 168-192 |
| | S1 | 8.12 | 2,823-3,226 | 229-262 |
| WEM | So | 8.73 | 1,913-2,187 | 167-191 |
| | S1 | 9.21 | 2,684-3,067 | 247-282 |
| PW | So | 11.38 | 2,981-3,906 | 339-388 |
| | S1 | 10.74 | 3,603-4,118 | 387-442 |
| | | | | |

Finally, the authors insist that beyond the selection of high protein content ears, it is more interesting to select through the amount of protein produced per hectare by the inbred. Then it is necessary to select jointly by high yields and high protein content. In most cases, protein content is negatively related to yield, but in some genotypes such as FW, such negative correlation does not seem to exist, and then it is possible to carry out a selection process that contemplates both traits with certain success.

Waxy maizes from Argentina. II. Endosperm protein content of inbreds

--Corcuera, VR; Naranjo, CA

As part of a maize breeding program with the purpose of obtaining waxy maize commercial hybrids, a selection process by high and low endosperm protein content in and among inbreds was carried out.

This program was initiated in 1990 at the IFSC with the collaboration of CITIM. Four waxy maize foundational populations (SCV1, SCV2, WEM and FW) were used and characterized. S1, S2 and S3 inbreds were obtained from these populations using

¹Average values followed by the same letter do not differ significantly at 0.05% within the same population.

Gn = generation

¹Average values followed by the same letter do not differ significantly at 0.05% within the same population.
Gn= generation

¹Average values followed by the same letter do not differ significantly at 0.05% within the same population.
Gn= generation.

the ear-to-row method developed by Shull, and applying a divergent selection procedure. This way, ears with high and low endosperm protein content are identified in each generation, similarly to Illinois Selections. In our case, each generation of inbreds is obtained by selfings and their endosperm protein content is measured at the laboratory using the microKjeldahl procedure (A.O.A.C., 1981). Values over 10.1% are considered high protein content in endosperm defatted flours, whilst minor values are considered low.

On the other hand, crosses of low endosperm protein content inbreds x high and the reciprocal crosses were done. A similar process, but using heterozygotes, was also done. The F1's obtained, as well as both parents of the crosses, were measured for their endosperm protein content. The results obtained let us confirm our previous hypothesis about the inheritance mechanism for the trait under study and set down an elucidative genetic model (see MNL 69, 1995).

In Table 1, for each population studied, as well as for the Sn inbred lines derived from them, the average endosperm protein content, range of variation, standard deviation and variance are shown. In each early inbred generation there are low and high endosperm protein content genotypes. This confirms the heterozygous condition for the trait of the original populations. Then, and according to the genetic model opportunely presented, the populations SCV2 and WEM will denote in their endosperm the genotype a/a/A, as they show low protein content in the endosperm, but the S1 inbreds derived show variation for the trait. This way, the polar cells of the original populations could be aa or AA whilst the second antherozoid genotype will be a or A. The possible genotypes of the S1 generation obtained by selfing plants of the original populations could be: a/a/a; a/a/A; a/A/A or A/A/A. This fact would explain the variation found for the trait in S1 inbred lines.

Table 1. Descriptive statistics for endosperm protein content in the populations (So) and the inbreds derived from them.

| Material | Gn | Average | S.D. | Variance | Range of Variation |
|----------|----|---------|------|----------|--------------------|
| SCV1 | So | 10.80 | | | |
| | S1 | 8.76 | 0.94 | 0.88 | 8.11-9.83 |
| SCV2 | So | 8.14 | | | |
| | S1 | 8.12 | 1.46 | 2.14 | 6.07-10.16 |
| | S2 | 10.22 | 1.09 | 1.19 | 8.25-11.65 |
| WEM | So | 8.73 | | | |
| | S1 | 9.21 | 4.60 | 21.19 | 5.96-12.47 |
| | S2 | 11.70 | 0.70 | 0.49 | 11.21-12.20 |
| PW | So | 11.38 | | | |
| | S1 | 10.74 | 2.50 | 6.27 | 8.28-14.79 |
| | S2 | 9.58 | 1.39 | 1.92 | 8.43-11.21 |
| | S3 | 11.37 | 2.43 | 5.92 | 9.65-13.09 |

The populations SCV1 and FW show high protein content. Thus, their endosperm genotype could only be a/A/A since in the S1 to S3 inbreds obtained by selfings it is possible to find individuals with high or low endosperm protein content.

It must be pointed out that in all the inbred generations derived from each original waxy maize population, individuals exist with a higher endosperm protein content than usual in commercial maize hybrids grown in Argentina. On the other hand, whether inbreeding normally causes depression for yields and plant traits, in the case of endosperm protein content it does not seem to do so. This is surely related to the inheritance mechanism of the trait and the divergent selection procedure used. Through consecutive selfing generations and selecting in and among the inbreds it is possible to increase the protein content in the endosperm.

Description of commercial hybrids and mutant inbreds incorporated into a waxy maize breeding plan in Argentina

-- Corcuera, VR; Naranjo, CA

During the growing season 1995/96 new materials were incorporated into a maize breeding plan carried out at the IFSC and in collaboration with CITIM.

By the end of 1994, a group of 18 mutant inbred lines was sent to the IFSC from the Maize Genetics COOP Stock Center (Urbana, Illinois, USA). These inbreds have mutant genes of interest for industrialization processes such as ones for modified starches and semolas with high protein quality. Such is the case with the genes wx1, o2, o5, o9, o12, o13 and ae.

Table 1 shows the list of the lines studied, their genotype and the average values found for the traits measured. The measurements were based on different descriptors grouped as follows:

A. PLANT TRAITS: stalk diameter in cm (SD), total leaf number (TLN) and number of leaves above the uppermost ear (NLUE).

B. PROLIFICITY TRAITS: ears per tiller (ET) and ears per plant (EP).

Table 1. Average values found for plant and prolificity traits measured in mutant inbreds sent by Maize Genetics Coop Stock Center in 1994.

| Inbred | Genotype | SD* | 田 | EI | ILN | NLUE |
|------------|-------------------|------|------|------|-------|------|
| 83-2205-2 | C1 sh1 wx1 AR | 1.36 | 2.00 | 2.00 | 10.80 | 4.80 |
| 87-3614-1 | c1 sh1 wx1+/v1 AR | 1.20 | 2.00 | 2.00 | 9.00 | 4.00 |
| 94W-140-5 | w23/L317/ ae1 td1 | 1.68 | 1.82 | 1.82 | 11.91 | 4.91 |
| 88-3553-8 | su1/su1 | 1.48 | 1.50 | 1.00 | 9.34 | 4.67 |
| 90-1863(4) | su1/su1 | 1.81 | 1.20 | 1.00 | 11.90 | 5.50 |
| 93W-1273-3 | 011 | 1.23 | 1.56 | 1.56 | 10.22 | 4.78 |
| 85-154-4 | 09 | 1.79 | 1.62 | 1.62 | 11.25 | 5.75 |
| 92-158-4 | +/05 | 1.64 | 1.82 | 1.82 | 12.53 | 5.47 |
| 94-1590-1 | +++/c1 sh1 wx1 | 1.57 | 1.40 | 1.40 | 11.00 | 4.50 |
| 92-1540(4) | +/ae1 0h43 | 1.39 | 1.00 | 1.00 | 9.86 | 4.86 |
| 94-1275-1 | a1 sh2/a1 sh2 | 1.49 | 2.86 | 2.86 | 11.14 | 4.43 |
| 93-1291(2) | wx1 +/d3 | 1.46 | 2.00 | 2.00 | 10.64 | 4.64 |
| 93-4042-1 | y1 Bh1 c1 sh1 wx1 | 1.22 | 1.87 | 1.87 | 9.87 | 4.25 |
| 86-1535-1 | wx1-a^Oh43 | 1.34 | 1.62 | 1.62 | 10.25 | 4.87 |
| 87-3533-2 | C1 wx1 ar1 AR | 1.32 | 1.22 | 1.22 | 10.67 | 4.44 |
| 93W-1275-4 | 013 | 1.00 | 1.40 | 1.40 | 9.60 | 3.60 |
| 93-3675-1 | +/012 | 1.51 | 2.38 | 2.38 | 12.77 | 5.69 |
| 92-1560-3 | 02^Oh43 | 1.50 | 2.00 | 2.00 | 10.60 | 5.00 |

*= see abbreviations In the text.

Except for the inbreds 90-1863(4), 92-1540(4) and 87-3533-2, the lines show excellent prolificity values. Evolutive cycle traits were not measured as these lines were sown in the greenhouse during 1995. Nevertheless, it could be concluded that they have normal or short evolutive cycle if the total leaf number is considered.

Two commercial hybrids, Zeneca 8340 (long evolutive cycle) and Zeneca 8543 (short evolutive cycle), were studied and used as testers of our waxy inbreds to measure the general combining ability of them through a comparative yield trial during 1996/97. Hybrids of different evolutive cycle length were chosen to make possible the crosses among them and the waxy inbred lines. Both testers were sown in two dates during October and November 1995.

Both hybrids were evaluated through the use of several descriptors grouped as follows:

- A. PLANT TRAITS: the same as measured in the mutant inbreds, and stalk number (SN).
- B. PROLIFICITY TRAITS: those measured in the mutant inbreds plus number of productive nodes per tiller (PN) and number of ears in the uppermost node (EUN).
- C. EAR TRAITS: ear length in cm (EL), number of rows (RN), ear

weight in grams (EW), cob percentage (%C) and humidity percentage of kernels at harvest (%H).

Tables 2 and 3 show the average values, standard deviation, variance, standard error and range of variation for each trait measured in both hybrids.

Table 2. Descriptive statistics for plant, prolificity and ear traits measured in Zeneca 8340.

| Trait* | Average Value ±S.D. | Variance | Standard Error | Range Of Variation |
|--------|---------------------|----------|----------------|---------------------------|
| DT | 2.18 ± 0.25 | 0.06 | 0.03 | 1.60 - 2.70 |
| NH | 14.33 ± 1.21 | 1.46 | 0.15 | 11.00 - 17.00 |
| NHEE | 6.21± 0.77 | 0.60 | 0.09 | 4.00 - 8.00 |
| NT | 1.00 ± 0.00 | 0.00 | 0.00 | 1.00 |
| NP | 2.29 ± 0.67 | 0.45 | 0.08 | 1.00-6.00 |
| ENS | 1.08 ± 0.27 | 0.07 | 0.03 | 1.00-2.00 |
| ET | 1.89 ± 0.53 | 0.28 | 0.06 | 1.00-3.00 |
| BP | 1.89 ± 0.50 | 0.25 | 1.41 | 10.00-22.00 |
| LE | 12.00± 6.78 | 46.00 | 0.60 | 12.00-18.00 |
| PESp. | 17.48 ± 48.03 | 2307.22 | 10.02 | 94.00-277.00 |
| %M | 17.68 ± 3.29 | 10.86 | 0.69 | 12.80-23.60 |
| 5H | 18.71± 2.82 | 7.93 | 0.59 | 14.00-24.40 |

^{* =} see abbreviations in the text.

Table 3. Descriptive statistics for plant, prolificity and ear traits measured in Zeneca 8543.

| Trait* | Average Value±S.D. | <u>Variance</u> | Standard Error | Range Of Variation |
|--------|--------------------|-----------------|----------------|--------------------|
| DT | 2.20±0.21 | 0.05 | 0.03 | 1.70-2.70 |
| NH | 12.80±0.84 | 0.71 | 0.10 | 10.00-14.00 |
| NHEE | 5.89±0.53 | 0.28 | 0.06 | 5.00-7.00 |
| NT | 1.00±0.00 | 0.00 | 0.00 | 1.00 |
| NP | 2.27±0.63 | 0.39 | 0.07 | 1.00-4.00 |
| ENS | 1.03±0.17 | 0.03 | 0.02 | 1.00-2.00 |
| ET | 1.88±0.71 | 0.51 | 0.09 | 1.00-3.00 |
| ₽ | 20.36±2.75 | 7.55 | 0.55 | 12.50-23.00 |
| LE | 14.08±1.47 | 2.16 | 0.29 | 12.00-18.00 |
| Pesp | 225.64±53.69 | 2882.32 | 10.74 | 103.00-333.00 |
| %M | 12.59±1.21 | 1.47 | 0.24 | 10.70-15.20 |
| 5H | 14.90±0.96 | 0.91 | 0.19 | 13.70-17.20 |

^{* =} see abbreviations in the text.

In Table 4, the statistical differences found between the two hybrids for the traits measured can be seen. There are significant differences for total leaf number. This is reasonable, as the hybrids studied have different evolutive cycle lengths. There are not significant statistical differences when prolificity traits are analyzed. Nevertheless, there exist significant differences for ear traits between Zeneca 8340 and Zeneca 8543.

Table 4. Statistical differences between both hybrids used as tester.

| | | | Plant An | d Prolificity | Traits | | | |
|----------|-------|--------|----------|---------------|--------|--------|------|---------|
| Tester | SD* | TLN | NLUE | SN | PN | EUN | EI | 田 |
| ZENECA | 2.18a | 14.33a | 6.21a | 1.00a | 2.29a | 1.08a | 1.89 | a 1.89a |
| 8340 | | | | | | | | |
| ZENECA | 2.20a | 12.80b | 5.89b | 1.00a | 2.27a | 1.03a | 1.88 | a 1.88a |
| 8543 | | | | | | | | |
| | | | E | ar Traits | | | | |
| Tester | | | EL | BN | EW | % | C | %H |
| ZENECA 8 | 340 | | 12.00a | 17.48a | 197.0 | 14a 17 | .68a | 18.71a |
| ZENECA 8 | 543 | | 20.36b | 14.08b | 225.6 | 4a 12 | .59b | 14.90b |
| | | | | | | | | |

^{*=} see abbreviations in the text.

Evaluation of combining ability of inbred maize lines for precocity and yield

-- Aulicino, MB; Naranjo, CA

Over the last few years there has been an increase in short-cycle maize harvesting. On the one hand precocious maize has allowed for the broadening of the cultivated area to marginal regions, while semiprecocious types have shown operative and commercial advantages when compared with normal cycle hybrids in typical traditional areas of the Argentine maize belt. Within the

breeding plan to obtain precocious materials, which has been under development since 1988, the differential behavior of testers and lines has been determined through the evaluation of their hybrid combinations, revealing crosses (lines x tester) which stood out against the rest, as regards precocity and yield. Two short-cycle materials with flint grain were used as pollinators, tester 1 (t1) and 2 (t2). Tester t2 is Gaspé variety with extreme precocity and t1 is a selected inbred line. They were crossed with seven different lines: B73 and A632 (USA), P465, P1338 and H38 (EEA INTA, Pergamino), AFE and AW (IFSC, UNLP). During the 1993-94 and 1994-95 campaign (F1's) hybrids were evaluated using a randomized complete-block design with 3 replications. The traits considered were: EL- Ear length, in cm, NKR- Number of kernels per row, NRE- Number of rows per ear, KY- Kernel yield per plot (kg ha⁻¹), HUT- Heat units to tasseling in °C. A variance analysis was carried out using a hierarchical factorial pattern, Table 1. The difference between tester and lines estimates the general combining ability (GCA) directly. It was estimated as follows: $l_i = x_i - x_i$ and $l_j = x_j - x_i$; where x_i is Line mean, x_i is Tester mean, and $x_{...}$ is Grand mean. The interaction tester x line determined the specific combining ability (SCA). A multiple comparisons test, using least significant differences (LSD, 5%), allowed a test of the significance of the individual GCA and SCA (Tables 2, 3, 4).

Table 1. F values for each of the traits: EL, NKR, NRE, KY and HUT.

| S. of Variation | GL | EL | NKR | NRE | KY | HUT |
|------------------|----|--------|---------|---------|-----------|---------|
| Tester | 1 | 2.61NS | 0.03NS | 9.52NS | 145,000** | 17.64NS |
| Line | 6 | 5.11* | 2.10NS | 11.53** | 0.61NS | 7.68* |
| Tester X Year | 1 | 6.16* | 23.10** | 7.16** | 0.00NS | 1.48NS |
| Line X Year | 6 | 1.48NS | 3.50** | 1.09NS | 1.73NS | 2.16NS |
| Tester X Line | 6 | 4.46* | 1.28NS | 1.98NS | 1.09NS | 2.08NS |
| Linextesterxyear | 6 | 0.59NS | 1.27NS | 1.34NS | 1.47NS | 1.17NS |
| Error | 54 | | | | | |

^{*, **:} Significant at 1 and 5 % respectively. NS: Not significant.

Table 2. Means, LSD 5%. and GCA for lines (I;).

| | Lines | Means | LSD: 0.62 | 1 |
|-----|-------|-------|-----------|--------|
| NRE | B73 | 13.86 | a | 0.935 |
| | P465 | 13.29 | a b | 0.365 |
| | AFE | 13.16 | b c | 0.235 |
| | P1338 | 12.96 | b c | 0.035 |
| | AW | 12.65 | b c | -0.275 |
| | A632 | 12.60 | c d | -0.325 |
| | H38 | 11.93 | ď | -0.995 |
| | Lines | Means | LSD: 1.11 | ¥. |
| EL | P1338 | 20.03 | а | 1.615 |
| | H38 | 20.02 | а | 1.605 |
| | A632 | 18.40 | b | -0.015 |
| | B73 | 17.87 | b c | -0.545 |
| | AW | 17.82 | b c | -0.595 |
| | P465 | 17.53 | b c | -0.885 |
| | AFE | 17.23 | C | -1.185 |
| | Lines | Means | LSD: 44.7 | ¥. |
| HUT | AFE | 1,197 | а | 70 |
| | AW | 1,167 | a b | 40 |
| | P1338 | 1,140 | b c | 13 |
| | P465 | 1,135 | b c | 8 |
| | H38 | 1,117 | C | -10 |
| | B73 | 1,067 | d | -60 |
| | A632 | 1,066 | d | -61 |

Table 3. Means, LSD 5%, and GCA for the Tester (Ij)

| | Tester | Means | LSD:0.71 | ¥. |
|----|--------|-------|----------|-------|
| KY | t1 | 2.46 | a | 0.23 |
| | t2 | 1.99 | b | -0.23 |

¹⁼ average values followed by the same letter do not differ significantly at 0.01% level.

Table 4. Means, LSD 5% and GCA for the hybrids.

| | Hybrid | Means | LSD: 1.08 |
|----|------------|-------|-----------|
| EL | H38 x t1 | 21.84 | a |
| | P1338 x t1 | 21.09 | а |
| | P1338 x t2 | 18.97 | b |
| | A632 x t1 | 18.07 | bc |
| | P465 x t1 | 18.56 | bc |
| | H38 x t2 | 18.19 | bс |
| | A632 x 12 | 18.13 | bс |
| | AW x t1 | 17.93 | bс |
| | B73 x t1 | 17.92 | bc |
| | B73 x t2 | 17.82 | C |
| | AW x t2 | 17.71 | C |
| | AFE x 11 | 17.35 | c d |
| | AFE x t2 | 17.12 | c d |
| | P465 x t2 | 16.49 | d |

It was concluded that for HUT, the lines B73 and A632 were those that offered the highest number of alleles for early flowering, the lines P1338 and H38 were significantly different from the rest for EL, and the B73 line offered the highest NRE. Given that the error means square for tester x line interaction depends on the effects of dominance, these would be non-existent for most of the evaluated traits, with the exception of EL. P1338 x t1 and H38 x t1 were the combinations that significantly surpassed the rest (Table 4). It was shown that the behavior of the hybrids may be adequately explained by the GCA, which depends exclusively on the additive and possibly epistatic effects. This would guarantee advancement in selection, in cases where some hybrids may participate in the formation of a synthetic population.

Tester t1 proved to be the one which offers higher grain yield per plot compared with its hybrid combinations. Nevertheless, when considering HUT, this does not show any difference from t2 (of extreme precocity) (Table 1). In conclusion, it has been shown that t1 is the most adequate for use in breeding plans for precocity and grain.

Preliminary studies to determine the degree of resistance to Ustilago maydis D.C. Corda in wild and cultivated species of the genus Zea

--Astiz Gassó, MM; Molina, MC

The purpose of the current work is to determine the degree of resistance of wild and cultivated species of the genus *Zea* to *Ustilago maydis* D.C. Corda (Um), which will subsequently be used in crosses to obtain new forage plants (Molina M.C. and Rosales T.P., Rev. Facultad Agr. 54:579-586, 1984). The host materials used were the population cv. Colorado Klein and the inbreds SC66, B73 and E642A88 of *Zea mays* ssp. *mays* as well as clones of *Z. diploperennis* and *Z. perennis*.

All the materials were inoculated with a strain of Um belonging to the collection of the Phytopathology Lab. of the Instituto Fitotécnico de Sta. Catalina. The strain was cultivated in a liquid medium of 2% C.P.G, under shaking for 8 days running at 25 C. The pathogen was inoculated using a hypodermic syringe. The trial involved three replications, in which 60-30 seedlings were inoculated (Hirschhorn, E., Las Ustilaginales de la Flora Argentina CIC 530 p., 1986). The plants were evaluated using a reaction scale (Table 1) to determine the mean percent infection of the Um fungus.

The results obtained point out that the population cv. Colorado Klein and the inbred SC66 (*Z. mays* ssp. *mays*), as well as the clones of *Z. perennis* and *Z. diploperennis*, are the most tolerant and show a good response to Um. Some plants of the inbreds B73 and E642A88 were lightly susceptible or susceptible (Table 2).

Table 1. Reaction scale in hosts.

| Behaviour | Host reaction |
|------------------------|---|
| Immune (0) | No reaction |
| Resistant(1) | Chlorosis partial |
| Medium resistant (2) | chlorosis accent and /or presence stripe or anthocyanin stain |
| Medium susceptible (3) | Necrosis and diminution growth in plants |
| Susceptibles (4) | Formation of tumors (galls) |
| | |

Table 2. Result of the reaction in species of Gro. Zea with isolates of U. maydis. (Sta. Catalina) in Argentina.

| Hosts | No. Plants | Total plants Infected | | | | fected | Means of the reaction in plants (%) | | | | |
|-------------------|------------|-----------------------|----|----|----|--------|-------------------------------------|------|------|------|------|
| | | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| Colorado Klein | 60 | 5 | 25 | 26 | 4 | 0 | 1.66 | 8.33 | 8.66 | 1.33 | 0.00 |
| SC66 | 60 | 5 | 25 | 25 | 4 | 0 | 1.66 | 8.33 | 8.33 | 1.33 | 0.00 |
| B73 | 60 | 4 | 19 | 20 | 10 | 7 | 1.33 | 6.33 | 6.66 | 3.33 | 2.33 |
| E642A88 | 60 | 1 | 17 | 22 | 16 | 4 | 0.33 | 5.66 | 7.33 | 5.33 | 1.33 |
| Zea perennis | 30 | 15 | 4 | 8 | 2 | 1 | 5.00 | 1.33 | 2.66 | 0.66 | 0.33 |
| Zea diploperennis | 30 | 11 | 7 | 10 | 1 | 1 | 3.66 | 2.33 | 3.33 | 0.33 | 0.33 |
| Testigo | 30 | 0 | 0 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

LOMAS DE ZAMORA, ARGENTINA

Inst. Fitotécnico Sta. Catalina and Centro de Inv. Genéticas

Pathogenic behaviour of spontaneus populations of *U. maydis* (DC) Corda on maize commercial hybrids

--Sandoval, MC

Field studies to determine the pathogenic behaviour of spontaneous populations of *Ustilago maydis* (Um) from several regions of Argentina were carried out. These populations were artificially inoculated on maize commercial hybrids (Hirschhorn, E. Las Ustilaginales de la Flora Argentina. CIC. 530 p., 1986).

The statistical analysis through Anova-1 and Tukey test showed the existence of significant differences for pathogenicity among the Um populations tested.

The response obtained with the maize hybrids inoculated let us group the 5 Um populations into 2 population groups (Table 1). The first group only includes the Um population named Necochea, in which greater degree of disease resulted in less vigorous and smaller maize plants (level 2 of the scale of pathogenicity). The second group, including the Um populations Pergamino, Paraná, Balcarce and Sta. Catalina, produced fewer symptoms on the maize hybrids inoculated (levels 0 and 1 of the same scale).

Table 1. Results of inoculation of commercial hybrids of corn with 5 U. maydis populations.

| Treatment | Means ¹ | Homogeneous Groups ² |
|--------------------------|--------------------|---------------------------------|
| 1. Necochea | 15.00 | a |
| 2. Pergamino | 4.286 | b |
| 3. Sta. Catalina | 2.143 | b |
| 4. Balcarce | 1.286 | b |
| Paraná | 0.000 | b |

¹Affected plants

The Um populations artificially inoculated showed a loss of pathogenicity compared to the spontaneous populations of the fungi. This phenomenon is surely due to the presence of mutations determining pathogenicity in the inoculated populations.

Cytogenetic study of diploid and triploid F1 hybrids of Zea mays ssp. mays x Zea parviglumis

--Molina, M del C; García, MD

Diploid and triploid hybrids were obtained from crosses between diploid or tetraploid Z. mays ssp. mays (2n=20 and 2n=40,

² Significant at P <0.05 (Tukey test)

respectively) and *Z. parviglumis* (2n = 20). The maize tetraploid lines used in these experiments were N103A and N107B, obtained from the Maize Genetics Cooperation Stock Centre. *Z. parviglumis* seeds were kindly supplied by Dr. Bird (CIMMYT, Mexico).

Diploid hybrid plants were fertile with a chromosome number of 2n = 20; their meiotic analysis is shown in Table 1. Chromosomes of both species were paired in 73.45 % of the cells in pachytene and a small reciprocal translocation was observed between 2 chromosomes of both species. 10% of the cells in anaphase showed chromatid bridges. The pollen fertility ranged from 80 to 94% and 80% of seeds were fertile.

Table 1. Meiotic configurations of F1 diploid hybrids of Z. mays ssp. mays x Z. parviglumis.

| No. of cells | 1 | Ш | Ш | IV | % |
|--------------|---|----|---|----|-------|
| 130 | | 10 | | | 73.45 |
| 26 | | 8 | | 1 | 14.69 |
| 26 16 | 2 | 9 | | | 9.04 |
| 2 | 2 | 7 | 1 | 2 | 1.13 |
| 2 | 4 | 8 | | | 1.13 |
| 1 | 1 | 6 | 1 | 1 | 0.56 |
| T = 177 | | | | | |

Triploid hybrids were obtained by embryo rescue because of the endosperm failure, about 14 days after pollination. The meiotic configurations of triploid hybrids are shown in Table 2. Pollen fertility ranged between 46 and 86% and the seed set was very low.

Table 2. Meiotic configurations of F1 triploid hybrids of Z. mays ssp. mays x Z. parviglumis.

| No. of cells | 1 | П | ш | % |
|--------------|----|----|---|-------|
| 38 | 5 | 5 | 5 | 31.66 |
| 26 | 6 | 6 | 4 | 21.66 |
| 26 20 | 7 | 7 | 3 | 16.66 |
| 15 | 8 | 8 | 2 | 12.50 |
| 10 | 4 | 4 | 6 | 8.33 |
| 5 | 9 | 9 | 1 | 4.16 |
| 2 | 10 | 10 | | 1.66 |
| 2 | 8 | 5 | 4 | 1.66 |
| 1 | 2 | 2 | 8 | 0.83 |
| 1 | 7 | 10 | 1 | 0.83 |
| T = 120 | | | | |
| | | | | |

The results from the cytogenetic studies of diploid and triploid F1 hybrids of *Z. mays* ssp. *mays* x *Z. parviglumis* suggest the following conclusions:

-Chromosomes of both species were paired in 73% of the diploid hybrid cells, which indicates that maize and *Z. parviglumis* chromosomes are homologous or homoeologous.

-The meiotic configuration 5I (Zp) + 5II (2 Zm) + 5III (2 Zm +1Zp) found in 30% of the triploid hybrid cells confirmed that 5 chromosomes of both species are homologous or highly homoeologous and the other 5 are partially homoeologous.

Different behavior of alloplasmic lines of *Zea mays* under tissue culture

--Galian, LR; Hirschhorn, E; Caso, O

Callus generation and regeneration frequency in corn (*Zea mays* L.) depends on plant donor cultivars and is under genetic control. Frequency is also affected by environmental conditions of mother plants, and later in vitro culture conditions for the explants. Many workers have defined methods for modifying in vitro conditions, to obtain new adapted genotypes. But the effort had limited results. A factor which is not considered often enough is cytoplasm influence on in vitro culture adaptation. Mazoti (1978, 1987) has identified nucleus-cytoplasm interactions, using corn lines of two distinct cytoplasms. These interactions were called

heritable cytoplasmic actions. This means that the cytoplasm conditions the expression of the genotype. One inbred line was improved in two different cytoplasms, called E (*Euchlaena* cytoplasm) and Z (*Zea mays* ssp. *mays* cytoplasm). The inbreds E and Z respond favorably to N6 salts with 3% sucrose. It may be deduced that the most appropriate 2,4-D concentration for the inbred E is 0.5 mg/l without kinetin. On the other hand, the inbred Z needs kinetin in the culture medium to originate regenerative callus.

LONDON, ONTARIO University of Western Ontario

Modifications to the Langdale protocol for in situ hybridization of antisense RNA

-- Greyson, RI; Yang, Z; Walden, DB

We reported (Greyson et al., Dev. Genet. 18:244-253, 1996) on the distribution of mRNA (and the protein) of the 18-kDa hsp gene family in maize radicles and plumules as revealed by in situ antisense RNA hybridization technology. To optimize the recovery of DIG labeled RNA and the reproducibility of our studies, we made some modifications to the Langdale protocol (In situ hybridization, In Freeling, M., Walbot, V. Eds. The Maize Handbook. NY Springer-Verlag, p. 165-180).

A set of thirty slides was loaded into a metal slide rack (Mercer Glass Works, Inc., Brooklyn, NY) and dewaxed in xylene, then hydrated in an ethanol series prepared with autoclaved, demineralized H₂O.

 The hydrochloric acid treatment was omitted from later runs. (NOTE: In early experiments when a hydrochloric acid wash was used, slides were transferred to glass slide holders and then re-transferred to the metal holder for subsequent steps. Metal slide holders were etched by hydrochloric acid).

2) 2X SSC was prepared from 20X SSC (Boehringer Mannheim).

3) 4% formaldehyde was prepared from formaldehyde solution (36.5-38% BDH, Toronto) and PBS.

4) Slides prepared for hybridization (22mm² coverslip, coated with Sigmacote, Sigma, St. Louis, MO) were ringed with rubber cement and placed in a hybridization cassette (Bios Corp., New Haven, CT), which was submerged in a water bath at 50 C overnight.

5) Following hybridization and removal of the coverslip, the formamide WASH BUFFER was used at 0.5X to 0.1X salt concentration.

6) Following the post-hybridization washes, the sections were covered with $200\mu l$ of substrate solution for (a) 15-18h in early experiments, and (b) 6-8h in later experiments. Slides were dehydrated through an ethanol series to xylene.

7) Coverslips were mounted with PRO-TEXX Mounting Medium (American Scientific Products, McGraw Park, II).

The induction and localization of mRNA from the 18 kDA HSP genes in metal-ion insulted maize radicles

--Yang, Z; Walden, DB

Environmental stress treatments, such as heavy metal and heat shock treatments, lead to altered gene expression in plants. Different metal-ions have been found to induce some, or all proteins induced by heat shock (heat shock proteins, or hsps) in dif-

ferent plants. The best-characterized environmental response, at the molecular level, is the heat shock response (Vierling, Annu Rev Plant Physiol Plant Mol Biol 42, 1991). Heat shock response regulation has been found at the transcription, RNA processing and translation levels (Lindquist, Ann Rev Biochem 55, 1986). In maize, a group of 18 kDa hsps referred to as the small hsps or shsps are induced when seedlings are exposed to methomyl (Rees et al., Plant Physiol 90, 1989) and metal-ions (Rees, Ph.D thesis. London, Ontario: UWO, 1989).

To detect and localize the mRNA from the 18 kDa hsp genes in metal-ion insulted maize radicles, four-day-old seedlings grown at 27 C were immersed in the insult solution and incubated at 27 C for 3 h. Controls were immersed either in ddH2O and incubated at 27 C or incubated at 27 C for 3 h. The insult solutions were cadmium chloride (CdCl₂, 10mM), zinc sulphate (ZnSO₄ 100 mM), potassium chloride (KCl, 100 mM), sodium chloride (NaCl, 100 mM), and copper sulphate (CuSO₄, 0.1 mM). The radicles of insulted seedlings were fixed (FAA), dehydrated (TBA series), embedded in wax, cut on a microtome, and mounted on slides. DIGlabeled 18 kDa hsp common ORF probe antisense RNA in situ hybridization was carried out as reported above in this volume. Quantitative estimates of hybridization intensity were made from black and white images of longitudinal sections viewed through a microscope via a video monitor and digitized using Northern Exposure Analysis Software (ver. 2.5) (Image Expert, Mississauga, Ontario). Black and white images (255 grey levels) were captured, and average relative video intensities of areas of the overall, the epidermis region, the cortex region, and the stele region of the proximal meristematic region of the root-tip were prepared separately. Duncan's Multiple Range (DMR) tests were performed with the average relative video intensity of overall, epidermis, cortex, and stele region of the meristematic region of the root-tip and are presented in Table 1. Means connected by a bar are not significantly different (p=0.05).

Table 1. DMR tests of mean relative video intensity* of overall area (a), epidermis region (b), cortex region (c), and stelle region (d) of the meristematic region of the metal-ion insulted maize root-tios.

| (a) | | | | | | | | |
|-------------------|-----|-----|-----|------------------|---------|-------|---------|-------|
| Means (overall) | 184 | 189 | 203 | 209 | 216 | 223 | 225 | 227 |
| Insult metal-ions | Zn | Cd | K | H ₂ O | Control | Cu | Sense | Na |
| (b) | | _ | = | = | _ | _ | | |
| Means (epidermis) | 134 | 138 | 175 | 181 | 184 | 192 | 194 | 195 |
| Insult metal-ions | Cd | Zn | K | H_2O | Cu | Na C | Control | Sense |
| (c) | | | _ | | _ | - | | |
| Means (cortex) | 173 | 184 | 196 | 206 | 208 | 221 | 221 | 222 |
| Insult metal-ions | Cd | Zn | K | H_2O | Control | Cu | Sense | Na |
| (d) | | - | = | _ | _ | | | |
| Means (stele) | 190 | 214 | 222 | 225 | 229 | 236 | 236 | 240 |
| Insult metal-ions | Zn | Cd | K | H_2O | Control | Sense | e Na | Cu |
| | | | _ | | | | | |

*Lower mean relative video intensity is attributable to greater concentration of the DIG-labelled antisense RNA in situ hybridization.

Note in the overall area (Table 1a) and cortex regions (Table 1c) of the meristematic regions of root-tips, means of relative video intensity of cadmium, zinc, and potassium insults are not sig-

nificantly different, but are significantly different from those of copper and sodium insults, control, and water treatments. In the epidermis region (Table 1b), means of relative video intensity of cadmium and zinc are significantly different from those of other insults, water and control treatments. These results indicate that cadmium insult is restricted to the epidermis and cortex regions and zinc insult can be mediated through the epidermis to the stele in the meristematic region of maize root-tips.

Immunogold localization of the 18-kDa hsp in Zea mays L. radicles

-- Denison, SJ; Smith, RJ; Walden, DB

Heat-shock proteins (hsps), induced by temperature shifts and other stresses, are among the most highly conserved proteins. In plant species, the low-molecular weight heat-shock proteins (LMW hsps) are among the most abundant and are highly conserved, in contrast to mammals in which the LMW hsps make up only a small fraction of total hsp content.

Immuno-gold cytochemistry and transmission electron microscopy were used to examine the distribution, subcellular and cellular localization of the 18-kDa hsp in inbred Oh43 heat-shocked and non-heat-shocked radicles. A recombinant protein, from our UWO 10 cDNA clone, produced in the pTrcHisB expression vector, was used to raise polyclonal antibodies (Greyson et al., Dev. Genet. 18:244-253, 1996). These antibodies, specific to the 18-kDa hsp proteins, were used to probe ultra-thin silver-gold sections of radicles. Colloidal gold particles (15 nm) conjugated to goat-anti-rabbit IgG secondary antibodies were employed to detect the presence of antibody-hsp-18 complexes. From these studies, we report five observations:

- 1) Research has suggested that the α -crystallin protein (a class of small hsp) is involved in the regulation of osmolyte transport (Kegel et al., Am. J. Physiol. 270:C903-C909, 1996). The 18-kDa maize hsp is known to contain an α-crystallin moiety. Under heat-stress conditions, water in the cell expands and may cause deformation of cell membranes and walls. Gold labelling of the hsp 18-antibody complexes reveals an increased density of label in the intercellular spaces of heat-shocked tissue. Localization of hsp 18 to the intercellular spaces suggests that it also may be involved in the regulation of osmotic pressure or osmolyte transport, thereby preventing damage to walls. Additionally, the coldstress induced antifreeze proteins, which are proteins that have the ability to retard ice crystal growth, were identified recently as the most abundant apoplastic proteins in cold-acclimated winter rye (Secale cereale L.) leaves (Hon, W. C. et al., Plant Physiol. 109:879-889, 1995).
- 2) Observations reveal greater density of labelling in 'thick' cell walls of heat-shocked radicles. We observed that the 'thin' cell walls have gold label at the plasmalemma membrane, whereas in the thicker walls label is observed within the wall. Different biochemical composition of thicker walls may be the cause of this differential labelling.
- 3) hsp 18-antibody complexes are abundant in cytoplasmically dense cell types relative to less cytoplasmically dense cells (Figure 1). The cytoplasmically dense cell types are those cells typically engaged in rapid cell division and therefore meristematic. It can be seen further that granular bodies, described as heat-shock granules (HSGs) by Nover et al., (Mol. Cell Biol. 3:1648-1655, 1983) have the greatest labelling density within these cells. Heat-

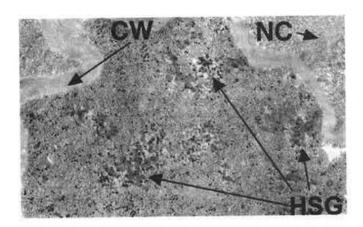


Figure 1. Electron micrograph of a heat-shocked radicle, probed with primary antibody and gold labelled, demonstrating the preferential gold labelling of the cytoplasmically dense cell types of the root meristem (x30,000). Note the absence of gold label in the cytoplasmically less dense neighbouring cells (NC). Cell wall (CW), heat shock granules (HSG).

shock granules contain RNA synthesized immediately preceding the heat-shock, as well as distinct subsets of HSPs. It is suggested, probably in connection with cytoskeletal elements, that HSGs serve as mRNA storage sites (Nover et al., 1983).

4) Gold labelling to membrane structures (see the companion article below - Denison et al., MNL 71, 1997), provide additional support for the role of the small hsps as 'molecular chaperones'.

5)Companion studies in our lab, using non-radioactive in-situ anti-sense RNA hybridization, to determine the localization of the mRNA for the 18-kDa gene, reveal similar distribution patterns for the 18-kDa hsp mRNA relative to the hsp 18 (Greyson et al., 1996).

In conclusion, localization of the hsp 18-antibody complexes to the intercellular spaces, membraneous whorl structures, thick cell walls, and cytoplasmically dense cells of maize radicles suggest multiple roles for members of the 18-kDa hsp gene family, ranging from the osmotic pressure or osmolyte transport regulation to serving as a component in the storage of mRNAs in the meristematic regions of the root.

18-kDa hsp localization to membraneous whorl structures

-- Denison, SJ; Smith, RJ; Walden, DB

Most organs of maize seedlings respond to brief heat-shock exposure with the synthesis of a variety of heat-shock proteins (hsps), particularly a group of 18-kDa hsps referred to as the small hsps (shsp). To date there is little known about the cellular 'chaperone' function of the shsps. The cytological localization of individual hsps may suggest possible functional roles and also exclude certain functions.

cDNAs from three members of the maize 18-kDa gene family have been sequenced and characterized (Atkinson et al., Genome 31:698-704, 1989; Goping et al., Plant Mol. Biol. 16:699-711, 1991) and stage specific constitutive expression of two of these has been detected during microsporogenesis (Atkinson et al., Dev. Genet. 14:15-26., 1993; Bouchard et al., Maydica 38:135-144, 1993). See also contribution from Wooster, Ohio, this volume.

We present observations made on the localization of the 18-kDa to unique membrane containing structures in heat-shocked root-tips of inbred Oh43, utilizing immunogold labelling of the 18-kDa hsp-antibody complex.

These membraneous structures, which are poorly represented

in the literature, are found close to cell walls (Figure 1). These structures appear to be comprised of numerous membranes with a whorl-type conformation. These structures may be involved in 'autophagic' digestion (Gunning and Steer, Ultrastructure and the Biology of Plant Cells, pp. 183-280, 1975), or may be the result of fixation.

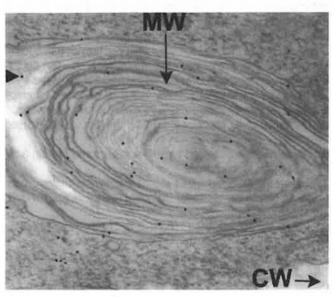


Figure 1. Electron micrograph of a membraneous whorl structure in Zea mays L. heat-shocked radicle (x75,000). Note the increase in gold label to this structure relative to other components of the cell. Cell wall (CW), membraneous whorl structure (MW).

With chemically fixed tissue one compromises structure to retain antigenicity and vice-versa. The presence of gold-label in these membraneous whorl structures indicates that the membranes retain their antigenicity. If, in fact, fixation resulted in poor structure maintenance then these membranes are probably components of the plasma membrane based on their association with cell walls. However, it should be noted that other organelle membranes in the vicinity of these structures are undamaged, which suggests that these whorls are not fixation artifacts. These structures are not found in non-heat-shocked tissues.

Under heat-stress conditions, tissues and proteins may be damaged and therefore no longer useful. These structures, if involved in 'autophagic' digestion, may aid in the degradation of these materials. The hsp 18, described as a 'chaperone' molecule, may assist this process by the transportation of damaged material to these structures for the purposes of degradation.

A method for preparing metaphase chromosomes from tapetal cells of maize

-- Maillet, DS; Walden, DB

Most studies of maize metaphase chromosomes utilize root-tip cells. With this approach it has been possible to detect evidence of somatic association of homologous chromosomes during interphase (Horn and Walden, Genetics 88:181-200, 1978). Recent studies of the arrangement of chromatin in differentiated mammalian neurons indicate that the different classes of cells have different characteristic patterns of chromatin (Manuelidis, Science 250:1533-40, 1991), suggesting that the developmental state of a cell may be related to the form/pattern in which the chromatin is arranged.

It is possible that different cell types of maize may exhibit characteristic arrangements of chromatin. The tapetum of maize is composed of highly specialized cells that are responsible for a number of processes that are necessary for the production of viable pollen. The tapetum is an ideal tissue within which to examine chromatin organization. Our goal is to determine if there is order within tapetal nuclei by examining them during different stages of their development, including metaphase and interphase, and to determine if there are differences / similarities between root-tip and tapetal nuclei organization. An anther can provide hundreds of synchronized cells that can be studied in metaphase using the following protocol for the recovery of tapetal nuclei in metaphase:

- 1. The upper portion of a sexually immature plant was removed and the leaves were trimmed to make the plant a manageable size.
- 2. The explant was kept at 4 C for 24 h with the stalk immersed in dd H_2O .
- 3. The tassel was dissected out of the explant and fixed in 3:1 ethanol: acetic acid overnight.
- Anthers were staged with a standard propionocarmine smear technique (Burnham, Maize for Biological Research, pp. 107-118, 1982).
- Anthers were then placed in dd H₂O for 3 X 10 min followed by 20 min in 0.01 M citrate buffer, pH 4.7.
- The anthers were cut in two and digested with 0.25% (w/v) beta glucuronidase (Sigma G0251) and 0.002 % (v/v) pectinase (Sigma P9179) in citrate buffer for 2-3 h at 37 C.
- Anther pieces were rinsed in citrate buffer, placed in dd H₂O for 15 min then transferred to a microscope slide in a drop of fixative.
- 8. After maceration the cells were spread and allowed to dry for 2 h and placed in 100 % ethanol overnight.
- 9. Slides were dried for 1 h followed by staining with propionocarmine (wet mounted) or C-banded (Jewell et al., The Maize Handbook, pp. 484-93) followed by mounting in Pro-Texx mounting media (America Scientific Products M7635-1).

The threshold temperature for the thermal shift induction of mRNA from 18 kDA HSP genes in maize radicles

--Yang, Z; Greyson, RI; Walden, DB

Plants start to up-regulate the transcription and/or translation of the heat-shock genes and down-regulate most other genes when shifted to temperatures five or more degrees above the optimal growing temperature (Vierling, Ann Rev Plant Physiol Plant Mol Biol 42, 1991). Maize seedlings respond to a brief heat-shock exposure with the synthesis of a variety of hsps, particularly a group of 18 kDa hsps referred to as the small hsps (shsps) and their mRNAs (Baszczynski et al., Can J Biochem 60, 1982; Baszczynski et al., Can J Biochem Cell Biol 61, 1983). Three members of the maize 18 kDa gene family have been characterized and sequenced (Atkinson et al., Genome 31, 1989; Goping et al., Plant Mol Biol 16, 1991), and they bear high identity (over 90%) in their open reading frames (ORFs). One common ORF fragment (18-9-2) can recognize all three genes (Atkinson et al., Dev Genet 14, 1993). Maize hsp induction in response to different growing temperatures, and thermal shifts within and beyond the normal growing temperature, have been studied. The major factors that may affect maize heat-shock response include: 1) the initial growing or "preshift" temperature; 2) the thermal shift increment; and 3) the duration of the thermal shift (Baszczynski et al., in Changes in Eucaryotic Gene Expression in Response to Environment Stress,

Academic Press, New York, 1985). In maize plumules, the 18 kDa hsps are not induced when maize seedlings are shifted from 15 C, 20 C, or 25 C to 25 C, 30 C, or 35 C respectively, but they are synthesized when seedlings are shifted from 30 C to 40 C, and from one of the preshift temperatures (15 C, 20 C, 25 C, or 30 C) to 42 C (Baszczynski et al., 1985).

Antisense RNA in situ hybridization can detect mRNAs of low abundance present only in a few cells and permits localization of specific mRNA transcripts at the cell level. The 18 kDa hsp mRNA localizes to the meristematic regions of root- and shoottips, the vasculature, and the young leaves (Greyson et al., Dev Genet 18, 1996).

To determine the threshold temperature for induction of 18 kDa hsp mRNA, four-day-old maize seedlings grown at either 22 C or 27 C were shifted to one of several temperatures (33 C, 35 C, 38 C, 40 C or 43 C), and incubated at that shift temperature for 2 h. Controls were kept at the preshift temperatures. The radicles of "non-thermal shifted" and the "thermal-shifted" seedlings were fixed (FAA), dehydrated (TBA series), embedded in wax, cut on microtome, and mounted on slides. DIG-labeled 18 kDa hsp common ORF (18-9-2) probe antisense RNA in situ hybridization with the Hybaid Omnislide System was carried out as described elsewhere in this contribution. Quantitative estimates of hybridization intensity were made from black and white images of longitudinal sections viewed through a microscope via a video monitor and digitized using Northern Exposure Analysis Software (ver. 2.5) (Image Expert, Mississauga, Ontario). Black and white images (255 grey levels) were captured, and average relative video intensities of areas which would include the guiescent zone and the proximal meristem of the root-tip were prepared (Figure 1).

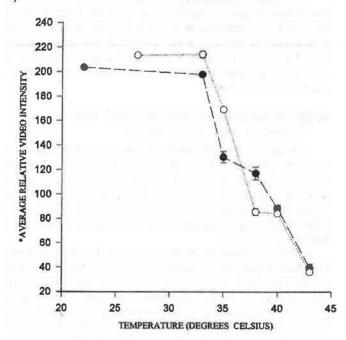


Fig. 1. Relative intensity of DiG-labelled antisense RNA in situ hybridization of radicles which underwent a thermal shift from one preshift temperature (22 C, black dots or 27 C, open dots) to one of several shift temperatures. Means of five observations are plotted. error bar = standard deviation.

*Lower average relative video intensity is attributable to greater concentration of the DIG-labelled antisense RNA in situ hybridization. Hybridization signal was observed when the shift temperature was at and above 35 C from either of the preshift temperatures. At 35 C, hybridization was found only in cells near the cap initials and in the immature stele region of the meristem. These results suggest that the threshhold temperature for heat shock induction of 18 kDa hsp mRNA in maize radicles is 35±1 C, and cells near the cap initials and in the immature stele region of the meristem respond to heat shock first.

LONDON, ONTARIO University of Western Ontario BUFFALO, NEW YORK State University of Western Ontario at Buffalo

A non-dehiscent mutant (ndh1)

--Walden, DB; Cheng, PC

A non-dehiscent phenotype (Figures 1 and 2) was identified in Oh43 stock and has been observed in each of six generations following self-pollination. The pollinations have been made by collecting the anthers in a tassel bag, cutting/macerating the anthers and applying the resultant mix of pollen and cut anthers with the knife onto appropriate silks. Most such pollinations were done in the afternoon, long after pollen would normally have been shed. Seed set was sparse and scattered; several hundred non-dehiscent Oh43 plants have been observed and the trait has been observed in other inbreds after pollen transfer as described above. The trait can be transferred through the female. In all studies, segregation patterns predict a single recessive, nuclear allele for

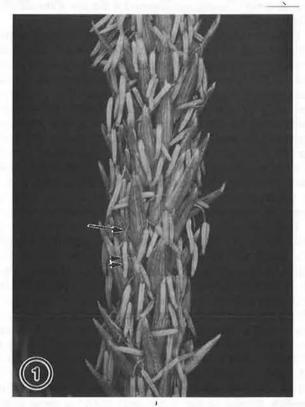


Figure 1. Close-up view of center rachts showing non-dehiscing spikelets. Arrow indicates a shriveled filament which has elongated many hours earlier; however, the anther (double arrow head) has failed to open.



Figure 2. Image shows an Incomplete anther dehiscent process caused by the failure of pore opening in the homozygous plant. The spikelets 1 and 2 are in the process of dehiscence. (Note the elongating filament); arrow indicates a shriveled filament which has elongated many hours earlier; however, the anther (double arrow head) has falled to open.

this trait. Mapping studies are not yet complete. Proposed symbol: *ndh1*. The expression of the non-dehiscent phenotype has been observed in the field (winter nursery in Hawaii and summer nursery in London, Ontario), in the glasshouse and in growth chamber (under both 12 hr/12 hr and 16 hr/8 hr light cycle, 25C condition) grown materials.

Specimens for microscopy were collected in our 1993 Hawaii winter nursery and from growth chamber grown material. The specimens were fixed in a tri-aldehyde fixative (Cheng, Walden and Greyson, Natl Sci Counc Monthly, ROC 10:1000-1007, 1979), postfixed in OsO₄, dehydrated in acetone and critical point dried with CO₂. Surface was sputtered with AuPd and examined with SEM.

In wild type material, at anthesis, the lodicules swell and pry apart the glume and lemma allowing the anthers to extrude. Soon after extrusion, pores at the tip of the anther break open and pollen is liberated. As described (Cheng, Greyson and Walden, Can J Bot 57:578-596, 1978), maize anther dehiscence consists of three major processes: (1) the swelling of lodicules to push the glume to open followed by the elongation of filaments; (2) separation of intermicrosporangial stripe 1 (IMS₁) from the underlying parenchyma in the anther (Figure 3); and (3) the opening of an anther pore along the distal end of the IMS₁ following the collapsing of epidermal cells. Figures 1 and 2 show that the filament elongation and opening of spikelets proceed normally in the homozygous mutant plant; however, the separation of intermicrosporangial stripe 1 (IMS₁) from the underlying parenchyma of the anther connective never occurs; as a result, the anther pores fail to open (Figure 4). This work was supported by NSERC (DBW), the

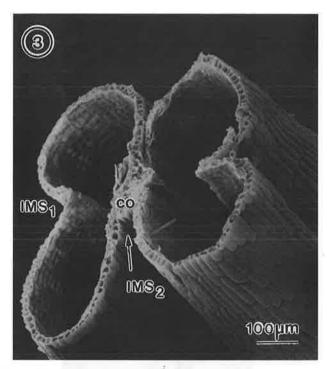


Figure 3. Cross-section of a wild-type anther showing the separation of IMS, from underlying anther connective (co). This process does not occur in the homozygous.

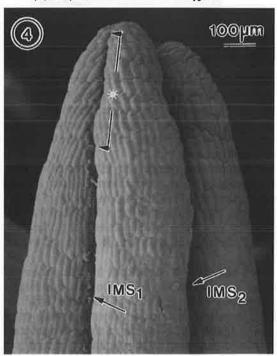


Figure 4. Scanning electron microscopic image shows the distal portion of an anther from a homozygous plant. The anther was taken from a spikelet which had undergone anthesis for at least three hours; the IMS₁ did not separate from the underlying connective tissue. The region indicated by the "" represents the approximate range where the IMS₁ should split open to form the anther pore. IMS₂: intermicrosporangial stripe 2.

National Science Council of the Republic of China (NSC83-02110-B-00100-001)(PCC) and Academic Development Fund of SUNY (PCC).

MANHATTAN, KANSAS Kansas State University RALEIGH, NORTH CAROLINA USDA-ARS, North Carolina State University

A gene conferring a hypersensitive reaction to the rice bacterial streak pathogen in maize

--Hulbert, SH; Drake, J; Leach, JE; Senior, L; Stuber, CW

Xanthomonas oryzae pathovars cause two serious diseases of rice. X. oryzae pv. oryzae causes the bacterial blight disease and X. o. pv. oryzicola causes bacterial streak. No single-gene resistances to bacterial streak have been identified in rice. The disease is a significant problem in China, and many of the elite hybrid rice cultivars are particularly susceptible (Xie, et al., Chinese J. of Rice Science 4:127-132, 1990).

We tested a number of maize inbred lines to determine their reaction to X.o. pv. oryzicola (strain BLS222). Bacteria (109 cfu/ml) suspended in sterile water were infiltrated into seedling leaves, which were then examined one to five days after inoculation. All the maize lines tested were scored as highly resistant to the rice pathogen because none of the lines had any water soaked lesions typical of susceptible rice. Two different reactions were observed. Most of the maize inbreds tested (A188, A619, B14, B73, CM37, K301, Mo20W, N6, Oh545, R168) exhibited a rapid hypersensitive reaction. The intensity of the hypersensitive reaction varied from complete necrosis of the region infiltrated with the bacteria by 48 hours after inoculation, to patches of necrosis throughout the infiltrated regions. Other inbreds, such as H95, Mo17 and Pa405, showed no noticeable necrosis after inoculation. At most, a mild chlorosis was observed at the site of inoculation in these lines.

Inoculation of two small backcross families indicated a dominant gene controlled the necrotic reaction. These families were made previously for the purpose of mapping two rust resistance genes, Rp4 and Rp7. The Rp4 and Rp7 lines both showed the necrotic reactions to X. o. pv. oryzicola. The Rp4 and Rp7 populations were made by crossing the Rp4 or Rp7 lines to H95, and then backcrossing the F1s to the H95 parent. The Rp4 population segregated 27 necrotic to 28 nonnecrotic, and the Rp7 population segregated 22 necrotic to 30 nonnecrotic. Subsequent inoculations of the same seedlings with common rust (Puccinia sorghi) indicated that the gene conferring the necrotic reaction to X. o. pv. oryzicola segregated independently of the Rp4 and Rp7 genes. The locus was designated rxo1, for 'reaction to X. oryzae'.

To determine the map position of the *rxo1* gene, recombinant inbred lines from a B73 X Mo17 population (developed at NCSU) were tested with the *X. o.* pv. *oryzicola* strain. Of 150 lines tested, 74 showed the strong necrotic reaction of the B73 parent, 75 showed the minimal response of the Mo17 parent, and one family segregated for the reaction. Analysis of *rxo1* segregation with the molecular markers already mapped in this population indicated the gene mapped approximately one map unit from *umc85*. The genomic area around *umc85* carries a number of resistance genes (McMullen & Simcox, Mol. Plant-Microbe Interact. 8:811-815, 1995), including genes for resistance to at least two viruses, and one fungal disease (southern corn leaf blight). The *rxo1* gene, therefore, appears to map to this same cluster of genes conferring resistance to a diverse variety of pathogens.

MEXICO CITY, MEXICO CIMMYT, International

Development of PCR-based markers to facilitate large-scale screening in molecular maize breeding

--Hu, X; Ribaut, JM; González de León, D

RFLPs are the most commonly used and informative molecular markers in cereal genome mapping and have been used to locate genes governing both qualitative and quantitative traits. RFLP analysis, however, requires relatively large quantities of DNA, and once the DNA has been transferred onto membranes by Southern blotting, the analysis is cost-effective only if these membranes are reprobed multiple times. In marker assisted selection (MAS) experiments, however, a subset of individuals may be selected after genotyping a single locus of interest and all individuals that do not carry the desired allele at that locus can be eliminated; at each subsequent selection step at other loci, the population is reduced further. This can only be achieved effectively by using PCRbased marker systems as described in Ribaut et al. (Plant Mol Biol Rep, submitted). Here we describe the set of PCR-based markers used in an ongoing MAS experiment for the improvement of drought stress tolerance in a CIMMYT tropical maize line.

Conversion of RFLP markers to PCR-based sequence tagged sites (STSs) offers several advantages over conventional RFLP analysis. These include the relative ease and rapidity of PCR reactions, the potential for rapidly screening very large populations (> 1000 individuals), the convenience of sharing primer sequences. and the relatively small amount of fairly crude genomic DNA needed for amplification (Erpelding et al., Genome 39: 802-810, 1996). More recently, PCR-based microsatellite or simple sequence repeat (SSR) markers have been developed in several crops (Roder et al., Mol Gen Genet 246: 327-333, 1995: Saghai Maroof et al., PNAS 91: 5466-5470, 1994; Wu and Tanksley, Mol Gen Genet 241: 225-235, 1993), as an alternative method to detect DNA polymorphisms. In maize, many SSR markers have been identified and mapped, and more than 200 SSR primer sets are available for public use (Taramino and Tingey, Genome 39: 277-287, 1996; MaizeDB, 1996). The major limitation of STS- and SSR-PCR analysis is that it requires extensive sequence information in order to design appropriate primers.

A number of loci at QTL peaks detected in mapping experiments involving a donor and a recipient line were investigated for the development of PCR-based markers for the transfer of the corresponding genomic segments from the donor to the recipient line.

A set of 9 pairs of primers was synthesized based on existing sequence information for eight RFLP probes (Table 1), and were evaluated using genomic DNA of the tropical maize lines Ac7643S5 (donor) and CML 247 (recipient). Among nine STS-PCR primer sets, only the primer set for umc67 sts amplified polymorphic products between the two lines at an annealing temperature of 56 C without subsequent restriction digestion (Fig. 1). One fragment (~1000 bp) was amplified from Ac7643S5 and two (~600 bp and ~400 bp) from CML247. Tests of individual F2 plants showed that the 1000 bp fragment from Ac643S5 and the 400 bp fragment from CML247 could be consistently scored as a codominant STS locus marker (Fig. 1). Because the ends of the PCR products are defined by the primer sequences, the amplified length polymorphism produced by the primer set for umc67 sts must represent insertions/deletions in the DNA be-

Table 1. Sequences of STS primers based on clone sequence data obtained from MaizeDB.

| Clone | Locus chrom. location | Primer name | Sequence (5'-3') |
|---------------|-----------------------------|-------------|-----------------------|
| umc67 | 1 | umc67F | GCTTGCTTGCCGCTAATA |
| SALEVOLIDO VI | 1000 | umc67R | GACCCTGATGTTCTCCTG |
| umc49 | 2 | umc49F | TATAATCTCTTTGTTCTTTTT |
| | | umc49R | CTTCCTTTGACCACCACCTTG |
| umc124 | 8 | umc124F | AGATCGCCGCCGCCTACACG |
| | | umc124R | GCCTCTGCCACAATGACACG |
| umc32 | 8 | umc32F | CGGCCAGGGGTTTCTTAGG |
| | | umc32R | TGGCTCTTTGACATCTTCC |
| bn17.08 | 8 | bnl7.08F | AAACTGGCATCATAAACATC |
| | | bn17.08R | ATACAAAAGCATCAACAAGC |
| umc64 | 10 | umc64F1 | ATGTTGTCGCCTGCTGTGC |
| | | umc64R1 | AAACTTATTGTCTTCTCCA |
| | | umc64F2 | ACTTCCGTGTCTGTTTCCAA |
| | | umc64R2 | CTGATGTTCTCCTGCTGCTA |
| umc130 | 10 | umc130F | AGGGGGAAGAAGGTCATCAT |
| | | umc130R | GGCAGAGGAGGGGAAGGAG |
| bnl7.49 | 10 | bn17.49F | CCCGATGAAACAATGAAACT |
| | | bnl7.49R | CGGAGAGGATGGTGATGAGC |

M 1 2 3 4 5 6 7 8 9 1011 12131415 1617



Fig. 1. PCR amplification of genomic DNA of maize inbred lines Ac7643S5 (lane 1), CML247 (lane 2), and 15 F2 plants (lanes 3-17) from the cross of these two lines with a primer set for umc67 sts. M, size marker of 100 bp ladder. Amplification products were fractionated on 1.5% SeaKem agarose gel in 1X TBE buffer.

tween the primer sequences. The rest of the primer sets amplified monomorphic products, single or multiple copy, between the two lines. The single or two copy products of primer sets for umc32, umc49, bnl7.08, and bnl7.49 were digested with ten 4- or 5-base-recognizing restriction enzymes (Avall, Haelll, Hhal, Hinfl, Rsal, Alul, Mspl, Taql, Mbol, and Msel). Polymorphisms were observed between the two maize lines when the PCR products of the primer sets for umc32 and bnl7.08 were digested with HaellI and Hhal, respectively. However, none of the digestions of PCR products of the primer sets for umc49, bnl7.08, and bnl7.49 revealed any polymorphisms between the two lines. The low level of polymorphism detected by STS markers is in sharp contrast to the high levels detected by the corresponding clones when used as RFLP probes and demonstrates at least one important limitation of STS markers. Indeed, PCR amplification detects polymorphisms only within the region between the primer sequences, while hybridization-based RFLP analysis detects polymorphisms beyond the hybridization site of the probe. The STS-PCR primer sets which revealed polymorphisms between the two maize lines before or after enzyme digestion were used to screen 72 F2 individuals from the cross of the two maize lines. As expected, most markers revealed loci that co-segregated with the corresponding RFLP loci. The umc67 patterns in particular, which did not require digestion, were easy to score and reliable, and were used in MAS of the genomic segment marked by locus umc67. The map positions of *umc32* sts and *bnl7.08* sts could not be determined because the polymorphic bands produced by these two primer sets were faint and difficult to score; better primer sets might need to be designed in such cases.

Microsatellite or SSR markers provide another powerful tool to detect polymorphisms at the DNA level through simple PCR amplification. Six published SSR primer sets, detecting loci at positions of interest, were used to amplify the genomic DNA from the same two maize lines; five of these amplified polymorphisms between the two lines. All five polymorphic SSRs were mapped to positions expected from published data (Taramino and Tingey, 1996, Genome 39: 277-287; MaizeDB, 1996). Although it may be safer to verify the location of the corresponding loci in any given cross. SSR loci seem to be conserved among different maize lines. The increasing availability of SSR primer sequences for maize loci and their seemingly higher polymorphism as compared to STS markers, should make them ideal tools for large-scale PCR-based MAS experiments. However, due to the small size of their amplification products, SSR marker polymorphisms may need to be resolved by polyacrylamide gel electrophoresis, which is more time consuming and technically demanding than simple agarose separation, or by electrophoresis using high resolution agaroses (such as Metaphor™), which are significantly more expensive than regular ones.

For cost-effective MAS from a large population, rapid and reliable techniques that require small amounts of DNA, less time and low-cost inputs involved in PCR reactions and gel electrophoresis, are preferred. We developed a technique for simultaneous amplification of more than one STS and/or SSR polymorphism in a single PCR reaction the products of which can be visualized in the same gel or in different gels by loading part of the amplification products from the same PCR reaction. Two factors are critical for success. First, different primer sets should be able to share common PCR amplification conditions for effective co-amplification of their respective products; finding the appropriate annealing temperature is therefore crucial. Second, the chosen markers should yield products that differ in size if effective scoring of the corresponding loci is to be achieved. After testing a range of annealing temperatures, it was found that for most double combinations of SSR and/or STS primer pairs, 56°C would give adequate or better results. Figure 2 shows the PCR products amplified with primer sets for umc67 sts and MAG1A01 in a single PCR reaction, and separated in 3% agarose gel (2% MetaPhor and 1% SeaKem). Since there was no size overlap in the respective products of each primer set, the polymorphisms corresponding to each



Fig. 2. PCR amplification of genomic DNA of two malze inbred lines Ac7643S5 and CML247, and 6 F2 plants from the cross of these two lines with two primer sets (for *umc67* sts and MAG1A01) in the same PCR reaction. M, *Hae*III - φ X174 slze marker. The amplification products were fractionated in 3% MetaPhor agarose gel in 1X TBE buffer.

of the two loci of interest could be easily scored in the same agarose gel. Because STS products are usually large and thus do not overlap with SSR products in size, STSs are very useful in combination with SSRs in multiplex PCR reactions in the same reaction tube. The PCR-products from the same reaction can be separated in the same or different gels depending on the size difference of the polymorphic fragments. Two people can comfortably screen two loci (whenever these can be visualized in the same electrophoretic gel) in a population of more than 2000 individuals in less than a week using this simple methodology. The cost-effectiveness, time-saving and large scale applications of this simple PCR-based methodology are obvious when compared with hybridization-based methods such as RFLP analysis. Eventually, allele-specific amplicons, giving simple presence/absence polymorphisms, and not requiring gel-based detection methods should prove to be the long-awaited method of choice for MAS from large populations. In any case, once the population size has been trimmed down to those individuals bearing the genomic segments of interest from the donor parent, the screening of other genomic regions for selection against the donor genome can then be most effectively achieved using RFLP analysis.

SSR fingerprints of CIMMYT and other inbred lines

--Bird, R McK; González de León, D; Hoisington, D

We have DNA fingerprinted 56 CIMMYT and 12 other inbred maize lines, using 19 microsatellite (SSR) markers. These were selected from the list of SSR markers defined by Pioneer HiBred International, Inc. (for loci denominated phi...) and by North Carolina State University (nc...) (Senior et al. MNL 70:50-54; 70:112-117). We have remapped these markers, and most of the 32 others available at CIMMYT, in tropical maize populations with no discrepancies compared to published data.

We fingerprinted a set of 17 CIMMYT lines (CMLs) presently being studied for heterotic relationships in 11 environments by Javier Betrán, 12 CMLs representing 4 tropical heterotic groups, and 37 lines already fingerprinted using RFLP markers (González de León et al. Agron Abstr 1989). Four comparative materials were placed in external lanes, B73, Mo17, a Huayleño (Peruvian highland flour) accession and a new, unique teosinte from Nicaragua.

The 19 SSR markers we used were the more promising ones when examined for clarity and amount of polymorphism on agarose gels using 14 inbred lines. We tested varying proportions of glycerol, MgCl2, primer, target DNA and nucleotides, and determined optimal annealing temperatures. We are examining the potential for multiplexing (joint PCR amplification) of markers, so tried to use uniform conditions.

The agarose was a 1:1 mixture of Metaphor and SeaKem, at 3%. Two CIMMYT- designed internal markers, 66 and 300 base pairs long, were used in all lanes. The PhiX174/HaelII size marker in lane 15 of the two 30-lane combs served to estimate base pair numbers for amplified products.

Lane bands were scored using enlarged acetate xerograms of the gel photos and a Numonics digitizer, and were recorded using the HyperBlot data entry and analysis system (Hoisington et al. Agron Abstr 1989). HyperBlot then estimated the numbers of nucleotides in each band entry (Southern Anal Bioch 100: 319-323 1979). Bands were clustered into morphs defined by migration distance (see following note) using average cluster linkage. The number of final clusters can be controlled by presetting the mini-

mum cluster distance -- we used 20-35 thousandths-of-an-inch (0.51-0.89 mm) depending on the marker. We found 2-5 morphs per SSR marker. Some morphs seem to contain a small array of "sub-morphs", probably differing by multiples of SSR motif repeats, not distinguishable in agarose gels. Many morphs differ by much more than a few repeats of the motif(s), perhaps involving insertions/deletions in regions between the primed sequences and the SSR itself. Such differences ranged up to 21, 28 and 36 bases. Variation in gel conditions, reading variance and differences between combs must be factors controlling some intramorph variation, probably resolvable by using polyacrylamide gels.

These data can be used to choose SSR markers for Marker Assisted Selection projects, but so far have not proved useful in

relating lines into meaningful patterns.

Designation of SSR morphs

--Bird, RMcK; González de León, D; Hoisington, D

In the process of SSR fingerprinting 68 CIMMYT and other inbred lines (note above), we have found that often we can define clearly separate "morphs" or clusters of bands defined by migration distance. The small-scale shifts in numbers of SSR repeats that are expected in SSR fingerprinting can be seen, but easily defined morphs are clearly a major feature. Given this situation, we need to designate morph identifiers in such a format that labs can compare results, and future improvements in definition and understanding will be easily incorporated into the system.

The results of classification and sorting of estimated migration distances for the p-phi114 SSR probe at the oec17 locus (bin 7.02) illustrate a simple situation. We have accepted the HyperBlot analysis which, for a set of bands ranging ca. 133-199 bp, provides 3 morphs, with bands of ca. 133 to 144 bp, 163 to 176 bp and 199 bp. It is rather easy to name these by their mean sizes: phi114-a139, phi114-a170 and phi114-a199 (the "a" means agarose). Like species definitions, even though, as one studies more materials, there can be changes in the balance of readings for a morph, it should be best to maintain the original designations as long as sensible.

How can we avoid having to completely redesignate the morphs if, with better resolution, we were to subdivide morphs here defined? If polyacrylamide gels were used, one might just name a new series of morphs starting with "p", plus the more narrowly defined size estimates. On the other hand, a slash and a letter could be appended to the present morph designations. Letters could be chosen which indicate relative sizes and anticipate the discovery of more submorphs. phi114-a137 might end up with three submorphs, phi114-a137/b, phi114-a137/e and phi114-a137/h, while phi114-a169 might be divided into phi114-a169/k, --/n, --/q and --/t. Such subdivisions need to made with the possible number of microsatellite repeat motifs in mind. With phi114 this would be tough -- between the primer sites there are three repeat motifs in GenBank accession Z26824: (CCG)3, (TGCC)4 and (TA)4, so there are a great many possible permutations.

wide range of methods appears.

We hope this note elicits some constructive discussion before a

MUENCHEN, GERMANY Ludwig-Maximilians-University

Isolation and mapping of mus1, a putative maize MutS-homolog --Horwath, M; Kunze, R

All organisms have a number of DNA repair mechanisms to maintain genetic continuity. Among the most highly conserved is the mismatch repair system (MMR), which corrects replication errors, contributes to the stabilization of simple sequence repeats. and is involved in preventing recombination between not perfectly identical sequences. Moreover, in humans, defects in one of the MMR genes are correlated with an increased probability to develop certain cancers.

In E. coli, the methyl directed MMR system consists of the three proteins MutH, MutL and MutS. The MutS-protein recognizes and binds to mispaired nucleotides, small insertions and deletions, and subsequently recruits MutL and MutH. MutS-homologous (MSH) and MutL-homologous proteins were found in many prokaryotes, yeast, insects, and vertebrates. In eukaryotes the organization of the MMR system is more complex than in E. coli. In yeast, for example, six msh genes have been identified.

To date, the genes and proteins of the plant MMR system are not known. As a first step towards the investigation of the plant MMR system, we have isolated a mutS-homologous gene from maize. As in all known MutS-homologous proteins certain amino acid sequence motifs and the distance between them are highly conserved, we used degenerate oligonucleotides (Fig. 1) as primers for RT-PCR on maize seedling mRNA. The RT-PCR products were gel-fractionated and a band of the expected size was eluted and ligated into an M13 vector. Among 16 seguenced inserts we found two putative msh sequences, that hybridized to maize single copy sequences on a Southern blot. One of these also hybridized to a weakly expressed transcript on a Northern blot.

With the latter probe we isolated a 3 kb cDNA clone from a maize seedling cDNA library (kindly supplied by Monika Frey, Technical University of Munich). Sequencing of this cDNA clone confirmed it as a putative maize mutS-homolog; we termed the gene mus1. The cDNA clone included a poly(A)-sequence at the 3'-end, and the 5'-end of the transcript was determined by 5'-RACE. The mus1 transcript has the coding capacity for a 942 amino acid protein that is most similar and colinear with the MSH2 protein from S. cerevisiae. Figure 1 shows the alignment of the most highly conserved segments of MutS-homologous proteins from various organisms with the MUS1 protein. The positions of the degenerate primers used for the initial RT-PCR are highlighted by bold letters.

In collaboration with Monika Frey (Technical University of Munich) we RFLP-mapped the mus1 gene by using a recombinant inbred population (Burr et al., Genetics 118:519-526, 1988; Burr and Burr, TIG 7:55-60, 1991). mus1 maps on chromosome 7L, by coincidence with the b32B locus [rip1] that encodes a ribosome inactivation protein.

EcoMutS StyMutS SpneuHexA BsubMutS AviMutS HinfMutS TaquaMutS Swi4 Spel1 mRep3 mMSH2 RnorvMSH2 hMSH2 yMSH2 yMSH2 RMLIITGPNMGGKSTYMRQTALIALMAYIGSYVPAQKVEIGPIDRIFTRVGAADDLASGR
RMLIITGPNMGGKSTYMRQTALIALLAYIGSYVPAQNVEIGPIDRIFTRVGAADDLASGR
RMLIITGPNMSGKSTYMRQTALIALLAYIGSYVPABSAHLPIFDAIFTRIGAADDLASGR
QMLITGPNMSGKSTYMRQTALISIMAQIGCFVPAKKAVLPIFDQIFTRIGAADDLISGQ
RMLIITGPNMGGKSTYMRQTALIVLLAHIGSFVPAQSCELSLVDRIFTRIGSSDDLAGGR
HLLVITGPNMGGKSTYMRQTALIVLLAHIGSFVPAQSCELSLVDRIFTRIGASDDLAGGR
RCLVLITGPNMGGKSTFLRQTALIALLAQVGSFVPAEBAHLPLFDGIPTRIGASDDLAGGR
RCLLITGPNMGGKSTFLRQTALIALLAQVGSFVPAEBAHLPLFDGIPTRIGASDDLAGGR
RCLLITGPNMGGKSTYIRSVGTAVLMAHIGAFVPCSLATISMVDSILGRVGASDNIIKGL
RVMIITGPNMGGKSTYIRSVGTAVLMAHIGAFVPCSLATISMVDSILGRVGASDNIIKGL
RVMIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGV
MFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGV
MFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGV
MFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGV
MFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCEBAEIAIVDAILCRVGAGDSQLKGV
WFQITTGPNMGGKSTYIRQTGVIVLMAQIGCFVPCEBAEIAIVDAILCRVGAGDSQLKGV
WFQITTGPNMGGKSTFIRQVGVNVLMAQVGSFVPCDQASISVRDCIFARVGAGDSQLKGV

EcoMuts StyMuts SpneuHexA BsubMuts AviMuts HinfMuts TaquaMuts Swi4 Spel1 mRep3 mMSH2 RnorvMSH2 hMSH2 yMSH2 zmmus1 STFMVEMTETANILHNATEYSLVLMDEIGRGTSTYDGLSLAWACAENLANKIKALTLFAT STFMVEMTETANILHNATENSLVLMDEIGRGTSTYDGLSLAWACAENLANKIKALTLFAT STFMVEMTETANILHNATENSLVLMDEIGRGTSTYDGMALAQSIIEYIHEHIGAKTLFAT STFMVEMEAKNAIVNATKNSLILFDEIGRGTSTYDGMALAQAIIEYVHDHIGAKTLFAT STFMVEMSETANILHNASERSLVLMDEVGRGTSFTDGLSLAWAABEHLAG-LRAWTLFAT STFMVEMSETANILHNASERSLVLLDEVGRGTSTYDGLSLAWAABEHLAG-LRAWTLFAT STFMVEMTEMANILHQATAQSLVLLDEVGRGTSTYDGLSLAWAABEHLAG-LRAWTLFAT STFMVEMLETKEVLSKATEKSMVIIDELGRGTSTYDGLSLAWAABEHLAG-HRAYTLFAT STFMVEMLETKEVLSKATEKSMVIIDELGRGTSTYDGGAISYAVLHYLNQYIKSYLLFVT STFMVEMLETSGIIRTATDKSLVIIDELGRGTSTYDGGAISYAVLHYLNQYIKSYLLFVT STFMVEMLETSGIIRTATDKSLVIIDELGRGTSTYDGGGLAWAISHAHLAKETKCFTLFAT STFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISHYIATKIGAFCMFAT STFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISHYIATKIGAFCMFAT STFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISHYIATKIGAFCMFAT STFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISHYIATKIGAFCMFAT STFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISHYIATKIGAFCMFAT STFMVELIETASILKNASKNSLIIUDELGRGTSTYDGFGLAWAISHIASKIGFCMFAT STFMVELIETASILKNASKNSLIIUDELGRGTSTYDGFGLAWAICHLMEVTRAPTLFAT STFMQEMLETASILKGASDKSLIIUDELGRGTSTYDGFGLAWAICHLMEVTRAPTLFAT

* * * .. ** **** . . * . . .

Figure 1. Alignment of the highly conserved C-terminal region of various MutS homologous proteins. The putative maize homolog, MUS1, is underlined.

EcoMutS - E. coli, StyMutS - Salmonella typhimurium, SpneuHexA - Streptococcus pneumoniae, BsubMutS - Bacillus subtilis, AviMutS - Acetobacter vinelandii, HinfMutS - Haemophilus influenzae, TaquaMutS - Thermus aquaticus, yMSH2 - Saccharomyces cerevisiae, Swi4 - Schizosaccharomyces pombe, Spel1 - Drosophila melanogaster, mMSH2 and mRep3 - Mus musculus, RnorvMSH2 - Rattus norvegicus, hMSH2 - Homo saplens, zmmus1 - Zea mays

MUENCHEN, GERMANY Technical University

Biosynthetic origin of oxygen atoms in DIMBOA

--Glawischnig, E; Eisenreich, W; Bacher, A; Frey, M; Gierl, A

In maize, 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one (DIMBOA) is important for resistance against insects, fungi and bacteria. DIMBOA is synthesized in large quantities during the first days after germination and stored in the vacuole in a glucosylated form. Two reactions of the DIMBOA pathway, i. e. the C2-hydroxylation of the benzoxazinone and the N-hydroxylation of the 2-hydroxy-benzoxazinone (HBOA) have been studied in vitro (Bailey and Larson, Plant Physiol. (1991) 95:792-796; Kumar et al., Phytochemistry (1994) 36: 893-898). They both are characterized as cytochrome P450 monooxygenase catalyzed reactions. We have demonstrated that all four oxygen atoms bonded to carbon atoms of DIMBOA are introduced by monooxygenases (Glawischnig et al., Phytochemistry, in press).

Maize seeds were germinated in a gastight container filled with a mixture of \$^{16}O_2\$ and \$^{18}O_2\$ in a ratio of 1:2 (v/v). DIMBOA was isolated and NMR spectra were recorded in deuterated methanol. The presence of \$^{18}O_2\$ induces an upfield isotope shift of the natural abundance \$^{13}C\$ NMR signal of adjacent carbon atoms. The signals for carbon atoms 3, 7, 8a and the methyl group (Figure 1) show upfield satellites indicating the binding to \$^{18}O\$. The \$^{13}C\$ signal for C2 appears with two upfield satellites indicating the presence of isotopomers with one and two \$^{18}O\$ atoms bound at C2. This indicates that all four oxygen atoms bound to carbon atoms of DIMBOA acquired isotope labeling from molecular \$^{18}O\$. The relative intensities of the non-shifted signals (i. e. with adjacent \$^{16}O\$ isotope) and the shifted signals (i. e. with adjacent \$^{18}O\$) are in the

Fig. 1: DIMBOA

range of 1:2, thus reflecting faithfully the $^{16}O_2/^{18}O_2$ composition in the growth chamber, indicating that O_2 is the immediate source for the O-atoms. Therefore, it is likely that all oxygen atoms of DIMBOA are introduced via cytochrome P450 monooxygenases.

MUMBAI, INDIA Tata Institute of Fundamental Research

Diversity of GTP-binding proteins (GPs) in corn and sorghum --Panigrahi, KC: Johri, MM

In plants, GTP-binding proteins have been suggested to mediate the signal transduction process in response to external stimuli. Towards this ultimate aim, we have examined the dark-grown coleoptiles of corn and sorghum for the presence of various GPs using immunological and biochemical methods. Based on the binding of $^{32}[P]\text{-}\alpha\text{-}GTP$ to the polypeptides on the western blots, cholera toxin (CT) catalysed ADP-ribosylation in the presence of GTP, and cross reactivity with anti-G- $\alpha\text{-}common$ antibodies, three classes of GPs were observed (Table 1). These are: i) low Mr GPs Table 1. Different types of GTP-binding proteins from corn and sorghum.

| Size (KDa) | Detection methods | Sub-cellular localization | Plant species |
|--------------|-------------------|------------------------------------|---------------|
| 74 | Ab | Cytosol | S |
| 68 | Ab, AR | Organelles | S |
| 56-60 | Ab, AR | Organelles | C, S |
| 44-45 | Ab. AR | Microsomes and cytosol | C, S |
| 41-43 | Ab, AR | Organelles, microsomes and cytosol | C, S |
| 35-38.5 | Ab | Microsomes and cytosol | C, S |
| 32 | Ab | Microsomes and cytosol | C |
| 29,27,25*,18 | Ab, 32P-GTP | Microsomes | C |
| 29,26,18 | Ab. 32P-GTP | Chloroplasts | C, S |

Ab - Polypeptides recognised by G- α common antibodies; AR - Polypeptides ADP-ribosylated; 32 P-GTP - Polypeptides binding labelled GTP. C - com and S - sorghum. *Small Mr GPs not detected by the GTP-binding method.

of 18-29 kDa, ii) high Mr GPs of 32-45 KDa which seem to be the $\alpha\textsc{-subunits}$ of heterotrimeric GPs and iii) another class of high Mr GPs of 56-74 KDa. The G- $\alpha\textsc{-common}$ polyclonal antibodies were produced using the GTP-binding domain peptide conjugated to KLH.

These studies show that the antibodies cross react with all classes of GPs and thus the immunodetection method is far more versatile as compared to the other two methods. Most of the low Mr GPs were detectable when GTP-binding method was used. while only a subset of high Mr GPs were detectable on the basis of CT-enhanced ADP ribosylation. Most of the GPs seem to be conserved and only a few are species-specific. Whereas corn shows a single high Mr GP (56-60 kDa), sorghum contained three different ones of which two were also ADP-ribosylated. The ADP-ribosylation of the α -subunit of heterotrimeric GPs is well known and such heterotrimeric GPs are localized in the plasmamembrane. In the present studies these GPs were found to be present not only in the microsomal pellet but also with the cytosolic and organellar fractions. At least four different ones were observed in corn and of these two were ADP ribosylated in the presence of CT. The sizes of corn GPs found in present studies are comparable to that reported earlier (see Ma, Plant Mol. Biol. 26:1611, 1994). The sorghum on the other hand has only three types of α -subunits.

We are trying to determine if the low Mr GPs belong to the Ras, Rho or Ypt/Rab class, which are known to play a role in secretion, intracellular transport or the restructuring of cytoskeleton during development and differentiation. In addition to the α -subunit of heterotrimeric GPs, the present studies suggest that some of the GPs of still higher Mr (56-60 and 68 kDa) can also be ADP-ribosylated in vitro. High Mr GPs have earlier been reported in mammalian systems; for instance, a 67 KDa GP has been reported to be coupled to insulin receptor and plays a role in the signal transduction process. A similar function for the higher Mr GP in plants cannot be ruled out.

NEW HAVEN, CONNECTICUT Yale University

The root and leaf isoforms of NADP-dependent malic enzyme are encoded by distinct genes

-- Tausta, SL; Nelson, T

NADP-dependent malic enzymes (NADP-ME; EC1.1.1.40) have been implicated in a wide range of metabolic pathways in the plastids and cytosol of plant cells. In maize, the chloroplastic leaf isoform of NADP-ME is a component of the C4 pathway which delivers carbon dioxide to ribulose bisphosphate carboxylase. A second NADP-ME isoform of uncertain function is found in maize roots

and can be distinguished from the leaf isoform by molecular weight, pl, Km, and pH activity profiles. To assign these distinct enzymatic activities to specific genetic/physical loci, we obtained isoform-specific clones by screening two root cDNA libraries with the previously cloned leaf NADP-ME cDNA (Rothermel and Nelson, J. Biol. Chem. 264:19587-19592, 1989).

Two distinct cDNAs (94% DNA identical) encoding root NADP-ME isoforms were identified, indicating that the root NADP-ME is encoded by one or two separate genes different from the leaf NADP-ME gene. The two root ME cDNAs are 85% identical at the nucleotide level and 80% identical at the amino acid level to the leaf ME cDNA.

Both root cDNAs contain putative chloroplast transit peptide coding sequences. However, there are no chloroplasts in the root and the root NADP-ME has C3-like enzymatic characteristics and most C3 NADP-MEs are cytosolic (Nishikido and Wada, Biochem. Biophys. Res. Commun. 61:243-249, 1974). To test the functionality of these sequences, we used a chloroplast import assay. One full length root cDNA was transcribed and translated using a coupled reticulocyte lysate system (TNT kit: Promega, Madison WI). The resulting (35S)Met labeled protein was subjected to a chloroplast import assay using isolated pea chloroplasts (Bruce, Perry, Froehlich and Keegstra. In SB Gelvin, RA Schilperoort. eds, Plant Molecular Biology Manual, vol J1. Kluwer Academic Publishers, pp 1-15, 1994). The preprotein (73 kD) was imported and the chloroplast transit peptide cleaved off resulting in a 68 kD protein band (Figure 1), suggesting the root NADP-ME is plastid localized.

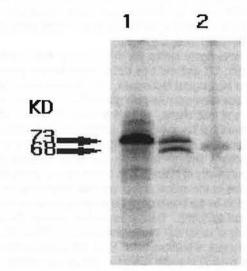


Figure 1. Chloroplast import of the root ME preprotein. 1) Translational product from the full length root ME cDNA clone pRC14. Sample loaded on the gel represents 3% of the total *in* vitro reaction product. 2) Intact purified chloroplasts representing 12% of the original import reaction. 3) Intact purified chloroplasts treated with the protease thermolysin and representing 12% of the original import reaction. Thermolysin treatment caused the disappearance of the preprotein, but not the processed protein, indicating the 68 kD protein is imported into and protected by the chloroplasts.

We utilized the leaf- and root-specific ME cDNAs to start to determine genetic map positions. Currently, there are three known loci associated with ME activities or coding sequences. A locus, *me1*, has been mapped to chromosome 3 (bin 3.08) and is associated with an electrophoretically defined isoform of NADP-ME which accumulates in coleoptiles, but does not hybridize with our

ME probes under stringent conditions (Goodman et al., Genetics 96:697-710, 1980). The leaf ME cDNA sequence has been mapped to the gene locus *me3* on chromosome 3 (bin 3.03) (Rothermel and Nelson, 1989; Keith et al., Plant Physiol. 101:329-332, 1993). A third locus, *me2*, is found on the long arm of chromosome 6 (bin 6.05) and is probably one of the root MEs (Rothermel and Burr). Because of their extreme sequence similarity, it has been difficult to map the two root NADP-MEs separately. It is intriguing that the C4 (leaf) ME is encoded by a gene embedded in a block of chromosome 3 homeologous to a block on chromosome 8 and a non-C4 (root) ME gene appears to be on a block of chromosome 6, which is also homeologous to chromosome 8 (Helentjaris MNL 69:67, 1995). If the second root cDNA corresponds to another ME gene, it seems likely that it will be found on chromosome 8.

NEW HAVEN, CONNECTICUT Yale University COLD SPRING HARBOR, NEW YORK Cold Spring Harbor Laboratory

Leafbladeless1 is allelic to raggedseedling1 and is required for dorsal cell identity

--Timmermans, M; Schultes, N; Martienssen, R; Nelson, T

The *leafbladeless1* (*lbl1*) mutation was isolated by Donald Miles from selfed *Mu*-active stocks. A preliminary characterization of the *lbl1* mutant phenotype (Miles, MNL 63:66-67, 1989) revealed that homozygous *lbl1* plants showed a complete or partial loss of leaf lamina development. In the most extreme phenotype, leaves formed no lamina tissue but developed as radial, thread-like organs. In the weaker phenotype, leaf lamina development varied and leaves often bifurcated along the midrib.

In order to investigate further the IbI1 defect in leaf initiation and development, Ibl1 and wild type sibling plants were examined using SEM and histological techniques. The adaxial/dorsal epidermis of wild type leaves can be distinguished by the presence of bulliform cells and macrohairs. SEM of severely affected, radial Ib/1 leaves showed that the epidermis of such leaves lacks bulliform cells and macrohairs and appeared abaxial/ventral in nature. Transverse sections through wild type leaf blade illustrate the dorsoventral asymmetry of maize leaves by the adaxial positioning of lamina relative to the midrib and of vascular xylem relative to phloem, as well as by the presence of the relatively larger bulliform cells in the dorsal epidermis. Transverse sections through the distal blade regions of radial IbI1 leaves showed a complete loss of asymmetry and a lack of bulliform cells. Such Ibl1 leaves appeared to consist of a central, irregular vascular cylinder surrounded by concentric rings of bundle sheath, mesophyll, and ventral epidermis.

These observations suggest that the loss of lamina development in *lbl1* plants could result from a defect in the establishment of dorsal cell identity. Recently, a similar mutation has been described in *Antirrhinum* (Waites and Hudson, Development 121:2143-2154, 1995). Because *lbl1* is a recessive mutation, the ventral nature of radial *lbl1* leaves indicates that dorsal cell identity is imposed on the primordia. In the absence of LBL1 activity, the epidermis adopts a ventral identity.

Consistent with the idea that LbI1 is required for dorsal cell identity, adaxial ectopic margins were found on weakly phenotypic

IbI1 leaves. Loss of *LbI1* function later in leaf development would result in patches of ventral cells adjacent to dorsal cells. Because marginal outgrowth occurs at the boundary between dorsal and ventral cell types, patches of ventral cells on the dorsal side of the leaf result in the formation of extra margins.

Dorsoventrality of the maize leaf is in part established during the recruitment of founder cells into the primordia. SEM and transverse sections of apices of *lbl1* plants showed a strong reduction in primordial leaf width as compared to normal sibs, consistent with a role for *Lbl1* in founder cell recruitment.

As noted above, *lbl1* leaves often bifurcate. SEM of *lbl1* shoot apices showed the bifurcation of leaf primordia as early as plastochron stage 3. Such bifurcated primordia appear to establish multiple proximodistal axes, each determining its own sheath/blade boundary. In addition, a single leaf was found that had developed a midrib in each half leaf, presumably the result of bifurcation prior to midrib differentiation. If the establishment of dorsoventrality is required for leaf initiation and outgrowth, patchy *Lbl1* expression may result in the formation of multiple proximodistal axes per plastochron.

In many respects the *lbl1* phenotype resembles the phenotype of *ragged seedling1* (*rgd1*) (Kramer, MNL 31:120-121, 1957). Preliminary observation indicated linkage to *white endosperm* (*y1*) (6L), and further RFLP linkage analysis placed *lbl1* on chromosome arm 6S near *rgd1* (Fig. 1). Allelism tests have confirmed that *lbl1* is an allele of *rgd1*.

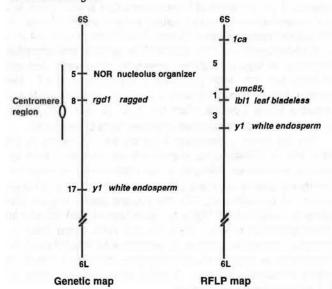


Figure 1. Chromosomal location of *lbi1* and *rgd1*. The genetic map was based upon information presented in the MNL of 1993 and 1996; numbers indicate map positions. The RFLP linkage map was obtained using *lbi1/lbi1* individuals of selfed progeny from outcrosses of *lbi1/l+* slblings to B73. Numbers in this case represent map distances in cM.

NORMAL, ILLINOIS Ilinois State University

Haploids and monosomics are produced by maize plants containing telocentrics for both arms of chromosome 3 and B chromosomes

--L'Heureux, TA; Muzumdar, DA; Schneerman, MC; Weber, DF

Rose and Staub (MNL 64:94-95, 1990) discovered a centromere breakage event in maize that produced stable telocentric chromosomes for both arms of chromosome 3. They also indicated

(K. L. Rose and R. W. Staub, Carleton College, personal communication) that in the presence of B chromosomes, one or the other of the telocentric chromosomes was sometimes not transmitted to the zygote through the pollen due to a non-disjunctional event at the second microspore division. When such gametes (that possess only one of the two telocentric chromosomes) are fertilized by normal haploid gametes, plants containing one normal homolog plus a telocentric for one of the two chromosome arms are formed. Such an individual is a monotelodisomic (p. 4, G. Khush, Cytogenetics of Aneuploids, Academic Press, NY, 1973).

A chromosome 3 double-ditelocentric stock (DDT-3, having two copies of the 3S telocentric, two copies of the 3L telocentric, and no normal chromosome 3) was generously provided to us by K. L. Rose and R. W. Staub. We examined several plants of this stock cytologically and confirmed them to be double-ditelocentrics for chromosome 3. These were crossed as male parents by Black Mexican sweet corn that contained about 10 B chromosomes. The F1 progeny (that contained a normal chromosome 3, a telocentric for 3S, a telocentric for 3L, and about 5 B chromosomes) were crossed as male parents by female tester parents with recessive seedling-expressed mutations in either the short (g2 or d1) or the long (Ig2 and et1) arm. Numerous progeny were recovered from these crosses that expressed each of the mutant phenotypes, and we have examined meiotic cells from some of these plants. From a cross where the F1 was crossed as a male parent to a g2/g2 female parent, two different plants expressing the g2 phenotype were found to possess one normal chromosome 3 plus a telocentric for the long arm of chromosome 3. Also, from a cross where the F1 was crossed as a male parent to a lg2 et1/lg2 et1 female parent, two different plants expressing the Ig2 and et1 phenotypes were found to contain one normal chromosome 3 plus a telocentric for the short arm of chromosome 3. Thus, we have recovered and cytologically confirmed monotelodisomics for both arms of chromosome 3. We have used these to map the position of the centromere of chromosome 3 on the maize RFLP map (reported elsewhere).

Previously, Chi, Fowler, and Freeling (MNL 68:16, 1994) reported that they crossed a plant carrying telocentrics for 3S and 3L as a male to female parents which carried mutants on the short arm (heterozygous for *v19*) or the long arm (homozygous for *y3*) of chromosome 3, and recovered plants that expressed these mutants. They analyzed these utilizing RFLP probes, and in all cases, they indicated that the predicted number and intensity of bands was consistent with the plants being monotelodisomics for 3S and 3L. However, they did not indicate if the plant carrying telocentrics for 3S and 3L contained B chromosomes or if it was homozygous or heterozygous for the telocentrics.

We also crossed DDT-3 plants as female parents with W22 plants that contained approximately 10 B chromosomes, and the F1s were crossed as male parents to *Ig2 et1* female parents. Forty-nine of 3809 (1.29%) seedlings germinated in a sandbench expressed both the *Ig2* and *et1* phenotypes. We have cytologically analyzed root-tips of seven of these to date. Two were found to be diploids (which could have been produced if the tester was fertilized by contaminating tester pollen), one was a haploid with no B chromosomes, three were monosomics with no B chromosomes, and one was a monotelodisomic for the short arm and contained 2 B chromosomes. The haploid was a maternal haploid because it expressed the *Ig2* and *et1* phenotypes; however, the origin of the monosomics is less clear. Thus, this system not only produces

monotelodisomics, it also produces haploids and monosomics. We are analyzing additional progeny to further characterize this interesting genetic system.

NORMAL, ILLINOIS Illinois State University COLUMBIA, MISSOURI University of Missouri

Identification of an isochromosome for the long arm of chromosome 7 in maize

--Muzumdar, DA; Schneerman, MC; Doyle, GG; Weber, DF

Doyle (1988, MNL 62:49-50) analyzed large numbers of selfed progeny of maize primary trisomics and identified "presumptive telocentrics" for several chromosome arms on the basis of deviant genetic ratios. Numerous crosses involving two presumptive telocentrics for 7L have been analyzed by DW and MS, and one of these (telocentric 7La) gave genetic ratios that were consistent with the presence of a telocentric for 7L. Pachytene in the original genetic background was examined but found to be unsuitable. This telocentric (and others) was crossed with KYS, an inbred with exceptionally favorable meiotic cytology. Pachytene cells in these revealed that the extra chromosome had two arms, and work with it was temporarily abandoned. Recently, we (DM, MS, and DW) re-examined it and recognized that the additional chromosome was an isochromosome. It is interesting to note that of the 5 presumptive telocentrics that were recovered by Doyle and cytologically confirmed, there were two independently isolated isochromosomes for 4S, two independently isolated telocentrics for 6L, and an isochromosome for 7L. In addition to these, telocentrics for both arms of chromosome 3 were recovered by Rose and Staub (MNL 64:94-95, 1990), and we have examined these extensively and confirmed that these are indeed telocentric for both arms of this chromosome.

Rhoades (1933, PNAS 19:1031-1038; 1936, Genetics 21:491-502; 1938, Genetics 23:163-164; 1940, Genetics 25:163-164) previously recovered and extensively studied a telocentric for the short arm of chromosome 5 (5S) in maize from a trisomic-5 plant. He (Rhoades 1933, 1940) found that the telocentric-5S gave rise to an isochromosome with a low but consistent frequency. The origin of this chromosome was hypothesized to be a transverse misdivision of the centromere during a pollen grain mitosis leaving the two arms attached. Unfortunately, this telocentric and isochromosomes derived from it have been lost (E. Dempsey, Indiana Univ., personal communication). It is not known if the isochromosomes for 4S and 7L originated as telocentrics which subsequently gave rise to isochromosomes or if they originated as isochromosomes. These telocentrics and others which may be recovered are powerful tools for analyzing the maize genome.

NORWICH, U.K. John Innes Center

Isolation and characterization of the maize mitochondrial RNA polymerase

--Young, DA; Allen, R; Lonsdale, DM

To date only the mitochondrial RNA polymerases (mtRNAP) of the fungi Saccharomyces cerevisiae and Neurospora crassa have

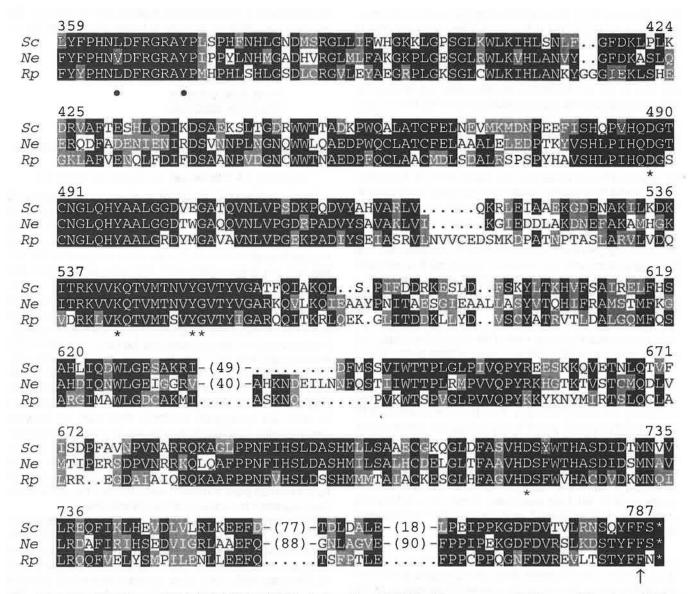


Figure 1. Prettybox amino acid alignment of the mitochondrial DNA-dependent RNA poly merases of yeast RPO41 (Sc) and Neurospora crassa cyt-5 (Ne) genes, with the maize rpomt (Rp) gene. Amino acids which are conserved between the majority of genes are black in verted, while similar amino acids are grey inverted. The figure is numbered relative to the known rpomt polypeptide. The five amino acids marked with an asterisk are those invariant in all DNA-directed polymerases, except multi-subunit RNA polymerases and RNA-directed polymerases. The phenylalanine (F786) residue marked with an arrow is conserved in all single-subunit RNA polymerases. This residue is proposed to interact with the incoming rNTP. Marked between dot symbols are the residues that constitute the hDhRGRhY domain which is conserved in all single-subunit DNA-directed RNA polymerases.

been isolated (Greenleaf et al., Proc Natl Acad Sci USA 83:3391, 1986; Chen et al., J Biol Chem 271:6537, 1996). The identification of rice ESTs with homology to the fungal polymerases provided the means to isolate the gene (*rpomt*) from maize. Using the rice EST clones and RT- PCR, maize cDNAs were isolated which were homologous to the yeast and *Neurospora* mitochondrial RNA polymerases. By employing a variety of 5'-RACE PCR techniques we have so far isolated approximately 3000 nucleotides of the maize cDNA, which we expect to be approximately 4000 nucleotides in length. We have yet to identify the 5'-terminus of the cDNA.

The known cDNA encodes 787 amino acids, which would produce a peptide of 89.2 kDa. Almost all 22 codons are used; codon usage and low G-C content of the cDNA suggests that the transcript is translated inefficiently. The amino alignment of the maize rpomt with the fungal polymerases is shown in Figure 1. The re-

gions marked with symbols are domains that are highly conserved in many polymerases. Residues defined with an asterisk are found in all single-subunit polymerases while the domain marked between circles (hDhRGRhY; h=hydrophobic) is found in only DNA-directed RNA polymerases (Delarue et al., Protein Eng 3:461, 1990).

Southern analysis predicts that the gene is present as a single copy (Fig. 2). Screening of a genomic library resulted in the isolation of a number of clones which when mapped using restriction endonucleases fell into a single group. This supported the Southern analysis result. Approximately 20 kb of the *rpomt* locus have been sequenced. The cDNA is spread over 17.2 kb and is divided into 19 exons. The presence of a poly-A tail on the transcript and the identification of a partial PREM-1 retro-element sequence within an intron of the gene confirms that it is nuclear and not mitochondrially encoded.

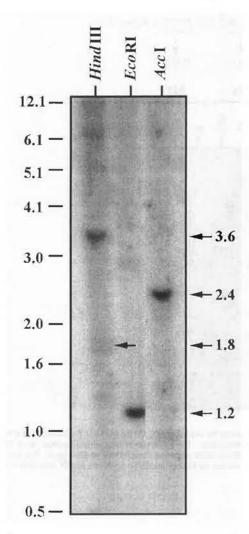


Figure 2. Genomic Southern analysis of B73N maize DNA. Each lane contains 20 g of DNA digested with the restriction endonuclease labelled above the corresponding lane. The Southern transferred get was hybridised with a 1.7 kb. rpomt genomic probe, and washed to a linal stringency of 1 x SSC, 0.1 % SDS at 65 C. The sizes of positively hybridising bands are indicated by arrows. The molecular weights were deduced by comparison to a lane containing 1 kb ladder (BRL). The arrows inside the body of the figure indicate the locations of a faint positively hybridising HindIII band at 1.8 kb.

Further experiments are in progress to isolate the 5' region of the gene. This will be followed by the determination of the *rpomt* encoded proteins subcellular location. Although the protein is highly homologous to the fungal polymerases (see Fig. 1) and such a gene is found in achloroplastic organisms (see Cermakian et al., Nucleic Acids Res 24:648, 1996) it has been proposed that a T3/T7-like RNA polymerase is also involved in chloroplast transcription (Allison et al., EMBO J 15:2802, 1996; Lerbs- Marche, Proc Natl Acad Sci USA 90:5509, 1993).

Evidence that plant mitochondrial transcription requires promoter-specific factors

--Young, DA; Lonsdale, DM

Transcription of the fungal and animal mitochondrial genome requires two protein components: the mitochondrial RNA polymerase (mtRNAP) and a single transcription factor which confers promoter specificity upon the polymerase. Neither protein has been isolated from plants to date. In maize the development of an

in vitro transcription system (Rapp and Stern, EMBO J. 11:1065, 1992) along with nuclease protection and G-capping experiments has failed to identify a conserved consensus proximate to transcription initiation nucleotides; though in a relatively high proportion of the known transcription initiation sites the tetranucleotide, 5'-CRTA-3', is present.

Transcription has been shown to initiate at several sites for most maize mitochondrial genes. Transcription of the *cox3* gene occurs at three sites which all possess the CRTA tetranucleotide sequence proximate to the transcription initiation nucleotide (Mulligan et al., Proc. Natl. Acad. Sci. USA 85:7998, 1988). Two of these three sites (at -360 and -320 relative to the ATG) show extensive homology to each other over a 21 nucleotide stretch; this repeated sequence can therefore be defined as a minimal promoter sequence. The close proximity of the two promoters allowed for the simple construction of a probe for use in mobility shift assays to identify the proteins involved in plant mitochondrial transcription.

Initial studies with the double-promoter probe and a S-100 mitochondrial protein extract produced a complex retardation pattern that was presumed to be due to the binding of a number of proteins to both promoter elements. After dividing the probe into its constituent promoter elements, -360 and -320, and using each promoter element individually in mobility shift assays the retardation pattern was simplified. The retardation patterns of each promoter were identical displaying two slowly migrating complexes, named A and B. These retardation complexes were stable in high-salt, 500 mM KCl, and in the presence of a 2000fold excess of non-specific competitor DNA demonstrating the specificity of the interactions. Linker-scanning mutagenesis of the cox3-360 promoter identified the sequence CRT of the 5'-CRTA-3' motif as being essential for complex-A formation (Fig. 1). Furthermore sequences both upstream and downstream of the CRTA motif were shown to be required for optimal complex-A formation (Fig. 1).

Mobility shift competition studies were performed to address the question as to whether complex-A formation was specific to cox3 or whether the binding factor could recognise other mitochondrial promoter sequences. The principle of these experiments was to add an excess of unlabelled specific competitor DNA to a reaction containing the radiolabelled cox3-360 probe and the S-100 mitochondrial protein extract. If the competitor DNA was capable of sequestering the complex-A binding factor then a reduction in the radiolabelled complex-A would be observed. Figure 2 shows the result of such an experiment. When the competitor DNA added to the reaction was either of the cox3 promoter sequences (-360 or -320) the formation of complex-A was greatly reduced due to the sequestration of factor 'A' by these DNAs. However when the competitor DNA used was another characterised maize mitochondrial promoter sequence (see figure legend for details) complex-A could still be visualised. These results therefore indicate that factor 'A' binds specifically to the cox3 promoter elements at -320 and -360. This result is surprising and strongly suggests that mitochondrial transcription requires 'promoter-specific' factors. A permutation of different promoter sequences upstream of genes will reduce the reliance of gene transcription on the abundance of any one factor. The recent proposal of gene-specific transcription of the cox2 gene in Zea perennis associated with the MCT locus (Newton et al., EMBO J. 14:585, 1995) supports this theory that each plant mitochondrial

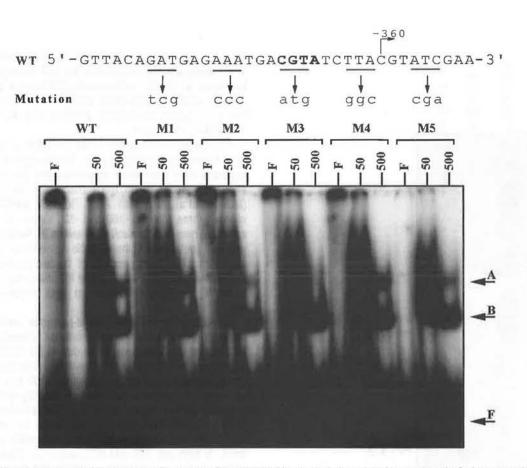


Figure 1. Mobility shift experiment using mutant cox3-360 probes. The sequence of the cox3-360 'wild type' probe is shown above the autoradiograph. On the sequence the nucleotide at which transcription initiation occurs is indicated with an arrow. Underlined are the triplet sequences that were mutated to form the mutant probes. The sequences of these mutated probes (M1 to M5) are shown in lower case above their corresponding lanes. Each probe was labelled with [α .32P] dATP and [α .32P] dTTP, and 12,000 cpm of the probe were loaded in each of three lanes. The first of the three lanes F contained only probe, the second and third (50 and 500 respectively) contained 15 µg S100-mitochon drial protein and the labelled quantity (in ng) of non-specific competitor DNA (polydldC, polydldC). The arrows labelled A, B and F refer to bound complex-A, complex-B and free probe respectively.

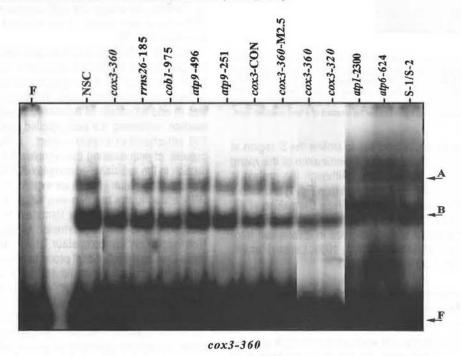


Figure 2. Competition study using various 'cold' mitochondrial promoter fragments versus radiolabelled cox3-360. All lanes contained 12,000 cpm of the cox3-360 probe and all, except lane F (free probe), contained 15 µg \$100-mitochondrial protein and 500 ng polydidC. DolydidC. Unlabelled competitor double-stranded oligonucletides, named above their corresponding lanes, encompass other maize mitochondrial promoter sequences, except where noted below, and were present in the reactions in a 50-fold excess relative to the labelled probe. NSC, no specific-competitor; cob1-975 is a rice mitochondrial promoter; cox3-CON is a 5' flanking region of the maize cox3 gene that contains a CRTA motif yet does not sequester factor 'A'; cox3-360-M2.5 is a TGA-mutation between mutations M2 and M3 (see Fig. 1) and \$5-1/\$-2 is the promoter present in the terminal inverted repeat of the maize cms-S plasmids.

gene may require its own factor for accurate transcription initiation.

NOTTINGHAM, UNITED KINGDOM University of Nottingham

Effect of silver nitrate and culture vessel on growth and quality of Type II callus of the A188 genotype

--Southgate, EM; Power, JB; Davey, MR

Using the point counting method of Mottley and Keen (Plant Cell Rep. 6: 389-392, 1987), the effects of both culture vessel and silver nitrate on white, friable Type II callus growth (percentage increase in size) of inbred A188 were assessed. In addition, the effect on callus quality (in terms of root production, yellowing or non-Type II characteristics) was ascertained. Deep, vented 9 cm tissue culture dishes (Corning, Bibby-Sterilin) and 9 cm petri dishes (Bibby-Sterilin) were compared and, in order to investigate ethylene accumulation in the two types of culture vessels, half of the callus maintenance medium was supplemented with 10 mgl⁻¹ AgNO₃. The callus maintenance medium consisted of Chu N6 basal salts (Chu et al. Sci. Sinica 18: 659-668, 1975) supplemented with 25mM proline, 1 mgl-1 thiamine-HCl, 0.5 mgl-1 nicotinic acid, 0.5 mgl-1 pyridoxine-HCl, 20 gl-1 sucrose and 1.5 mgl-1 2,4-D at pH 5.8. Twenty-five calli (approx. 100 mg pieces; 5 calli per dish/5 replicates per treatment) were placed onto maintenance medium in the two types of culture vessel, with or without silver nitrate. For each treatment, the mean percentage increase in size was calculated over a 14 d period. In addition, the proportion of calli exhibiting yellowed regions (>10% coverage of callus), roots and non-Type II characteristics (>10% coverage of callus) after 14 d of culture was recorded. The use of silver nitrate in the culture medium of A188 Type II callus significantly (p<0.05) enhanced growth when using both types of culture vessel and this was most noticeable when the culture vessel was smaller and not vented (petri dishes). The assessment of callus quality (in terms of minimising yellowed or non-Type II callus and root growth), demonstrated that the presence of Type II callus was not affected

by AgNO₃ supplementation of the medium or the culture vessel. However, in the absence of AgNO₃, the use of vented tissue culture dishes reduced the number of calli exhibiting rhizogenesis.

Table 1. Effect of AgNO₃ and culture vessel on growth and quality of A188 Type II callus over a 14 d culture period.

| | | | Att | 88 Type II calli exhib | iting response | (%)° |
|--------------------------------|-------------------|-------------------------------|----------------|------------------------|----------------------|----------------------|
| Culture vessel ¹ | AgNO ₃ | Mean increase in size (%)3 | Type II callus | Yellowed callus | Root pro- duction | Non-Type I callus |
| TC | + | 134.5 (11.2) a | 97.5 (2.5) | 52.5 (9.2) | 20.0 (6.5) | 12.5 (3.7) |
| | | 104.0 (9.7) bc | 97.5 (2.5) | 47.5 (10.0) | 5.0 (5.0) | 10.0 (5.3) |
| P | + | 129.4 (19.6) ab | 97.1 (2.9) | 51.4 (12.9) | 17.1 (5.2) | 14.3 (8.4) |
| | | 80.5 (7.4) c | 95.0 (3.3) | 60.0 (10.7) | 20.0 (8.5) | 27.5 (11.9) |

 1 TC = tissue culture dish, P = petri dish. 2 Percentage of calli (5 replicated dishes of 5 explants) exhibiting response. Data are a mean of 25 replicates \pm SE. 3 Percentage values followed by the same suffix are not significantly different at p<0.05 (determined by the Kruskal-Wallis and Mood's Median test for non-parametric data).

PUSA, BIHAR, INDIA Bajendra Agricultural University

Comparative cytotoxicity of four herbicides on mitosis and meiosis in maize

--Kumar, M; Kumar, H; Nasar, S

Four herbicides, namely Atrazine, Oxidiazon, Oxyflourfen and Pendiamethaline, were evaluated for their cytotoxic effect on root tip mitosis and PMC meiosis. Two concentrations of aqueous solutions of each herbicide, 5 and 50 ppm, were used for seed treatment for 6 and 12 h. In mitotic studies, 4 h of recovery period was also given, whereas in meiotic studies, treated seeds were directly sown in the field and immature tassels were collected to study PMCs. Root and tassel samples were fixed in 3:1 alcohol-acetic acid and standard 2% acetocarmine squash preparations were made to observe the division rate as well as various chromosomal abnormalities.

Mitotic index was severely affected, and was directly proportional to dose and duration of treatment. Frequency of chromosomal abnormalities varied in different treatments, maximum in Pendiamethaline, followed by Oxyflourfen, Atrazine and Oxidiazon.

Table 1. Comparative efficacy of different herbicides in maize root tips.

| Herbicides | | | Meta- | Total | | Ty | rpes c | of Abr | orma | liti | es | | | | | |
|-------------|----------------------------------|-------|---------|-------------------------|----------------|----------------|--------|--------|------|------|----------------------|------------|------|-----|-----|------------------|
| | tion/Reco- very ppm/hrs/hr | | % phase | abnor- malities % | Ge | Lg | FrC | Frn | Pl | Cm | Des | Мр | St | Bn | Mn | Br |
| Control | Zero | 19.77 | 7.42 | 0.75 | - |) - | + | - | - | + | - | - | - | - | - | • |
| Atrazine | 5/6/4 | 9.22 | 3.59 | 2.29 | - | - | + | - | - | + | + | - | - | - | | - |
| | 50/12/4 | 3.35 | 1.07 | 1.19 | + | - | - | - | - | - | - | - | 73- | - | + | |
| ncsellazon | 5/6/4 | 9.20 | 3.13 | 1.15 | - | - | - | - | - | - | • | - | - | + | - | |
| | 50/12/4 | 5.99 | 1.64 | 1.69 | + | - | - | - | ~ | - | - | - | - | - | - | |
| exytlourfer | 5/6/4 | 9.41 | 3.62 | 3.63 | | - | + | 7.0 | + | + | + | - | | - | • | |
| | 50/12/4 | 4.30 | 1.25 | 5.86 | 3 | - | + | - | + | | ++ | - | | - | - | - |
| Pendime- | 5/6/4 | 4.95 | 2.3 | 11.96 | - | - | + | + | + | + | + | + | ++ | - | - | - |
| | 50/12/4 | NILL | NIL | 37.99 | - | + | ++ | ++ | ++ | + | + | + | ++++ | + | + | + |
| | Cale : | | | Fro = Fra | gment | atio | n of | c | m a | c-mi | tostu | - | | 3 | n - | Pinestante sell |
| | + - presen | t | | ch: | Omoso | mea | | D | 999 | Desp | irall | zazi | on | 21 | n = | Sulphorters of |
| | - = absent | | | Frn = Frg | gment 1 mis | atlo | n of | | | | | and Arrest | | е в | c - | Bridge formition |
| | Lg= Laggar | | | Pl = rul | ver1s omoso | | n of | 3 | | | 10 10 10 10 10 10 | | | | | |

Table 2. Comparative efficacy of different herbicides on maize pollen mother cell meiosis

| S1. No. | Herbicides | Concen- tration (ppm) | ment period | No.of | index (x) | Abnor- mal PMC: (%) | Meiosis I (%) | Helosis II (%) | Pollen ster- lity (%) | | | | Stic- kiness | Bridge forma- tion | Multiva- lent for- mation | Univa- lent | Disorien- ted Metophase | Unequal separation of Chromosome |
|------------|--------------|-----------------------------|----------------|-------|--------------|---------------------|---------------------|----------------------|--------------------------------|-------|-------|-------|-----------------|--------------------------|---------------------------------|----------------|-------------------------------|---|
| 1. | Control | Zero (0) | zero (0) | 2228 | 99.60 | 0.40 | 56.73 | 42.92 | 0.54 | 14,45 | 15.31 | | | | | • | - | - |
| 2. | Atrazina | 5 | 6 | 2410 | 96,80 | 3.30 | 56.57 | 40.21 | 0.50 | 28.63 | 22.03 | + | ٠ | | ٠ | - | • | - |
| | | 50 | 12 | 2398 | 88.32 | 11.69 | 54.19 | 34.15 | 2.73 | 12.93 | 15.47 | ** | ••• | • | *** | ٠ | | |
| 3. | 7xy'lour fen | 4 | | 2036 | 97.51 | 2,49 | 67.02 | 10.49 | 0.57 | 22,53 | :3.07 | | | | | - | | ** |
| | | 50 | 12 | 2042 | 93.24 | 6.76 | 94.72 | 8.52 | 3.16 | 13.48 | :.27 | *** " | *** | - | | - | - | * |
| 4. | Oxadiazon | 5 | 6. | 2412 | 96.10 | 3.90 | 68,29 | 27.82 | 1.13 | 10.76 | 14.09 | | ٠ | - | | • | | |
| | | 50 | 12 | 2437 | 87.82 | 12.19 | 52,94 | 34.89 | 2.52 | 23.89 | 15.82 | ** | *** | • | ••• | ٠ | • | |
| 5. | Pendimetha- | 5 | 6 | 2272 | 95.82 | 4.19 | 69.49 | 27.33 | 1.29 | 31.47 | 15.54 | | | | | - | - | *: |
| | lin | i) 1 | 1 2 | 2307 | 87.12 | 12.27 | 52.45 | 24.66 | 7.32 | 26.05 | :5.71 | | *** | - | *** | - | | |

Colei

Both clastogenic and turbagenic types of anomalies were induced by Pendiamethaline, Oxyflourfen and Atrazine, but Oxidiazon induced only binucleate and giant cells (Table 1). Pendiamethaline produced the highest frequency and type of abnormal cells.

The effect of these herbicides on PMCs revealed a dose dependent decrease in cells entering meiotic division (Table 2). The relative efficacy of these herbicides in producing meiotic abnormalities was Pendiamethaline > Oxidiazon > Atrazine > Oxyflourfen. Pollen sterility was also highest in Pendiamethaline treatments. These herbicide treatments led to accumulation of more cells in meiosis I than meiosis II. Arrest of cells at metaphase I and metaphase II was another significant effect observed.

Thus, it appears that the division process in general has been affected by such treatments due to disturbances in normal progression of the nuclear cycle and/or disturbances in the spindle apparatus.

RALEIGH, NORTH CAROLINA USDA-ARS AMES, IOWA Iowa State University

Polymorphic microsatellites found in cDNA clones

-Senior, L; Lee, M

To date, 33 polymorphic microsatellite markers have been developed by screening sequenced RFLP probes obtained from the UMC, BNL, NPI and PHP public probe libraries (Senior and Chin). An additional 32 putative microsatellite-containing probes were identified from the same libraries by dot-blot hybridizations, but have not yet been sequenced. Given the large number of probes available to the public, screening RFLP probe libraries should provide a rapid method for the development of microsatellite markers. All of the probes screened thus far were derived from maize genomic libraries, however, many of the publicly available RFLP probes are derived from cDNA libraries. Although cDNA probes may contain microsatellite regions, we would not expect them to be polymorphic. To test this hypothesis, 122 cDNA clones

from the ISU clone library (Pereira et al., Genome 37:236-243, 1994) were probed with the following oligos: CTx10, GAAx5, CCTx5 and GTCx5. The screening was done by PCR amplification of the cDNA probe insert from the plasmid using an aliquot of the bacterial culture as template, followed by electrophoresis of the products on a 0.8% agarose gel. A Southern blot of the gel was made and used in the hybridizations. The blots were probed with a mixture of all four oligos. Three probes showed a strong positive hybridization signal and were subsequently sequenced. The probes contained the following repeats: ISU62 (AGC x5), ISU76 (GGT x 5) and ISU89 (AG x 5, AC x 5, GTGTC x 3, GTC x 5). Primers were designed to flank each of the six potential microsatellite regions as described in Senior and Heun (Genome 36:884-889, 1993). Amplification of the microsatellite region was then performed as described in Senior et al. (Crop Sci 36:1676-1683, 1996). No amplification products were obtained from the GTC repeat in ISU89. A monomorphic product was obtained from the GGT repeat in ISU76. The remaining 4 microsatellites looked promising after the initial screen and were subsequently run against a panel of 96 elite US maize inbreds and sized on 4% Metaphor agarose gels. The loci were then mapped using a Mo17 x B73 recombinant inbred population (seed provided by C.W. Stuber). Locus names were assigned following the conventions described in Senior et al. (1996). Results of the screening and mapping are shown in Table 1. Primer sequences are shown in Table 2. Although this study is preliminary, it shows that polymorphic microsatellite regions do occur in maize cDNA clones and can provide another source for the development of microsatellite-based markers.

Table 1. Number of alleles found, product size range and map location for microsatellites derived from ISU cDNA probes.

| Locus name | Source | Bin No. | Repeat type | No. of alleles | Product size (bp) |
|---------------|--------|---------|-------------|-------------------|----------------------|
| nc130 | 1SU62 | 5.00 | AGC/GCT | 2 | 140-143 |
| nc131 | ISU89 | 2.04 | AC/GT | 4 | 135-147 |
| nc132 | ISU89 | 2.04 | AG/CT | 3 | 214-234 |
| nc133 | ISU89 | 2.04 | GTGTC/GACAC | 3 | 110-120 |

i a bread

^{- -} absent

Table 2. Primer sequences for microsatellites derived from ISU cDNA probes.

| Locus name | Forward Primer (5' - 3') | Reverse Primer (5' -3') |
|-------------------|--------------------------|-------------------------|
| nc130 | GCACATGAAGATCCTGCTGA | TGTGGATGACGGTGATGC |
| nc131 | TTTCTTCGATCCCATGTCAC | TAGTGTGCTAGAACGTGCGC |
| nc132 | TCATCTTGCTCTGATGCTCG | TGTGGGGGCACGTTAATTAC |
| nc133 | AATCAAACACACACCTTGCG | GCAAGGGAATAAGGTGACGA |
| 5/4/2-5/01 (CR54) | | |

ST. PAUL, MINNESOTA University of Minnesota

RFLP mapping of Semi-dwarf1

-- Vladutu, CI; Phillips, RL

Semi-dwarf1 (Sdw1) is a semi-dominant EMS-induced mutation affecting whole-plant morphology. As described by R.M. Bird and M.G. Neuffer (MNL 59:42), the reduction of height in heterozygous mutants varies from 1/4 to 1/3 of the wild-type plants and is due to internode shortening and not decreased node number. In mutants, the leaves and tassel branches are erect and shorter, and ears are smaller than in wild-type plants. In the complex genetic background of our material we did not notice wilting in Sdw1 heterozygotes. However, among the progeny of a selfed Sdw1 heterozygote, several very short plants with erect leaves and tassel branches (which likely were homozygous Sdw1) showed wilting. Also, the interval between anthesis and silking was generally longer and tillering was suppressed in Sdw1 heterozygotes. Using the waxy reciprocal translocation series, Bird and Neuffer located Sdw1 on the long arm of chromosome 8 about 8 map units from the 8L.35 breakpoint. Mo17 was the source of the pollen subjected to EMS mutagenesis (Bird and Neuffer, MNL 58:71). Because of its deleterious effects, Sdw1 has been maintained as a heterozygote.

Seed provided by P. Stinard (Maize Genetics Stock Center) resulted from a cross between Sdw1/+ (+ designates a wild-type allele from either Mo20Y or W23) and a wild-type single cross (L317 x W23). We used bulked segregant analysis to identify useful polymorphic patterns (i.e. distinct bands linked in cis with Sdw1) between heterozygous Sdw1 and wild-type plants segregating from the above cross. DNA was extracted from bulked leaf tissue of six wild-type plants, four mutants, and L317 and W23. The four DNA samples were digested with BamHI, HindIII, EcoRI and EcoRV, Southern-blotted, and probed with 10 genomic and cDNA sequences previously mapped on chr.8: umc124, ucbanp1 (from J. Vogel), pge11 (from S. Hake), umc89, umc12, bnl12.3, csu31, umc93, umc48 and umc30. umc89 (with BamHI and EcoRV) and ucbanp1 (with HindIII) each detected a band present in the mutants and absent in the wild-type controls. umc30 (with BamHI) revealed a more intense band in mutants compared with the wild-type segregants (the respective band was not detected in either L317 or W23).

In 1994 the four *Sdw1* heterozygotes were selfed or testcrossed with wild-type segregants. A self and a testcross progeny, involving the same *Sdw1* heterozygote, were planted in 1995. Neither the mutant nor the wild-type plant used in the testcross were recombinant for the informative. *umc89*, *ucbanp1* and *umc30* fragments. We could not unambiguously score all the phenotypes of the self progeny; however, the testcross progeny segregated in two distinct classes (35 wild-type: 31 mutant). DNA was extracted from the 66 testcross individuals and digested with *BamHI* and *HindIII*. No recombination occurred between *Sdw1* and *umc89* and *ucbanp1*. Three recombinants occurred between *Sdw1* and *umc30* (4.5±2.6% recombination).

These results indicate considerable shrinkage of recombination values on chromosome 8L in this material compared with other mapping populations. In the UMC 1995 map (Coe et al. MNL69:164) the <code>umc89-umc30</code> interval is ~25 cM. In a N28 x N28E F2 population (88 individuals) <code>ucbanp1</code> mapped ~ 10 cM toward the centromere from <code>umc89</code> (Vladutu, M. S. thesis, 1996). Localized factors such as inversions, heterozygosity for knobs or general background or environmental effects could account for the reduction in recombination values. Alternatively, the recombination values may be underestimated due to the small population size used in this mapping experiment.

In conclusion, Sdw1 is confirmed by RFLP mapping to be on chromosome 8L, close to umc89 and ucbanp1.

SARATOV, RUSSIA Saratov State University

Producing of parthenogenetic forms of maize

--Tyrnov, VS

Fifteen years ago we produced the line AT-1. It had the following characteristics:

- 1. It gave matroclinous haploids with a frequency of 90-100%. The frequency of haploidy could be higher than 100% because of the occurrence of twins and triplets like n-n and n-n-n types.
- 2. All haploids were produced by pollination. Without pollination the endosperm developed only to a certain stage, and its development was not completed. By the method of genetic marking it was shown that normal endosperm was formed only after fertilization. We suppose that this is connected with the necessity for the endosperm to have ploidy equal to 3n or divisible by 3. Consequently, according to cytological data, the line is characterized by autonomous reduced parthenogenesis, but genetic data show that pseudogamous haploid parthenogenesis takes place.
- Side by side with haploids, diploid parthenogenetic individuals were found. Their origin has not been ascertained for the present, but it is connected, most probably, with spontaneous diploidization of haploids.
- 4. The highest frequency of haploidy was observed by delay of pollination (5-10 days). In connection with this, the idea seems attractive to create apomictic lines which, by super early pollination (for example, before emergence of silks), would keep the possibility of hybridization and selection by traditional methods.
- 5. AT-1 has purple colour of leaves, seeds and roots, which is controlled, probably, by genes A C R B Pl. This makes the diagnosis of matroclinous individuals difficult, but allows discovery of androgenic plants easily. The latter were produced by crossing with Mangelsdorf's tester and other lines, having genes *lg*, *gl*.

During the first four years there was a constant danger of losing this line, because only haploids arose and diploids were rare. In addition the plants were strongly affected by *Ustilago maydis*. So we were obliged to make selection on resistance and on decrease of haploidy level. At the present time we have selected a resistant form. The frequency of haploidy, by early dates of pollination, lies in the limits of 2-3%. It can be increased ten times by delay of pollination. Together with this, pollination delay leads to decrease of seed productivity, probably as a result of anomalous autonomous endospermogenesis.

We ascertained that ability for parthenogenesis is a nuclearencoded trait. It can be transmitted to other lines by egg or pollen. It shall allow in the future combining of different traits with parthenogenesis in the same genome. Taking this into account, at the present time we have accomplished the following:

 AT-3 line, the analogue of AT-1 line, but having yellow seeds, green leaves, and white roots, was produced. It allows diagnosis of apomixis with purple, brown and embryo markers. In hybrid progeny from crossing of this line with Mangelsdorf's tester, lines were selected with different marker genes. It is not excluded that it can be possible to determine the chromosomes controlling parthenogenesis.

By backcrossing the analogues with CMS of T, S and Ctypes, it was shown that these cytoplasms did not influence manifestation of parthenogenesis. In the future male-sterile lines can

be required to exclude selection on sexual reproduction.

We produced hybrids between AT-3 and line-donors of some non-reduction genes. On this basis we suppose to test the possibility of production of unreduced apomicts.

4. Most wild apomicts are polyploids. So we also carry out

work on production of polyploid apomicts.

5. Crosses of AT-3 with *Tripsacum dactyloides* were made. We propose to test the possibility of (1) introgression from *T. dactyloides* to AT-3 of non-reduction genes only, (2) increasing of ability for apomixis in hybrids and (3) decreasing of quantity of non-desirable linked wild characters resulting from "borrowing" of parthenogenesis from maize.

Thus, we have demonstrated the possibility of occurrence of autonomous reduced parthenogenesis, conditioned by nuclear genes, which produced (and produces now) unique material for further work on synthesis of apomictic forms. This work was supported in part by a grant from the Russian Foundation for Basic

Research.

Development of seeds with haploid embryo on haploid plants of parthenogenetic line

--Tyrnov, VS

It is known that normal seeds can develop by pollination of maize haploids by pollen of diploid plants. We observed this phenomenon in haploids of parthenogenetic line AT-1. The frequency

of haploidy varied from 1 to 26 seeds on an ear.

In 1986, 60 haploids of AT-1 line were pollinated by Mangelsdorf's tester pollen. 840 seeds were produced. Among them 72 matroclinous haploids, one androgenic haploid and 16 twins were discovered. 10 twins were of 2n-n type, 6 of n-n type. One twin of n-n type had both matroclinous and androgenic seedlings. Next year, in 1987, 390 seeds were produced from 40 haploids, developed from haploids. 48 matroclinous haploids and 9 twins were discovered among them. In 1988-1990 we continued to discover haploids formed on haploids of previous generations. The frequency of haploidy reached 10-20%.

So, during 5 generations, monoploid individuals served as sporophyte. This can be considered as an experimental system of seed reproduction on the haploid level. By a comparatively small number of plants (50-100) it can be maintained for a long time.

Later (1990-1996) we pollinated by diploid pollen haploids of another line, AT-3, which regularly appeared in the field. Haploids similarly developed on them. Subsequently haploid level was maintained during 2-3 generations by presence of 5-8 plants only.

We suppose, that development of haploids on haploids can be a diagnostic trait of parthenogenesis. This work was supported in part by a grant from the Russian Foundation for Basic Research.

Cytological manifestation of apomixis in AT-1 plants of corn

-- Enaleeva, NCh; Tyrnov, VS

The results of embryological investigations of AT-1 line (16 plants) and 6 hybrids (19 plants) are presented. Two hybrids were produced by crosses of AT-1 line as female with DPL-1 and Mangelsdorf's tester (MT) lines. Four hybrids were derived from crosses of AT-1 plants as males with plants of DPL-1, DPL-2, DPL-3, and MT lines. The DPL-1, DPL-2, DPL-3 and MT lines were shown in preliminary investigations to have no apomictic traits. The ears, previously isolated by parchment bags, were fixed in ethanol-acetic acid, 1:3, 7-8 days after silk emergence. Embryo sacs (ESs) were extracted under a stereo microscope from ovules stained with acetocarmine and macerated with cytase. 2025 ESs were examined in the course of the investigation.

In AT-1 plants proembryos were observed in a significant part of ESs without signs of pollen tube penetration. The proembryo stages varied from two-cell to globular. The cytological structure of proembryos in most cases was similar to normal zygotic ones, though in some ESs abnormal proembryos were found. The most frequent irregularity was that the egg cell divided not transversely but longitudinally resulting in two equal cells. These two cells gave rise to two equal proembryos. In some cases the following divisions in each of the "cleavage" embryos proceeded asynchronously, and as a result the two proembryos differed in size. "Cleavage" twin proembryos also were found in which one of the proembryo initials failed to divide. This cytological proembryo type is presumed to be a prerequisite of twin seedlings (2n-n), occurring rather often in offspring of the AT-1 line under pollination.

Other abnormalities of haploid embryogenesis also were observed: lack of cytokinesis after first or second egg cell division; oblique wall formation after first mitosis; an asymmetrical first division resulting in a large apical cell and a small basal one. The frequency of ESs with parthenogenetic embryogenesis varied from 17.1 to 82.6%. In 10 of 16 plants studied the frequency of such ESs comprised more than 50%.

In ESs of 11 plants an autonomous endosperm was registered. In the most cases it was accompanied by autonomous embryogenesis. The cytological course of endospermogenesis varied significantly. The first and second divisions, as a rule, happened typically, but further development passed abnormally. Disturbances were expressed in irregular nuclear location (nuclei were concentrated in several regions), in different nuclear sizes, and in premature cellularization in the presence of a low number of nuclei. The cells were of different size and contained several nuclei. In no case was the autonomous endospermogenesis found to be normal. Later this endosperm degenerated. The frequency of ESs with autonomous endosperm development in AT-1 plants ranged from 0 to 59.4%.

The analysis of hybrids derived from crosses when AT-1 plants were used as females showed that in 6 of 7 hybrids analyzed, autonomous embryogenesis occurred. In one hybrid plant autonomous endospermogenesis was revealed. The greatest amount of ESs with embryo or with endospermogenesis per plant was found to be 13.2% and 1.0% respectively. In hybrids derived from crosses when AT-1 line was used as males, parthenogenetic embryo development was observed in all 12 hybrid plants, and autonomous endospermogenesis in 5 plants. The greatest amount of ESs with proembryo or with endosperm per plant was 12.3% and 1.4% respectively. The cytoembryological patterns of embryo and

endospermogenesis in hybrids were similar to the ones described above for AT-1 plants.

The results allow us to reach the following conclusions:

(1) AT-1 line is characterized by constant expression of autonomous haploid embryogenesis. Since its frequency in some plants amounted to up to 82.6%, the fundamental possibility of increasing the average percentage exists.

(2) The genetic system controlling autonomous embryogenesis can be transmitted either through female or male gametes, and can

be expressed in different genetic backgrounds.

(3) Because of the lack of essential difference in frequency of embryogenesis between reciprocal hybrids, the cytoplasm obviously does not take part in determination of this phenomenon.

(4) The expression of autonomous endospermogenesis in AT-1 line is less frequent, and characterized by strong cytological abnormalities. It is not inconceivable that divisions both of the egg cell and of the central cell are induced by the same factor. However, owing to specific peculiarities of these cells, the cytological effects of induction are different. The reason for endospermogenesis abnormality is most likely related to a diploid chromosome set in the primary endosperm nucleus. It is known that for many species including *Zea mays* L., the endosperm develops normally under the condition that its chromosome set number is divisible by three.

Therefore, it appears to be promising to transfer the AT-1 line genome at the triploid level. This work was supported by a

grant from the Russian Foundation for basic Research.

Possibilities of diagnosis of parthenogenesis by culture in vitro of unpollinated ovaries

-- Alatortseva, TA; Tyrnov, VS

For seven years we have carried out comparative investigations of lines reproducing by sexual mode, and forms able to undergo parthenogenesis (AT-1 and F1 from AT-1 x sexual forms) (Alatortseva and Tyrnov, Biol Cult Cell and Biotechnology, Proc Int Conf, Alma-Ata, p.139, 1989 (in Russian); Reproductive Biol and Plant Breeding, XIII EUCARPIA Congr., p. 329-330, 1992; Apomixis in Angiosperms: problems and perspectives of investigation, Proc Int Symp, Saratov, Russia, p. 8-9, 1994 (in Russian); Alatortseva, Tyrnov, and Suchanov, Embryology and Seed Reproduction, Proc XI Int Symp Leningrad, USSR, 1990, St.Petersburg Nauka, 1992, p. 29-30; Tyrnov and Alatortseva, Devel Genet, Tashkent, p.164-166, 1990 (in Russian). It has been ascertained, that in the same conditions in vitro on nutrient medium MS with addition of sucrose (9%), 2,4-D (2.0 mg/l) and vitamins the difference was distinct in the reaction of ovaries, which were taken from the donors with different modes of reproduction. The difference is expressed in transition of the egg to division with formation of a proembryo in apomicts, while in female sexual cells of amphimictic plants (16 lines) there are not visible changes, and soon they degenerate. In ovaries of apomictic lines the beginning of autonomous division of the egg without pollination falls 8-10 days after the moment of silk appearance, including the time as explants in nutrient medium. In 3-4 week ovaries, on the surface of apomictic proembryo numerous embryoids appear, which either produce daughter embryoids or form haploid regenerants after 2-3 months of cultivation. Egg division and formation of globular proembryo can take place on hormoneless medium with lower concentrations of sucrose (2-3%).

The influence of endosperm on the fate of the proembryo was

also investigated. In unpollinated ovaries of apomictic lines in vivo either autonomous development of proembryo and defective endosperm or only proembryo or only endosperm occur. By cultivation in vitro, regeneration of plantlets happens through embryoor embryoidogenesis in the absence of endosperm. In the presence of endosperm the process of differentiation of embryos and reproduction of embryoids in early developmental stages is inhibited. Thus, the presence of endosperm in unpollinated ovaries is not a positive factor for formation of apomictic proembryos and regeneration. It can not be excluded that endosperm can play a negative role in sexual forms, as in cases of its development without fertilization its ploidy and other traits will be similar with characteristics of apomictic lines. The absence of endosperm in ovaries is compensated by nutrient medium. This explains the ability of the embryo for further development in vitro, whereas in vivo it is excluded. Under cultivation of unpollinated ovaries, formation of plant-regenerants happens by two ways: through direct embryogenesis or by means of embryoidogenesis. However, in the case of direct embryogenesis the developing embryo frequently degenerates. Regenerants form mainly from embryoids. Sometimes in tissues of the maternal embryo, side by side with centres of embryoidogenesis, formation of absolutely autonomous elements of the conductive system, roots, leaf-like structures and also teratologous structures, giving ugly plants, can be observed. However, they do not inhibit production of normal embryoids and plants.

It has been determined that autonomous parthenogenesis can be manifested in vitro in hybrids between line AT-1 and common amphimictic lines, although with lower frequency than in pure line AT-1. Thus, use of in vitro techniques in parthenogenetic lines allows the possibility of producing haploid plants in mass quantity, and of carrying out diagnosis and selection of apomictic forms. This work was supported in part by a grant from the Russian

Foundation for Basic Research.

SENDAI, JAPAN Tohoku University JOETSU, JAPAN Hokuriku Natl. Agr. Exp.Stn.

Visualization of methylated cytosine in maize somatic chromosomes --Fluminhan, A; Ohmido, N; Fukui, K; Mizugaki, M; Kameya, T

Methylation of DNA is considered to be a determinant of gene expression and its role in growth and development has been extensively studied in many systems. Diverse evidences have implicated the changes in the pattern and degree of DNA methylation in the control of a number of cellular processes, including gene activity, cellular differentiation, chromatin structure, DNA replication, transcription and repair. Methylation interferes with DNA-protein interactions and thus may play a crucial role in all processes involving such interactions. However, much remains to be elucidated about this role and about the distribution of methylated bases at the DNA and chromosome levels. The DNA of higher plants contains a considerable proportion of cytosine residues modified to 5-methylcytosine (5-MeC). Up to 30% of the cytosine residues are methylated in certain plant species, compared to 3 to 8% in vertebrates (Adams and Burdon, Molecular Biology of DNA Methylation, Springer-Verlag, 1985). Virtually, all of the 5-MeC found in plant genomes is confined to the symmetrical sequences CpG and CpNpG, which are distributed non-randomly in the DNA.

Many studies have demonstrated that certain genes are undermethylated in tissues where they are actively expressed, but highly methylated in tissues where they are silent, suggesting that methylation of DNA is a possible mechanism by which gene activity is regulated during development. The relationship between the degree of DNA methylation and gene expression has been found primarily in transgenic plants (Amasino et al., MGG 197: 437-446, 1984) and transposon insertions (Chandler and Walbot, PNAS 83:1767-1771, 1986; Schwartz and Dennis, MGG 205:476-482, 1986).

Immunological detection of 5-MeC has been used in studies with mammalian cells to detect different levels of methylation between euchromatin and heterochromatin, and between the heterochromatic regions of different chromosomes in the same complement (Miller et al., Nature 251:636-637, 1974; Schnedl et al., Chromosoma 59:59-66, 1975). High levels of 5-MeC are found in unexpressed regions of human and mouse genomes, such as centromeric and heterochromatic regions, several types of satellite DNA, and inactive mammalian X-chromosomes. We describe here an indirect immunofluorescence procedure based on the use of anti-5-MeC antisera for the analysis of the distribution of methylated cytosine in somatic chromosomes of maize. Chromosome preparations from long-term cultured cells and root meristems of germinating aged seeds were employed. Single-stranded DNA was produced either by UV irradiation or by heating in a formamide solution. In the first case, slides were placed cell side up in a plastic Petri dish and covered with a layer about 1 cm deep of PBS solution (phosphate-buffered saline: 20g NaCl, 85 ml 0.25M Na₂HPO₄, 15 ml 0.25M KH₂PO₄ in 2400 ml distilled water, pH 7.2 - 7.4). The dish was placed 32 cm from two 15 W germicidal lamps (Hitachi) and exposed to UV irradiation for 18 h at room temperature. For the heating treatment, chromosome preparations were incubated in a 90% formamide solution with 0.25% formaldehyde for 60 min at 65 C. Slides were then covered with an appropriate dilution of specific anti-5-MeC antisera (elicited in mouse by immunization with nucleoside-protein conjugates) and incubated in a moist chamber for 30 min at 37 C. The preparations were rinsed with PBS solution, layered with an appropriate dilution of specifically purified goat anti-mouse immunoglobin tagged with biotin and incubated for additional 30 min at 37 C. After staining with fluorescein-isothiocyanate (FITC)-avidin conjugate, signals were amplified by applying a biotinylated anti-avidin solution to the preparations, followed by incubation with a fluoresceinavidin solution. Chromosomes were then counter-stained with DAPI, and examined by fluorescence microscopy. Images were captured by a cooled CCD camera (Photometrics) mounted on the microscope. Digitized images were photographed by a color image recorder (Nippon Avionics).

Our results are consistent with the binding of anti-5-MeC antisera on single-stranded DNA in fixed chromosomes. The antibody employed in the present study was observed to bind intensely to specific regions in maize somatic chromosomes, reflecting a non-random distribution of 5-methylcytosine in the nuclear DNA of this species. Brilliant fluorescence, indicating extensive antibody binding, was mostly observed in knob heterochromatic regions (Figure 1). The degree of fluorescence and the size of the fluorescent regions tended to vary in different cells and in the two homologues of the same complement. The intense fluorescence of heterochromatic knobs partly reflects the greater concentration of 5-MeC at the knob repetitive sequences. We have also ob-

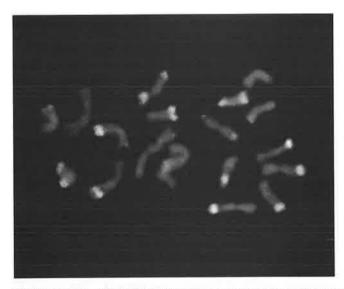


Figure 1. Metaphase chromosomes of a 2-year-old maize embryogenic callus culture after indirect immunofluorescence detection of 5-methylcytosine (5-MeC). Higher levels of methylated cytosine are observed at the heterochromatic knobs.

served a moderately bright fluorescence in the distal portion of several chromosome arms, and in the nucleolus-organizing region. The fluorescent pattern observed with anti-5-MeC antisera might reveal the possible location of CpG and CpNpG-rich regions of the maize genome. Consistently, Dennis and Peacock (J. Mol. Evol. 20:341-350, 1984) have reported that the 180 bp knob-specific repeats could show up to ten sites with CpG dinucleotides and/or CpNpG trinucleotides among the different clones sequenced. It is recognized that the binding pattern of the anti-5-MeC antibody might also reflect differences in regional base composition, extent of denaturation of DNA, density of nucleoproteins, tertiary structure or protein binding. Furthermore, current evidence suggests that 5-methylcytosine is not connected with maintenance of the stability of eukaryote DNA or DNA synthesis. Thus, DNA from different tissues of the same plant (or different developmental stages of the same tissue) may have a differing content of 5-MeC.

The observation of an increased presence of methylated cytosine at knob heterochromatin is consistent with the proposition that changes in the degree and pattern of DNA methylation could be an underlying cause of chromosomal abnormalities commonly observed in maize tissue cultures and their regenerants (Phillips et al., Proc. 7th Intl. Cong. Plant Tissue Cell Cult. pp. 131-141, 1990). These findings have prompted us to investigate the role of this biological modification in chromosomal abnormalities currently observed in cultured cells and other systems, such as for example: a) at first mitosis in germinating aged seeds (Fluminhan and Kameya, Genome - in press) and b) at the second microspore mitosis in a specific genetic background where the combination of B chromosomes and knobs leads to chromatin loss (Rhoades et al., PNAS 57:1626-1632, 1967; Rhoades and Dempsey, Genetics 71:73-96, 1972; Rhoades and Dempsey, J. Hered. 64:12-18, 1973).

We can conclude that indirect immunofluorescence procedures should therefore be very useful in analyzing the nature of the DNA present in the chromosomes, especially because of the high degree of base specificity shown by antinucleoside antibodies. Our results clearly show the applicability of the method for the direct visualization of methylated cytosine in maize chromosomes and may

represent a promising approach to the molecular structure of nuclear genomes and the evolution of highly repetitive sequences.

SIMNIC - CRAIOVA, ROMANIA Agricultural Research Station

The theoretical significance of a mutant in the phylogeny of the species Zea mays L.

--Radu, A; Urechean, V; Naidin, C; Motorga, V

"The whole living world has had one single start point. Theoretically speaking, if we would have studied this point, it is possible to find out several genes that have been kept just as they were for three billion years." (Maximilian C., Doina Maria loan, Dict Enc Genet, Stiint Enc Bucharest, 1984)

The origin of corn is still submitted to scientific controversy because of the morphological changes that led to the appearance of the corn plant as it is known today, deriving from the ancestral, wild extinct forms (Galinat, pp. 1-4 in Sprague, GF, Corn and Corn Improvement, Amer Soc Agron, Madison, 1977). The Maydeae tribe includes seven genera: two of American origin, Zea and Tripsacum, and another five of Eastern origin, Coix, Chionachne, Sclerachne, Trilobachne and Polytoca. It is generally agreed that the greatest role was played by the American genera Zea and Tripsacum in corn phylogeny, whilst the Eastern ones played only a small role. It is accepted that the Coix genus might have occurred in a certain stage of the phylogenetic development of the species Zea mays L. There are a number of characters that show phenotypic expression and penetration according to which we presume that the Manisuris genus, belonging to the Andropogoneae, might have contributed to the phylogenetic tree of final development of Zea mays L.

Cytogenetic studies at the Zea x Tripsacum hybrid level (Mangelsdorf and Reeves, Adv Genet 1947) confirmed the presence of several Tripsacum chromosomes, on which the supernumerary B chromosome existence relies and that can be found in the current corn genotypes (Galinat, 1977). Undoubtedly, the origin of Zea mays relies on an ancestral extinct corn form with genome (2n = 20 MM) and many recessive genes that led to the appearance of a new sterile hybrid F1 (2n = 10 M + 18 T) by natural hybridization to Tripsacum dactyloides (2n = 36 TT).

By natural diploidization a new hybrid form F1 (2n = 20 MM + 18 T) has been obtained, which led to the appearance of the old corn forms by introgressive hybridization to the ancestral corn form (2n = 20 MM) under the influence of empirical selection. This model of hypothetical development has been checked and confirmed (Galinat, 1977), but using as a starting point old American corn varieties. The ancestral corn form that initiated the phylogenetic development of *Zea mays* by natural introgression is unknown today. The various natural mutants that appear with *Zea mays* do not show special scientific interest as far as the origin of corn is concerned, the mutant gene expression being within the limits of intraspecific variation. Up to now (February 1996) there were no reports about corn mutants that might explain the development of the *Zea* genes by the contribution of certain species belonging to

Getting haploids of wheat, by breeding wheat and corn (Barclay, 1975; Inagaki, 1985, 1988; Comeau et al., 1988; Ahmad, Comeau, 1990 -- cited by Giura, Cerc Genet Veg Anim III, 17-26 1994) indirectly proved that the genus Zea is able to pollinate

genera outside the Maydeae.

with the genus *Triticum*. So, in corn phylogeny it has been possible for species belonging to other tribes and genera (Andropogoneae - *Manisurus*; Gramineae - *Triticum*) to contribute to the appearance and development of the genus Zea, especially to the species *Zea mays* L., by gene introgression.

Getting a corn mutant through somatic embryogenesis at the Agricultural Research Station, Simnic - Craiova, Dolj, Romania (Urechean, Com Pers Ses Anu Ref ICCPT Fundul, 1996) having characters much more different than the species *Zea mays* seems to represent the lost transpecies that Mangelsdorf, Reeves and Galinat theoretically placed at the beginning of the species *Zea mays* as well as *Tripsacum dactyloides*.

This report shows a series of theoretical considerations, starting with the expression of some phenotypic characters obvious in the resulting mutant as scientific arguments for the possibility of recovering some latent genetical information within the genome of *Zea mays* by somatic embryogenesis.

Caryopses belonging to the genotype S3 x A188 were collected 10 days after pollination and were sterilized in 70% ethyl alcohol and 1% mercuric chloride (HgCl₂). The excised immature embryos inoculated on MS callus medium (Murashige and Skoog, 1962), 30 g/l saccharose, 2 mg/l 2,4-D; pH 5.8, were incubated in darkness at 27-28 C. Passage 1 was done on NBM medium (N6 macroelements, Chu, 1978; B5 microelements, Gamborg, 1968; M vitamins, Murashige and Skoog) without hormones with 12 g/l saccharose, pH 5.8, callus cultivation at a photoperiod of 16 h light and 8 h dark, at 28-30 C. Rooting was done on MS medium with 2.0 mg/l 2,4-D and 30 g/l saccharose, followed by transfer on sterile soil and cultivation in vegetation plots under laboratory conditions. One single seedling of 50 transferred tillers on root medium survived to maturity, representing the mutant described in this work.

Using the chromosomal maps of Neuffer, Jones and Zuber (Mutants of Maize, Crop Sci Soc Amer, 1968) and those filed by Micu (Genet Issled Kuk, Stiint Chis 211-225, 1981), the known mutants whose phenotypic expression was obvious in the case of the mutant, "hermaphroditic grassy small plant", were identified. The mutant is phenotypically characterized as follows: miniature grassy plant, 8-10 cm height, showing tillers and branches like teosinte type (Figure 1); stem with short internodes, lacking the





Figure 1. Hermaphroditic grassy miniature plant. Figure 2. Terminal hermaphroditic flowers.





Figure 3. The mutant seeds - caryopses enclosed in glumes and nude seeds compared to a normal wheat seed (x 5).

Figure 4. Leaves with hairy sheath; uniaxial tassel; hermaphrodltic simple flowers.

Table 1. Genetical expression modifications of Zea mays L. in the mutant *hermaphroditic grassy miniature plant*.

| Specification | Position mutant locus | Symbol mutant gene | Description of the phenotipical character |
|-------------------------|-----------------------------|--------------------------|--|
| | | rd1 | reduced plant |
| Chromosome | 119 | TS3 | tassel seed |
| 1 | 124 | tb | teosinte branched |
| | 158 | TS6 | tassel seed |
| Chromosome 2 | 0 | WS3 | white sheath |
| | :•: | mn1 | miniature seed |
| | 0 | cr1 | crinkly leaf |
| Chromosome | 31 | Cg | corn grass |
| 3 | 55 | ts4 | tassel seed |
| | 72 | ba1 | barren stalk |
| Chromosome | 0 | de, | defective endosperm |
| 4 | 107 | Tu | tunicata |
| Chromosome | 22 | bt, | brittle endosperm |
| 5 | 27 | bv, | brevis |
| Chromosome | 0 | Hs | hairy sheath |
| 7 | 26 | CP ₁ | colapsed |
| Unknown placed genes | | ig | indeterminate gametophytic |
| on chromosomes | | ub | unbraced tassel |

characteristic concavity at the female flower insertion area; tassel with simple, hermaphroditic flowers (Figures 2, 4); seeds like caryopses having small dimensions 0.6/0.3 mm, not viable, completely closed in glumes (Figure 3).

The appearance of a mutant can be induced by: somaclonal variability that appears in somatic embryogenesis, induced by the

culture medium; simultaneous modifications, synchronous in one direction, belonging to a large number of genes (Table 1); activation of some transposons within chromosomes 1, 2, 3, 4 and 7 induced by culture stress (Chomet et al., EMBO J 6:295-302, 1987; Schwartz, PNAS 86, 1989); or epigenetic heredity mechanisms that modify the expression of a large number of homeotic genes (involved in morphogenesis), by modification of methylation patterns.

The mutant phenotypically recalls the genera *Manisuris*, *Andropogonea as* well as *Coix*, *Tripsacum* and even *Triticum* by its flowers, spikelets and seeds, differing basically from the current species, *Zea mays*. The mutant represents a far ancestor of *Zea mays* L. kept latent in the species genome and reactivated through somatic embryogenesis. The appearance of this mutant opens again the scientific hypothesis concerning the origin and phylogenetic dynamics of corn and might provide scientific proof that there was an hermaphroditic ancestor in the origin of *Zea mays*, confirming the hypothesis of Mangelsdorf, Reeves and Galinat. The ancestor would be the starting point for the phylogenetic tree from which the current crop corn has evolved. The species *Zea mays* L. seems to have been developed based on certain sudden macromutations; the morphological-physiological changes incurred became hereditary and entropically preserved.

SOFIA, BULGARIA Institute of Genetics, Bulgarian Academy of Sciences

Seeds with two bilaterally situated embryos and endosperms

-- Dankov, T; Kruleva, M; Krapchev, B

In connection with the study of heterofertilization during 1995 in the crosses (sweet corn X dent with embryo marker + sweet corn) and (sweet corn X line 502 + sweet corn) out of a total of 5832 kernels, except for some ones with typical heterofertilization, two seeds with independent bilaterally situated embryos without marker and their respective normal endosperms had on one hand dent form but on the other hand sweet corn (as if two kernels of sweet corn and dent form are stuck together). An attempt at seed germination and obtaining progeny was made, but we failed. The germs perished while still in petri dishes.

Our explanation is: probably one of the synergids participated in obtaining the second independent embryo. We don't know what part of the embryo sac participated in the second independent endosperm and what kind of nucleus they had. We suppose that two sperms participated in forming two embyos and endosperms. In the first case, it doesn't show if they are from pollen grains of two different pollen parents, and in the second case--it is indisputable.

Increase the percentage of maternal haploids by effect of herbicide basagran

-- Dankov, T; Kruleva, M; Dimitrov, B; Krapchev, B

Genetic lines, which as pollen parents increase the percentage of spontaneously obtained haploids in maize with a frequency of 3.24% (Stock 6) are known (Coe, 1959). In 1993 (MNL) we reported a line obtained by us, named later PX-32hh, which induced 13.88% haploid seeds as a pollen parent. Into the line was transferred our original marker "spot on the embryo".

Cut off tassels from PX-32hh were submerged into 0.1% solution of herbicide basagran (bentason + 2,4DP) for 24h as a

pollen test of the herbicide effect. Tassels submerged in tap water were used as control. On the next day five ears from line B-73 were pollinated. Only treated tassels increased the percentage of the obtained haploid seeds by 4-5%. After electrophoretic analysis it was established that these seeds were female and it concerned maternal haploidy.

We conclude that an effect on the high haploid line by 0.1% solution of herbicide basagran can increase the percentage of ob-

tained maternal haploid seeds.

STANTON, MINNESOTA Novartis Seeds, Inc. ST. PAUL, MINNESOTA University of Minnesota

Differential expression of chitinase in maize kernels

-- Hanten, J; Kendra, D; Mettler, I; Harlander, S; Fulcher, RG

Several different plant species (i.e., corn, peas, wheat, barley, beans, etc.) contain significant levels of chitinases in seed and vegetative tissues (Collinge et al., Plant J 3:31-40, 1993). The physiological role of plant chitinases is uncertain because plants do not possess an endogenous chitin substrate (β-1,4 linked N-acetylglucosamine). However, it has been shown that plant chitinases act as defensive agents responding to environmental stress factors such as chitin containing fungal pathogens and certain abiotic elicitors (Boller, Dev Plant Path 2:391-397, 1993). An assessment of the level of chitinase present in maize kernels at two different stages of maturity was performed in order to establish relative abundance in these tissues.

Maize kernel chitinase was partially purified by 60% ammonium sulfate precipitation from shrunken-2 (sh2) and field corn kernels at 21 days after pollination (dap)-(R3 stage) and at physiological maturity (R6 stage). Comparisons in hydrolytic activity towards chitin substrate were made between R3 and R6 kernels with a colorimetric microtiter plate assay. Significant increases in chitinase activity were apparent in mature sh2 and field corn kernels (Table

Table 1. Chitinase activity in sh2 and field corn kernels. The amount of enzyme yielding 1 mol s-1 of N-acetylglucosamine is defined as a Katal (kat). Each column of data represents the mean values of two replicates of 10 kernel samples. sh2 mature kernels were F1 hybrid seed and 21 dap kernels were progeny of the F1 seed (F2). Field corn kernels at 21 dap and maturity were inbreds.

| Chitinase Specific Activity (µkat/mg sol. protein) | 21 dap - (R3) | Mature - (R6) |
|---|------------------|-------------------|
| Fieldcom | 53.95 (±9.15) | 1355.93 (±17.28) |
| Sweetcorn (sh2) | 39.60 (±8.7) | 525.91 (±4.13) |
| Chilinase Activity (µkat/gm dry weight) | 21 dap - (R3) | Mature - (R6) |
| Fieldcom | 783.30 (±126.16) | 1493.81 (±284.44) |
| Sweetcorn (sh2) | 536.70 (±90.63) | 1211.73 (±254.38) |

These results indicate that chitinase expression increases during maturation of the kernel. Additional studies also provide evidence that chitinase expression levels increase during seed maturation and germination. For example, chitinase mRNA levels are higher in mature kernels as compared to immature embryos (Huynh et al., J Biol Chem 267:6635-6640, 1992). In addition, chitinase expression levels increase dramatically during early germination of soybean seeds (Teichgraber et al., Agro-Food Ind Hi-Tech 3:11-14, 1991). The physiological significance of such differential expression is not completely understood. It is believed that the

regulation of chitinases and a whole host of additional enzymes and secondary metabolites is controlled by internal and external growth signals, contributing to the defense response. Therefore, it is plausible that chitinase expression would increase during kernel maturation and early embryonic development to ensure survival against the numerous pathogens encountered.

> TOLEDO, OHIO University of Toledo COLUMBIA, MISSOURI University of Missouri MUNCIE, INDIANA **Ball State University**

Localization of autonomous pairing sites in maize

--Goldman, SL; Dovle, GG; Blakev, CA

The existence of autonomous pairing sequences (APS) which function in the process of homologous recognition have been postulated by many scientists working to define the mechanism of homologue sorting. Using the convention established by Sybenga (Genetica 37:186, 1966), the term zygomere is used to describe those genetic elements, i.e., nucleotide sequences, specialized to function in the initial phases of pairing. Given this, we are identifying the segments associated with zygomere activity using the technique of Restriction Fragment Length Polymorphism (RFLP) mapping. This is made possible by the fact that trisomic stocks segregating heterologous zygomeres have been mapped to chromosome 3 (Doyle, TAG 79:145, 1990). These autonomous pairing sites have been recognized following changes in preferential pairing and should associate with concomitant alterations in RFLP profiles subsequent to Southern analysis. As a result, an unambiguous linkage should exist between the phenomenon of preferential pairing and the segregation of heterologous zygomeres. In principle, this association is detected when seed lines exhibiting marker segregation distortion cosegregate with (a) defined RFLP(s).

The inbreds B41, Hy, and 38-11 have been shown to be marked with distinctive APSs. Therefore, if the F1 hybrid (standard a1 sh2/inbred A1 Sh2), for example, is crossed to the standard trisome (a1 sh2/a1 sh2/a1 sh2), A1 and Sh2 will segregate from their respective APSs in the progeny. This conclusion is based on the fact that at least one autonomous pairing site and A1 Sh2 region should recombine freely if these synaptic initiator sequences map to the subterminal ends of chromosome 3 as has already been shown. As such, it will be possible to recover chromosomes marked by A1 Sh2 whose pairing kinetics have changed. The location of putative autonomous pairing sites on chromosome 3 has been recognized following changes in preferential pairing, and they associate with concomitant alterations in RFLP profiles subsequent to Southern analysis. We are in the process of analyzing the segregation pattern of each of the 10 RFLP core markers of chromo-

some 3 in lines segregating preferential pairing.

Plants of the inbred lines (B41, Hy, and 38-11) have been crossed with the multiple marked stock noted above. The hybrids obtained will be heterozygous for all gene loci and crossed as the pollen parent with standard trisome 3 which is homozygous for al and sh2 and the other six dominant alleles. These trisome 3 heterozygous (A1 Sh2/a1 sh2/a1 sh2) are crossed as the pollen parent onto a1 sh2 testers to determine the presence or absence of preferential pairing as indicated by changes in the ratios of *A1 Sh2* to *a1 sh2* in the progenies. In addition, each tested trisome is self-fertilized to determine which chromosome was donated by the diploid hybrid parent. In this way, linkages between preferential pairing factors (heterologous zygomeres) and genetic markers can be established. Following the mapping, we will expand these lines in order to obtain greater quantities of fresh material. For the initial study only *A1 Sh2* markers will be used as illustrated in Figure 1.

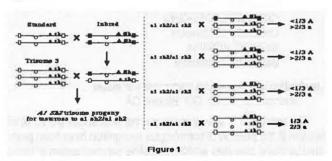


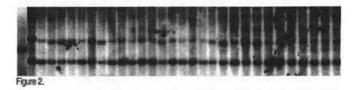
Figure 1 outlines a simplified series of crosses designed to illustrate the correspondence between zygomere activity and preferential pairing. The black and white squares represent zygomeres in different mutational states. For the purpose of discussion their placement is arbitrary. The only difference in the four test crosses represented is expected to be a function of heterologous zygomere expression. This will be determined by showing a correspondence between seed having modified genetic ratios and (a) defined RFLP(s). Specifically, plants from test crosses 1, 2 and 3 that give preferential pairing should segregate different RFLPs than those found in test cross 4 where pairing is at random. The diploid standard/inbred hybrid was also crossed to a diploid tester (a1/a1 or sh2/sh2) as a control to rule out the presence of gametophyte factors, differential viability of gametes or zygotes, or anything that would disturb the genetic ratios. Here, as expected 50% transmission of A1 Sh2 was observed.

DNA from the inbred lines and the standard stocks segregating the chromosome 3 markers as well as the resulting F₁s have been restricted with *EcoR1*, *HindIII* and *EcoRV*. As a first screen, this DNA was probed, in turn, with RFLP markers mapping to the subterminal ends of linkage group 3. These enzymes have been proven to be particularly effective in elucidating polymorphisms in maize in the past (Hoisington and Coe, Gene Manipulation and Plant Improvement, p. 331-352, 1990). This simple test has discriminated the RFLPs of interest as they exist in this region of chromosome 3. Similar results have been obtained with the RFLP markers that span the length of chromosome 3.

Significantly, the material segregating the different zy-gomeres is highly polymorphic based on experiments using three restriction enzymes that include *EcoRV*, *EcoRI*, and *BamHI* and 10 different RFLP makers. Given this, the morphs that are associated with each inbred on a marker by marker basis can be delineated from those segregating in the standard. RFLP markers have been used to screen DNA isolated from inbreds, from standard/inbred hybrids, from trisomes, and from tester lines. Significantly, leaves from approximately 800 plants have already been isolated from the progeny of stocks that express preferential pairing and from the progeny of stocks that do not based on the transmission of *A1 Sh2*. In addition, DNA has already been

isolated from a limited number test cross progeny using the cross standard zygomere/standard zygomere X B41 zygomere/standard zygomere.

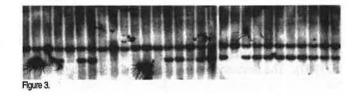
DNA from 67 plants representing three different families where preferential pairing is expressed based on seed marker segregation has been isolated, cut with *EcoRV* and probed for *umc32*. This marker is linked both to the proximal subtelomeric region of chromosome 3 (*umc32a*) and to the centric region of chromosome 8 (*umc32b*). When DNA from the inbred B41 is cut with *EcoRV* a single band is discriminated as is shown in lane 1 of Figure 2. When DNA is isolated from tester-lines segregating standard zygomeres and probed with *umc32* two bands are distinguished as is shown in lane 2. Lanes 3 through 32 show the segregation pattern of B41:standard when DNA is isolated from individual plants obtained from a single ear. In this connection, B41 segregates from standard in a ratio of 0:29. Specifically, the B41 allele is not transmitted and evidence of RFLP segregation is absent.



When data from these 67 plants are pooled, the segregation pattern of B41:standard is also of interest. Specifically, B41 segregates from standard in the ratio 10 to 57. This distribution differs significantly from the expected 1 B41:2 standard when preferential pairing is absent. Differences in degree of preferential pairing could reflect the number of different heterologous zygomeres segregating in a population. Under the assumption that there is a minimum of one zygomere in each chromosome arm, three different populations of individuals expressing distinctive patterns of preferential segregation are possible as is shown in Figure 1.

DNA from these same 67 plants was probed using the marker umc161. umc161 maps to the centric region of chromosome 3. A portion of this data is shown in Figure 3 and corresponds precisely to the plant DNA probed in Figure 2. When B41 is cut with EcoRI and probed with umc161 a single band is discriminated as is shown in lane 1. When the tester lines are probed a single distinctive signal is likewise identified. B41 segregates from standard in a ratio of 18:11 in this family. The presence of both morphs provides evidence that this family constitutes a segregating population. This observation is particularly important, since RFLP segregation was absent when this DNA was probed with umc32. Among the 67 plants scored B41 segregates from standard in a 36:31 ratio.

DNA has also been isolated from plants where preferential pairing is absent. When B41 is cut with *EcoRV* and probed with *umc32*, a single band is discriminated as is shown in lane 2 of Figure 4. When the tester lines segregating standard zygomeres are cut with *EcoRV* two bands are identified as is shown in lane 1.



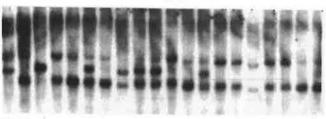


Figure 4.

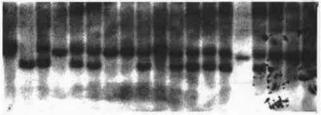


Figure 5.

Lanes 3 through 20 are the segregants of the RFLP variants from the cross a1 sh2 (standard)/a1 sh2 (standard) X A1 Sh2 (B41)/a1 sh2 (standard)/a1 sh2 (standard). In this connection the B41 morph segregates from standard in a ratio of 7 to 10. When this blot is reprobed with umc161 the morphs segregate in a similar manner, see Figure 5. Based on these most preliminary findings the segregation distortion associated with preferential pairing appears to be region specific and linked to the subtelomeric region of chromosome 3. These data lend credence to the hypothesis that if the zygomeres linked to chromosome 3 are marked by an RFLP, it should be possible to map these structures. Specifically, it should be possible to identify the RFLP(s) that co-segregates with preferential pairing and thereby establish the map position of the zygomere.

Work is in progress to demonstrate the expression of zygomere activity cytologically. In principle, trisomes manifest two general pairing modes that include either a bivalent and a univalent or a trivalent. Given this, four types of distinctive trivalents have been discriminated. These include "chains", "frying pans", "triradials", and "birdcages". Since preferential pairing is associated with the inbreds B41, Hy, and 38-11 but not with W23, there should be a reduction in the frequency of trivalents and shifts in the relative frequencies of each of the four types of trivalent. As predicted, the frequency of bivalents plus univalents rises from 38% in the control to 43%, to 49%, and to 47% in 38-11, B41, and Hy respectively. For details see Table 1.

From a study of chromosome pairing in the plant kingdom, it may be concluded that a complex of different mechanisms may function to delineate homologous recognition. For example, the prescriptive global response observed in allohexaploid wheat restricts recognition to intragenomic homologues in the presence of the *Ph* gene (Sears and Okamoto, Proc. X Int. Congr. Genet. 2:258, 1958; Riley et al., J Hered 52:22, 1961). When this gene is deleted, the pairing kinetics change and homeologous associations form leading to a rise in multivalent formation and a concomitant decrease in the frequency of fertile gametes. Formally then, in the absence of *Ph* this alloploid is changed to an autoploid with attendant problems associated with fertility.

Given this, the RFLP mapping of zygomeres in maize will have enormous practical consequences for plant breeders. For example through the manipulation of zygomeres it may prove possible to

Table 1. Effect of preferential pairing on pairing configurations in trisomes.

| | B+ (Bivalent + | | T (Trivalent) | | | | |
|-------------------|-------------------|------------|------------------|------------|-----------|-----|-------|
| | | - | | 0- | 3 | ٨ | Total |
| Std/Std/Std | No. | 43 38.0 | 22 19,5 | 38 33.6 | 9 8.0 | 0,9 | 113 |
| B41/Std/Std | No. | 26 49.1 | 7 13.2 | 11 20.8 | 8 15.1 | 0.9 | 243 |
| Hy/Std/Std | No. | 32 47.1 | 11 16,2 | 19 27.9 | 6 8.8 | 0.0 | 117 |
| 38- 11/Std/Std | No. | 34 43.6 | 17 21.8 | 20 25.6 | 6 7.7 | 1 | 217 |

turn a maize autotetraploid into an allotetraploid. Autotetraploids segregate a high frequency of aneuploids, which decrease the fitness of the populations (Doyle, TAG 54:103, 1979a; TAG 54:161, 1979b; TAG 61:81, 1982; TAG 71:585, 1986). Strictly speaking, an allotetraploid is a true breeding hybrid and would have few aneuploids. This said, considerable progress has already been made on the allotetraploidization of maize through the mutation of zygomeres (Doyle, 1979a, b; 1982; 1986; 1990). Specifically, the sequestering of homeologous chromosomes one from the other in these lines can not be attributed to chromosome aberrations as no cytogenetic anomalies have been observed. When the zygomeres are identified and mapped, restructured lines using conventional breeding protocols, could be synthesized with less difficulty as has been suggested by Sybenga (Euphytica 22:433, 1973), leading to the production of maize allotetraploids.

TUCSON, ARIZONA University of Arizona

Allele-specific PCR in maize

-- Natividad, M; Winkler, RG

Allele-specific PCR is a commonly applied method for the speedy detection of known single-base polymorphisms in DNA utilizing specially designed oligonucleotides. It requires the design of a specific oligonucleotide that selectively amplifies one allele over another. Specificity is achieved by designing the oligonucleotide to match the desired allele but mismatch other alleles near the 3' end of the allele-specific oligonucleotide. The mismatch between the DNA and the oligonucleotide results in specific amplification of the desired allele and little or no amplification of the undesired alleles by preventing elongation at the 3' end by the enzyme.

We and others have found that designing allele-specific oligonucleotides is not trivial. Our work has involved designing allele-specific oligonucleotides for the GC-rich d3 gene in maize. The gene's high GC content makes it inherently predisposed to generate non-specific PCR product. Amplification of the gene produced non-specific banding, making it necessary for us to optimize. After having considered the numerous methods published for increasing the specificity of PCR products, we have realized a new approach to obtaining specific PCR product. We have successfully optimized for the desired point mutation by varying the oligonucleotide, MgCl₂, DNA, and enzyme concentrations simultaneously.

There have been numerous reports suggesting ways of enhanc-

ing the specificity of allele-specific PCR:

It has been found that the use of dimethylsulfoxide (DMSO) (Winship et al., Nucl Acids Res 17:1266, 1989) and nonionic detergents (Bachmann et al., Nucl Acids Res 18:1309, 1990) improves the DNA sequencing reaction possibly by decreasing interand intrastrand reannealing. DMSO has also been found to improve amplification of the retinoblastoma gene (Bookstein et al., Nucl Acids Res 18:1666, 1990). However, DMSO has also been found to reduce DNA synthesis by Taq polymerase by 50% in PCR assays (Gelfand and White, in PCR Protocols--A Guide to Methods and Applications, p. 137, 1990).

Tetramethylammonium chloride (TMAC) is another reagent that is proven to increase PCR specificity (Hung et al., Nucl Acids Res 18:4953, 1990). TMAC has been found to reduce potential DNA/RNA mismatch. Titration studies showed that TMAC used at concentrations of 1X10⁻⁴ M - 1X10⁻⁵M can effectively eliminate non-specific amplification without any inhibitory effects on Taq

polymerase.

The absence of KCl has been reported to be optimal for the amplification of DNA molecules in the range of 3-6 kbp (Ponce et

al., Nucl Acids Res 20:623, 1991).

E.coli single-stranded DNA binding protein (SSB) has been found to facilitate the amplification of genomic sequences by PCR (Oshima, BioTechniques 13:188, 1992). Its stability at temperatures of up to 100 C makes it suitable for use in PCR (Weiner et al., J Biol Chem 25:1972-1980, 1975).

The use of formamide has been reported to improve specificity of PCR products particularly in GC rich genes (Sarkar et al., Nucl Acids Res 18:7465, 1990).

It has also been found that increasing the denaturation temperature for GC rich genes improves specificity (Dutton et al., Nucl Acids Res 21:2953-2954, 1993).

A "touchdown" method for decreasing annealing temperatures improves specificity for some genes (Don et al., Nucl Acids Res 19:4008, 1991).

It has been reported that diluting DNA 10-fold of the standard genomic DNA concentration, lowering $MgCl_2$ concentration below 1.5 mM, and lowering the amount of enzyme to 0.2-0.3 U/25 μ l can increase specificity of PCR product (Bottema et al., Meth

Enzymol 218:388-402, 1994).

All PCR reactions were performed in a final volume of 50 µL containing PCR buffer (50 mM Tris pH 9.0, 250 mM KCl, 0.5% gelatin, 0.5% Triton X-100) (Triton X-100 was later eliminated since it inhibited transfer to membrane; the elimination of Triton X-100 had no effect on the results of the experiments). Original 1X concentrations from which optimizations were performed are as follows: 50 µM dNTP's; 200 ng DNA; 1 µM oligonucleotide; and 0.5 U enzyme /50 µL reaction. PCR reactions were contained in 0.2 mL thin walled tubes. PCR was conducted using a PTC-100 HB Programmable Thermal Controller (MJ Research, Watertown, MA, USA) with the following parameters: initial denaturation at 95 C for 1 min.; and 35 cycles of denaturation at 94 C for 30 s; annealing at 60 C for 30 s; and extension at 72 C for 2 min. PCR products were electrophoresed on 2% IBI Agarose (Eastman Chemical Company, New Haven, CT, USA) in 1X TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA) and analyzed by ethidium staining. PCR product was confirmed by dot blot.

Specific PCR product for the GC rich d3 gene was achieved with DNA (1X), 0.3 mM MgCl₂, 0.25X oligonucleotide concentra

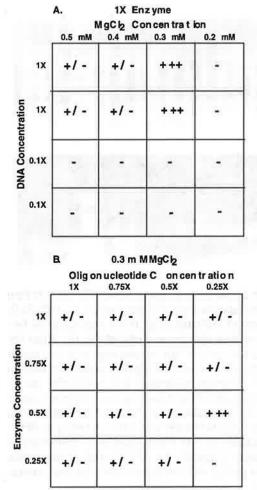


Figure 1. Optimization table used to achieve specific PCR product. A) DNA and MgCl₂ were simultaneously optimized with 1X enzyme in order to identify specific PCR product. B) The optimized DNA and MgCl₂ concentrations were then used to optimize for the proper oligonucleotide and enzyme concentrations that would produce specific PCR product. +++ denotes that product was present and specific; +/- denotes that product was present but not specific; encountered that product was present but not specific; encountered were as follows: 50 μM dNTP's; 200 ng DNA; 1 μM oligonucleotide; 0.5 U enzyme/50 μL reaction.

tion, and 0.5X enzyme concentration. Lowering the DNA concentration by one tenth its original concentration did not produce product. Lowering MgCl₂, oligonucleotide, and enzyme improved specificity, however exceedingly low concentrations did not produce product. Optimizing for each reagent in combination showed some improvement over optimizing for each reagent individually. However, when the conditions were optimized in combination, the effects were not additive or synergistic on increasing specificity.

Initial experiments proved that specificity of PCR product was increased when reagent concentrations were lowered. After optimizing for each reagent and still encountering non specificity, we decided to optimize the reagents simultaneously. Under the assumption that optimizing the reagents would produce specific PCR product, we were surprised to find no additive or synergistic effect on improving specificity. This suggests that optimizing DNA, MgCl₂, oligonucleotide, and enzyme concentrations contribute the same specificity effect possibly through the same or similar mechanisms. In future endeavors we would concentrate on identifying a component that attacks a different mechanism to achieve specific PCR product.

URBANA, ILLINOIS

Maize Genetics Cooperation Stock Center, USDA/ARS and University of Illinois

Bn1 status of selected inbred lines

--Stinard, P

As reported in the 1995 MNL (MNL 69:129-130), the Maize COOP has undertaken a survey of its most commonly used inbred lines to determine which ones carry *Bn1*, a mutation that conditions brown aleurone. This mutant is most easily observed on homozygous *y1* endosperms, and gives them a pale yellow appearance. Homozygous *bn1* on *y1* gives a pure white endosperm appearance. *Bn1* can mask the appearance of certain carotenoid mutants such as *y9*, and for that reason, lines carrying the *bn1* allele are desirable for the propagation of these mutants.

The experiment was set up as follows: Each inbred line studied was crossed to a homozygous *Bn1-Ref y1* line. The F1 was back-crossed by a homozygous *bn1 y1* line. If the inbred line carries *Bn1*, all of the kernels in the *y1* class will be pale yellow (half *Bn1-Ref Bn1-Ref bn1* aleurones and half *Bn1-Inbred Bn1-Inbred bn1* aleurones). If the inbred line is *bn1*, half of the kernels in the *y1* class will be pale yellow (*Bn1-Ref Bn1-Ref bn1* aleurones), and half will be white (*bn1 bn1 bn1* aleurones). The results, presented in Table 1, indicate that the following inbred lines are homozygous for *bn1*: B77, H99, KYS, L317, M14, Mo17, and Q66. The inbred lines A632, A636, B73, B79, Oh43, Q67, W22, and W23 are homozygous for *Bn1*. The *bn1* lines will be used in our breeding program for carotenoid mutants.

Table 1. Counts of pale yellow and white kernels from the cross (inbred line X Bn1 y1) X bn1 y1.

| Inbred Line | pale yellow | white | Bn1 status of inbred | |
|-------------|-------------|-------|----------------------|--|
| A632 | 320 | 0 | Bn1 | |
| A636 | 852 | 0 | Bn1 | |
| B73 | 947 | 0 | Bn1 | |
| B77 | 299 | 312 | bn1 | |
| B79 | 921 | 0 | Bn1 | |
| H99 | 225 | 212 | bn1 | |
| KYS | 167 | 161 | bn1 | |
| L317 | 340 | 343 | bn1 | |
| M14 | 218 | 236 | bn1 | |
| Mo17 | 189 | 192 | bn1 | |
| Oh43 | 537 | 0 | Bn1 | |
| Q66 | 351 | 356 | bn1 | |
| Q67 | 866 | 0 | Bn1 | |
| W22 | 984 | 0 | Bn1 | |
| W23 | 531 | 0 | Bn1 | |
| | | | | |

gl7 and v17 map to the short arm of chromosome 4 --Stinard, P

The COOP's chromosome 4 linkage stock su1 gl7 v17 bm3 was crossed by TB-4Sa and TB-4Lf in order to place the unmapped mutants gl7 and v17 to chromosome arm. Sand bench plantings of kernels from four separate crosses of su1 gl7 v17 bm3 by TB-4Sa segregated for seedlings that were both virescent and glossy. Since the kernels that were planted for these seedling tests were non-sugary, the mutant seedlings could not have been the result of self-contamination. Therefore we conclude that these two mutants are located on the short arm of chromosome 4. Four-point linkage tests of su1, gl7, v17, and bm3 are in progress.

Isolation of a new su3 allele

--Stinard, P

The endosperm mutant su3 conditions a sugary-shrunken en-

dosperm and affects debranching enzyme activity in developing maize endosperms (Oliver Nelson, personal communication). su3 shows reduced frequencies of transmission and expression and possible duplicate factor inheritance, but the genetics of su3 have not been completely worked out yet. The only known alleles of su3 (su3-ref; MNL 66:4-5 and su3-89-1303-18; MNL 68:107-108) have arisen from Mutator populations. If su3 is indeed a duplicate factor pair, it is conceivable that at least some Mutator lines are mutant for one of the factors. We report the isolation of a third su3 allele from a Mutator population.

A single ear segregating for a low frequency of sugary-shrunken kernels was found among a group of self-pollinated ears from a Robertson's *Mutator* population. The sugary-shrunken phenotype of the mutant kernels is similar to that of *su3*, so mutant kernels from this ear, 94-4079-6@, were planted last summer and the resulting plants were crossed with plants homozygous for *su3-ref*. The resulting ears had all sugary-shrunken kernels, indicating allelism. This new allele of *su3* has been designated *su3-94-4079-6*.

The COOP's rd3 is allelic to ct2

--Stinard, P

The COOP has been maintaining a mutant named *rd3* for many years. This reduced plant height mutant conditions shortened internodes and bifurcated ears. This mutant was obtained from Allan Caspar, and first grown by the Maize COOP in 1962. The compact plant mutant *ct2* has a similar phenotype, and crosses were made in 1994 between plants homozygous for the two mutants. F1's were grown in the COOP's 1994 winter nursery, and all progeny had the compact plant phenotype, indicating allelism. Subsequent pedigree searches indicated that *ct2* also originated from Allan Caspar, so the COOP's *rd3* and *ct2* alleles may actually be identical. Until this is resolved, it is suggested that these mutants be kept separate, and that the COOP's *rd3* be redesignated *ct2-rd3*.

It should be noted that *ct2-rd3* is not at all related to the locus more recently named *rd3* by Matz et al. (MNL 65:104-105).

Pale aleurone-Brawn (Pa*-Brawn) is allelic to in1 --Stinard, P

A dominant pale aleurone mutant designated Pale aleurone-Brawn has been maintained by the COOP for many years. It was obtained from R. Brink and first grown by the COOP in 1959. No allelism tests or mapping had been done with it, so we set up allelism tests with the known aleurone color mutants. Tests with a1, a2, c1, c2, r1, bz1, and bz2 proved to be negative. An allelism test with In1 was set up as follows: Plants homozygous for In1-D and Pa*-Brawn were crossed with each other. The F1's were backcrossed by our M14/W22 hybrid ACR line, which is nonmutant at the In1 locus. All of the progeny kernels (n = 1780) were pale, indicating either very tight linkage of the two loci, or more likely, allelism. The inheritance and expression of Pa*-Brawn are very similar to that of In1-D, so it is conceivable that Pa*-Brawn is simply a reisolation of In1-D from another source. Only a molecular analysis will resolve this question. For now, we are redesignating Pa*-Brawn as In1-Brawn.

ws1-Coop is allelic to ws1-Pawnee ws2-Pawnee

--Stinard, P

As reported in the 1995 MNL (MNL 69:130-131), two different lines called ws1 have been maintained by the COOP, ws1-Pawnee ws2-Pawnee, which traces back to a line obtained from E. G. Anderson in 1954, and ws1-Coop ws2-Coop, which was obtained from Charles Burnham and first grown by the COOP in 1953. Both mutants have a white leaf sheath phenotype and show duplicate factor inheritance, but the two mutants were never tested for allelism during the years they were maintained by the COOP. Last summer, plants homozygous for the two mutants were crossed with each other, and the F1 placed in our winter nursery for observation. All of the progeny plants had white sheaths, indicating allelism. Nothing is known about the origin of these two sets of mutants, so it is conceivable that they could both be the same ws1 ws2 mutant alleles described by Kempton (J Hered 12:224-226, 1929). Until this is resolved, the two mutant lines will be maintained separately.

Clarification on ws1, ws2, and g1

--Stinard, P

Some confusion has arisen concerning the relationship between the mutants ws1, ws2, and g1. This confusion arose because a Coop mutant stock that was labeled genotypically as "ws2" (obtained from Charles Burnham in the early 1950s) turned out to be allelic to g1. This allele was subsequently designated g1-ws2 (Stinard, MNL 69:130-131, 1995). The Coop also has stocks that carry the duplicate factor pair ws1 and ws2. The latter ws2 is different from g1-ws2, and should not be confused with it. The ws2 allele in the ws1 ws2 stocks is the authentic duplicate factor of ws1 and is not at all related to, or a duplicate factor of g1. ws1, ws2, and g1 are three distinct loci.

dat is on 10L

--Stinard, P

Little is known about the pale aleurone color mutant da1 (dilute aleurone1), first described by Eyster (J Hered 22:224-225, 1931). Linkage studies reported by Emerson, Beadle, and Fraser (Cornell Univ Agric Exp Stn Mem 180, 1935) place da1 to chromosome 9, but the data are inconsistent. Nothing is known about the interactions of da1 with other aleurone color factors. During the course of propagating the COOP's stocks of da1, it became clear that da1 was not linked to wx1 as was expected (p = .21 according to Emerson et al.). From a two-point coupling backcross linkage test of da1 and wx1, we obtained the following data: 100 Da1 Wx1, 98 da1 wx1, 118 Da1 wx1, and 97 da1 Wx1. This does not differ significantly from a 1:1:1:1 ratio, and indicates that the two mutants are not linked. Similar results were obtained from other

Since da1 did not map where it was expected on chromosome 9, allelism tests were conducted between da1 and other aleurone color factors. Tests with a1, a2, c1, c2, bz1, and bz2 all yielded kernels with full purple aleurone color. However, crosses with r1 gave a range of pale to colorless kernels, indicating possible allelism, albeit with variable expression.

Subsequent crosses of da1 stocks by TB-10L19 yielded ears segregating for small pale and colorless kernels with purple scutellum (putative hypoploid da1 endosperms with hyperploid embryos) as would be expected if da1 is located on 10L.

Reciprocal crosses of da1 with R1-r A1 A2 C1 C2 gave R1 mottling when the da1 stock was used as a female, and full color kernels when da1 was used as a male. Reciprocal crosses made with an R1-scm2 line gave full color kernels whether da1 was used as a female or as a male.

Allelism of da1 and r1 seemed certain except for one anomalous result. When the heterozygous da1 / r1 kernels from the allelism test ears were planted and the resulting plants crossed again by r1, the ears that were obtained segregated for a consistent, but a low percentage of fully colored kernels (around 10%, see Table 1). This percentage seems extraordinarily high to be accounted for by intragenic recombination. Additional crosses are planned to determine the cause of this phenomenon.

Table 1. Counts of full colored (CI) and pale or colorless kernels (cI) on ears from the cross [rf X da1] X r1 and its reciprocal. The differences in percentage of full colored kernels between male and female outcrosses of the heterozygotes are not significant.

| r1 X da1 parent | r1 X da | 1 parent as | male | r1 X da1 parent as female | | | | |
|------------------------------|---------|-------------|------|---------------------------|-------|------|--|--|
| Activities of the Particular | No. CI | No. cl | % CI | No. CI | No. d | % CI | | |
| 96-690-1 | 41 | 375 | 9.9 | 49 | 366 | 11.8 | | |
| 96-690-3 | 58 | 491 | 10.6 | 40 | 482 | 7.7 | | |
| 96-690-6 | 49 | 302 | 14.0 | 59 | 421 | 12.3 | | |
| 96-690-8 | 23 | 258 | 8.2 | 52 | 559 | 8.5 | | |
| 96-690-10 | 47 | 412 | 10.2 | 36 | 468 | 7.1 | | |
| Totals | 218 | 1838 | 10.6 | 236 | 2296 | 9.3 | | |
| | | | | | | | | |

Three-point linkage data for Ig1 gl2 et2 on 2S

--Stinard, P

The results of a three-point linkage test for lg1, gl2, and et2 on chromosome 2 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, the resulting plants self-pollinated, and kernel samples from each self-pollinated ear planted in the sand bench. Seedlings grown from these kernels were scored for lg1, gl2, and the albino seedling phenotype conditioned by et2, which is more reliable to score than kernel etching. The following linkage relationship was established: lg1 -15.4 - gl2 - 19.8 - et2. The lg1 gl2 distance of 15.4 cm is shorter than the value of 22 cM from the current genetic map (possibly due to undetected double crossovers in this interval), but the order of the three genes is clear, and gl2 - et2 distance of 19.8 cm places et2 in the vicinity of b1 on chromosome 2.

Table 1. Three-point linkage data for Ig1 - gl2 - el2. Testcross: Lg1 Gl2 Et2 X (lg1 gl2 Et2 / Lg1 Gl2 et2).

| Reg. | Phenotype | No. | Totals |
|------|-------------|-----|--------|
| 0 | lg1 gl2 + | 105 | |
| | + + et2 | 86 | 191 |
| 1 | lg1 + et2 | 24 | |
| | + gl2 + | 20 | 44 |
| 2 | lg1 gl2 et2 | 27 | |
| | + + + | 30 | 57 |
| 1+2 | lq1 + + | 1 | |
| | + gl2 et2 | 0 | 1 |

% recombination lg1--gl2 = 15.4 \pm 2.1 % recombination gl2--el2 = 19.8 \pm 2.3

% recombination lg1--et2 = 35.2 \pm 2.8

URBANA, ILLINOIS University of Illinois RASTATT, GERMANY SüdWestdeutsche Saatzucht

Effect of different carbohydrates in the corn anther culture medium

--Beaumont, VH; Miao, SH; Widholm, JM

Higher yield was reported when sucrose was replaced with maltose for barley, rye and wheat anther culture. The purpose of our study was to compare different sugars in corn anther culture. HF1, a diplohaploid obtained through anther culture of the cross H99 x FR16, was chosen for this experiment because of its high ability to produce embryos in anther culture (around 50 embryos/100 anthers). Plants were grown in the field (Champaign, Illinois) during the summer of 1993, or in our growth chamber (16 hours of light: intensity 700 mEm⁻²s⁻¹ one meter above the floor, 29 C, 60% RH; 8 hours of darkness, 19 C, 80%RH; fertilization every three days). The anther culture protocol has been described elsewhere (Beaumont et al., MNL66: 114-115, 1992). Each tassel was plated both in 10 ml of the control medium and 10 ml of one of the modified media (30 anthers/Petri dish). Because of this design, no bias existed from the plant to plant variations usually observed in corn tissue culture. About 17 tassels were plated for each treatment. The number of responding plants (%RESP.P:) and the number of embryos produced from 100 anthers plated (E/100A) was recorded.

Table 1. Composition of the different media tested

| Treatment | Composition |
|-----------|--|
| Control | YP medium + 60 g/l sucrose (0.18 M) |
| TRE | YP medium + 66g/l trehalose (0.18 M) |
| TOL | YP medium + 32 g/l mannitol (0.18 M) |
| TRI | YP medium + 88 g/l maltotriose (0.18 M) |
| MAL | YP medium + 63 g/l maltose (0.18 M) |
| ST | YP medium + 30 g/l soluble starch + 32 g/l mannitol (0.18 M) |
| TOL15 | As TOL but 10 ml of control medium was added after 15 days |

The results obtained with the different carbohydrates are presented in Figure 1. No plant responded to produce embryos in the medium containing mannitol, even when complemented with sucrose after two weeks. Since mannitol cannot be metabolised by the cells, the result shows that a source of carbohydrate is needed for the pollen grain to induce division and that the anther tissue cannot supply this carbohydrate.

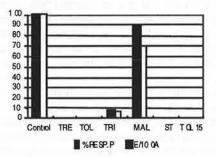


Figure 1. Effect of different carbohydrates on the yield in anther culture. The results are expressed in percentage of the control and are the average of 12 (MAL) to 25 (ST) replications. See Table 1 for the different treatments

Starch (supplemented with 55 g/l mannitol to maintain 0.18 M osmotic pressure in the medium), trehalose and maltotriose when used as unique sources of carbon in the medium, failed to induce the production of embryos from pollen grains. The yield obtained with maltose was close but did not reach that observed with sucrose (control). The results found here suggest that the corn microspore does not react in the same way as microspores of other cereal crops.

The excellent technical assistance of James Hageman, Véronique Villalba and Xiuping Sun is greatly acknowledged. This note is dedicated to the memory of S.H. Miao, a pioneer in corn anther culture.

WALTHAM, MASSACHUSETTS University of Massachusetts

The ${\rm O_2\text{-}CO_2}$ cycle and the development of trait technology for yield enhancement in maize: Umbrella canopy adjacent to the ear sink

-- Galinat, WC

We and our food plants are a small part of a general symbiotic balance within all of life on planet earth. It results from complementation between the hemoglobin of animals and the chlorophyll of plants that allows survival of both by mutually supplying each other's lack of raw materials. This mutual exchange is reflected in every breath of air we take. Simplistically, the hemoglobin in the lungs' capillaries captures the plants' O_2 waste from the chlorophyllic photosynthesis of carbohydrates. The O_2 now fixed in red blood cells then circulates with insulin into a sequence of known reactions involved in cellular metabolism. The animal's body wastes, CO_2 and excrement, are released to planet earth where they then find a way back to the food plants as raw material to make more photosynthetic food.

Success of these symbiotic exchanges depends partly on a population balance between food plants and animals. When people invented agriculture and plant breeding, they allowed a great increase in human populations. In some areas now the symbiosis has become unbalanced as expanding human populations exceed the capacity of the fixed plant populations to feed them and this situation is expected to spread. By increasing the efficiency and productivity of the food plants, we may increase their carrying capacity for human populations.

Perhaps more like the architecture of banana, a new arrangement possibly effecting yield enhancement in maize might be to have the energy sink ear immediately below an umbrella canopy of broad, erect leaves together with a reduced tassel (Fig. 1). This combination of traits was constructed by recombination of umb (umbrella), which is an allele at the *abp* (abnormal phyllotaxy) locus with BI (broad leaves) and es (eye stabber, erect leaves) together with *ub* (unbranched tassel). While all of these traits can behave as single genes, they do occur at compound loci, sometimes called QTL loci, and do interact with modifier genes (suppressors and enhancers). These genes must work together in perfect harmony to build the beautiful maize symphony of the future.

The story of yield enhancement starts with the identification of those precious traits within the total diversity that could contribute to yield. Especially non-agronomic or exotic races must be scrutinized. Such maize that survives, despite severe genetic and/or environmental handicaps and their interplay, would have fil-

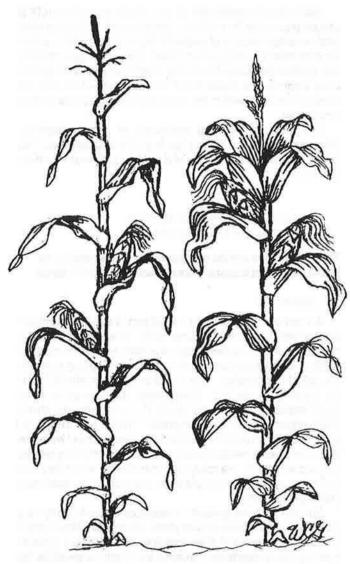


Figure 1. On left, control normal maize. On right, yield enhanced maize with umb, Bl, es, ub, as described in the text.

tered out some powerfully beautiful yield enhancers. The selected enhancers may then be put to important use in the elite backgrounds with the added power of their accumulation through recombination. All of this occurs rapidly under the guidance and direction of the most important people in the world, the plant breeders - the cultural descendants of those who converted the wild ancestor teosinte into the first maize many thousands of years ago in their gardens of more useful forms. Then as now, as each splendid new mutation comes along, the breeder-conductor must recombine it in exquisite harmony with the other melodious mutations in order for the yield of maize to maintain its symbiotic harmony with volume increases in human populations.

Evolution from either natural or domestic selection depends upon two kinds of raw material. First, selection and bringing together the special beautiful mutations that are carried in the total racial diversity and second, selection of the recombination harmonies from interactions between these mutations. Under domestication evolution is more rapid because both kinds of raw material are selected deliberately by the plant breeder from artificially controlled pollinations. The types selected by the breeder may be

adapted "not to the plant's own good, but to man's use or fancy" (Darwin, C. 1859. The Origin of Species by Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life.) In contrast, as Darwin described, in nature progress is slower but over vast expanses of time and the adaptation is to the wild plant's own good by means of selection for intricate specialization for specific natural environments.

The great evolutionary success of mindless, unplanned, trial and error in bringing order out of chaos by natural selection operating over immense vistas of geological time is obvious. But we humans are armed only with dubious, sporadic intelligence and communication skills, and we have only a little time left to conduct the symphony of evolution and the fate of our species on earth and in the universe. Therefore, let us use our minds to plan with the wisdom of forethought to create a beautiful future, a future in which our species smiles on for ever and ever.

Female spikelet architecture, outer glume induration and rachilla elongation

-- Galinat, WC

The first observation of a factor on the short arm of chromosome 4 that would transform the female spikelet-rachis architecture and increase the glume induration of maize into that of teosinte was made by Mangelsdorf and Reeves (Tex Agric Exp Sta Bull 574:1-315, 1939) in terms of their ill-fated hypothesis of an origin of teosinte by Tripsacum introgression into maize. The position as close to the Su-su locus was confirmed by Rogers (Genetics 35:541-558, 1950). Although a research associate of Mangelsdorf at the time over 33 years ago, Galinat (Econ Bot 17:51-59, 1963) made a unique series of drawings, reproduced here in Figure 1, illustrating this transformation only in the reverse direction starting with Tripsacum and teosinte and terminating in modern maize, with intermediates reconstructed from experimental known introgression. Apparently the significance of the sequence concealed in the beauty of my art work escaped Mangelsdorf at the time. Recently a genetic symbol of Tga (teosinte glume architecture) was assigned to this chromosome 4S factor by Dorweiler et al. (Science 262:233-235, 1993). Although useful, the symbol is inaccurate because the change in architecture is the upward orientation of the whole spikelet - not just the outer glume - except for the degree of outer glume induration. Furthermore, the direction of the evolution is now accepted as from teosinte to maize so that I have coined the symbol of msa (maize spikelet architecture) for the recessive mutant allele leading toward maize because the genetic effect of a recessive gene is the traditional basis for coining a new symbol rather than the original dominant wild type represented here by teosinte.

Since all known races of extant maize carry the *msa* allele and all teosinte species the *Tga* allele, this is an important key trait for their separation. From the oldest known archaeological cobs from Tehuacan, Mexico, to modem cobs, the *msa* allele causes the female spikelet to be reflexed down to a right angle position from the rachis, the outer glume to have reduced induration and the rachilla elongate, all of which combine to expose the grain and make it threshable. Rather than by mutation of teosinte's *Tga* allele to maize's *msa* allele, similar phenotypic changes could have been made, in a more unstable manner, by the powerful modifying action upon *Tga* expression of a different gene, an intermediate tunicate allele down on the long arm of chromosome 4 (Beadle, Field Mus Nat Hist Bull 43:2-11, 1972; Mangelsdorf and Galinat, Proc Nat Acad Sci

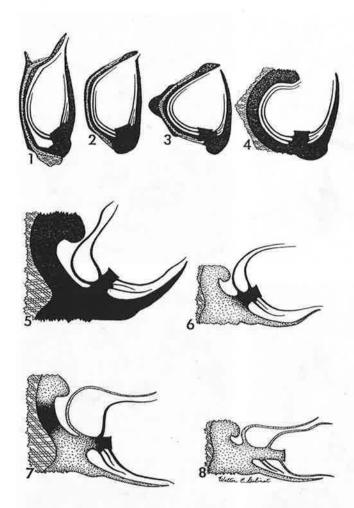


Figure 1. Transitional specimens in longitudinal view extending from *Tripsacum* (1), leosinles (Florida 2, Nobogame 3), a maize-teosinte hybrid (4) to their homologues in modern maize (8) created by experimental introgression of known teosinte segments into inbred A15s modern maize (5,6,7). The *Tga* gene is manifest in specimen 5 while its allele *msa* in specimen 8, as described in the text. Specimen 6 (teosinte chromosome 1) has a reduced rachilla; Specimen 7 (teosinte chromosome 9) an elongate cupule increasing ear length. Solid black indicates teosinte effects. (drawing by W. C. Galinat from Economic Botany 17:51-59, 1963.)

USA 51:147-150, 1964) but this course of evolution now seems to be ruled out in the origin of maize.

WOOSTER, OHIO The College of Wooster LONDON, ONTARIO The University of Western Ontario

Localization of shsp mRNA synthesis in maize spikelets

--Bouchard, RA; Greyson, RI; Walden, DB

As we have shown recently using in situ techniques, heat-induced accumulation of RNA detected by an ORF probe specific for members of the gene family of the small heat-shock protein (shsp) genes of maize is localized to specific cell and tissue-types in the radicle and plumule of seedlings (Greyson et al., Devel Genet 18:244-253, 1996). We are now extending these studies to developing spikelets containing anthers whose microspores are in the meiotic prophase or early uninucleate stages of development, since we have previously demonstrated both developmental and heat shock induction of shsp transcript accumulation in such anthers using RNA-dot hybridization (Bouchard et al., Maydica 38:135-144, 1993).

In these initial experiments, field-grown plants of inbred Ohio 43 were used. The tassels of plants at the appropriate stage were heat shocked by maintaining them between 41 C and 44 C in the field for two hours, using a system of plastic jackets containing heated water; a control tassel was harvested from an untreated sibling of the same stage in the same accession. Upon harvest, tassels were fixed immediately in FAA using vacuum infiltration and shaken overnight, then stored in 70% ethanol at -20 C. Spikelets were dehydrated, embedded, and processed for in situ hybridization as in the seedling study.

Based on our initial observations, vigorous and localized shsp transcript accumulation in cross-sections of heat shocked spikelets is seen in both early prophase and late prophase/early uninucleate anthers. The strongest signal is in the dense cytoplasm of the tapetum, but transcript accumulation also appears to occur in the developing microsporocytes, and also to some degree in the immature vascular bundles of the anthers. Interestingly, when the lower-level developmental accumulation that exists in untreated material is examined using spikelets from the control plant, it appears to be localized to the same regions.

WUHAN, CHINA Wuhan University

Physical location of maize (Zea mays L.) cdc2 and prh1 genes by in situ hybridization

--Ren, N; Song, YC; Bi, XZ; Ding, Y; Liu, LH

A biotin-labeling in situ hybridization technique was used to physically map two single copy genes, cdc2 and prh1 in maize. The product of the cdc2 gene is the p34cdc2 protein kinase (ser/thr kinase), which is believed to be a central component controlling cell division in eukaryotes (Colasanti et al., Proc Natl Acad Sci USA 88:3377-3381, 1991). This protein kinase is a part of the Mphase promoting factor (MPF), which is the key regulation factor of the cell cycle (Nurse, Nature 344:503-507, 1990). The product of the gene prh1 is ser/thr phosphatase (type 1 protein phosphatase, PP1). It is speculated that plant PP1 could be involved in the regulation of a number of cellular processes including mitosis. chromosome separation, transcription, and protein synthesis (Smith et al., Plant Physiol 97:677-683, 1991). The reversible phosphorylation of proteins is a major mechanism by which metabolic and developmental processes are regulated in eukaryotic cells (Cohen et al., J Biol Chem 264:21435-21438, 1989). Therefore, these two genes are metabolically interrelated.

The full-length cDNA clones cdc2 ZmA and ZmPP1 of genes cdc2 and prh1 were adopted as the probes. They are 1.3 and 1.7 kb in size respectively. Clone cdc2 ZmA was physically mapped on the long arms of chromosomes 4, 8, and 9 (Fig. 1 A, B, C). The percent distances from centromere to detection site were 57.87 \pm 2.68, 28.42 \pm 1.45 and 88.16 \pm 3.26 (Table 1). The detection rates were 10.07%, 3.13% and 8.33% respectively. Clone ZmPP1 was physically mapped on the long arms of chromosomes 4, 6 and 8 (Fig. 1 E, F, H). The percent distances were 53.62 \pm 1.17, 60.77 \pm 2.90 and 17.10 \pm 1.61. The detection ratios were 12.07%, 5.17% and 6.17% respectively (Table 1).

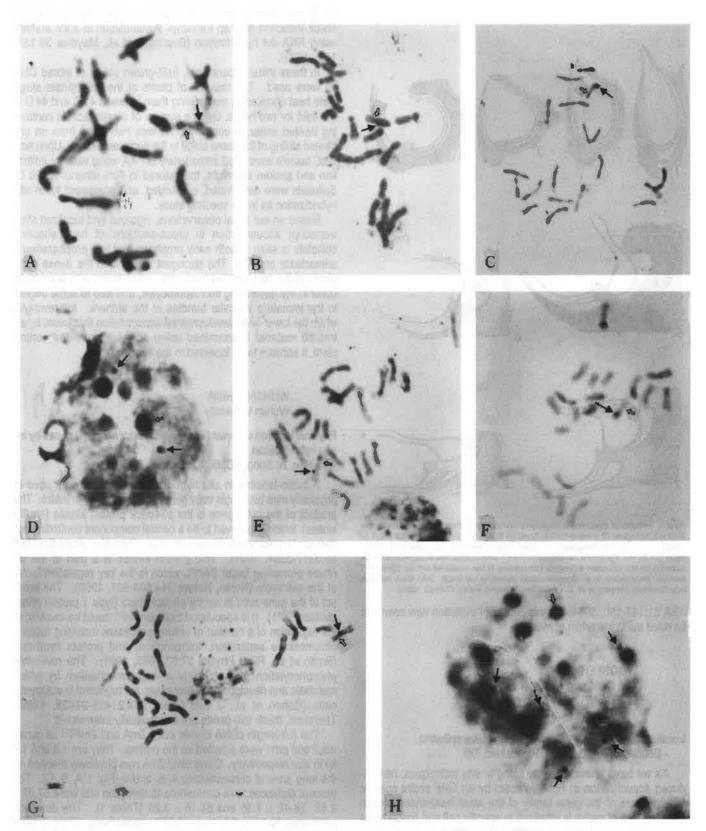


Figure 1. The hybridized sites of the probes cdc2 ZmA and ZmPP1. The black arrows show the hybridization signals in all figures, the white arrows show the chromocenters in d and h, and centromeres in the other figures. (A) The hybridization signal of cdc2 ZmA is in the long arm of chromosome 4. (B) The hybridization signal of cdc2 ZmA is in the long arm of chromosome 8. (C) The hybridization signal of cdc2 ZmA is in the long arm of chromosome 9. (D) The interphase cell has two hybridization signal of ZmPP1 is in the long arm of chromosome 4. (F) The hybridization signal of ZmPP1 is in the long arm of chromosome 8. (H) The interphase cell has five hybridization signals of ZmPP1.

Table 1. Average arm ratios and signal location in the chromosome in the maize genome.

| Probe | Chr | Detections (No.) | Ratio of long to short arm | Signal location (%) |
|-------|-----|------------------|----------------------------|---------------------|
| cdc2 | 4 | 29 | 1.72±0.05 | 57.87±2.68 |
| | 8 | 9 | 2.81 ± 0.07 | 28.42±1.45 |
| | 9 | 24 | 2.01±0.05 | 88.16±3.26 |
| prh1 | 4 | 28 | 1.80±0.07 | 53.62±1.17 |
| | 6 | 12 | 2.70±0.16 | 60.77±2.90 |
| | R | 15 | 2.75+0.10 | 17.10±1.61 |

For the hybridization of the *cdc2* ZmA probe there were differences in detection rates among the different chromosomes. We think there must be differences in homology between the probe and the DNA sequence among different hybridization sites. The greater the degree of homology, the greater the chance that the probes were hybridized onto the chromosomes, and the more tightly the probes combine with the chromosomes. This suggests that the gene *cdc2* is located on chromosomes 4 and 9, and that there is a gene for another protein kinase that has homology with the gene *cdc2*.

The facts that not only the genes cdc2 and prh1 are metabolically interrelated genes, but also that their physical locations are on chromosome 4 and near each other (Fig. 2), lead us to think about the relationship between the location and function of genes on a chromosome. As we know, the homeotic genes of Drosophila in a cluster on a chromosome are arranged in the order in which they are expressed along the body axis (Harding et al., Science 229:1236-1242, 1985; Akam, Development 101:1-22, 1987) and it has been proved by Blumenthal et al. that there may also be operon structure in eukaryotes. They reported that the nematode, Caenorhabditis elegans, adopted both the prokaryotic and the eukaryotic patterns of gene organization and transcription and at least a quarter of the genes seem to be organized into operons (Zorio et al., Nature 372:270-272, 1994). Whether the fact that the genes cdc2 and prh1 are linked closely represents the general trend that metabolically interrelated genes in eukaryotes, including plants, have an operon structure or an organization that resembles operons or not, is a significant problem which remains to be studied further.

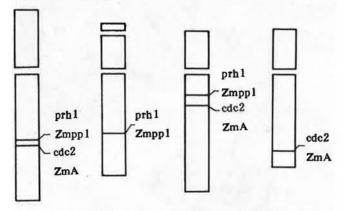


Figure 2. Ideograms of hybridization sites of cdc2 ZmA and prh1 ZmPP1 probes.

WUHAN, CHINA HuaZhong Agricultural University XIAMEN, CHINA XiaMen University

RFLP analysis of maize mtDNA using important function genes as probes

--Jiang, YH; Zhang, FD; Xia, T; Zheng, YL

Cytoplasmic male sterility (CMS) in maize has been classified into three groups (T, C and S) based on the differential restoration by nuclear fertility restorer genes. Unique chimeric genes in mitochondria are always thought to be responsible for the male sterility phenotype. *T-urf13* and *atp6-C* are postulated to be the causes of CMS-T and CMS-C correspondingly and they are both chimeric genes: *T-urf13* is rearranged in *rrn26* and an unknown nucleotide sequence; *atp6-C* is a triple gene fusion product comprised of DNAs derived from *atp9*, *atp6* and an open reading frame of unknown origin. CMS-S is the most sufficient cytoplasm in CMS of maize, but there is less knowledge whether it is due to chimeric genes.

To identify mitochondrial genes that are potentially rearranged with respect to the N cytoplasm, RFLP analysis was conducted using available clones of mitochondrial genes which have important functions. The mtDNAs from 18 kinds of maize including N (normal) and CMS-T, C, S were digested with HindIII, BamHI, EcoRI, Pstl and hybridized with mitochondrial gene probes: coxl, coxII, cob, atp6, atp9, atp-alpha, rrn26, rrn5-18. All of these genes are frequently involved in chimeric genes according to the results from rice, rapeseed, sorghum, sunflower, etc. RFLP maps show abundant polymorphisms among different cytoplasms. Analysis of data suggests that there may be two copies of atp-alpha in N and CMS-S, one copy from CMS-S may be the same as that from N, but the others display significant differences. CMS-C and CMS-T mitochondria only possess one copy of atp-alpha. When mtDNAs were digested with HindIII and hybridized with cob. special bands about 4.4kb appeared in CMS-S (Figure 1). In coxl/HindIII, bands about 4.4kb also were found specially in CMS-S (Figure 2). These three genes: coxl, cob, atp-alpha, were assumed to be related with CMS-S. atp6 gene was reported to be involved in CMS-C. It is interesting that variance was observed among the CMS-C group in atp6/EcoRI combination (Figure 3). This provides a probable way to elucidate the nature of subgrouping.

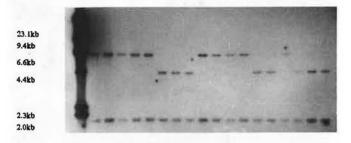


Figure 1. RFLP maps of cob/H/ndill. From left to right the order is: Mo17-N, T group (T), C group (RB, EL, C), S group (TANGXU, SHUANG, J) and 77-N, T group (T), C group (EL, C), S group (VG, TANGXU, SHUANG, JANG, S, RJIN). DNA markers lambda/H/ndill are located at the left of the photo. The arrows show the 4.4kb bands in S group and the 6.5kb bands in the other cytoplasms.

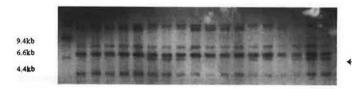


Figure 2. RFLP map of coxf/Hindill. The arrow shows the special bands about 4.4kb in S group.

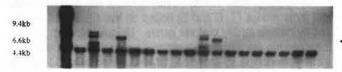


Figure 3. The arrow shows bands in Mo17-EL and 77-EL among C group in atp6/EcoRI combination.

ZARIA, NIGERIA Ahmadu Bello University

Induced chlorophyll deficient mutants in maize

--Adamu, A. Kasim

Air dried seed of an inbred line of corn ($Zea\ mays\ L$.) was irradiated with a gamma cell 220 cobalt 60 (^{60}Co) source, at 10 to 400 Gray, and thermal neutrons (flux 1.5x10⁻⁴ n.cm⁻² S⁻¹) for 25 minutes to 12 hrs. The irradiated seeds were sown in the field and pots in a complete randomized block design (CRBD) with four replications.

Among the dominant seedling mutants are sectors of chlorophyll mutants in M1 and M2 plants. Most of these mutant characters were lethal or unfavourable to growth. The type of chlorophyll deficient mutants found are albina, xantha, virescent, lutescent, chlorina (Fig. 1) and striata. Among the chlorophyll deficient mutants albina and xantha were rare, while lutescent and virescent were the most frequent. In the M1 generation, of the total number



Figure 1. Chlorophyll mutant in maize (i.e. chlorina).

of mutants observed, 2.73% were albina, 10.38% xantha, 22.95% lutescent, 34.42% chlorina, and 7.38% were striate. However, in the M2 generation, of the number obtained 5.11% were albina, 9.85% xantha, 12.41% lutescent, 44.16% virescent, 16.42% chlorina, and 12.05% striata. When the two mutagens are compared in both the M1 and M2 generation, gamma rays produced higher chlorophyll deficient mutants in the M1 generation than thermal neutrons, while in the M2 generation thermal neutrons produced more chlorophyll deficient mutants than gamma rays cobalt 60 source.

Induced tiller formation in maize

Adamu, A. Kasim

Air dried seed of an inbred line of corn (*Zea mays* L.) was irradiated with gamma rays from a cobalt 60 (⁶⁰Co) source, and thermal neutrons (Flux 1.5x10⁴ n.cm² S⁻¹) at different doses. The irradiated seed were planted in the field and pots in a complete randomized block design (CRBD) with four (4) replications.

One of the partially dominant mutants observed, resulting from gamma rays (⁶⁰Co) and thermal neutrons, was the formation of tillers. The result demonstrated variability in the relative frequency of the number of tillers formed following treatment with different doses of the same and different mutagens. Plants observed were classified according to the number of tillers produced, namely one tiller, two tillers, three tillers (Fig. 1) and more than three tillers (Fig. 2). The number of tillers increases with the dose in both gamma rays and thermal neutrons, with the highest percentages of 40% and 17.33% obtained at the highest doses of gamma rays and thermal neutrons respectively. However, less than 1% of the tillers formed in the M1 generation were recovered in the M2 generation.



Figure 1. An induced tiller mutant with 2-3 tillers.



Figure 2. An induced tiller mutant with more than three (3) tillers.

From the Columbia Missourian, 25 Aug 96

From an article by Walter Nicholls, Washington Post, re rise in butter prices -

"...the price of grain - that's relevant," says Alan Levitt, a senior analyst for the Jerry Dryer Group, Chicago-based marketing consultant to the food and dairy industry. "When it comes right down to it, milk is liquid grain," he says.

"It's not just butter," says dairy economist Mary Ledman. "The entire dairy industry is driven by the production of corn and soybeans."

III. USING MAIZE IN K-12 CLASSROOMS

Below are four submissions for ways that maize has be used in the K-12 classroom. Thanks to everyone who submitted their ideas and activities!

Your ideas and activities are needed for future editions. Our plan is to have this section be a regular part of the maize newsletter. If you have visited classrooms, worked with teachers, or in any other way used maize in the K-12 classroom, your colleagues would love to hear about it. Also, if you make use of ideas submitted in the newsletter, we would like to hear about it.

Vicki Chandler will act as the occordinator to assemble this section. Your ideas and reports can be submitted via email, FAX, diskette, or old-fashioned mail. It would be best to send the information by February 1st each year. However, material can be submitted anytime during the year.

Vicki Chandler (after May 1, 1997) Dept. of Plant Sciences 303 Forbes Hall University of Arizona Tucson, AZ 85721 (520) 626-8725 phone (520) 621-7186 fax email: chandler@ag.arizona.edu

Providing maize ears for local high schools and junior colleges

--Vicki Chandler, University of Oregon, Eugene, Oregon; After May 1, 1997: Dept. of Plant Sciences, 303 Forbes Hall, University of Arizona, Tucson, AZ 85721, chandler@ag.arizona.edu

In Oregon, as in many states, budgets for education are being slashed. This makes it very difficult for high schools to purchase maize ears from supply companies to use for genetic demonstrations. However, most high school and junior college teachers that I have interacted with, would very much like to use maize ears to demonstrate genetic concepts. Each field season, I have tried to generate several hundred ears for use in the classroom. This is easy to do using the large variety of linked anthocyanin and starch synthesis genes. Typically, I will do a few self pollinations and outcrosses to generate 1:1, 3:1, 9:3:3:1 ears. One can use a1 sh2 and bz1 sh1 stocks to demonstrate linkage as well. The teachers have made excellent use of ears with transposons. I like to use P-vv (Ac in the P locus) with a Ds in an anthocyanin gene (B-Peru or R). This genotype results in stripes in the pericarp and spots on the seeds. Reciprocal crosses can be used to demonstrate maternal inheritance, and ear sectors can be used to discuss clonal analyses and development. Since the maize ears last for quite a long time, the donation will be used for many years and by numerous stu-

Using kernel color in maize to explore Mendelian genetics

--Ken Kubo and Nicholas Burger, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, 520-626-4664, http://biotech.biology.arizona.edu, email: kmkubo@U.Arizona.EDU

In order to promote hands-on inquiry into the concepts of Mendelian genetics, we have developed a maize genetics activity for the high school classroom. We are currently using this in Tucson, AZ classrooms. In this activity students investigate the inheritance patterns of two alleles of the b gene, which regulates the synthesis of anthocyanin pigments in maize. One of the b alleles, B-Peru, produces dark purple kernels, whereas the second allele, B', produces very little or no detectable kernel pigment. By observing kernel color through several generations in maize pedi-

grees, students can explore how physical characteristics are inherited.

The students examine ears from *B-Peru* and *B'* maize stocks and record their observations regarding kernel color. The students then use their observations to analyze pedigrees of *B-Peru* and *B'*, in which the two maize stocks were intercrossed through controlled pollinations and resulting ears saved for analysis. In one pedigree, the *B-Peru* and *B'* maize stocks were cross-pollinated, producing ears that contain all dark purple kernels. In the next generation, plants grown from these dark kernels (F1) were crossed with the original *B'* parental stock. The resulting ears contain dark and light kernels in a 1:1 ratio. In another lineage, plants grown the F1 kernels were intercrossed, producing ears with dark and light kernels in a 3:1 ratio.

Working in groups, the students count the number of kernels of each color on the maize ears from the pedigrees. After determining the color frequencies on the maize ears, the students are asked to explain how these patterns (1:1 and 3:1) arise. As the students generate alternative explanations and test some of them using the pedigrees, the teacher can introduce several principles of Mendelian genetics, and the students can apply them to the pedigrees. Students learn how to integrate their understanding of genetic concepts such as genes, gametes, dominant and recessive traits, and independent assortment.

Students have responded very positively in their evaluations of this activity, especially since maize provides a visually appealing demonstration of inheritance patterns. They also appreciate how their understanding of maize genetics can be applied to the inheritance of human genetic diseases, such as cystic fibrosis and Tay-Sachs disease.

Developmental experiments for 6th, 7th and 8th grade classrooms
--Sarah Hake, maizesh@nature.Berkeley.EDU, Plant Gene

Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA

A) Epidermal impressions of maize leaves using superglue.

This easy and cheap experiment demonstrates how maize cells are in files, the presence of different cell types, including stomates, and differences between blade and sheath. It is good to

do the experiment in comparison with some dicot leaves such as mint.

1) drop of glue on slide

2) place a piece of leaf on glue

- 3) wait a few minutes until glue dries, peel off leaf
- 4) examine under microscope

B) Pollen tube growth.

I have plated fresh pollen on media to examine pollen tube growth. In vitro pollen germination is described by D.B. Walden in The Maize Handbook.

C) Genetic segregation of Adh1 nulls.

This experiment can be used to demonstrate segregation ratios, gene activity and protein function. Each student has a few seeds from an ear that segregates for an *Adh1* null and they slice a piece of the scutellum into a microtiter dish. They need to keep track of seed and slice. If they hold the seed in pliers, they don't run the risk of hurting themselves. A drop of *adh1* activity stain is added to the microtiter dish and the dish is placed in the dark for 10 minutes. The appearance of the blue color is close to a miracle for most of the students. One of the controls can be absence of alcohol in the stain mixture. I have strains in which *adh1* is linked to albinism. These seeds can then be planted, demonstrating genetic linkage.

Growth response of maize root seedlings under varying temperature conditions: the use in teaching upper grade students

--Lyudmila Makarova, Yuri Konstantinov, Siberian Institute of Plant Physiology & Biochemistry, P.B. Box 1243, Irkutsk, 664033, RUSSIA, Tel: (39-52)-46-07-21, FAX: (39-52)-31-07-54. E-mail: konst@sifibr.irkutsk.su

In the course of long-term studies the workers of our institute have found some phenomena, which make it possible to compare the growth response of different maize genotypes to temperature. We would like to propose demonstration experiments to assess the temperature response of elongating cells of different genotypes in teaching upper grade students.

Maize seedlings grown in cuvettes on filter paper soaked with tap water are taken for comparison. The cuvette should be lightproof and tightly closed from above with a plastic lightproof cap.

The procedure of growing initial seedlings starts by placing presoaked seeds on filter paper dampened by tap water. The cuvette containing these seeds is kept at room temperature for germination to start. In two days the seedling roots are marked with India ink at two points at a distance of 2 and 4 cm from the root tip. No less than 30 seedlings should be used in each treatment. It is the root segment between these two marks that contain elongating cells. The seedlings are divided into two groups, placed into two cuvettes on filter paper dampened with tap water and covered with caps. The first cuvette is kept at room temperature and the other one is put into a household refrigerator (+8 -+10 C). The distance between the two marked points is measured at regular intervals. The seedlings growing at room temperature are measured every hour for 6-8 hours until ceasing growth of the marked region. The seedlings growing in the refrigerator should be measured every 12-24 hour for 2-4 days also until ceasing growth of the marked region. These measurements, the number of which should be not less than three, are easy to perform with construction paper. The average growth rate expressed in mm/hr is calculated for all seedlings in each treatment using the distance between the marks.

Thus, by determining growth rates of the marked region in these two treatments one can compare the root growth response to the effect of low temperature. The correlation between growth rate and temperature can then be compared in different varieties of maize. We have observed a genotype dependent 6-12-fold difference in reduction of rootgrowth rate response. A similar experiment can also be used to demonstrate genotype responses to the effect of other environmental factors (high temperature, salt and drought resistance, etc.).

IV. ADDRESS LIST

```
Abedon, Bruce; Dept of Agronomy; 1575 Linden Dr.; Univ of Wisconsin; Madison WI 53706; 608-262-3660; Fax: 608-262-5217; bgabedon@students.wisc.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; Fax: 234 69 50891
       Agrama, Hesham A; Agricultural Research Centre; 42 Memphis Street; Camp Caesar; Alexandria 21321; EGYPT; 203-59-70525; Fax: 203-59-63042;
     Augrama@rusys.eg.net
Ainsworth, Thomas C.; Ainsworth Seed Company; Mason City IL 62664; 217-482-3281; Fax: 217-482-3283; CORNDOCTOR@AOL.COM
Akamatsu, Toyokazu; Sakata Seed Corp; Plant Biotechnology Center; 358 Uchikoshi Sodegaura City Chiba; JAPAN; 0438-75-2369; Fax: 0438-75-2594
Albertsen, Marc C.; Pioneer Hi-Bred International; 7250 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1731; 515-270-3648; Fax: 515-253-2149;
ALBERTSNM@PHIBRED.COM
    Alexander, Danny; Ciba Agricultural Biotech.; 3054 Cornwallis Rd.; Research Triangle Park NC 27709-2257
Alexander, Larry; Mycogen Plant Sciences; 720 St. Croix St.; Prescott WI 54021
Alfenito, Mark R.; Dept Biol Science; Stanford Univ; Stanford CA 94305-5020; 415-723-2609; Fax: 415-725-8221; alfenito@leland.stanford.edu
Alleman, Mary; 253 Mellon Hall; Duquesne University; Pittsburgh PA 15282; 412-434-1660; Fax: 412-434-5907; ALLEMAN@DUQ3.CC.DUQ.EDU
Allen, Jim; 235 Jordan Hall; Indiana University; Bloomington IN 47405
Altosaar, Illimar; Department of Biochemistry; University of Ottawa; 40 Marie Curie Private; Ottawa, Ontario K1N 6N5; CANADA; 1-613-562-5800 Ext. 6374; Fax: 1-613-562-5100; etc. Canada, C
 Altosan; Illimar; Department of Biochemistry; University of Ottawa; 40 Marie Curie Private; Ottawa, Ontario K1N 6N5; CANADA; 1-613-562-5800 Ext. 6374; Fax: 1-613-562-5180; altosaar@oreo.chem.uottawa.ca
Alvey, David; 237 Myrtle Drive; W. Lafayette IN 47906; 317-567-2115; Fax: 317-567-4046
Amano, Etsuo; Joint FAO/IAEA Division, IAEA; Wagramerstrasse 5; P.O. Box 200; A-1400 Vienna; AUSTRIA; 43 1 2360 ext. 1616; Fax: (43)-1-234564
Ananiev, Evgenii; Academy of Sciences; N.I. Vavilov Inst. Gen. Genet; Gubkin Street 3; Moscow; RUSSIA; anani001@gold.tc.umn.edu
Andersen, Tim; 7300 NW 62nd Ave; Box 129; Johnston IA 50131-0129; 515-276-6541; Fax: 515-270-4312; andersent@phibred.com
Armstrong, Charles L.; Monsanto, GG4H; 700 Chesterfield Parkway North; St. Louis MO 63198; 314-537-7229; Fax: 314-537-6567; clarms@ccmail.monsanto.com
Armold, Jerry M.; Asgrow Seed Co.; 9866 Highway 66; Newburgh IN 47630
Arpat, Hacimurat; Ohio State University; OARDC, Dept of Agronomy; 1680 Madison Avenue; Wooster OH 44691-4096; 216-263-2882; Fax: 216-263-3658
Arruda, Paulo; Univ. Estadual de Campinas; Cidade Univ. Zeferino Vaz; Distrito de Barao Geraldo; Campinas - SP; BRAZIL; (192)398351; Fax: (192)394717
Arthur, Lane; Genetics Dept.; Univ. of Georgia; Athens GA 30602; (706)542-1857; arthur@dogwood.botany.uga.edu
Artim-Moore, Lori; Ciba-Ag Biotechn; PO Box 12257; Research Triangle Park NC 27709
Astwood, James D.; Dept. of Plant Science; Faculty of Agriculture; University of Manitoba; Winnipeg, Manitoba R3T 2N2; CANADA; 204-474-6064; Fax: 204-275-5128; Atanassov, A.; Institute of Genetic Engineering; 2232 Kostinbrod 2; BULGARIA; +359.0721.2552; Fax: +359.721.4985; atanos@icgeb.trieste.it
Attewell, John; 2166 Forrest Green Drive; Decatur IL 62621; (217)422-8146
Auger, Donald L.; Biol Sci; Tucker Hall; Univ Missouri; Columbia Mo 65211; idauger@biosci.mbp.missouri.edu
Auyeung, Man Ting; Pioneer Overseas Corporation; 2729-1 Kohyama, Matsubase Machi; Shimomashiki-Gun; Kumamoto Pref, 869-05; JAPAN; 964-33-7666; Fax: 964-33-1677
   Ayala-Osuna, Juan; Rua Dos Trabalhadores 906; 14870.000 Jaboticabal-SP; BRAZIL; 016-3220260; Fax: 016-3224275
Aycock, Harold S; 305 Montefiore St, #305; Lafayette IN 47905-5519; 317-477-7758
Ayers, John E; Dept Plant Pathology; 308 Buckhout Lab; Pennsylvania State Univ; University Park PA 16802; 814-865-7776; Fax: 814-863-7217; jea@psu.edu
Azanza, Fermin; 12 Chemin de L'Hobit; B.P. 27; Hilleshog NK; 31790 Saint-Sauveur; FRANCE; (+33) 61-37-68-09; Fax: (+33) 61-37-38-83;
   fermin.azanza.gwa.sandoz.com

Badenhorst, C; Salfola Box 36; Boskop 2528; SOUTH AFRICA

Bai, Yongyan; Inst. of Plant Physiol.; 300 Fengling Road; Shanghai 200032; CHINA

Bailey-Serres, Julia; Botany & Plant Sciences; Batchelor Hall; Univ. Calif. Riverside; Riverside CA 92521; (714)787-3738; Fax: (714)787-4437; SERRES@UCRAC1.UCR.EDU
     Bairoch, Amos; Centre Medicale Universitaire; 1211 Geneva 4; Geneva; SWITZERLAND; 41-22-784-40-82; BAIROCH@cmu.unige.ch
    Baker, Raymond; 3524 Grand Avenue, Apt. 208; Des Moines IA 50312; 515-271-6803
Baker, Barbara; USDA; Plant Gene Expression Center; 800 Buchanan Street; Albany CA 94710; 510-559-5912
Baker, Barbara; USDA; Plant Gene Expression Center; 800 Buchanan Street; Albany CA 94710; 510-559-5912
Baker, Frank; Plant Biology Dept.; UC Berkeley; 111 Koshland Hall; Berkeley CA 94720-3102
Balconi, C.; Ist Sper Cereal; Via Stezzano 24; 24100 Bergamo; ITALY
Ball, Dale W.; Wilson Seeds, Inc.; P.O. Box 391; Harlan IA 51537; 712-755-3841; Fax: 712-755-5261; dballmaize@aol.com
Banuett, F.; Dept Biochem Biophys; Univ of California; San Francisco CA 94143-0448; 415-476-4985; Fax: 415-476-0943
Bar-Zur, Avri; Moshava; P.O. Box 32; Yokneam 20600; ISRAEL; 972-4-9590854; Fax: 972-4-9894026; abar@cramel.zvulun.k12.ll
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; 503-346-5145; Fax: 503-346-5891; abarkan@molbio.uoregon.edu
Barker, Tom; Pioneer Hi-Bred Internat., Inc.; Plant Breeding Division; RR1, Box 90A; Princeton IN 47670; 812-385-2935; Fax: 812-385-8502; barkertc@phibred.com
Barnes, Wayne M.; Dept. Biochemistry 8231; Washington University Medical School; 660 South Euclid Ave.; St. Louis MO 63110
Barros, MEC; PO Box 395; Pretoria, 0001; SOUTH AFRICA; 27-12-841-3221
Baskin, Tobias; 109 Tucker Hall; Div. Biol. Sci.; University of Missouri; Columbia MO 65211; 573-882-0173; baskin@biosci.mbp.missouri.edu
Bass, Hank W.; Dept Molecular and Cell Biology; 345 LSA; Univ California-Berkeley; Berkeley CA 94720; 510-643-8277; Fax: 510-643-6791; bass@candelab.berkeley.edu
Baszczynski, C. L.; Pioneer Hi-Bred International, Inc.; 7300 N.W. 62nd Avenue; P.O. Box 1004; Johnston IA 50131-1004; (515)270-3693; Fax: (515)270-3367; BASZCZYNSKI@PHIBRED.COM
Bates, Lynn S.; Alteca Ltd.; 731 McCall Road; Manhattan KS 66502; 913-537-9773; Fax: 913-537-1800
  Bayliss, Michael W.; ICI Seeds; 2369 330th St.; P.O. Box 500; Slater IA 50244; 515-685-3574; Fax: 515-685-2548
Baysdorfer, Chris; Biological Sciences; California State U; Hayward CA 94542; 510-885-3459; Fax: 510-888-4747; 72652.662@CompuServe.COM
Beach, Larry; Pioneer Hi-Bred Internall; Specialty Grain Traits; PO Box 1004, 7300 NW 62nd Ave; Johnston IA 50131-9410; 515-270-3798; Fax: 515-270-3367; beachl@phibred.com
  Beavis, W. D.; Pioneer Hi-Bred International; 7300 NW 62nd Ave.; P.O. Box 129; Johnston IA 50131-0129; 515-270-3618; Fax: 515-270-4312; beavis@phibred.com Beckett, M.; INRA Station D'Amelioration des Plantes; 63039 Clermont Ferrand; FRANCE; (33)73 624319; Fax: (33)73 624453 Beckett, Jack; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2674 Becraft, Philip; Zoology & Genetics/Agronomy Depts; 2116 Molecular Biology Bldg; Iowa State University; Ames IA 50011; 515-294-2903; Fax: 515-294-0345; becraft@iastate.edu
    Bedinger, Patricia; Biology Dept; Colorado State Univ.; ; Fort Collins CO 80523; 303-491-2879; Fax: 303-491-0649; bedinger@lamar.colostate.edu Behr, Fritz; 222 W. Pearl St.; Geneseo IL 61254; (309)944-6500
   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; Fax: (517)486-2631
Benito, Maria-Ines; Biology Dept.; Herrin Bldg.; Stanford University; Stanford CA 94305-5020
Benner, Michael; Science and Technology Center; Rider University; Lawrenceville NJ 08648-3099; 609-896-5096; Fax: 609-895-5782; benner@enigma.rider.edu
Bennett, Michael; Jodrell Laboratory; Royal Botanic Gardens; Kew, Richmond; Surrey, TW9 3AB; UNITED KINGDOM; 44-1181-332-5000; Fax: 44-1181-332-5310;
                     M.Bennett@rbgkew.org.uk
    Bennetzen, Jeff; Dept of Biological Sciences; Purdue University; W. Lafayette IN 47906; 317-494-4763; Fax: 317-496-1496; 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; Fax: 515-253-2149; BENSENRJ@PHIBRED.COM
```

```
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
Bergstrom, Gary C.; Dept. Plant Pathology; 316 Plant Science Bldg.; Cornell University; Ithaca NY 14853-5908; 607-255-7849; Fax: 607-255-4471; gcb3@cornell.edu
Bergvinson, David; CIMMYT; Apdo. Postal 6-641; 06600 Mexico, DF; MEXICO; 595-544-00; Fax: 595-544-25; DBERGVINSON@ALPHAC.CIMMYT.MX
Berke, Terry; Box 42; Shanhua; Tainan, Taiwan 741; REPUBLIC OF CHINA; 8866 583 7801; Fax: 8866 583 0009; TERRY@NETRA.AVRDC.ORG.TW
Berlyn, Mary; Dept. of Biology; Yale University; New Haven CT 06520; 203-432-3365; Fax: 203-432-33854; mary@fetalpig.biology.yale.edu
Bernardo, Rex; Limagrain Genetics; 4805 West Old Chruch Rd.; Champaign IL 61821; (217)398-8968; Fax: (217)398-8976; bernardo@prairienet.org
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 67 61 74 65; Fax: 33 67 54 89 00;
BERTHAUD@ORSTOM.FR
   BERTHAUD@ORSTOM.FR
Bertrand, Ralph L.; 1040 N. Nevada St.; Dept. of Biology; Colorado College; Colorado Springs CO 80903; (719)389-6402; Fax: (719)389-6940
Berville, A.; INRA; Station d'Amelior des Plantes; 2 Place Viala; 34060 Montpellier Cedex; FRANCE
Bhaltramakki, Dinakar; 348 Manter Hall; PO Box 880118; University of Nebraska; Lincoln NE 68588-0118; 402-472-6243; Fax: 402-472-2083; ddh@unlinfo.unl.edu
Bianchi, Angelo; Ist Sper Cerealicoltura; Via Cassia 176; 00191 Rome; ITALY; 06-3295705; Fax: 06-3630-6022
Bigwood, Doug; National Agricultural Library; 10301 Baltimore Blvd; Beltsville MD 20705; 301-504-6689; Fax: 301-504-7473; dbigwood@gig.usda.gov
Biradar, Madhuri; 320 ERML, Crop Sci; Univ. of Illinios; 1201 W. Gregory; Urbana IL 61801
Birchler, James; Biological Sciences; Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-4905; Fax: 573-882-0123; birchler@biosci.mbp.missouri.edu
Bird, Robert; CIMMYT; Lisboa 27, Apdo Postal 6-641; CP06600; Mexico, D.F.; MEXICO; 415-833-6655 ext. 1392; Fax: 415-833-6656; rbird@CIMMYT.MX
Bjamason, Magni; Semences Cargill; IM Aufeld 5; D-77815 Buehl; GERMANY; +49-7227-5691; Fax: +49-7227-5691
Blakey, Cynthia Ann; Cooper Science Bldg; Dept. of Biology; Ball State University; Muncie IN 47306; 317-285-8820; Fax: 317-285-2351; 01CABLAKEY@BSUVC.BSU.EDU
Blewitt. Michael: Brookhayen National Lab: Bldg. 463: Upton NY 11973
Blakey, Cynthia Ann; Cooper Science Bldg; Dept. of Biology; Ball State University; Muncie IN 47306; 317-285-8820; Fax: 317-285-2351; 01CABLAKEY@BSUVC.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
Bodeau, John; Dept Pomology; UC-Davis; Davis CA 95616; Fax: (916)752-8502
Bogorad, Lawrence; Blol Labs; Harvard Univ; 16 Divinity Ave; Cambridge MA 02138; 617-495-4292; Fax: 617-495-4292; bogorad@biosun.harvard.edu
Bohn, Martin; Institut fur Pflanzenzuchtung; Universitat Hohenheim; 70593 Stuttgart; GERMANY; 0049711/459-2334; Fax: 0049711/459-2343
Bokde, S.; Tecnagri International Inc.; CMC Maputo Branch; C.P. 4602; Maputo; MoZAMBIQUE; 2581-456-109,356,316,456; Fax: 258-1-450-335,490
Bollman, Krista; Dept. of Biology; Plant Science Institute; University of Pennsylvania; Philadelphia PA 19104-6018
Bommineni, Venkat R; USDA-ARS, NCSL; State University Station; Fargo ND 58105-5677; 701-239-3131; Fax: 701-239-31369; bomminen@badlands.nodak.edu
Bongard-Pierce, Deverie K; Dept Molee Biology; Wellman 11; Massachusetts General Hosp; Boston MA 02114; 617-726-5938; Fax: 617-726-6893; DEVERIE.BONGARD-PIERCE@MOLBIO.MGH.HARVARD.EDU
Borovskii, Genadii; Siberian Inst Plant Physiol Biochem; P.O. Box 1243; Irkutsk-33, 664033; RUSSIA; root@silibr.irkutsk.su
Bosch, Lluis; Escola Superior d'Agricultura; Comte d'Urgell, 187; 08036 Barcelona; SPAIN; 3-4304207; Fax: 3-4192601
Boston, Rebecca S.; Box 7612; Dept Botany; North Carolina State Univ; Raleigh NC 27695-7612; (919)515-2727; Fax: (919)515-3436
Bouchard, Robert A.; College of Wooster; Biology Dept.; Wooster OH 44691; 216-263-2433; Fax: 216-263-24378; rbouchard@acs.wooster.edu
Bowen, Ben; Pioneer Hi-Bred Internati; 7300 NW 62nd Avenue; P.O. Box 1004; Johnston IA 50131-940; (515)270-3647; Fax: (515)270-3367; bowenb@phibred.com
Boyer, Charles D.; Dept. of Horticulture; Oregon State University; of Missouri; Columbia MO 65211; 573-882-0123; braun@biosci.
      Bridgers, Shelley; Department of Biology; Univ North Carolina at Pembroke; Pembroke NC 28372; 910-521-6377; bridgers@nat.pembroke.edu
Briggs, Steven; Pioneer Hi-Bred International; 7300 NW 62nd Ave; P.O. Box 1004; Johnston IA 50131-1004; 515-270-4143; Fax: 515-253-2149;
BRIGGS@PHIBRED.COM
       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; Fax: 515-254-2619; BRIGGSKK@PHIBRED.COM
   254-2619; BRIGGSKK@PHIBRED.COM

Briggs, Robert W.; Crow's Hybrid Corn Company; Box 306; Milford IL 60953; 815-889-4151; Fax: 815-889-5253

Brinkman, Mark; Mycogen Seeds; 809 4th Street; Gothenburg NE 69138; 308-537-2938; Fax: 308-537-2960; brinkman@mycogen.com

Britt, Anne Bagg; Section of Plant Biology; U of CA; Davis CA 95616; 916-752-0699; Fax: 916-752-5410; abbritt@ucdavis.edu

Brockman, Laura L.; Cargill Hybrid Seeds; 2600 W. Galena Blvd; Aurora IL 60506; 708-801-2352

Brooke, Glenn; Pioneer Hi-Bred International; 7300 NW 62nd Ave; P.O. Box 129; Johnston IA 50131-0129; (515)270-4030; Fax: (515)270-4312; brookerg@phibred.com

Brown, Steven; 2493 Pinch Road; Manheim PA 17545-9466

Brown, R. P.; 9555 Johnstown-Utica Rd; Johnstown OH 43031; 614-893-2501

Brown, Sherri; Monsanto; AA2G; 700 Chesterfield Village Pkwy; St. Louis MO 63198; (314)537-7244; Fax: (314)537-6759

Brown, Robert L; USDA/ARS/SRRC; 1100 Robert E. Lee Blvd; New Orleans LA 70125; 504-286-4359; Fax: 504-286-4419; rbrown@nola.srrc.usda.gov

Brown, Douglas; Dept of Biology; Bishops University; Lennoxville J1M 1Z7 Quebec; CANADA; 819-822-9632; Fax: 819-822-9661

Bruce, Wes; Pioneer Hi-Bred International, Inc.; 7300 NW 62nd Ave.; P.O. Box 1004; Johnston IA 50131; (515)270-4167; Fax: (515)270-4167; Brucewb@phibred.com

Brutnell, Thomas P.; Dept Plant Sci; University of Oxford; South Parks Road; Oxford, OX1 3RB; ENGLAND; 011-44-1865-275030; Fax: 011-44-1865-275147; tom.brutnell@plant-sciences.oxford.ac.uk
                                   tom.brutnell@plant-sciences.oxford.ac.uk
      Bubeck, David; Northrup King Co.; 317 330th St.; Stanton MN 55018-4308; (507)663-7666
Buckler, Edward; 105 Tucker Hall; University of Missouri; Columbia MO 65211
Buckner, Brent; Div. Science, Science Hall; Truman State Univ; 100 East Normal; Kirksville MO 63501-4221; 816-785-4083; Fax: 816-785-4045; bbuckner@truman.edu
Buescher, Pat; Custom Farm Seed; Box 160; Momence IL 60954; 815-472-2433; Fax: 815-472-3890
Bueter, Bernd; Swiss Federal Inst. Sci. (ETH) Zurich; Inst. Plant Sci.; ETH - Eschikon 33; CH - 8315 Lindau; SWITZERLAND; 41-52-33-92-83; Fax: 41-52-33-27-06;
   Bueter, Bernd; Swiss Federal Inst. Sci. (ETH) Zurich; Inst. Plant Sci.; ETH - Eschikon 33; CH - 8315 Lindau; SWITZERLAND; 41-52-33-92-83; Fax: 41-52-33-27-06; BUETERB@UNIX.DAT ACOMM.CH
Bullock, W. Paul; ICI Seeds, Inc.; Hwy 210; P.O. Box 500; Slater IA 50244
Bureau, Thomas; Dept. of Genetics; University of Georgia; Athens GA 30602
Burr, Benjamin; Biology Dept; Brookhaven National Lab; Upton NY 11973; 516-282-3396; Fax: 516-282-3407; burr@bnlux1.bnl.gov
Butler, Lou; Dept of Agronomy; Curtis Hall; Univ of Missouri; Columbia MO 65211; 573-882-7818; lou@teosinte.agron.missouri.edu
Butnaru, Gallia; Univ Stiinte Agricole A Banatului; Disciplina de Genetica; C. Post 136, O.P. 1; Timisoara 1900; RÖMANIA; 40-96-14142
Byrne, Mary; Cold Spring Harbor Laboratory; PO Box 100; Cold Spring Harbor NY 11724; 516-367-8827; Fax: 516-367-8369; byrne@cshl.org
Byrne, Patrick; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-0832; Fax: 573-884-7850; byrne@teosinte.agron.missouri.edu
Byron, Dennis; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004
Cabulea, I.; Library; Agric Res Station - Turda; Str. Agriculturii 27; 3350 Turda; ROMANIA; 040-95-311680; Fax: 040-95-311134
Calderon-Urrea, Alejandro; Osborn Memorial Laboratories; Dept of Biology; Yale University, Box 6666; New Haven CT 06511-7444; (203)432-3894; Fax: (203)432-3879; Calalea@YALEVM.ycc.yale.edu
Caldwell, Elizabeth E. Oberthur; 4505 Regal Ave NE; Cedar Rapids IA 52402-2143; 319-335-8333
Callaway, Brett; P.O. Box 3131; Thomasville GA 31799
```

```
Callis, Judy; Section of Molecular and Cellular Biol.; Univ of CA; Davis CA 95616; (916)752-1015; Fax: (916)752-3085; JCallis@ucdavis.edu
Camara-Hernandez, J.; Altolaguirre 1295; Buenos Aires 1427; ARGENTINA; 54-1-521-6464; Fax: 54-1-522-1687
Camargo, Luiz; Depto. Fitopatologia -ESALQ/USP; C.P. 09; 13418-900 Piracicaba-SP; BRAZIL; 55-194-294124; Fax: 55-194-344839; 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; Fax: 906-487-3355;
      Campbell, Wilbur H.; Dept. of WCAMPBEL@MTU.EDU
   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; 510-642-1669; Fax: 510-643-6791
Cardinal, Andrea J.; Agronomy Hall 1571; Iowa State Univ; Arnes IA 50011; 515-294-8734; Fax: 515-294-3163; cardinal@iastate.edu
Carle-Urioste, Jose; Dept of Biological Sciences; Stanford University; Stanford CA 94305-5020
Carlson, Wayne; Botany Department; University of Iowa; Iowa City IA 52242; (319)335-1316; Fax: (319)335-3620; wayne-carlson@uiowa.edu
Carlson, Lawrence A.; 7 North Winthrop Street; St. Paul MN 55119-4674; 612-738-8812; Lawrence.A.Carlson-1@tc.umn.edu
Carreiro, Newton; University of Arizona; Dept. of Plant Sciences; Bldg. 36; Tucson AZ 85721
Carson, Martin L.; Dept. Plant Pathology; Box 7616; NCSU; Raleigh NC 27695-7616; (919)515-3516; Fax: (919)515-7716
Carson, Chris; 105 Tucker Hall; Biological Sciences; University of Missouri; Columbia MO 65211; 573-882-4871; carson@biosci.mbp.missouri.edu
Cartinhour, Sam; Crop Biotechnology Center; Texas A&M Univ; College Station TX 77843; 409-862-3495; Fax: 409-862-4790; scarlinh@coyote.tamu.edu
Carvalho, Carlos Roberto de; Depto. Biologia Geral; Universidade Federal de Vicosa; 36570.000; Vicosa - MG; BRAZIL; 31-899-2568; Fax: 31-899-2203
Causse, Mathilde; Station de Genetique Vegetale; Ferme du Moulon; 91190 Gilf/Vette; FRANCE; 33 1 6941 6727; Fax: 33 1 6941 2790; causse@moulon.inra.fr
Cavanaugh, Kevin; Beck's Superior Hybrids; 6767 E. 276th St.; Atianta IN 46031
Chalyk, S. T.; Institute of Genetics; Lesnaya str. 20; Kishinev 277002; REPUBLIC OF MOLDOVA; 037-32-53-83-67; Fax: 037-32-55-61-80
Chan, Annette; Dept. of Plant Biology; 111 Koshland Hall; University of California; Berkeley CA 94720-3102; ACHAN@UCLINK3.BERKELEY.EDU
Chandler, Vicki; Department of Biology; Institute of Molecular Blology; University of Oregon; Eugene OR 97403; 503-346-5136; Fax: 503-346-5891; chandler@molbio.uoregon.edu
                                     chandler@molbio.uoregon.edu
      Chandrakanth, E.; Center for Plant Mol. Biol.; Osmania University; Hyderabad 500007; INDIA
Chang, Ru-Ying; Plant Path Dept; 1453 Fifield Hall; Univ Florida; Gainseville FL 32611; 904-392-9469
Chang, Ming-Tang; ExSeeds Genetics L.L.C.; 1567 Food Sciences Bldg.; Iowa State Univ.; Ames IA 50011-1061; 515-294-2993; Fax: 515-294-2644;
Chang, Ming-Tang; ExSeeds Genetics L.L.C.; 1567 Food Sciences Bldg.; lowa State Univ.; Ames IA 50011-1061; 515-294-2993; Fax: 515-294-2644; mingchang@ames.net
Chang, S. C.; 13, LN36, Chung Shan Rd.; Chiayi, ROC; TAIWAN; 05-278-4603
Chao, Shiaoman; Inst. Botany, Rm. 114; Academia Sinica; Nankang, Taipei; TAIWAN; (02) 789-9590 ext. 114; Fax: 886-2-782-7954; schao@ccvax.sinica.edu.tw
Char, Bharar; Plant Gene Expression Center; 800 Buchanan St.; Albany CA 94/10
Charlton, Wayne; Rodney Porter Building; Dept. of Plant Sciences; University of Oxford, South Parks Road; Oxford, OX1 3RB; UNITED KINGDOM; 44-1865-275814;
Fax: 44-1865-275805; WCHARLTON@GLYCOB.OX.AC. UK
Chase, Sherret S.; Chase Road; P.O. Box 193; Shokan NY 12481; 914-657-2392
Chase, Christine D.; Horticultural Sciences Dept.; 1143 Fifield Hall; Univ of Florida; Gainesville FL 32611-0690; 904-392-1928x316; Fax: 904-392-6479; ctdc@gnv.ifas.ufl.edu
Chechak, C.; Stokes Seeds Limited; 39 James Street; P.O. Box 10, St. Catharines; Ontario L2R 6R6; CANADA; 905-688-4300; Fax: 905-684-3022
Chen, Zengjian; Department of Agronomy & Plant Genetics; 411 Borlaug Halt; University of Minnesota; St. Paul MN 55108; (612)625-5793; Fax: (612)628-1268
Chen, Yun-Chia; Plant Gene Expression Center; 800 Buchanan St.; Albany CA 94/710
Chen, Rei Yang; Nankai University; Biology Division; Plant Molecular Biology Dept.; Tianjin 300071; CHINA
Chen, Shouyi; Academia Sinica; Institute of Genetics; Datun Road, Andingmen Wal; Beijing, 100101; CHINA
Chen, Welcheng; Dept. of Agronomy; Henan Agric. University; ZhengZhou 45000; CHINA
Chen, Jychian; Institute of Genetics; Datun Road, Andingmen Wal; Beijing, 100101; CHINA
Chen, Welcheng; Dept. of Agronomy; Henan Agric. University; TengZhou 45000; CHINA
Chen, Welcheng; Dept. of Malecular Biology Division; Plant Molecular Biology Dept. Plant Plant Plant Industry; National Pingtung Polytechnic Inst; Neipu, Pingtung 91207; TAIWAN; 886-8-774-0267; Fax: 886-8-774-0264
Cheng, Ping-chin; Advanced Microscopy and Imaging Lab; Dept Elect Comp Eng. 201 Bell
     Chojecki, Jan; Zeneca Seeds; Jealott's Hill Hesearch Sta; Brackhell Berks HG42 6E1; UNITED KINGDOM; 44-1344-414814; Fax: 44-1344-414996
Chomet, Paul; DeKalb Plant Genetics; 62 Maritime Drive; Mystic CT 06355; 203-572-5224; Fax: 203-572-5240; 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; Fax: 5160963
Chopra, Surinder; 2116 Molecular Biology Building; Iowa State University; Ames IA 50011; (515)294-5054; Fax: (515)294-0345; chopra@iastate.edu
Chourey, Prem; USDA-ARS; Plant Pathology Department; University of Florida; Gainesville FL 32611-0680; 904-392-2806; Fax: 904-392-6532;
PSCH@GNV.IFAS.UFL.EDU
 Chourey, Prefix USDA-ARS, Plant Pathology Department, University of Plotta, Gamesville Pt. 32611-0660, 904-392-2606; Pax. 904-392-2606, Pax. 904-995. Pax. 904-392-2606, Pax. 904-995. Pax. 904-392-2606, Pax. 904-995. Pax. 904
      Coors, James G.; Department of Agronomy; University of Wisconsin; 1575 Linden Drive; Madison Wi 53706; 608-262-7959; COORS@MACC.WISC.EDU
Corke, Harold; 45 Highwest; 142 Pokfulam Road; Hong Kong; HONG KONG; 011 852 2859 2820; Fax: 011 852 2858 3477; harold@hkuxa.hku.hk
Cormack, Jean; DeKalb Genetics Corp.; 3100 Sycamore Rd; DeKalb IL 60115; 815-758-9682; Fax: 815-758-4106; jcormack@dekalb.com
Comu, A.; Sta d'Amelior des Plantes; B V 1540; 21034 Dijon; FRANCE; 80-63-3159; Fax: 80-63-3263
Cowan, Kyra; University of Ottawa; 40 Marie Curie Private; Ottawa, Ontario K1N 984; CANADA; (613)564-5869; Fax: (613)564-9562; altosaar@acadvm1.uottawa.ca
```

```
Cowen, Neil; DowElanco Discovery Research; 9410 Zionsville Rd., Cl/#245; Indianapolis IN 46268-1053; 317-337-3684; Fax: 317-337-3228
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; Fax: (515)253-2149; CRANEVC@PHIBRED.COM
Creech, Roy G.; Plant & Soil Sciences; Box 9555; Mississippi State University; Mississippi State MS 39762; 601-325-2699; Fax: 601-325-8742; rgcreech@arm.msstate.edu
  Creech, Roy G.; Plant & Soil Sciences; Box 9555; Mississippi State University; Mississippi State MS 39762; 601-325-2699; Fax: 601-325-8742; rgcreech@ra.msstate.edu
Cross, John; Instrumentation and Instr Devel Program; National Science Foundation; 4201 Wilson Blvd; Arlington VA 22230; ; jwcross@nsf.gov
Cuany, Robin L.; Department of Agronomy; Colorado State University; Fort Collins CO 80523; 303-491-6832; Fax: 303-491-0564
Cui, Xianqin; B420 Agronomy Hali; Iowa State University; Ames IA 50011
Culley, David; Dept of Plant Pathology; Washington State Univ; Pullman WA 99164-6430; 509-335-3751; Fax: 509-335-9581; dculley@wsu.edu
Curmings, D. P.; Dekalb Genetics Corporation; P.O. Box 367; 908 North Independence; Windfall IN 46076-0367; 317-945-7125; Fax: 317-945-7152
Currie, Randall; SWREC; 4500 E. Mary; Garden City KS 67842; 316-276-8288; Fax: 316-276-6028; RCURRIE@COXNET.KSU.EDU
Curtis, Christine A.; Dept Med & Molec Genetics, IB 264; Indiana University Medical Center; 975 W. Walnut St.; Indianapolis IN 46202; 317-274-1060
D'Halluin, Kathleen; Plant Genetic Systems N.V.; Jozef Plateaustraat 22-8 9000; Gent; BELGIUM; (32) (9):2358486; Fax: (32) (9):2240694; pgs@pgsgent.be
da Costa e Silva, O; Inst Allgemeine Botanik; Univ Hamburg; Ohnhorststr. 18; 22609 Hamburg; GERMANY; 49-40-822-82-82-391; Fax: 49-40-822-82-503;
FB5A111@rrz-cip-1.rrz.uni-hamburg.de
Dahleen, Lynn; USDA-ARS-NCSL; 1307 N. 18th St; P.O. Box 5677, S.U. Sta.; Fargo ND 58105-5677; 701-239-1384; Fax: 701-239-1389; dahleen@badlands.NoDak.edu
Damerval, Eatherine; Station de Genetique Vegetale; Ferme du Moulon; 91190 Gif Sur Yvette; FRANCE; (1)69 41 67 24; Fax: 301(1)69 41 27 90
Dankov, Toma; Ivan Assen Str. 93; Sofia 1124; BULGARIA; 43-82-73
Dannenhofter, Joanne; Central Michigan Univ; Dept of Biology; 217 Brooks Hall; Mr. Pleasant Ml 48859; 517-774-2509
Daohong, Xie; Maize Research Institute; Jilin Acad.; 5 W. Xing Hua Street; Gongahuling, Jilin, P.R. 136100; CHINA; (86)-04441-215179; Fax: (86)-04441-214884
Darrah, Larry; 110A Curtis Hall; University of Mi
       day@aesop.rutgers.edu

De Leon, Carlos; CIMMYT; P.O. Box 9-188; Bangkok 10900; THAILAND; 662-561-4057; Fax: 662-579-4858; cimmyt@netserv.chula.ac.th

De Oliveira, Antonio Costa; Rua 15 de Novembro, 1030; Pelotas - RS 96100; BRAZIL; 317-743-3052; Fax: 317-496-1496; acosta@bilbo.bio.purdue.edu

Dean, Caroline; BBSRC; John Innes Centre; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 011-44-1603-452571; Fax: 011-44-1603-505725;
De Onleyfar, Alfondo Costar, fuel or en Voerfind's Colley Lane; Norvich BNR211; 317-49-3052; Pack 317-49-1995; acrolline; BBSRC; John Innes Centre; Collney Lane; Norvich BNR2 70H; UNITED KINGDOM; 011-44-1603-452571; Fax: 011-44-1603-505725; ARABIDOPSIS @bbsrc.AC.UX
Deboo, Gulsham; 154 Johnson Hall; Horticulture and Landscape Architecture; Pullman WA 99164-8414
DeBroux, Steve; 700 E. Butler Ave; Delaware Valley College; Doylestown PA 18901; 215-345-1500; Fax: 215-345-5277
Delannay, Xavier; Monsand-Ceregen; 700 Chesterfield Pkwy Norty; St. Louis Mo 63198; 314-537-6017; xxdela@ccmail.monsanlo.com
Dellaporta, Steve; Department of Biology; 356 OML; Yale University; New Haven CT 06511; 203-432-3895; sdellap@yalevm.ycc.yale.edu
Dempsey, Ellen; 7 Prospect St; Comwall-on-Hudson NY 12520; 914-534-5265
Dennis, Elizabelti; CSIRO; Division of Plant Industry; P.O. Box 1600; Canberra, ACT 2601; AUSTRALLA; 618-246-5061; Fax: 61-6-246-5000; liz@pican.pi.csiro.au
Deutsch, James A; ICI Seeds, Inc.; RPI2; Box 16; Marshal Mo 65340; 818-88-6836; 7as: 816-886-8977
Dewald, Chester L.; ARS-USDA; 2000 18th St; Woodward OK 73801; 405-258-7449; Fax: 405-258-1322
Dhilon, B. S.; CIMMYT, Maize Program; Apartado Postal 6-641; C.P. 06600 Mexico, D.F.; MEXICO; (5)726-90-91; Fax: (595)410-69
Dietrich, Chuck; B420 Agronomy Halt; 1002 South Goodwin Ave; Urbana IL 61801; 217-244-3388; Fax: 217-333-9817; DIJKHUIZ@UX1.CSO.UIUC.EDU
Dijke, John E.; Winthrop College Biology Dept: Rook Hill SC 28733; 803-323-2411; Fax: 803-323-3448; dille@winthrop.edu
Dilworth, Mach; NSF Div BiO/IBN, Room 6855; 4201 Wilson Blvd.; Arlington VA 22230; 703-3361-423; mdllwort@nst.gov
Dincher, Sander; PO Box 12257; CIEV-GEIGY Corp.; Research Triangle Park NC 27709
Ding, Qun-Xing; Plant Biology Department; University of Milano; Via Celoria Egi. 2013 Milano; ITALY; 39 2 26605; Fax: 39 2-684551
Dolfini, Silvana Faccio, Dipartimento di Genetica; University of Milano; Via Celoria Egi. 2013 Milano; ITALY; 39 2 26605; Fax: 59-39-39-99; Fax: 54-82-16-85
Dombrink-Kurtzman, Mary Ann; N
                                  ARABIDOPSIS@bbsrc.AC.UK
                                drdunct@ccmail.monsanto.com
  Durkovicova, Viera; Prachaticka 43; SK-96001 Zvolen; SLOVAK REPUBLIC
Duvick, Susan; 1501 Agronomy; Iowa State University; Ames IA 50011; 515-294-5545
Duvick, Donald N.; P.O. Box 446; Johnston IA 50131; 515-278-0861; Fax: 515-253-2125; duvick@phibred.com
Duvick, Jonathan P.; Pioneer Hi-Bred Internati; Dept of Biotechnology Res; 7300 N.W. 62nd Ave; Johnston IA 50131-1732; 515-270-3176; Fax: 515-253-2147
Earle, Elizabeth D.; Dept Plant Breed & Biom; Cornell University; 252 Emerson Hall; Ithaca NY 14853-1902; 607-255-3102; Fax: 607-255-6683; ede3@cornell.edu
Eathington, Samuel R.; 910 Gaskill; Ames IA 50014; 515-685-5141; Fax: 515-685-5080
Eaton, Dana; Asgrow Seed Co; 32545 Galena Sassafras Rd; Galena MD 21635; 410-827-9240; Fax: 410-827-9763
Edwards, Marlin; Northrup King Co.; 317 330th Street; Stanton MN 55018-4308; 507-663-7623; Fax: 507-645-7519
Edwards, Keith; Cell Biol Dept; IACR-Long Ashton Res Stn; Univ Bristol, Long Ashton; Bristol BS18 9AF; UNITED KINGDOM; 44-0-1275-392181; Fax: 44-0-1275-394281; KEITH.EDWARDS@BBSRC.AC.UK
Efremov, Alexander; Max-Planck-Institute; Carl-von-Linne-Weg 10; D-50829 Koln (Vogelsang); GERMANY
Eggleston, Bill; Department of Biology; Virginia Commonwealth University; 218 Park Ave; Richmond VA 23284; 804-828-1562; Fax: 804-828-0503; weggles@cabell.VCU.edu
        Durkovicova, Viera; Prachaticka 43; SK-96001 Zvolen; SLOVAK REPUBLIC
                                weggles@cabell.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; Fax: (612)625-1268; peggy@bioscl.cbs.umn.edu
Eichholtz, David A.; Monsanto, BB4D; 700 Chesterfield Village Pkwy; St. Louis MO 63198; 314-537-6227; Fax: 314-537-6047; DAEICH@CCMAIL.MONSANTO.COM
```

Eisses, John; Dept. of Biological Sciences; University of Idaho; Moscow ID 83844-30521

```
Elsing, Evan; Pioneer Hi-Bred Intl Inc.; Kekaha Research Sta; PO Box 596; Kekaha, Kauai HI 96752-0596
England, Dan; Curits Halt; Univ of Missouri; Columbia MO 65211; 573-882-7818
Erturk, Neval; Dept. 6 fiology; Virginia Polytech Inst & State Univ; Blacksburg VA 24061-0406
Esen, Asim; Dept Biology; Va Polytech Inst & State Univ; Blacksburg VA 24061; 231-5894; Fax: 231-9307; aevatan@vt.edu
Eubanks, Mary W.; Dept Botany; Duke Univ; Durham NC 27708-0338; 919-684-4314; Fax: 919-684-5412; eubanks@duke.edu
Evans, Mathew; Laboratory of Genetics; 445 Herny Malt; University of Wisconsin; Madison WI 53706; 608-262-4934; Fax: 608-262-2976; mmevans@facstaff.wisc.edu
Evans, Mike; Dept Plant Biology; Ohio State Univ; Columbus OH 43210; 614-292-9162; Fax: 614-292-6345; evans.20@osu.edu
Evarsole, Kellye; Eversole Associates; 3208 Park View Road; Chevy Chase MD 20815; 301-951-3345; Fax: 301-951-1846
Evola, S. V.; Novartis Seeds Biotechnol Res Unit; P.O. Box 18300; Greensboro NC 27419-8300; 910-547-1038; Fax: 910-547-1030; evolas@a1.abru.cg.com
Falbel, Tanya; Department of Horticulture; University of Wisconsin; 1575 Linden Dr.; Madison WI 53705; 608-262-1622; Fax: 608-262-4743; faibel@macc.wisc.edu
Fan, Yun-Liu; Chinese Academy of Agricultural Sciences; Blotechnology Research Center; 30 Baishiqiao Lu; Beijing 100081; CHINA
Farish, Guy; Biology Dept; Adams State College; Alamosa CO 81102; 719-589-7969; Fax: 719-589-7522; GFARISH@CC4.ADAMS.EDU
Fauron, Christiane; University of Utah; 2100 Eccles Genetics Bldg; Salt Lake City UT 84112; 801-589-1435; Fax: 801-585-1777; christiane.fauron@genetics.utah.edu
Feix, Gunter; Institut Fur Biologie III; Universitat Freiburg; 79104 Freiburg; GERMANY; 0761-2032724; Fax: 0761-2032745; FEIX@SUN1.RUF.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
Fennoy, Sheila; Botany & Plant Sciences; Unc. Riverside; Riverside Ag5221
Fergason, Virgit; Custom Farm Seed; 100 East Shafer, P.O. Bo
 Fluminhan, Antonio Jr.; Institute of Genetic Ecology; Tohoku Univ - Katahira Campus; Katahira 2-1-1, Aoba-Ku; 980-77 Sendai; JAPAN; 81-22-21/-5683; Pax: 81-22-203-9845; antonio@bansui.ige.tohoku.ac.jp
Foley, Terry; Holden's Foundation Seeds Inc; 201 North Maplewood; P.O. Box 839; Williamsburg IA 52361; 319-668-1100; Fax: 319-668-2453; terry@holdens.com
Foley, Rhonda; Dept. of Biology; Univ of California - Los Angeles; 405 Hilgard Ave; Los Angeles CA 90024-1606; Fax: 310-206-3987
Foreman, Daphne; 4085 Kraus Hall; Department of Biology; University of Michigan; Ann Arbor MI 48109-1048; (313)764-3579; Fax: (313)747-0884
Foster, Toshi; Plant Gene Expression Center; USDA; 800 Buchanan St.; Albany CA 94710
Fowler, John; Dept. of Biology - CB#3280; University of North Carolina; Chapel Hill NC 27599-3280; 919-962-0778; Fax: 919-962-0778
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. 1; Arva, Ont NOM 1C0; CANADA; 519-461-0072; Fax: 519-461-0275
Frank, Mary; Dept. of Biology; MC0116; Univ. of California - San Diego; La Jolla CA 92093-7108
Franken, P.; Max-Planck Inst Zuchtungsf; Carl-von Linne-Weg 10; D 5000 Koln 30; GERMANY
Franklin, Amie; Dept Molec Cell Biol; 345 LSA; Univ of California; Berkeley CA 94720-0001
Freeling, Michael; Dept of Plant Biology; 111 Genetics & Plant Biology Bldg; Univ of California; Berkeley CA 94720; 510-642-8058, 1107; Fax: 510-642-4995; freeling@nature.berkeley.edu
  Freeling, Michael; Dept of Plant Biology; 111 Genetics & Plant Biology Bldg; Univ of California; Berkeley CA 94720; 510-642-8058, 1107; Fax: 510-642-4995; freeling@nature.berkeley.edu
Frei, Mark; Winkelgasse 2; 4512 Bellach; SWITZERLAND; (41)65 38 17 97
Frey, Ruth; Dept. of Biological Sciences; University of Idaho; Moscow ID 83843
Frey, Monika; Lehrstuhl fur Gentechnik Hoherer Pflanzen; TUM; Lichtenbergstrabe 4; 85747 Garching; GERMANY; +49-89-3209 3532; Fax: +49-89-3209-2892; frey@c2h5oh.org.chemie.tu-muenchen.de
Freymark, Peter J.; 16 Cameron Road; P.O. Borrowdale; Harare; ZIMBABWE; 011-263-4-726061; Fax: 011-263-4-726061; FREYMARKP@PHIBRED.COM
Friedman, Robert B.; American Maize-Products Co; 1100 Indianapolis Blvd; Hammond IN 46320-1094; 219-659-2000 ext 390; Fax: 219-473-6607
Friedrich, James W.; Maize Genetic Resources, Inc; 10570 Hwy. 50 North; Angier NC 27501; 919-894-5594; Fax: 919-894-5660
Frolich, Walter; Oberriexingerstr. 32; D-74343 Sachsenheim; GERMANY
Fromm, Michael; Monsanto Co. GG3A; 700 Chesterfield Village Pkwy; St. Louis MO 63198; 314-537-6048; Fax: 314-537-6759; mefrom@ccmail.monsanto.com
Frova, Carla; Dept of Genetics & Microbiology; University of Milano; Via Celoria 26; 20133 Milano; ITALY; 02/26605.201; Fax: 02/2664551; CAMELOT@IMIUCCA.CSI.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; Fax: 86-
    Fu, Xi-Qin; Hunan Academy of Agricultural Sciences; Hybrid Rice Research Center; Mapoling, Dong Jiao, Changsha; Hunan 410125; CHINA; 86-731-469-1443; Fax: 86-731-469-1877
     Fuerstenberg, Susan; 1206 Orkney Drive; Ann Arbor MI 48103
    Fung, Jennifer; Dept Biochem Biophys; Box0554 HSE; UC San Francisco; San Francisco CA 94143
Furtek, D. B.; Inst. Molecular Biology Labs; Pennsylvania State Univ.; 214 Borland Lab; University Park PA 16802-2505; 814-863-7785; Fax: 814-863-6123;
                        dbf1@psu.edu
    Gabay-Laughnan, Susan; Plant Biology/265 Morrill Hall; University of Illinois; 505 S. Goodwin Avenue; Urbana IL 61801; 217-333-2919; Fax: 217-244-7246 Gaillard, Antoine; Maisadour; Route de Saint-Sever-Haut Mauco; BP 27; 40001 Mont-de-Marsan; FRANCE; 58 05 84 84; Fax: 58 05 84 99 Gale, Michael; John Innes Centre; Norwich Research Park; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM
Gale, Michael; John Innes Centre; Norwich Research Park; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM
Galinat, Walton C.; Eastern Agric. Center, U. Mass.; 240 Beaver Street; Waltham MA 02154-8096; 617-891-0650; Fax: 617-899-6054
Gallie, Daniel; Dept. of Biochemistry; University of California; Riverside CA 92521; (909)787-7298; Fax: (909)787-3590; 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; Fax: 34-1-3365757
Gardiner, Jack; Biology Building; University of Iowa; Iowa City IA 52242; 319-335-1095; Fax: 319-335-1069; zeamays@biovax.biology.uiowa.edu
Gardiner, Michele; Rogers NK Seed Co; 6338 Highway 20-26; Nampa ID 83687; (208)466-0319; Fax: (208)467-4559
Gardner, C. A. C.; Pioneer Hybrid Internatl; Highway 65 South, PO Box 218; Carrollton MO 64633; 816-542-3780
Garnaat, Carl W.; Pioneer Hi-Bred Int't; Department of Biotechnology Research; 7300 N. W. 62nd Ave-P.O. Box 1004; Johnston IA 50131-1004; (515)253-2251; Fax: (515)270-3367; GARNAATC@PHIBRED.COM
Garwood, D. L.; Garwood Seed Company; RR 1, Box 20; Stonington IL 62567; 217-325-3715
Gavazzi, Giuseppe; Universita de Milano; Dip Fisiol Pianta Coltivate Chim Agrar; Via Celoria 2; 20133 Milano; ITALY; +39-2-70630856; Fax: +39-2-2663057
Gay, Philippe; CIBA-GEIGY Ltd; Agricultural Division; CH-4002 Basel; SWITZERLAND; 41-61-697-4457; Fax: 41-61-697-6194
Geadelmann, Jon L.; Holden's Foundation Seeds, Inc; 2440 Highway 19 Blvd; Stanton MN 55018-7220; 507-263-3476; Fax: 507-263-4839; jon@holdens.com
Gebauer, Juan E.; Corn Breeding; Casilla 190; Buin; CHILE; 011(56-2)821-1552; Fax: 011(56-2)821-3564
Geiger, Hartwig H.; Univ Hohenheim; 350 Inst Pflanzenzucht; Seed Sci and Pop Genetics; D-70593 Stuttgart; GERMANY; 49-711-459-2644; Fax: 49-711-459-2343; PZHHG@RS1.RZ.UNI-HOHENHEIM.DE
Gengenbach, Burle G.; Agron & Plant Genetics; Univ of Mi
    Gengenbach, Burle G.; Agron & Plant Genetics; Univ of Minnesota; 1991 Upper Buford Cir.; St Paul MN 55108; 612-625-6282; Fax: 612-625-1268;
                        burle@molbio.cbs.umn.edu
    Getschman, R. J.; Dekalb Plant Genetics; Box 408; Dayton IA 50530; 515-547-2550; Fax: 515-547-2552
    Gierl, Alfons; Lehrstuhl fur Genetik; Technische Universitat Munchen; 85747 Garching; GERMANY; 49-89-3209-2890; Fax: 49-89-3209-2892; gierl@gen.chemie.tu-
                        muenchen.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; Fax: 61-2-351-4771; cgillies@extro.ucc.su.oz.au
Gillikin, Jeff; Dept. Botany; NCSU; Box 7612; Raleigh NC 27695-7612; 919-515-3570; Combip@unity.NCSU.edu
Giorio, Giovanni; c/o Metapontum Agrobios; SS. Ionica Km 448.2; I-75010 Metaponto (MT); ITALY; 39-835-740276; Fax: 39-835-745306;
AGROBIOS@MVX36.CSATA.IT
```

```
Girard, Lisa; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102
Giroux, Michael; Dept. of Horticultural Sciences; Univ of Florida; 1255 Fifield Hall; Gainesville FL 32611; 904-392-0091
Glover, David V.; Dept of Agronomy; Purdue University; W. Lafayette IN 47907; 317-494-8067; Fax: 317-494-6508; 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, Stephen; Dept of Biology; University of Toledo; Toledo OH 43606; 419-530-4197; Fax: 419-530-7737
Goldman, Irwin; Department of Horticulture; Univ. of Wisconsin; 1575 Linden Drive; Madison WI 53706; (608)262-7781; Fax: (608)262-4743
Golubovskaya, Inna; Dept of Genetics; N I Vavilov Res Inst Plant Ind; Hertzen Street, 44; 190 000 St Petersburg; RUSSIA
Gong, Zuxun; Academia Sinica; Shanghai Inst. of Biochem.; 320 Yue-Yang Road; Shanghai 200031; CHINA
Gonzalez de Leon, Diego; CIMMYT, INT; Lisboa #27, APDO. POSTAL 6-641; 06600 Mexico, D. F.; MEXICO; (52)(5)955-4400/4410; Fax: (52)(5)955-4425;
DGDELEON@CIMMYT.MX
Goodman, Major M.: Department of Crop Sciences; North Carolina State Linky P.O. Rev 7600; Relative N.O. 27005, 240. 240. 240.
        Goodman, Major M.; Department of Crop Sciences; North Carolina State Univ; P.O. Box 7620; Raleigh NC 27695; 919-515-7039; Fax: 919-515-7959 Gordon, P. N.; CT Forest & Park Assoc.; 16 Meriden Rd.; Rockfall CT 06481-2961; (203)346-2372; Fax: 203-347-7463 Gordon-Kamm, William; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave., PO Box 1004; Johnston IA 50131-1004
        Gouesnard, Brig.; INRA Centre de Montpellier; Sta Genet Amel Plantes; domaine de Melguell; 34130 Mauguio; FRANCE; ; Fax: 67 29 39 90; gouesnar@montpellier.inra.fr Gracen, V. E.; Cargill Hi-Bred Seed; Box 9300; Minneapolis MN 55440; 612-742-7244; Fax: 612-742-7235
      Grafi, Gideon; Univ of Arizona; Dept of Plant Sciences; Tucson AZ 85721; 520-621-9154
Grafi, Gideon; Univ of Arizona; Dept of Plant Sciences; Tucson AZ 85721; 520-621-9154
Grant, David; G304 Agronomy Hall; lowa State Univ; Ames IA 50011-1010; 515-294-1205; Fax: 515-294-2299; dgrant@iastate.edu
Gray, John; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-1412; Fax: 573-884-7850; gray@teosinte.agron.missouri.edu
Grier, Stephen L.; Novartis Seeds; 317 330th St.; Stanton MN 55018-4308; 507-663-7662; Fax: 507-645-7519
Griffin, Daniel; 745 Beach Ave; Beachwood NJ 08722; 908-244-6721; grriffin@rider.edu
     Griffin, Daniel; 745 Beach Ave; Beachwood NJ 08722; 908-244-6721; grriffin@rider.edu
Griffor, Matthew; DeKalb Genetics; 62 Maritime Road; Mystic CT 06355
Grimanelli, Daniel; ORSTOM - CIMMYT; Apdo Postal 6-641; 06600 Mexico D.F.; MEXICO; (52)595-54400; Fax: (52)595-54425; dgrimanelli@cimmyt.mx
Grobman, Alexander; Semillas Penta del Peru S.A.; Apartado 270227; Lima 27; PERU; (5114)426375; Fax: (5114)425465
Gronwald, J. W.; Agronomy & Plant Genetics; Univ of Minnesota; St. Paul MN 55108
Grossniklaus, Ueli; Cold Spring Harbor Laboratory; PO Box 100; Cold Spring Harbor NY 11724; 516-367-8825; Fax: 516-367-8369; grossnik@cshl.org
Grotewold, Erich; Cold Spring Harbor Lab; 1 Bungtown Rd; Cold Spring Harbor NY 11724; (516)367-6860; Fax: (516)367-8369; GROTEWOL@CSHL.ORG
Gu, Ming-Hong; Jiangsu Agricultural College; Dept. of Agronomy; Yangzhou; Jiangsu 225001; CHINA
Guiltinan, Mark; Penn State Biotechnology Institute; 306 Wartik Lab; Dept of Horticulture; Univ. Park PA 16802; 814-863-7957; Fax: 814-863-6139;
                                mjg@psupen.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; Fax: 55196-72-2424 Guo, Baozhu; USDA/ARS/IBPMRL; PO Box 748; Tifton GA 31793-0748; 912-387-2326; Fax: 912-387-2321; bguo@tifton.cpes.peachnet.edu Guo, Mei; 105 Tucker; Division of Biol. Sciences; University of Missouri; Columbia MO 65211; 573-882-4871; Fax: 573-882-0123; mguo@biosci.mbp.missouri.edu
      Guo, Jun-Yuan; Academia Sinica; South China Institute of Botany; Guiangzhou 510650; CHINA
Gupta, Manju; Mycogen; 9330 Zionsville Rd; Building 306/C-1; Indianapolis IN 46268-1054; 317-337-5980; Fax: 317-337-5989; mgupta@dowelanco.com
Guilerrez, Osman A.; Dept Agronomy; 1150 Lilly Hall of Life Sciences; Purdue University; West Lafayette IN 47907-1150; ; ogutierrez@dept.agry.purdue.edu
Gutierrez, Lucero; Biology Dept; Herrin Bldg; Stanford Univ; Stanford CA 94305-5020
Haag, Wayne L.; Sasakawa Global 2000; C. P. 4247; Maptuo; MOZAMBIQUE
     Hadal, Wayne L.; Sasakawa Global 2000; C. P. 4247; Maptuo; MOZAMBIQUE
Hable, Whitney E; 528 N. Olsen Ave.; Tucson AZ 85711
Hadad, Robert G.; 317 Stonegate Way; Lexington KY 40503
Hake, S.; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5907; Fax: 510-559-5678; maizesh@nature.berkeley.edu
Hakiza, J.; OARDC; Dept. of Agronomy; 1680 Madison Ave.; Wooster OH 44691
Hall, Lisa Naomi; Oxford Univ; Dept Plant Sci; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; 865-275030; Fax: 865-275147
Hallauer, Arnel R.; Agronomy Building; 1505 Agronomy Hall; lowa State University; Ames IA 50010; 515-294-3052; Fax: 515-294-3163
Hamilton, R. I.; Agriculture and Agri-Food Canada; Eastern Cereal and Oilseed Res Ctr; Bldg # 121; Ottawa, Ontario K1A 0C6; CANADA; 613-759-1616; Fax: 613-952-
9295; Hamiltonr@em.agr.ca
Han, Changdeok: Gyeongsang National University: Chiniu 660-701; KOREA: 82-591-751-5188; Fax: 82-591-759-9363; cdhan@nongae.gspu.ac.kr
  9295; Hamiltonr@em.agr.ca

Han, Changdeok; Gyeongsang National University; Chinju 660-701; KOREA; 82-591-751-5188; Fax: 82-591-759-9363; cdhan@nongae.gsnu.ac.kr

Hancock, Denis C.; 213 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-1722; Fax: 573-884-7850; dhancock@teosinte.agron.missouri.edu

Hannah, Curtis; Veg Crops Dept; Univ of Florida, IFAS; Gainesville FL 32611; 904-392-1928x315; Fax: 904-392-6479; Hannah@GNV.IFAS.UFL.EDU

Hanning, Gary; Anheuser Busch; 1 Busch Place; St. Louis MO 63118; 314-577-7250

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; Fax: 515-294-0453; jdhansen@iastate.edu

Hansen, Leon A.; Rogers Seed Co.; 6338 Highway 20-26; Nampa ID 83687; 208-466-0319; Fax: 208-467-4559

Hansen, Maureen R.; Section Genetics/Develop.; Cornell Univ.; Biotech Bldg; Ithaca NY 14853; 607-254-4833; Fax: 607-255-2428

Hanten, John; Northrup King Co.; 317 330th St.; Stanton MN 55018; 507-663-7649; Fax: 507-645-7519

Hardeman, Kristine; Dept Bot Plant Path; Cordley 2082; Oregon State Univ; Corvallis OR 97331-2902

Harper, Lisa; Dept. Plant Biology; Genet. & Plant Biol., Bldg.; University of CA; Berkeley CA 94720; 510-841-1539; Fax: 510-642-7948; ligule@nature.berkeley.edu

Harris, John W.; Dept of Biology; Tennessee Tech Univ; Cookeville TN 38505; 615-372-3143; Fax: 615-528-4097

Harris, Linda J.; Plant Research Centre; Agriculture Canada; Bldg. #21, Central Exp. Farm; Ottawa, Ontario K1A 0C6; CANADA; (613)995-3700 ext.7647; Fax: (613)992-7909; harrisl@ncccot2.agr.ca
Harris, John W.; Dept of Biology; Tennessee Tech Univ; Cookeville TN 38505; 615-372-3143; Fax: 615-528-4097
Harris, Linda J.; Plant Research Centre; Agriculture Canada; Bldg. #21, Central Exp. Farm; Ottawa, Ontario K1A 0C6; CANADA; (613)995-3700 ext.7647; Fax: (613)992-7909; harrisl@ncccot2.agr.ca
Harrison, Virginia; Dept of Science; 470 E Lockwood Av; Webster University; St. Louis MO 63119-3194; 314-968-7079; Fax: 314-968-6909
Harry, David; DataGenetics Corp; 1933 Davis St., Suite 207; San Leandro CA 94577; 510-729-0720; deh@s27w007.pswfs.gov
Harrings, H.; Ist Sper Cereal; Via Stezzano 24; 24100 Bergamo; ITALY
Hartman, Carl; 155 South St. Rd. 2; Valparaiso IN 46383; 219-462-1927
Harvikkala, Ilkka; Genesis R & D Corp. Ltd.; P.O. Box 50; Auckland; NEW ZEALAND; +64-9-373-5600; Fax: +64-9-373-5601; i.havukkala@genesis.co.nz
Hawk, James A.; Dept Plant & Soil Sciences; University of Delaware; Newark DE 19717-1303; 302-831-1379; Fax: 302-831-3651; jhawk@strauss.udel.edu
He, Zuhua; Zhejiang Agricultural University; Biotechnology institute; Hangzhou; Zhejiang 31029; 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; Fax: 619-554-6330; heinlein@scripps.edu
Helentjairs; 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; Fax: 515-270-4312; helentjartg@phibred.com
Heller, Stephen R.; Bidg 005, Room 337; BARC-W; Beltsville MD 20705-2350; 301-504-6029; Fax: 301-504-6054; SRHELLER@ORIGIN.NALUSDA.GOV
Heredia-Diaz, Oscar; P.O. Box 313; Arlington WI 53911-0313; 608-635-8653
Hershberger, R. Jane; Dept. of Biological Sciences; Stanford University; Stanford CA 94305-5020; (415)723-2609; Fax: (415)725-8221
Hershberger, R. Jane; Dept. of Biology; University of Freiburg; Schlanzlestr, 1; 79104 Freiburg; GERMANY
Heun, Manfred; Institue of Biology; University of Freiburg; Schlanzlestr, 1; 79104 Freiburg; GERMANY
Heun, Manfred; Max-Planck Inst Zuchtungsforsch;
```

```
hodges@btny.purdue.edu
Hoegemeyer, Thomas C.; Hoegemeyer Hybrids Inc; Rt 2; Hooper NE 68031; 402-654-3399; Fax: 402-654-3342
Hoekenga, Owen; 101 Tucker Hall; University of Missouri; Columbia Mo 65211
Hoisington, David; CIMMYT; Lisboa 27, Aptdo. Postal 6-641; 06600 Mexico, D. F.; MEXICO; 525-726-7575; Fax: 525-726-7558; DHOISINGTON@ALPHAC.CIMMYT.MX
Hole, David; Plant Soils & Biometeorology Dept.; Utah State Univ.; Logan UT 84322-4820; (801)750-2235; Fax: (801)750-3376; dhole@mendel.usu.edu
Holland, Greg; Asgrow Seed Co; Box 460 Hwy 20 W; Parkersburg IA 50665; 319-347-6633; Fax: 319-347-2720
Holley, Randall; 340 Southside Dr.; Henderson KY 42420
Hollick, Jay; Institute of Molecular Biology; University of Oregon; Eugene OR 97403-1229
Hong, Guo-Fan; National Center for Gene Research; Chinese Academy of Science; 500 Cao Bao Road; Shanghai 200233; CHINA; 86-21-482-2885; Fax: 86-21-482-5775
Hong, Meng-Min; Academia Sinica; Shanghai Inst. of Plant Physiol.; 300 Fonglin Road; Shanghai 200032; CHINA; 2kxu@fudan.ihep.ac.cn
Houchins, Kate; 302 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2033; Kate@teosinte.agron.missouri.edu
Howard, John; Prodigene; 1500 Research Pkwy Suite 220; College Station TX 77845; 409-862-8537; Fax: 409-862-8538
Hsia, An-Ping; B420 Agronomy Hall; lowa State University; Ames IA 50011
Hu, Jianping; Dept of Botany; University of Georgia; Athens GA 30602
Hu, Gongshe; Dept Plant Path; 414 Throckmorton Hall; Kansas State Univ; Manhattan KS 66506
Huang, Bing-Quan; Biology Dept; Univ of North Dakota; Grand Forks ND 58202; 701-777-4479; Fax: 701-772-5041; bhuang@badlands.nodak.edu
Huang, Wei-Da; Fudan University; Dept. of Biochem; Handan Road 220; Shanghai 200433; CHINA
Huang, Wei-Da; Fudan University; Dept. of Biochem; Handan Road 220; Shanghai 200433; CHINA
Huang, China National Rice Research Institute; Hangzhou; Zhejiang 310006; CHINA
Huang, Yih-Ching; Department of Agronomy, National Taiwan University; Taipei; TAIWAN; (02)363-0231 ext. 2727; Fax: (02)362-0879
Hubbard, Lauren; USDA-ARS
                          hodges@btny.purdue.edu
  HUFFMANG@PHISHED.COM
Hulbert, Scot; Kansas State University; Dept. of Plant Pathology; Throckmorton Hall; Manhattan KS 66506-5502; 913-532-1392; Fax: 913-532-5692; shulbert@plantpath.ksu.edu
Hultquist, Marc A; Brown University; Box 1445; Providence RI 02912; 401-274-6486
Hunsperger, John P.; P.O. Box 2217; Gilroy CA 95021-2217; (408)848-1161
Hunter, Clifford; ICI Seeds; P.O. Box 500; Slater IA 50244
Hunter, Brenda; Dept Plant Sci; Forbes Hall, Room 303; Univ of Arizona; Tucson AZ 85721
Hussey: Patrick I.: Plant Sci; Forbes Hall, Room 303; Univ of Arizona; Tucson AZ 85721
   Hussey, Patrick J.; University of London; Royal Holloway New College; Dept. Blochem., Egham Hill; Egham, Surrey TW20 OEX; UNITED KINGDOM lida, Shigeru; Dept Biol Science & Technology; Science Univ of Tokyo; 2641 Yamazaki, Noda-shi; Chiba 278; JAPAN; 0471-24-1501 ex.4400; Fax: 0471-25-1841 lm, Kyung Hoan; Dept Plant Path; Univ of Florida; Gainesville FL 32611 lngham, Erika; 105 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-1168
   Innes, R. L.; DeKalb Canada Inc.; R.R. 2; Glanworth, Ont. NOL 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; Fax: 319-335-3620; erin-irish@uiowa.edu
Isaac, Peter; The Nickerson Seed Co Ltd; Cambridge Science Park; Milton Road; Cambridge CB4 4GZ; UNITED KINGDOM; 44-1223-423933; Fax: 44-1223-420925;
  Isaac, Peter; The Nickerson Seed Co Ltd; Cambridge Science Park; Milton Road; Cambridge CB4 4GZ; UNITED KINGDOM; 44-1223-423933; Fax: 44-1223-420925; mbnis @segnet.daresbury.ac.uk
shige, Teruo; Ministry Agric, For, Fisheries; Kasumigaseki, Chiyodaku; Tokyo; JAPAN; 81-3-3502-8111x5051; Fax: 81-3-3507-8794; tishige@s.affrc.go.jp
Ishikawa, Ryuji; Faculity of Agriculture; Hirosaki Univ; Hirosaki Aomori 036; JAPAN; 510-642-8058; ishikawa@cc.hirosaki-u.ac.jp
Ishizaki, Mary; Mycogen Plant Sciences; 5649 East Buckeye Rd; Madison WI 53716; 608-221-5000; Fax: 608-221-5008x364
Islam-Faridi, M. Nurul; Dept Soil and Crop Sci; Texas A&M Univ; College Station TX 77843-2474
Jackson, Janet Day; Crop Sci, AW-101 Turner; University of Illinois; 1102 S. Goodwin Ave; Urbana IL 61801; 217-333-6331; Fax: 217-333-6064; j-day@uiuc.edu
Jackson, Paul J.; Life Sci Div; LSZ MS M880; Los Alarmos Nat Lab; Los Alarmos NM 87545; 505-667-2775; Fax: 505-665-3024; JACKSON@telomere.lanl.gov
Jackson, David; USDA Plant Gene Expression Centre; 800 Buchanan St; Albany CA 94710; 510-559-5976; Fax: 510-559-5648; dJackson@nature.berkeley.edu
James Martha G.; Dept. of Biochemistry & Biophysics; Molecular Biology Building, Room 2152; Iowa State University; Ames IA 50011; 515-294-3818; Fax: 515-294-0453; mgjames@lastate.edu
  mgjames@iastate.edu

Jampatong, Sansern; Curtis Hall; University of Missouri; Columbia MO 65211

Jarboe, Sue G.; Purdue University; Department of Agronomy; 1150 Lilly Hall of Life Sciences; West Lafayette IN 47907-1150; (317)494-4772; Fax: (317)496-1368

Jayachandran, Sanjay; Botany & Plant Sciences; UC Riverside; Riverside CA 92521

Jeffries-Griffor, Joanne; DeKalb Genetics; 62 Maritime Rd; Mystic CT 06355

Jegla, Dorothy; Dept Blol 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; ; Fax: 510-642-4995; Ijesaiti@nature.berkeley.edu

Jewell@CGNET.com

Joanin, Philippe: Inst Biol Molec Plantes CNBS: Univ Louis Pasteur: 121 Bue du General Simmer: Strausbourg 67000; FBANCE
     Joanin, Philippe; Inst Biol Molec Plantes CNRS; Univ Louis Pasteur; 121 Rue du General Simmer; Strausbourg 67000; FRANCE
Jobling, Steve; Unilever Research; Colworth House; Sharnbrook; Bedford MK44 1LQ; UNITED KINGDOM; 44 1234 222575; Fax: 44 1234 222552;
STEVE.JOBLING@URCGB.SPRINT.COM
   Johal, Guri; Agronomy Dept; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-0342; Fax: 573-884-7850; AGROGJ@MIZZOU1.MISSOURI.EDU Johns, Mitrick A.; Dept Biological Sciences; Northern Illinois University; DeKalb IL 60115; 815-753-7836; Fax: 815-753-0461
Johnson, M. W.; Dept of Agronomy; Pennsylvania State Univ; University Park PA 16802; 814-865-0324; Fax: 825-863-7043
Johnson, Scott; Mycogen Plant Sciences; 301 Campus Dr; P.O. Box 280; Huxley IA 50124; 515-597-3284; Fax: 515-597-2875
  Johnson, Scott, Mycogen Plant Sciences; 301 Campus Dr. P.O. Box 280; Huxley IA 50124; 515-597-2875
Johnson, Lane; Agricultural Research; Green Giant Co; 1201 North 4th St; Le Sueur MN 56058; 612-665-3515; Fax: 612-665-2682
Johnson, Elmer C.; 1525 Vine St.; Belmont CA 94002
Johnson, William K.; University of California, Davis; CEPRAP; 1930 5th Street; Davis CA 95616; 916/757-3045
Johnson, Elizabeth; CIBA-GEIGY Corporation; P.O. Box 1830; Kaunakakai HI 96748; 808-567-6146; Fax: 808-567-6753
Johri, M. M.; Molecular Biology Unit; Tata Inst Fundamental Res; Homi Bhabha Road; Mumbai 400 005; INDIA; 215-2971; Fax: 091-22-215-2110; MMJOHRI@tifrvax.tifr.res.in
MMJOHRI@tifrvax.tifr.res.in

Jondle, Doug; Cargill Hybrid Seeds; 1502 N Gault; St. Peter MN 56082; 507-931-2940; Fax: 507-931-9691

Jones, Todd; E. I. DuPont de Nemours & Co; Experimental Station; P.O. Box 80402; Wilmington DE 19880-0402; 302-695-4372; Fax: 302-695-4296; jonest@esvax.dnet.dupont.com

Jones, John Edward; 8429 Meadow Green Way; Gaithersburg MD 20877

Jones, Mark; Selby Hall, O.A.R.D.C, USDA-ARS; 1680 Madison Ave; Wooster OH 44691; (216)263-3838; Fax: (216)263-3841; jones.390@osu.edu

Jones, John K.; Dept Agric Bot; University of Reading; Whiteknights; Reading RG6 2AS; UNITED KINGDOM; 44-1734-875123 EXT 7950; Fax: 44-1734-750630

Jones, Jonathan; Sainsbury Laboratory; John Innes Centre; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44-1603-452571; Fax: 250024; JONESJ@bbsrc.AC.UK

Julstrom, Paul; c/o CIMMYT; Lisboa 27, Apdo. Postal 6-641; 06600 Mexico, D.F.; MEXICO; 52 5 726 9091; Fax: 52 5 726 7558

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; Fax: 217-333-4777; j-juvik@uiuc.edu

Kaeppler, Shawn; Dept of Agronomy; University of Wisconsin; 1575 Linden Drive; Madison WI 53706; 608-262-9571; smkaeppl@facstaff.wisc.edu

Kaeppler, Heidi F.; USDA/ARS; 344 Keim Hall P.O. Box 830937; Univ of Nebraska; Lincoln NE 68583-0937; 402-472-9640; Fax: 402-437-5254;

AGRO237@UNLVM.UNL.EDU

Kahler, Alex; Biogenetic Services, Inc; 2308 6th Street E.; P.O. Box 710; Brookings SD 57006; 605-697-8500; Fax: 605-697-8507
```

Kahler, Alex; Biogenetic Services, Inc; 2308 6th Street E.; P.O. Box 710; Brookings SD 57006; 605-697-8500; Fax: 605-697-8507

```
Kaleikau, Ed; USDA, NRICGP; 901 D. Street, SW, Room 323; Washington DC 20250; 202-401-1901; Fax: 202-401-6488; EKALEIKAU@REEUSDA.GOV Kalia, V.; Regional Research Station; Dhaulakuan-173001; Distt Sirmur (H. P.); INDIA Kang, M. S.; Department of Agronomy; Louisiana State University; Baton Rouge LA 70803; 504-388-2110; Fax: 504-388-1403 Kang, Chun-Lin; Hunan Agricultural College; Dong Jiao; Changsa; Hunan 410128; CHINA Kannenberg, L. W.; Dept of Crop Science; University of Guelph; Guelph, Ontario N1G 2W1; CANADA; 519-824-4120 EXT 2506; Fax: 519-763-8933; LKANNENB@CROP.UOGUELPH.CA
  Kantety, Ramesh; Dept of Agronomy; 1150 Lilly Hall; Purdue University; West Lafayette IN 47907-1150; 317-496-2729; Fax: 317--494-6508; RKANTETY@DEPT.AGRY.PURDUE.EDU
RKANTETY®DEPT.AGRY.PURDUE.EDU

Karpoff, Arnold; Dept of Biology; Univ of Louisville; Louisville KY 40292; 502-852-5934; Fax: 502-852-0725; AJKARP01@homer.Louisville.edu

Kasha, Ken; Dept of Crop Science; Univ of Guelph, Guelph, Ontario N1G 2W1; CANADA; 519-824-4120 EXT 2507; Fax: 519-763-8933

Kass, Lee; L. H. Bailey Hortorium; 462 Mann Library; Cornell Univ; Ithaca NY 14853; 607-255-2131; LBKBHWON@aol.com

Kaszas, Etienne; Division of Biological Sciences; Tucker Halt; University of Missouri; Columbia MO 65211; 573-882-4871; Fax: 573-882-0123

Katayose, Yuichi; Rice Genome Res Program, STAFF Inst; 446-1, Ippaizuka, Kamiyokoba; Ippaizuka, Tsukuba-shi; Ibaraki-ken 305; JAPAN; +81-298-38-2199; Fax: +81-298-38-2302; katayose@abr.affrc.go.jp

Kato, Akio; Lab. Mutation Induct. Techn.; Inst. Radiation Breeding, NIAR; P.O. Box-3, Ohmiya-machi-Naka-gun; Ibaraki-ken, 319-22; JAPAN; 02955 (2) 1138

Kato Y., T. A.; Centro de Genetica; Colegio de Postgraduados; Chapingo 56230; MEXICO; (595)4-52-65; Fax: (595)4-57-23

Katt, Maria L.; Pioneer Hi-Bred Intl., Inc.; 7300 N.W. 62nd Avenue; P.O. Box 1008; Johnston IA 50131; 515-270-4370; Fax: 515-270-3444

Keddie, James; Plant Biology Dept; UC Berkeley; Berkeley CA 94720

Keeling, Peter; ExSeeds Genetics L.L.C.; 1567 Food Sci Bldg; Iowa State Univ; Ames IA 50011-1061; 515-294-3259; Fax: 515-294-2644; pkeeling@iastate.edu

Keim, Kent R.; Sunseeds; 1200 Anderson Corner Road; Parma ID 83660; 208-674-2208; Fax: 208-674-2372

Keith, Carlyn S.; Dept. Biol. Sciences; 105 Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-8033; ckeith@blosci.mbp.missouri.edu

Kellogg @oeb.harvard.edu

Kellogg @oeb.harvard.edu

Kendall, Tim; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave., PO Box 1004; Johnston IA 50131-1004; 515-270-5952; Fax: 515-270-3367; KENDALLTL@PHIBRED.COM
              KENDALLTL@PHIBRED.COM
  Kendra, David F.; Northrup King Company; 317 330th Street; Stanton MN 55018; 507/663-7636; Fax: 507/645-7519; dkendra@MR.Net Kent, Beth; 220 Biological Sciences Center; 1445 Gortner Ave.; St. Paul MN 55108; (612)625-0271; Kent0021@maroon.tc.umn.edu Kermicle, Jerry; 218 Genetics Dept; 445 Henry Mall; University of Wisconsin; Madison WI 53706; 608-262-1253; Fax: 608-262-2976
  Kerstetter, Randall; Plant Gene Expression Center, USDA; 800 Buchanan Street; Albany CA 94710; (510)559-5922; Fax: (510)559-5648; RAND@NATURE.BERKELEY.EDU
HAND@NATURE.BERKELEY.EDU
Khairaillah, Mireille; CIMMYT; Apdo. Postal 6-641; Mexico, D.F. 06600; MEXICO; 415-833-6655; Fax: 415-833-6656; mkhairaillah@cimmyt.mx
Khanna, Anupama; Illinois State University; 4120 Biological Sciences; Normal IL 61790-4120; 309-438-3088; Fax: 309-438-3722; AKHANN@acadcomp.cmp.ilstu.edu
Khavkin, Emil E.; Inst Agric Biotech; 42 Timiryazevskaya ul.; Moscow, 127550; RUSSIA; (7-095)976-6544; Fax: (7-095)977-0947; 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, Byung Dong; Dept of Horticulture; Seoul National Univ; 103 Seodoon-dong; Suwon 441-744; KOREA; 82-331-290-2563; Fax: 82-331-290-2563;
Kim Song Kwon; Internat Inst Tropical Agr P M B, 5320; Ibadan; NIGERIA; Fax: 44-81-681-8583
  Kim, Soon Kwon; Internat Inst Tropical Ag; P.M.B. 5320; Ibadan; NIGERIA; ; Fax: 44-81-681-8583
Kindiger, Bryan; Southern Plains Research Stn.; 2000 18th St.; Woodward OK 73801; (405)256-7449; Fax: (405)256-1322; bkindige@ag.gov Kiss, Charles; 18 Avenue Gallieni; 49130 Les Ponts de Ce; FRANCE; 33-2-41-44-97-97; Fax: 33-2-41-44-98-69 Kleese, Roger; 6700 80th Ave. N.; Brooklyn Park MN 55445; 612-566-3561; Fax: 515-254-2744 Klein, Anita S.; Department of Biochemistry; Spaulding Life Science Bldg; University of New Hampshire; Durham NH 03824; 603-862-2455; Fax: 603-862-4013;
              klein@Colsa.UNH.EDU
  Kloeckener, Barbara; Univ. of CA - Berkeley; Dept. of Plant Biology; 111 Koshland Hall; Berkeley CA 94720; 510-642-7085; Fax: 510-642-4995; bkg@nature.berkeley.edu
 Knapp, Steve; Department of Crop & Soil Science; Oregon State University; Corvalis OR 97331-2902 Ko, Chris; Dept Biol Sci; Stanford Univ; Stanford CA 94305-5020
 Koch, Karen E.; 2147 Fifield Hall; Horticulture Dept, Plant Molec Cell Biol; University of Florida; Gainesville FL 32611; 352-392-4711 ext 309; Fax: 352-392-6479; kek@gnv.ifas.ufl.edu
Kek & gnv. Itas. uni. edu
Kochert, Gary; University of Georgia; Department of Botany; Plant Sciences building; Athens GA 30602
Koester, Ruth; Genetic Design, Inc.; 7017 Albert Pick Rd.; Greensboro NC 27409; 919-668-1432; Fax: 919-665-3966
Kohashi, Josue; Dept of Botany; Colegio de Postgrad; Chapingo, Edo de Mex; MEXICO; (595)4-22-00 ext.5294; Fax: (595)428-73
Koinuma, Keiichi; Kyusyu Natl. Agric. Exp. Stn.; Yokoichi; Miyakonojo; Miyazaki 885; JAPAN; 0986-22-1506; Fax: 0986-23-1168
Konstantinov, Yuri; Siberian Inst Plant Phys Bioch; Russian Academy of Sciences; P.O. Box 1243; Irkutsk-33 664033; RUSSIA; 39-52-46-09-03; Fax: 7-39-52-46-16-03; root@sifibr.irkutsk.su
Konstantinov, Kosana; Maize Research Institute; P.O. Box 89; 11081 Zemun; YUGOSLAVIA; (381)11-617-434; Fax: (381)11-197-890
Kowalewski, Shirley; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2674; Fax: 573-884-7850; 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; Fax: 507-457-1633; DKOWLES@REX.MNSMC.EDU
 Kravchenko, O. A.; Sprinchenogia Stz. 1; Institute of Genetics; Kishinev, MD 2028; MOLDOVA
Krawetz, Julie; Dept of Botany; Box 7612; North Carolina State Univ; Raleigh NC 27695; 919-515-3570; KRAWETZ@UNITY.NCSU.EDU
Krebbers, Enno; DuPont de Nemours & Co.; Agricultural Biotechnology; Experimental Station 402/2253; Wilmington DE 19880-0402; (302)695-8577; Fax: (302)695-
              7361; krebbers@esvax.dnet.dupont.com
Kreisman, Lori S; Dept Biol Sci; Gibb Hall; Univ of Idaho; Moscow ID 83844; ; krei9540@uidaho.edu
Krivov, Nicolai V.; Institute of Genetics, of AS RM.; Padurie str.; 20, Kishinev-277002; MOLDOVA; (0422)622068; Fax: (3732)556180; crivov@centr.moldova.su
Kriz, Al; DeKalb Plant Genetics; 62 Maritime Dr.; Mystic CT 06355-1958; (203)572-5217; Fax: (203)572-5240; akriz@dekalb.com
Krone, Todd; Asgrow Seed Co; 634 E. Lincoln Way; Ames IA 50010-6598; 515-232-7170; Fax: 515-232-6905;
Krueger, Roger W.; Monsanto; 800 N. Lindbergh Blvd. C2NA; St. Louis MO 63167; 314-694-3677; Fax: 314-694-5926; RWKRUE@ccmail.monsanto.com
Kuhn, William E; Mid-Corn Bell/Specialty Res Dir.; Pioneer Hi-Bred Internatl., Inc.; 7300 NW 62d Ave., PO Box 1004; Johnston IA 50131-1004; 515-270-3362; Fax: 515-
              253-2288
Kumar, M.; Department of Genetics; Rajendra Agric. Univ., Bihar; Pusa (Samastipur)-848125; INDIA
Kumar, Sushil; Central Inst. of Medicinal & Aromatic Plants; PO CIMAP; Lucknow 226015; INDIA
Kunze, Reinhard; Inst of Genetics; Univ of Munich; Maria-Ward-Str. 1a; 80638 Muenchen; GERMANY; 49-89-17 91 98-68; Fax: 49-89-17 91 98-20;
UJ44208@SUNMAIL.LRZ-MUENCHEN.DE
  Kurata, Nori; Rice Genome Res Program, STAFF Inst; 446-1, Ippaizuka, Kamiyokoba; Ippaizuka, Tsukuba-shi; Ibaraki-ken 305; JAPAN; +81-298-38-2199; Fax: +81-298-
              38-2302
Kuzmin, Evgeny V.; Dept Biol Sci; Univ of Missouri; Columbia MO 65211; 573-882-8033; ekuzmin@biosci.mbp.missouri.edu

Kyetere, Denis T.; OARDC, Dept of Agronomy; 1680 Madison Avenue; Wooster OH 44691

Labate, Joanne; Iowa State Univ; Dept of Agronomy; Ames IA 50011; 515-294-8704; Fax: 515-294-9359; jlabate@iastate.edu

Laccetti, Lucille B.; DeKalb Plant Genetics; 62 Maritime Park; Mystic CT 06355; 203-572-5247; Fax: 203-572-5240

Lai, Jinsheng; Lab Molec Genetics; College of Biology; Beijing Agricultural University; Beijing 100094; CHINA; 86(1)2582244x374; Fax: 86(1)2582332; chengxu@bepc2.ihep.ac.cn
```

Lakra, Alejandro Blanco; CINVESTAV; Apdo Postal 629; Irapuato, GTO; MEXICO; ; Fax: (462)51282

```
Lambert, Antoine; Verneuil Recherche; 33, rue de la Croix Blanche; 49630 Corne; FRANCE; 41-66-17-00; Fax: 41-45-02-61 Lamkey, Kendall; Agronomy Building; Iowa State University; Ames IA 50011; 515-294-7826; Fax: 515-294-9359; KRLAMKEY@IASTATE.EDU Lampoh, E.; Crops Res Inst; PO Box 3785 Kumasi; Ashanti Region; GHANA; 6221 Lanahan, Michael; c/o Ciba; P.O. Box 12257; Research Triangle NC 27709-2257; 919-541-8513
     Lane, Barbara; Department of Plant Biology; 351 Koshland Hall; University of California; Berkeley CA 94720; 510-642-8058; Fax: 510-642-4995;
    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; ENGLAND; 011-44-865-275099; Fax: 011-44-865-275147;
    jane.langdale@plants.ox.ac.uk
Larkin, John C.; Dept. Genetics Cell Biol.; 1445 Gortner Ave.; University of Minnesota; St. Paul MN 55108-1095; 612-624-3607; Fax: 612-625-5754; jlarkin@molbio.cbs.umn.edu
   Larkins, Brian; Dept Plant Sciences; University of Arizona; Building #36; Tucson AZ 85721; (520)621-9958; Fax: (520)621-3692; LARKINS@ag.arizona.edu
Last, Robert; Boyce Thompson Inst Plant Res, Inc.; Tower Road; Ithaca NY 14850; 607-254-1325; Fax: 607-254-1242; RLL3@cornell.edu
Laudencia-Chingcuanco, Debbie; Plant Gene Expression Center; USDA-ARS; 800 Buchanan St.; Albany CA 94710; 510-559-5968; Fax: 510-559-5678
Laurie, David; JJ Centre for Plant Science Res.; Colney Lane; Norwich; Norfolk NR4 7UJ; UNITED KINGDOM; 44-1603- 52571; Fax: 44-1603-502241;
LAURIED@BBSRC.AC.UK
   Lawson, Emily; Plant Science Institute; Dept of Biology; Univ of Pennsylvana; Philadelphia PA 19104-6018
Lawton, Amy; 105 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-1168
Lazarowitz, Sondra G.; Dept. of Microbiology; U. of III./131 Burrill Hall; 407 S. Goodwin Av.; Urbana IL 61801; 217-333-0390; Fax: 217-244-6697;
                   Lazarowitz@UIUC.EDU
     Leaver, Chris; Dept Plant Sciences; University of Oxford; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; 01865 275143; Fax: 01865 275144;
Leaver, Chris; Dept Plant Sciences; University of Oxford; South Parks Road; Oxford OX1 3RB; UNITED KINGDOM; 01865 275143; Fax: 01865 275144; chris.leaver@plants.ox.ac.uk
Ledger, Elizabeth; NMSU; Biology Dept; Box3AF Dept 30001; Las Cruces NM 88003; 505-646-7963; Fax: 505-646-5665; eledger@nmsu.edu
Lee, Elizabeth A.; Marsh Life Sciences Bldg.; University of Vermont; Burlington VT 05405-0086; (802)656-0894; Fax: (802)656-0440; elee@moose.uvm.edu
Lee, Michael; Department of Agronomy; lowa State University; Ames IA 50011; 515-294-3052; Fax: 515-294-3163; mlee@lastate.edu
Lee, Warren; 4120 Dept. of Biol. Sciences; Illinois State University; Normal IL 61790-4120; 309-438-3088; Fax: 309-438-3722
Lee, Hsueh-Sheng; Zoology Dept; University of Texas; Austin TX 78712-1064
Lee, Danny; Section of Plant Biology; Robbins Hall c/o Neellima Sinha; UC Davis; Davis CA 95616
Lee, Hee Bong; Dept Agronomy. Coll Agriculture; Chungnam Natl Univ; 220 Gung-Dong, Yusung-Gu; Taejon 305-764; KOREA; 82-042-821-5721; Fax: 82-042-823-8050
Lee, In-sup; Department of Biology; College of Science; Kyungsung University; Pusan, 608-736; SOUTH KOREA; 051-620-4647; Fax: 051-627-4115
Leland, Tim; CIBA Biotechnology Res; P.O. Box 12257; Research Triangle Park NC 27709-2257; 919-541-8680; Fax: 919-541-8557
Lemaux, Peggy; Department of Plant Biology; 111 Koshland Hall; University of California, Berkeley; Berkeley CA 94720; 510-642-1589; Fax: 510-642-7356; lemauxgg@nature.berkeley.edu
Leon, Patricia; Dept Biol Sci; Stanford Univ; Stanford CA 94305-5020
Lemer, David; Biology Dept 0116; UC San Diego; La Jolla CA 92093-0116; 619-534-2514; Fax: 619-534-7108; drlerner@ucsd.edu
Leroy, Philippe; INRA; Domaine De Crouelle; 63039 Clermont-Ferrand; Cedex 2; FRANCE; 33 73 624337; Fax: 33 73 624453; leroy@valmont.clermont.inra.fr
Lesnick, Marc; Institute of Molecular Biology; University of Oregon; Eugene OR 97403; lesnickembloi.uuregon.edu
Letovsky, Stan; 117 Woodlawn Rd; Ballimore MD 21210; 410-614-1061; letovsky@gdb.org
Levites, E. V.; Inst Cytol Genetics; Novosibi
                    chris.leaver@plants.ox.ac.uk
 Li, Yuxing; 2312 Food Sciences; Iowa State Univ; Ames IA 50011
Li, Xianggan; 2116 MBB; Dept. of Genetics; Iowa State University; Ames IA 50011; (515)294-5054; Fax: (515)294-0345; shonlee@iastate.edu
Li, De-Bao; Zhejiang Agricultural University; Biotechnology Institute; Hangzhou; Zhejiang 310029; CHINA
Li, Xiao-Fang; Guangdong Academy of Agric. Sciences; Rice Research Institute; Wushan, Guangzhou; Guangdong 510640; CHINA
Li, Ping; Sichuan Agricultural University; Rice Research Institute; Yaan; Sichuan 625014; CHINA
Li, Qing; China Natl. Cente Biotech. Development; P.O. Box 8118; Beljing 10008; CHINA
Li, Jiansheng; Maize Research Lab; Department of Agronomy; Huazhong Agricultural University; Wuhan, Hubei 430070; CHINA; 86-27-7815681-8545; Fax: 86-27-7815057
7815057

Li, Yongzhong; Maize Inst., Jilin Acad. Agric. Sciences; 6-West Xinghua Street; Gongzhuling; Jilin, P. R., 136100; CHINA; 04441-215179; Fax: 04441-214884

Lijegren, Sarah; Dept of Biology 0116; UC San liego; La Jolla CA 92093-7108

Lin, Liang-Shiou; Room 323 Aerospace Bldg.; 901 D St. SW; Washington DC 20250-2241; 202-401-5042; Fax: 202-401-6488; Ilin@reeusda.gov

Lin, Robert; Oklahoma State University; Dept of Agronomy; Stillwater OK 74078-0507

Lin, Bor-yaw; Institute of Molecular Biology; National Chung Hsing University; Taichung 402; TAIWAN; (04)285-1885; Fax: (04)287-4879; bylin@dragon.nchu.edu.tw

Lincoln, Cindy; USDA Plant Gene Expression Center; 800 Buchanan St.; Albany CA 94710; 510-559-5968; Fax: 510-559-5648; clincoln@nature.berkeley.edu

Ling, Peng; 700 Experiment Station Road; Lake Alfred FL 33850; 813-956-1151 ext.301

Lisch, Damon; Dept of Plant Biology; University of California; Berkeley CA 94720; 501-642-7085

Liu, Yanhong; Botany Dept.; Univ. Georgia; Athens GA 30602; (706)542-1857; Fax: (706)542-1805; yanhong@dogwood.botany.uga.edu

Liu, QinQin; Dept of Biology, College Sci Eng; 317 Life Sci Bldg; 10 University Drive; Duluth MN 55812-2496; 218-726-7271; Fax: 218-726-8142; qliu1@d.umn.edu

Liu, Aimin; Jiangsu Academy of Agric. Science; Institute of Agrobiol. Genet. & Prodeding Popt.; Hangzhou 310006; CHINA

Liu, Liang-Shi; Zhongshan University; Biotechnology Research Center; Guangzhou 510275; CHINA

Liu, Liang-Shi; Zhongshan University; Biotechnology Research Center; Guangzhou 510275; CHINA
   Liu, Llang-Shi; Zhongshan University; Biotechnology Research Center; Guangzhou 510275; CHINA
Liu, Xiangdong; South China Agricultural University; Agronomy Dept.; Wushan, Tianhe, Guangzhou; Guangdong 510642; CHINA
Lonsdale, David M.; Cambridge Laboratory; IPSR; Colney Lane; Norwich NR4 7UH; UNITED KINGDOM; 44 1603 452571; Fax: 44 1603 505725;
   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; Fax: 011-
   55-31-773-9252; mauricio@cnpms.embrapa.br
Loraine, Ann; Plant Biology Dept; UC Berkeley; Berkeley CA 94720
Lorenzen Dahl, Lisa; Pioneer Hi-Bred Internat., Inc.; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004
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; Fax: (523)599283; genetica@ipcucsc.bitnet
Lorio, Julio C.; Biology Dept.; 324 Tucker Hall; University of Missouri; Columbia MO 65211
Lorz, Horst; Institut Allgemeine Botanik; Universitat Hamburg; Ohnhorststrasse 18; 22609 Hamburg; GERMANY; 49-40-82282-420; Fax: 49-40-82282-229
Loukides, Cynthia; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102
Lowe, Brenda; 204 Old Evarts Lane; Mystic CT 06355-2153; 203-572-5226; Fax: 203-572-5240; blowe@minerva.cis.yale.edu
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, Yingtang; Dept. of Plant Biology; 111 Koshland Hall; Univ. of Cal.; Berkeley CA 94720; ; yingtang@nature.berkeley.edu
Lu, Xiaowu; Department of Horticulture; University of Hawaii; Honolulu HI 96822; ; xiao@hawaii.edu
Lu, Xiaowu; Dept of Horticulture; Univ of Hawaii; 3190 Maile Way; Honolulu HI 96822-2279; 808-956-2156; xiao@hawaii.edu
Lu, Yong-Gen; South China Agricultural University; Guangzhou 510642; CHINA
```

Lambert, Robert J.; Crop Science, Tumer Hall; Univ of Illinois; 1102 S. Goodwin Ave.; Urbana IL 61801-4798

```
Ludwig, Steven; 709 E. Capitol St., S.E.; Washington DC 20003
   Luebberstedt, Thomas; Inst fur Pflanzenzuchtung; Universitat Hohenheim; 70593 Stuttgart; GERMANY; ; Fax: Ge-711-4592343 Luo, Rui Bai; Biology Dept, Lehman College; 250 Bedford Park Blvd West; CUNY; Bronx NY 10468; ; RBLLC@CUNYVM.CUNY.EDU
 Luo, Rui Bai; Biology Dept, Lehman College; 250 Bedford Park Blvd West; CUNY; Bronx NY 10468; ; RBLLC@CUNYVM.CUNY.EDU
Luo, Ming; Sichuan Agricultural University; Rice Research Institute; Yaan; Sichuan 62500; CHINA
Luthe, Dawn S; Dept Biochemistry; Miss State Univ; Mississippi State MS 39762; 601-325-7733; Fax: 601-325-8664; dsluthe@ra.msstate.edu
Lutz, Sheila; Dept Agron Plant Genetics; 411 Borlaug Hall, 1991 Butord Circle; Univ of Minnesota; St. Paul MN 55108
Lynch, Margaret; 1883 21st Ave; San Francisco CA 94122
Lysikov, V. N.; Inst. of Genet. of AS RM.; st. Paudurie 20; Kishinev-277002; MOLDOVA; (0422)622068; Fax: 3732-556180
Ma, Hong; Cold Spring Harbor Laboratories; P.O. Box 100; Cold Spring Harbor NY 11724; 516-367-8309; Fax: 516-367-8369; mah@cshl.org
MacDonald, M. V.; Plant Breeding International; Maris Lane; Trumpington, Cambridge CB2 2LQ; UNITED KINGDOM; 01223-840411; Fax: 01223-844425
Machado, Joaquim A.; Rua D. Pedro I 424 Ap 71D; 13065075 Campinas, SP; BRAZIL; 0192-427501; Fax: 016-6511321; jmachado@convex.com.br
MacRae, Amy F.; Biology Dept; Univ of Missouri - St. Louis; 8001 Natural Bridge Rd; St. Louis MO 63121; 314-516-6205; Fax: 314-516-6233; SAFMACR@JINX.UMSL.EDU
Maddock, Sheila E: Pioneer Hi-Bred Int Inc.; 7300 NW 62nd Ave.: PO Box 1004; Johnston IA 50131-1004; 515-270-4047; Fax: 515-270-3444; maddocks@phibred.com
  Maddock, Sheila E; Pioneer Hi-Bred Int Inc.; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004; 515-270-4047; Fax: 515-270-3444; 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; Fax: 515-253-2149;
             maddoxj@phibred.com
   Magill, Clint; Texas A & M University; Dept. of Plant Pathology; College Station TX 77843; 409-845-8250; Fax: 409-845-6483; c-magill@tamu.edu
  Maguire, Marjorie; Zoology Department; University of Texas; Austin TX 78712-1064; 512-471-7451; Fax: 512-471-9651; marjm@mail.ulexas.edu Maheshwari, J. K.; National Botanical Res Inst; H.I.G.-130, Sector 'E'; Aliganj Extension, Aliganj; 226 020, U.P. - Lucknow; INDIA; 72655; Fax: 244330
   Maillet, Dan; Dept Plant Sciences; Univ of Western Ontario; London, Ontario N6A 5B7; CANADA
   Maluf, Mirian; Crop Science; Turner Hall, 1102 S. Goodwin Ave.; University of Illinois; Urbana IL 61801
  Mangano, Mary Lou; DeKalb Genetics Corp.; 62 Maritime Dr.; Mystic CT 06355
Manjunath, Sivalinganna; Dept Bot & Plant Sci; Batchelor Hall; Univ California; Riverside CA 92521-0124; 909-787-6376; manju@ucrac1.ucr.edu
   Manzocchi, Lucia A.; Consiglio Nazionale Ricerche; Istituto Biosintesi Vegetali; Via Bassini 15; 20133 Milano; ITALY; 0039-2-70600170; Fax: 0039-2-2362946;
             luman@icm.mi.cnr.it
  Marillonnet, Sylvestre; Dept of Botany; 2502 Plant Sciences; Univ of Georgia; Athens GA 30602-7271; 706-542-1857; Fax: 706-542-1805; MARILLONNET@BSCR.UGA.EDU
  Marocco, Adriano; Universita Cattolica S. Cuore; Istituto Di Genetica Vegetale; Via E. Parmense 84; 29100 Piacenza; ITALY; 39-523-599207; Fax: 39-523-599283;
Marocco, Adriano; Universita Cattolica S. Cuore; Istituto Di Genetica Vegetale; Via E. Parmense 84; 29100 Piacenza; ITALY; 39-523-599207; Fax: 39-523-599283; amarocco @pc.unicatt.it

Marshall, Lori; Holden's Foundation Seeds, Inc.; P.O. Box 839; 201 N. Maplewood Ave; Williamsburg IA 52361; 319-668-1100; Fax: 319-668-2453

Martienssen, R. A.; Cold Spring Harbor Labs; P.O. Box 100; Cold Spring Harbor NY 11724-2212; 516-367-8322; MARTIENS@CSHL.ORG

Martin, Michael J.; ICI Seeds; P.O. Box 500; Slater IA 50244; (515)685-3574; Fax: (515)685-3828

Martin Sanchez, J. A.; UdL-IRTA Centro R S D; Alcalde Rovira Roure 177; 25006 Lerida; SPAIN; 34-73-702569; Fax: 34-73-238301

Mascarenhas, Joseph P.; Department of Biol Sci; State University of New York; Albany NY 12222; 518/442-4388; Fax: 518/442-4354; jm558@cnsunix.albany.edu

Mascia, Peter N.; Cargill Hybrid Seeds; RR 3, Box 751; Aurora IL 60506; 708-801-2304; Fax: 708-801-2345; peter_mascia@cargill.com

Mather, Diane E.; Plant Sci Dept - Macdonald Col; McGill Univ - 21111 Lakeshore; Ste-Anne-de-Bellevue; Quebec H9X 3V9; CANADA; (514) 398-7851x7854; Fax: (514)398-7897; MATHER@AGRADM.LAN.MCGILL.CA

Mathem, Julie: USDA-ARS-PGEC: 800 Buchanan Street: Albany CA 94710: (510)559-5907; Fax: (510)559-5678; imatherm@nature.berkelev.edu
 Mathern, Julie; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; (510)559-5907; Fax: (510)559-5678; jmathem@nature.berkeley.edu Mathur, D. S.; Div. of Genetics; Indian Agr Res Inst; New Delhi-110012; INDIA; 581481

Matias, Dayana; Lehman College, CUNY; Biology Dept c/o Elli Wurtzel; 250 Bedford Park Blvd West; Bronx NY 10468

Matthews, Paul; Lehman College, CUNY; 250 Bedford Park Blvd West; Bronx NY 10468
 Matz, Eileen C.; Dept of Biology; Building 463; Brookhaven National Laboratory; Upton NY 11973; 516-344-3396; Fax: 516-344-3407; matz@bnlux1.bnl.gov Mawgood, Ahmed L. Abdel; 31 Saada St; Karmous; Alexandria; EGYPT; (203)492-1718; mawgood@Alex.eun.eg
 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; Fax: 302-695-7361;
            mazur@esvax.dnet.dupont.com
 McCarthy, Susan A.; National Agricultural Library; 10301 Baltimore Blvd; Beltsville MD 20705; 301-504-6875; Fax: 301-504-7098; SMCCARTH@ASRR.ARSUSDA.GOV
 McCarty, Donald R.; Vegetable Crops Department; 1255 Fifield Hall; University of Florida; Gainesville FL 32611; 352-392-1928 ext 322; Fax: 352-392-6479;
             drm@gnv.ifas.ufl.edu
drm@gnv.itas.ufl.edu

McConnell, R. L.; Research & Product Development; Pioneer Hi-Bred International; Box 85; Johnston IA 50131; (515)270-3363; Fax: (515)253-2125; McConnell, @phibred.com

McConnell, D. J.; Genetics, Trinity College; University of Dublin; Dublin 2; IRELAND; 353-1-7021140; Fax: 353-1-714968

McCormick, Sheila; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710; 510-559-5906; Fax: 510-559-5678; sheilamc@mendel.berkeley.edu

McCurlough, Andrew; Dept. of Biochemistry; Baylor College of Medicine; Houston TX 77030; 713-798-4622; andrewm@bcm.tmc.edu

McCurdy, Leroy; P.O. Box 77; McCurdy Seed Co; Fremont IA 52561; 515-933-4291

McElver, John; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave.; PO Box 1004; Johnston IA 50131-1004

McFerson, John; Asgrow, Seed Co; 534 E, Lincoln Way: Ames IA 50010; (515)232-7170; Fax: (515)232-6905
 McFerson, John; Asgrow Seed Co.; 634 E. Lincoln Way; Ames IA 50010; (515)232-7170; Fax: (515)232-6905
McLaren, James S.; Inverizon Int Inc; 2214 Stoneridge Terrace Court; St. Louis MO 63017-7120; 314-530-6943; Fax: 314-530-6945; mclaren@inverizon.com
McMullen, Michael; Agronomy Dept; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-7606; Fax: 573-884-7850; mcmullen@teosinte.agron.missouri.edu McWhirter, Ken; 127 Victoria Road; W. Pennant Hills NSW 2125; AUSTRALIA; 02-484-7417

Mead, Doug; Northrup King Co.; 317 330th St.; Stanton MN 55018-4308; (507)663-7623; Fax: (507)645-7519

Meeley, Robert; Trait and Technology Dev.; Pioneer Hi-Bred Int'l; 7300 NW 62nd Ave. - Box 1004; Johnston IA 50131-1004; 515-270-3770; Fax: 515-253-2149;
             MeeleyRB@phibred.Com
 Meghji, Moez; CIBA-GEIGY Corp; RR13/Box166; Rt 51 S at Hendrix Rd; Bloomington IL 61704; 309-823-8578; Fax: 309-823-9468; meghjim@AM.abru.cg.com Mei, Mantong; South China Agricultural University; Genetic Engineering Laboratory; Guangzhou 510642; CHINA Melchinger, Albrecht E.; Universitat Hohenheim; Institut für Pflanzenzuchtung; Postfach 70 05 62 (350/1); D-70593 Stuttgart; GERMANY; 0711-459-2334; Fax: 0711-459-2343
459-2343
Melia-Hancock, Susan; Curtis Hall - Agronomy; University of Missouri; Columbia MO 65211; 573-882-2674; Fax: 573-884-7850
Mella-Sampayo, Tristao; R. Padre Francisco 16, 5.F.; 1300 Lisboa; PORTUGAL
Mena, Montana; UC San Diego; Dept of Biology, 0116; La Jolla CA 92093-7108
Messing, Jo; Rutgers, The State Univ; Waksman Institute; P.O. Box 759; Piscataway NJ 08855-0759; 908-445-4256; Fax: 908-445-0072; messing@mbcl.rutgers.edu
Messmer, MM; Univ Hohenheim; Inst Pflanzenzuchtung; Postfach 70 05 62 (350/1); D-7000 Stuttgart 70; GERMANY
Mettler, I. J.; Northrup King Co.; 317 330th Street; Stanton MN 55018-4300; 507-663-7643; Fax: 507-645-7519; mettler@mr.net
Meyer, Terry; Pioneer Hi-Bred International; 7300 N.W. 62nd Avenue; P. O. Box 1004; Johnston IA 50131-1004; 515-270-3962; Fax: 515-270-3367;
MEYERTE@PHIBRED.COM
MEYERTE@PRIBRED.COM
Meyerowitz, Elliot; Biology Dept 156-29; California Inst Tech; Pasadena CA 91125; 818-395-6889; Fax: 818-449-0756; meyerowitze@starbase1.caltech.edu
Michelini, Luiz Antonio; R. Ayrton Playsant, 21; Ponta Grossa; Parana 84100-550; BRAZIL; 55-42-223-2774; Fax: 55-42-223-2774
Michiels, Frank; Plant Genetic Systems; Jozef Plateaustraat 22; Gent B-9000; BELGIUM; 32-9-2358475; Fax: 32-9-2240694; FRAL@PGSGENT.BE
Micu, V. E.; Scientific Res Inst Maize & Sorghum; Pashcani; Criuleni; 278336 Moldova; MOLDOVA; (3732)-22-24-78; Fax: (3732)-22-73-02
Mies, David; Northrup King; 306 Meadow Drive - Box 710; St. Joseph IL 61873; 217-469-2746; Fax: 217-469-2407
Mihm, John A.; French Agricultural Research; RR2, Box 294; Lamberton MN 56152; 507-752-7274; Fax: 507-752-6132; JMihm@Prairie.lakes.com
Mikula, Bernard C.; Defiance College; 901 College Drive; Defiance OH 43512; 419-784-4010 EXT 426; Fax: 419-784-0426
```

```
Milach, Sandra; 1991 Buford Circle; Room 411 Borlang Hall; St. Paul MN 55108; (612)625-6223; Fax: (612)625-1268
Milas, Donald; Tucker Hall; Div. Biological Sciences; University of Missouri; Columbia MO 65211; 573-882-7933; milas@ biosci.mbp.missouri.edu
Millard, Mark; USDA-ARS-NCPP; State and Mortensen Rd; Ames IA 50010; 515-292-6500; Fax: 515-292-6509; nc7mm@ars-grin.gov
Min, Reiquang; Dept Soil Crop Sci; Texas A&M Lohiv; College Station TX 77843; 409-862-2459; Fax: 20-845-0456; reiguan@tam2000.tamu.edu
Minnang; Dept Soil Crop Sci; Texas A&M Lohiv; College Station TX 77843; 409-862-2459; Fax: 39-845-0456; reiguan@tam2000.tamu.edu
Minnan; Phil; PO Box 25217; Monument Park; Protoria 0105; SOUTH AFRICA; ; philmin@solo, pipex.co.za
Miranda, Luiz; Eugenio C 6r; Av B de Itapura; 1481; CX Postal 28; Campinas P 13001-970; BRAZIL; 019-23154222150
Miura, Y; Maize Breeding Station; Hokkaido Natl. Agr. Exp. Sln.; Hitsuligaoka; Sapporo 062; JAPAN
Miyoshi, K.; Sakata Seed Corp.; Kakegawa Breed, STN, Yoshioka 1743-2; Nakegawa City, Shizukoka City 436-01; JAPAN
Modena, Stephen; Dept. of Crop Sciences, North Carolina State Univ.; Box 7620; Raleigh NC 27695-7620; 919-515-2246; Fax: 919-515-7959; ab4el@Cybernetics.NET
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
Mononey, Mark; UC Berkeley; Plant Biology Dept; 111 Koshland Hall; Berkeley CA 94720-3102; mmooney@nature.berkeley.edu
Moore, Kathy, Molec & Cell Biology-116; 1800, Cold Glamp In Harbor NY 11724
Mooney, Mark; UC Berkeley; Plant Biology Dept; 111 Koshland Hall; Berkeley CA 94720-3102; mmooney@nature.berkeley.edu
Morei, Marky, Molec & Cell Biology-116; 1800, Glamp In Univ. of California - San Diego; Ucason A 28792; In 1800-848-5020
Moore, Kathy, Molec & Cell Biology-116; 1800, Glamp In Univ. of California - San Diego; Ucason A 28792; In 1800-848-5020
Moore, Marky, Molec & Cell Biology-116; 1800, Glamp In 1800, Glamp In 1800, Plant In 1800, Plant
       murigneux@cicc.univ-bpclermont.fr

Murphy, Charles F; USDA NPS; Bldg 005, Rm. 239; BARC-W; Beltsville MD 20705; 301-504-5560; Fax: 301-504-5467; cfm@ars.usda.gov

Murray, Michael G.; Mycogen Plant Sciences; 5649 E Buckeye Rd; Madison WI 53716; 608-221-5000

Musket, Theresa; Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-2033; Fax: 573-884-7850; musket@teosinte.agron.missouri.edu
         Mustell, Robert A.; National Corn Growers Assoc.; 1000 Executive Parkway, Suite 105; St. Louis MO 63141; 314-275-9915x113; Fax: 314-275-7061 Muszyriski, Michael G.; Pioneer Hi-Bred Intl.; Agronomic Traits/TTD; 7300 NW 62nd Ave, POB 1004; Johnston IA 50131-1004; 515-254-2637; Fax: 515-254-2608;
    muszynskimg@phibred.com

Muzumdar, Daiva; Illinois State University; 4120 Biological Sciences; Normal IL 61790-4120; 309-438-3088; damuzum@ilstu.edu

Myers, A. M.; Dept Biochem & Biophys; 2110 Molecular Biology Bldg; lowa State University; Ames IA 50011;; ammyers@iastate.edu

Nagamura, Yoshiaki; Rice Genome Res Program, STAFF Inst; 446-1, Ippaizuka, Kamiyokoba; Ippaizuka, Tsukuba-shi; Ibaraki-ken 305; JAPAN; +81-298-38-2199; Fax: +81-298-38-2302; nagamura@staff.or.jp

Nagle, B. J.; Dekalb Plant Genetics; Box 33; Mason City IL 62664; 217-482-3213

Nakagawa, Yoichi; Takii & Company LTD; P.O. Box 7; Kyoto C. P. O.; JAPAN; (075)365-0123; Fax: (075)365-0110

Nan, Ren; College of Life Science; Wuhan University; Wuhan 430072; CHINA

Naranjo, Carlos; Inst Fitotecnico de Santa Catalina (UNLP); C.C.4 (1836) Llavallol; Buenos Aires; ARGENTINA

Natti, Thomas A.; Harris Moran Seed Co.; 1832 Garrity Blvd.; P.O. Box 980; Nampa ID 83653; 208-467-6341; Fax: 208-466-8188

Negrotto, David; CIBA-Geigy Corp; 3054 Cornwallis Rd; Research Triangle Park NC 27709-2257

Nel, P. M.; Dept of Genetics; Univ of The Witwatersrand; P.O. WITS; Transvaal 2050; SOUTH AFRICA; 011-716-2154; Fax: 011-716-8030

Nelsen, Terry C; USDA-ARS; 1815 N. University; Peoria IL 61604

Nelson, Timothy; Department of Biology; PO Box 208104; Yale University; New Haven CT 06520-8104; 203-432-3860; Fax: 203-432-5632; TIMOTHY.NELSON@YALE.edu

Nelson, Oliver E.; Department of Genetics; 445 Henry Hall; University of Wisconsin; Madison WI 53706; 608-265-4636; Fax: 608-262-2976; nelsonoe@macc.wisc.edu
                                   muszynskimg@phibred.com
    TIMOTHY.NELSON@YALE.edu
Nelson, Oliver E.; Department of Genetics; 445 Henry Hall; University of Wisconsin; Madison WI 53706; 608-265-4636; Fax: 608-262-2976; nelsonoe@macc.wisc.edu
Nelson, Jennifer; UC Berkeley; Plant Blology Dept; 111 Koshland Hall; Berkeley CA 94720-3102
Nemeth, J; Gabonater Kutatointezet; Also Kikoto sor 9; Szeged; HUNGARY; 62-54-555; Fax: 62-54-588
Neuffer, M. G.; 202 Curtis Hall; University of Missouri; Columbia MO 65211; 573-882-7735; Fax: 573-884-7850; Gneuffer@aol.com
Newhouse, Keith; ICI Seeds; Highway 210, P.O. Box 500; Slater IA 50244; 515-685-3574; Fax: 515-685-2548
Newman, Thomas C; MSU-DOE Plant Res Lab; Plant Biology Bldg; East Lansing MI 48824-1312; 517-353-0854; Fax: 517-353-9168; 22313TCN@IBM.CL.MSU.EDU
Newman, T. S.; Wyffels Hybrids, Inc; P.O. Box 246; Atkinson IL 61235; 309-936-7833; Fax: 309-936-7930
Newton, Kathleen; Dept Biol Sci; University of Missouri; Columbia MO 65211; 573-882-4049; Newton@bioscl.mbp.missouri.edu
Nguyen, Mai Ngoc; 105 Tucker Hall; University of Missori; Columbia MO 65211; 573-882-4049; Newton@bioscl.mbp.missouri.edu
Nguyen, Henry T.; Dept of Plant & Soil Science & Inst for Biotech; Texas Tech University; Lubbock TX 79409-2122; 806-742-1622; Fax: 806-742-0775; bwhtn@ttacs1.ttv.edu
                                   bwhtn@ttacs1.ttu.edu
       Nikolau, Basil; Dept. Biochem. & Biophys.; Iowa State Univ.; Ames IA 50010; 515-294-9423; dimmas@iastate.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; Fax: 515-270-4314; NOBLES@PHIBRED.COM
   NOBLES@PHIBRED.COM

Norton, Robert A.; USDA, ARS, NCAUR Mycotoxin R.U.; 1815 N. University; Peoria IL 61604; 309-681-6251; Fax: 309-671-7814

Notani, N. K.; Bhabha Atomic Res Centre; Biology Division, Mod Lab; Trombay, Bombay 400085; INDIA; 5510323; Fax: 91-22-5560750

Nourse, Sarah; Dept. of Horticulture; University of Hawaii; 3190 Maile Way; Honolulu HI 96822; ; nourse@hawaii.edu

Nowell, David C; Pannar (Pty) Ltd; PO Box 19; Greytown 3500; SOUTH AFRICA; 27-334-31131; Fax: 27-334-71208; dnowell@iafrica.com

Nutter, Robert; Pioneer Hi-Bred; 7300 NW 62nd Street; Johnston IA 50131-1004; (515)270-3349; Fax: (515)270-3367

Ober, Eric; 1-87 Agriculture Bidg.; University of Missouri; Columbia MO 65211; 573-882-6832; Fax: 573-882-1469; agroeric@mizzou1.missouri.edu

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, Ron; Dept Environm Hort; UC Davis; Davis CA 95616; 916-752-6202; RJOKAGAKI@UCDAVIS.EDU

Okuno, Kazutoshi; National Inst of Agrobiol Resources; 2-1-2 Kannondai; Tsukuba; Ibaraki 305; JAPAN; 81-298-38-7458; Fax: 81-298-38-7408; 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 55108; 612-625-9258; olho0002@Gold.tc.umn.edu

Orr, Alan R.; Department of Biology; University of Northern lowa; Cedar Falls IA 50614; 319/273-2150; Fax: 319/273-2893; ORR@UNI.EDU
```

```
Osterman, John C.; School of Life Sciences; University of Nebraska-Lincoln; Lincoln NE 68588; 402-472-5129; Fax: 402-472-2083; josterman@unl.edu
Ostrander, Brad; Dept of Agronomy; Univ of Wisconsin; 1575 Linden Drive; Madison WI 53706; 608-262-5087; OSTRANDE@CALSHP.CALS.WISC.EDU
Ottoboni, Laura; CBMEG Inst. Biol.; UNICAMP/Caixa Postal 6109; CEP 13083-970; Campinas SP; BRAZIL; 55-192-397030; Fax: 55-192-39-4717;
OTTOBONI@TURING.UNICAMP.BR
   Pace, Gary M.; Ciba-Geigy; POB 12257; 3054 Corn Wallis Road; Research Triangle Park NC 27709; 919-541-8582; paceg@am.abru.cg.com 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; Fax: 515-276-9360

Pages, Montserrat; Jordi Girona Salgado 18-26; 08034 Barcelona; SPAIN; 34-3-2040600; Fax: 34-3-2045904;
   Palmer, Reid; USDA-ARS-MWA; Iowa State University; Agronomy Department, Room G301; Ames IA 50011
Palmer, K. E.; Dept. of Microbiology; University of Cape Town; Private Bag; Rondebosch 7700; SOUTH AFRICA; 21-650-3269; Fax: 21-650-4320; KENNETH@MOLBIOL.UCT.AC.ZA
  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; Fax: 317-337-3228
Parlov, Dragomir; Inst Breeding & Prod Field Crops; Marulicev trg 5; 41000 Zagreb; YUGOSLAVIA; 041-750-311; Fax: 41-750-523
Parsons, Ronald; UC San Diego; Dept of Biology, 0116; La Jolla CA 92093-7108
Partas, E. C.; Maize and Sorghum Res. Inst.; Pashcani; Criuleni; 278336 Moldova; MOLDOVA
Passas, Hilli; Dept of Biology, CB# 3280; 317 Coker Hall; Univ of North Carolina; Chapel Hill NC 27599-3280; 919-962-5655; Fax: 919-962-1625;
 Pataky, Jerald K.; Crop Science, Turner Hall; 1102 S. Goodwin Ave; University of Illinois; Urbana IL 61801; 217-333-6606; Fax: 217-244-1230; j-pataky@uiuc.edu
Paterson, Andrew H; Dept Soil & Crop Sci; Texas A&M Univ; College Station TX 77843-2474; 409-845-3773; Fax: 409-845-0456; ahp2343@bloch.tamu.edu
Patil, M. S.; Agric. Research Station; Gulburua 585101; INDIA; 21120; Fax: 091-08472-21120
Patterson, Earl; Crop Sci, S-118 Turner Hall; University of Illinois; 1102 S. Goodwin Avenue; Urbana IL 61801; 217-333-6631; Fax: 217-333-6064; maize@uiuc.edu
Paul, Anna-Lisa; Dept Horticultural Sciences; 1255 Fifield Hall; Univ Florida; Gainesville FL 32611; 904-392-4711x313; Fax: 904-392-6479;
ALP@NERVM.NERDC.UFL.EDU
Pawar, S. E.; Nuclear Agriculture Division; Bhabha Atomic Research Center; Trombay Bombay 400085; INDIA; 5563060 ext 2638; Fax: 9122-556-0750
              2@kmail.ksc.nasa.gov
  Pierozzi, Neiva; S. Citologia - (IAC); Av B de Itapura 1481; Cx Postal 28; Campinas SP 13001-970; BRAZIL; 019-2315422; Fax: 012-2314943
Pixley, Kevin V; PO Box MP 163; Mount Pleasant; Harare; ZIMBABWE; 263-4-301807; Fax: 263-4-301327; k.pixley@cgnet.com
Plehn, Steve J.; Cargill Hybrid Seeds; P.O. Box 762; Mt. Vernon IN 47620; (812)838-5218; Fax: (812)838-8864
Plett, Stanley; Brandon Research Station; Agriculture Canada; P.O. Box 1000A RR #3; Brandon, Manitoba R7A 5Y3; CANADA; 204-726-7650; Fax: 204-728-3858;
Plunkett, Dave; Green Giant Company, M.S. 9921; 330 University Ave. S.E.; Minneapolis MN 55408; 612-330-8007; Fax: 612-330-8064
Podolskaya, Anna P.; N. I. Vaviiov Ali Union Inst Plant Industry; 44 Herzen Street; 190000, St. Petersburg; RUSSIA; 311-99-45; Fax: 311-8762
Poethig, R. Scott; Biology Department; Leidy Labs, Univ Penn; Philadelphia PA 19104-6018; 215-898-8915; Fax: 215-898-8780; SPOETHIG@MAIL.SAS.UPENN.EDU
Poggio, Lidia; Inst Fitotecnico de Santa Catalina (UNLP); C.C.4 (1836) Llavallol; Buenos Aires; ARGENTINA
Polacco, Mary; Curtis Hall; University of Missouri; Columbia MO 65211; 573-884-7873; Fax: 573-884-7850; maryp@teosinte.agron.missouri.edu
Pollacsek, M.; Station Amelior PI-INRA; 63039 Clermont Ferrand; FRANCE; 73-62-43-01; Fax: 73-62-44-53; jaques.bordes@clermont.inra.fr
Pollak, Linda; USDA-ARS; Dept. Agronomy; lowa State Univ; Ames IA 50011; 515-294-7831; Fax: 515-294-9359; Impollak@iastate.edu
Pollmer, W. G.; Universitat Hohenheim; Egilolfstr. 25; D-70599 Stuttgart; GERMANY; +49-711-4586315; Fax: +49-711-4569008
Poneleit, Charles G.; Agronomy; University of Kentucky; Lexington KY 40546-0091; 606-257-4934; Fax: 606-258-1952; AGR021@UKCC.UKY.EDU
Porter, Hedera L.; RR 1; Box 81; Revere MN 56166; 507-752-7012; Fax:
Prasanna, B.M.; Division of Genetics; Indian Agricultural Research Institue; New Delhi-110 012; INDIA
Pratt, Richard C.; Dept. Hort. & Crop Science; OSU/CARDC; 1680 Madison Avenue; Wooster OH 44691; 330-263-3972; Fax: 330-263-3887; pratt.3@osu.edu
Price, Carl; Waksman Inst; Rutgers University; Piscataway NJ 08855-0759; 908-445-2920; Fax: 908-445-5735; Price@mbcl.rutgers.edu
Pring, Daryl R.; Dept of Plant Pathology; 1453 Fifield Hall; University of Florida; Gainesville FL 32611; 904-392-3638; Fax: 904-392-6532; DRPG@ICBR.IFAS.UFL.EDU
Prioli, Laudenir M.; Depto. Genetics; Ib/CBMEG, Univ Estad Campinas; CP 6109; Campinas 13083-970; BRAZIL; 55-192-397030; Fax: 55-192-394717; LAUDENIR@CCVAX.UNICAMP.BR
Prioul, Jean-Louis; IBP Bat 630; Universite de Paris-Sud; 91405 Orsay Cedex; FRANCE; 331693
   Prioul, Jean-Louis; IBP Bat 630; Universite de Paris-Sud; 91405 Orsay Cedex; FRANCE; 33169336373; Fax: 33169336424; prioul@ibp.u-psud.fr
  Prosen, Dennis; Harris Moran Seed Co.; 100 Breen Rd.; San Jun Bautista CA 94045; (408)623-4323; Fax: (408)623-2260 Prostko, Michael; Dept Biol Sci; Univ of Wisconsin; Kenosha WI 53141
  Pryor, Tony; Plant Industry CSIRO; PO Box 1600; Canberra ACT 2601; AUSTRALIA; (61)(6)2465494; Fax: 2465000; poss@pican.pi.csiro.au Puigdomenech, Pedro; Centro de Investigacion y Desarrollo; c/ Girona Salgado, 18-26; 08034 Barcelona; SPAIN; 34-3-400-61-29; Fax: 34-3-204-59-04;
               pprgmp@cid.csic.es
  Purugganan, Michael; Dept of Genetics; Box 7614; NC State University; Raleigh NC 27695-7614; ; michaelp@unity.ncsu.edu
Qin, Minmin; USDA-ARS-PGEC; 800 Buchanan Street; Albany CA 94710
Qin, Lu; Institute of Genetics and Crop Breeding; Fuzhou 0591; CHINA
  Qiu, Lijuan; G403 Agronomy Hall; Iowa State Univ; Ames IA 50011
Qu, Feng; Institute of Genetics; Lab No. 801; Beijing 100101; CHINA
   Qualset, Calvin O.; Genetic Resources Cons Program; Univ of California; Davis CA 95616-8602; 916-757-8921; Fax: 916-757-8755; coqualset@ucdavis.edu
  Quarrie, Steve; Cambridge Lab; John Innes Centre; Colney Lane; Norwich NR4 7UJ; UNITED KINGDOM; 44-1603-452571; Fax: 44-1603-502241; QUARRIE@BBSRC.AC.UK
```

Quayle, Tom; Dept. of Biological Sciences; 900 Wood Road, Box 2000; University of Wisconsin-Parkside; Kenosha WI 53141-2000; 414-595-2172; Fax: 414-595-2056;

```
Quayle@cs.uwp.edu
Quebedeaux, Bruno; Horticulture Dept.; Univ of MD; College Park MD 20742; 301-405-4336; Fax: 301-314-9308; 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; Fax: 208-397-4165; 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; Fax: 301-903-1003;
Racchi, Milvia L.; Genetics Unit; Univ of Florence; Via San Bonaventura 13; 50145 Firenze; ITALY; 55-573201; Fax: 55-580341; gene_agr@cesit1.unifi.it
Radicella, Pablo; Room 56-730; Department of Biology; Massachusetts Institute of Technology; Cambridge MA 02139; (617)253-4724
Ragot, Michel; Ciba-Geigy Limited; R-1096.2.17; CH-4002 Basel; SWITZERLAND; 41(61)697 8621; Fax: 41(61)697 4256; RAGOTM@ABRU.CG.COM
Raizada, Manish; Dept Biol Sci; Stanford Univ; Stanford CA 94305-5020
Ralston, Ed; DNA Plant Technology Corp; 6701 San Pablo Avenue; Oakland CA 94608; 510-547-2395; Fax: 510-547-2817; 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; Fax: 314-553-6233; swdrapp@umslvma.umsl.edu
    Rayburn, A. Lane; Crop Sci, 320 ERML; 1201 W. Gregory; Univ of Illinois; Urbana IL 61801; (217)333-4777; Fax: (217)333-9817; arayburn@uiuc.edu
Rayburn, A. Lane; Crop Sci, 320 ERML; 1201 W. Gregory; Univ of Illinois; Urbana IL 61801; (217)333-4777; Fax: (217)333-9817; arayburn@uiuc.edu
Reardon, Ellen; Waksman Institute; Piscataway NJ 08855-0759; 908-445-3512; Fax: 908-445-5735; reardon@mbcl.rutgers.edu
Reddy, Gurjal; Department of Genetics; Osmania University; Hyderabad-500007; INDIA; 868951 Ext. 375; Fax: 91-0842-868087
Reddy, A. R.; School of Life Sci; Univ of Hyderabad; Hyderabad-500001; INDIA; 253901, ext. 226
Redinbaugh, Margaret; USDA-ARS; Utah State Univ; Forage & Range Research Lab; Logan UT 84322-6300
Reid, L. M.; Bldg 121; Plant Research Centre; Agriculture Canada; Ottawa, Ontario K1A 0C6; CANADA
Retief, AE; Dept of Genetics; Univ of Stellenbosch; Stellenbosch; Totology, South AFRICA; 027-21-808-4369; Fax: 027-21-808-4336; AER@MATIES.SUN.AC.ZA
     Reynolds, James O.; Department of Biological Sciences; University of Idaho; Moscow ID 83843; 208-885-7477; Fax: 208-885-7905
Rhee, Seung; Carnegie Institution of Washington; 290 Panama St.; Stanford CA 94305
Rhodes, C. A.; 219 Bay Tree Rd.; San Carlos CA 94070; 415-598-9469; Fax: 415-857-1125
Ribaut, Jean-Marcel; CIMMYT; Apdo. Postal 6-641; 06600 Mexico D.F.; MEXICO; 595-54400/54410; Fax: 595-54425; JRIBAUT@ALPHAC.CIMMYT.MX
Rice, Doug; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 22; Johnston IA 50131-1004
Richards, Fric: Biology Dept.: Washington University: St. Louis MO 63130: 314-935-7196; richards@biodec.wustl.edu
  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; Fax: (913)532-5692; trichter@ksuvm.ksu.edu
Richter, Todd E.; Plant Path. Dept.; Throckmorton Hall; Manhattan KS 66506; (913)532-6176; Fax: (913)532-5692; trichter@ksuvm.ksu.edu
Richter, Todd E.; Plant Path. Dept., Gronomy & Plant Genetics; Univ. Minnesota; 411 Borlaug Hall, 1991 Buford Circle; St. Paul MN 55108-6026; (612)625-6223; Fax: (612)625-1268; rier0001@gold.tc.umn.edu
Richter, Karl; Box 187; Marshalltown IA 50158; 515-752-2170
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; Fax: 317-538-3600
Roberts, Jean L.; DowElanco Discovery Research; P.O. Box 68955; 9330 Zionsville Road/D1; Indianapolis IN 46268-1053; 317-337-3126
Robertson, Donald S.; 1707 Woodhaven Circle; Ames IA 50010-4198; 515-232-2892; Fax: 515-294-0453; donrrs@aol.com
Robison, Glenn; DeKalb Plant Genetics; 3100 Sycamore Road; DeKalb IL 60115; 815-758-9531; Fax: 815-758-4106
Rocheford, Torbert R.; Crop Sci, AW-100 Turner Hall; University of Illinois; 1102 S. Goodwin Av.; Urbana IL 61801; 217-333-9643; Fax: 217-333-9817; trochefo@uiuc.edu
                              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; Fax: 515-294-1337; S1SRR@ISUVAX.BITNET
Roebbelen, G.; Tuckermannweg 9; D-37085 Goettingen; GERMANY; 0049-551-394361; Fax: 0049-551-394601
Rogers, Suzanne; Salem-Teikyo University; Salem WV 26426-0520
Rogowsky, Peter; RCAP; ENS-Lyon; 46 Allee d'Italie; F-69364 Lyon cedex 07; FRANCE; +33 72 72 86 07; Fax: +33 72 72 86 00; Peter.Rogowsky@popserver.ens-
       Romero-Severson, Jeanne; Linkage Genetics; PO Box 157; Mazomanie WI 53560; 608-767-3237; Fax: 608-795-4563; romeros@mailbag.com
Ronchi, Angela; University of Milan; Dept. of Genetics & Microbiology; Via Celoria 26; 20133 Milan; ITALY; +39-2-26605235; Fax: +39-2-2664551;
Ronchi, Angela; University of Milan; Dept. of Genetics & Microbiology; Via Celoria 26; 20133 Milan; ITALY; +39-2-26605235; Fax: +39-2-2664551; tonelli@milucac.st.unimi.it

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

Rosichan, Jeffrey L.; Northrup King Co.; Research Center; 317 330th St.; Stanton MN 55018-4308; 507-663-7642; Fax: 507-645-7519

Rosielle, A.; Asgrow Seed Co; 634 E. Lincoln Way; Ames IA 50010; 515-232-7170; Fax: 515-232-6905

Roth, Brad; Pioneer Hi-Bred Internat, Inc; 7300 NW 62nd Ave.; Box 1004; Johnston IA 50131-1004; [515)270-3789; Fax: (515)270-3367; rothba@phibred.com

Rubenstein, Irwin; 220 Bloscience Center; Dept Plant Biology; University of Minnesota; St Paul MN 55108; (612)624-2755

Rubinstein, Amy; Dept. of Biology; Colorado State Univ; Ft Collins CO 80521; 303-491-3348; rubins@lamar.colostate.edu

Ruferer, Keilti; ICI Seeds; Hwy 210; P.O. Box 500; Slater IA 50244

Ruff, Thomas G.; Monsanto-Ceregen; 700 Chesterfield Pkwy North; AA3E; St. Louis MO 63198; 314-537-7023; Fax: 314-537-6759; tgruff@ccmail.monsanto.com

Russell, Doug; Agracetus, Inc.; 8520 University Green; Middleton WI 53562; 608-836-7300; Fax: 608-836-9710; darussel@unix.midplains.net

Russell, Kelly; Institute of Molec Biol; University of Oregon; Eugene OR 97403

Saab, Imad; Crop Sci, Turner Hall; 1102 S. Goodwin Ave; Univ of Illinois; Urbana IL 61801; i-saab@uiuc.edu

Sacher, Robert; Hunt-Wesson; Research and Development; 1645 W. Valencia Drive; Fullerton CA 92634; 714-680-2811; Fax: 714-449-5166

Sachs, Marty; USDA/ARS; S108 Turner Hall; 1102 S. Goodwin Ave; Urbana IL 61801; (217)244-0864/333-9743lab; Fax: (217)333-6064; msachs@uiuc.edu

Saghai Marcot, M. A.; CSES Department; VPI & SU; Blacksburg VA 24061; 540-231-313-315; MARROOF@VT:EDU

Sainz, Manuel; Institute of Molecular Biol; Univ of Oregon; Eugene OR 97403-1229; (503)346-5123; Fax: (503)346-5891; sainz@molbio.uoregon.edu

Salerno, Juan C.; PJE. San Sebasti
                              tonelli@imiucca.csi.unimi.it
    San Miguel, Phillip; HANS 339; Dept. of Biological Sciences; Purdue University; West Lafayette IN 47907; (317)494-4919; pmiguel@bilbo.bio.purdue.edu
Santos, M. A.; Dept Genetica Molecular; Centro Invest Desarrollo; Gorge Girona 18-24; 08034 Barcelona; SPAIN; 34-3-4006100; Fax: 34-3-2045904
Sarkar, Kumud R.; Division of Genetics; Indian Agric Res Inst; New Delhi 110012; INDIA; 91-011-5783077; Fax: 91-011-5752006
Sasaki, Takumi; Rice Genome Research Program; STAFF Institute, 446-1, Ippaizuka; Kamiyokoba, Tsukuba; Ibaraki 305; JAPAN; 81-298-38-2199; Fax: 81-298-38-1780;
                            tsasaki@abr.affrc.go.jp
    tsasaki@abr.affrc.go.jp
Satarova, Tatjana N; Research Institute of Maize; Dzerzhinskaya st., 14; Dniepropetrovsk; UKRAINE
Saura, Fulgencio; Dpto Tecnologia; Univ. Nacional Lujan; CC 221-6700 Lujan(Bs As); ARGENTINA
Savidan, Yves H.; APONET/CIMMYT; Aptdo. Postal 6-641; 06600 Mexico D.F.; MEXICO; (52)5-726.90.91; Fax: (52)5-7267558; Yves.Savidan@mpl.orstom.fr
Scandalios, John; Dept. of Genetics; North Carolina State Univ.; Box 7614; Raleigh NC 27695-7614; (919)515-7079; Fax: (919)515-3355
Scanlon, Mike; Dept of Plant Biology; 111 Genetics & Plant Biology Bldg; Univ of California; Berkeley CA 94720; ; mjscanlo@nature.berkeley.edu
Scheffler, Brian E.; Inst. Allgem. Bot.; Angew. Molek.; Pflanzen, AMP I, Ohnhorststrafie 18; D-2000 Hamburg 52; GERMANY; (49) 40 822 82 381
Schichnes, Denise E.; Dept of Plant Biology; 111 Koshland Hall; Univ California Berkeley; Berkeley CA 94720; (510)642-8058; Fax: (415)642-4995;
Schichne@nature.berkeley.edu
```

```
Schiefelbein, John W.; 4085 Natural Science Bldg.; University of Michigan; Ann Arbor MI 48109; 313-764-3579; Fax: 313-747-0884; 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; San Diego CA 92093; 619-534-1636; Fax: 619-534-7108; rschmidt@ucsd.edu Schnable, Patrick; Dept of Agronomy; G302 Agronomy Hall; Iowa State Univ; Ames IA 50011; (515)294-0975; Fax: (515)294-2299; Schnable@iastate.edu Schneeberger, Richard G.; Dept. of Plant Biology; 111 Koshland Hall; University of California; Berkeley CA 94720-3102; 510-642-7948; Fax: 510-642-4995;
                schnee@nature.berkeley.edu
  Schneerman, Martha C; Illinois State University; 4120 Biological Sciences; Normal IL 61790-4120; 309-438-3088; Fax: 309-438-3722; MCSCHNEE@ilstu.edu Schnicker, Bruce; Cornnuts; 1000 S. Edgewood Ave.; P.O. Box 830; Urbana OH 43078; 513-652-1321; Fax: 513-653-3675
Scholl, Randy; Arabidopsis Biol Resource Center; Ohio State; 1735 Neil Ave.; Columbus OH 43210; 614-292-0603; Fax: 614-292-0603; scholl.1@osu.edu Schramke, Mary; Bio-Rad Laboratories, Life Sciences Group; 2000 Alfred Nobel Drive; Hercules CA 94547; 510-741-6717; Fax: 510-741-1051
  Schulman, Alan H.; Univ Helsinki; Institute of Biotechnology; P.O. Box 56, Viikinkaari 9; 00014 Helsinki; FINLAND; 358-0-708-59366; Fax: 358-0-708-59422;
               aschulman@operoni.helsinki.fi
  Schultes, Neil; Dept Biochem & Genetics; Conn Agric Exper Sta; 123 Huntington St; New Haven CT 06511; 203-789-6912; Fax: 203-789-7232; NPSCHULT@PCNET.COM
Schwall, Michael; Suedwestdeutsche Saatzucht; Im Rheinfeld 1-13; 76437 Rastatt; GERMANY; 49-7222-7707-0; Fax: 49-7222-770777; schwall@sun1.ruf.ur freiburg.de
Schwartz, Drew; Biology Dept; Indiana University; Bloomington IN 47405; 812-855-6060; Fax: 812-855-6705; Schwartz@bio.indiana.edu
Schwer, Joseph F.; Mycogen Plant Sciences; 5649 E. Buckeye Road; Madison WI 53716
Scott, Paul; 1407 Agronomy; USDA-ARS; Iowa State University; Ames IA 50011; 515-294-7825; Fax: 515-294-9359; pscott@iastate.edu
Scott, Lu Ann; Dept Biol Sci; University of Idaho; Moscow ID 83844-3051
Selinger, David; Institute Molec Biol; University of Oregon; Eugene OR 97403-1229; 541-346-5123; dselinger@morel.uoregon.edu
Selzer, Gerald; Room 615; National Science Foundation; 4201 Wilson Blvd.; Arlington VA 22230
Sendo, Shigeyuki; Hokkaido Plant Genet Res S Ctr; 363-2 Minami Takinokawa; Takikawa City 073; Hokkaido; JAPAN; 0125-23-3195; Fax: 0125-24-3877
Senechal, Nell; NPBL 256; Box 2140C; South Dakota State Univ; Brookings SD 57007; 605-688-4502; PX67@SDSUMUS.SDSTATE.EDU
Senior, Lynn; USDA-ARS; N. C. State University; Box 7620; Raleigh NC 27695-7620; (919)515-4087; Fax: (919)515-7959; marker@unity.ncsu.edu
Seo, Beom-Seok; G412 Agronomy Dept; Iowa State Univ; Ames IA 50011
Setter, Tim L.; Department of Soil Crop and Atm. Sci.; 519 Bradfield Hall; Cornell University; Ithaca NY 14853; 607-255-1701; Fax: 607-255-2644; tls1@Cornell.edu
Settles, Mark; Cold Spring Harbor Lab; PO Box 100; Cold Spring Harbor NY 11724; 516-367-8827; Fax: 516-367-8369; settles@cshl.org
Sevilla P., Ricardo; Programa de Maiz; Univ Nacional Agraria; Aptdo 456, La Molina; Lima; PERU
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
  Schwall, Michael; Suedwestdeutsche Saatzucht; Im Rheinfeld 1-13; 76437 Rastatt; GERMANY; 49-7222-7707-0; Fax: 49-7222-770777; schwall@sun1.ruf.uni-
  Shamina, Ziata; Academy of Sciences; K. A. Timiryazev Inst. Plant Phys.; Botanicheskaya 35; 127276 Moscow; RUSSIA
Shams, Henry L.; USDA-ARS-NPS; Bidg 005 BARC-W; Beltsville MD 20705; 301-504-5059; Fax: 301-504-6699; shands@sun.ars-grin.gov
Shao, Qi-Quan; Academia Sinica; Genetics Institute 917 Bidg.; 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; Fax: 61-46-512-578;
                pbic00@angis.su.oz.au
  Shaver, Jonathan; 1845 W. Larpenteur Apt. 6; St. Paul MN 55113; 612-625-5215
Shaver, Donald L; Plant Br Dept, Cornnuts; 24 Winham St; Salinas CA 93901; 408-424-1023; Fax: 408-424-5091
Shaw, Janine; Univ of Florida; P.O. Box 110690; Gainesville FL 32611; 352-392-1928
Shaw, Janine; Univ of Florida; P.O. Box 110690; Gainesville FL 32611; 352-392-1928
Shcherbak, V. S.; Corn Breeding Dept; Krasnodar Res Inst Agric; 350012 Krasnodar; RUSSIA
Sheen, Jen; Dept. of Molecular Biology; Wellman 11, MGH; Boston MA 02114; 617-726-5916; Fax: 617-726-6893; sheen@frodo.mgh.harvard.edu
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; Fax: (302)695-4296; shenjb@esvax.dnet.dupont.com
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; Fax: (302)695-4296; shenjb@esvax.dnet.dupont.com
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; Fax: (302)695-4296; shenjb@esvax.dnet.dupont.com
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; Fax: (302)695-4296; shenjb@esvax.dnet.dupont.com
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; (302)695-1246; Fax: (302)695-4296; shenjb@esvax.dnet.dupont.com
Shen, Jennie; P.O. Box 80402, E402/4255; DuPont Co.; Wilmington DE 19880-0402; CHINA
Shen, Zhejiang Agricultural University; Dept. of Agronomy; Hangzhou; Zejiang 310029; CHINA
Shen, 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; 701-777-4479 or -4705; Fax: 701-777-2623
Sheridan, W. F.; D20 Princeton Park; Grand Forks ND 58203
Shiga, Toshio; Sakata Seed Corp; Plant Bio Center, SAKATA SEED Corp; 358 Uchikoshi Sodegaura; Chiba 299-02; JAPAN; 438-75-2369; sscbio@leo.bekkoame.or.jp
Shigemori, I.; Chusin Agr. Exp. Stn.; Sooga Shiojiri; Nagano 399-64; JAPAN
Short, Kent E.; Carnia Seed (Ply) Ltd.; P.O. Box 7424; Petit 1512; SOUTH AFRICA; (011)965-1905; Fax: (011)965-1906
Shotwell, Mark A; Dept of Biology; 123 Vincent Science Hall; Slippery Rock Univ; Slippery Rock PA 16057-1326; 412-738-2476; Fax: 412-738-2188; mark.shotwell@svu.edu
               mark.shotwell@svu.edu
  Siddiqui, Khushnood A.; Plant Genetics Division; A.E.A.R.C.; Tandojam Sind; PAKISTAN; 40468 02233-837; Fax: 02233-284 Sidorenko, Lyuda; 2292-1 Molec Biol; Iowa State Univ; Ames IA 50011
  Silva, WJ da; Univ Estadual Campinas; Inst Biol; Caixa Postal 6109; BF-13081-970 Campinas, SP; BRAZIL; ; dasilva@turing.unicamp.br
  Simcox, Kevin; OARDC/OSU Dept Hort Crop Sci; 216 Williams Hall; 1680 Madison Ave; Wooster OH 44691; 330-263-3876; Fax: 330-263-3887; simcox.3@osu.edu 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; Fax: 515-270-3367; SIMSL@PHIBRED.com
  Singh, Karam; Dept Mol, Cell & Devel Biol; 405 Hilgard Avenue; UCLA; Los Angeles CA 90095-1606; 310-206-8259; Fax: 310-206-3987; Ksingh@ucla.edu
Sinha, Neelima; Section of Plant Biology; Division of Biological Sciences; University of California; Davis CA 95616; 916-754-8441; Fax: 916-752-5410;
               NRSINHA@UCDAVIS.EDU
  Sinibaldi, Ralph; 1780 Acacio Ct.; Fremont CA 94536; 510-794-6410
Sinibaldi, Ralph; 1780 Acaclo Ct.; Fremont CA 94536; 510-794-6410
Sisco, Paul; USDA-ARS, Dept. Crop Science; North Carolina State Univ; P.O. Box 7620; Raleigh NC 27695-7620; (919)515-3309; Fax: (919)515-7959; Sisco@ncsu.edu
Sleper, David A.; Agronomy Dept; 210 Waters Hall; University of Missouri; Columbia MO 65211; 573-882-7320; Fax: 573-882-1467
Smith, Margaret E.; Cornell Univ; 252 Emerson Hall; Dept of Plant Breeding; Ithaca NY 14853; 607-255-1654; Fax: 607-255-6683; mes25@cornell.edu
Smith, Laurie G.; Dept of Biology - Coker Hall; University of North Carolina; Chapel Hill NC 27599-3280; 919-962-6943; Fax: 919-962-1625
Smith, Oscar S.; Pioneer Hi-Bred International; 7300 NW 62nd Ave.; P.O. Box 85; Johnston IA 50131-0129; 515-270-3539; Fax: 515-270-4312; SMITHO@Phibred.com
Smith, Steve; Pioneer Hi-Bred Internatl; P.O. Box 1004; Johnston IA 50131-9410; 515-270-3353; Fax: 515-270-4312; SMITHS@PHIBRED.COM
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, James D.; PO Box 2132; Department Soil & Crop Sci; Texas A & M University; College Station TX 77843; 409-845-8276
Snape, John W.; Cambridge Laboratory; JI Centre for Plant Science Research; Colney Lane; Norwich NR4 7UJ; UNITED KINGDOM; 44-1603-452571; Fax: 44-1603-502270
               502270
  Sobral, Bruno W S; National Center for Genome Resources; 1800 Old Pecos Trail; Santa Fe NM 87505; 505-982-7840; Fax: 505-982-7690; bws@ncgr.org
  Sokolov, Victor A.; Institute of Cytology and Genetics; Russian Academy of Sciences; Lavrentjev str., 10; Novosibirsk 630090; RUSSIA; 3832-355-713; Fax: 3832-356-
                558; sokolov@cgi.nsk.su
  Soller, M.; Department of Genetics; The Hebrew University; Jerusalem 91 904; ISRAEL; 972-2-585104; Fax: 972-2-586975; SOLLER@HUJIVMS.Bitnet
  Somers, David A.; Dept Agron & Plant Genet; University of Minnesota; 1991 Upper Buford Cir.; St. Paul MN 55108; 612-625-5769; Fax: 612-625-1268;
                somer001@maroon.tc.umn.edu
  Somerville, Chris; Plant Biology Dept; Carnegie Institution; 290 Panama St; Stanford CA 94305; 415-325-1521; Fax: 415-325-4133; crs@andrew.stanford.edu
Song, Xiangfu; Chrina 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; Fax: 7813833
Songstad, David; Pioneer Hi-Bred Int'l; 7300 NW 62nd Ave; P.O. Box 1004; Johnston IA 50131-1004; 515-270-3642; Fax: 515-270-3444;
SONGSTADDD@PHIBRED.COM
  Sotomayor-Rios, A.; Tropical Agric Res Stn; P.O. Box 70; Mayaguez PR 00709
```

```
Southgate, E. M.; Plant Genetic Manipulation Group; Department of Life Science; Univ of Nottingham; Nottingham NG7 2RD; UNITED KINGDOM; 44-1602-515151 ext.
                   847314; Fax: 44-1602-513251
    S47314; Fax: 44-1002-513251
Sprague, George F.; Washington Abbey; 494 West 10th St.; ; Eugene OR 97401; 503-465-8920
Spray, Clive R.; Dept. of Biology; 405 Hilgard Av; UCLA; Los Angeles CA 90024-1606; 310-825-3177; Fax: 310-825-3177
Springer, Patricia; Cold Spring Harbor Lab; P.O. Box 100; Cold Spring Harbor NY 11724; (516)367-8827; Fax: (516)367-8369; SPRINGER@CSHL.ORG
Srinivasan, Ganesan; ClMMYT Maize Program; Lisboa 27, Aptdo Postal 6-641; 06600 Mexico, D. F.; MEXICO; 52-5-726-9091 Ext. 1116; Fax: 52-5-726-7558/59;
GSRINIVASAN@ALPHAC.CIMMYT.MX
St. Clair Grape Piencer Hisboral Inc. Trait & Technology Dept. 7300 NW 62nd Ave. PO Roy 1004: Johnston IA 50131-1004
     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; For Collins CO 80523-1878; 970-491-6802; Fax: 970-491-0649; sstack@lamar.colostate.edu Staebell, Mark S; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004; 515-253-2148; Fax: 515-254-2619
Staiger, Chris; Dept. of Biological Sciences; Purdue University; 321A Hansen Bldg.; West Lafayette IN 47907; 317-496-1769; Fax: 317-496-1496; CSTAIGER@BILBO.BIO.PURDUE.EDU
     Stapleton, Ann E.; Dept Biol Envir Sci; Univ Tenn Chattanooga; 615 McCallie Ave; Chattanooga TN 37403-2598; 423-755-4397; Fax: 423-785-2285; Ann-
                   Stapleton@utc.edu
  Stapleton@utc.edu
Start, William; DeKalb Genetics; 62 Maritime Drive; Mystic CT 06355; 860-572-5223; Fax: 860-572-5240; dekalb@conix.com
Steffensen, D. M.; 506 Morrill Hall, Cell Biol; 505 S. Goodwin Ave; University of Illinois; Urbana IL 61801; 217-333-3087; Fax: 217-244-1648
Stein, Nils; Univ Hohenheim; Plant Breeding Biotech Inst; D70593 Stuttgart; GERMANY
Stelly, David M.; Dept Soil & Crop Science; Texas A & M Univ; College Station TX 77843-2474
Stern, David B.; Boyce Thompson Inst. Plant Res. Inc.; Tower Road; Ithaca NY 14853-1801; (607)254-1306; Fax: (607)254-1242; ds28@cornell.edu
Stettner, Cornelia; LS Gentechnik Hoherer Pflanzen; Tu-Munchen; Lichtenbergstrasse 4; 85747 Garching; GERMANY
Stiles, J. I.; Dept. of Pl & Molec Physiology; Univ. Hawaii at Manoa; Honolulu HI 96822; 808-956-7354; Fax: 808-956-3542; stiles@hawaii.edu
Stinard, Philip; USDA/ARS; S123 Turner Hall; 1102 S. Goodwin Ave.; Urbana IL 61801-4798; (217)333-6631; Fax: (217)333-6064; pstinard@uiuc.edu
Strom, Daniel; Dept Bot Plant Path; Cordley 2082; Oregon State Univ; Corvallis OR 97331-2902
Stuber, Charles W.: Dept of Genetics: North Carolina State Univ; Box 7614: 3513 Gardner Hall: Raleigh NC 27695-7614: 919-515-5834: Fax:
    Stuber, Charles W.; Dept of Genetics; North Carolina State Univ Box 7614; 3513 Gardner Hall; Raleigh NC 27695-7614; 919-515-5834; Fax: 919-515-3355;
                   cstuber@ncsu.edu
    Stucker, Robert; Agronomy & Plant Genetics; 411 Borlaug Hall; Univ of Minnesota; St. Paul MN 55108; 612-625-2738; Fax: 612-625-1268 Styles, E. D.; Biology; Univ of Victoria; Victoria; British Columbia V8W 2Y2; CANADA; 604-721-7101 Subbaiah, Chalivendra; University of Illinois; Crop Sci, S-27 Turner Hall; 1102 S. Goodwin Ave.; Urbana IL 61801; ; subbaiah@uiuc.edu Subramanian, Alap Raman; Dept Biochemistry; BioScience West; Univ of Arizona; Tucson AZ 85721-0001
    Sudupak, Mehmet Ali; Illinois Institute of Technology; Life Sciences - Dept. of Biology; 3101 S. Dearborn #286; Chicago IL 60616; ; SUDUMEH@karl.iit.edu Sughroue, Jay; Great Lakes Hybrids; 2201 229th Place; Boone IA 50036; 515-292-1957; Fax: 515-292-7010 Sul, III-Wan; Biol Sci; 324 Tucker Hall; Univ of Missouri; Columbia MO 65211
  Sullivan, Sue; ICI Seeds; 2369 330th St.; Slater IA 50244; 515-685-3574; Fax: 515-685-2548; TDSULLVN@MACC.WISC.EDU
Sullivan, Tom; Laboratory of Genetics; 445 Henry Mall; University of Wisconsin; Madison WI 53706; 608-262-4934; Fax: 608-262-2976; tdsulliv@facstaff.wisc.edu
Sun, Chongrong; Fudan University; Dept. of Biochem.; Handan Road 220; Shanghai 200433; CHINA
Sun, Zongxiu; China National Rice Research Institute; Genetics and Breeding Dept.; Tiyuchang Road 171; Hangzhou 310006; CHINA
Sundaresan, V.; Institute of Molecular Agrobiology; National University of Singapore; 59A The Fleming, 1 Science Park Dr; Singapore 118240; INDIA; 65-8723339; Fax:
65-8725349; imav1@nus.sg
    Sundberg, Marshall D.; Department of Botany; 502 Life Science Bldg; Louisiana State University; Baton Rouge LA 70803-1705; 504-388-8563; Fax: 504-388-8459; BTMARSH@LSUVM.LSU.EDU
Sundberg, Marshall D.; Department of Botany; 502 Life Science Bidg; Louisiana State University; Baton Rouge LA 70803-1705; 504-388-8563; Fax: 504-388-8459 BTMARSH@LSUVM.LSU.EDU

Sung, Tong Ming; Dept of Agronomy; Beijing Agric Univ; 912 Research Building; Beijing 100094; CHINA; 2582223-0274; Fax: 2582332 Suprasanna, P.; Plant Biotechnology Division; Bhabha Atomic Research Centre; Trombay, Bombay 400 085; INDIA; 91-22-556-3060x2349/3276; Fax: 91-22-556-0750 Suresh, Jayanti; Dept. of Agronomy; 513 Borlaug Hall; 1991 Buford Cr.; St. Paul MN 55108; (612)625-1208

Sussex, Ian M.; Dept Plant Biology; Univ of California; Berkeley CA 94720; 510-642-7759; Fax: 510-642-4995; SUSSEX@NATURE.BERKELEY.EDU
Suttie, Janet; CIBA; PO Box 12257; 3054 Cornwallis Rd; Research Triangle Park NC 27709-2257

Sutton, Teresa R.; Ploneer Hi-Bred; 7250 NW 62nd Ave; P.O. Box 1004; Johnston In A 50131-1004; (515)270-3319; Fax: (515)270-4312

Swiecicki, W.K.; Polish Academy of Sciences; Institute of Plant Genetics; ul. Strzeszynska 34; 60-479 Poznan; POLAND; 233-511; Fax: (48-61)221-122

Sylvester, Anne W.; Dept of Biological Sciences; University of Idaho; Moscow ID 83843; 208-885-7477; Fax: 208-885-7905; annes@uidaho.edu

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; Fax: 217-333-9817

Tagliani, Laura; Pioneer Hi-Bred Internat Inc; PO Box 1004; Johnston IA 50131-1004; 515-270-4188; Fax: 515-270-3367; TAGLIANILA@PHIBRED.COM

Tan, C. C. (Jia Zheng); Fudan University; Inst. of Genet.; Shanghai 20043; CHINA

Tang, Xi-Hua; Academic Sinica; Shanghai Inst. of Plant Physiol.; 300 Fonglin Road; Shanghai 200032; CHINA

Tanksley, Steven D.; Dept. Plant Breeding; Cornell University; 252 Emerson Hall; Ilhaca NY 14853

Tao, Quanzhou; 320 Yue-Yang Road; Shanghai 200031; CHINA; 86-21-4374430; Fax: 86-21-4378357

Taramino, Graziana; Depart Genet Biol Microrg; Univ of Milan; Via Celoria 26; I-20133 Milano; ITALY

Taylor, Broy Fax: Ad
   Theuri, James; Dept of Agronomy; Univ of Missouri; Columbia MO 65211; 573-882-1412
Thompson, Steven A.; United Agriseeds; 103 Tomaras Ave; Savoy IL 61874
Thompson, R. D.; Max Planck Inst Zuchtungsf; Abt Pflanzenz Ertragsphysiol; D-50829 Koeln; GERMANY; +49-221-5062-440/441; Fax: +49-221-5062-413;
    thompson@mpiz-koeln.mpg.de

Thombury, David W.; S-305 Ag Science Center N; Dept Plant Path; University of Kentucky; Lexington KY 40546; 606-257-4819; Fax: 606-323-1961; THORN@UKCC.UKY.EDU
 THORN@UKCC.UKY.EDU
Thornsberry, Jeffry; Biol Sci; 324 Tucker Hall; Univ of Missouri; Columbia MO 65211; ; 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; Fax: (507)625-6446; TIFFANYD@PHIBRED.COM
Till, Bradley; Inst Molec Biol; Univ of Oregon; Eugene OR 97403
Timmermans, Marja; Dept of Biology; Yale Univ; PO Box 208104; New Haven CT 06520-8104; 203-432-3862; Fax: 203-432-5632; marja.timmermans@yale.edu
Ting, Yu-Chen; Biology, Boston College; Chestnut Hill 67; Boston MA 02167; 617-552-2736; Fax: 617-552-2011
Tingey, Scott V.; Du Pont Company; P.O. Box 80402; Wilmington DE 19880-0402; (302)695-7252; Fax: (302)695-4296; TINGEYSV@ESVAX.DNET.DUPONT.COM
Toennissen, Gary; The Rockefeller Foundation; New York NY 10018
Tomes, Dwight T.; Pioneer Hi-Bred Intl; 7300 NW 62nd Ave; Johnston IA 50131-1004; 515-270-3646; Fax: 515-270-3444; tomesd@phibred.com
Tonelli, Chiara; University of Milan; Dept. of Genetics & Microbiology; Via Celoria 26; 20133 Milano; ITALY; 39-2-26605210; Fax: 39-2-2664551; tonelli@imiucca.csi.unimi.t
                 tonelli@imiucca.csi.unimi.it
  Tossberg, John; Pioneer Hi-Bred Internat Inc; Reid Research Center; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004
Tracy, William; Department of Agronomy; 1575 Linden Drive; University of Wisconsin; Madison WI 53706; 608-262-2587; wttracy@facstaff.wisc.edu
Trimnell, Mary; Pioneer Hi-Bred International; PO Box 85; Johnston IA 50131-0085; 515-270-3657; Fax: 515-270-3667; trimnellm@phibred.com
Troxell, Cynthia; Dept. MCD Biology; Campus Box 347; University of Colorado; Boulder CO 80309-0347; 303-492-8534; Fax: 303-492-7744;
```

```
troxell@beagle.colorado.edu
Troyer, A. Forrest; Cargill Hybrid Seeds; Blackberry Creek Facility; 2600 W. Galena Blvd; Aurora IL 60506; 708-801-2302; Fax: 708-801-2345
Tsai, C. Y.; Dept Bot Plant Pathol; Purdue University; W. Lafayette IN 47907; 317-494-4640
Tu, Zeng-Ping; Guangdong Acad. of Agric. Sciences; Rice Research Institute; Wushan, Guangzhou; Guangdong 510640; CHINA
Tuerck, Jutta; ATC; 210 Science Park; Milton Road; Cambridge CB4 4WA; UNITED KINGDON; 44-1223-420284
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; ; tyersome@nature.berkeley.edu
Tyrnov, Valery; Genetics Dept; 83 Astrakhanskaya Str; Saratov State University; Saratov 410071; RUSSIA; ; Fax: (845-2)240446; postmaster@scnit.saratov.su
Uhr, David V.; Northrup King Co.; 340 Southside Drive; Henderson KY 42420; (502)827-5787; Fax: (502)827-5703
Ujile, Katsuhiro; HOKUREN Naganuma Res. Stn.; Minami-2, Higashi-9; Naganuma-Cho, Yuubari-Gun; Hokkaido 069-13; JAPAN; 01238-8-330; Fax: 01238-8-3200
Ulrich, Valentin; P.O. Box 451; Morgantown WV 26506; 304-292-5262
Usami, Satoru; Plant Brdg Genetics Res Lab; Japan Tobacco Inc; 700 Higashibara Toyoda-cho; Iwata-gun Shizuoka ken438; JAPAN; 81-538-32-7111; Fax: 81-538-32-8700; LDJ00277@niftyserve.or.jp
Vain, Philippe; Cent. Rech. Plantes Physiol. Veg. Molec.; B'aiment 430; Universit'e de Paris Sud; 91 405 Orsay Cedex; FRANCE; 33-1-69-41-77-08; Fax: 33-1-69-85-37-15; quetier@citi2.fr
                                      troxell@beagle.colorado.edu
Vain, Philippe; Cent. Rech. Plantes Physiol. Veg. Molec.; B'airment 430; Universit'e de Paris Sud; 91 405 Orsay Cedex; FRANCE; 33-1-69-41-77-08; Fax: 33-1-69-85-37-15; quetler @citiz.!r

Valdez, Gregorio; Lehman College CUNY; Biology Dept c/o Elli Wurtzel; 250 Bedford Park Blvd West; Bronx NY 10468

Vallejos, Ruben H.; CEFOBI; Suipacha 531; 2000 Rosario; ARGENTINA; +54-41-371955; Fax: +54-41-370044; RNCEFOBI@ARCRIDE.EDU.AR

Van Heeckeren, Wim; Inst Molec Biol; Univ of Oregon; Eugene OR 97403-1229; wim@more.uorgon.edu

Van Montagu, M.; Lab Genetics; K L Ledeganckstr 35; B-9000 Gent; BELGIUM; 32-91-645170; Fax: 32-91-645349; jecop@gengenp.rug.ac.be

van Nocker, Steve; Dept Horticulture; Univ of Wisconsin; Madison WI 53706

Van Schaik, N.; Dept Genetics/U Witwatersrand; PO Wits 2050; SOUTH AFRICA; (011) 716-2125; Fax: 27-11-403-1733

Vanderslice, Olin L.; Vanderslice Enterprises; RD #3, Box 194; Gallatin MO 64640; 816-663-2946

Varagona, Rita; Department of Biology; Box 30001/Dept 3AF; NMSU; Las Cruces NM 88003-0001; (505)646-7963; Fax: (505)646-5665; MVARAGON@NMSU.EDU

Vasal, S. K.; CIMMYT, Lisboa 27; Apartado Postal 6-641; Mexico 6, D.F.; MEXICO; 52-5-726-0991x1206; Fax: 52-5-726-558; SVASAL @ALPHAC.CIMMYT.MX

Vasil, Indra K; Laboratory of Plant Cell & Molecular Biol; 1143 Filigled Hall; University of Florida; Gainesville FL 32611; 904-392-1193; Fax: 904-392-9366

Veit, Bruce; Dept of Biology 0116; Univ of California-San Diego; La Jolla CA 92093-0116; 619-534-7298; bveit@jeeves.ucsd.edu

Veltdboom, Lance; PO Box 839; Holden's Foundation Seeds, Inc.; Williamsburg IA 52361; 319-668-1100; Fax: 319-668-2453; lance@holdens.com

Verbeke, Judith A.; Developmental Mechanisms Program; National Science Foundation; 4201 Wilson Boulevard; Arington VA 22230

Vergne, Phillippe; RCAP; ENS-Lyon; 64 Allee d'altalie; 69364 Lyon cedex 07; FRANCE; 4372-72-86-08; Fax: 439/27 27 86 00; Philippe, Vergne@ens-lyon.fr

Vermerris, Wilfred; Dept. Crop Science; Box 7620; North Carolina State Univ; Raleigh NC 27695-7620; 919-515-2704; Fax: 9
                                        15; quetier@citi2.fr
 VogelJM@ESVAX.DNET.DuPONT.COM

Vollbrecht, Erik, Dept. of Plant Biology; University of California; Berkeley CA 94720; 510-559-5922; Fax: 510-559-5648; erikv@uclink2.berkeley.edu

Von Bulow, J. F. W.; Rua Sa Ferreira, 172; Apt. 601; 22071-100 Rio de Janeiro, RJ; BRAZIL

Von Weltstein, D.; Dept Crop Soil Sci, Genet & Cell Biol; Washington State Univ; Pullman WA 99164-6420; 509-335-3635; Fax: 509-335-8674; diter@wsu.edu

Voytas, D.; Dept. of Botany; lowa State University; Ames IA 50011; ; voytas@iastate.edu

Waines, J. Giles; Dept of Botany; lowa State University of California; Riverside CA 92521; 909-787-3706; Fax: 909-787-4437

Walbot, V.; Dept Biol Sci; Stanford Univ; Stanford CA 94305-5020; 415-723-2227; Fax: 415-725-8221; walbot@leland.stanford.edu

Walden, D. B.; Dept of Plant Sciences; Univ of Western Ontario; London N6A 587; CANADA; 519-661-3103; Fax: 519-661-3935

Walker, Elsbeth L.; Biology Dept; Morrill Science Center; Univ of Mass, Amherst; Amherts MA 01003; 413-545-0861; Fax: 413-545-3243; ewalker@bio.umass.edu

Walker, John; Biol Sci; 308 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-3583; Fax: 573-882-0123; jcw@biosci.mbp.mlssouri.edu

Walker, Nigel; Plant Biol Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720-3102; nigel@mendel.berkeley.edu

Walko, Richard; Pharmacia LKB Biotechnology; Alameda CA 94501

Walsh, Laura C.; Mycogen Plant Sciences; 720 St. Croix Street; Prescott WI 54021; 800-321-2867; Fax: (715)262-3996

Walsh, Laura C.; Mycogen Plant Sciences; 720 St. Croix Street; Prescott WI 54021; 800-321-2867; Fax: (715)262-3996

Walsh, Linkage Genetics; 2411 South 1070 West; Sulle B; Salt Lake City UT 84119; 801-975-1188; Fax: 801-975-1244; wallonm@linkgene.com

Wan, Yuechun; Monsanto Agricultural Group; 700 N Chesterfield Pkwy - GG4H; St. Louis MO 63198; 314-537-6734; ywan@ccmail.monsanto.com

Wang, Zhendong; Waksman Institute; Rudgers Univ; PO Box 759; Piscataway NJ 08855-0759

Wang, Xun; Ploneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004

Wang
      Wang, Liming; Maize Research Institute; Shandong Academy Agric Sci; Jinan, Shandong 250100; CHINA; 011-86-531-8963725 ext 2313; Fax: 011-86-531-8962303 Wang, Ya-hui; Academia Sinica; Inst. of Cell Biol.; 320 Yo-Yang Road; Shanghai 200031; CHINA Wang, Bin; Academia Sinica; Institute of Genetics; Beijing 100101; CHINA Wang, Qinnan; National Natural Science Foundation; Dept. of Life Science; Beijing 100083; CHINA Wanous, Michael; Biol Sci; 117 Tucker Hall; Univ of Missouri; Columbia MO 65211; 573-882-4871; Fax: 573-882-0123; mwanous@biosci.mbp.missouri.edu
      Warner, Todd; Northrup King Co; 317 330th St; Stanton MN 55018-4308
Warner, Jana; Pioneer Hi-Bred Internat Inc; 7300 NW 62nd Ave; PO Box 1004; Johnston IA 50131-1004
Wasserman, Bruce; Food Science Dept; Rutgers Univ; New Brunswick NJ 08903-0231; 908-932-9611 ext 220; Fax: 908-932-6776; Wasserman@AESOP.RUTGERS.EDU
     Weaver, Sam; Quaker Oats Company; 617 W. Main St.; Barrington IL 60010-4199; 708-304-2135; Fax: 708-304-2166
Weber, David; Illinois State Univ; 4120 Biological Sciences; Normal IL 61761; 309-438-2685; Fax: 309-438-3722; dfweber@rs6000.cmp.ilstu.edu
Weber, Gerd; Institut für Pflanzenzuchtung; Universitat Hohenheim; D 70593 Stuttgart; GERMANY; 49 (711)459 2341; Fax: 49 (711)459 2343; weberg@rs1.rz.uni-hohenheim.de
 hohenheim.de

Weck, Edward A.; IAEA; POB 100; 1400 Vienna; AUSTRIA; 43-1-2060-21626; Fax: 43-1-20607; weck@ripo1.iaea.or.at

Weil, Cliff; Dept. of Biological Sciences; University of Idaho; Moscow ID 83843; (208)885-6370; Fax: (208)885-7905; cweil@uidaho.edu

Wen, Tsui-Jung; B422 Agronomy Hall; Iowa State University; Ames IA 50011; 515-294-1659; Fax: 515-294-2299; TJWEN@IASTATE.EDU

Wendel, Jonathan F.; Department of Botany; Bessey Hall; Iowa State University; Ames IA 50011; 515-294-7172; Fax: 515-294-1337; 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; Fax: +49 221 470 5164; Wower@Gen1.Genetik_Uni-Koeln.de

Wessler, Sue; University of Georgia; Dept of Genetics; Life Sciences Bldg; Athens GA 30602; 706-542-1870; Fax: 706-542-3910; sue@dogwood.botany.uga.edu

West, Dennis R.; Dept Plant and Soil Sci; Univ of Tennessee; Knoxville TN 37901-1071; 423-974-8826; Fax: 423-974-7997; DWEST3@UTK.EDU

Westhoff, Peter; Ins. Ent. Mol. Bio. Pflanzen; Heinrich-Heine-Univ Dusseldorf; D-40225 Dusseldorf; GERMANY; 49-211-311-2338; Fax: 49-222-311-4871; West@Uni-
```

```
Duesseldorf.de
       Wetzel, Carolyn; Dept of Botany; Bessey Hall; Ames IA 50011; 515-294-7724; Fax: 515-294-1337; cmwetzel@iastate.edu
Whalen, R. H.; Dept of Biology; South Dakota State Univ; Brookings SD 57007; 605-688-4553
White, Shawn; Dept of Plant9iology; 220 Biological Science; 1445 Gortner Avenue; St. Paul MN 55108; 612-625-0271; white090@tc.umn.edu
Whitwood, W.; Robson Seed Farms; 1 Seneca Circle; Hall NY 14463; 716-526-5879; Fax: 716-526-5350
       Wildholm, Jack M.; Crop Science; Univ of Illinois; PABL, 1201 W. Gregory; Urbana IL 61801; 217-333-9462; Fax: 217-333-4777; Wildholm@UIUC.EDU Widstrom, Neil W.; Coastal Plain Exp Sta; PO Box 748; Tifton GA 31793; 912-387-2341; Fax: 912-387-2321; nwidstro@tifton.cpes.peachnet.edu Wienand, Udo; Inst. Allge. Bot.; Angew. Molek.; Pflanzen, AMP I, Ohnhorststrasse 18; D-22609 Hamburg; GERMANY; (49)40 822 82 501; Fax: (49)40 882 82 503; FBSA114@RRZ-CIP-1.RRZ.UNI-HAMBURG.DE
         Wilkes, H. Garrison; Biology-College II; Univ of Mass/Boston; 100 Morrissey Blvd; Boston MA 02125-3393; 617-287-6600; Fax: 617-287-6650; WILKES@UMBSKY.CC.UMB.EDU
    WILKES@UMBSKY.CC.UMB.EDU

Willcox, Martha; CIMMYT; Apartado Postal 6-641; Mexico, D.F. 06600; MEXICO; 52(5)726-9091 ext.1128; Fax: 52(5)726-7559; MWillcox@CIMMYT.MX

Williams, Terrill E.; Pioneer Hi-Bred Internatl; P.O. Box 128; New Holland PA 17557; 717-354-6044; Fax: 717-355-2445; Williamst@phibred.com

Williams, Mark; DuPont de Nemours & Co Agric Biotech; Stine-Haskell Res Cent 210N/253; 1090 Elkton Rd; Newark DE 19714; 302-366-5102; Fax: 302-451-4832; WILLIAME@ESVAX.DNET.DUPONT.COM

Williams, W. Paul; Crop Sci Res Lab; PO Box 9555; Miss. State MS 39762; 601-325-2735; Fax: 601-325-8441

Williams, Robert E.; PO Box 294; Pittsfield IL 62363; 217-285-2530

Williams, Claire G.; Texas A&M Univ; Dept of Forest Science; College Station TX 77843-2135; 409-845-6049; Fax: 409-862-3745; claire_williams@tamu.edu

Williams, Rosalind; Plant Biology Dept; 111 Koshland Hall; UC Berkeley; Berkeley CA 94720

Willman, Mark R.; Hunt-Wesson Inc; 463 U.S. Hwy. 30 East; Valparaiso IN 46383; 219/477-2233; Fax: 219/477-2232

Wing, Rod; Dept of Agronomy; 270 Poole Ag Ctr; Clemson University; Clemson SC 29631; 864-656-7288; Fax: 864-646-5682; RWING@CLEMSON.EDU

Winkler, Rodney G.; University of Arizona; Plant Science (C795424); Forbes Bldg Rm 303; Tucson AZ 85721; 520-621-8746; Fax: 520-621-7186; RWINKLER@AG.ARIZONA.edu

Winter-Vann. Ann Marie: CIBA Ag Biotech: PO Box 12257; Research Triangle Park NC 27709-2257
   Winter-Vann, Ann Marie; CIBA Ag Biotech; PO Box 12257; Research Triangle Park NC 27709-2257
Wirtz, Uwe; I.G. S. Biotec GmbH; Hauptstr. 8; D06408 Biendorf; GERMANY; 49-34722-400-11; Fax: 49-34722-400-20
Wise, Roger; USDA-ARS; Dept. Plant Pathology; lowa State Univ.; Ames IA 50011-1020; 515-294-9756; Fax: 515-294-9420; rpwise@iastate.edu
Wolfe, Kenneth; Dept. of Genetics; University of Dublin; Trinity College; Dublin 2; IRELAND; 353-1-702-1253; Fax: 353-1-679-8558; KHWOLFE@VAX1.TCD.IE
Woo, Claudine; 2667 Parker St; Berkeley CA 94704
Woodman, Wendy; Dept. of Agronomy; lowa State Univ.; Ames IA 50011; 515-294-3635; Fax: 515-294-3163; wlwoodma@iastate.edu
Woodruff, Dorde; 6366 Cobblerock Lane; Salt Lake City UT 84121-2304; 801-277-5526
Woodward, Wendy; Biol Sci; T10 Tucker Hall; Univ of Missouri; Columbia MO 65211
Woody, Laura; Biol Sci; 110 Tucker Hall; Univ of Missouri; Columbia MO 65211
Wright, James; Pioneer Hi-Bred Intl; PO Box 245; ; Wilson NC 27894-0245
Wright, Allen; ExSeed Genetics; 1515 West 2200 South, Suite C; Salt Lake City UT 84119; (801) 975-1188; Fax: (801)975-1244
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; Fax: 718-960-8236; etwic@cunyvm.cuny.edu
Xia, Yiji; B420 Agronomy Hall; lowa State Univ; Ames IA 50011
Xia, Zhen-Ao; Academia Sinica; Shanghai Inst. of Plant Physiol.; 300 Fonglin Road; Shanghai 200433; CHINA
Xiao, Yongli; 2292-1 Molecular Biology Bidg; lowa State University; Ames IA 50010; 515-294-3277; yxiao@iastate.edu
Xie, You-Ju; College of Biology; China Agricultural University; Ames IA 50010; 515-294-3277; yxiao@iastate.edu
Xie, You-Ju; College of Biology; China Agricultural University; Ames IA 50011
Xia Zhen-Ao; Academia Sinica; Shanghai Inst. of Plant Physiol.; 300 Fonglin Road; Shanghai 200433; CHINA
Xiao, Yongli; 2292-1 Molecular Biology Bidg; lowa State University; Ames IA 50010; 515-294-3277; yxiao@iastate.e
Xie, You-Ju; College of Biology; China Agricultural University; Beljing 100094; CHINA; 86 (10) 62631895; Fax: 0086-1-2582332; <daimin@public3.bta.net.cn>
Xiong, Cheminir, China National Rice Research Institute; Ti Yu Chang Road 171; Hangzhou; Zhejiang 310006; CHINA
Xu, Xiaojie; B420 Agronomy Pall; Iowa State Univ; Amse Ia 50011
Xu, Wenwei; DeKalb Genetics Corp; 3100 Sycamore Road; DeKalb IL. 60115; 815-758-9507; Fax: 815-758-1257; WXU@DEKALB.COM
Xu, Guilin; Agronomy Dept.; 3002 Curtis Halt; University of Missouri; Columbia MO 65211; 573-882-2033
Xu, Yun-Bi; Zhejiang Agricultural University; Dept. of Agronomy; Hangzhou; Zhejiang 310029; CHINA
Xu, Zhi-Hong; Shanghai Inst. of Plant Physiol; 300 Fenglin road; Shanghai 200032; CHINA
Xu, Zhi-Hong; Shanghai Inst. of Plant Physiol; 300 Fenglin road; Shanghai 200032; CHINA
Xu, Zhi-Hong; Shanghai Inst. of Plant Physiol; 300 Fenglin road; Shanghai 200032; CHINA
Xu, Zang-Fu; Zhongsan Univ; Biotechnology Res. Center; 135 West Xingang Road, Guangzhou; Guangdong 510275; CHINA
Yakin, Paul; Pioneer Hi-Bred Internat Inc; Trait & Technol Devel; 7300 NW 62nd Ave, PO Box 1004; Johnston IA 50131-1004
Yamada, Timoru; STAFF; Sankaido Bldg, 7F; Akasaka 1-9-13, Minato-ku; Tokyo 107; JAPAN; (03)3586-8644; Fax: (03)3586-8277
Yamaguchi, Judy; Plant Gene Expression Center; USDA-ARS; 800 Buchanan St; Albany CA 94710
Yamamato, Kirniko; Rice Genome Res Program, STAFF Inst; 446-1, Ippalzuka, Kamiyokoba; Ippalzuka, Tsukuba-shi; Ibaraki-ken 305; JAPAN; ; yamamoto@staff.or.jp
Yamad, Tom; Biol Sci; 324 Tucker Hali; Univo filissouri; Columbia MC 65211
Yang, Jin Shuí; Fudan University; Institute of Genetics; Shanghai 200433; CHINA
Yang, Hen-Cui; Fujian Agricultural College; Helerosis Ullization Lab.; Chinmen, Fuzhou; Fujian 350002; CHINA
Yang, Hong; Chinses Academy of Agric. Sciences; Biotech. Research Centre; Belijing 100081; CHINA
Yang, Hong; Chinses Academy of Agric. Sciences; Biotech. Research Centre; Belijing 100081; CHINA
Yang, Hong; Chinses Academy of Agric. Sciences; Biotech. Research; Hokuri
   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
You, Chong-biao; Chinese Academy of Agric. Sciences; IAAE, Dept. of Biotechnology; P.O. Box 5109; Beijing 100094; CHINA
Young, Todd; Botany Dept; UC Riverside; Riverside CA 92521-0129
Yu, Jia; Dept. Biological Science; Lehman College; 250 Bedford Park Blvd. West; Bronx NY 10468; 212-960-4994; Fax: 212-960-8227; ARYLC@CUNYVM.CUNY.EDU
Yu, Yong Gang; CSES VPI & SU; Blacksburg VA 24061-0404; [703]231-3701; YGYU@VTVM1.CC.VT.EDU
Yu, Li; China National Rice Research Institute; Library; Tiyuchang Road No. 171, Hangzhou; Zhejiang 310006; CHINA
Yu, Di-qiu; Zhongsan(Sun Yat-Sen)University; Biotechnology Research Center; Guangzhou; Guangdong 510275; CHINA
Yu, Yuhua; Souh China Inst. Bot.; Academia Sinica; Guangzhou 510650; CHINA
Zabala, Gracia; Plant Biol/265 Morrill Hall; Univ Illinois; 505 S. Goodwin Avenue; Urbana IL 61801; 217-333-3736; Fax: 217-244-7246; Gracia.Zabala@Qms1.Life.uiuc.edu
Zaitlin, David; 4112 Yurna Drive; Madison WI 53711; 510-729-0720; Fax: 510-562-9215
Zavalishina, Alexandra; Genetics Dept; Saratov State University; 83, Astrakhanskaya St.; 410071, Saratov; RUSSIA; ; Fax: 845-2-240446; POSTMASTER@SCNIT.SARATOV.SU
Zehr, Brent; Maharashtra Hyb Seeds Co; Rehsam Bhavan, 4th Floor; 78, Veer Nariman Road; Mumbai 400020; INDIA; ; mahycord@bom2.vsnl.net.in
      Zehr, Brent; Maharashtra Hyb Seeds Co; Rehsam Bhavan, 4th Floor; 78, Veer Nariman Road; Mumbai 400020; INDIA; ; mahycord@bom2.vsnl.net.in Zeng, Zhaomei; Washington University; Dept. of Biology; Campus Box 1137; St. Louis MO 63130; 314-935-6826
```

Zeng, Mengqian; Institute of Genetics; Academia Sinica; 3 Datun Rd.; 100101 Beijing; CHINA; 64917283; Fax: 8610-64914896; root@igt.ac.cn
Zhang, Fan; Department of Botany; Box 7612; NCSU; Raleigh NC 27695-7612; ; Fax: 919-515-3570; Fan_Zhang@ncsu.edu
Zhang, Jianbo; 2288 Molecular Biology Building; Iowa State University; Ames IA 50010; (515)294-2922; jzhang@iastate.edu
Zhang, Peifen; 2288 Molecular Biology Bldg; Iowa State University; Ames IA 50011; 515-294-2922; zpf@iastate.edu
Zhang, Deyu; Jiangsu Academy of Agricultural Sci.; Inst. of Genet. and Physiol.; Nanjing 210014; CHINA
Zhang, Gui-Quang; South China Agricultural University; Dept. of Agronomy; Guangzhou 510642; CHINA
Zhang, Qifa; Huazhong Agricultural University; Nat Key Lab Crop Genet Imp; Wuhan 430070; CHINA; 86-27-7393392; Fax: 86-27-7393392; qifazh@public.wh.hb.cn
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; Fax: 515-270-3444;

zhaoz@phibred.com
Zhao, Qiquan; Zhejiang Agricultural University; Dept. of Tea Science; Hangzhou; Zhejiang 32100; CHINA
Zhen, Zhu; Academia Sinica; Institute of Genetics; Beijing 100101; CHINA
Zheng, Zhenwei; Waksman Institute; Rutgers Univ; PO Box 759; Piscataway NJ 08855; 908-445-2307; Fax: 908-445-5735; zzheng@ocelot.rutgers.edu
Zheng, Zhenwei; Waksman Institute; Rutgers Univ; PO Box 759; Piscataway NJ 08855; 908-445-2307; Fax: 908-445-5735; zzheng@ocelot.rutgers.edu
Zheng, Xin-Zhou; Div Biol Sci; 117 Tucker Hall; University of Missouri; Columbia MO 65211; 573-882-4871; Fax: 573-882-0123; yzheng@biosci.mbp.missouri.edu
Zheng, Kangle; China National Rice Research Institute; 171 Ti Yu Chang Road; Hangzhou 310006; CHINA
Zhixian, Liu; 11 Sangyuan Road; Maize Research Institute; 171 Ti Yu Chang Road; Hangzhou 310006; CHINA
Zhou-Ping; Fujian Agricultural College; Dept. of Agronomy; Fuzhou; Fujian 350002; CHINA; (0531)8963721-313; Fax: (0531)862303
Zhong, Zhen-Ping; Fujian Agricultural College; Dept. of Agronomy; Fuzhou; Fujian 350002; CHINA
Zhou, Li; Dept Molec Biol; Wellman 11, MGH; Boston MA 02114; 617-726-5916; Fax: 617-726-6893; zhou@frodo.mgh.harvard.edu
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
Zhou, Hongsheng; Institute of Crop Breeding and Cultiv; Chinese Acad Agric Sci; 30, Bai Shi Qiao Lu; Beijing 100081; CHINA; 86-1-8351731; Fax: 86-1-8316545;
dyhzhs@public3.bta.net.cn
Zhu, Li-Hong: Naniino Agric, University: Dept. of Agronomy: Naniing: Jiangsu 210014; CHINA

dyhzhs @public3.bta.net.cn
Zhu, Li-Hong; Nanjing Agric. University; Dept. of Agronomy; Nanjing; Jiangsu 210014; CHINA
Zhu, Z. P.; Shanghai Inst. of Plant Physiol.; 300 Fengling Road; Shanghai 200032; CHINA
Zhu, Ying-Guo; Wuhan University; Genetics Dept; Wuchang; Hubei 430072; CHINA; 27-7822712-4560; Fax: 27-7812661
Zhu, Li-huang; Academia Sinica; Institute of Genetics; Datun Road, Andingmen Wai; Beijing 100101; CHINA; 86-1-2033491; Fax: 86-1-4913428
Zhu, Xiaoyang; Inst Crop Germplasm Resources; Chinese Academy of Agric Sci; 30 Bai Shi Qiao Road; Beijing, 100081; CHINA; 86-10-62186647; Fax: 86-10-62174142
Zimmer, Elizabeth; Lab of Molecular Systematics MRC 534; Support Ctr. Nat'l Museum Nat. History; Smithsonian Inst; Washington DC 20560; 301-238-3025; Fax: 301-238-3059; zimmer@onyx.si.edu

"In a sense, NSF gives grants to people who pose probing and insightful questions and then relentlessly test the answers until they create new kernels of knowledge and understanding. These kernels are like pebbles tossed into a pool of water. They generate an ever expanding series of circles -- circles of open knowledge for others to contemplate, add to, and combine for amazingly varied and practical uses"

-- Dr. Neal Lane, Director, NSF, speaking at the Arlington VA Rotary Club, July 25, 1996

V. MAIZE GENETICS COOPERATION STOCK CENTER



Maize Genetics Cooperation • Stock Center

USDA/ARS/MWA - Plant Physiology and 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]

During 1996, 2254 seed samples were supplied in response to 295 requests. Of these, a total of 53 requests were received from 19 foreign countries. Approximately three fourths of our requests were received by electronic mail or through our order form on the World-Wide Web.

Approximately 5 acres of nursery were grown this summer. Although early rains delayed planting, growing conditions were nearly optimal, and with modest supplemental irrigation we obtained good increases of numerous stocks that were in low supply and new stocks from the collections of Robert Brawn, Nina Fedoroff, Barbara McClintock, Gerry Neuffer, Marcus Rhoades, Donald Robertson and others. Special plantings were made of several categories of stocks, with special attention given to the vast collection of mutants that we are in the process of receiving from Dr. Neuffer. We had a much improved winter nursery at the USDA facility in Isabella, Puerto Rico compared to the previous year. Unfortunately, the USDA's winter nursery program was discontinued and we will be growing our winter nursery this year for the first time at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico.

Greenhouse sandbench plantings were made to determine or confirm genotypes relative to seedling traits. Field plantings were also grown to develop pedigree information with regard to mature plant traits. Such information is used to determine or verify genetic constitutions of sources used to paraetists and supply seed requests.

stitutions of sources used to perpetuate stocks and supply seed requests.

We have obtained additional stocks from the collections of Marc Albertsen, Jack Beckett, Vicki Chandler, Ed Coe, Nina Fedoroff, Michael Freeling, Jerry Kermicle, Bob Lambert, John Laughnan, Gerry Neuffer, Donald Robertson, Don Shaver, Virginia Walbot, and others. We expect to receive additional large accessions of stocks from Gerry Neuffer and other maize geneticists within the upcoming year.

During the early years of maize genetics, it was common practice to maintain different mutant alleles of a locus without designating the alleles or keeping them separate, especially if there were no visible phenotypic differences. Many single gene mutant stocks were maintained this way at the Stock Center. This year, extensive pedigree searches were made for each source envelope for the stocks for which multiple alleles were known or suspected to exist, and the alleles were separated and given unique stock numbers. The glossy stocks were the most extensively affected by these changes. Furthermore, many bad sources of *glossy6* were discovered and the stocks either discarded or pulled aside for allelism testing. Authenticated sources of *gl6* will continue to be provided.

We set up a WWW home page in March of 1995 that allows us to receive requests over the 'Web' from users with software such as Mosaic, Internet Explorer or Netscape. We are continuing to enter data into our internal database. We have also been continuing our collaboration with Ed Coe's efforts in the growing Maize Genome Database (MaizeDB). Up-to-date information about our stocks is presently in MaizeDB. A list of available stocks will continue to be published annually as part of the Maize Genetics Cooperation • Newsletter.

Marty Sachs Director Philip Stinard Curator Janet Day Jackson Senior Research Specialist

CATALOG OF STOCKS

| CHROMOSOME 1 | 118N Adh1-IL14H; su1 | 129E ptd1-Mu1568 | 219J B1-I; A1 A2 C1 C2 PI1-Rhoades |
|--|--|---|--|
| | 118O Adh1-Cm 118P Adh1-FCm | 129F dek*-MS2115 129G dek*-MS6214 | r1-g 219K B1-S; pl1-McClintock R1-g |
| 101A sr1 zb4 P1-ww 101B sr1 P1-wr 101C sr1 P1-ww 101D sr1 P1-rr 101F sr1 ts2 P1-rr 102F ms28 103D vp5 103DA vp5-mu3076 103DB vp5-86GN4 103DC vp5-86GN3 103DD vp5-86GN11 103DF vp5-86GN11 103DF vp5-86GN11 103DF vp5-Mumm#1 103E zb4 ms17 P1-ww 104F ms*_6034 | 118Q Adh1-Ct | 130A 010-N1356 | 219L B1-S; pl1-McClintock R1-r |
| 101C sr1 P1-ww | 119A Adh1-1S; Adh2-1P | | 220A Les1-N843 |
| 101D sr1 P1-rr | | CHROMOSOME 2 | 220B ws3 lg1 gl2; Alien Addition T2- |
| 101F sr1 ts2 P1-rr | 119C gs1 | | Tripsacum |
| 102P (11526) | 119D gs1 bm2 119E Ts6 | 201F ws3 lg1 gl2 b1 | 220F os1 221A gs2 |
| 103DA vp5-mu3076 | 119F bm2 | 203BA al1-Brawn | 221C wlv1-N1860 Ch1 |
| 103DB vp5-86GN4 | 119H Adh1-FkF(gamma)25; Adh2-N | 203BB al1-y3 | 221G wlv1-N1860 |
| 103DC vp5-86GN3 | 119J Adh1-Fm335::Ds1 | 203D al1 lg1 | 221I B1-Peru; TB-2Sa |
| 103DD vp5-86GN6 | 119K Adh1-Fm335RV1 | 203G al1-y3 gl2 | 221J TB-2Sb |
| 103DE vp5-86GN11 | 119L Adn1-2F11::US2 | 205A 811 Ig1 g12 | 222A TB-1Sb-2L4464 222B TB-3La-2S6270 |
| 103E zb4 ms17 P1-ww | 120A id1 | 205C lg1 gl2 | 223A trisomic 2 |
| 104F ms*-6034 | 120B nec2 | 205G al1 gl2 B1 | 224AB w*-017-14-A |
| 104G ms*-6044 105A zb4 P1-ww | 119K Adh1-Fm335RV1 119L Adh1-2F11::Ds2 119M Adh1-1F725 120A id1 120B nec2 120C ms9 120CA ms9-6032 120CB ms9-6037 120CC ms9-6042 120D ms12 120E v22-055-4 bm2 120F MpI1-Siscol | 201F ws3 lg1 gl2 b1 203B al1 203BA al1-Brawn 203BA al1-y3 203D al1 lg1 203G al1-y3 gl2 205A al1 lg1 gl2 205B lg1 205C lg1 gl2 205G al1 gl2 B1 206A lg1 gl2 B1 206A lg1 gl2 B1 sk1 208C lg1 gl2 b1 208H gl2-Salamini 209F lg1 gl2 b1 sk1 | 224B v*-5537 |
| 105A zb4 P1-ww | 120CA ms9-6032 | 208B lg1 gl2 B1 sk1 | 224H whp1; A1 A2 C1 c2 R1 |
| 105B zb4 P1-wr 105C zb4 P1-ww br1 | 120CB MS9-6037 | 2080 ig1 gi2 B1 sk1 v4 | 2241 ws3-7752 224J ijmos*-7335 |
| 105E ms17 P1-wr | 120D ms12 | 208E la1 al2 b1 | 224K glnec*-8495 |
| 105F ms17 P1-ww | 120E v22-055-4 bm2 | 208E lg1 gl2 b1 208H gl2-Salamini | 224L ws3-8949 |
| 106B ts2 P1-rr | 120F Mpl1-Sisco | 209E lg1 gl2 b1 sk1 | 224M ws3-8991 |
| IU/A F I-CI | 120G MDI (-1 leelii lg | 2091 gl2-Parker's Flint | 224N ws3-8945 |
| 107B P1-rr | 121A ms14 | 210E gl2-Pl3050-3 | 225A TB-3La-2L7285 (2L.26 |
| 107C P1-rw 107D P1-cw | 121A ms14 121AA ms14-6005 121B br2-mi8043 121C D8 121D lls1 121E ty*-8446 121G ct2 121GA ct2-rd3 122A TB-1La | 209E lg1 gl2 b1 sk1 209I gl2-Parker's Flint 210E gl2-Pl3050-3 210F gl2-Pl200291 210G gl2-Pl239114 210H gl2-Pl251009 210I gl2-Pl251885 210J gl2-Pl251930 210K gl2-Pl262474 210L gl2-Pl262493 210M gl2-Pl267186 | 3L.39) 225B TB-1Sb-2Lc (1S.05; 2L.33) |
| 107E P1-mm | 121C D8 | 210H gl2-Pl251009 | 227A dek3-N1289 |
| 107F P1-vv::Ac | 121D lls1 | 210l gi2-Pl251885 | 227B dek4-N1024A |
| 107G P1-or | 121E ty*-8446 | 210J gl2-Pl251930 | 227C dek16-N1414 |
| 107H P1-ww | 121G ct2 | 210K gi2-Pi262474 | 227D dek23-N1428 |
| 109D P1-rr ad1 bm2 109E P1-wr br1 f1 | 121GA CI2-103 | 210L giz-Pi262493 210M gi2-Pi267186 | 227E Les4-N1375 227I nec4-N516B |
| 110A P1-wr an1 Kn1 bm2 110D P1-wr an1 bm2 110E P1-wr ad1 bm2 | 122B TB-1Sb (1S.05; B) 122C P1-wr; R1-nj TB-1Lc Y1 | 210M gl2-Pl267186 211A lg1 gl2 b1 fl1 211H gl2 wt1 | 227K et2-2352 |
| 110D P1-wr an1 bm2 | 122C P1-wr; R1-nj TB-1Lc Y1 | 211H gl2 wt1 | 0071 -40 01-6000 06 |
| 110E P1-wr ad1 bm2 | 122C P1-wr; H1-nj IB-1Lc Y1 124A v*-5688 124B j*-5828 124C w*-8345 124CB w*-8245 124D v*-5588 124E w*-018-3 124F w*-4791 124G w*-6577 124H w*-8054 124I v*-032-3 | 212B Ig1 gi2 b1 fi1 v4 | 228A I18-N1940 |
| 110F P1-wr br1 Vg1 | 1248 "-5828 | 212D ig1 gl2 b1 v4 213B ig1 gl2 wt1 213F ig1 B1-V Ch1 | 228B spt1-N464 |
| 110H P1-Wr Dr1 11 Dm2 | 124C W -0345 | | 228C v26-N453A 228E B1-Bh |
| 110E P1-wr aci om2 110F P1-wr br1 Vg1 110H P1-wr br1 f1 bm2 110K P1-wr br1 111G rs2 111H Les5-N1449 | 124D v*-5588 | 213H lg1 gl2 B1-V 214B lg1 b1 gs2 214C d5 | 228F ms*-6019 |
| 111H Les5-N1449 | 124E w*-018-3 | 214B lg1 b1 gs2 | 228G ms*-6024 |
| 1126 P1-WW DIT II DI112 | 124F w*-4791 | | 228H ms*-6029 |
| 1125 851 | 124G w*-6577 | 214D gl11 B1 214E B1 ts1 | 228l ms*-6038 |
| 112H P1-WW DF1 | 124H W*-8054 | 2142 B1 IS1 214J B1 sk1 | 228J ms*-6041 229A rf3 Ch1 |
| 112K an1 as1 bm2 | 124J v*-8943 | 214L lg1 gl2 mn1 | 229B v24-N424 |
| 112H P1-ww br1 112l P1-ww br1 gs1 bm2 112K an1 gs1 bm2 113A as1 br2 | 124G W'-5577 124H w'-8054 124I v'-032-3 124J v'-8943 124K yg'-8574 124L w'-6474 125A Les2-N845A 125B Mpl1-Jenkins | 215A gl14 | 229C w3 rf3 Ch1 |
| 1100101 | 124L w*-6474 | 215B gl11 | 229E emp2-Mu1047 |
| 113BA rd1-Wasnok | 125A Les2-N845A | 215C wt1 | 229F dek*-MS1365 |
| 113C br1 f1 113E br1 f1 Kn1 | 125B Mpl1-Jenkins 126A bz2 gs1 bm2; A1 A2 Bz1 C1 C2 | 215D mn1 215E fl1 | 229G dek*-MS4160 |
| 113K hm1; hm2 | R1 | 215EA fl1-04 | CHROMOSOME 3 |
| 113L Hm1; hrn2 | 126F o13 | 215G fl1 v4 | |
| 114C br1 bm2 | 126G P1-vv::Ac bz2-m::Ds; A1 A2 Bz1 | | 301A cr1 |
| 114D Vg1 | C1 C2 R1 TB-1Sb (1S.05; B) | 216A fl1 v4 Ch1 | 302A d1-6016 |
| 114F br2 hm1; Hm2 114G br2 hm1; hm2 | 126H P1-vv::Ac bz2-m::Ds 126I P1-vv::Ac | 216D fl1 w3 216E fl1 v4 w3 | 302B d1 rt1 302E d1-tall |
| 115C v22-8983 | 126J P1-ww-1112 | 216G fl1 v4 w3 Ch1 | 303A d1 rt1 Lg3-O |
| 115CA v22-055-4 | 126K P1-ovov-1114 | 217A ts1 | 303F g2 |
| 115J bz2-m::Ds; A1 A2 C1 C2 Pr1 R1 | 126L P1-rr-4B2 | 217B v4 | 303FA g2-pg14::I |
| 116A bz2-m::Ds; A1 A2 Ac C1 C2 Pr1 | 126M P1-vv-5145 | 217G v4 Ch1 | 303FB g2-v19 |
| R1 116C an1 bm2 | 127A bz2 zb7-N101 bm2 127B dek1-N792 | 217H ba2 v4 218A w3 | 303FC g2-Funk 303FD g2-56-3040-14 |
| 116D an1-bz2-6923; A1 A2 Bz1 C1 C2 | 127C dek2-N1315A | 218C w3 Ch1 | 303FE g2-59-2097 |
| Pr1 R1 | 127D dek22-N1113A | 218D Ht1-GE440 | 303FF g2-94-1478 |
| 116G an1 | 127E f1 | 218DA Ht1-Ladyfinger | 303G g2 d1 |
| 116GA an1-93W1189 | 127F Msc1-N791A | 218E ba2 | 304A d1 ys3 |
| 116l bz2 gs1 bm2 Ts6; A1 A2 Bz1 C1 C2 R1 | 127G Tir1-N1590 127i gt1 | 218G B1-Peru; A1 A2 C1 C2 r1-r 218H w3-8686 | 304F d1 Lg3-O ys3 304G Lg3-O Rg1 |
| 117A br2 | 128A ij2-N8 | 218l w3-86GN12 | 304l d1 h1 |
| 117D tb1 | 128B I16-N515 | 219A B1-Peru; A1 A2 C1 C2 r1-g | 305A d1 Lg3-O |
| 117DA tb1-8963 | 128C 117-N544 | 219B b1; A1 A2 C1 C2 r1-g | 305B d1 Lg3-O gl6 |
| 117E Kn1 | 128D pg15-N340B | 219C Ch1 | 305D d1 Rg1 |
| 118B Kn1 bm2 118C W1 | 128E pg16-N219 128F v25-N17 | 219D Ht1 Ch1 219G B1-Bolivia-706B; A1 A2 C1 C2 | 305K d1 cl1; Clm1-4 306D d1 Rg1 ts4 |
| 118CA lw1-3108 | 128G py2-N521A | | 307C pm1 |
| 118J Adh1-3F1124r53 | 129A w18 | r1-g 219H B1-Bolivia; A1 A2 C1 C2 Pl1 Pr1 | 308B d1 ts4 |
| 118K Adh1-1S5657; Adh2-33 | 129B wlu5-N266A | r1-g | 308E ra2 |
| 1101 A II 4 0E1101 I - | | | |
| 118L Adh1-3F1124::Mu3 118M Adh1-3F1124r17 | 129C zb7-N101 129D emp1-R | 219I B1-I; A1 A2 C1 C2 PI1-Rhoades r1-r | 308F ra2 Rg1 310C ra2 lg2 |

| 310D Cg1 |
|--|
| 311A d1 |
| 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 |
| 311F ys3 311G La3-O ys3 |
| 312D Lg3-O |
| 312G bm1-R |
| 312H g2 brn1-R 312l brn1-R cr1 |
| 312J bm1-R ra2 lg2 |
| 312K brn1-Nelson |
| 312L brn1-3071 |
| 312M ms23 313A gl6 |
| 313AA gl6-gl7 |
| 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 |
| 315C Rg1 |
| 315D A1-b(P415); A2 C1 C2 R1 316A ts4 |
| 317l a1-m1-5996-4m; Spm |
| 317J a1-m2::Spm-s; Spm-w |
| 317K a1-m2-7991A::Spm-s |
| 317L a1-m2-8004::dSpm 317M a1-m2-8010::Spm-s |
| 317N a1-m2-8011::Spm-w |
| 3170 a1-m2-8012 |
| 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 317Y a1-m2-8409-2 |
| 317Y a1-m2-8409-2 317Z A1 def-1260 |
| 318A ig1 |
| 318B ba1 |
| 318H vp1-Mc#2 |
| 318l 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 320A lg2 |
| 320C lg2 na1 |
| 320E et1 |
| 320F A1 sh2; A2 b1 C1 C2 pl1 R1 320K sh2-94-1001-11 |
| 320L sh2-94-1001-11 |
| 320M sh2-94-1001-1003 |
| 320N a3-Styles; B1-b Pl1-Rhoades |
| r1-g 3200 a3-Styles; B1-b Pl1-Rhoades |
| R1-nj |
| 320P TB-1La-3Le (1L.20; 3L.45) |
| 320Q TB-5La-3L(1) 320R TB-5La-3L(2) |
| |
| 320S TB-5La-3L(3) |
| 320S TB-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 |
| 320S TB-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 321B lg2 a1; A2 C1 C2 dt1 R1 |
| 320S TB-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 |
| 320S 1B-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 321B Ig2 a1; A2 C1 C2 dt1 R1 321C Ig2 A1-b(P415) et1; A2 C1 C2 dt1 R1 321D a1-m4::Ds: A2 C1 C2 R1 |
| 320S 1B-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 321B Ig2 a1; A2 C1 C2 dt1 R1 321C Ig2 A1-b(P415) et1; A2 C1 C2 dt1 R1 321D a1-m4::Ds; A2 C1 C2 R1 321E a1-rUq; A2 C1 C2 R1 |
| 320S 1B-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 321B Ig2 a1; A2 C1 C2 dt1 R1 321C Ig2 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 |
| 320S 1B-5La-3L(3) 321A A1-d31; A2 C1 C2 R1 321B Ig2 a1; A2 C1 C2 dt1 R1 321C Ig2 A1-b(P415) et1; A2 C1 C2 dt1 R1 321D a1-m4::Ds; A2 C1 C2 R1 321E a1-rUq; 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 C2 dt1 pl1 R1
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-m:rDt (Neuffer); A2 C1 C2
Dt1 R1
 323H a1-st; A2 C1 C2 dt1 Mrh R1
 3231 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
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::Mu1; A2 C1 C2 R1
324K a1-Mus1; A2 C1 C2 R1
324K 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
325C a1-x1; A2 C1 C2 R1
325D a1-x3; A2 C1 C2 R1
 325E A1 ga7; A2 C1 C2 R1
 325G a3
 325l a1-p; A2 C1 C2 Dt1 R1
325J a1-p; A2 C1 C2 Pt1 R1
325K a1-m3 sh2-m1::Ds; A2 Ac C1 C2
 326A sh2-Elmore
326B vp1
326BA vp1-Mum3
326BC vp1-86N6
326BD vp1-86GN14
326BF vp1-86GN19
326BG vp1-Mum2
326C Rp3
326D te1-1
326DA te1-Forester
327A TB-3La
327B TB-3Sb
327C TB-3Lc
 327D TB-3Ld
 328A trisomic 3
 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
329l pg2
329K yel*-8630
329L yel*-5787
329Z T3-B(La); T3-B(Sb)
 330A h1
330G a1-mrh; A2 C1 C2 Mrh R1
330H A1-b(P415) Ring 3; A2 C1 C2
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; Mrh
331A TB-1La-3L5267
331B TB-1La-3L4759-3
331C TB-1La-3L5242 (1L.90; 3L.65)
331E TB-3Lf
331F TB-3Lg
331G TB-3Lh
 331H TB-3Li
 3311 TB-3Lj
331J TB-3Lk
331K TB-3LI
```

331L TB-3Lm

```
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
CHROMOSOME 4
401A Rp4-a
401B Ga1
401C Ga1 su1
401D Ga1-S
401E Ga1-S; y1
401l ga1 su1
401J Ga1-M
401K Ga1-S su1
402A st1
402D Ts5
403A Ts5 fl2
403B Ts5 su1
405B la1-Pl239110
405D la1-R su1 gl3
405G la1-R su1 gl4
406C fl2
406CA fl2-Mu9234
406D fl2 su1
407D su1
407DA su1-N86
407DB su1-N2316
407DC su1-BKG489-13
407DD su1-Pl::Mu
407E su1-am
407F su1-am; du1
408B bm3-Burnham su1
408C su1 zb6
408E bm3-91598-3
408J su1 ra3
408K su1; se1
409A su1 zb6 Tu1
410D su1 zb6 gl3
411A su1 gl4 j2
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-ldf1(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
414B gl4
414BA gl4-Stadler
414BB gl4-gl16
414C gl4 o1
414E de*-414E
415A j2
416A Tu1-A158
416B Tu1-l(1st)
416C Tu1-l(2nd)
416D Tu1-d
416E Tu1-md
416F Tu1 gl3
417A j2 gl3
417B v8
417C gl3
417D o1 gl3
417G j2 gl3 c2; A1 A2 C1 R1
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 Df6 gl3 C2; a1-m A2 C1 R1 419H c2-m1::Spm; A1 A2 C1 R1 419I c2-m2::dSpm c2-m3::Mpi1 419J c2-Mum1 420A su1 Dt4 C2; a1-m A2 C1 R1 420B TB-9Sb-4L6504 420C nec*-rd 420D yel*-8957 420F dp*-4301-43 420G w*-9005 420H Dt4 C2; a1-m A2 C1 R1 4201 TB-9Sb-4L6222 421A TB-4Sa 421B TB-1La-4L4692 421C TB-7Lb-4L4698 422A trisomic 4 423A TB-4Lb 423B TB-4Lc 423C TB-4Ld 423D TB-4Le 423E TB-4Lf 424C gl3-64-4 424D gl3-56-3120-2 424E gl3-56-3129-27 424H gl3-Pl251928 424l gl3-Pl251938 424J gl3-Pl254858 424K gl3-Pl267180 424L gl3-Pl267219 424M gl3-Pl-311517 425A TB-4Sg 425B TB-4Lh 425C TB-4Li 427A cp2-o12 427A cp2-012 427AA cp2-N211C 427AB cp2-N1875A 427AC cp2-MS2608 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 427l Sos1-ref 428A gl5 Su1; gl20 428C nec5-N642 428D spt2-N1269A 428E wt2-N10 428F lw4; Lw3 428G bx1 428H gl5 su1; gl20 428I Dt6; TB-4Sa CHROMOSOME 5 501A am1 a2; A1 C1 C2 R1 501B lu1

501A am1 a2; A1 C1 C2 R1
501B lu1
501D ms13
501E gl17
501G gl17 a2; A1 C1 C2 R1
501l am1
502B A2 ps1-Sprague pr1; A1 C1 C2
R1
502D A2 bm1 pr1; A1 C1 C2 R1
502F NI2-N1445
502G A2 ga10; Bt1
503A A2 bm1 pr1 ys1; A1 C1 C2 R1
504A A2 bt1 pr1; A1 C1 C2 R1
504A A2 bt1 pr1; A1 C1 C2 R1
504A A2 bt1; A1 C1 C2 R1
505B A2 pr1 ys1; A1 C1 C2 R1
505B A2 pr1 ys1; A1 C1 C2 R1
505B A2 pr1; A1 C1 C2 R1
506A A2 v3 pr1; A1 C1 C2 R1
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
506C A2 pr1 v12; A1 C1 C2 R1

| 507A a2; A1 C1 C2 R1 | 520F A2 Dap1; A1 C1 C2 R1 | 607A y1 Pl1-Bh1; A1 A2 c1 C2 R1 sh1 | 707EA vp9-3111 |
|---|---|---|--|
| 507AA a2-Mus2; A1 C1 C2 R1 507AB a2-Mus3; A1 C1 C2 R1 | 520G A2 pr1 Dap1; A1 C1 C2 R1 520H Dap1-2 | wx1 607C y1 su2 | 707EB vp9-86GN9 707EC vp9-86GN15 |
| 507AC a2-Mus1; A1 C1 C2 R1 | 521A nec3 | 607E y1 pl1 su2 v7 | 707F y8 gl1 |
| 507F a2 bm1 bt1 ga*-Rhoades; A1 C1 | 521B Nec*-3-9c | 607H y1 Pl1-Bh1; A1 A2 c1 C2 R1 sh1 | 707G in1 gl1; A1 A2 C1 C2 Pr1 R1 |
| C2 R1 | 521C nec*-8624 | Wx1 607I y1 PI1-Bh1; A1 A2 c1 C2 R1 sh1 | 708A ra1 708G y8 |
| 507G a2 bm1 bt1; A1 C1 C2 R1 507H A2 bt1 pr1; A1 C1 C2 R1 | 521D nec*-5-9(5614) 521E nec*-7476 | | 709A gl1 |
| 507l a2-m4::Ds; wx1-m7::Ac7 | 521F nec*-6853 | skb1 wx1 608B Y1 I12 608F Y1 pl1 w1 608G Y1 I11 609A Y1 pb4 609D Y1 su2_89_1273 | 709AA gl1-56-3013-20 |
| 508A a2 bm1 bt1 pr1; A1 C1 C2 R1 | 521G nec*-7281 | 608F y1 pl1 w1 | 709AB gl1-56-3122-7 |
| 508C a2 bt1 bv1 pr1; A1 C1 C2 R1 508F a2 bm1 pr1 ys1; A1 C1 C2 R1 | 521H Rec*-8376 521L v*-6373 | 6094 V1 nh4 | 709AC gl1-Pl183644 709AD gl1-Pl218043 |
| 508H a2-Mum1 | 521J yg*-8951 | 609D Y1 su2 | 709AE gl1-Pl251652 |
| 508l a2-Mum2 | 521K lw3; lw4 | 000DA 11 302-00-1270 | 709AF gl1-Pl257507 |
| 508J a2-Mum3 | 521G nec*-7281 521H nec*-8376 521I v*-6373 521J yg*-8951 521K lw3; lw4 521L w*-021-7 521NA inec*-8549 521P lw3; Lw4 | 609F ms1-Albertsen 610B Dt2 Pt1; a1-m A2 C1 C2 R1 | 709AG gl1-Istra 709AH gl1-BMS |
| 509G a2-muiii4 509G a2-m1::dSpm: Bt1 | 521P lw3: Lw4 | 610C pl1 sm1; P1-rr | 709Al gil-7L |
| 508J a2-Mum3 508K a2-Mum4 509G a2-m1::dSpm; Bt1 509H a2-m1(II)::dSpm(class II) 509I pr1-m1 | 522A TB-5La | 610F Y1 pl1 su2 v7 | 709AJ gl1-9:COOP |
| 509l pr1-m1 | 522B TB-5Lb | 610H Y1 Dt2 pl1; a1-m A2 C1 C2 R1 | 709C gl1-m |
| 510A a2 hm1 nr1 v2: A1 C1 C2 R1 | 5220 1B-550 522D TB-51 d | 611A Pl1 sm1; P1-rr 611D Pt1 | 710A gl1 Tp1 710B gl1 mn2 |
| 510G a2 bm1 pr1 eg1; A1 C1 C2 R1 | 521NA Inec*-8549 521NA Inec*-8549 521P Iw3; Lw4 522A TB-5La 522B TB-5Lb 522C TB-5Sc 522D TB-5Ld 527A dek18-N931A 527B dek9-N1365 527C dek26-N1331 527D dek27-N1380A 527E grt1 527F nec7-N756B 527H Msc2-N1124B | 611E Y1 pl1 w1 | 710E o5 gl1 |
| 511C a2 bt1 pr1; A1 C1 C2 R1 | 527B dek9-N1365 | 611I sm1 py1; P1-rr | 710l gl1 Bn1 |
| 511F a2 bt1 Pr1; A1 C1 C2 H1 | 527C dek25-N1331 527D dek27-N1380A | 611K Y1 PH W1 | 711A Tp1 711B ij1-ref::Ds |
| 512C a2 bt1 pr1 ga*-Rhoades: A1 C1 | 527E grt1 | 6111 sm1 py1; P1-rr 611K Y1 P11 w1 611L w1; I1 611M afd1 612A w14 612B po1 | 711G ts*-br |
| C2 R1 | 527F nec7-N756B | 612A w14 | 712A ms7 |
| | 527H Msc2-N1124B 527I ppg1-N199 | 612B po1 | 712AA ms7-6007 |
| 513C a2 pr1 v2; A1 C1 C2 R1 513D A2 pr1 sh4; A1 C1 C2 R1 | 5271 ppg1-N199 527J nec6-N493 | 612C I*-4923 | 712B ms7 gl1 713A Bn1 |
| 513E a2 pr1 v12; A1 C1 C2 R1 | 528A Hsf1-N1595 | 612D oro1 | 713E Bn1 bd1 |
| 514A a2 bm1 pr1; A1 C1 C2 R1 | 528B wgs1-N206B | 612DA oro1-6474 | 713H Bn1 ij1 |
| 515A vp2 515AA vp2-Mu5180 | 527J nec6-N493 528A Hsf1-N1595 528B wgs1-N206B 528C ani1-N1643 528D TB-1La-558041 528E prg1-Mu8186 528F ren1-Mu807 | 612B po1 612BA po1-ms6 612C 1*-4923 612D oro1 612DA oro1-6474 612l py1 612J w14-8657 612K w14-8050 | 713I bd1 Pn1 714A Pn1 |
| 515AB a2 vp2-green mosaic; A1 C1 C2 | 528E prg1-Mu8186 | 612K w14-8050 | 714B o5 |
| R1 | 528F ren1-Mu807 | 612L w14-6853 | 714BA o5-Mu3038 |
| 515C ps1-Sprague | CHBOMOSOME 6 | 612M w14-025-12 612N w14-1-7(4302-31) | 714D va1 |
| 515CA ps1-8776 515CB ps1-881565-2M | CHROMOSOME 6 | 613A 2NOR; A1 a2 bm1 C1 C2 pr1 R1 | 715A Dt3; a1-m A2 C1 C2 R1 715C gl1 Dt3; a1-m A2 C1 C2 R1 |
| 515D bm1 | 601C rgd1 y1 | v2 | 716A v*-8647 |
| 515CB ps1-881565-2M 515D bm1 516B bt1-R 516BA bt1-Elmore 516BB bt1-C103 516BC bt1-Singleton 516BE bt1-sh5 516BE bt1-6-783-7 516BH bt1-6-783-7 516BJ bt1-T 516C ms5 516D td1 ae1 516G A2 bm1 pr1 yg1; A1 C1 C2 R1 517AB v3-8982 517B ae1 | 601D rgd1 Y1 | 613D vms*-8522 | 716B yel*-7748 |
| 516BR bt1-C103 | 601F po1-ms6 y1 pl1 601H rhm1 rgd1 y1 | 613F w14-8613 | 716F Les9-N2008 717A TB-7Lb |
| 516BC bt1-Singleton | 601l rhm1 y1 l11 | 613F w14-0613 613I tus*-5267 613J gm*-6372 613L w*-8954 613M yel*-039-13 613N yel*-7285 | 718A trisomic 7 |
| 516BD bt1-sh3 | 601J Wsm1 Mdm1; Wsm2 Wsm3 | 613L w*-8954 | 719A TB-7Sc |
| 516BE bt1-sh5 | 601K wsm1 mdm1; wsm2 Wsm3 601L Mdm1 y1 | 613M yel*-039-13 | 720A Dt3; a1-m1::rdt TB-7Lb 727A dek11-N788 |
| 516Bl bt1-Vineyard | 602A po1-ms6 wi1 y1 | 613P yel*-8631 | 727B wlu2-N543A |
| 516BJ bt1-T | 602C y1 | 613T pg11-6656; pg12-6656 | 727E gl1-cgl |
| 516C ms5 | 602D rhm1 Y1 | 614A TB-6Lb 614B TB-6Sa | 727F Ås4-N1606 727G Rs1-O o2 v5 ra1 gl1 |
| 516G A2 bm1 pr1 vg1: A1 C1 C2 R1 | 602J y1-w-mut 602K y1-gbl | 614C TB-6Lc | 727H ms*-6004 |
| 517A v3 | 602L y1-pb1 | 615A trisomic 6 | 727I ms*-6010 |
| 517AB v3-8982 | 602M y1-8549 | 627A dek28-N1307A | 727J ms*-6013 |
| 517B ae1 517BA ae1-EMS | 602N y1-Caspar 602O y1-0317 | 627B dek19-N1296A 627C vp*-5111 | 727K ms*-6014 728A Px3-6 |
| 517BB ae1-Mu12 | 603A y1 110 | 627D hcf26 | 728B ptd2-Mu3193 |
| 517BC ae1-Mu13 | 603AÁ y1 I10-1359 | 627E Dt2; a1-m A2 C1 C2 R1 TB-6Lc | 728C cp1 |
| 517BD ae1-Mu14 | 603B y1 111-4120 | 627G dek*-MS1104; I*-1104 | 728D sh6-8601 728E sh6-N1295 |
| 517BE ae1-Mu15 517BF ae1-Mu16 | 603C y1 112-4920 603D w15-8896 y1 | CHROMOSOME 7 | 728F ren2-Mu326 |
| 517E ae1 pr1 gl8 | 603H mn3-1184 ý1 | | 728G dek*-MS2082 |
| 518A sh4 | 604D y1 115 | 701B In1-D | 728H dek*-MS5153 |
| 518AA sh4-Rhoades 518AB sh4-o9 | 604F y1 si1-mssi 604FA y1 si1-ts8 | 701D o2 701E o2-Mum1 | CHROMOSOME 8 |
| 518B gl8-Salamini | 604H y1 ms1 | 701F Hs1 | or in tolling contact |
| 518BB gl8-6:COOP | 604HA ms1-Robertson y1 | 702A v5 o2 | 801A gl18-g |
| 518BC gl8-6:Salamini 518BD gl8-10:COOP | 604I Y1 ms1 604IA ms1-6050 | 702B o2 v5 ra1 gl1 702I In1-Brawn | 801B v16 801l yel*-024-5 |
| 518BE gl8-Pl180167 | 605A wi1 y1 | 703A o2 v5 gl1 | 801K v16 ms8 |
| 518C na2 | 605C y1 pg11; pg12 Wx1 | 703D o2 ra1 gl1 | 802G ms43 |
| 518D lw2 519A ys1 | 605E wi1 Y1 PI1 605F wi1 Y1 pI1 | 703J Rs1-0 703JA Rs1-1025::Mu6/7 | 802H gl18-Pl262473 802l gl18-Pl262490 |
| 519AA ys1-W23 | 606A Y1 pg11-4484; pg12-4484 | 7035A AST-1025Wu077 | 803A ms8 |
| 519AB ys1-5344 | Wx1 | 704B o2 ra1 gl1 sl1 | 803B nec1-025-4 |
| 519B eg1 | 606AA pg11-8925; pg12-8925 | 705B o2 gl1 sl1 | 803D gl18-g ms8 |
| 519C v2 519D yg1 | 606AC pg11-8563; pg12-8563 606AD pg11-8322; pg12-8322 | 705D o2 bd1 706A o2 sl1 | 803F nec1-7748 803G nec1-6697 |
| 519E A2 pr1 yg1; A1 C1 C2 R1 | 606B y1 pg11; pg12 wx1 | 707A y8 v5 gl1 | 804A v21-A552 |
| 519F A2 pr1 gl8; A1 C1 C2 R1 | 606C Y1 pg11; pg12 wx1 | 707B in1; A1 A2 C1 C2 pr1 R1 | 804B dp*-8925 |
| 519G zb3 520B v12 | 606E y1 pl1 606F y1 Pl1 | 707C in1 gl1; A1 A2 C1 C2 pr1 R1 707D v5 | 804C tb*-poey1013 804D wh*-053-4 |
| 520C br3 | 606l y1 pg11 su2; pg12 Wx1 | 707E vp9 | 804E w*-017-14-B |

| 804F w*-034-16 804G w*-8635 804H w*-8963 805A fl3 805C gl18-g v21-A552 805E el1 805G ms8 j1 806A TB-8La 806B TB-8Lb 807A trisomic 8 808A ct1 808B Lg4-O 808C Hin1 809A TB-8Lc 810A v16 j1; l1 810B j1 810C gl18-g v21-A552 j1 827A dek20-N1392A 827B dek29-N1387A 827C Bif1-N1440 827D Sdw1-N1592 827E Cit1-N1985 |
|--|
| 827D Sdw1-N1592 827E Clt1-N985 |
| 827J wlu3-N203A 827K pro1 827L pro1-Tracy 828A ats1 |
| |

CHROMOSOME 9 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-I; 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; A1 A2 C2 902D yg2 c1 sh1 Bz1 wx1 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 904D C1 wx1 ar1; A1 A2 C2 R1 904F C1 sh1 bz1 gl15 bm4; A1 A2 C2 905A C1 sh1 wx1 K9S-I; A1 A2 C2 R1 905C C1 bz1 Wx1; A1 A2 C2 R1 905D C1 sh1 wx1 K9S-I; A1 A2 C2 K10 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 R1-906A 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 907A C1 wx1; A1 A2 C2 R1 907E C1-I wx1; A1 A2 C2 R1 y1 907G c1-p; A1 A2 C2 R1 B1-b pl1 907H c1-n; A1 A2 b1 C2 pl1 R1 907l 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 y1 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 910H C1 sh1-bz1-x3; A1 A2 C2 R1 910l sh1-bb1981 bz1-m4::Ds

910L yg2-str 911A c1 wx1; A1 A2 C2 R1 911B c1 wx1 v1; A1 A2 C2 R1 911C c1 wx1 gl15; A1 A2 C2 R1 912A sh1 912AA sh1-1746 912AB sh1-9026-11 912AD sh1-60-155 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 I7 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 913M yg2-Mum9 913N yg2-Mum9 913O yg2-Mu83-106-3 913P yg2-Mu83-106-5 914A wx1 d3-COOP 914K Wc1-ly; Y1 914L bz1-Mus1 914M bz1-Mus2 914N bz1-Mus3 9140 bz1-Mus5 914Q bz1-Mus7 914R bz1-Mus10 915A wx1 915B wx1-a 915C w11 915E wx1-Alexander 916A wx1 v1 916C wx1 bk2 916E wx1 v1 gl15 917A wx1 Bf1-ref 917C v1 917D ms2 917DA ms2-6002 917DB ms2-6012 917E gl15-Sprague 917EA gl15-Lambert 917F d3-COOP 917FC d3-072-7 917FD d3-8054 917FF d3-d2-Harberd 917FG d3-d2-Phillips 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 I6 919D 17 919G 16; I1 919I Bf1-Mu-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

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 921A TB-9La 921B TB-9Sb 921C TB-9Lc 922A trisomic 9 922B Wc1; TB-9Lc 922C C1-I; TB-9Sb 922D TB-9Sd 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 9230 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-l 923X wx1-J 923Y wx1-M 923Z wx1-M1 923ZA wx1-M6R 923ZB wx1-M6NR 923ZC wx1-M8 923ZD wx1-P60 923ZE wx1-R 923ZF wx1-Stonor 924A Ring 9S Wd1 C1-l; wd1 C1; A1 A2 C2 R1 924B Ring 9S C1-I; A1 A2 C2 R1 924C yg2 924D wd1 924E wd1 C1 sh1 bz1 924F tiny fragment 9 Sh1 Bz1; C1 sh1 bz1 wx1 924G C1-I Bz1; Ac DsI 924H c1 sh1 bz1 wx1; Ac 925A bz1-m1::Ds wx1-m9::Ac 925B wx1-m9::Ds; Ac 925C bz1-m2::Ac 925D Wx1-m9r1 925E bz1-m2(DII)::Ds wx1-m6 925F C1 sh1 bz1 wx1-m8::Spm-l8 925H bz1-m2(DI)::Ds wx1; R1-sc 925l c1-m2::Ds Wx1; Ac 927A dek12-N873 927B dek13-N744 927C dek30-N1391 927D Les8-N2005 927E Zb8-N1443 927H C1 Dt7; a1-r A2 C2 R1 9271 G6-N1585 927K Rld1-N1990 928A v28-N27 928B wlu4-N41A

928G c1-m5::Spm wx1-m8::Spm-l8; A1

A2 C2 R1

919U bz1-Mum11

928H wx1-m7::Ac7 928I C1 bz1-mut::Mut; A1 A2 Bz2 C2 928J C1 bz1-(r)d; A1 A2 C2 R1 928K C1 Sh1 bz1-s; Mut A1 A2 C2 R1 928L ms*-6006 928M ms*-6011 9280 ms*-6021 928P ms*-6022 928Q ms*-6027 928R ms*-6031 928S ms*-6046 928T ms*-6047 929A IsoB9-9 isochromosome Type 1 929B IsoB9-9 isochromosome Type 2 929C T9-B(La); T9-B(Sb) 929D IsoB9-9 soct isochromosome (original)
929E Dp9
929F T9-B (La + Sb)
929G T9-8(4453); TB-9Sb
929H T9-3(6722); TB-9Sb 9291 TB-9Sb-1866 929J TB-9Sb-1852 929K TB-9Sb-2150 929L TB-9Sb-14 929M TB-9Sb-2010 930A wx1-Mum1 930B wx1-Mum2 930C wx1-Mum3 930D wx1-Mum4 930E wx1-Mum5 930F wx1-Mum6 930G wx1-Mum7 930H wx1-Mum8 930l wx1-Mum9 930J wx1-Mum10 930K wx1-Mum11 930L wx1-Mu16 930M wx1-Mus181 930N wx1-Mus215 **CHROMOSOME 10** 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 X03A sr3 X03B Og1 X03D Og1 R1; A1 A2 C1 C2 X03E oy1 y9 X04A Og1 du1 R1; A1 A2 C1 C2 X04B ms11 X04D bf2 X05A zn1 bf2 X05E bf2 sr2 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

X08F li1

X09F ms10

X10A du1

X09FA ms10-6001

X09FB ms10-6035

X10AA du1-Mu1

X08FA li1-IL90-243Tco

X09EA g1-g4 X09EB g1-56-3004-24 X09EC g1-1-7(X-55-16) X09ED g1-68-609-13 X09EE g1-ws2

X09G li1 g1 r1; A1 A2 C1 C2

X09B li1 g1 R1; A1 A2 C1 C2

| X10AC du1-Mu3 | |
|--|-----|
| X10AD du1-Mu6 | |
| 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 | |
| X11E g1 R1 sr2; A1 A2 C1 C2 X11F g1 r1; A1 A2 C1 C2 X11H zn1 R1-r; 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; Å1 A2 C1 C2 X13D g1 r1-r sr2; 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 Isr1-El; A1 A2 C1 C2 | |
| X14E r1; A1 A2 C1 C2 wx1 | |
| X14F v18 r1; A1 A2 C1 C2 X14I r1-m3::Ds | |
| X15B I1 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:Pl302369 | |
| X16B abnormal-10 r1; A1 A2 C1 C2 X16C R1-ch; A1 A2 C1 C2 Pl1 | |
| X16D r1 sr2; 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 | |
| X19B w2 X19BA w2-Burnham | |
| X19BB w2-2221 | |
| X19C I1 w2 | |
| X19D o7 X19F r1 w2 | |
| X19G r1-n19 Lc1; b1 | |
| X19H r1-g:e Lc1; b1 X20B I1 | |
| X20C v18 | |
| X20F yel*-8721 | |
| X20F yel*-8721 X20H yel*-5344 X20HA yel*-8793 | |
| X20HB vg*-8962 | |
| X20HB yg*-8962 X21A TB-10La X21B TB-10L19 | |
| X21B TB-10L19 X21C TB-10Ld | |
| X22A TB-10Sc | |
| X22R T11 a-R-101 18 | |
| X22C TB-10Lb X23A trisomic 10 | |
| X24A cm1 | |
| X24B lep*-8691 | |
| 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 | |
| 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 wx | 1 |
| X26E R1-scm2; A1 A2 C1 C2 pr1 wx X26F R1-scm2; A1 A2 C1 C2 in1-D | Š. |
| X26G R1-scm2; A1 A2 C1 c | 2 - |
| 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 119-N425 X27J 113-N59A X27K v29 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-Mu1339 X29B dek*-MS2181 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 X311 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 X321 TB-10L26 X32J TB-10L27 X32K TB-10L28 X33A TB-10L29 X33B TB-10L30 X33C TB-10L31 X33D TB-10L32 X33F TB-10L34 X33G TB-10L35 X33H TB-10L36 X34A TB-10L37 X34B TB-10L38 **UNPLACED GENES**

U140C 14 U140E 13 U140F Fas1 U140G ms22 U140H ms24 U240A Les7-N1461 U240B vp10 U240BA vp10-86GN5 U240BB vp10-TX8552 U240C v13 U240D o11 U340B zb1 U340C zb2 U340D ws1-COOP; ws2-COOP U340DA ws1-Pawnee; ws2-Pawnee U340E y11 U340G oro2 U340H oro4 U440B gl13 U440C zn2 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

MULTIPLE GENE M141A A1 A2 B1 C1 C2 PI1 Pr1 R1-g M141B A1 A2 B1 C1 C2 PI1 Pr1 R1-g M141D A1 A2 b1 C1 C2 pl1 R1-g M241A A1 A2 B1 C1 C2 Pl1 Pr1 r1-g M340A 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 M341C A1 A2 b1 C1 C2 Pl1 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 M441D A1 A2 B1 C1 C2 PI1 Pr1 r1-r M441F A1 A2 b1 C1 C2 pl1 Pr1 R1-g wx1 M541F a1 A2 C1 C2 R1-nj M541G A1 a2 C1 C2 R1-n M541H A1 A2 c1 C2 R1-n M541I A1 A2 C1-I C2 R1-nj M541J A1 A2 C1 c2 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 in1 gl1 R1-nj M641C A1 A2 b1 C1 C2 pl1 Pr1 R1-r 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 M741C Stock 6; A1 A2 B1 C1 C2 pl1 R1-r M741F Stock 6; A1 A2 C1 C2 pl1 R1-g M741G Stock 6; A1 A2 C1-I C2 pl1 R1-g wx1 y1 M741H Stock 6; A1 A2 B1 C1 C2 PI1 R1-nj M7411 Stock 6; A1 A2 C1 C2 R1 M841A A1 A2 C1 C2 pr1 R1 su1 M941A A1 A2 C1 C2 Pr1 R1 wx1 y1 MX40A Mangelsdorf's tester; a1 bm2 g1 gl1 j1 lg1 pr1 su1 wx1 y1 MX40C a1 Dt1 gl2 lg1 wt1 MX40D gl1 wx1 y1 MX40E gl8 wx1 y1 MX41A A1 A2 C1 C2 gl1 pr1 R1 wx1 y1 MX41B A1 A2 C1 C2 gl1 pr1 R1 su1 MX41C a1 a2 bz1 bz2 c1 c2 pr1 r1 wx1 MX41D a1 A2 C1 C2 gl1 pr1 R1 su1

wx1-m8::Spm-l8 **B-CHROMOSOME**

wx1 v1

B542A Black Mexican Sweet, B chromosomes present B542B Black Mexican Sweet, B chromosomes absent

MX41E a1-m1-n::dSpm A2 C1 C2 R1

TETRAPLOID

N102A Autotetraploid; A1 A2 B1 C1 C2 Pl1 Pr1 R1 N102C Autotetraploid; a1-m A2 C1 C2 **Dt1 R1** N102D Autotetraploid; A1 A2 C1 C2 N102E Autotetraploid; B chromosomes present N102EA Autotetraploid; chromosomes present

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

N102F Autotetraploid; A1 a2 C1 C2

CYTOPLASMIC STERILE/RESTORER C736A R213; mito-N Rf1 rf2 C736AB R213 sterile (T); cms-T Rf1 C736B Ky21; mito-N Rf1 Rf2 Rf3 RfC C736C B37; mito-N rf1 Rf2 rf3 rfC C736CA B37 sterile (T); cms-T rf1 Rf2 C736E Tr; mito-N rf1 rf2 Rf3 rfC C736F W23; mito-N rf1 Rf2 rf3 rfC C736G B73; mito-N rf1 Rf2 rf3 rfC C836A Wf9 Sterile (T); cms-T rf1 rf2 C836B Wf9; mito-N rf1 rf2 rf3 rfC C836E Mo17 Sterile (T); cms-T rf1 Rf2 rf3 rfC C836F Mo17; mito-N rf1 Rf2 rf3 rfC C836G Mo17 Sterile (C); cms-C rf1 Rf2 rf3 rfC C836H Mo17 Sterile (S); cms-S rf1 Rf2 rf3 rfC C936D K55; mito-N Rf1 Rf2 rf3 RfC C936F N6; mito-N rf1 Rf2 rf3 rfC CYTOPLASMIC TRAIT C337A NCS2 **C337B NCS3**

T318AA lg1 ig1; R1-nj TB-3Ld

TOOLKIT

T318AB cms-L; ig1 R1-nj T318AC cms-MY; ig1 R1-nj T318AD cms-ME; ig1 R1-nj T318AD cms-NE; ig1 R1-nj T318AE cms-Sp; ig1 R1-nj T318AF cms-Sp; ig1 R1-nj T318AH cms-CA; ig1 R1-nj T318AI cms-C; ig1 R1-nj T318AJ cms-C; ig1 R1-nj T318AJ cms-C; ig1 R1-nj T940A Hi-II Parent A (for producing embryogenic callus cultures)
T940B Hi-II Parent B (for producing embryogenic callus cultures)
T940C Hi-II A x B (for producing embryogenic callus cultures)
T940D KYS (for chromosome observations in pachytene microsporocytes) 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 | RECIPE |
|---|-------------------------|
| T3307G trAc8183; T3-9c (3L.09; 9L.12) wx1 T3308A trAc8200; T4-9g (4S.27; | wx01A T |
| 9L.27) wx1 T3308B trAc6076; T5-9a (5L.69; | wx01B wx1 |
| 9S.17) wx1 T3308C trAc6076; T5-9c (5S.07; | wx02A wx1 |
| 9L.10) wx1 T3308D trAc8175; T5-9c (5S.07; | wx02AA wx1 |
| 9L.10) wx1 T3308E trAc8193; T5-9c (5S.07; | wx03A wx1 |
| 9L.10) wx1 T3308F trAc8179; T5-9a (5L.69; | wx04A 7 |
| 9S.17) wx1 T3308G trAc8181; T5-9a (5L.69; | wx06A 7 |
| 9S.17) wx1 T3308H trAc8186; T5-9a (5L.69; | wx1 |
| 9S.17) wx1 T3309A trAc8196; T5-9a (5L.69; | wx09A wx1 |
| 9S.17) wx1 T3309B trAc6062; T6-9b (6L.10; | wx10A 7 |
| 9S.37) wx1 T3309C trAc6063; T6-9b (6L.10; | wx12A 7 |
| 9S.37) wx1 T3309D trAc8172; T6-9b (6L.10; | wx13A 1 |
| 9S.37) wx1 T3309E trAc8184; T6-9b (6L.10; | wx15A |
| 9S.37) wx1 T3310A trAc8161; T7-9(4363) | WX1 |
| (7ctr; 9ctr) wx1 T3310B trAc8173; T7-9(4363) | WX17A T |
| (7ctr; 9ctr) wx1 T3310C trAc8173; T7-9a (7L.63; | wx1 wx19A T |
| 9S.07) wx1 T3310D trAc8190: T7-9(4363) | wx20A T wx21A |
| (7ctr; 9ctr) wx1 T3310E trAc8194; T7-9(4363) (7ctr; 9ctr) wx1 | wx22A |
| (7ctr; 9ctr) wx1 T3310F trAc8185; T7-9a (7L.63; | wx23A T |
| 9S.07) wx1 T3311A trAc8162; T8-9d (8L.09; | wx25A wx1 |
| 9S.16) wx1 T3311B trAc8182; T8-9d (8L.09; | wx26B 9S.3 |
| 9S.16) wx1 T3311C trAc8182; T8-9(6673) | wx28A wx1 |
| (8L.35; 9S.31) wx1 T3311D trAc6059; T9-10b (9S.13; | Wx30A T |
| 10S.40) wx1 T3311E trAc6059; T9-10(8630) | Wx30C |
| (9S.28; 10L.37) wx1 T3311F trAc8180; T9-10b (9S.13; | Wx1 Wx31A |
| 10S.40) wx1 T3311G trAc8180; T9-10(8630) | Wx31B 7 Wx32A Wx1 |
| (9S.28; 10L.37) wx1 INVERSION | Wx32B Wx1 |
| | Wx32C 3 Wx33A |
| 1143B Inv1c (1S.30-1L.01) 1143C Inv1d (1L.55-1L.92) 1143D Inv1k (1L.46-1L.82) | Wx33B Wx1 |
| 1243A Inv2b (2S.06-2L.05) 1243B Inv2h (2L.13-2L.51) | Wx33C Wx34B |
| 1343A Inv3a (3L.38-3L.95) 1343B Inv3b (3L.21-3L.70) | Wx1 Wx34C |
| 1343C Inv3c (3L.05-3L.95) 1344A Inv9a (9S.69-9L.90) | Wx35A Wx1 |
| 1443A Inv4b (4S.10-4L.12) 1443B Inv4c (4S.89-4L.62) | Wx35B 7 Wx35C |

ROCAL TRANSLOCATION 1 and Wx1 marked) T1-9c (1S.47; 9L.22); wx1 T1-9(5622) (1L.10; 9L.12); T1-9(4995) (1L.19; 9S.20); T1-9(4995) (1L.19; 9S.20); T1-9(8389) (1L.74; 9L.13); T2-9c (2S.48; 9S.33); wx1 T2-9b (2S.17; 9L.22); wx1 T2-9d (2L.83; 9L.270); wx1 T3-9(8447) (3S.44; 9L.14); T3-9c (3L.09; 9L.12); wx1 T3-9(8562) (3L.65; 9L.22); T4-9e (4S.53; 9L.26); wx1 T4-9g (4S.27; 9L.27); wx1 T4-9(5657) (4L.33; 9S.25); T4-9b (4L.90; 9L.29); wx1 T5-9(022-11) (5S.29; 9L.27); T5-9(4817) (5L.06; 9S.07); T5-9d (5L.14; 9L.10); wx1 T5-9a (5L.69; 9S.17); wx1 T6-9(4778) (6S.80; 9L.30); T6-9a (6S.79; 9L.40); wx1 T6-9b (6L.10; 9S.37); wx1 y1 T6-9(4505) (6L.13; 9ctr); T7-9(4363) (7ctr; 9ctr); wx1 T7-9a (7L.63; 9S.07); wx1 T8-9d (8L.09; 9S.16); wx1 T8-9(6673) (8L.35; 9S.31); T9-10(059-10) (10L.53; 31); wx1 T5-9(8386) (5L.87; 9S.13); T1-9c (1S.47; 9L.22); Wx1 T1-9(4995) (1L.19; 9S.20); T1-9(8389) (1L.749; 9L.13); T2-9c (2S.48; 9S.33); Wx1 T2-9b (2S.17; 9L.22); Wx1 T3-9(8447) (3S.44; 9L.14); T3-9(8562) (3L.65; 9L.22); T3-9c (3L.09; 9L.12); Wx1 T4-9e (4S.53; 9L.26); Wx1 T4-9(5657) (4L.33; 9S.25); T4-9g (4S.27; 9L.27); Wx1 T5-9(4817) (5L.06; 9S.07); T4-9b (4L.90; 9L.29); Wx1 T5-9(8386) (5L.87; 9S.13); Wx35B T5-9a (5L.69; 9S.17); Wx1 Wx35C T5-9d (5L.14; 9L.10); Wx1 Wx36A T6-9(4778) (6S.80; 9L.30); Wx1 Wx37A T6-9(8768) (6L.89; 9S.60); Wx1 Wx37B T7-9(4363) (7ctr; 9ctr); Wx1 Wx37C T6-9(4505) (6L.13; 9ctr); Wx1

Wx39B T9-10b (10S.40; 9S.13); Wx1

504

BOX/2 H.K. HAYS

Letters sent to Doctors H. K. Hayes, R. A. Brink, E. W. Lindstrom, M. T. Jenkins, A. M. Brunson, P. C. Mangelsdorf, G. F. Sprague, W. H. Eyster, D. F. Jones, M. Demerec, J. H. Kempton.

November 18, 1931

To Corn Geneticists :-

the corn linkage data ready for publication. In fact considerable progress has already bean made in organising the materials. If you have data not heretofore furnished us which you are willing to send us, we shall be glad to receive them at an early date. As in previous years, we prefer that you publish your own records or have them ready for publication when we get them, so that we can give proper credit and citations to the literature. But, if you have data showing either linkage or independent inheritance, which you do not expect to publish this winter, we shall be glad to get them now. Such records should be sent to Dr. C. E. Beadle, California.

Sincerely,

RAESE

R. A. Emerson

V. MAIZE GENOME DATABASE http://www.agron.missouri.edu

MaizeDB currently presents 14,000 loci, including quantitative trait loci and gene candidates; 2,227 references; 3,618 colleague addresses; 486 traits; 2,479 genetic stocks; 5,449 elite germplasm pedigrees; 21,399 variations with 3000 images and 763 phenotypes; 46,000 links to external databases from 10,000 records; SwissProt and GenBank (Entrez format) link reciprocally to MaizeDB pages. The WWW page 'Previous What's New' provides an historical listing of data and design changes. Since Jan 1995 there have been 1,455,677 accesses, with 108,658 in Feb 1997. A more detailed usage analysis is accessible from the database homepage.

Of Loci, Probes and Maps

MaizeDB maintains information on 1109 mapped genes (to chromosome or better) and 2839 probed sites (RFLP, SSR). There are 1627 public EST's (partially sequenced cDNA's or expressed sequence tags), and 288 have been placed, as probed sites and/or genes, onto public maps. Of 1284 designated genes, there are 794 with some map data, 601 with described phenotypes, and 429 with sequence information, including 160 EST's and 33 simple sequence repeats (SSR). Of the probed sites that do not include designated genes, 462 have probes with sequence information and there are 312 where the sequence is an EST; 160 probed sites have SSRs.

FINDING MAP INFORMATION -- or "why are there so many maps?"

Loci may be initially placed onto any of the following: classical genetic maps (837 loci), the cytogenetic maps (2307 loci), and genetic maps derived from individually defined mapping populations (2614 loci on either/or the UMC and BNL mapping populations (see Table 1). The 1935 genetic map has expanded from 718 centimorgans to some 1800, and is in agreement with the total centimorgans for the maps based on map score data from defined mapping populations (see Table 2).

Integration. The bins maps were first presented in in MNL 66:127 (1992) and currently represent some 6750 loci that have been placed on public maps. However, within a bin, order needs to be ascertained by examining other maps; most loci have not been ordered on more than one map. For example, of 2614 loci placed on either the BNL or UMC map populations, only 371 are on both maps. Of these 371, only 53 are on the genetic map, although some 195 loci on the genetic maps are on either a BNL or a UMC map. See Table 1 for more details.

Other maps in MaizeDB. (1) mitochondrial; (2) maps for tropical or mixed subspecies germplasm; (3) Early releases of maps (BNL, UMC, genetic) for historical and data tracking. To see a full list of the maps in MaizeDB, type a % in the Map Name field and then retrieve.

Retrieval of map score data. Complete sets of map scores for a population may be retrieved using the WWW focussed query form called "Map Score Tables". Subsets, constrained by a range of bins, may be retrieved from "[Map Scores]. By Bins" focussed query page. Alternatively, individual locus pages list all the map scores for a locus, distinguished from the recombination data used for the genetic map.

TABLE 1. NUMBERS OF LOCI SHARED BY DIFFERENT MAPS

| MAP | Bin ¹ | | | | | | |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Bin | 6750 | Cyt ² | | | | | |
| Cyt | 2305 | 2307 | Gen ³ | | | CAL CLOSE | |
| Gen | 836 | 96 | 837 | BNL ⁴ | | | |
| BNL | 1816 | 2 | 114 | 1897 | UMC ⁵ | | |
| UMC | 1087 | 1 | 132 | 371 | 1088 | BxM ⁶ | |
| BxM | 200 | 10 | 24 | 143 | 119 | 204 | MxH ⁷ |
| MxH | 212 | 212 | 0 | 25 | 116 | 92 | 307 |

¹Bin, bins map, <u>coordinates are bins numbers</u>, styled chr#.bin#. A bin is an interval between two fixed Core Marker loci (MNL 69:230, 1995) and includes the beginning (leftmost or top) Marker. It is statistically defined (rather than absolute) only in the sense that chance can lead to inaccurate left-right placement of loci: Some placement to bins is dependent only on the Core Markers, for example, and order may be determined only by 3-point data. That is just the classical mapping order problem. In the case of multipoint data, order can be called with increasing certainty as markers increase in number or populations increase.

²Cyt, cytogenetic map, <u>coordinates vary from -1.0 (short arm) to 1.0 (long chromosome arm)</u> and represent proportional physical distances along a chromosome arm.

³Gen, classical genetic maps.

⁴BNL recombinant inbreds(RI) from CO159 x Tx303 and/or T232 x Cm37.

⁵UMC immortal F2 from Tx303 x CO159.

⁶BxM B73 x Mo17 RI.

⁷MxH Mo17 x H99 RI ^{3,4,5,6,7} centimorgan coordinates.

TABLE 2. COMPARISON OF LINKAGE GROUP SIZES FOR SELECTED MAPS.

| Мар: | Bins | Genetic 1997 | Genetic 1935 | BNL 95/96 | UMC 1995 | UMC 1996 |
|-------|------|--------------|-----------------|-----------|----------|----------|
| Chr | bins | am | am | am | am | am |
| 1 | 12 | 258 | 128 | 284 | 260 | 218 |
| 2 | 10 | 224 | 71 | 203 | 219 | 181 |
| 3 | 10 | 216 | 103 | 182 | 215 | 148 |
| 4 | 11 | 172 | 111 | 196 | 212 | 164 |
| 5 | 09 | 185 | 72 | 220 | 183 | 166 |
| 6 | 08 | 144 | 61 | 159 | 151 | 144 |
| 7 | 06 | 128 | 52 | 180 | 122 | 145 |
| 8 | 09 | 177 | 18 | 171 | 171 | 154 |
| 9 | 08 | 178 | 70 | 145 | 174 | 151 |
| 10 | 07 | 174 | 32 | 140 | 177 | 137 |
| Total | 79 | 1856 | 718 | 1880 | 1884 | 1608 |

Mary Polacco

VII. MAIZE PROBE BANK

DNA clones in the public bank and distribution center at the UMC Maize RFLP Lab now total over 6400, of which we distribute more than 4700 (Table 1). This includes the large sets of sequenced cDNAs generated by Chris Baysdorfer, California State University -Hayward (designated csu), and by Tim Helentjaris (designated uaz or 5C, 6C, etc.), which represent the largest collection of candidate genes publicly available for maize. Over the last four years the UMC Maize Probe Bank has filled more than 540 requests for more than 16,000 clones to 31 countries, including 71 sets of the UMC Core Marker probes (Table 2), which have become the standard landmark probe set for gene and QTL trait mapping in maize. The UMC Core Marker set has become the standard landmark set for mapping genes, phenotypes and QTLs in maize. We are particularly pleased with the growth of interest in using maize clones in "developing" countries. During the period 1993-1996 we received a total of 69 probe requests, including 15 requests for core marker probe sets, from scientists in 15 "developing" countries for a total of 3338 probes distributed. China (PRC) led the list with a total of 1601 probes requested and received. The number of probes sent to scientists in China has increased each year, indicative of the continuing demand for probe distribution. Brazil was second with 979 probes requested. Third in probes requested was Mexico. In addition to the probe banking and distribution functions, the UMC RFLP Lab will place on the UMC Maize map any clones of defined function contributed by individual scientists. This greatly enriches the UMC Maize Genetic Map as a tool to define correlations of seguence to function. To date, we have placed 250 loci from 193 different "contributed" probes. Information about genes, probes, and maps is constantly updated on MaizeDB. Most of the sets of clones of other species being held by us are a backup to their primary storage site and for our use in crossspecies mapping experiments.

Three major, current research efforts will have a major impact on probe resources for maize. The first is the development of SSR probes. The current total of available SSR primer sets for maize is 195. This number is increasing due to the efforts of Lynn Senior, USDA-ARS; Ben Burr, Brookhaven Natl. Labs., and others. As this number expands, coverage of the genome will soon reach a stage that some of the applications currently conducted with RFLPs may be replaced with SSRs. The second development is the expanded use of AFLP technology. For many private and large scale public efforts the cost effectiveness of AFLPs is very attractive. The major drawback for maize is the lack of codominant information for most AFLP alleles. The third development is the initiation of large-scale EST sequencing projects for maize. Four different private concerns are reportedly sequencing greater than 100,000 ESTs for maize. While the sequences and clones are currently being held as proprietary information by the companies, these efforts or a public EST effort in maize have the potential to place into the public sector a number of defined sequence probes much greater than currently available.

The activities of the RFLP Lab and the Probe Bank are meaningful as they contribute to the research needs of the community. The easiest avenue to request clones is through the "PROBE Requests" form in MaizeDB http://www.agron.missouri.edu or by email to musket@teosinte.agron.missouri.edu.

To contribute clones for distribution or to be mapped onto the UMC Maize Genetic Map contact Theresa Musket at the email listed above and she will inform you of the information and materials needed.

These activities are supported by USDA-ARS CRIS projects "Genetic Mechanisms and Molecular Genetic Resources for Corn" and "Maize Genome Database"; supplemental support from USDA Plant Genome and Germplasm Programs; USDA-NRI Plant Genome Panel Grant, "Maize cDNAs and Mutants Mapped in Concert; FAO/IAEA Coordinated Research Program, "Molecular Markers for Maize, Rye and Rice", and helpful contributions from CIMMYT, DeKalb Genetics Corporation, Monsanto-Ceregen, Mycogen Plant Sciences, and Pioneer Hi-Bred International Inc.

| Table 1. Clones maintained by UMC RFLP La Clone Set | Abbreviation | Type of Clone | Total Number in Set | Distribution by UMC |
|--|---|-------------------|---------------------|---------------------|
| Asgrow | \$\(\text{0.000}\) \(\text{0.000}\) \(\text{0.000}\) \(\text{0.000}\) | maize genomic | 85 | Yes |
| Brookhaven National Laboratories | asg bnl | maize genomic | 109 | Yes |
| Brookhaven National Laboratories | bni | maize cDNA | 12 | Yes |
| California State University-Hayward | csu | maize cDNA | 1197 | Yes |
| Cornell University | bcd, cdo, rg, rz | barley, oat, rice | 151 | No |
| Iowa State University | isu | maize cDNA | 136 | Yes |
| Mycogen Plant Sciences | agr | maize cDNA | 413 | Yes |
| Northrup King | npi | maize | 30 | No |
| Contributed Clones | 2000 | mostly maize | 165 | 49 Yes, 116 No |
| Pioneer Hi-Bred International | php | maize genomic | 161 | Yes |
| Pioneer Hi-Bred International | npi | maize both | 236 | Yes |
| Rice Genome Research Program | rgp | rice | 371 | No |
| Texas A&M University | txs | sorghum genomic | 145 | No |
| University of Arizona | uaz 5C, 6C, 7C | maize cDNA | 1920 | Yes |
| University of Hohenheim | Ch6S | maize genomic | 339 | No |
| University of Minnesota | umn | oat | 29 | No |
| University of Missouri | umc | maize both | 249 | 237 Yes, 12 No |
| University of Missouri-Rice | pOs ScG | rice genomic | 18 | Yes |
| University of Missouri-Rye | | rye genomic | 171 | Yes |
| University of Missouri-Tripsacum | tda | tripsacum genomic | 476 | 20 Yes, 456 No |
| | Total Number of Clones: | | 6413 | |
| | Total Distributable Clones: | | 4752 | |

| Table 2. Clone of | distribution. | | | | | | | | | | | |
|-------------------|---------------|--------|-----------|----------------|--------|-----------|--------------|--------|-----------|----------|---------|-----------|
| | | 1993 | | | 1994 | | | 1995 | | | 1996 | |
| | No. of | No. of | No. of | No. of | No. of | No. of | No. of | No. of | No. of | No. of | No. of | No. of |
| Country | Requests | Clones | Core Sets | Requests | Clones | Core Sets | Requests | Clones | Core Sets | Requests | Clones | Core Sets |
| Argentina | 0 | | | 1 | 12 | | 2 | | | 1 | 1 | |
| Australia | | | | 1 | 2 | | | | | 7 | 18 | |
| Belgium | | | | 1 | 214 | | 1 | 7 | | | | |
| Brazil | 1 | 210 | | 1 | 214 | | 4 | 387 | 3 | 7 | 168 | 1 |
| Canada | | | | 1 | 2 | | 1 | 2 | 75 | 3 | 4 | |
| China | 7 | 277 | 1 | 2 | 354 | 2 | 5 | 426 | 3 | 9 | 544 | 3 |
| Colombia | | | | - 2 | | | ĭ | 1 | | 1 | 1 | |
| Costa Rica | | | | | | | | 1.5 | | • • | i | |
| Cuba | | | | | | | | | | - 1 | 48 | |
| England | | | | | | | 1 | 2 | | | 40 | |
| France | 6 | 298 | 1 | 3 | 446 | 2 | 12 | 572 | 3 | 12 | 150 | |
| Germany | U | 230 | | 3 | 128 | 1 | 12 | 147 | 1 | 3 | 4 | |
| | 1 | 118 | | 3 | 120 | | 12 | 147 | | 1 | 7 | |
| Hungary India | 1 | 110 | | | | | | | | 929 | - 4 | |
| Indonesia | | | | | | | | | | | 20 | |
| | | 440 | | 4 | | | • | 110 | | 1 | 20 | |
| Israel | 4 | 118 | | 1 | 4 | | 2 2 11 | 113 | ! | 1 | 92 | |
| Italy | 1 | 7 | | 1 | 13 | | 2 | 207 | 1 | 3 | 56 | - |
| Japan | | | | | | | 11 | 624 | 4 | 9 | 292 | 1 |
| Korea | 1000 | | | | | | 2 5 | 183 | 1 | 3 | 4 | |
| Mexico | 4 | 193 | | 1 | 50 | | 5 | 31 | | 5 | 64 | |
| Netherlands | | | | | | | | | | 1 | 100 | |
| Poland | | | | | | | 774275 | 12721 | | 1 | 10 | |
| South Africa | | | | | | | 3 | 25 | | 5,020 | 0.00 | |
| Spain | | | | | | | 1 | 1 | | 2 2 | 10 2 | |
| Switzerland | | | | | | | 2 | 2 | | 2 | 2 | |
| Taiwan | 1 | 5 | | 1 | 4 | | 1 | 112 | 1 | | | |
| Thailand | | | | | | | 1 | 90 | 1 | | | |
| United Kingdom | 1 | 92 | | 2 | 134 | 1 | 2 | 17 | | 6 | 101 | - 1 |
| Uruguay | | | | | | | | | | 1 | 1 | |
| USĂ | 43 | 2093 | | 58 | 1940 | 9 | 134 | 2506 | 17 | 121 | 1905 | 12 |
| Yugoslavia | | | | - | | | | | | 1 | 90 | 1 |
| TOTALS: | 66 | 3411 | -2 | 77 | 3517 | 15 | 203 | 5455 | 36 | 203 | 3688 | 18 |

Theresa Musket, Georgia Davis, Mary Polacco, Mike McMullen, and Ed Coe

VIII. SYMBOL INDEX

| 1ca (ynh61) 66 a1 27 52 79 abp 85 Ac 2 3 13 14 ad*-N590C 2 ad*-N647 2 adh1 44 ae1 52 ar1 52 ar1 52 atp-alpha 89 atp6-C 89 atp9 89 B-chr 45 47 48 66 b1 21 B1-Peru 24 b32B 63 ba1 78 bh1 52 bm3 83 bn1 83 bn14.36 26 bn15.71 26 bn17.08 61 bn17.49 61 bn112.30a 73 bp1 27 bt1 78 bv1 78 cr1 26 27 bc2 26 cc1 21 27 52 C1-McC 25 c1-W22 25 cc2 27 cc-m1 13 cg1 78 cms-CA 45 cm | cr4-N590 3 cr4-N647 3 csu4 16 csu31 73 csu133 16 ct2-rd3 83 cytopl-E 55 d1 67 d3 52 81 da1 84 DDT-3 67 de*-N1122A 24 de1 78 Ds 2 3 En 13 et1 67 et2 84 g1 84 g2 67 g11 73 gl2 13 84 gl3-m1 13 gl3-m2 13 gl3-m2 13 gl3-m3 12 gl7 83 gl8 13 glossy* 30 GUS 3 12 hs1 78 hsp18 56 57 58 ig1 45 78 in1 27 In1-Brawn 83 In1-D 83 iso-4S 67 iso-7L 67 isu62 72 isu89 72 knox11 73 lb11 66 Les*-A7145 24 Les8-1442 24 Les8-1442 24 Les8-1442 24 Les8-1442 24 Les21 24 lg1 73 84 lg2 67 Irt1 35 mct1 69 me1 65 me2 66 me3 66 mn1 78 ms1 8 ms2 8 ms41 8 ms44 8 msa 86 | mus1 63 nc130 72 nc131 72 nc132 72 nc133 72 ndh1 59 npi393 26 nuc 2 02 13 14 22 49 52 05 52 09 52 011 52 012 52 013 52 0ec17 63 p1 3 21 27 P1-ovov-1114 3 P1-rr 21 Pa*-Brawn 83 pge11 73 phi114-a139 63 phi114-a199 63 phi114-a199 63 phi114-a199 63 phi114-a190 63 phi152 pri 26 px* 7 rr 21 31 84 rr-m3::Ds 3 R1-nj 41 45 Rab 65 Rab2 36 Ras 65 rd1 78 rd3 83 rem1 28 rf4 10 rf5 10 rf6 10 rgd1 66 Rho 65 Ring2S 24 rip1 63 rp4 60 rp7 60 rpomt 68 rrn5-18 89 rrn26 89 rxo1 60 sh1 52 sh2 52 79 shsp 87 sn1 14 | su1 8 52 83 86 su3-89-1303-18 83 su3-94-4079-6 83 T-urf13 89 T9-10(8630) 24 T9-10b 24 TB-3Ld 45 TB-4Lb 38 TB-4Lf 83 TB-4Lf 83 TB-4Sa 38 83 TB-9La 37 TB-9Sb 37 TB-10L19 84 TB-10Lb 38 TB-10Lb 38 TB-10Sc 38 tb1 78 td1 52 telo-3L 67 telo-3S 67 telo-6L 67 telo-7La 67 tga1 86 tlk 29 trisome-5 67 ts3 78 ts4 78 ts6 78 tu1 78 tub4 26 ub1 78 ubi1 12 ucb(anp1) 73 ufo1 21 umc12 73 umc30 73 umc31 16 umc32 61 umc32 61 umc32 80 umc32b 80 umc49 61 73 umc64 61 umc67 61 umc67 61 umc67 61 umc685 60 66 umc89 73 umc90 26 umc93 73 umc95 26 umc105a 27 umc107b 26 umc109 26 umc109 26 umc109 26 umc109 26 umc124 61 73 umc126 26 umc128 26 umc128 26 umc128 26 umc130 61 umc161 80 | v17 83 v19 67 whp1 27 ws1-Coop 84 ws2-Coop 84 ws2-Pawnee 84 ws3 78 wx1 7 26 27 42 51 52 84 wx1-a 52 wx1-m7 13 y1 8 66 83 y3 67 ynh61 (1ca) 66 Ypt 65 ypt 36 zb1 23 zb2 23 zb4 23 zb7 23 zm38 27 |
|--|---|--|--|---|
| cr4-H624 3 cr4-H651 3 | msa 86 msx 8 | sn1 14 sod2 44 | umc161 80 umc193 16 | |
| cr4-H1231 3 | Mu1 30 83 | Spm 13 | v1 8 | |

IX. AUTHOR AND NAME INDEX (* identifies articles authored in this newsletter)

Adams, RLP 75 Adamu, A. Kasim 90* Ahmad, F 77 Akam, ME 89 Alatortseva, TA 75* Albertsen, M 1 112 Allard, RW 29 Allen, R 67* Allison, LA 69 Amasino, RM 76 Anderson, EG 84 Arziev, AS 40* Astiz Gassó, MM 54* Athma, P3 Atkinson, BG 57 58 Auger, JA 47 Aulicino, MB 49* 53* Bacher, A 64* Bachmann, B 82 Bailey, BA 64 Balconi, C 10* 11* 12* Ball, Y1 Barclay, IR 77 Basandrai, AK 32* Baszczynski, CL 58 Battey, NH 2 Baysdorfer, C 122 Beadle, GW 84 86 Beaumont, VH 85* Beckett, JB 24 38 112 Becraft, PW 2* Bennett, B 1 Bennett, MD 47 49 Bertrand-Garcia, R 23* Betrán, FJ 62 Bhat, NK 41 Bhawalkar, JD 16 Bi, XZ 87' Bianchi, A 12 Bird, R McK 55 62* 63* 73 Blakey, CA 79* Blumenthal, T 89 Bockholt, AJ 22* Boller, T 79 Bookstein, R 82 Bosio, D 10* 11* Bottema, CDK 82 Bouchard, RA 57 87* Bowen, B 1 Boyer, CD 12 Brawn, RI 112 Brink, R 83 Bruce, B 65 Burdon, RH 75 Burger, N 92* Burilkov, VK 44* Burnham, C 21 84 Burr, B 63 66 122 Burr, FA 27 Byrne, PF 26* 27* Cámara Hernández, J 45* Carlson, WR 37* 48

Casarini, E 15*

Caserta, M 39 Caso, O 55* Caspar, A 83 Cermakian, N 69 Chandler, VL 76 92* 112 Chang, MT 41 Chen, B 68 Cheng, PC 16* 18* 59* Chernousova, NM 33* Chernov, AA 44* Chi, Y 67 Chiavarino, AM 45* 47* 48* Chin, ECL 72 Chomet, PS 78 Chourey, PS 11 Chu 71 Chumak, MV 45 Coe, EH 1* 12 26* 27* 28 41 73 78 80 112 123* Cohen, P 87 Colasanti, J 87 Collinge, DB 79 Collins, GB 33 Comas, C 49* Comeau, A 77 Cone, K 1 25* Confalonieri, V 49* Conti, S 15* 15* Cooper, P 25* Corcuera, VR 50* 51* 52* Curtis, CA 37 Dai, J 3* Dankov, T 78* Darwin, C 86 Davey, MR 71* Davis, G 123* Dawe, K1 Delarue, M 68 Dempsey, E 67 76 Deng, Y 8* Denison, SJ 56* 57* Denk, W 16 Dennis, ES 76 Dewald, C 19* Dimitrov, B 78* Ding, Q 3* Ding, Y 87* Doebley, J 34 49 Don, RH 82 Donini, G 12* Dorweiler, J 86 Doyle, GG 67* 79* Dragan, Al 45 Drake, J 60* Dutton, CM 82 Echeverria, M 39

Eisenreich, W 64*

Enaleeva, NCh 74*

Eubanks, MW 19 34*

Emerson, RA 84

England, D 24*

Erpelding, JE 61

Eyster, WH 84

Fabry, J 36 Fedenko, VS 32* Fedoroff, N 32 112 Feix, G 35* Ferl, RJ 36* Flint, S 26* Fluminhan, A 75* Fowler, J 67 Franken, P 27 Fraser, AC 84 Freeling, M 67 112 Frey, M 63 64* Froehlich, J 65 Frommer, M 13 Fukui, K 75* Fulcher, RG 79* Galian, LR 55* Galinat, WC 34 77 85* 86* García, MD 54* Gardiner, J 23 Gelfand, DH 82 Gengenbach, B 11 Genovesi, AD 33 Gierl, A 64* Gifford, RM 11 Giura, A 77 Glawischnig, E 64* Goldman, SL 79* González de León, D 61* 62* 63* Gonzalez,G 49* Goodman, MM 66 Goping, IS 57 58 Gostimsky, SA 45 Green, CE 42 Greenleaf, AL 68 Greyson, RI 55* 58* 87* Grotewold, E 3 21* Gulko, EA 42 Gunning 57 Gustafson, JP 28* Guzmán, MR 35 Hageman, J 85 Hake, S 2* 73 92* Hancock, D 24 Hannah, LC 1 Hanten, J 79* Hanway 15 Harding, K 89 Harlander, S 79* Harper, L 3 Helentjaris, T 29 36 66 122 Heun, M 72 Hirschhorn 54 Hirschhorn, E 55* Hochholdinger, F 35* Hoisington, DA 23 28 62* 63* 80 Hon, WC 56 Horn, JD 57 Horwath, M 63* Hu, X 61* Hudson, A 66 Hulbert, SH 60*

Hung, T 82 Huynh, QK 79 Iltis, HH 34 49 Inagaki, M 77 Inamdar, NM 14 Jablonka, E 32 Jackson, JD 112* Jackson, LL 19 Jewell, D 58 Jiang, W 8* Jiang, YH 89* Johri, MM 64* Jones, L 77 Juniper, BE 12 Kahn, RA 36 Kalia, V 32* Kameya, T 75* Kaparthi, R 19* Kappen, T 31* Kass, L1 Kato, A 36* Keegstra, K 65 Keen 71 Kegel, KB 56 Keith, CS 66 Kempton, JH 84 Kendra, D 79* Kenton, A 50 Kermicle, JL 45 112 Kessler, S 30* Khrapunov, SN 45 Khush, G 67 Kindiger, B 45 Kloeckener, B 1 Konstantinov, YM 39* 40* 93* Kowalewski, S 1 Kramer, H 66 Krapchev, B 78* Kravchenko, OA 42* Kriete, A 16 Krochik, GM 45 Kruleva, M 78* Kubo, K 92* Kumar 64 Kumar, H71* Kumar, M 71* Kunze, R 14 63* Kuo, Z 3* L'Heureux, TA 66* Lal 32 Lamb, MJ 32 Lambert, R 112 Landi, P 15* Langdale, J 1 Larkin, JC 21 Larson, RL 64 Laudencia-Chingcuanco, D 2* Laughnan, J 112 Laughner, BH 36* Laurie, DA 47 Leach, JE 60* Lebreton, C 15 Lechelt, C 3

Lee, EA 26* 27* Lee, M 72* Lerbs-Marche, S 69 Levites, EV 44 Li, CH 1 Li, J 8* Li, S 7* Lin, KR 22* Lindquist, J 56 Liou, WS 16* 18* Liu, LH 87* Liu, Q 34* Liu, X 5* Liu, Y 5* 7* Liu, Z 41* Lloyd, AM 21 Longley, AE 48 Lonsdale, DM 67* 69* Lupotto, E 12* Lyndon 2 Lysikov, VN 42* 43* 44* Ma, H 21* Maddaloni, M 12* Maillet, DS 57* Makarova, L 93* Maliga, P 42 Mangelsdorf, PC 19 35 77 86 Manuelidis, L 57 Marrs, K 26 Martienssen, R 66* Martin, JT 12 Mather, DE 28 Matz, E 83 Maximilian C., DMI 77 Mazoti, LB 55 McClintock, B 1 112 McMullen, MD 1 12 26* 27* 60 123* Meisner 39 Melchinger, AE 15 Mettler, I 79* Meyer, P 13 Mi, J 3* Miao, SH 85* Michel, M 13 Micu, VE 77 Mihailov, ME 44* Mikula, BC 31* Miles, D 66 Miller, ME 11 Miller, OJ 76 Misra 11 Mizugaki, M 75* Molina, M del C 50 54* Motorga, V 77* Mottley 71 Motto, M 10* 11* 12* 13* 14* Muraoka, S 41 Murashige, T 42 Musket, TA 15 26* 27* 123* Muzumdar, DA 66* 67* Naidin, C 77 Naranjo, CA 45* 47* 48* 49* 50* 51* 52* 53* Nasar, S 71* Natividad, M 81*

Nelson, OE 83 Nelson, T 65* 66* Neuffer, MG 2 24* 73 77 112 Nguyen, DQ 20* Nishikido, T 65 Nitsch, C 11 Nitsch, JP 11 Nover, L 56 Nurse, P 87 Oaks, A 11 Ohmido, N 75* Okamoto, M 81 Okamuro, JK 2 Orr, A 19* Oshima, RG 82 Pai, EF 36 Palii, AF 42* Pan, SJ 16* 18* Panigrahi, KC 64* Park, MS 18* Park, YD 13 Parsad, PN 18 Paschenko, VM 44* Patterson, EB 8 Payak, MM 32 Paz-Ares, J 26 Peacock, WJ 76 Pellegrini, L 13* 14* Pereira, MG 72 Perry, S 65 Peterson, T 3* Phillips, RL 42 73* 76 Poggio, L 45* 47* 48* 49* Polacco, M 1 24 121* 123* Ponce, MR 82 Power, JB 71* Provine, W 1 Rabinowicz, P 21* Radu, A 77* Ragot, M 28 Rayburn, AL 47 49 Reali, A 12* Rees, CAB 56 Reeves, RG 35 77 86 Reggiani, R 11 Reiter, RS 28 Ren, N 87* Rhoades, MM 67 76 112 Ribaut, JM 61* Riley, R 81 Rivin, C 29* Rizzi, E 10 Robertson, DS 83 112 Roder, MS 61 Roe, JL 29 Rogers, JS 86 Romanova, IM 43* Ronchi, A 13 14 Rosales, TP 54 Rosato, M 45* 47* 48* 49* Rose, KL 66 Roseman, R 48 Rosi, P 48* Rossi, V 13* 14* Rothermel, BA 65 66 Sachan, JKS 32

Sachs, MM 1 112* Saghai Maroof, MA 61 Salamini, F 12* Salvi, S 15* Sandoval, MC 54* Sanguineti, MC 15* Saravitz, CH 12 Sarkar, G 82 Satarova, TN 33* Saxena, KN 32 Schnable, PS 30 35* Schnedl, W 76 Schneerman, MC 66* 67* Schultes, N 66* Schwartz, D 76 78 Sears, ER 81 Senior, ML 60* 62 72* 122 Sharma, RC 32 Shatskaya, OA 45* Shaver, D 112 Shcherbak, VS 45* Sheridan, W 1 Shih, A 16* 18* Shull, GH 50 52 Sidorov, VA 42 Simcox, K 60 Singh, A 32* Sinha, N 30* Sisco, P1 Sitailo, SZ 40 Skoog, F 42 Slater, EC 41 Smith 49 Smith, JD 22* Smith, L3 Smith, RD 87 Smith, RJ 56* 57* Snape, JW 28* Snook, ME 26* 27* Solinas, G 14* Song, YC 87* Southgate, EM 71° Splett, M 34* Stafford, HA 26 Staiger, CJ 14 Stam, P 15 Starlinger, P 14 Staub, RW 66 Steer 57 Stinard, P 2 73 83* 84* 112* Struzhko, VS 32* Stuber, CW 44 60* 72 Subota, IY 40* Suchanov 75 Sun , R 8* Sun, X 85 Sundberg, M 19* Surdam, D 23* Sybenga, J 79 Ta, CT 10 Tacke, E 13 Tanhuanpaa, PK 27 Tanksley, SD 61 Taramino, G 61 Tarasenko, VI 39* Tausta, SL 65*

Taylor, BA 28 Teichgraber 79 Thind, BS 32 Tikhomirov, AYe 32* Timmermans, M 66* Ting, YC 20° Tingey, S 61 Tito, CM 48 49 Tuberosa, R 15* Tyrnov, VS 73* 74* 75* Urechean, V 77* Utz, HF 15 Vain, P12 Valencia, A 36 Vancetovic, J 10* Vidakovic, M 10* Vierling, E 56 58 Villalba, V 85 Vladutu, Cl 73* Vogel, J 173 von Wettstein-Knowles, P 12 Wada, T 65 Waites, R 66 Walbot, V 32 76 112 Walden, DB 18 55* 56* 57* 58* 59* 87* Wang, G 18* Wang, LH 13 Wanous, MK 28* Weber, DF 66* 67* Weil, C 1 Weiner, JH 82 Wendel, JF 44 White, TH 82 Widholm, JM 85* Widstrom, NW 26* 27* Williams, JG 28 Wilson, DB 36 Wilson, MP 36 Winkler, RG 81* Winship, PR 82 Wiseman, BR 26* 27* Wu, KS 61 Xia, T 89* Xiao, Y 3* Xie, G 60 Xie, Y 3* Xu, G 26* Yang, T 5* 7* Yang, Z 55* 58* Ye, GN 12 Yoon, E 29* Young, DA 67* 69* Zabirova, ER 45* Zeng, M 5* 7* Zhang, FD 89* Zheng, Y-Z 37* Zheng, YL 89* Zhizina, GP 45 Zhou, H 8* Zorio, DA 89 Zuber, MS 77

| 24 | | |
|------|---|---|
| | | |
| | | |
| | | |
| ì | | |
| | | |
| | | |
| | | |
| 100 | | |
| | | |
| 94 | | |
| ě | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| 100 | | * |
| | | |
| 9 | | |
| | | |
| 10 | | |
| | | |
| | 8 | |
| | | |
| OL, | | |
| £.79 | | |
| 2 | | |
| | | |
| | | |
| ŭ. | | |
| | | |
| - 2 | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| 9 2 | × | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| 124 | | |

CLONE INFORMATION SHEET -- PLEASE SUPPLY FOR EACH CLONE--FORM IS ALSO AT THE FOLLOWING URL: http://www.agron.missouri.edu/Coop/clonesheet96.html

| | DES | | |
|--|-----|--|--|
| | | | |
| | | | |

ISOLATING LAB/PERSON:

IS THIS A KNOWN SEQUENCE CLONE (circle one)? Yes No

PROPOSED GENE SYMBOL:

WHAT PRODUCT OR FUNCTION?

PRODUCT ACRONYM:

EC NO .:

CLONE TYPE (genomic, cDNA, etc.):

ISOLATED FROM WHAT ORGANISM:

REFERENCE:

Restriction Map/Sequence Information (please specify GENBANK, EMBL, EST, SWISSPROT NOS. if possible):

SOUTHERN BLOT INFORMATION

LINE ANALYZED

ENZYME(S) TRIED

BANDS SEEN

APPROX. MW

NORTHERN BLOT INFORMATION

TISSUE(S)

CONDITION(S)

BANDS SEEN

APPROX. MW

CHROMOSOME ARM, IF KNOWN: NEAREST MARKERS, IF KNOWN:

If you already have map information for this clone, please submit mapscores and mapping population information in typed or electronic format with this form for inclusion in the Maize Genome Database.

IT IS OPTIMAL FOR US TO RECEIVE A STAB (ELSE 10µg OF DRIED PLASMID WOULD BE ACCCEPTABLE).

HOST OF SUPPLIED STAB CULTURE:

AMT. OF PURIFIED PLASMID:

VECTOR:

SELECTIVE AGENT:

ENZYME(S) TO CUT OUT INSERT:

INSERT SIZE:

CAN THE INSERT BY PCR'D?

Yes No

PRIMER SEQUENCE:

SPECIAL CONDITIONS NEEDED FOR PCR:

MAY WE FREELY DISTRIBUTE THIS CLONE NOW?

Yes No

AFTER PUBLICATION OR ONE YEAR?

CONTACT PERSON REGARDING CLONE:

Yes No

NAME:

ADDRESS:

PHONE:

FAX:

E-MAIL:

SEND CLONES AND INFORMATION TO:

MS THERESA MUSKET 302 CURTIS HALL UNIVERSITY OF MISSOURI COLUMBIA, MISSOURI 65211 PHONE: 573/882-2033 FAX: 573/884-7850

EMAIL: MUSKET@teosinte.agron.missouri.edu

AMOUNT TO STATE OF THE STATE OF

EMAIL:

SUBSCRIPTION AND INFORMATION FORM MAIZE GENETICS COOPERATION NEWSLETTER and MAIZE GENOME DATABASE

Please complete both sides and return. Your cooperation in providing this information is needed, whether you subscribe to the Maize Newsletter or not, to keep the database and mailing lists current. Phone, FAX, and E-MAIL addresses are particular aids to Cooperation today.

Subscription has been increased because of increased costs, and may be paid as follows:

| Subscription | n has been increas | ed because of increas | sed costs, and r | nay be paid as follows: | |
|--|--|-----------------------|------------------|---|--------------------------------|
| (mark) | | Non-student | Student | Advisor's Signature | |
| (mark) | 1998 issue | 12.00 | 6.00 | | |
| | 1999 issue | 12.00 | 6.00 | | |
| | 2000 issue | 12.00 | 6.00 | | |
| ••••• | 2001 issue | 12.00 | 6.00 | | |
| | 2002 issue | 12.00 | 6.00 | | |
| \$10.00 per The TIN fo Most back | issue. r the University of issues are availa | Missouri is 43-6003 | 8859. | estantially; if you wish to receive the N | The state of |
| available at | t \$15.00. | | | Code - De- | |
| | | | | ; or by instruments such as postal mone pank 80-86/815; or by credit card with | |
| Name o | f card holder | | Discover o | r Visa or MasterCard? | |
| Address | of card holder | | | | |
| 71001000 | o or our a riola or | | | | |
| | | F | | | |
| Account | No | | Exp. Date | | |
| S | ignature | | | × 1 - 1, 1, - 1, - 1, - 1 | |
| Amount | enclosed or to be | charged: \$ | | | |
| I reques | st Relief from Subs | cription Fee as Follo | ws (mark): | | |
| F | Financial or Exchange Limitations | | | Public Library, Cannot Afford | |
| Please prov | vide your address, | phone, FAX, and E-I | MAIL (please ty | pe or print carefully), and other informa | tion requested on the reverse. |
| NAME: | | | | | |
| ADDRESS | : | | | | |
| | | | | | |
| PHONE: FAX: | | | | | |

| To receive the annual "Call and | | for Maizo Noveletter | VOC | no | |
|--|--------------|-------------------------|------------------------------|--|--------------------|
| To receive the annual Notice for | | yes | | | |
| To receive a diskette, annually, | | yes | | | |
| (Available only to Lifetime contribut | | | | | or 5" disk: MS-DOS |
| Mac; and Microsoft Word, Word P | | one runa picase see se | ion, speeny when | ner you need a c | or or disk, Wo boo |
| Please identify yourself as follows | (mark): | | | | |
| Individual subscriber: | yes no | | | | |
| Research scientist: | yes no | | | | |
| Teacher: | yes no | | | | |
| Corporate research group: | yes no | | | | |
| Public library subscriber: | yes no | | | | |
| Private library subscriber: | yes no | | | | |
| Other subscriber (Please list) | yes no | | | | |
| Interests (please circle): | | | | | |
| | anipulation | Breeding/Selection | Biochemistry | | lopment/Biology |
| | equencing | field corn | photosynthe | | oiology |
| molecular transposable elements cytogenetics transposon tagging | | sweet corn | growth regulation flavonoids | ulators cell cycle/kinetics reproductive biol | |
| | n/expression | industrial food corn | carotenoids | | osis |
| evolution transfor | | Physiology | storage pro | | |
| fine structure | illation | Stress | carbohydrat | | yolo |
| QTLs Gemplas | n | Pests/Diseases | oil content | | |

The Endowment Fund, established with contributions from individuals, corporations and other institutions, underwrites distribution of the Newsletter to deserving institutions and libraries (e.g., in public or Third World institutions) and reduces annual subscription fees to the extent possible. One special way to support the Newsletter some Cooperators have taken, and you may wish to consider, is a gift Lifetime Subscription to a student, postdoc, or technician. Corporate donations have been particularly helpful to date, and continue to be solicited.

Your Gift to the Maize Genetics Newsletter Endowment (confers Lifetime Subscription; donors will be acknowledged in the Newsletter):

| Kernel Endowment | \$ 150.00 | | |
|-----------------------|-----------|--|--|
| Ear Endowment | 250.00 | | |
| Whole Plant Endowment | 1000.00 | | |
| Nursery Patron | 5000.00 | | |
| Hectare Patron | 10000.00 | | |

Disease identify (see all) whether we wish

Donor name as you wish it to be listed (professional titles will not be used; corporate or institutional donors may add the name of an individual in parentheses if desired):

The Notes in this Newsletter are cooperatively shared information. 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

Notes for the 1998 Maize Genetics Cooperation Newsletter need to be in the editor's hands before January 1. Be concise, not formal, but include specific data, tables, observations and methods. A double-spaced, letter-quality copy of your text is needed, and an electronic version. Please follow the simple style used in this issue (title; --authors; tab paragraphs; use minimal citations in the form Corner, BA et al., J Hered 35:35, 1995). Figures, charts and tables should be compact and camera-ready, and provided not only in hard copy but in electronic form (jpg or gif) if possible. Please use tabs instead of spaces to separate columns in tables. Send your MNL Notes, hard copy and electronic, anytime; they will go on the Web verbatim, and will be printed in the annual issue. Electronically, either (1) attach to an email addressed to me; (2) send by FTP to teosinte.agron.missouri.edu (pub/mnl_subunit director; see MaizeDB for details), and alert us with an email to ed@teosinte.agron.missouri.edu; or (3) send a diskette. By all means forward a hard copy. Send to:

Ed Coe 210 Curtis Hall University of Missouri Columbia, MO 65211

SEND YOUR ITEMS ANYTIME; NOW IS YOUR BEST TIME

Author and Name Indexes (and see MaizeDB)

Nos. 3 through 43 Nos. 44 through 50 Nos. 51 to date

Symbol Indexes (and see MaizeDB)

Nos. 12 through 35 Nos. 36 through 53 Nos. 54 to date

Stock Catalogs Marker Stocks Translocations

Rules of Nomenclature (1995)

Cytogenetic Working Maps Gene List

Clone List Working Linkage Maps Plastid Genetic Map

Mitochondrial Genetic Maps

Appendix to MNL 44, 1970 (copies available)

MNL 50:157 Annual in each issue

Appendix to MNL 36, 1962 (copies available)

MNL 53:153 Annual in each issue

In this issue and MaizeDB MNL 55:140 and MaizeDB MNL69:182 and MaizeDB

MNL 52:129-145; 59:159; 60:149 and MaizeDB

MNL69:191; 70:99 and MaizeDB

MNL 65:106; 65:145; 69:232 and MaizeDB

MNL69:191; 70:118 and MaizeDB MNL 69:268 and MaizeDB 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 have reported 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 of the stocks for research when you publish, and advice or help you have received in development of your research project.

MaizeDB needs Cooperators (this means you) to:

- (1) Look at the entries in MaizeDB (see section IX in this Newsletter) for "your favorite genes" and send refinements and updates to maryp@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) Probe or primer information per the information sheet in the back of this issue; fingerprint data indicating enzyme and fragment sizes and defining mapped as well as unmapped fragments.

Cooperators, Clone Home! Each functionally defined clone enhances the map, and mapping information enhances further exploration of the function. Your clone is wanted; please see http://www.agron.missouri.edu/Coop/clonesheet96.html, or the Clone Information Sheet in the back of this issue. EC

