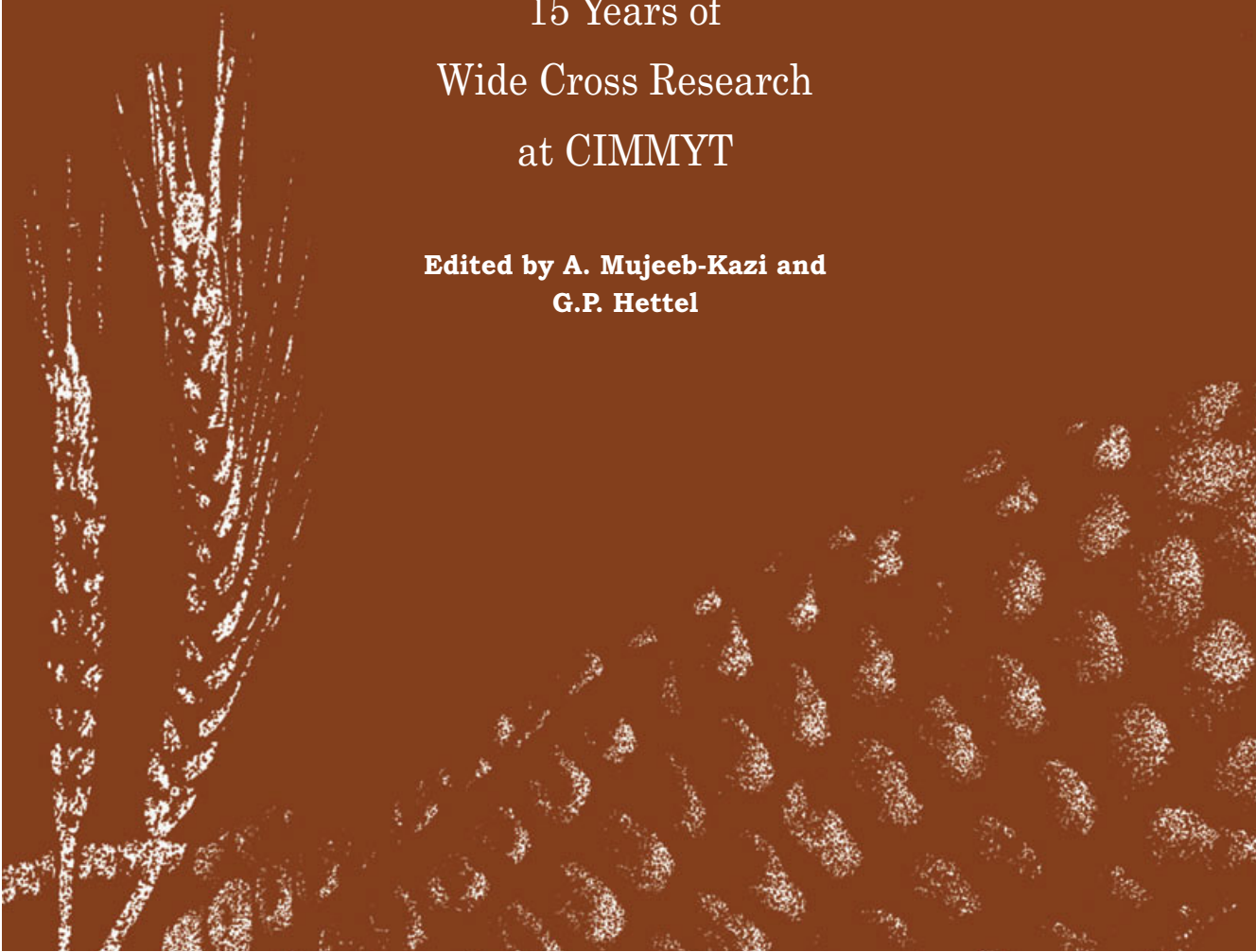
CIMMYT MR

Research Report No. 2

Utilizing Wild Grass Biodiversity in Wheat Improvement:

15 Years of Wide Cross Research at CIMMYT

Edited by A. Mujeeb-Kazi and G.P. Hettel



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CIMMYT is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center is engaged in a worldwide research program for maize, wheat, and triticale, with emphasis on improving the productivity of agricultural resources in developing countries. It is one of 16 nonprofit international agricultural research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). The CGIAR consists of a combination of 40 donor countries, international and regional organizations, and private foundations.

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The nine chapters of this report were sent to external reviewers noted for their respective expertises: Roland von Bothmer, John Carman, André Comeau, Ardeshir Damania, Bob Forsberg, Pat Heslop-Harrison, Howard Rines, and J. Valkoun. For their comments and suggestions, which enabled us to better present complicated topics more clearly, we are highly appreciative. The internal review by George Varughese provided added insight and is also appreciated.

Throughout this 15-year research effort, the Wheat Program directing staff teams of Glenn Anderson/Art Klatt, Byrd Curtis/Art Klatt, and Tony Fischer/George Varughese are gratefully recognized for their support and guidance.

However, the individual who truly made this program possible is N.E. Borlaug, whose vision jump-started the wide crosses research effort at CIMMYT during the early 1970s, which led to my support at Kansas State (1974-1979) and later employment at CIMMYT. Norm has been an ardent supporter of our wide cross efforts and his continuing research discussions up to the present are fondly acknowledged.

Abdul Mujeeb-Kazi
El Batan, Mexico
May 30, 1994

ABBREVIATIONS/ACRONYMS

α–AMY—α-amylase.
ADH—Alcohol dehydrogenase.
ß-AMY—ß-amylase.
BAP—6-benzylaminopurine.
BC—Backcross.
CS—Chinese Spring.
DNA—Deoxyribonucleic acid.
EST—Seed esterase.
FISH—Fluorescent *In situ* hybridization.
GA_3—Gibberellic acid.
GOT—Glutamate oxaloacetate transaminase.
GPI—Glucose phosphate isomerase.
HMW—High molecular weight.
IAA—Indole-3-acetic acid.
IEF—Isoelectric focusing.
ITMI—International Triticeae Mapping Initiative.
KB—Karnal bunt.
LS—Linsmaeier and Skoog (1965) medium.
MDH—Malate dehydrogenase.
MS—Murashige and Skoog (1962) basal medium.
MTT—3-(4,5 dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide.
NAD—B-nicotinamide adenine dinucleotide.
NADP—B-nicotinamide adenine dinucleotide phosphate.
PAGE--Polyacrylamide gel electrophoresis.
PCR—Polymerase chain reaction.
PGM—Phosphoglucomutase.
pI—Isoelectric point.
PMS—Phenazine-methosulfate.
RAPD—Randomly amplified polymorphic DNA sequence.
RFLP—Restriction fragment length polymorphism.
SDS—Sodium dodecyl sulfate.
SKDH—Shikimate dehydrogenase.
SOD—Superoxide dismutase.
TCCP—Tissue Culture for Crops Project.
TEMED—Tetramethylenediamine.
2,4-D—2,4-dichlorophenoxyacetic acid.

PREFACE

The CIMMYT Research Report Series documents specific CIMMYT research efforts and is directed toward technical audiences. Each publication synthesizes the results of research that has usually occurred over an extended period. This report, the second installment in the series, relates the accomplishments of 15 years of wheat wide cross research at CIMMYT. This body of work has been conducted and accumulated by Dr. Abdul Mujeeb-Kazi, head of the Wheat Wide Crosses Section, in conjunction with specialists, who have spent time at CIMMYT or who have collaborated in various ways through their respective centers of excellence in developed and developing countries.

The CIMMYT Wheat Wide Crosses Section assists our cereal breeding programs in adding new variability to the wheat gene pool by making crosses between: 1) wheat and the annual grasses within the genus *Triticum* (interspecific) and 2) wheat and more distant relatives in other genera of the Triticeae tribe (intergeneric). CIMMYT has achieved an international reputation for the production and characterization of these two types of wide hybrids through basic and applied research and by significant scientific reporting in peer-reviewed publications and at international symposia. Although relying on established plant breeding techniques, the Section makes use of emerging technologies to better understand genetic relationships and to introgress genetic variability.

The research has shown that an alien grass species can be screened for a specific resistance or tolerance to a disease or abiotic stress and then be hybridized with wheat to introgress the desired trait. Alternatively, an alien species can be first hybridized to wheat and then the derivative plants can be screened for the trait. The key to either strategy is wide hybrid production, which normally involves embryo rescue and chemical treatment to double the chromosome number of the resulting hybrid plants. These plants are the critical base from which alien genetic material can be utilized by CIMMYT breeders and others in cereal improvement programs.

This report details the outstanding contributions-to-date of new knowledge and techniques emanating from our wide crossing efforts. These include unique approaches to production of wheat polyhaploids involving crosses with maize and *Tripsacum*, application of tissue culture to demonstrate the potential of inducing genetic variability or alien introgressions, and the use of biochemical and molecular markers to confirm the introgression of alien chromosomes. As explained in the report, we are using isozyme analyses, randomly amplified polymorphic DNA sequences (RAPDs), and *in situ* hybridization techniques to detect the presence in wheat of inserted chromosome segments and entire alien chromosomes.

At the onset of our wide crosses effort in 1979, we did not anticipate producing on-the-farm products as rapidly as we have. Recent releases in Pakistan of salt- and drought-tolerant wheat varieties, derived from CIMMYT's alien genetic material, are products already in the hands of farmers. Wheat lines—arising from our wide crosses work to obtain resistance to spot blotch,

a devastating disease of wheat in warmer areas—are being field-tested in a number of countries and will soon be available to farmers. These lines, together with accessions of goat grass (*Triticum tauschii*) and advanced derivatives of wheat with immune responses to Karnal bunt disease, have been registered as genetic stocks with the Crop Science Society of America. Over the last four years, we have also produced an impressive number (nearly 525) of synthetic bread wheats (durum wheat x *Triticum tauschii*), which represent another major contribution toward increasing variability in the wheat gene pool.

I believe that this Research Report is a major document that provides relevant information in a very challenging area of research and trust that it will stimulate discussion among researchers in the related disciplines.

Donald Winkelmann
Director General

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Executive Summary

In their effort to meet the increasing worldwide demand for food, plant breeders are finding less and less appropriate germplasm with desired traits among cultivated crops themselves with which to make needed improvements. Fortunately, useful genetic resources (i.e., important traits for use in crop improvement) are being found among uncultivated plants in the wild. As stated in Chapter 1, the challenge is to be able to incorporate this "new" germplasm routinely into existing food crops through a technique called wide crossing.

Wide Crosses

Most efforts to transfer alien germplasm from wild plants into cultivated crops have involved the *Triticum* grass species—with the greatest emphasis being placed on improving bread wheat (*T. aestivum* L.). Over the past 15 years, CIMMYT has been a part of this endeavor as it has vigorously pursued bread wheat improvement not only with interspecific hybridization (crosses made among annual grasses within the *Triticum* / *Aegilops* group), but also with intergeneric hybridization (crosses with wheat using some of the 250 perennial grasses in the Triticeae tribe). The perennials are important because their natural habitats provide the possibility that they could be potent sources of resistance for several biotic and abiotic stresses. Chapter 2 sets the stage for our wide cross efforts by describing the complex genome make-up of the perennials and annuals in the Triticeae. As described in Chapter 2, some perennial species have recently undergone taxonomic readjustments based on new knowledge about their genomes.

Interspecific hybridization (Chapter 3)

When utilizing the varied gene pools within other *Triticum* species for improving bread wheat, a high priority for breeders is to utilize the many alien accessions among these species that have genomes similar to the A, B, or D genomes of bread wheat. This not only makes accomplishing alien gene introgression easier because of genomic similarities, but it is also compatible with field research and sets the stage for a high success rate in accomplishing multiple-gene (polygenic) transfers. Thus, our interspecific work for bread wheat improvement focuses on using these closely related genomes.

CIMMYT has been concentrating on exploiting accessions of a wild relative of wheat called goat grass (*Triticum tauschii* syn. *Aegilops squarrosa*). We believe this wild relative's diversity and distribution across Eurasia provide a unique opportunity for exploiting novel genetic variability for bread wheat improvement. In addition, *T. tauschii*, unequivocally accepted as being the grass that contributed the D genome to bread wheat, is attributed to have a wide range of resistances and tolerances to diseases and abiotic stresses.

As described in Chapter 3, the best way to exploit *T. tauschii* variability is to first reliably screen the accessions for desired resistances or tolerances. The selected accessions can then be crossed directly with bread wheat (*T. aestivum*)—if a program has embryo rescue and chromosome doubling capabilities—or crossed with durum wheat (*T. turgidum*)—to produce what are called synthetic hexaploids, which can in turn be easily crossed with bread wheat by any conventional breeding program. We have made successful

crosses between Karnal bunt (*Tilletia indica*)-susceptible bread wheats and several KB-resistant *T. tauschii* accessions. We have identified synthetics that are resistant to spot blotch (*Helminthosporium sativum*), *Septoria tritici*, and scab (*Fusarium graminearum*), which can then be crossed to bread wheat. Several synthetics have shown tolerance to salt stress in initial field screening at La Paz, Baja California Sur, Mexico. To date, we have produced nearly 525 synthetic hexaploids—most involving a unique *T. tauschii* accession (see Appendix 2)—for use in crosses with bread wheat.

Intergeneric hybridization (Chapter 4)

The different gene pools within the annual and perennial species of the Triticeae can provide tremendous genetic variability for wheat improvement. However, in contrast to the *Triticum*/*Aegilops* group, the species we deal with in our intergeneric crosses are quite diverse genomically and rather difficult to cross with wheat. Even when successfully combined, the resulting hybrids exhibit little or no intergenomic chromosome association. Despite these limitations, significant successes and advancements have been made by centers of excellence over the past 20 years. CIMMYT's principal objectives in intergeneric crosses have been to obtain tolerances to toxic levels of aluminum and salt; copper uptake efficiency; and resistances to *H. sativum*, *F. graminearum*, and *T. indica*. We also anticipate the eventual transfer of other traits such as resistance to barley yellow dwarf virus (BYDV), *S. tritici*, and Russian wheat aphid (*Diuraphis noxia*).

Hybrids and, in most cases, amphiploids have been produced in crosses between species of the genera *Hordeum, Agropyron, Elymus, Secale*, *Taeniantherum*, *Eremopyrum*, and *Haynaldia*. The range of new hybrids with more distantly related species is constantly increasing and it is expected that a greater range of genotypes will become available for introgressing novel genetic variability into wheat.

Search for New Applications and Knowledge

Although the CIMMYT Wheat Wide Crosses Section's primary thrust has been to assist CIMMYT breeders in adding new variability to the wheat gene pool through interspecific and intergeneric crosses, it has also contributed a significant body of knowledge on new techniques and applications in plant biotechnology.

Polyhaploid production (Chapter 5)

Over the last four years, we have been able to produce high frequencies of polyhaploid wheat plants using either maize or *Tripsacum* pollen. Polyhaploid plants are important in our efforts to reduce the number of generations it takes to fix the homozygosity of wheat and other cereal plants. Homozygosity is required in basic research projects, such as our collaborative work with Cornell University to produce RFLP maps of the wheat and barley genomes.

CIMMYT has obtained a high recovery of polyhaploid wheat plants from crosses between the wheat cultivar 'Morocco' and CIMMYT maize population 'Pool 9A'. The taxonomic proximity of *Tripsacum dactyloides* (L.) to maize has encouraged us to evaluate cross combinations involving *Tripsacum* and wheat (*T. aestivum* and *T. turgidum*) and *T. turgidum* x *T. tauschii* amphiploids. We felt that *Tripsacum* could serve as a novel and alternate sexual route for the production of cereal polyhaploids and, indeed, wheat x *Tripsacum* crosses have resulted in the production of polyhaploid wheat plants of various genotypes.

Unlike wheat anther culture or sexual hybridization of wheat with *H. bulbosum*, genotypic specificity and aneuploidy are absent in maize- and *Tripsacum*-mediated polyhaploid production, which makes them both superior systems. The potential of stored maize and *Tripsacum* pollen needs to be explored because it could be a significant factor in extending the use of the methodology to countries where cropping cycles are separated or where adequate facilities are lacking for growing plants under controlled conditions.

Tissue culture (Chapter 6)

Tissue culture applications have been essential to the production of complex hybrids within the Triticeae. These techniques will presumably even widen the existing range of hybridization possibilities. CIMMYT has exploited long-term callus culture and regeneration to demonstrate the potential of inducing variability within various groups of the Triticeae for morphological, biochemical, and cytological characteristics. Two operational constraints in intergeneric hybridization are associated with alien gene introgression and amphiploid induction. Callus culture methodology has significantly helped us to overcome these constraints by enhancing chromosome pairing analogous to that characteristic of the *Ph* locus on chromosome 5B and by inducing amphiploidy in two intergeneric hybrid combinations.

Callus culture also: 1) provides advantages in inducing variability in euploid wheat cultivars, 2) facilitates *in vitro* screening for stress- or toxin-producing pathogens, and 3) furnishes the capacity to alter chromosomes structurally. In addition, callus culture might be used to modify recombination frequencies in otherwise low-pairing complex hybrids as well as facilitate recovery of hybrid derivatives with double the number of chromosomes.

Confirming alien introgressions (Chapters 7 and 8)

Although in some cases we have been using a radical methodology that purposely de-emphasizes the confirmation of alien gene introgressions and chromosomal interchanges, the Wide Crosses Section has been somewhat involved in looking at biochemical and molecular markers as means to accomplish such confirmation when it is feasible and/or desirable to do so. Initial identification and characterization of alien introgressions can be done by using relatively inexpensive, less complicated cytological techniques and biochemical markers. Once the material is characterized, molecular markers could be established and subsequently used to detect the presence of minute and harder to detect chromosomal interchanges.

Biochemical markers—These marker techniques, which utilize isozymes and seed storage proteins, are applicable in distinguishing alien chromosomes in wheat for both intergeneric and interspecific hybridizations. More than 100 structural genes for isozyme markers have been identified and located on different chromosomal segments in wheat. The major advantage to using isozymes is the speed with which material can be screened because there is adequate polymorphism. Information regarding the homoeology of the alien chromosomes in the addition lines can be ascertained by identifying the genes they possess that are orthologous to sets of *T. aestivum* genes of which the chromosomal locations are known.

This can also be done by studying the ability of the alien chromosomes to substitute for and pair with specific wheat chromosomes.

When biochemical markers are first identified, it is necessary to study the banding profiles for a particular enzyme system in the two parental species. When using these markers, analysis of the two parental species and the amphiploid is important in characterizing the alien genetic material. Sometimes the alien species may show a certain degree of polymorphism for a particular enzyme system that results from allelic differences and/or the mutually incompatible nature of the alien species. We can identify markers when the two parental species show remarkably different banding profiles.

Molecular markers—Molecular markers are becoming increasingly important in detecting alien introgressions and chromosomal interchanges—especially those involving small segments of alien chromatin in wheat backgrounds. The molecular techniques that CIMMYT uses in the wheat wide crosses laboratory currently include *in situ* hybridization and Randomly Amplified Polymorphic DNA sequences (RAPDs), which are based on polymerase chain reactions (PCRs). Wide adaptability of *in situ* hybridization procedures was made possible by the development of nonradioactive labeling techniques.

Since it is an important source of salinity tolerance, CIMMYT has used *Thinopyrum bessarabicum* to produce disomic addition lines. Its combination with bread wheat has allowed us to characterize quite a few biochemical markers. With the objective to develop molecular markers for tracking *Th. bessarabicum* chromatin in wheat backgrounds and subsequently detect subtle introgressions, we have conducted genomic *in situ* hybridizations using the amphiploids of *T. aestivum* cv. Chinese Spring (CS) x *Th. bessarabicum*. More recently, we have begun successfully exploiting the fluorescent *in situ* hybridization technique (FISH) to detect alien DNA.

Conclusions

We believe that our radical approach of advancing wide hybrids is serving as an important rapid mechanism to get needed germplasm to CIMMYT breeders. With this strategy of leaving scientific questions unanswered—at least for the time being—we have been able to distribute germplasm with needed attributes that had not been obtained by breeders in their conventional programs. To a considerable extent, slower basic research will address questions that we leave unanswered. However, over the last four or five years, we have embarked on a more meticulous methodology with our intergeneric hybridizations that may allow for more basic research later.

The structure of the CIMMYT Wide Crosses Section is designed to link plant-level manipulation with cellular and molecular approaches—two aspects that are essential to the program's function and effectiveness. We anticipate that a number of very desirable approaches will subsequently emerge to aid cereal crop improvement. When these research breakthroughs are refined and made applicable, they will find complementary use in wheat improvement and may even have the potential to replace several conventional stages of genetic manipulation.

We anticipate that the successful use of wheat polyhaploids will receive greater application in our program as well as in the breeding/molecular areas. Viable stored pollen may provide an additional boost to the application of the wheat x maize or wheat x *Tripsacum* techniques for producing polyhaploids. We also expect to obtain wheat polyhaploids—as well as desirable diversification—from sexual crosses with sorghum. And we may be able to extend the procedure to range grasses where analysis of the resulting polyhaploids could help clarify some complicated genomic relationships in the Triticeae.

In our 15 years of investigations, we have progressed to a stage that allows us to project a prosperous future. Historically, wide crosses at CIMMYT were not anticipated to yield on-the-farm products in a short time frame nor to provide answers for each and every aspect of development. However, we have had notable achievements in these areas. So, we are optimistic about additional achievements over the next five years.

Resumen

En el esfuerzo por satisfacer la creciente demanda de alimentos, los fitomejoradores encuentran que entre las especies cultivadas hay cada vez menos germoplasma adecuado con las características que requieren para mejorar los cultivos. Afortunadamente, en la actualidad se están encontrando recursos genéticos (es decir, características útiles en el fitomejoramiento) entre las plantas no cultivadas. Como se menciona en el Capítulo 1, el reto es elaborar, mediante una técnica denominada cruzas amplias, un procedimiento sistemático que permita incorporar este germoplasma "nuevo" en los cultivos alimentarios existentes.

Cruzas amplias

Las especies del género *Triticum* han formado parte de la mayoría de los trabajos orientados a transferir genes de otras especies, particularmente, al trigo harinero (*T. aestivum* L.). En los últimos 15 años, el CIMMYT ha participado en ese esfuerzo, tratando vigorosamente de mejorar el trigo harinero no sólo mediante la hibridización interespecífica (cruzas entre gramíneas anuales del grupo *Triticum*/*Aegilops*), sino también la intergenérica (cruzas con trigo en las que han participado algunas de las 250 gramíneas perennes de la tribu Triticeae). Las gramíneas perennes son de crítica importancia debido a que, gracias a los hábitats donde se originaron, podrían ser fuentes de fuerte resistencia a varios factores bióticos y abióticos adversos. El Capítulo 2 establece el contexto de nuestro trabajo con cruzas amplias al describir la compleja configuración genómica de las especies perennes y anuales de las Triticeae. Como se indica en ese capítulo, la clasificación taxonómica de algunas de las especies perennes se ha reajustado con base en nuevos conocimientos de su configuración genómica.

Hibridización interespecífica (Capítulo 3)

Cuando los mejoradores utilizan los variados complejos genéticos de otras especies *Triticum* para mejorar el trigo harinero, una de sus prioridades es utilizar en cruzas interespecíficas las innumerables accesiones extrañas cuyos genomios son muy semejantes a los genomios A, B o D del trigo harinero. Esto no sólo facilita la transferencia de genes extraños debido a las similitudes genómicas, sino que también resulta compatible con la investigación en campo y, además, permite alcanzar un alto grado de éxito en las transferencias de genes múltiples (poligénicas). Así pues, en los trabajos interespecíficos orientados al mejoramiento de trigo harinero nos centramos en utilizar genomios muy afines.

En el CIMMYT nos hemos concentrado en la explotación de las accesiones de *Triticum tauschii*, sin. *Aegilops squarrosa*, pariente silvestre del trigo, porque creemos que su gran diversidad y distribución en Europa y Asia. nos brindan una oportunidad única de utilizar variabilidad genética nueva en el mejoramiento del trigo. Por otra parte, se le atribuye a *T. tauschii* (que se acepta sin lugar a dudas como la especie que donó el genomio D al trigo harinero) una gran diversidad de resistencias y tolerancias a factores adversos, tanto bióticos como abióticos, que podrían contribuir a mejorar el trigo harinero.

Como se describe en el Capítulo 3, la técnica ideal para explotar la variabilidad de *T. tauschii* en el mejoramiento del trigo harinero requiere que se haga una selección eficaz para encontrar

accesiones con distintas resistencias o tolerancias deseables. Las accesiones seleccionadas entonces se pueden cruzar directamente con trigo harinero (*T. aestivum)* —siempre que el programa tenga la capacidad de efectuar rescate de embriones y duplicar cromosomas—o con trigo durum (*T. turgidum*) a fin de producir lo que denominamos haploides sintéticos, que a su vez pueden ser fácilmente cruzados con trigo harinero utilizando un programa fitotécnico convencional. Hemos efectuado cruzas entre trigos harineros susceptibles al carbón parcial (*Tilletia indica*) y varias accesiones de *T. tauschii* resistentes a ese hongo. Hemos identificado trigos sintéticos que son resistentes al tizón foliar (*Helminthosporium sativum), Septoria tritici* y roña de la espiga (*Fusarium graminearum) y* que posteriormente se podrán cruzar con trigo harinero. Varios de ellos mostraron tolerancia a la sal en la selección inicial en campo realizada en La Paz, Baja California Sur, México. Hasta la fecha, hemos producido casi 525 hexaploides sintéticos, la mayoría de ellos a partir de una accesión única de *T. tauschii* (véase el Apéndice 2), para usarlos en cruzas con trigo harinero.

Hibridización intergenérica (Capítulo 4)

Los distintos complejos genéticos de las gramíneas anuales o perennes de la tribu Triticeae pueden aportar una enorme variabilidad genética al mejoramiento de trigo. Sin embargo, en contraste con lo que ocurre en el grupo *Triticum*/*Aegilops*, las especies que participan en las cruzas intergenéricas son genómicamente muy diversas y bastante difíciles de cruzar con el trigo. Incluso cuando se logra combinarlas, los híbridos que producen muestran poca o ninguna asociación cromosómica intergenómica. A pesar de estas limitaciones, algunos centros de excelencia han logrado avances significativos en los últimos 20 años. Los principales objetivos del CIMMYT en los trabajos intergenéricos han sido obtener tolerancia a niveles tóxicos de aluminio y sal; captación eficiente del cobre, y resistencia a *H. sativum, F. graminearum* y *Tilletia indica*. Asimismo, a la larga esperamos poder transferir otras características como la resistencia al virus del enanismo amarillo de la cebada (BYDV), a *Septoria tritici* y al pulgón ruso del trigo (*Diuraphis noxia*).

Se han producido híbridos y, en la mayoría de los casos, anfiploides entre especies de los géneros *Hordeum, Agropyron, Elymus, Secale, Taeniatherum, Eremopyrum* y *Haynaldia*. Va en aumento la cantidad de híbridos nuevos que son producto de cruzas con parientes más lejanos y se espera que pronto habrá un número mayor de genotipos que podrán utilizarse para introducir variabilidad genética nueva en el trigo.

La búsqueda de nuevos conocimientos y aplicaciones

Aunque el trabajo primordial de la Sección de Cruzas Amplias de Trigo del CIMMYT ha sido ayudar a los mejoradores del mismo Centro agregando nueva variabilidad al complejo genético del trigo mediante cruzas interespecíficas e intergenéricas, también ha contribuido un acervo considerable de nuevas técnicas y aplicaciones en el área de la biotecnología vegetal.

Producción de polihaploides (Capítulo 5)

En los últimos cuatro años, hemos podido lograr altas frecuencias de plantas polihaploides utilizando polen de maíz o de *Tripsacum*. Las plantas polihaploides son útiles en nuestros esfuerzos por reducir el número de generaciones necesarias para fijar la homocigocidad del trigo y otros cereales. Se requiere homocigocidad en proyectos de investigación básica, como nuestro

trabajo conjunto con la Universidad de Cornell, cuyo objeto es elaborar mapas de los RFLPs de los genomios del trigo y de la cebada.

El CIMMYT ha logrado un alto grado de recuperación de trigos polihaploides en las cruzas entre la variedad de trigo Morocco y el Pool 9A, una población de maíz del CIMMYT. La proximidad taxonómica de *Tripsacum dactyloides* (L.) al maíz nos ha llevado a evaluar distintas combinaciones de cruzas entre *Tripsacum* y el trigo (*T. aestivum* y *T. turgidum*) y anfiploides de *T. turgidum* x *T. tauschii.* Pensábamos que *Tripsacum* podría proporcionar una forma sexual novedosa de producir polihaploides de cereales y así ha sido, pues las cruzas entre el trigo y *Tripsacum* han conducido a la producción de trigos polihaploides de distintos genotipos.

A diferencia de lo que sucede en el cultivo de anteras de trigo o la hibridización sexual del trigo con *H. bulbosum*, no hay especificidad genotípica ni aneuploidía en la producción de polihaploides mediada por el maíz y el *Tripsacum*, lo cual los hace sistemas superiores. Es necesario explorar el potencial del polen de maíz y de *Tripsacum* que ha estado almacenado porque podría ser un factor significativo para extender el uso de la metodología a países donde los ciclos de cultivo no son sucesivos o donde no existen instalaciones adecuadas para el cultivo de plantas bajo condiciones controladas.

Cultivo de tejidos (Capítulo 6)

Las aplicaciones del cultivo de tejido han sido de vital importancia para la producción de híbridos complejos de las especies Triticeae. Su manipulación presumiblemente ampliará las hibridizaciones posibles actualmente. El CIMMYT ha explotado el cultivo y regeneración de callos a largo plazo a fin de demostrar las posibilidades de inducir variabilidad para características morfológicas, bioquímicas y citológicas en varios grupos de las especies Triticeae. Existen dos limitaciones operacionales en la hibridización intergenérica que están relacionadas con la introgresión de genes ajenos y la inducción de anfiploides. La metodología del cultivo de callos nos ha ayudado grandemente a superar ambas limitaciones gracias a que ha aumentado el apareamiento cromosómico análogo a aquel que es característico del locus *Ph* en el cromosoma 5B e inducido anfiploidía en dos combinaciones híbridas intergenéricas.

Asimismo, el cultivo de callos: 1) nos proporciona ventajas en la inducción de variabilidad en variedades de trigo euploides, 2) facilita la selección *in vitro* para factores de estrés o patógenos que producen toxinas, y 3) nos da la capacidad de alterar la estructura de los cromosomas. Por otra parte, es posible que el procedimiento modifique las frecuencias de recombinación en híbridos complejos que de otra forma tienen bajas frecuencias de apareamiento y facilite también la recuperación de derivados híbridos con el doble de cromosomas.

Confirmación de la introgresión de genes (Capítulos 7 y 8)

Si bien en algunos casos hemos utilizado metodologías radicales que deliberadamente no dan importancia a la confirmación de la introgresión de genes de otras especies y los intercambios cromosómicos, la Sección de Cruzas Amplias hasta cierto punto ha estado explorando los marcadores bioquímicos y moleculares como medios de efectuar tal confirmación cuando resulte factible y/o conveniente hacerlo. La identificación y caracterización iniciales de introgresiones extrañas pueden realizarse con técnicas citológicas y marcadores bioquímicos que son menos complicados y relativamente baratos. Una vez que se ha caracterizado el material, los

marcadores moleculares pueden establecerse y después usarse para detectar la presencia de pequeñísimos intercambios de cromosomas con otras especies que son más difíciles se detectar.

Los marcadores bioquímicos. Estos marcadores, que utilizan isoenzimas y proteínas almacenadas en la semilla que le permiten a ésta germinar, pueden usarse para distinguir cromosomas extraños en el trigo en hibridizaciones tanto intergenéricas como interespecíficas. Más de 100 genes estructurales para los marcadores isoenzimáticos han sido identificados y localizados en distintos segmentos cromosómicos del trigo. La mayor ventaja de utilizar los isoenzimas es la rapidez con la que el material puede ser seleccionado debido a que hay polimorfismo adecuado. La información sobre la homoeología de los cromosomas extraños en las líneas de adición puede confirmarse identificando los genes que poseen y que son ortólogos a conjuntos de genes de *T. aestivum* cuyas ubicaciones en los cromosomas se conocen. Esto también puede hacerse estudiando la capacidad de los cromosomas extraños de remplazar cromosomas específicos del trigo.

Cuando los marcadores bioquímicos son identificados por primera vez, es necesario estudiar los perfiles de las bandas correspondientes a un sistema enzimático determinado utilizando las dos especies progenitoras. El análisis de las dos especies progenitoras y del anfiploide es importante cuando se utilizan estos marcadores para caracterizar materiales genéticos extraños. A veces las especies extrañas muestran cierto grado de polimorfismo para un determinado sistema enzimático como resultado de diferencias alélicas y/o porque no son compatibles entre sí. Podemos identificar marcadores cuando las dos especies progenitoras muestran perfiles de bandas notablemente distintos.

Marcadores moleculares. Los marcadores moleculares se están volviendo cada vez más importantes en detectar introgresiones de genes extraños e intercambios cromosómicos, especialmente cuando se trata de pequeños segmentos de cromatina extraña en una configuración de trigo. Las técnicas moleculares que actualmente se utilizan en el laboratorio de cruzas amplias de trigo del CIMMYT incluyen la hibridización *in situ* y las secuencias de ADN polimórfico amplificado al azar (RAPD) basadas en las reacciones en cadena de polimerasa (PCR). La amplia adaptabilidad de los procedimientos de hibridización *in situ* es posible gracias a la generación de técnicas no radiactivas de marcado.

Debido a que constituye una fuente importante de tolerancia a la salinidad, *Thinopyrum bessarabicum* se ha utilizado en el CIMMYT para producir líneas de adición disómicas. Su combinación con trigo harinero nos ha permitido caracterizar un gran número de marcadores bioquímicos. Con el objeto de desarrollar marcadores moleculares que rastreen cromatina de *Th. bessarabicum* en configuraciones de trigo y, posteriormente, detectar introgresiones sutiles, hemos realizado hibridizaciones genómicas *in situ* utilizando anfiploides de *T. aestivum* cv. Chinese Spring x *Th. bessarabicum*. Más recientemente, hemos comenzado a emplear la técnica fluorescente de hibridización *in situ* (FISH) para detectar ADN extraño, con excelentes resultados.

Conclusiones

Estamos convencidos de que nuestro enfoque radical para avanzar en la producción de híbridos mediante cruzas amplias, es un mecanismo rápido importante que permite entregar a los mejoradores del CIMMYT el germoplasma que necesitan. Siguiendo nuestra

estrategia de dejar interrogantes científicos sin responder (al menos por el momento), hemos podido distribuir germoplasma con determinados atributos que los mejoradores no habían podido obtener con los programas fitotécnicos tradicionales. Las investigaciones básicas realizadas a un ritmo menos acelerado responderán en gran medida a los interrogantes que hemos dejado pendientes. No obstante, cabe mencionar que en los últimos cuatro o cinco años hemos adoptado una metodología más meticulosa para realizar nuestras hibridizaciones intergenéricas que quizá permita que posteriormente efectuemos investigaciones más básicas.

La sección de cruzas amplias de trigo del CIMMYT está estructurada de tal manera que vincula la experimentación con plantas con métodos moleculares y celulares —dos aspectos que son esenciales para el buen funcionamiento y efectividad del programa. Confiamos en que más adelante también surgirán métodos muy eficaces que ayudarán a mejorar los cereales. Cuando esos avances científicos se perfeccionen y sean aplicables, tendrán usos complementarios en el mejoramiento de trigo y quizá hasta lleguen a remplazar por completo algunas etapas convencionales de la manipulación genética.

Creemos que se encontrarán otras formas de aplicar los trigos polihaploides tanto en nuestro programa como en las áreas fitotécnica y molecular. Es posible que el uso de polen que ha estado almacenado y que sigue siendo viable dé un impulso adicional a la aplicación de las técnicas trigo x maíz o trigo x *Tripsacum* que inducen polihaploidía. Esperamos producir polihaploides de trigo—así como lograr diversificación— a partir de cruzas sexuales con sorgo. También es posible que podamos utilizar el procedimiento de los polihaploides con pastos naturales y esclarecer, mediante el análisis de los polihaploides resultantes, las complejas relaciones genómicas entre las especies Triticeae.

En los 15 años que llevamos en la investigación, hemos progresado hasta un punto en que podemos predecir un futuro próspero para nuestra sección. Cabe mencionar que no se esperaba que la sección de cruzas amplias del CIMMYT fuera a generar productos para el campo en un corto plazo ni proporcionar las soluciones requeridas en cada aspecto de su generación. No obstante, hemos logrado avances notables en esas áreas y, por tanto, somos optimistas respecto a lo que lograremos en los próximos cinco años.

CHAPTER 1

Introduction: 15 Years of Progress in Wheat Wide Crosses at CIMMYT

Abdul Mujeeb-Kazi

Experts predict today's worldwide population of 5.5 billion people will grow by 1 billion over the next decade, and double to 11 billion in 40 years (Beamish 1994). By 2050, 12 billion people will crowd the planet, with more than 90% of the growth occurring in developing nations. These ominous circumstances are placing a formidable task before agricultural scientists and the food management sector. On one front where plant breeders are involved in crop improvement efforts to meet the ever-increasing demand for food, they are finding less and less appropriate germplasm with desired traits among cultivated crops themselves with which to make the needed improvements (Harlan and deWet 1971). Fortunately, new and useful genetic resources are being found in wild, uncultivated plants. The challenge is to incorporate this germplasm into existing food crops.

Wide Crosses in the Triticeae

Because cereal crops provide the structural base for world food production, it is fortuitous that most alien genetic transfers, to date, have involved the *Triticum* grass species within the tribe Triticeae—where the greatest emphasis has been placed on using these introgressions to improve bread wheat (*T. aestivum* L.). Wheat has received the most attention because of its global importance and because genetic manipulation techniques have become well established for wheat and its relatives. Using wide crosses to improve bread wheat is an area that CIMMYT has pursued vigorously over the past 15 years. Our research efforts and active collaborations involving wide crosses are detailed in this research report.

Of the approximately 325 perennial and annual grasses within the Triticeae tribe, relatively few have been hybridized with wheat. Perennials used have been predominantly among *Thinopyrum* spp. Among the 75 or so annuals, particular successes have been achieved with *Aegilops*, *Hordeum*, and *Secale* spp. Over the last decade and a half, noteworthy successes at CIMMYT and various other laboratories have been achieved in the production of complex hybrids among species in the Triticeae, which now provide a potential stock of invaluable alien germplasm. CIMMYT is continually adding to this stock with its growing number of A genome hexaploids and D genome synthetic hexaploids, which are being developed through crosses between durum wheat and a number of diploid grass species (see Chapter 3 and **Appendices 2 and 3**), and other hybrid combinations produced by crossing wheat with various perennial species in the Triticeae (See Chapter 4 and **Appendices 4-6**).

Utility of Wide Hybrids

There are different methodologies for transferring desired resistances or tolerances from the alien species to wheat (Chapters 3 and 4). Irrespective of the procedure adopted, production of hybrids (intergeneric or

interspecific) is the key to accomplishing useful genetic transfers. There are two ways to accomplish this: 1) alien species can be screened for specific resistances or tolerances and then be hybridized with wheat or 2) the alien species are first hybridized with wheat and then the advanced derivatives can be screened.

Wide hybrids provide cytological data and evolutionary information about the parental species, as well as the practical motivation to improve wheat by transferring significant characteristics from alien species. Alien transfers can diversify variability for both dynamic biotic situations and static abiotic circumstances. Alien sources of variability are normally inaccessible to breeders who work in conventional crop improvement programs and hence wide hybridization is considered additive to traditional plant breeding efforts.

Intergeneric vs interspecific

Actual successes of incorporating usable alien genetic variation have been relatively few (Sharma and Gill 1983a, Mujeeb-Kazi and Kimber 1985) and for the most part have involved simply inherited genetic traits. So, in order to ensure faster practical returns to agriculture, we believe that simply inherited traits should be the major emphasis when making intergeneric crosses (see Chapter 4), while traits with complex heritability (involving the introgression of several genes simultaneously) and ill-defined genetic information should be limited to less complex interspecific crosses (see Chapter 3). Some of the complex traits receiving our attention are associated with resistances or tolerances to *Helminthosporium sativum*, *Fusarium graminearum*, *Tilletia indica* (syn. *Neovossia indica*), and salinity.

As major problems that limit the use of wide hybrids, i.e., crossability and embryo development, continue to be solved, it has encouraged researchers to examine the possibilities of yet even wider hybrids, e.g., crossing wheat with maize, millet, sorghum, teosinte, and *Tripsacum* (see Chapter 5).

Evolution of Wide Cross Research at CIMMYT

In the late 1970s, the research mandate for wheat wide crossing at CIMMYT was highly specific in its emphasis to refrain from activities that emulated those of other established centers of excellence. We were not to set our investigations towards highly specific cytogenetic research. Our efforts were to focus upon exploiting the applied advantages for CIMMYT's mandate of wheat crop improvement. Presumably, we would lose some precise scientific explanations, but, through opening up collaborative linkages with basic research centers of excellence, it would be their job to unravel the "hows" and "whys" of the new germplasm we were developing. This was essentially "CIMMYT's look toward year 2000"—a wheat wide crosses investigative framework to span two decades (1980-2000).

It was unlikely that there would be speedy pay-offs from such a risky endeavor. Further, instead of handling a restrictively narrow objective through one hybrid combination (wheat x one alien source), the two-decade span would allow us the time to exploit a wide array of alien germplasm. It would also permit us to adapt readily to new situations that would most likely emerge with significant scientific discoveries, a changing research environment, budgetary decreases or increases, and client needs.

At the onset of the current wide crosses program in 1979, the goals were to:

- Produce classical intergeneric hybrids with cytological validation over a three-year duration.
- Design a breeding methodology that permitted advance of the hybrids for field evaluations with elite plant types that dispensed with or minimized chromosome analysis to confirm actual introgression.
- Emphasize complex genes and polygenically controlled traits instead of simply inherited traits.
- Select and stabilize the advanced hybrid derivatives by transferring improved germplasm to CIMMYT base and national breeding programs.
- Proceed with advances of F1 hybrids and basic research activities along classical cytogenetical lines, preferably through collaborative research, which would require scientific communication through various media.

Subsequently, a changing research environment after 1987 placed considerable emphasis upon basic and strategic research—a new stage that the wide crosses program needed to assess accordingly. Hence, there emerged a slight reduction in the original research structure and some additional modifications that involved:

- Initiating an interspecific hybridization program based upon genomic proximity of the closely related Triticeae species with the three genomes (A, B, and D) of wheat. This offered an avenue for relatively rapid, short-term returns.
- Transferring targeted traits from distant wheat relatives via intergeneric crosses.
- Producing polyhaploids in wheat using a sexual cross system (wheat x maize) in efforts to generate doubled haploids for the wheat RFLP (restriction fragment length polymorphism) mapping project (see Chapter 5). These techniques would have subsequent ramifications for future cytogenetics and breeding activities.
- Using tissue culture to facilitate alien introductions (see Chapter 6).
- Exploring the application of biochemical and molecular markers to enhance the detection of alien introgressions (see Chapters 7 and 8).
- Publishing results in refereed scientific journals.

Prerequisites for Success

Two major prerequisites for success in wide cross research are long-term commitment and collaboration among specialists and institutions.

Long-term commitment

Over the years, certain advances in wide cross research have set the stage for current support. Some of these well-recognized global endeavors include:

- Nearly a century of accomplishments with wheat x rye (triticale) and wheat x barley crosses.
- Nearly 50 years of astounding successes (by the late E.R. Sears) with wheat cytogenetic stocks.
- Some 20 years of successes (by the late E. Sebesta) in the production of wheat/alien translocations such as 1AL/1RS. This translocation and the spontaneous 1BL/1RS translocation are undoubtedly the most significant examples of successful alien introgressions in wheat breeding; the 1BL/1RS contribution (more than 5 million

hectares planted to such wheat varieties) is believed—by some—to be the most important natural genetic manipulation made to date.
- Some 15 years of work involving alien genetic stock developments of *Dasypyrum villosum*, *Thinopyrum elongatum, Th. bessarabicum*, *Hordeum vulgare*, and *Triticum tauschii*—in each case by independent researchers working in major programs.

In addition to the above examples, the known complexities that are firmly established in the scientific literature show that long-term commitments must prevail for such involved research to be successful. Regardless of the financial investment, both researchers and administrators must recognize this inescapable element.

Collaboration among specialists and institutions

A successful wide crosses program like the one at CIMMYT requires the commitment and cooperation of literally hundreds of specialists located at CIMMYT and research centers worldwide. Interconnected disciplines include genetics, cytogenetics, pathology, entomology, physiology, biotechnology, breeding, nutrition, and agronomy. For a who's who listing of specialists involved, just scan the references cited in this research report.

Collaboration with other research centers has been particularly important to recent successes at CIMMYT. Just as one example, scientists at the USDA-ARS Forage and Range Research Laboratory, Logan, Utah, have provided germplasm of many species, facilitated crosses with Triticeae species in the grass nursery maintained at Logan, and shared knowledge on genome compositions of various Triticeae species. Other institutions have provided valuable information on sources of various disease resistances and environmental stress tolerances.

Strengths of other Laboratories

We recognize that non-CIMMYT workers with basic research strengths are located in other laboratories and we do not pretend to compete. With diversified mandates, some are most likely more ideally suited than CIMMYT's applied program to conduct specific sophisticated aspects of wide cross work aimed at global crop improvement. We acknowledge—but do not identify—these researchers in this particular forum.

CHAPTER 2

Perennial and Annual Wheat Relatives in the Triticeae

Abdul Mujeeb-Kazi and Richard R.-C. Wang

Of the approximately 325 species in the tribe Triticeae, about 250 are perennials and 75 are annuals (Dewey 1984). Relatively few perennials have been intergenerically crossed with wheat because of the complexity of doing so and embryo rescue/regeneration constraints. The perennials, which include many important forage grasses, have the potential to serve as a vital genetic reservoir for the improvement of the annual grasses, which include the major cereals (bread wheat, durum wheat, triticale, barley, and rye). Perennials that have been successfully utilized for improving wheat are predominantly in the *Thinopyrum* group.

Wide hybrids in the Triticeae have been attempted and studied for more than 100 years. The first such hybrid was between wheat and rye (Wilson 1876). Rimpau (1891) described 12 plants recovered from seed of a wheat-rye hybrid that represented the first triticale. Farrer (1904) made similar early studies of wheat-barley hybridization; however, Shepherd and Islam (1981) concluded that it is improbable that these were true hybrids. Several perennial grasses were hybridized with wheat as early as the 1930s with the objectives of transferring disease resistance and perenniality into annual crops (Tsitsin 1960, 1975). Many hybrids involving *Triticum* and several *Aegilops* species were made during the 1920s and 1930s (Kihara 1937) from which the genomic relationships of the two genera were derived (Lilienfeld 1951). The large-scale practical use of the hybrids, however, was delayed until the advent of colchicine treatment (Eigsti and Dustin 1955) in the late 1930s. The ability to double the chromosome number of hybrids using colchicine had both practical and theoretical consequences. The production of fertile amphiploids provided the way to develop triticale as a new cereal crop (Gupta and Priyadarshan 1982) and also advanced evolutionary studies when McFadden and Sears (1946) resynthesized *T. aestivum* and thus discovered *T. tauschii* to be the D genome donor to bread wheat.

With the advancement of hybridization techniques (Kruse 1973) and embryo culture (Murashige 1974), wide hybridization became a more common practice and involved more perennial species. In reviews of the progress of wide hybridization, Dewey (1984) and Wang (1989) clearly showed intense interest among breeding programs in utilizing the genetic resources available in the perennial Triticeae for cereal improvement.

Since 1980, CIMMYT has been conducting a vigorous gene transfer program in which species of the perennial Triticeae are utilized for wheat improvement. We have emphasized the perennials over the annuals because of greater biotic/abiotic resistances that the perennials most likely possess because of their habitats. In the quest to acquire genetic diversity, we have found that obtaining usable alien characters requires precise transfer of the controlling genes of a desirable character that comes from a donor

species with different genomes. An ideal gene transfer involves normal introgression of the alien material without negative background effects on grain yield and quality. To utilize effectively the Triticeae gene reservoir, we need to know:

- The genome constitutions of the donor species;
- The genomic relationships between the donor and recipient species;
- The chromosomal location(s) of the desirable gene(s);
- The number of the gene(s) conferring the desirable trait and the mode of inheritance;
- Whether the donor's gene(s) can be expressed in the recipient species; and
- Whether any negative effects ensue from the transfer.

CIMMYT is meeting the above prerequisites with regard to various desirable characteristics, including disease resistances and abiotic stress tolerances.

Genome Make-Up in the Triticeae

Perennial species

In the genomic system of classification of the perennial Triticeae (Dewey 1984), 13 genera with defined genomes or genome combinations are recognized. Due to a lack of sufficient information, we do not consider two of these: *Hordelymus* and *Festucopsis*. However, the remaining 11 genera with their type species and genome compositions are: *Agropyron* (*A. cristatum*; P), *Australopyrum* (*A. pectinatum*; W), *Pseudoroegneria* (*P. strigosa*; S), *Psathyrostachys* (*Ps. lanuginosa*; N), *Critesion* (*C. jubatum*; H; also *Hordeum*), *Thinopyrum* (*Th. junceum;* J-E), *Elytrigia* (*E. repens*; SX), *Elymus* (*E. sibiricus*; SHY) *Leymus* (*L. arenarius*; XN), *Pascopyrum* (*Pa. smithii*; SHXN), and *Secale* (*S. montanum*; R).

As new genomic compositions were found in the Triticeae species (Wang et al. 1986; Liu and Wang 1989; Jensen 1990a,b; Torabinajad and Mueller 1993; Assadi and Runemark 1994), the genomic classifications were modified. The closeness between the J and E genomes also necessitated a genome symbol change from E to J^e (Wang and Hsiao 1989). With these changes, a more detailed system became warranted. **Table 2.1** lists the perennial genera under the new genomic system where specific genome combinations are assigned to known species.

Agropyron remains a small genus consisting of P-genome species at three ploidy levels. *Psathyrostachys* is comprised of N-genome diploids, although autotetraploid cytotypes have been both discovered and synthesized. Pending new information, the genome make-ups of the genera *Pascopyrum*, *Australopyrum*, and *Secale* presently remain unchanged. See the Chapter 7 discussion of the genomic status of tetraploid *Leymus racemosus*.

With the discovery of natural species having the SSPP genomes, i.e., *Pseudoroegneria tauri* (Wang et al. 1986) and *P. deweyi* (Jensen et al. 1992), *Pseudoroegneria* now has a section named *Pseudopyron* to accommodate these SSPP species. These species exemplify the inadequacy of morphology alone and the added importance of genome analysis when studying species relationships. Similarly, only genome analysis can separate species within the genus *Elymus* into the SH, SHY, SY, and SYP groups with any certainty. Chinese taxonomists (Keng 1965, Yen and Yang 1990) have given genus names to the SSYY and SSYYPP species (i.e., *Roegneria* and *Kengyilia*, respectively). However, we have kept them at the sectional level to avoid massive name changes since *Elymus* is the largest genus

Table 2.1. The genomes and ploidy levels of the perennial genera of the tribe Triticeae.[a,b]

Genus	Section	Genome(s)/ Ploidy level(s)	Species
Agropyron		PP	*A. cristatum, A. mongolicum, A. fragile*
		PPPP	*A. desertorum, A. michnoi*
		PPPPPP	*A. cristatum*
Pseudoroegneria		SS, SSSS	*P. strigosa, P. libanotica, P. stipifolia, P. spicata*
	Pseudopyron	SSPP	*P. tauri, P. deweyii*
Elymus		SSHH	*E. sibiricum, E. canadensis, E. arizonicus, E. caninus, E. vaillantianus*
		SSHHYY	*E. dahuricus, E. drobovii, E. tsukushiensis, E. kamoji*
		SSYYWW	*E. scaburus, E. rectiselus*
	Roegneria	SSYY	*E. ciliaris, E. parviglume, E. longearistatus, E. strictus, E. gmelinii, E. pendulinus, E. abolinii, E. panormitanus, E. shandongensis, E. ugamicus*
	Kengyilia	SSYYPP	*E. alatavicus, E. batalinii, E. grandiglumis, E. kengii*
Psathyrostachys		NN	*P. lanuginosa, P. juncea, P. fragilis, P. huashanica, P. kronenburgii.*
Hordeum	*Critesion*	From HH to HHHHHH (i.e., 2x to 6x)	*H. bogdanii, H. violaceum, H. jubatum, H. brevisubulatum, H. iranicum, etc.*
Thinopyrum	*Junceum*	JJJJEE	*Th. junceum,*
		JJ	*Th. bessarabicum*
		JJEE	*Th. junceiforme, Th. sartorii, Th. distichum*
	Elongatum	EE	*Th. elongatum*
		EEEE	*Th. scirpeum*
		JJJJ	*Th. curvifolium*
		JJJJEEEE	*Th. turcicum*
		JJJJEEEEEE	*Th. ponticum*
	Intermedium	EESS	*Th. caespitosum, Th. nodosum, Th. scythicum*
		JJJJSS/EEEESS/ JJEESS	*Th. intermedium*
Elytrigia		SSSSHH	*E. repens*
Leymus		NNNNXXXX	*L. arenarius, L. racemosus,*
		NNXX	*L. mollis, L. triticoides, L. salinus, L. cinereus, L. innovatus, L. chinensis*
		NNNNNNXXXXXX	*L. angustus*
Pascopyrum		SSHHNNXX	*P. smithii*
Australopyrum		WW	*A. pectinatum*
Secale		RR	*S. montanum*

[a] This table does not provide details of genera hybridized in the CIMMYT program that are elaborated in Chapter 4. For a more detailed listing of species, readers should refer to Dewey (1984).

[b] For simplicity, the J^b and J^e genomes (discussed in the text) are replaced by J and E, respectively, in this table.

in the Triticeae consisting of approximately 150 species. Only a small number of these species has been genomically analyzed (Lu and von Bothmer 1993).

Although the diploid species in *Australopyrum* contain the W genome (Hsiao et al. 1986), the polyploid species in some Australian *Elymus* species appear to be allopolyploids that contain other genomes in combination with W. Therefore, *Elymus* may include some other genomic combinations presently unknown to us. For example, *E. rectisetus* and *E. scabrus* both appear to have the genome composition SSYYWW (Torabinajad and Mueller 1993).

Critesion is now recognized as a section of *Hordeum* (Bothmer et al. 1986). Genetic regulators (promoters and suppressors) of chromosome pairing might have confused the make-up and earlier classification of the *Hordeum* genus, which contains both perennial and annual species (Dewey 1984). For example, a gene has been shown to be responsible for the low pairing between *H. violaceum* and *H. bogdanii* (Wang et al. 1991). It may also have led to misinterpretation of some cytogenetic data since the number of hybrid plants obtained from certain combinations has been very low. The bivalentization mechanism may also cause an autopolyploid to behave like an allopolyploid (Wang and Hsiao 1989). Further research being pursued by Bothmer and his colleagues should lead to a better understanding of the genomic relationships of the perennials in *Hordeum*.

The genus *Thinopyrum* is embroiled in the most recent controversy. The two diploid species, *Th. bessarabicum* and *Th. elongatum*, were originally given the genome symbols J and E, respectively (Löve 1984). Lately, cytogenetic data have demonstrated the closeness between the two genomes. Thus, they have been merged into a single, basic genome symbol as proposed by Dvorak (1981), Dewey (1984), McGuire (1984), Wang (1985), Pienaar et al. (1988), and Wang and Hsiao (1989). See the discussion on *Th. bessarabicum* in Chapter 7. Different opinions do exist (Jauhar 1988, 1990). Liu and Wang 1993a) suggest that *Th. junceum*, the type species ($J^bJ^bJ^e$), possesses two J^b genomes and a modified J^e genome, which is also present in *Th. sartorii*.

On the other hand, *Th. junceiforme* (J^bJ^{e2}) and *Th. scirpeum* (J^eJ^{e2}) share another modified genome (J^{e2}), differing from the respective second genomes, which are J^b and J^e. The two modified J^e genomes probably arose from recombination between J^b and J^e through the pivotal-differential evolution of the polyploid species. As a result, J^{e1} is closer to J^e while J^{e2} is closer to J^b. Therefore, all the triploid and tetraploid hybrids involving *Thinopyrum* species have meiotic pairing patterns closer to those for autoploid plants than those for strict alloploids. The fertility in the hybrids of *Th. scirpeum* / 2**Th. bessarabicum*/*Th. elongatum*, which have the genome constitution $J^eJ^{e2}J^bJ^e$ (Wang 1992), further supports the pivotal-differential hypothesis.

Liu and Wang (1989) have shown the presence of an S genome in *Th. caespitosum* and the J^eJ^eSS genome composition has since been found in *Th. nodosum* and *Th. scythicum* (Liu and Wang 1993b). Depending on the accessions, *Th. intermedium* can be represented by the genome formula $J^eJ^eJ^xJ^xSS$, where J^x can be any version of the J genome.

Annual species

The annual plants of the Triticeae are confined largely to the *Triticum* and *Aegilops* species; some notable exceptions include species from *Hordeum*, *Secale*, *Haynaldia*, *Eremopyrum*,

Heteraunthelium, Taeniantherum, and *Henrardia*. The large number of generic and specific names of the interrelated *Triticum* and *Aegilops* groups has led to considerable confusion over the years. The multitude of names not only expresses the whims of various taxonomists, but also represents the diversity of the species themselves. To reduce some of the confusion, Kimber and Feldman (1987) have compiled a synonym list of the most commonly used names among the *Triticum*/*Aegilops* groups (**Table 2.2**).

Table 2.2. A synonym list of annual *Aegilops*/*Triticum* species (Kimber and Feldman 1987).

Ae. aucheri	=	*T. speltoides (aucheri)*
Ae. bicornis	=	*T. bicorne*
Ae. biuncialis	=	*T. machrochaetum*
Ae. caudata	=	*T. dichasians*
Ae. columnaris	=	*T. columnare*
Ae. comosum	=	*T. comosum*
Ae. crassa	=	*T. crassum*
Ae. cylindrica	=	*T. cylindricum*
Ae. geniculata	=	*T. ovatum*
Ae. heldreichii	=	*T. comosum*
Ae. juvenalis	=	*T. juvenale*
Ae. kotschyi	=	*T. kotschyi*
Ae. ligustica	=	*T. speltoides (ligustica)*
Ae. longissima	=	*T. longissimum*
Ae. longissima var. *sharonensis*	=	*T. sharonense*
Ae. lorentii	=	*T. macrochaetum*
Ae. markgrafii	=	*T. dichasians*
Ae. mutica	=	*T. tripsacoides*
Ae. neglecta	=	*T. neglecta, T. triaristatum* (4x)
Ae. ovata	=	*T. ovatum*
Ae. peregrina	=	*T. peregrinum*
Ae. persica	=	*T. triunciale*
Ae. recta	=	*T. recta, T. tiaristatum* (6x)
Ae. searsii	=	*T. searsii*
Ae. sharonensis	=	*T. sharonense*
Ae. speltoides	=	*T. speltoides (ligustica)*
Ae. squarrosa	=	*T. tauschii*
Ae. tauschii	=	*T. tauschii*
Ae. triaristata (4x)	=	*T. neglecta*
Ae. triatistata (6x)	=	*T. recta*
Ae. tripsacoides	=	*T. tripsacoides*
Ae. triuncialis	=	*T. triunciale*
Ae. turcomanica	=	*T. juvenale*
Ae. umbellulata	=	*T. umbellulatum*
Ae. uniaristata	=	*T. uniaristatum*
Ae. variabilis	=	*T. peregrinum*
Ae. vavilovi	=	*T. syriacum*
Ae. ventricosa	=	*T. ventricosum*
T. aegilops	=	*T. tauschii*
T. aegilopoides	=	*T. monococcum*
T. araraticum	=	*T. timopheevii*
T. bicorne	=	*Ae. bicornis*
T. boeoticum	=	*T. monococcum*
T. carthlicum	=	*T. turgidum*
T. columnare	=	*Ae. columnaris*
T. comosum	=	*Ae. comosa, Ae. heldreichii*
T. crassum	=	*Ae. crassa, Ae. vavilovi*
T. cylindricum	=	*Ae. cylindrica*
T. dichasians	=	*Ae. caudata, Ae. markgrafii*
T. dicoccoides	=	*T. turgidum*
T. dicoccum	=	*T. turgidum*
T. durum	=	*T. turgidum*
T. juvenale	=	*Ae. juvenalis, Ae. turcomanica*
T. kotschyi	=	*Ae. kotschyi*
T. longissimum	=	*Ae. longissima*
T. macrochaetum	=	*Ae. biuncialis, Ae. lorentii*
T. monococcum	=	*T. aegilopoides, T. boeoticum, T. urartu*
T. neglecta	=	*Ae. neglecta, Ae. triaristata* (4x)
T. ovatum	=	*Ae. ovata, Ae. geniculata*
T. peregrinum	=	*Ae. peregrina, Ae. variabilis*
T. persicum	=	*T. turgidum*
T. polonicum	=	*T. turgidum*
T. recta	=	*Ae. triaristata* (6x)
T. searsii	=	*Ae. searsii*
T. sharonense	=	*Ae. sharonensis,*
	=	*Ae. longissima var. sharonensis*
T. speltoides (aucheri)	=	*Ae. aucheri*
T. speltoides (lingustica)	=	*Ae. speltoides, Ae. ligustica*
T. syriacum	=	*Ae. vavilovi, Ae. crassa var. vavilovi or var. palaestina*
T. tauschii	=	*Ae. squarrosa*
T. timopheevii	=	*T. timopheevii, T. araraticum*
	=	*T. timopheevii var. zhukovskyi*
T. timopheevii var. *zhukovskyi*	=	*T. timopheevii*
T. triaristatum	=	*Ae. triaristata, Ae. recta, Ae. neglecta, T. rectum*
T. tripsacoides	=	*Ae. mutica*
T. triunciale	=	*Ae. triuncialis*
T. turgidum	=	*T. carthlicum, T. dicoccoides, T. dicoccum, T. durum, T. persicum, T. polonicum*
T. umbellulatum	=	*Ae. umbellulata*
T. uniaristatum	=	*Ae. uniaristata*
T. urartu	=	*T. monococcum*
T. ventricosum	=	*T. ventricosa*

Despite its limitations, analysis of chromosome pairing, which has evolved from the "analyzer method" of H. Kihara (Lilienfeld 1951), can provide an insight into genomic relationships among the annuals. Although universal acceptance on usage may not exist, those interested in in-depth treatment of this topic are referred to Morris and Sears (1967), Bowden (1959, 1966), and proceedings of recent International Wheat Genetics Symposia (Sakamoto 1983, Miller and Koebner 1988, IWGS 1993).

In general, hybrids, in which the alien parent is the diploid genome donor, exhibit approximately seven bivalents because the Triticeae tribe has a basic chromosome number of seven. All hybrids involving nondonor diploid "analyzers" should have 21 or more univalents at meiotic metaphase I. This approach has worked quite well in enabling cytogenetists to assign genome designations to the annuals (**Tables 2.3** and **2.4**). Only a few cytogenetic studies have resulted in any significant changes to the genome designations made using the analyzer method.

Genome Relationships among Perennial and Annual Species

Increased understanding of genome relationships among the perennial and annual species of the Triticeae would contribute much to accomplishing successful genetic transfers. Unfortunately, researchers have done very little work on these relationships. However, extrapolated interpretations of meiotic data from polyploid hybrids suggest very low homology

Table 2.3. Genome designations of the diploid *Triticum*/*Aegilops* species, according to various workers.

Diploid *Triticum*/*Aegilops* species	Kihara, in Lilienfeld (1951)	Kimber & Sears (1983)	Kimber & Tsunewaki (1988)
T. monococcum L.	A		
T. urartu Tum.			A
Ae speltoides Tausch	S		
Ae. bicorne (Forsk.) Jaub. & Sp.	S^b		
Ae. longissimum Schweinf. & Muschli in Muschli	S^l		
Ae. sharonensis Eig			S^l
Ae. searsii Feldman & Kislev		S^s	
Ae. mutica Boiss.	Mt*		T
Ae. tauschii Coss.	D		
Ae. comosa Sibth. & Sm.	M		
Ae. uniaristata Vis.	M^t	Un	N
Ae. caudata L.	C		
Ae. umbellulata Zhuk.	C^u	U	
———————-	B		
———————-	G		

* Ohta (1990) proposed that the genome Mt be changed to S^m.

between any of the genomes (P, S, N, H, J, W, X, and Y) in the perennials and some of the genomes (A, B, D, G, M, T, and U) in the annuals. Most early alien gene transfers to wheat involved species of *Thinopyrum*. This suggests a closer homoeologous relationship between the *Thinopyrum* genomes and the wheat genomes than that present in the other perennial genomes. However, the chromosome pairing between these two genomic groups in the absence of the *Ph1* gene is still much lower than the pairing among the A, B, and D genomes of wheat alone. Therefore, gene transfer attempts require complex, long-term genetic manipulations to facilitate recombination and alien introgression.

Hybridization between Wheat and Perennial Triticeae

Since Wang (1989) reviewed intergeneric crosses involving perennial Triticeae, a few new hybrids have been obtained. All genomes of the perennial Triticeae have been combined, either singly or in combination, with the A, B, and D genomes of bread wheat (Wang 1989, Mujeeb-Kazi et al. 1994b). To date, there have been 89 different combinations made involving the three genomes of wheat and the eight genomes of the perennial species. Of these, 52 involve hexaploid wheats, 30 tetraploid wheats, and seven diploid primitive wheats.

Table 2.4. Genome designations of polyploid *Triticum*/*Aegilops* species.

Polyploid *Triticum*/*Aegilops* species	Kihara, in Lilienfeld (1951)	Kimber & Sears (1983)	Latest changes
T. dicoccoides Körn	AB		
T. dicoccum Schrank	AB		
T. durum Desf.	AB		
T. turgidum L.	AB		
T. persicum (Percival) Vavilov ex Zhukovsky	AB		
T. aestivum L. em. Thell.	ABD		
T. spelta L.	ABD		
T. compactum Host	ABD		
T. sphaerococcum Perc.	ABD		
T. macha Dek. et Men.	ABD		
T. timopheevii Zhuk.	AG		m
T. zhukovskyi Men. et Er.		AAG	$AA^{u}G$
Ae. ovata L.	$C^{u}M^{o}$	UM	UM
Ae. biuncialis Vis.	$C^{u}M^{b}$	UM	
Ae. columnaris Zhuk.	$C^{u}M^{c}$	UM	
Ae. triaristata Willd.	$C^{u}M^{t}$	UM	UM
Ae. recta (Zhuk.) Chen.	$C^{u}M^{t}X$	UMUn	UMX
Ae. variabilis Eig	$C^{u}S^{v}$	US	US^{l}
Ae. triuncialis L.	$C^{u}C$	UC	
Ae. cylindrica Host	CD		
Ae. crassa (4x) Boiss.	DJ	DM	$D^{C}X$
Ae. crassa (6x) Boiss.	DJX	DDM	$D^{C}XS^{s}$
Ae. vavilovi (Zhuk.) Chen.		DMS	$C^{c}XS^{s}$
Ae. ventricosa Tausch	DM^{v}	DUn	DN
Ae. juvenale (Thell.) Eig		DMU	

Additional *Triticum*/*Agropyron* hybrids include those from crosses of *T. aestivum* and *A. mongolicum* (2n=2x=14, PP), *A. desertorum* or *A. michnoi* (2n=4x=28, PPPP) (Chen et al. 1990); and crosses of *T. aestivum* and *A. cristatum* (2n=4x=28, PPPP) (Jauhar 1992). New *Triticum*/*Thinopyrum* hybrids are crosses of *T. durum* and *Th. bessarabicum* (2n=2x=14, JJ) (Jauhar 1991), *Th. scirpeum* (4x=28, $J^{e}J^{e}J^{e}J^{e}$), and *Th. junceiforme* (4x=28, $J^{b}J^{b}J^{e}J^{e}$) (Liu and Wang 1993c).

Newly obtained *Triticum*/*Elymus* combinations include *E. shandongensis* x *T. aestivum* (SYABD; Lu and Bothmer 1989), *E. kamoji* x *T. aestivum* (SHYABD and SHYAABBDD; Weng and Liu 1989), *T. turgidum* x *Ps. juncea* (Mujeeb-Kazi et al. 1994e) and *T. aestivum* x *E. rectisetus*

(ABDSSYYWW; Wang et al. 1993). Meiotic pairing data were published for previously obtained hybrids *T. aestivum* x *A. cristatum* (ABDP and ABDPP; Limin and Fowler 1990) *T. aestivum* x *A. desertorum* (ABDPP; Li and Dong 1991a), *T. aestivum* x *A. michnoi* (ABDPP; Li and Dong 1991b), and *T. aestivum* x *Ps. juncea* (ABDN and ABDNN; Plourde et al. 1990).

The wide array of genetic variability residing in the above Triticeae relatives supplies a superb arsenal of new defenses against biotic and abiotic stresses in cereals. Inevitably, the use of this variation has its constraints since genomic homoeology does not offer a satisfactory level of chromosomal association in the F1 hybrids to promote alien gene transfers. Use of the *ph1* locus may provide a way to overcome the recombination constraint, but the ensuing complexities to obtain backcross derivatives and stable advanced progenies are going to be fairly long-term. Other genetic manipulations exist that progressively revolve around production of alien disomic chromosome additions or substitutions, which could lead to translocations or subtle exchanges through cytogenetic and novel manipulative procedures. Although the process will be slow, the benefits of incorporating these diverse genetic resources into wheat will be extremely high.

Hybridization between Wheat and the Annual Triticeae

Production of interspecific hybrids involves the diploid *Triticum* / *Aegilops* spp. In Chapter 3, we elaborate on the utilization of these diploids that have high genetic proximity to the A, B, and D genomes of bread wheat—particularly *Triticum tauschii* (*Aegilops squarrosa*). Although variation in the annual species resides predominantly in the diploids, it does extend somewhat to the tetraploids and hexaploids. *Triticum*/ *Aegilops* spp. with different genomic make-ups (Tables 2.3 and 2.4) offer an additional gene pool also worth considering in our intergeneric crossing efforts.

Kimber (1993) compiled a list (**Table 2.5**) of all the *Triticum* / *Aegilops* spp. available for alien transfer based on genome relationships determined by mathematical measurements made to date.

Table 2.5. The genomes of *Triticum*/*Aegilops*[a] and their availability for alien gene transfer (Kimber 1993).

Genome	Species	Optimum technique, difficulty, and availability
A	*T. monococcum*	Recombination, possible partially unreduced gametes, some genome repatterning[b], good availability
	T. turgidum	Recombination, some genome repatterning, good availability
	T. timopheevi	Recombination, genome repatterning, meiotic difficulty[c], available
	T. aestivum	Recombination from landraces, some genome repatterning, very good availability
C	*T. dichasians*	Pairing modification, meiotic difficulty, considerable genome repatterning, poor availability
	T. cylindricum	Pairing modification, meiotic difficulty, considerable genome repatterning, poor availability
D	*T. tauschii*	Recombination, possible partially unreduced gametes, good availability
	T. cylindricum *T. ventricosum*	Recombination, meiotic difficulty, genome repatterning, available
	T. crassum *T. juvenale* *T. syriacum*	Recombination, meiotic difficulty, considerable genomic repatterning, poor availability
G	*T. timopheevi*	Recombination, meiotic difficulty, genome repatteming, poor availability
M	*T. comosum* *T. columnare* *T. crassum* *T. juvenale* *T. macrochaetum* *T. ovatum* *T. syriacum* *T. triaristatum*	Pairing modification or ionizing radiation, meiotic difficulty, considerable genome repatterning, poor availability
N	*T. uniaristatum* *T. ventricosum*	Pairing modification or ionizing radiation, meiotic difficulty, considerable genome repatterning, poor availability
S[d]	*T. speltoides* *T. bicorne* *T. kotschyi* *T. longissimum* *T. searsii* *T. sharonensis*	Recombination and/or pairing modification (some S genome accessions contain suppressors of Ph), meiotic difficulty, considerable genome repatterning, available
T	*T. tripsadoides*	Recombination and/or pairing modification (some T genome accessions contain suppressors of Ph), meiotic difficulty, considerable genome repatterning, available
U	*T. umbellatum* *T. kotschyi* *T. columnare* *T. juvenale* *T. macrochaetum* *T. ovatum* *T. triaristatum* *T. triunciale*	Ionizing radiation, meiotic difficulty, considerable genomic repatterning, poor availability

[a] See Table 2.2 for *Triticum/Aegilops* synonyms.
[b] Genome repatterning indicates modifications of the genome in the donor.
[c] Meiotic difficulty indicates possible complications in the introduction of alien variation due to the presence of nonhomologous genomes or translocations.
[d] S genome also similar to the B genome designation of *T. speltoides*.

CHAPTER 3

Interspecific Crosses: Hybrid Production and Utilization

Abdul Mujeeb-Kazi

In conventional improvement of bread wheat (*Triticum aestivum* L.; 2n=6x=42; AABBDD), breeders have normally made crosses between varieties. Such crosses have few constraints and invariably all associations of parental traits and segregation are based on genetic recombination. The next step in bread wheat improvement is to tap into the varied gene pools of other *Triticum* species. In such interspecific crosses, breeders utilize the numerous alien accessions among these species that have genomes similar to the A, B, or D genomes of bread wheat. These crosses allow for relatively easy alien gene transfers, are compatible with normal field research, and set the stage for the successful introgression of several genes simultaneously (i.e., en-bloc transfers).

Germplasm Used

A number of accessions of diploid wild relatives, which have either the A, B, or D genomes, are potential candidates for use in interspecific crosses. We maintain working collections of wild grass accessions with these genomes. For the D genome, there is *T. tauschii* (**Appendix 2**); for the A genome, there is *T. monococcum* (= *T. urartu* and *T. boeoticum*; **Appendix 3**); and for the B (S) genome, there are the *T. speltoides* and related accessions. Kimber and Feldman (1987) discuss other potential sources. The procedures used to incorporate such alien variability and the choice of genome to work with differ among researchers.

Use of *T. tauschii* at CIMMYT

Why *T. tauschii*?

CIMMYT has been concentrating on exploiting accessions of the annual wild relative *T. tauschii* (goat grass) because we believe the wide diversity and distribution of this species across Eurasia (see Kimber and Feldman 1987) provide a unique opportunity for exploiting new genetic variability. *T. tauschii* has a wide range of resistances or tolerances to biotic or abiotic stresses (Valkoun et al. 1990), such as Karnal bunt (*Tilletia indica*), scab (*Fusarium graminearum),* spot blotch (*Helminthosporium sativum* syn. *Bipolaris sorokiniana)*, leaf rust (*Puccinia recondita*), stripe rust (*P. striiformis*), salinity, and drought. The wild grass also appears to be a potent source of new variability for important yield components such as 1000-grain weight and increased photosynthetic rate not to mention improved bread making quality.

In addition, *T. tauschii* (also called *Aegilops squarrosa*) is unequivocally accepted as being the donor of the D genome to bread wheat (Kimber and Feldman 1987). We consider crosses with *T. tauschii* to be interspecific instead of intergeneric because of *T. tauschii*'s diploid nature and its D genome status, i.e., 2n=2x=14, DD. Note that we consider most crosses with diploid *Triticum*/*Aegilops* spp. (see **Table 2.3**) to be interspecific because of their genomic similarity to bread wheat. As explained in Chapter 2, we place all other crosses with

different *Triticum*/*Aegilops* spp. having dissimilar genomes into the intergeneric category (see **Table 2.4**).

Sources of the accessions

For our work with the D genome, we obtained 490 *T. tauschii* accessions—mostly of winter habit—from CIMMYT's wheat germplasm bank and from researchers in Pakistan (N.I. Hashmi of the National Agricultural Research Council, Islamabad), the UK (C. Law, Institute of Plant Science Research, Norwich), and the USA (B.S. Gill, Kansas State University; G. Kimber, University of Missouri; R. Metzger, Oregon State University; and G. Waines, University of California, Riverside).

Plant generation

Seeds of selected *T. tauschii* accessions are planted in jiffy-7 peat pots and vernalized at 8°C with eight hours light for eight weeks. The seedlings are transplanted in pots containing a steam-sterilized mixture of soil, sand, and peat moss. Controlled greenhouse conditions involve 22/14°C day/night temperatures, approximately 60% relative humidity, and 14 hours of natural light. We vernalize the winter habit *T. tauschii* seedlings and transplant them during CIMMYT's normal wheat crop cycles at four Mexican locations:

- Ciudad Obregon (November to May) for crossing with *T. turgidum*;
- El Batan (May to October) for crossing with *T. turgidum*;
- Toluca (May to October) for *F. graminearum* screening; and
- Poza Rica (November to April) for *H. sativum* screening.

At Ciudad Obregon, vernalization of the *T. tauschii* accessions results in very vigorous growth; flowering takes place from 90 to 135 days of age. Growth is less vigorous at El Batan, Poza Rica, and Toluca; flowering is extremely delayed. We recognize that photoperiod response may have alleviated the late flowering, but logistically, this is possible only in Cd. Obregon.

Utilization

We are using the *T. tauschii* accessions in the following ways:

- Producing synthetic hexaploids by crossing *T. turgidum* cultivars with *T. tauschii* accessions.

- Crossing elite, but susceptible *T. aestivum* cultivars with resistant *T. tauschii* accessions and backcrossing the ABDD F1 hybrids with the elite *T. aestivum* cultivar used in the initial cross.

- Extracting the AABB genomes from commercial *T. aestivum* cultivars and then developing hexaploids by crossing with desired *T. tauschii* accessions.

For any of the above techniques, it is important that the desired traits in the D genome from *T. tauschii* be identified since genetic factors in the A and B genomes may mask or modify its expression. However, this may not be a general rule. For example, Multani et al. (1988) observed that synthetic hexaploids, which were produced with a KB-susceptible durum parent, expressed the KB resistance of the *T. tauschii* parent.

We can screen the *T. tauschii* accessions for their many desired attributes and then cross selected ones to *T. aestivum*. However, when screening the *T. tauschii* accessions is sometimes a major constraint, we can hybridize them with *T. turgidum* and then screen the resulting synthetic hexaploids.

Resistance screening of the *T. tauschii* accessions for *H. sativum* at Poza Rica and *F. graminearum* at Toluca have been inconclusive. Growing conditions at these two Mexican locations can adversely affect the alien species, but logistically the sites are ideal for disease resistance screening. Because of the screening constraints, winter habit, and the tendency for shattering (which could cause a weed problem at the stations) of the *T. tauschii* accessions themselves, we decided to indiscriminately cross *T. turgidum* with *T. tauschii* accessions. This has allowed us to screen the resulting synthetic hexaploids more adequately for our objectives without having to deal with vernalization. In addition, when we find a positive attribute (the durum parent being susceptible), the breeding program can immediately use the synthetic hexaploid. Even if the synthetic expresses diluted resistance, the end-product resistance is far superior to that encountered in the best wheat germplasm available.

Variability of *T. tauschii* accessions

See Chapter 7 for discussions on: 1) evaluation of the variability for seed storage proteins and isozymes associated with some *T. tauschii* accessions and 2) comparison of the wide variability of *T. tauschii* accessions with the variability of the synthetic hexaploids.

Synthetic Hexaploid Production

Methodology

The hybridization process is quite simple when using any of several manipulative crossing procedures (durums as the female parent; **Appendix 1**) described by Kruse (1973), Sharma and Gill (1983a), Mujeeb-Kazi and Asiedu (1990), or Riera-Lizarazu and Mujeeb-Kazi (1990). For the crossing cycles, we always plant the durum cultivars obtained from CIMMYT's durum section over at least three planting intervals so that *T. turgidum* flowering coincides with flowering of the *T. tauschii* accessions. Procedures for embryo rescue, embryo culture, and plantlet management are similar to those described by Mujeeb-Kazi et al. (1987). We transplant plantlets to a potted soil mix and maintain them in the greenhouse at El Batan.

The 21 chromosomes of the F1 hybrids are doubled (induced with colchicine or spontaneous) to produce 42-chromosome synthetic hexaploids (2n=6x=42, AABBDD; **Figure 3.1**; Appendix 2). If found to be resistant or tolerant after appropriate disease or stress

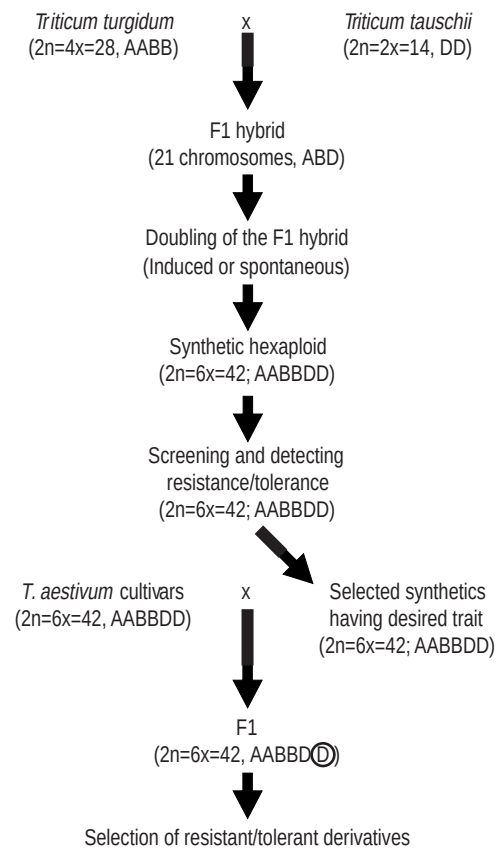


Figure 3.1. Schematic showing the production of synthetic hexaploids derived from crossing *Triticum turgidum* x *T. tauschii* and their utilization.

screening, the synthetic hexaploids can be crossed conventionally with other bread wheat cultivars.

Chromosome analysis (cytology)

To analyze the chromosomes in resulting hybrids, root tips are collected from young growing plants and processed according to the schedule of Mujeeb-Kazi and Miranda (1985). F1 hybrids with 2n=3x=21 chromosomes are treated with 0.1% colchicine + 2.0% dimethyl-sulfoxide for six hours via aerated root-treatment. We grow the colchicine-treated seedlings in the greenhouse and place a glassine bag over each spike after emergence from the boot. The seeds that set on such plants after this treatment are planted; after germination, they are analyzed for chromosome number. Resulting plants are maintained in the greenhouse and a glassine bag is placed over each spike. For each doubled fertile plant, we increase seed to a reserve of 50 g. We use surplus seed supplies for testing resistance and tolerance to biotic and abiotic stresses.

Results

We have produced, to date, nearly 525 synthetic hexaploids—most involving a unique *T. tauschii* accession—over several crossing cycles (Appendix 2). Whenever possible, we have screened them for selected resistances to diseases and abiotic stresses. We have identified synthetics resistant to *H. sativum* and *F. graminearum*. Several have shown tolerance to salt stress in initial field screening at La Paz, Baja California Sur, Mexico. When screening shows the synthetics to have these positive attributes, we must be cognizant of the interaction of the A and B genomes with the D genome as well as the dilution effect of the resistance or tolerance of the D genome in the hexaploid plant. Villareal et al. (1990) have studied agronomic and taxonomic traits of a few synthetics, such as days to anthesis, plant growth, maturity, biomass, harvest index, yield, pigmentation, and pubescence.

With the development of synthetic hexaploids in mind, all genuine F1 hybrids are stable for 2n=3x=21 chromosomes. After colchicine doubling, the seeds generally possess 42 chromosomes. Although there are some hypoploids and hyperploids among the resulting synthetics (**Figure 3.2**), they can be subsequently eliminated through additional chromosome analysis (cytology) and seed increase. We have identified synthetics that are resistant to *H. sativum* and *T. indica*. Synthetics resistant to *F. graminearum* and tolerant to salt await additional testing before we turn them over to the breeders.

Molecular applications of the synthetics

Scientists involved in the collaboration of CIMMYT, Cornell University, and the International Triticeae Mapping Initiative (ITMI) have found that some synthetic hexaploids resulting from the *T. turgidum* x *T. tauschii* crosses are highly polymorphic (M. Sorrells, pers. comm.). This is aiding molecular laboratories in the development of the RFLP linkage map for the D genome. The CIMMYT/Cornell collaboration has produced synthetics that have ultimately led to the development of doubled polyhaploid plants (see Chapter 5 for the significance of this). Other synthetics with desirable agricultural attributes can be subjected to molecular analysis.

New synthetics and AA genome hexaploids

We are producing new synthetics using additional *T. tauschii* accessions maintained in the CIMMYT wide crosses working collection. The CIMMYT Wide Crosses Laboratory has also produced about 155 A genome hexaploids by crossing durum wheat with A genome diploid species (Appendix 3). It is anticipated that these approaches will contribute to the availability of additional genetic variability for wheat breeding efforts, germplasm conservation, and global germplasm distribution. There is merit for international distribution of the synthetic and A genome hexaploids, which would enable national agricultural research programs with specific objectives to do their own screening of these accessions.

Direct Crossing of *T. tauschii* with Bread Wheat

The most ideal, efficient technique for exploiting *T. tauschii* variability for bread wheat improvement is to achieve direct transfers from resistant/tolerant *T. tauschii* accessions to bread wheat. This methodology rapidly produces improved BCI derivatives with the six genomes (AABBDD), five of which (AABBD) resemble the elite wheat cultivar used in the cross (**Figure 3.3**). With this methodology, we may have to contend with the ensuing aneuploidy in the BC derivatives and hence it may have lesser value in mapping programs for recognized quantitative traits. Cox et al. (1990) report on the numerous advantages.

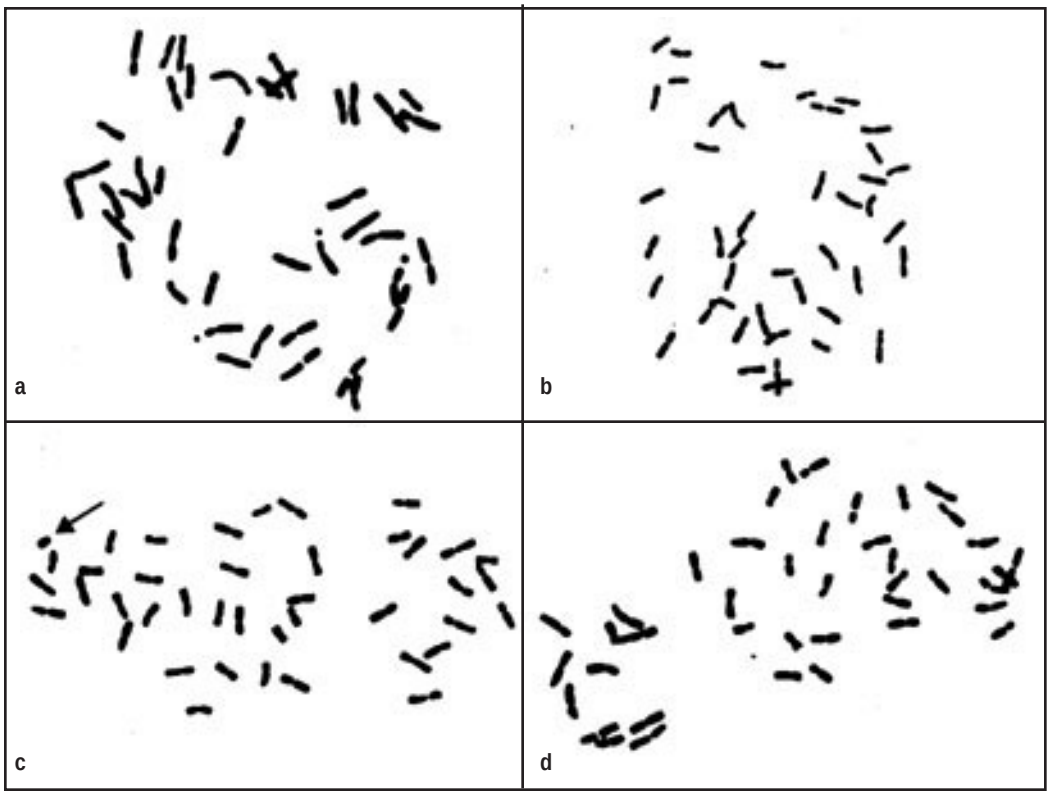


Figure 3.2. Somatic chromosome numbers of synthetic hexaploids from *Triticum turgidum* x *T. tauschii*: a) 2n=6x=42, b) Hyperploid with 43 chromosomes, c) 42 with a telocentric arrowed, and d) Hypoploid with 41 chromosomes.

Before crossing with bread wheat, reliable screening of the *T. tauschii* accessions for resistances and tolerances to diseases and abiotic stresses is critical. Alonso and Kimber (1984), Cox et al. (1990, 1991), and Gill and Raupp (1987) unequivocally placed priority on direct *T. tauschii* crossing with bread wheat cultivars. Based on the transfer of stem rust resistance from *T. tauschii* to the bread wheat cultivar Chinese Spring, Alonso and Kimber (1984) determined that one backcross onto the F1 hybrids re-instated 92% of the genotype of the recurrent parent.

When there are constraints to direct screening of *T. tauschii* accessions, such as less than reliable identification of resistance or tolerance to *H. sativum*, *F. graminearum*, *T. indica*, and salinity, we believe that screening of the synthetic hexaploids, resulting from *T. turgidum* x *T. tauschii* crosses, is an alternative (**Tables 3.1-3.4**) especially where the durums are susceptible. The information obtained from screening the synthetics allows us to target specific *T. tauschii* accessions for direct crossing with susceptible elite bread wheat cultivars, e.g., 'Ciano T 79' and 'Bacanora T 88' for *H. sativum* resistance, 'Seri M 82' and 'Opata M 85' for *F. graminearum* resistance, and 'Oasis F 86' for salt tolerance. Using these cultivars, we have duplicated the crossing successes that Alonso and Kimber (1984) had with the cultivar Chinese Spring. Several other options are also available for achieving additional crossing successes (Gill and Raupp 1987, Riera-Lizarazu and Mujeeb-Kazi 1990).

We have satisfactorily screened *T. tauschii* accessions for KB resistance (Warham et al. 1986, and unpublished data) and identified several 0% infection types. Successful crosses have been made between KB-susceptible bread wheat cultivars Seri M 82 and Bacanora T 88 and several of these *T. tauschii* accessions. The above procedure is highly efficient and, from about 270 F1 hybrids, 99% are normal with 2n=4x=28 chromosomes.

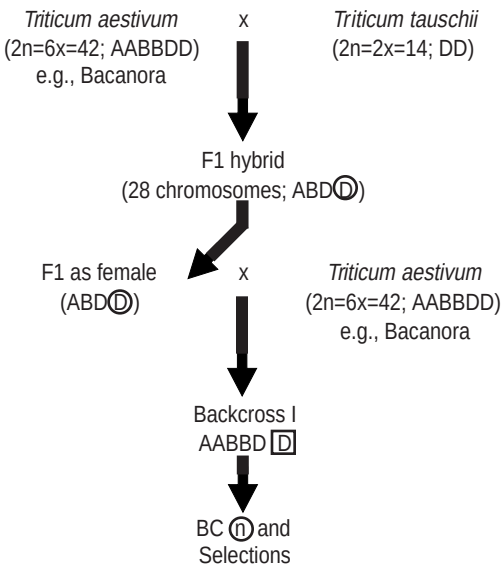


Figure 3.3. Schematic demonstrating alien transfers from *Triticum tauschii* (2n=2x=14, DD) to elite *T. aestivum* cultivars via direct crossing and backcrossing.

Extracting the AABB genomes

Extracting the AABB genomes from commercial *T. aestivum* cultivars and then developing hexaploids by crossing with desired *T. tauschii* accessions allow for very clear analysis of the genetic contribution of the alien D genome. There is negligible interference from recombinant segregation of the A and B genomes that is rampant in the first hybridization procedure (**Figure 3.4**). However, transmission of paternal chromosomes and aneuploidy in the backcross generations can complicate the process.

Table 3.1. Five synthetic hexaploids selected as resistant to *Helminthosporium sativum* compared with their durum wheat parents.

Durum wheat parent and Synthetic hexaploid	Seed source identifier (Synthetic ID no.[a])	Disease score	
		Leaf damage[b]	Seed damage[c]
CPI/Gediz'S'/3/Goo'S'//Jo'S'/CR'S'	—	99	4
CPI/Gediz'S'/3/Goo'S'//Jo'S'/CR'S'/4/*T. tauschii*	41	93	2
TK SN1081	—	98	4
TK SN1081/*T. tauschii*	54	93	2
Gan'S'	—	96	3
Gan'S'/*T. tauschii*	69	93	2
Decoy1	—	97	3
Decoy1/*T. tauschii*	114	93	2
Decoy1/*T. tauschii*	128	93	2
Ciano T 79 (susceptible bread wheat)	—	99	5
BH1146 (resistant bread wheat)	—	97	3

[a] See Appendix 2.
[b] Two-digit scoring system: first digit = height of infection; i.e., five = up to center of plant, 9 = upto the flag leaf; second digit = disease severity on infected leaves, 1 = low and 9 = total leaf destroyed.
[c] Grain infection scored as: 1 = low and 5 = high seed blemish at embryo points.

Table 3.2. Five synthetic hexaploids selected as resistant to *Fusarium graminearum* compared with their durum wheat parents.

Durum wheat parent and synthetic hexaploid	Seed source identifier (Synthetic I.D. no.[a])	Disease score on spikes[b]
Altar 84	—	5
Altar 84/*T. tauschii*	64	0
Laru'S'	—	5
Laru'S'/*T. tauschii*	82	0
Gan'S'	—	5
Gan'S'/*T. tauschii*	134	0
Crocethia_1'S'	—	5
Crocethia_1'S'/*T. tauschii*	145	0
Crocethia_1'S'/*T. tauschii*	161	0
Seri M 82 (Susceptible bread wheat)	—	5

[a] See Appendix 2.
[b] Fungal presence on spikes recorded as: 0 = no infection; and 5 = severe infection detected through pink colored fungal exudate on nodes and spikelets.

Table 3.3. Five synthetic hexaploids that have tested positive for the Na:K discrimination trait associated with salinity tolerance in hydroculture testing compared with their durum wheat parents.[a]

Durum wheat parent and synthetic hexaploid	Seed source identifier (Synthetic I.D no.[b])	K:Na ratios[c]
Rokel'S'/Kamilario'S'	—	1.2
Rokel'S'/Kamilario'S'//*T. tauschii*	39	7.7
PBW 34	—	1.2
PBW 114/*T.tauschii*[d]	—	13.3
CPI/Gediz/3/Goo//Jo'S'/CR'S'	—	1.1
CPI/Gediz/3/Goo/ /Jo'S'/CR'S'/4/*T. tauschii*	24	16.4
Scoop_1	—	1.5
Scoop_1/*T. tauschii*	111	17.7
Decoy1	—	0.7
Decoy1/*T. tauschii*	128	3.5

[a] Levels recorded 50 days after a 50 mol/m^3 NaCl concentration was reached.
[b] See Appendix 2.
[c] K:Na discrimination ratios; higher values are positive for salinity tolerance.
[d] Synthetic obtained from H. Dhaliwal. Instead of the durum PBW114, we have used PBW34 in the evaluation, since both are susceptible.

Table 3.4. Five synthetic hexaploids selected as resistant to Karnal bunt under the greenhouse screening test.

Durum wheat parent and synthetic hexaploid	Seed source identifier (Synthetic I.D. no[a])	Percent Karnal bunt infection
Sora	—	19.4
Sora/*T. tauschii*	14	0
Altar 84	—	7.4
Altar 84/ *T. tauschii*	23	0
D67.2/P66.270	—	25.5
D67.2/P66.270//*T.tauschii*	59	0
Yar'S'	—	64.2
Yar'S'/*T. tauschii*	125	0
68112/Ward	—	21.8
68112/Ward// *T.tauschii*	101	0

[a] See Appendix 2.

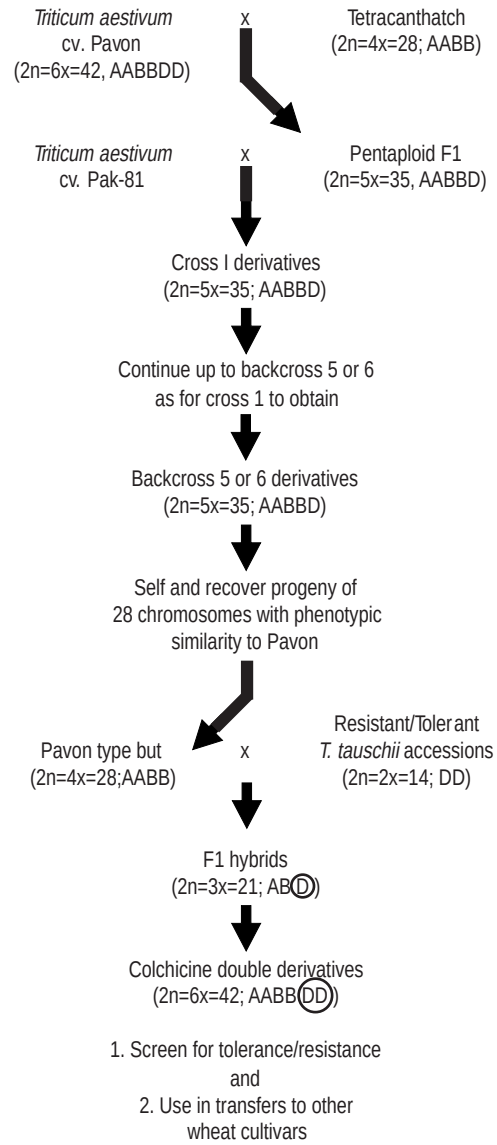


Figure 3.4. Schematic showing extraction of the AABB component from an elite hexaploid wheat cultivar, derivation of a synthetic hexaploid by crossing it to *Triticum tauschii* (2n=2x=14; DD), and utilization of the doubled derivative (2n=6x=42; AABBDD).

Chapter 4

Intergeneric Crosses: Hybrid Production and Utilization

Abdul Mujeeb-Kazi

The different gene pools within the annual and perennial grasses of the Triticeae Tribe can provide tremendous genetic variability (Dewey 1984) for improving wheat. However, in contrast to the *Triticum*/*Aegilops* spp. discussed in the previous chapter, the species we deal with in our intergeneric crosses are quite diverse genomically and rather difficult to cross with wheat. Even when successfully combined, the resulting hybrids exhibit little or no intergenomic chromosome association. Hence, accomplishing beneficial alien transfers through intergeneric hybridization is quite time-consuming. Despite these limitations, significant successes and advancements have been made over the past 20 years (Kruse 1973; Islam et al. 1981; Sharma and Gill 1983a,b,c; Mujeeb-Kazi and Kimber 1985; Mujeeb-Kazi et al. 1987, 1989; Mujeeb-Kazi and Asiedu 1989, 1990; Gill 1989). The CIMMYT Wide Crosses Section now has a significant number of hybrids derived from intergeneric crosses among its genetic stocks (**Appendices 4-6**).

This chapter describes the methodologies currently available for accomplishing successful intergeneric crosses within the Triticeae and using the resulting hybrids for wheat improvement—particularly in the areas of abiotic stress tolerance (e.g., aluminum, salt, and copper uptake efficiency). We also anticipate that greater emphasis will soon be attached to biotic traits such as resistances to spot blotch (*Helminthosporium sativum* syn. *Bipolaris sorokiniana*), scab (*Fusarium graminearum*), Karnal bunt (*Tilletia indica*), barley yellow dwarf virus (BYDV), *Septoria* spp., and Russian wheat aphid (*Diuraphis noxia*).

Hybrid Production

Germplasm used

The germplasm we utilize in our intergeneric hybridization program includes accessions of *Triticum turgidum*, *T. aestivum*, *Hordeum vulgare*, *Secale cereale* cv. Prolific, and numerous perennials mentioned in the sections below.

Methodology

Mujeeb-Kazi and Rodríguez (1984) described the procedures involving growth, hybridization, embryo culturing, somatic analyses, meiosis, backcross seed production, and amphiploid induction. The cytological techniques are similar to those employed by Mujeeb-Kazi and Miranda (1985). The chromosomal variations reported extend over investigations that have been in progress for more than a decade. Mujeeb-Kazi et al. (1984, 1987) describe the methodology for situations where the hybrids are produced under field conditions.

Application of the simplest techniques of emasculation and pollination used in conventional wheat breeding accomplished production of the earliest intergeneric hybrids. Many important hybrids are still produced with these techniques. The extensive series of crosses made by Kihara (1937), his colleagues, and

others can be cited as examples. Kimber and Abu Bakar (1979) provided a tabulation of hybrids involving wheat and its relatives. These contributions coupled with the pioneering work of Kruse (1967, 1969, 1973) led to an increased momentum in the area of intergeneric hybridization that extended the range of combinations among the Triticeae (Sharma and Gill 1983a,b,c; Mujeeb-Kazi and Bernard 1985a,b; Mujeeb-Kazi et al. 1987, 1989).

Some intergeneric hybrids, e.g., *T. aestivum* x *Aegilops cylindrica*, are quite easy to produce; while others, e.g., *Hordeum vulgare* x *T. aestivum*, are much more difficult. Some other difficult intergeneric combinations involve wheat crosses with *Psathyrostachys juncea*, all *Agropyron* species, and the small-anthered *Elymus* species. Regular production of intergeneric hybrids between *Hordeum* and *Triticum*, which may have been made as early as 1904 by Farrer, had to await the discovery by Kruse (1973) that embryos could be rescued by applying gibberellic acid to the developing ovule with subsequent culturing of the embryo on an artificial medium.

Choice of parents

With the intergeneric combinations that have already been made, choice of parents and cross direction become the paramount considerations in producing newer complex hybrids. The low frequency of the production of viable embryos in some hybrid combinations indicates the significance of placing together pollen and ovules that are genetically compatible. Presently, the only way to determine compatibility of the parents is actually to make a cross. Therefore, if difficulty is experienced with a particular hybrid combination, the only practical solution is to increase the range of parental genotypes involved and to attempt making reciprocal crosses (i.e., the alien species as the female parent).

Brink and Cooper (1940) described and discussed the effects of particular genotypes, ploidy level, and the species choice for the female parent in hybridization. In both interspecific and intergeneric crosses, it has been the general practice to use the higher ploidy species as the female parent because there appears to be less imbalance between the chromosome numbers of the embryo and endosperm and it is generally easier to emasculate the hexaploid *T. aestivum* than the tightly invested florets of most alien species. However, the early production of hybrids from wheat x barley crosses was accomplished with barley (lower chromosome number) as the female parent. Similarly, Sharma and Gill (1983a,b,c) used *Agropyron ciliare* and *A. yezoense* as the female parents in crosses with *T. aestivum*; and in the following crosses, the alien species was used as the female parent: *A. trachycaulum* x *T. aestivum* (Mujeeb-Kazi 1980), *A. fibrosum* x *T. aestivum* (Mujeeb-Kazi and Bernard 1982), and *Elymus canadensis* x *T. aestivum* (Mujeeb-Kazi and Bernard 1985a,b). So, it would appear that other hybrids, which have not been possible to date, may be recovered if we make the reciprocal combination. Fertility restoration in such combinations will vary.

Emasculation

Emasculation procedures can also affect hybrid production. At CIMMYT, we clip the tops of the glumes of the female parent and extract the anthers with forceps (Mujeeb-Kazi and Bernard 1985a,b). Other workers prefer not to clip the glume tops. At very high temperatures, there may be some advantage in not clipping, as it reduces the chance of drying the stigma, which can be a major problem in some of the wild species. It is almost always advantageous to remove the awns.

Male sterility

The development of cytoplasmic male sterility in *T. aestivum* eliminates the need for mechanical emasculation of the female parent. However, it is possible that the system producing the male sterility may also cause sterility in the hybrid or its derivatives. Male sterility has been induced in many species by cold treatment of the developing spike prior to microsporogenesis. The availability of chemicals that can induce male sterility without affecting ovule or embryo development may greatly enhance hybrid production.

Fertilization

Researchers have used pre-pollination chemical treatments with varying success to overcome fertilization barriers. These treatments likely induce pollen-tube growth, gynoecium longevity, micropylar barriers, and the delivery of the male gametes through the pollen tube. Treatments with immuno-suppressants (Bates et al. 1976); 2,4-dichlorophenoxyacetic acid (2,4-D) (Kruse 1974a,b); and gibberellic acid (GA3) (Larter and Chaubey 1965) have been reported. GA3 has emerged as the most popular treatment.

Characteristically, a rapid elongation of cells that produces a fluffy appearance in the stigmata of Triticeae species indicates receptiveness to pollination. In addition, the glumes of some species tend to gape at this time or slightly later. Pollination is usually done at this time; however, Mujeeb-Kazi et al. (1984, 1987, 1989) made previously unattainable hybrids by pollinating before the stigmata showed the visible receptivity indicator. It would seem that early pollination circumvents fertilization barriers that develop as the stigma matures (Sitch 1984). In addition, Kruse (pers. comm.) found that the first pollination with inactivated pollen followed by normal pollen enhanced the recovery frequency of hybrids of *T. aestivum* x *H. vulgare*. Kruse (1973) demonstrated the significance of a post-pollination treatment, which consisted of one application of a 75-ppm GA3 solution to the stigma and ovule walls to assist the developing embryoAlthough up to 10 daily post-pollination applications have been tried, the single GA3 application has been equally effective and, at the same time, it decreases labor and reduces the risk of the invasion of accidental out-pollination. In the divergent crosses of wheat with maize or *Tripsacum* for polyhaploid production (see Chapter 5), a 2,4-D post-pollination treatment is considered to be essential. We suggest a 2,4-D post-pollination treatment be tried in other wide hybrids. It is, however, unlikely that 2,4-D would modify the effect of crossability genes in such crosses.

Embryo rescue and culture

Embryo excision and culture on artificial media are the developments that have advanced the production and utilization of wide hybrids more than any other techniques. Although various media are employed in different laboratories, we use those developed by Murashige and Skoog (1962) and Taira and Larter (1978).

Embryo rescue and culture are aimed at removing the embryo aseptically as late as possible in its development, yet still early enough to allow its continued development on artificial media. Endosperm degeneration may start earlier and seems to be closely related to the cessation of embryo growth. Characteristically, embryo development in wide hybrids tends to slow down about eight days after pollination and, in 10 to 14 days, the embryo often ceases development. Embryos of the hybrid derived from *Hordeum vulgare* x *Secale cereale* (Fedak 1977a,b) were rescued and successfully cultured only 12 to 14 days after pollination; however,

most embryo rescues are accomplished 16 to 18 days after pollination under field conditions and around 20 days under growth chamber or greenhouse conditions.

Recently developed media (A. Comeau, per. comm.) will undoubtedly allow the recovery of even younger, less developed embryos. This area of study currently seems to attract little attention, yet the potential of increasing the range of producible wide hybrids is substantial. The difficulty of producing mature plants from hybrids of only a few cells is probably considerably less than that from artificially fused somatic cells because some level of compatibility has already been demonstrated by the fact that sexual fusion has taken place. Therefore, it would seem that the improvement of embryo-rescue techniques would present a greater potential for utilizing the alien genetic material found in distant, but related species.

Bridge crosses and polyploidy

We have also obtained wide hybrids through the use of bridge crosses and the contribution of polyploidy (Mujeeb-Kazi and Asiedu 1990). Examples of the former are possible hybridization of *T. aestivum* with *Heteranthelium* by using the F1 hybrid of the *Heteranthelium* x *Thinopyrum elongatum* cross and combining *T. aestivum* x *Agropyron desertorum* by crossing the amphiploid of the *A. desertorum* x *Th. repens* with *T. aestivum*. We have demonstrated the utility of polyploidy by obtaining the wheat combination with the *Psathyrostachys juncea* combination. With this, the alien-induced tetraploid source not only facilitates hybrid production, but also simplifies obtaining the amphiploid when the self-sterile F1 hybrid (2n=5x=35, ABDNN or 2n=4x=28, ABNN) is backcrossed to *T. aestivum* or *T. turgidum*, respectively.

Cytological verification

Production of an F1 plant does not necessarily mean a successful intergeneric cross has been made. Morphological recognition that a wide hybrid has actually been produced is usually very unreliable because important characters may be completely suppressed (Kimber 1983). Cytological verification is more convincing, but this too can result in erroneous conclusions due to:

- Technical problems in the collection and preparation of material;
- Unreliability of the chosen cytological technique;
- Somatic chromosome elimination or chimeras; and
- Misinterpretation of meiotic data.

Root tip preparations from plants in plant pots can provide the first indication of hybridity. We usually delay root tip collection from a suspected hybrid for cytological analysis until it is growing in a pot because of the small number (often only one) of roots produced in culture. After taking roots from the pots, the number of dividing cells is usually less than that found in normal seedlings that have germinated in a petri dish. Further, silica particles adhering to the roots can spoil the preparation of good squashes. Feulgen or carmine/orcein staining of prefixed cells validates that a genuine hybrid plant has been produced. A technique we developed (Mujeeb-Kazi and Miranda 1985) can result in very clear preparations from which we can determine chromosome number, arm ratios, secondary constrictions, and intergeneric differences in chromatid thickness (e.g., *Thinopyrum*/ *Secale*). If both parents of a hybrid have the same chromosome number, somatic chromosome counts, at best, can only give an indication of

hybridity if there are large and characteristic karyotypic differences between chromosomes of the two parents.

The reliability of a particular cytological technique must also be considered. If the parents differ in chromosome number, simple counting of somatic chromosome number can be reliable. If the somatic chromosome numbers are the same, the parents might still differ in their ability to show C or N bands. The reliability of hybrid recognition on the basis of chromosome banding is directly proportional to the number, intensity, and chromosomal distribution of the bands. If the arm ratio of the chromosomes of the parents is sufficiently different, this too may be used as an indication of hybridity. Again, the reliability of the arm ratio as an indicator of hybridity depends on the number of easily recognized differences between the parents. The presence or absence of secondary constrictions is not a good method of recognizing hybrids since amphiplasty does contribute to suppression. Resolution of secondary constrictions requires that superior somatic preparations be made and that no nucleolar organizer competition prevails. The length of somatic chromosomes generally provides a very poor method for the identification of hybridity. Low reliability can be attributed to: 1) the general absence of large differences in relative chromosome length in the Triticeae and 2) the inaccuracies involved in measuring chromosome length (Kimber 1970).

Even if the zygote of an intergeneric cross is recovered, it is still possible that the seedling may not be a hybrid due to chromosome elimination in the early zygotic divisions. Haploid barleys and polyhaploid wheats, produced by pollination of wheat by *Hordeum bulbosum* and *Zea mays*, are examples of this type (Kasha and Kao 1970, Barclay 1975; see upcoming section on genome elimination and Chapter 5). The spontaneous production of chimeras (cells with different chromosome numbers) may also hinder the recognition of hybrids or make their utilization more difficult. Several authors have recognized chimeras. For example, Kasha and Sadasivaiah (1971) recorded the expected chromosome number in only 40% of the somatic cells of a diploid hybrid of *Hordeum vulgare* x *H. bulbosum*.

Clearly, when such difficulties abound, any claim of hybridity must be accompanied by rigid chromosome analysis. The interpretation of such analysis is quite important because both species relationships and the choice of the most suitable method for the introduction of alien variation depend on the ability of the chromosomes to pair. In the earliest work, although judgments were largely subjective, correct conclusions were still often reached (Lilienfeld 1951). More recently, numerical methods for the analysis of meiosis in hybrids have provided some objectivity in determining genomic relationships (Kimber et al. 1981, Alonso and Kimber 1981, Kimber and Alonso 1981, Espinasse and Kimber 1981, Kimber and Pignone 1982).

Ideally, genomic analysis is conducted using a triploid hybrid that results from a cross between a tetraploid plant and a diploid analyzer; however, analyses are often made at other polyploid levels. In general, diploid hybrids can provide little if any genomic information, for there must be competition for chromosome pairing partners in order to recognize differences in the genomes present. The pairing patterns at higher levels of polyploidy can be very confusing because of the large number of pairing possibilities between both the homologous (if present) and homoeologous chromosomes

within any homoeologous group. These practical limitations result in a useful range of triploid to pentaploid hybrids from which we can reliably obtain information. If telocentric chromosomes are available, they can provide unequivocal information about the frequency with which particular chromosomes are pairing, but their usefulness is usually limited to measurements of relationships with the A, B, and D genomes of *T. aestivum*

Clear proof of hybridity can only come from chromosome analysis of the supposed hybrids. The investigation of species relationships from backcrossed hybrids can, in some cases, be accomplished; however, complications introduced by the production of unreduced gametes or the random elimination of chromosomes can give rise to incorrect interpretations.

Chromosomal variations

Chromosomal variations in intergeneric hybrids among the Triticeae occur fairly consistently and at various phases in the formation and the development of the hybrid. To a certain extent, the events are fortuitous, but in other situations the variation effects may pose a serious constraint in systematic wide crossing programs. Stable amphiploids are a prerequisite for the development of alien chromosome addition lines. The production of these lines could be hindered greatly if amphiploids cannot be produced. This forces researchers to adopt the F1 self-sterile based route to generate F1 backcross I (BCI) progeny that has the potential to induce alien structural chromosomal modifications. In the "shot-gun" approach, chromosomal variations have a decided breeding advantage, more so for complex polygenically controlled characters. The occurrence of these variations augments those associated with callus culture and mutagenesis. During the cytological analyses of some intergeneric hybrids or of their advanced derivatives, we have observed unique chromosomal variations.

Genome elimination

A normal F1 hybrid resulting from an intergeneric cross possesses half the chromosome number of each parent involved in the combination. In some situations, however, the alien genome may be totally or partially eliminated, which results in the production of polyhaploid or aneuploid F1 hybrids. This phenomenon has been observed in a number of hybrids resulting from intergeneric crosses using species within the Triticeae as both parents and in crosses where one parent is outside of the Triticeae.

Crosses within the Triticeae include: *Hordeum* x *Triticum* (Kruse 1974a,b; Mujeeb-Kazi et al. 1978), *Triticum* x *Hordeum* (Fedak 1980, Finch and Bennett 1980, Islam et al. 1981), *Hordeum* x *Secale* (Kruse 1967, Fedak 1977a), *Triticum* x *Elymus* (Mujeeb-Kazi and Bernard 1985a), *Agropyron* x *Triticum* (Mujeeb-Kazi and Bernard 1985a,b), and *Triticum* x *Agropyron* (Mujeeb-Kazi, unpublished). Crosses involving a parent outside the Triticeae include: *T. aestivum* x *Zea mays* (Laurie and Bennett 1986), *T. aestivum* x *Sorghum bicolor* (Laurie and Bennett 1988b, Ohkawa et al. 1992), *T. aestivum* x *Z. mays* ssp. *mexicana* (Ushiyama et al. 1991), and *T. aestivum* x *Tripsacum dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993).

The most widely documented and high frequency method of polyhaploid production involves the hybridization of *T. aestivum* with *Hordeum bulbosum* (Barclay 1975). Wheat polyhaploids were obtained from pollination of

T. aestivum cv. Chinese Spring (CS) by both diploid and tetraploid forms of *H. bulbosum*. Studies on the early embryo and endosperm development of these hybrids demonstrated that fertilization was followed by the elimination of the *H. bulbosum* chromosomes during subsequent divisions of the embryo and endosperm. The same phenomenon has been observed in both diploid and tetraploid reciprocal *H. vulgare* x *H. bulbosum* hybridizations, resulting in a high frequency of *H. vulgare* haploids (Davis 1960, Kao and Kasha 1969, Bennett et al. 1976).

We do not know what controls genome elimination in *T. aestivum* x *H. bulbosum* hybridizations. However, studies of chromosome elimination in *H. vulgare* x *H. bulbosum* hybridizations have revealed that many factors are involved. Three genes have been identified and located in chromosome arms 2S, 2L, and 3S of *H. vulgare*, which control *H. bulbosum* genome elimination (Barclay et al. 1972, Ho 1972, Ho and Kasha 1974). In addition, the *H. bulbosum* genotype (Pickering 1979, Simpson et al. 1980, Fukuyama and Hosoya 1983), the *H. vulgare* genotype (Simpson et al. 1980), and the balance between the parental genomes appear to be involved.

Pollination of *T. ventricosum* by tetraploid *H. bulbosum* has also resulted in polyhaploids of *T. ventricosum* because the *H. bulbosum* genomes were eliminated (Fedak 1983).

Low frequencies of genome elimination have been observed in other *Triticum* x *Hordeum* hybridizations, which involve species other than *H. bulbosum*. Within the 20 F1 progeny of hybridization between CS and *H. vulgare* cv. Betzes, Islam et al. (1981) identified three 21-chromosome plants that phenotypically resembled wheat polyhaploids, implicating the elimination of the *H. vulgare* genome. Fedak (1980) obtained one polyhaploid from the same hybrid combination. Finch and Bennett (1982) obtained a particularly high frequency (80%) of wheat polyhaploids among the progeny of a cross between *T. aestivum* cv. TH3929 and *H. vulgare* (2n=2x=14) Paavo Line P-4. The elimination of the *H. vulgare* genome was suspected due to variations in the somatic chromosome number between and within a number of nonviable embryo-derived plantlets; these variations ranged from the polyhaploid (n=3x=21) to the hybrid chromosome number (2n=4x=28).

Genome elimination and the consequent production of wheat polyhaploids have also been observed in the reciprocal hybridization, *H. vulgare* x *T. aestivum* (Kruse 1974a,b), in our own studies, and in the cross between *H. vulgare* x *T. turgidum* (Mujeeb-Kazi et al. 1978). Hybridizations between *H. vulgare* and *S. cereale* have also produced a low frequency of polyhaploid *H. vulgare* progeny, following the elimination of the rye genome. Kruse (1967) reported the production of one polyhaploid and two hybrid progenies from this intergeneric hybridization. Later, Fedak (1977a) noted the production of barley haploids at a frequency of less than 1% in barley x rye hybridizations.

We obtained a 21-chromosome F1 progeny with a wheat polyhaploid phenotype from intergeneric hybridizations of *T. aestivum* x *Elymus patagonicus* (Mujeeb-Kazi and Bernard 1985a,b). From the reciprocal cross of *E. fibrosus* x *T. aestivum*, we obtained a polyhaploid of *E. fibrosus* (Mujeeb-Kazi 1994a). Laurie and Bennett (1986) observed elimination of the maize genome six days after a *T. aestivum* x *Zea mays* hybridization, which has provided an alternative

for producing *T. aestivum* polyhaploids; this technique, which has had up to a 4.4% recovery of wheat polyhaploid plants (Laurie and Bennett 1988c), functions satisfactorily and is independent of the *kr1 kr2* loci (Laurie and Bennett 1987). A modification of this technique, which uses 2,4-D and an altered mode of application, has significantly improved the recovery of wheat polyhaploids (Inagaki and Tahir 1990, Riera-Lizarazu and Mujeeb-Kazi 1990, Suenaga and Nakajima 1989). Fertilization of *T. aestivum* by *S. bicolor* has had a 59% success rate following which elimination of the *S. bicolor* chromosomes occurs. Polyhaploid wheat plants have since been recovered (Ohkawa et al. 1992).

Aneuploid F1 progeny

Reports of the occurrence of aneuploid F1 progeny from intergeneric hybridizations involving the Triticeae are restricted to crosses between *T. aestivum* and *H. vulgare* (Islam et al. 1981). Meiotic instability within complete F1 hybrids has, however, been more widely reported, namely within *Triticum* x *Hordeum* reciprocal hybrids (Fedak 1977b, 1980; Islam et al. 1981; Mujeeb-Kazi and Rodríguez 1983a,b) and within *Hordeum* x *Secale* hybrids (Finch and Bennett 1980).

Islam et al. (1981) reported a high frequency of aneuploid F1 progeny from a cross between CS and *H. vulgare* cv. Betzes. Of 20 F1 progeny, 19 showed nonhybrid chromosome numbers ranging from 21 to 36. Meiotic analyses of these plants indicated that aneuploidy in five plants was associated with the addition of 1, 2, 3, 4, or 6 *H. vulgare* chromosomes to the polyhaploid wheat genome. Islam et al. (1981) postulated that this was due to spindle abnormalities at the early zygotic divisions of the hybrid embryo, causing the elimination of one or more *H. vulgare* chromosomes. In 11 plants, the duplication and/or deficiency of wheat and barley chromosomes were apparent. For example, one 35-chromosome plant showed a meiotic pairing pattern of 15II + 5I. Although the 28-chromosome F1 hybrid resulting from the wheat x barley cross exhibited stability in the somatic cells and in the majority of the pollen mother cells, some mosaic cells with aneuploid and polyploid chromosome numbers were observed, which indicated meiotic instability in this complete hybrid (Islam et al. 1981). Similarly, from an examination of the meiosis of complete *H. vulgare* x *T. aestivum* F1 hybrids, we found meiotic instability and the precocious separation of five or six chromosomes at meiosis (Mujeeb-Kazi and Rodríguez 1983a).

We also observed meiotic instability in *H. vulgare* x *T. turgidum* F1 hybrids (Mujeeb-Kazi and Rodríguez 1983b) in the form of hyperploid and hypoploid pollen mother cells, aneuploid cells showing trivalent and quadrivalent associations, and premature chromosome separation. We speculate that the chromosomes showing premature disjunction may undergo synapsis without the formation of chiasmata, resulting in more rapid chromosome separation. Fedak (1980) observed a differential degree of meiotic instability in reciprocal *H. vulgare* x *T. aestivum* hybrids, the frequency of euploid pollen mother cells being lower in F1 hybrids that possess *H. vulgare* cytoplasm. Fedak (1979) also observed a number of meiotic abnormalities, namely unequal disjunction, multipolar mitoses, and the presence of isochromosomes, which implies misdivision at anaphase I.

Finch and Bennett (1980) observed similar meiotic instability in hybrids derived from *H. jubatum* ssp. *breviaristatum* x *S. africanum*, which had hyperploid pollen mother cells containing 22 to 32 chromosomes and

occasionally 47 chromosomes. No aneuploid F1 hybrids have been reported in this or other *Hordeum x Secale* hybridizations. In a triploid hybrid (2n=3x=21) obtained from the cross of *H. vulgare* cv. Betzes x *S. vavilovii*, the mean somatic chromosome number was 19.7 with a range of 7 to 24 between individual cells. In meiocytes, the mean chromosome number was 18.3 with a range of 14 to 26 (Fedak and Nakamura 1982).

F1 hybrids produced from a cross between CS and the hybrid resulting from the intergeneric cross of *Thinopyrum repens*/*A. desertorum* also showed a high degree of aneuploidy. We found that chromosome numbers of the F1 progeny ranged from 2n=35 to 2n=57 where the expected complete chromosome complement was 2n=8x=56. In the 35-chromosome hybrids, the elimination of three genomes had presumably occurred (Mujeeb-Kazi et al. 1989).

Although rare, we found chromosome irregularities in F1 hybrids of *H. vulgare* x *E. canadensis* where meiocytes at metaphase I possessed chromosomal compositions exceeding the normal 2n=3x=21 complement (Mujeeb-Kazi and Rodríguez 1982).

Variations in backcross I (BCI) derivatives

Aneuploid BCI progeny—In wide crosses, the self-sterile F1 hybrids, upon colchicine treatment, classically result in fertile amphiploids that may then have practical utility. Triticale (X *Triticosecale* Wittmack) is a noteworthy result of such a process—both at the hexaploid and octoploid polyploidy levels. In other cases, the fertile amphiploids are sources of BCI derivatives (amphiploid/*Triticum* source), from which the eventual production of alien disomic addition lines—after cytogenetic manipulations—can lead to subtle alien genetic transfers. The BCI derivatives are generally expected to have normal wheat and normal alien chromosome complements—the former in a double dosage and the latter in a single dosage.

Alternatively, the F1 hybrid can be pollinated by wheat to produce the BCI derivatives in a low frequency. This occurs via fusion of the wheat pollen with an unreduced egg cell of the F1 hybrid. This common procedure is a rapid way of meeting applied research goals, although it is beset with considerable aneuploidy that is maternally contributed. The unreduced egg cell could be an assemblage of wheat/wheat, wheat/alien, or alien/alien translocations and may have drastic aneuploid changes to be expressed as hyperploid or hypoploid progeny in the BCI derivatives.

Hybridization of *T. aestivum* with decaploid (2n=10x=70) *Th. elongatum* (*Elytrigia pontica*) produced F1 hybrids with 56 chromosomes. Subsequent pollination of these F1s with *T. aestivum* was expected to produce BCI progeny with 77 chromosomes. The chromosome numbers in 143 BCI plants ranged from 42 to 62 chromosomes and N-band analyses indicated a random variation for the presence of specific maternal chromosomes. As expected, we obtained plants that were disomic for chromosome 5B, but several plants were mono-5B which, upon selfing or further backcrossing, may lead to selfed nulli-5B derivatives (**Table 4.1**). These derivatives may enhance recombination events between wheat and alien species. We found a similar trend in BCI derivatives of the hybrid of *T. aestivum*/*Aegilops variabilis* (Jewell and Mujeeb-Kazi 1982). Variation in chromosome number of the BCI appears to be common in other intergeneric hybrids (Mujeeb-Kazi and Bernard 1982,

1985a,b), but such events seem independent of alien genomic composition. We have found a high frequency of 56-chromosome BCI derivatives for the *T. aestivum*/*Th. scirpeum*//*T. aestivum* cross (Mujeeb-Kazi and Miranda 1984) where *Th. scirpeum* contributes the E1E2 genomes to the above BCI derivatives with the AABBDD wheat genomic combination; Sharma et al. (1987) obtained similar results. Replacing *Th. scirpeum* with the tetraploid *Th. curvifolium*, which also has the E1E2 genomes, in the above cross results in a far lower frequency of 56-chromosome BCI progeny. This not only creates doubt about the E1E2 genomic designation in *Th. scirpeum* and *Th. curvifolium*, but also presumably reflects the varied effects of wheat's regulator genes for meiotic pairing. The expected chromosome count of a BCI derivative should not be considered normal because there is ample opportunity for structural changes to arise in both the wheat and alien genome(s) contributed to the F1 hybrid. The applied aspects of a wide cross program are not hampered by this BCI variability because the chromosomal reorganizations may actually be advantageous for breeding. Most F1 hybrids derived from intergeneric crosses do not exhibit enough chromosome association to indicate a direct alien transfer. Aspects of induced pairing manipulation do promote such F1 *T. aestivum*/alien chromosomal associations, but the BCI derivatives (F1 hybrid x *T. aestivum*) are highly aneuploid (Asiedu et al. 1989) and extremely valuable—an example of exploitation of aneuploidy.

The BCI variations are often a constraint to producing alien addition lines. We believe that producing amphiploids prior to BCI formation can alleviate this problem (ter Kuile et al. 1988). However, disomic addition lines of *H. vulgare* to wheat have been produced (Islam et al. 1975, 1978, 1981) through the BCI approach when amphiploids of *T. aestivum*/*H. vulgare* could not be produced. Thus, each hybrid should be regarded as a unique entity, but we feel that so-called normal BCI derivatives will be obtained if a large BCI population is produced.

Apomixis—In traditional intergeneric crosses (Mujeeb-Kazi 1994b), the F1 hybrid is always self-sterile. Upon placing wheat pollen on the self-sterile F1 spikes, BCI seed set does occur at reasonable frequencies, which leads to normal or aneuploid progeny. In rare situations, the BCI progenies have a chromosomal complement identical to the F1 hybrid and are classified as apomictic (Mujeeb-Kazi 1981a,b). Such a phenomenon has occurred more often with the BCIs of *H. vulgare*/*T. turgidum* than with those of

Table 4.1. Some backcross derivatives (BCI) from *Triticum aestivum/Thinopyrum elongatum* (2n=10x=70)// *T. aestivum* with aneuploid chromosome numbers and some N-banded chromosome compositions.

BCI derivatives with chromosomal variations	Some N-banded chromosomes								
	1B	2B	3B	4B	5B	6B	7B	4A	7A
50 chromosomes	+	+	+	+	+	+	+	+	+
				+		+		+	+
40 chromosomes	+	+	+	+	+	+	+	+	+
			+	+	+	+			
49 chromosomes	+	+	+	+	+	+	+	+	+
	+	+		+		+		+	

H. vulgare/*T. aestivum* (**Table 4.2**). In our initial BCI crosses, we used the same wheat variety as was used in the production of the F1 hybrid, so we were not able to categorically state that the progenies were apomictic. There was a possibility that all the maternal wheat chromosomes were eliminated, then the so-called 'apomictic progeny' could have resulted from the fertilization of the egg-cell (with seven barley chromosomes) by wheat pollen. In subsequent BCI crosses, we used different wheat varieties and utilized awn markers to unequivocally demonstrate that apomixis was indeed occurring (Mujeeb-Kazi 1994b). We also used a trigeneric system to further document the findings in the *T. aestivum*/*Leymus racemosus*//*Th. elongatum* cross where, in addition to the expected 70-chromosome progeny (21+14+35), apomictic 35-chromosome plants resulted with 21+14 chromosomes whose banding and meiotic pairing were similar to those we found independently in the F1 hybrid of *T. aestivum*/*L. racemosus* (Mujeeb-Kazi et al. 1983).

Doubled egg cell—A doubled chromosome number in the egg cell of the hybrid is rare—considering the wide range of hybrids we produce (Mujeeb-Kazi 1984, Mujeeb-Kazi and Bernard 1982). However, we did observe it in a BCI of the *Th. elongatum*/2**S. cereale* combination. The F1 possessed 21 chromosomes where the seven rye chromosomes could be identified on the basis of their larger size, although C-banding also aided identification. We pollinated the F1 of *Th. elongatum*/*S. cereale* cross with diploid *S. cereale*; the resulting BCI derivative possessed 49 chromosomes (**Figures 4.1a,b**). We inferred this to be the product of the fertilization of a

Table 4.2. Apomictic progeny in backcross I derivatives of *Hordeum vulgare*/2* *Triticum turgidum* and *H. vulgare*/2* *T. aestivum* with cytological details.

Cross Combination	Somatic chromosome number	Meiotic association at metaphase I					
		I	II Rods	II Rings	III	IV	VI
H. vulgare/*T. turgidum*	21	19.3	0.4	0.5	0.02		
H. vulgare/2* *T. turgidum*	21	19.3	0.4	0.5	0.03		
H. vulgare/*T. aestivum*	28	23.9	1.4	0.5	0.1	0.03	0.01
H. vulgare/2* *T. aestivum*	28	23.9	1.4	0.4	0.1	0.04	

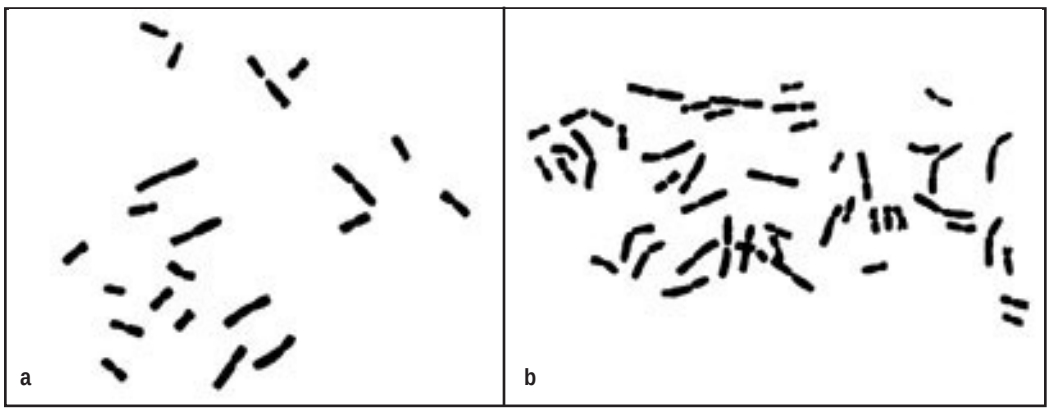


Figure 4.1. Somatic details at metaphase for: a) *Thinopyrum elongatum*/*Secale cereale* F1 (2n=3x=21), and b) *Th. elongatum*/2* *S. cereale* backcross I with 49 chromosomes (2n=7x=49).

spontaneously doubled egg cell (28+14) by seven-chromosome *S. cereale* pollen. Chromosome size and C-banding indicated this to be the case. Initially, determining chromosome size differences often led to erroneous conclusions (Mujeeb-Kazi and Kimber 1985), but a procedure we developed in 1985 eliminates this problem (Mujeeb-Kazi and Miranda 1985).

Selfed progenies of backcross I derivatives; BCIF1 and production of modified genomes

Complete synthetic genomes—The F1 hybrids of crosses between *T. aestivum* and *Th. junceiforme* (2n=4x=28), *Th. curvifolium* (2n=4x=28), and *Th. scirpeum* (2n=4x=28) possess 35 chromosomes and are self-sterile. Upon pollination of these F1s with wheat, seed set occurred; somatic analyses indicated a predominance of 56-chromosome progenies for *Th. scirpeum*; for *Th. junceiforme* and *Th. curvifolium*, there were some variations around the expected number of 56. Some spikes on each BCI derivative were covered with glassine bags; seed set took place on these spikes, proving them to be self-fertile (BCIF1). The seed set frequency was *Th. scirpeum* > *Th. curvifolium* = *Th. junceiforme*. Somatic analysis showed these seeds to possess 56 chromosomes (**Figure 4.2d**) or near this number (**Figures 4.2a, b, c**). Chromosomal associations ranged from high bivalency (**Figures 4.2e, f**) to 21 bivalents and 14 univalents. The meiosis in the 56-chromosome derivatives of *T. aestivum*/*Th. scirpeum*//*T. aestivum* was the most organized due to the high pairing. Sharma et al. (1987) further substantiated this where the multivalency they observed was attributed to genomic structural variations. Where 14 univalents were observed, extremely low seed set led to the perpetuation of this BCIF1 self-fertility.

This self-fertility phenomenon in the three partial autopolyploids is indicative of a genomic composition that may provide novel options for plant-level genetic manipulation. Repeated selfings of the partial autopolyploid-based BCIF1 derivatives could lead to synthetic genome formation, i.e., a means of aggregating complex polygenic recessive traits in a modified package (Mujeeb-Kazi and Miranda 1984).

After repeated selfings of the BCI, additional backcrosses to wheat resulted in derivatives that predominantly had 49 chromosomes but some variation as well in chromosome number, i.e., 48 (**Figure 4.3a**), associated as 19 bivalents + 7 univalents + 1 trivalent (**Figure 4.3b**), or as 20 bivalents + 8 univalents (**Figure 4.3c**) for *Th. scirpeum*-based progeny. In the near 49-chromosome *Th. curvifolium*- or *Th. junceiforme*-based progeny, aneuploidy was more pronounced. It is our contention that addition lines from selfed *Th. scirpeum* BCIF (n) will be different than those from where the BCI is directly advanced to BCII or BCIII, etc., i.e., devoid of several BCI selfing steps. We are currently testing this theory and we should be able to validate it in all tetraploids where aneuploidy is not noticeable. Other tetraploids that respond to the synthetic genome concept are hybrids of crosses between *T. aestivum* and *Th. distichum*, *Th. rechingeri*, *Th. scythicum*, and *Th. repens*/*A. desertorum* (a 35-chromosome F1) (Mujeeb-Kazi et al. 1987, 1989).

Asymmetric synthetic genomes—In segmental allohexaploids (*Th. junceum*; 2n=6x=42) or segmental autoallohexaploids (*Th. intermedium* and synonymous species), there are two related genomes (E_1E_2) and a distinctly different third genome (X or Z). Taking *Th. intermedium* and its relatives as an example, the genomic constitution of a hybrid resulting from a cross with wheat would be $ABDE_1E_2X$ or $ABDE_1E_2Z$. When such

self-sterile F1 hybrids are pollinated with wheat, the BCI derivatives usually possess 63 chromosomes and have a genomic make-up of $AABBDDE_1E_2X$. These are partially self-fertile and the BCIF1 progenies as well as progenies of additional selfings usually possess derivatives with 56 chromosomes. We believe that an asymmetric genome loss occurs in the BCI octoploid (2n=8x=56) yielding BCIF1 progeny. This prevalent phenomenon has advantages and disadvantages. It has been described as a mechanism known as "genome splitting" or "asymmetric genome reduction" by Gottschalk (1971), Cauderon (1977), Ladizinsky and Fainstein (1978), and Dewey (1980). These workers provided information concerning the extraction of different genomes of the complex polyploid *Agropyron* species, which facilitated

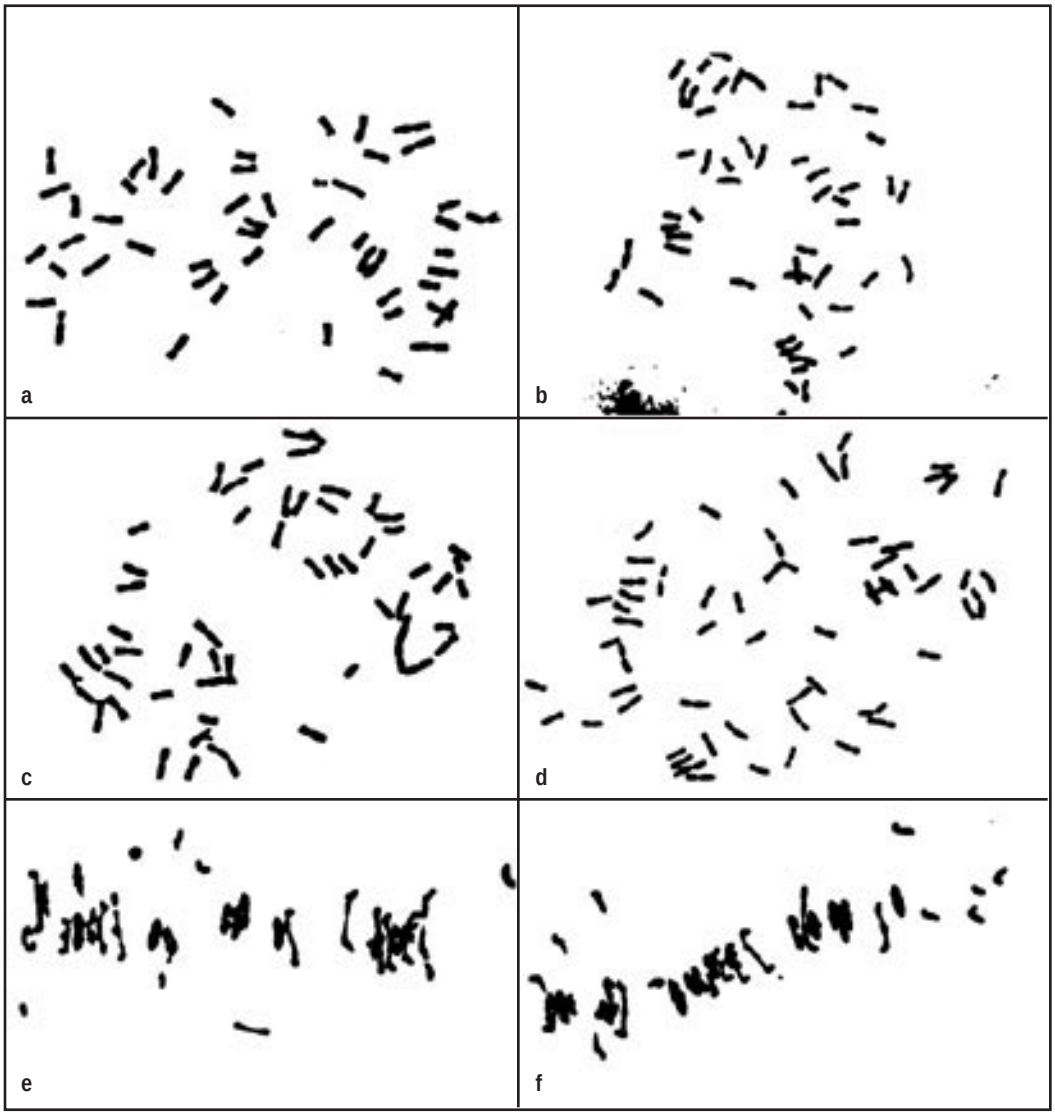


Figure 4.2. Mitotic and meiotic chromosomal details of some backcross I derivatives of *Triticum aestivum/ Thinopyrum scirpeum//T. aestivum* shown in a somatic cell with a) 51 chromsomes, b) 53 chromosomes, c) 56 chromosomes including a telocentric, and d) 56 chromosomes and in a meiocyte with e) 6 univalents + 12 rod bivalents + 13 bivalents at metaphase I and f) 6 univalents + 11 rod bivalents + 14 ring bivalents at metaphase I.

cytogenetic analysis as conventionally done for a diploid alien species. The data presented by the above workers show a range of variation that is explained by the genome splitting phenomenon, which we do not deal with here.

Variations in advanced backcross generations

In analyses of BC progeny of *T. aestivum/ L. racemosus$/n*T. aestivum*, unusual chromosome number variants were observed. In one instance, wheat polyhaploids originated as twin seedlings in the selfed progeny of a 44-chromosome double monosomic addition line. N-banding revealed 16 typical wheat chromosomes in the polyhaploids. Other variants in the backcross progeny included mixoploids with 43 and 86 chromosomes.

After selfing of the BC derivatives (with 49 chromosomes) of *T. aestivum/Th. junceiforme//2*T. aestivum*, 56-chromosome derivatives were recovered. One explanation implies fertilization of a 28-chromosome egg cell with pollen that maintained a high number of *Th. junceiforme* chromosomes in the transmission. Such events have also been observed in BCIIF1 derivatives of wheat crossed with *Th. rechingeri*, *Th. curvifolium*, and *Th. scirpeum*. In a similar way, Cauderon (1963, 1977) obtained a 56-chromosome amphiploid from a *T. aestivum/Th. intermedium//T. aestivum* selfed derivative, although the transmitted genome was unidentified. The occurrence is common in the 49-chromosome BCI derivatives of *T. aestivum/ Th. bessarabicum//T. aestivum*. These BCI plants are fairly self-fertile and, upon analysis, the BCIF1 progeny shows a range in chromosome number from 23 to 51. This provides an opportunity to obtain homologous alien chromosomes as multiple disomic additions (Mujeeb-Kazi et al. 1988); thereby complex polygenes can be accumulated; an advantage commonly associated with induced amphiploids or spontaneously doubled progeny.

Under certain conditions, individual alien chromosomes may be preferentially substituted for wheat chromosomes. Such chromosomes may compensate adequately for the wheat chromosome that is being replaced. Chromosome F of *L. racemosus* replaced 6B6B of wheat (Mujeeb-Kazi et al. 1983) and we later observed that chromosome M of *L. racemosus*

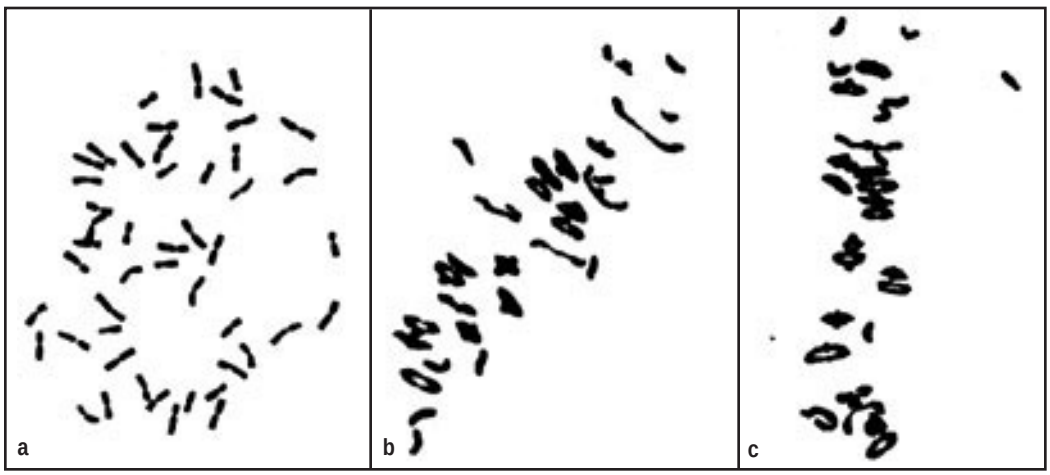


Figure 4.3. A hypoploid backcross II derivative from *Triticum aestivum/Thinopyrum scirpeum//2* T. aestivum* showing in: a) 48 somatic chromosomes, b) 7 univalents + 4 rod bivalents + 15 ring bivalents + 1 trivalent at metaphase I, and c) 8 univalents + 3 rod bivalents + 17 ring bivalents at metaphase I.

replaced chromosome 6D6D of wheat (**Figure 4.4**). We are critically analyzing these replacements using ditelocentric wheat stocks of chromosomes 6DS or 6DL. In another derivative, chromosome M disomically replaced 6A6A—this was verified by electrophoretic analysis (unpublished data).

Upon further backcrossing, the preferentially substituted chromosomes enable the development of two univalents in the derivative progeny (M+6A or M+6D) that could, via centric break and fusion, form new derivatives through translocation of the alien chromosomes to wheat. It was presumably such a univalent misdivision and its selfing that formed the natural homozygous 1BL/1RS translocation in *T. aestivum* varieties that has proven to be so valuable (Merker 1982, Mujeeb-Kazi 1982, Rajaram et al. 1983, Jahan et al. 1990).

Variations in amphiploid maintenance

There are not too many examples of fertile amphiploids in diverse wide crosses presumably because few researchers have an interest in pursuing their production. Also, there are some genetic constraints despite the availability of an efficient technique. Fertile amphiploids have been obtained in the following diverse crosses: *T. aestivum*/*Th. bessarabicum* (2x) (Forster and Miller 1985, Kimber and Sallee 1980), *T. aestivum*/*Hordeum chilense* (Chapman and

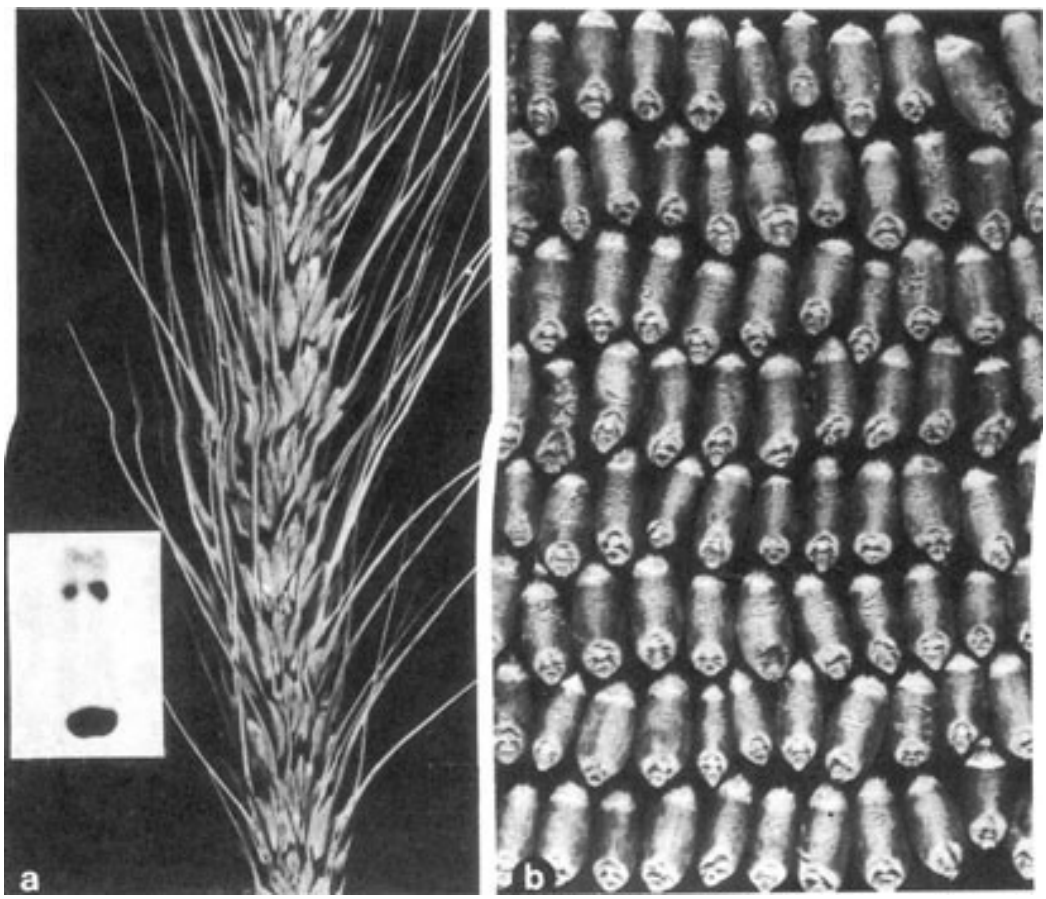


Figure 4.4. a) Spike showing a C-banded chromosome M of *Leymus racemosus* (inset) that spontaneously substitutes for chromosome 6A6A or 6D6D of *T. aestivum* and b) well developed grains produced as a result.

Miller 1978), *H. bogdanii*/*T. timopheevii* (Kimber and Sallee 1976), *T. aestivum*/*Thinopyrum distichum* (Pienaar 1980), *H. pubiflorum*/*Secale africanum* (Fedak 1985), and *H. californicum*/*T. aestivum* (Fedak, unpublished). Although numerous efforts failed to produce fertile amphiploids from the *T. aestivum*/*H. vulgare* cross (Fedak 1977b; Islam et al. 1975, 1978, 1981; Mujeeb-Kazi and Rodríguez 1983b), success was finally achieved (Molnar-Lang and Sutka 1993). Mujeeb-Kazi et al. (1987) successfully produced fertile amphiploids from the *T. aestivum*/*Th. ovchingeri* cross and earlier from *T. aestivum*/*Th. junceum* cross (Mujeeb-Kazi and Bernard 1985a). These amphiploids usually possessed 70 and 84 chromosomes, respectively, but in both amphiploids, aneuploidy was observed. Five amphiploid plants derived from the *T. aestivum*/*Th. junceum* cross had from 72 to 84 chromosomes. Despite a high degree of asynapsis (up to six univalents) in the *H. californicum*/*T. aestivum*-derived amphiploids, progenies studied so far have had the expected chromosome number of 2n=8x=56 (Fedak, unpublished). Recently, several primary triticale hexaploids have been produced that express a high degree of normal chromosome counts over three generations of selfing (unpublished data) and, contrastingly, we have reported several amphiploids derived from intergeneric crosses that show variable extents of aneuploidy (Mujeeb-Kazi et al. 1994c).

We found a rare example of an amphiploid derived from *Elymus fibrosus*/*T. turgidum* where the alien species was the maternal parent (Mujeeb-Kazi 1994a). *E. trachycaulum*, *E. ciliare*, and *E. caninus* have also been used as the female parents in intergeneric crosses with wheat, but no amphiploids have resulted after colchicine treatment (Sharma and Gill 1981, 1983a,b,c; Mujeeb-Kazi and Bernard 1985a; Sharma and Baenzinger 1986). The same is true for hybrids of *Hordeum vulgare*/*T. turgidum* and *H. vulgare*/*T. aestivum*

In essence, each hybrid combination must be treated individually and generalities should be avoided as illustrated above. The aneuploidy referred to above is for limited individual chromosomes (**Figures 4.5a-d**), but complete genome(s) may be lost in the advance to amphiploids. There are several examples in the range grasses (Dewey 1980) where 10- to 12-ploid amphiploids have spontaneously stabilized at the octoploid level of 56 chromosomes. A *T. turgidum*/*Leymus mollis*-derived amphiploid was stabilized at 42 chromosomes (N.V. Tsitsin; information to A. Mujeeb-Kazi via D. Dewey, Logan, Utah, USA, and A. Merker, Svalov, Sweden).

Utilization

Although, as mentioned earlier, there are numerous traits to be utilized from intergeneric crosses in the areas of biotic and abiotic stresses, we are particularly enthusiastic about introgressing tolerance to various abiotic stresses and emphasize this aspect in our discussion of the utilization of intergeneric hybrids. We briefy discuss some of our successes involving biotic (disease) stress resistances in the conclusion of this chapter and in the section on the radical approach in Chapter 9.

Substantial information is available on the contribution of alien species to stress tolerance in wheat (Mujeeb-Kazi et al. 1991a, Manyowa and Miller 1991, Paull et al. 1991). Many of these attributes require situation-specific improvement strategies. We have been specifically working with the transfer of traits related to salt tolerance, copper efficiency, and aluminum tolerance.

Salt tolerance

Through a collaborative research program involving CIMMYT; the Institute of Plant Science Research, Norwich, UK; and the laboratory at Bangor, Wales, we have been able to identify several salt-tolerant alien genera and conventional sources, further substantiated by other literature reports (**Table 4.3**). This cumulative information has led to the formulation of a comprehensive list of alien variability. Targeted alien species with salt tolerance include *Thinopyrum bessarabicum* and *Th. elongatum*; both are diploids (2n=2x=14). An additional source of salt tolerance includes selected accessions of *Triticum tauschii*. Mujeeb-Kazi et al. (1987, 1989) and Mujeeb-Kazi and Asiedu (1990) document the hybrid production and the advance procedures involved. We closely follow the hydroculture salinity screening methodology for advanced derivatives adopted in the Bangor Laboratory (Gorham et al. 1987, Gorham 1990).

Tolerance in conventional germplasm— CIMMYT has established a tester set that contains wheat cultivars that are either salt-tolerant (CS, Kharchia 65, Shorawaki, and Lu26S) or salt-susceptible (Yecora 70, Oasis F86, and PBW 34). Some of these entries like CS have a long history of being classified as salt-tolerant and have been extensively studied in several laboratories. Tolerant Shorawaki has had little publicity; some cultivars (Sakha 8, SNH-9, WH-157, and Candeal) still require rigid evaluations.

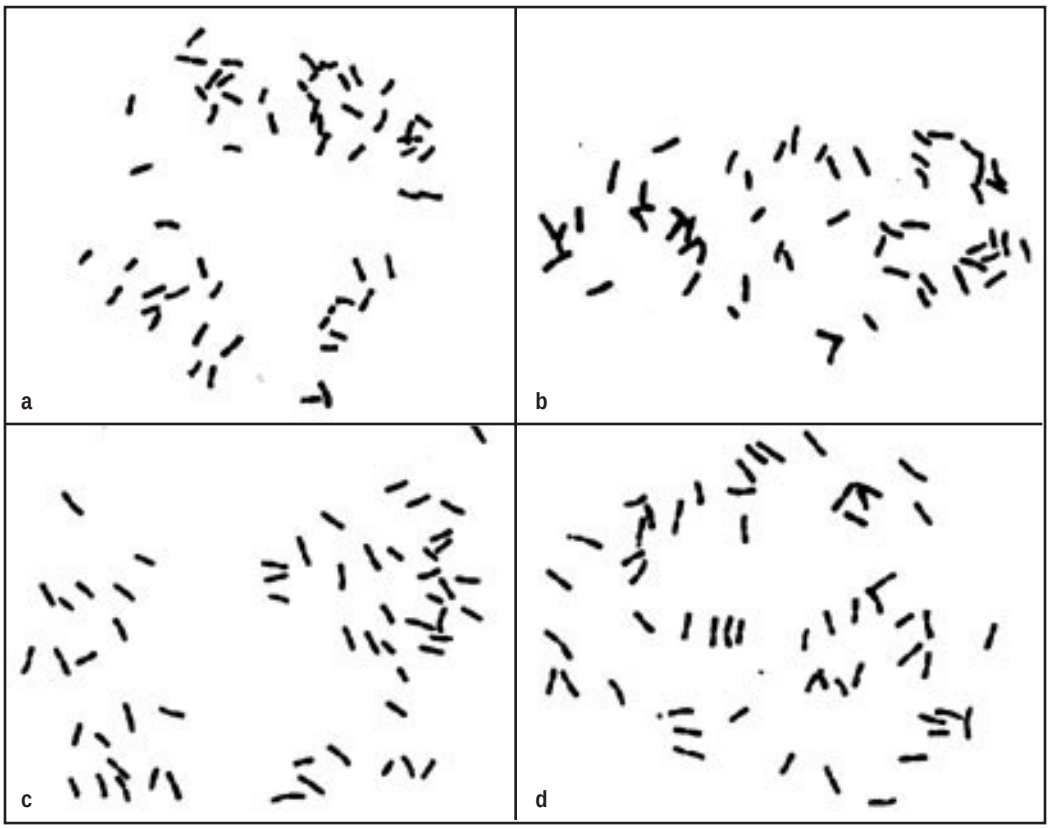


Figure 4.5. Aneuploidy in amphiploid advance of *Triticum turgidum* L. cv. Laru/*Aegilops variabilis*: a) 55 chromosomes + 1 telocentric, b) 56 chromosomes + 1 telocentric, c) 57 chromosomes, and d) 56 chromosomes. The normal chromosome number expectation of this amphiploid is 2n=8x=56.

We plan to study a new Indian release (KRL 1-4)—a derivative from a cross involving Kharchia 65, considered an elite cultivar for saline-sodic soils—reported to have superb growth and high yield even at pH 9.6 (K.N. Singh, pers. comm.).

Tolerance in alien germplasm—With such a limited source of salt tolerance in conventional germplasm, it is prudent to search for additional genetic diversity in the alien species. There has been a flurry of reports (especially over the past five years) about alien germplasm possessing salt tolerance. How close we might be in receiving benefits from use of such alien species is still an open question. However, we believe the prognosis is quite encouraging. As mentioned above, *Th. elongatum* (2n=2x=14) and *Th. bessarabicum* (2n=2x=14) are particular standouts (Gorham et al. 1985, Dvorak et al. 1988).

In hydroculture tests, we re-evaluated some salt-tolerant characteristics of these species and our observations support their potential as assessed through their amphiploids derived from crosses with *T. aestivum* cultivars (**Table 4.4**). Even though diploid alien sources are a priority, we

Table 4.3. Triticeae relatives with a promise for salinity tolerance based upon literature reports and collaborative research findings.

Germplasm designation	Origin or polyploidy	Reference
Conventional sources		
T. aestivum cultivars		
Candeal	Mexico	
Kharchia 65	India	
KRL 1-4	India	
Lu 26S	Pakistan	
Pasban-90	Pakistan	
Sakha-8	Egypt	
Shorawaki	Pakistan	
SNH-9	India	
WH-157	India	
Yecora 70, Oasis (susceptible checks)	Mexico	
Alien sources		
*Thinopyrum elongatum** (*Elytrigia elongata*)	2n=2x=14	McGuire & Dvorak (1981)
Th. scirpea (*E. scirpea*)	2n=2x=28	McGuire & Dvorak (1981)
Th. elongatum (*E. pontica*)	2n=10x=70	McGuire & Dvorak (1981)
Th. junceiforme (*E. junceiformis*)	2n=4x=28	McGuire & Dvorak (1981)
Th. distichum (*E. disticha*)	2n=4x=28	McGuire & Dvorak (1981)
*Th. bessarabicum**	2n=2x=14	Gorham et al. (1985)
Leymus racemosus (*E. giganteus*)	2n=4x=28	McGuire & Dvorak (1981)
*Aegilops squarrosa** (*Triticum tauschii*)	2n=2x=14	Gorham (1990)
Ae. umbellulata	2n=2x=14	Gorham (1990)
Ae. comosa	2n=2x=14	Gorham (1990)
Ae. mutica	2n=4x=28	Gorham (1990)
*Psathyrostachys juncea**	2n=4x=28	Dewey (1984)

* Priority source in CIMMYT's Wide Crossing Program.

recognize that, where diverse genomic distance prevails, the salt-tolerant trait may not be simply inherited. This is clear from the observations of Dvorak et al. (1988), who found three (3E, 4E, 7E) of seven disomic addition lines derived from *T. aestivum* (CS) / *Th. elongatum* to give positive salt tolerance responses. This poses several constraints for introgressing genes from these three addition lines into wheat and a further constraint in transferring from CS the tolerance into a commercial cultivar that will contribute to agricultural productivity in salt-prone areas. This will take time to accomplish. We anticipate more success in exploiting the alien cytoplasm by working with the reciprocal cross (*Th. elongatum* / *T. aestivum*). This may also facilitate obtaining derivatives in agronomically superior plant types. Because multiple alien chromosomes are involved, we will also produce multiple disomics, incorporate the maize-mediated polyhaploid system (see Chapter 5), and then attempt the alien introgression by Ph locus manipulation.

The disomic 5J addition of *Th. bessarabicum* (2n=2x=14) to CS imparts salt tolerance; its 2J addition is salt-susceptible while the amphiploid (2n=8x=56) is tolerant (Forster et al. 1987, 1988). Further verification of 5J's reported positive effect is warranted since our results have been varied (Mujeeb-Kazi et al. 1991a). We are skeptical as to whether just one alien chromosome, such as 5J, can contribute to acquiring a sufficient level of salt tolerance. So, we have proceeded to produce the complete addition set (seven chromosomes in total) in a background that is superior to CS. *Th. bessarabicum* is also believed to be a source of tolerance to copper, aluminum, and manganese toxicities (Mujeeb-Kazi et al. 1992) and the complete additive line set may contribute significantly to gaining more abiotic stress tolerance in wheat.

By backcrossing a commercial bread wheat cultivar (Genaro T 81) onto the F1 hybrid of CS / *Th. bessarabicum* and then selfing, we have selected several 44-chromosome derivatives.

Table 4.4. Dry weight (g) and Na and K cell sap values (mol/m^3) in some wheat cultivars and their alien derivatives under hydroculture at 50 mol/m^3, then at 200 mol/m^3 NaCl. Growth data measured 50 days after reaching 50 mol/m^3, Na + K measured in plants grown at 50 mol/m^3 NaCl.

Cultivar or line	Dry weight (g)	Na (mol/m^3 plant sap)	K (mol/m^3 plant sap)
Chinese Spring (CS)	4.42 ± 1.14	31 ± 3 (268 ± 21)	225 ± 5 (207 ± 4)*
CS/*Th. bessarabicum* (amphiploid)	2.30 ± 0.30	17 ± 2 (169 ± 28)	196 ± 7 (285 ± 8)*
Awnless + solid stem CS/*Th. bessarabicum*	3.42 ± 0.40	22 ± 2 (123 ± 15)	240 ± 9 (321 ± 9)*
Goshawk (GH'S')	1.85 ± 0.26	20 ± 3 (220 ± 27)	243 ± 1 (270 ± 9)*
Th. elongatum/GH'S'	5.33 ± 0.67	17 ± 2 (105 ± 20)	233 ± 7 (285 ± 17)*
CS/*Th. elongatum*	6.23 ± 0.86	18 ± 2 (99 ± 12)	263 ± 7 (290 ± 11)*

* Na and K measured in plants grown at 200 mol/m^3 NaCl.

These meiotically stable (22 bivalents) derivatives possess superior agronomic characters, are highly fertile, and—based upon morphological, cytological, and biochemical diagnostics—appear to have characteristics that are associated with each of the seven wheat homoeologous groups (**Table 4.5**). Through limited screening of some of these 44-chromosome derivatives (**Table 4.6**), we have identified a positive salt tolerance response for two lines and two multiple disomic derivatives. The latter two are a consequence of the selfing of BCI derivatives that possess 49 chromosomes (2n=7x=49; AABBDDJ). In the process of selfing,

Table 4.5. *Thinopyrum bessarabicum* (2n=2x=14, JJ) disomic addition lines identified on the basis of cytological, morphological, and biochemical markers and tentatively assigned to the seven homoeologous groups of *Triticum aestivum* L.

Tentative disomic line designation	Line characteristic		
	Cytological	Morphological	Biochemical
1J	44 (22 II)*		MDH and Glu
2J	44 (22 II)*	Tapering spike	SOD
3J	44 (22 II)*	Solid stem	EST
4J	44 (22 II)*	Blue aleurone	PGM (?)
5J	44 (22 II)*	Club-shaped spike	ß-AMY
6J	44 (22 II)*		GOT
7J	44 (22 II)*		α-AMY

* Mean relationships over 40 meiocytes estimated. Predominantly 22 bivalents observed.

Table 4.6. Salinity hydroculture screening of some promising wheat/*Thinopyrum bessarabicum* addition lines with 44 chromosomes and multiple disomics at 150 mol/m^3 NaCl measured after 50 days of full stress. Data tabulated for dry weight (g) and Na plus K from plant sap.

Amphiploid and addition lines	Dry weight (g)	Na (mol/m^3 plant sap)	K (mol/m^3 plant sap)
CS/*Th. bessarabicum* (Amphiploid)	3.65 ± 0.65	26 ± 9.0	240 ± 32
Addition lines			
91-103	1.41 ± 0.23	19 ± 6	303 ± 17
91-156	2.74 ± 0.64	49 ± 22	212 ± 17
91-164	2.07 ± 0.46	36 ± 8	264 ± 16
91-195	1.39 ± 0.27	8 ± 1	290 ± 19
Multiple disomics			
89-3171	1.77 ± 0.43	40 ± 11	231 ± 8
89-3179	2.39 ± 0.45	21 ± 6	264 ± 7
89-3186	3.16 ± 0.88	31 ± 5	218 ± 23

the alien chromosomes are paternally transmitted in a high enough frequency to obtain BCIF1 derivatives with chromosomal complements of more than one disomic alien addition (e.g., 23 bivalents). Subsequent genetic manipulation procedures using desirable single chromosome disomic additions or multiple disomic additions are underway.

The role of the CS *ph1b* genetic stock seems very crucial to acquiring complex characters like salt tolerance. Even though difficulties have been encountered in exploiting its full potential (Sharma and Gill 1986), the merits warrant additional effort. Since the report of Sharma and Gill (1986), we have advanced *ph1b* x alien species—although it has been difficult (Rosas and Mujeeb-Kazi 1990). We are looking at an alternate route involving the CS (*Ph*) x *Th. bessarabicum* hybrid (**Figure 4.6**), which we believe offers more promise. Results from the backcross to the *ph1b* source and subsequent manipulation promise to prove quite effective in handling alien transfers for salt tolerance. After establishing a methodology for genomic *in situ* hybridization with *Th. bessarabicum* (Rayburn et al. 1993), the exploitation of the *ph1b* locus should be a logical extension for its application aimed at detecting subtle wheat/alien homoeologous exchanges.

Copper uptake efficiency

It appears that copper (Cu) deficiency affects the wheat plant's reproductive stage more than its vegetative (Graham 1975). Graham (1978) studied the symptomology of copper (Cu) deficiency on triticale, wheat, and rye by evaluating the effects on grain yield of various Cu levels ranging from 0 to 4.0 mg/pot. This screening led him to identify *Secale cereale* cv. Imperial as a rye variety that can efficiently use available Cu. Chromosome 5R from *S. cereale*, disomically added to CS, was further identified as being responsive and subsequently the 5RL rye arm of this chromosome addition was positively associated with Cu use efficiency.

We obtained the 5AS/5RL translocation line from the Plant Breeding Institute in Cambridge and used it as the male parent in crosses onto some CIMMYT spring wheat cultivars for generating the 5A,5AS/5RL heterozygote F1 combinations. Subsequent backcrosses of these F1s with their respective recurrent parents, up to BCVIII followed by an eventual selfing of the best heterozygote BC derivative, should result in elite *T. aestivum* materials that are near-isogenic substitution lines with the 5AS/5RL chromosome disomic. The 5AS/5RL

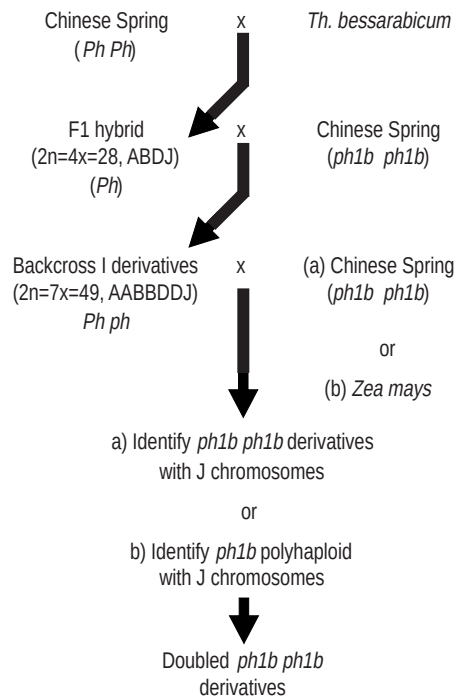


Figure 4.6. Schematic showing genetic manipulation option using the *ph1b* locus of *Triticum aestivum* cv. Chinese Spring by direct crossing or by the maize-mediated polyhaploid system.

heterozygote is identified at each BC through the morphological presence of the hairy peduncle (*hp*) marker mapped on 5RL, controlled by a dominant gene and also effective in the hemizygous stage. Differential C-banding checks on the heterozygote BC derivatives were integrated to ensure adequate accuracy in the generation advance procedures. We have delayed screening for Cu efficiency until the BC program, currently at BCIV, is completed.

Tolerance to copper toxicity

Since Cu is bound strongly to soil particles, Cu toxicity is rare, but over-fertilization with Cu in acid soils and the element's use in fungicide applications can sometimes create a toxicity problem. Although alternative remedial solutions exist, their integration with genetic tolerance does provide an advantage. Manyowa and Miller (1991) have identified *Th. bessarabicum* as a potent alien source to contribute tolerance genes. *S. cereale* is another identified source—specifically chromosome 2R. The partial availability of disomic addition lines allowed Manyowa and Miller (1991) to associate chromosomes 2J and 5J (also $2E^b$ and $5E^b$) as disomics that contribute to tolerance. The potent 5J/6J translocation ($5E^bL/6E^bL$) may be a positive contributor because of $5E^bL$. *Th. repens* has also been identified as a potential source, however, its hexaploid status may complicate its use. We are now focusing on *Th. bessarabicum*, then we will move on to *S. cereale* as a secondary source of tolerance.

Aluminum tolerance

Screening—Polle et al. (1978) and Takagi (1983) developed selection systems involving direct observation of wheat seedling roots under aluminum (Al) stress. López-Cesati et al. (1986) described a modified screening methodology employed at CIMMYT. The process, based on the fact that Al tolerance in wheat is largely a function of Al exclusion from the roots, involves:

- Immersing the roots in a nutrient solution containing 46 ppm Al;
- Staining the roots with a 0.2% aqueous hematoxylin solution;
- Observing any continued root growth; and
- Scoring on a 1-to-3 scale the corresponding Al tolerance based continued root growth.

Based upon the above screening schedule, we conducted experiments using: 1) conventional germplasm, 2) alien species with their wheat amphiploids, and 3) some *S. cereale* cultivars (**Table 4.7**). The Al test levels were 0 and 46 ppm for the conventional germplasm and alien species and 0, 46, 70, and 95 ppm for the *S. cereale* cultivars. Gustafson and Ross (1990) reported on the suppression of rye gene expression by wheat, which suggests that full expression of the rye genes may not occur. Continued root growth scores after hematoxylin staining allowed us to estimate Al tolerance at various concentration levels (Table 4.7).

Genetics and wide hybridization studies— *Genetic studies.* Earlier unpublished observations in our laboratory have provided information that Glennson M 81 is a highly susceptible cultivar at 46 ppm; CS is medium-tolerant; and Maringa and CNT-1 are highly tolerant. Al tolerance in wheat varies across cultivars. The genes controlling tolerance have been reported to: 1) range from one to the additive effect of two or more, 2) be dominant in action, and 3) be located genomically and chromosomally (see review and update by Manyowa and Miller 1991). Since a monosomic series is now available in Glennson M 81—highly susceptible to Al—we designed a monosomic study using CNT-1 as the tolerant

parent. Glennson 81 monosomic plants with 41 chromosomes were cytologically identified and crossed with CNT-1. The F1 seed obtained forms the basis of the monosomic analytical study that is currently underway.

Wide hybridization studies. With the effectiveness of the *ph1b* locus demonstrated in CS (Sears 1977), we have incorporated CS as the female parent in crosses with *S. cereale* cultivars and *Aegilops variabilis*, which has, in addition to superb resistance to Karnal bunt (see Chapter 6), excellent Al tolerance. First, we repeatedly screened *S. cereale* cultivars, and then grew tolerant seedlings for controlled seed increase. After at least near homozygosity was achieved, hybridizations were made with CS (*ph1b ph1b*). Mujeeb-Kazi and Miranda (1985) and Mujeeb-Kazi et al. (1987, 1989) describe *T. aestivum* / *Ae. variabilis* hybrid production, embryo excision, regeneration, transplanting, and cytological procedures for validation. The self-sterile F1 hybrids were advanced by crossing with wheat and obtaining BCI seed from which embryos had to be excised, presumably a consequence of the presence of the *ph* locus. Our subsequent backcrosses produced reasonably well filled seed and embryo culture was not necessary. Cytological analysis of the advanced BC derivatives is essential to facilitate selection of plants possessing 42 chromosomes and stable meiosis. However, we have not yet achieved such normal BC-selfed derivatives. Even though somatic counts are 42 or close to it, the meiotic relationships have been highly abnormal. We have also crossed *Ae. variabilis* with Pavon F 76, which has a *Ph*-dominant locus. Backcrosses to Pavon F 76 and eventual selfings have resulted in numerous plants with 44 chromosomes with bivalent meiosis. Once we complete seed increase, Al screening will hopefully help identify alien chromosome(s) associated with Al tolerance. Further incorporation, although time-consuming, will follow routine genetic manipulation procedures.

Table 4.7. Triticeae germplasm screened for aluminum tolerance under laboratory conditions in hydroculture with tolerance response (%).

Germplasm	Cultivars or Accesions	Al^{+++} concentration (ppm)			
		0	46	70	95
Conventional					
Triticum aestivum	Chinese Spring	100	24	–	–
	CNT-1	100	100	–	–
	Glennson M 81	100	0	–	–
	Maringa	100	100	–	–
	Pavon F 76	100	56	–	–
Alien					
Parents and amphiploids	Chinese Spring	100	20	–	–
	Aegilops variabilis	100	90	–	–
	T. turgidum cv. Laru	100	0	–	–
	CS/*Ae. variabilis*	100	82	–	–
	Laru/*Ae. variabilis*	100	0	–	–
Secale cereale	Short T-4776	100	100	95	65
	Sardev T-4777	100	100	100	90
	Doukala T-4778	100	100	100	95
	Turkey T-4779	100	100	100	90
	Prolific T-4781	100	100	100	90
	Blanco T-4783	100	100	95	95

Berzonsky and Kimber (1989) reported that alien species with the N genome possess Al tolerance. Although we could utilize *Aegilops* (*Triticum*) *ventricosum* (DDNN) or *T. rectum* (UUMMNN), we prefer to exploit *Ae.* (*Triticum*) *uniaristata* (NN) because of its diploid status. To the best of our knowledge, no direct hybrids exist between *Ae. uniaristata* and *T. aestivum*, but with the current successes of numerous divergent crosses like wheat x maize (Riera-Lizarazu et al. 1992), achieving this hybrid combination should be forthcoming.

Conclusions

It is now over 38 years since Sears (1956) introgressed the *Lr9* gene for leaf rust resistance from *Ae. umbellulata* into bread wheat by irradiation. Since then, the range and potential of the techniques available for this type of manipulation have increased dramatically. This improvement has been both in the production of wide hybrids and in the cytogenetic manipulations possible on the derivatives of the hybrids.

Most of the species of the *Triticum*/*Aegilops* group can now be easily hybridized with the cultivated wheats. Their genomic relationships are well understood (Kimber 1984b). Once desirable variation has been recognized in the wild species and its expression in a hybrid is established, then the choice of methodology for the introduction of the alien variation follows (Kimber 1984a), logically from measurements of the relative affinity of the chromosomes involved. This ability can only greatly increase the range of variation upon which plant breeders can exercise selection.

Hybrids and, in most cases, amphiploids have been produced in crosses between species of the genera *Hordeum*, *Agropyron*, *Elymus, Secale*, *Taeniatherum*, *Eremopyrum*, and *Haynaldia* and various species of the *Triticum*/*Aegilops* group as defined by Morris and Sears (1967), Sakamoto (1973), Mujeeb-Kazi (1982), Sharma and Gill (1983a,b,c). The range of new hybrids with more distantly related species is constantly increasing and it is to be expected that a greater range of genotypes will become available for introgressing novel genetic variability into wheat.

It is not possible to predict the future genetic demands that may be placed on wheat as new races of pathogens appear or as cultivation is extended into new areas. Consequently, a stock of alien genetic material introgressed from wide hybrids may prove to be of great value.

The CIMMYT program's structure and its linkage with other disciplines demonstrate what may be called a form of pre-breeding. After the complex production of hybrids, development of genetic stocks under controlled environments, and identification of derivatives with resistance and tolerance to biotic and abiotic stresses, the germplasm is transferred to CIMMYT's breeders and national program collaborators. Through this pre-breeding structure, germplasm derived from CIMMYT wide crosses has already resulted in varietal releases in Pakistan. Lines resistant to Karnal bunt and *H. sativum* have been registered as genetic stocks. During the 1993-94 cycle in Poza Rica, Mexico, 90% of the material selected by the wheat breeding program for its *H. sativum* resistance was derived from the wide crosses germplasm. This developmental scheme demonstrates how genetic resources used in a pre-breeding effort can provide novel variability for use in breeding programs. It also illustrates how our support infrastructure assists in the utilization of genetic stocks.

The practical potential of wide hybridization in the Triticeae is probably greater than in most other tribes of the Grass Family, partly because of the ease of hybridization, partly because of the clear understanding of the cytogenetical relationships, and partly because of the immense importance of wheat. Consequently, there may not be as much need to utilize techniques such as gene splicing or somatic cell fusion within the Triticeae as in other crops. Further, such techniques may have limitations in that the introgressed material may not integrate well with the wheat genotype. The introgression of genetic material from species with relatively close evolutionary ties to wheat would be expected to have the most potential. In addition, the ability to induce recombination between homoeologous chromosomes in the Triticeae would tend to place introgressed segments of the grass species in the best location within the recipient wheat chromosomes.

Chapter 5

Production of Polyhaploid Wheat Plants Using Maize and *Tripsacum*

Abdul Mujeeb-Kazi, Oscar Riera-Lizarazu,
and Manilal D.H.M. William

Several workers have successfully crossed *Triticum aestivum* L. with *Zea mays* L. (Zenkteler and Nitzsche 1984, Laurie and Bennett 1986) and *Tripsacum dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993), which has led to documented production of polyhaploid plants. There have also been successful crosses between *Z. mays* and *T. turgidum* L. as well as other *Triticum* and *Aegilops* spp. (O'Donoughue and Bennett 1988).

Successful fertilizations have also been accomplished in crosses between wheat and *Sorghum bicolor* L. Moench, sorghum (Laurie and Bennett 1988a,b); *Pennisetum glaucum* R. Br., pearl millet (Laurie 1989); *Z. mays* ssp. *mexicana*, teosinte (Ushiyama et al. 1991); *Hordeum vulgare* L., barley (Laurie and Bennett 1988c); and *Secale cereale* L., rye (Laurie et al. 1990).

Crosses between wheat and the above species (as the pollen parent) provide an alternative means of producing polyhaploid (haploid if the species is a diploid) wheat plants through the natural elimination of the pollen parent's chromosomes in the early stages of embryo development (see Chapter 4). Also, there is the possibility of exploiting the genetic variability of the diverse gene pools within these alien species for wheat improvement if, for instance, maize or *Tripsacum* chromosomes could actually be retained in a wheat background.

Over the last four years, we have been producing high frequencies of polyhaploid wheat plants in crosses using either maize or *Tripsacum* pollen. We believe that both of these polyhaploid production procedures for wheat are better than anther culture or wheat x *Hordeum bulbosum* crosses.

Polyhaploid plants are important in our efforts to reduce the number of generations it takes to fix the homozygosity of wheat and other cereal plants. A homozygous plant is obtained when a polyhaploid's chromosomes are doubled. This homozygosity is required in basic research projects such as our collaborative work with Cornell University and the International Triticeae Mapping Initiative (ITMI) to produce RFLP maps of the wheat and barley genomes.

After successful fertilization occurs in any of the above crosses, chromosomes of the male parent are eliminated very early, thus producing a polyhaploid embryo with the chromosomes of the female parent. Normally, the embryo soon aborts; however, exogenous treatment with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) promotes seed and embryo development until the embryo can be excised and plated onto a synthetic medium for continued growth and plantlet regeneration (Laurie et al. 1990).

Using this methodology, polyhaploid cereal plants have been recovered from crosses of bread wheat (*T. aestivum*) x maize (Comeau et al. 1988,

Laurie and Bennett 1988c, Suenaga and Nakajima 1989, Inagaki and Tahir 1990, Rines et al. 1990, Riera-Lizarazu and Mujeeb-Kazi 1990, Laurie and Reymondie 1991); durum wheat x maize (Riera-Lizarazu and Mujeeb-Kazi 1993); wheat x pearl millet (Ahmad and Comeau 1990); bread wheat x sorghum (Ohkawa et al. 1992); bread wheat x teosinte (Ushiyama et al. 1991); barley x maize (Furusho et al. 1991); and wheat x *Tripsacum* (Riera-Lizarazu and Mujeeb-Kazi 1993).

Until recently, polyhaploid production in the Triticeae had relied mostly on anther culture and sexual crossings with the perennial barley relative *Hordeum bulbosum* L. The occurrence of somaclonal variation, aneuploidy, and genotypic specificity (Picard 1989) are major limitations of anther culture in polyhaploid production. The homoeologous group 5 crossability loci (*Kr1*, *Kr2*, *Kr3)* are the major limiting factors of the *H. bulbosum* sexual crossings (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch and Snape 1986, 1987; Mujeeb-Kazi and Asiedu 1990). In order to avoid tissue culture-associated somaclonal variation, the sexual route to polyhaploid production seemed to be more desirable; however, we needed a substitute for the troublesome *H. bulbosum* technique. So, we have been exploring *Zea mays* L. (Laurie and Bennett 1986, 1988a,c; O'Donoughue and Bennett 1988; Laurie et al. 1990) and *Tripsacum dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993) as alternative sexual routes for polyhaploid production in the Triticeae.

Wheat x *Zea mays* Hybridization

Since maize pollen growth and fertilization activity appear to be insensitive to the *Kr* crossability alleles of wheat (Laurie and Bennett 1987), polyhaploids can be recovered across different genotypes (Suenaga and Nakajima 1989, Inagaki and Tahir 1990). This makes it superior to the *H. bulbosum* system. In addition, gametoclonal variation induced in doubled polyhaploid lines using the maize system was similar to that found in doubled polyhaploids obtained from wheat x *H. bulbosum* crosses (Laurie and Snape 1990).

The use of 2,4-D appears to be critical in promoting seed set and embryo formation in wheat x maize crosses (Laurie and Bennett 1988c, Inagaki and Tahir 1990). Techniques using 2,4-D treatment include: floret culture (Laurie and Bennett 1988c), tiller injection (Suenaga and Nakajima 1989, Inagaki and Tahir 1990), spike spraying (Rines et al. 1990), and floret treatment (Riera-Lizarazu and Mujeeb-Kazi 1990). Detached tillers (Riera-Lizarazu and Mujeeb-Kazi 1990) and detached spikelets (Laurie and Bennett 1988c) offer more flexibility because experimental material can be transferred to locations where conditions can be more easily controlled and monitored.

We first obtained a high recovery of wheat polyhaploids from crosses between the wheat cultivar 'Morocco' and CIMMYT maize population 'Pool 9A'. Subsequently, we achieved successful polyhaploid embryo production for additional *T. aestivum* and *T. turgidum* cultivars and for the *T. turgidum* x *T. tauschii* synthetic hexaploids, using a detached tiller culture method.

Plant material

We used two sets of plants that were field-grown at El Batan, CIMMYT, Mexico:

- *T. aestivum* cv. "Morocco" and *Z. mays* population "Pool 9A".
- *T. aestivum*, *T. turgidum*, *Secale cereale*, *T. turgidum*/*T. tauschii*-derived amphiploids, and *Z. mays* (bulk pollen sample from several cross-pollinating maize populations).

Crossing procedures and detached tiller culture

We hand-emasculated spikes before anthesis and covered them with glassine bags. When the stigmatic surface was receptive (three to four days after emasculation), the spikes were pollinated with fresh maize pollen. The tillers of pollinated spikes were detached 5 cm below the peduncular node and placed in a beaker with an aqueous solution of 100 mg 2,4-D/L. The basal halves of detached tillers were then surface-sterilized in a 20% (v/v) chlorine bleach (5.25% sodium hypochlorite) solution for 5 min., rinsed six times in sterile deionized water, and transferred to test tubes (45 ml) containing liquid MS (Murashige and Skoog 1962) basal medium components with 100 mg 2,4-D/L without agar (Riera-Lizarazu and Mujeeb-Kazi 1990). We placed the test tubes with detached tillers in a Styrofoam box containing ice-water in the greenhouse under regimes of 25/12°C (day/night), 16-hour photoperiod, and 45 to 60% relative humidity. Detached tillers were kept in the 2,4-D medium for 48 hours and then transferred to a growth regulator-free medium for 12 days.

Embryo rescue, plant regeneration, and transplanting

For each of the three crossing techniques, we collected seeds 14 days after pollination and sterilized them in a chlorine bleach solution (20% v/v) for 15 min. Embryos were excised under a stereomicroscope (2x) in a laminar flow hood decontaminated with 75% ethanol. Excised embryos were transferred to vials containing half strength MS basal medium supplemented with 20 g sucrose/L, 0.4 mg indole-3-acetic acid (IAA)/L, 0.1 mg 6-benzylaminopurine (BAP)/L, and 2 g Gelrite (Scott Laboratories, Inc., West Warwick, RI, USA)/L. Vials with embryos were kept in the dark at room temperature for 1 to 2 weeks. After germination, we transferred the regenerated plantlets to peat pots and eventually to soil in pots kept in the greenhouse.

Cytology

Somatic chromosome analysis of all regenerated plants was conducted according to the method of Mujeeb-Kazi and Miranda (1985). For meiotic analysis, the young spikes were fixed in 6:3:1 [ethanol (99%): chloroform: glacial acetic acid] for 48 hours and stored in 70% ethanol solution in the freezer (-10°C) until needed. Anthers at metaphase I were stained in alcoholic carmine (Snow 1963), then processed according to the modified procedure of Mujeeb-Kazi et al. (1994a) for high contrast, intense staining, and reduced stickiness. Mean metaphase I pairing associations were calculated from 25 meiocytes for some bread wheat and synthetic hexaploid polyhaploids.

Colchicine treatment

We treated cytologically identified polyhaploid plants with colchicine (Mujeeb-Kazi et al. 1987) in order to induce chromosome doubling. We presumed successful doubling had occurred if we observed seed set.

Protein separation

Some female wheat parents and their doubled polyhaploid progenies were analyzed by studying the banding profiles of their seed storage proteins (glutenin) and isozymes (Esterase, E.C. 3.1; and ß-Amylase, α-1,4-glucan maltohydrolase E.C. 3.2.1.2). The endosperm halves of mature kernels were used to analyze protein separation and the isozymes.

The high molecular weight glutenin subunits were separated by using a slight modification of the SDS-Polyacrylamide gel electrophoresis procedure of Ng et al. (1988). Stacking gels of 2 cm and 10% separation gels of 15.5 cm were

used. Thickness and width of the gels were 0.15 and 16 cm, respectively. Each gel was run at 20 mA constant current for 1 hour followed by 30 mA constant current for 4 hours on a Bio-rad protean II electrophoresis unit. The temperature was maintained at 15ºC during electrophoresis. Esterase and ß-Amylase isozymes were separated by isoelectric focusing using precast Pharmacia PAG plates with pH gradients of 3.5-9.5 for Esterase and 4-6.5 for ß-Amylase. The running conditions and the staining protocols were similar to those of William and Mujeeb-Kazi (1992).

Morocco x *Z. mays* pool 9A

Table 5.1 summarizes the data for the number of florets pollinated, embryos rescued, and plants regenerated for three 2,4-D treatment techniques: detached tiller culture, tiller injection, and floret spray (Riera-Lizarazu and Mujeeb-Kazi 1990). Plant regeneration frequencies, as a percentage of embryos excised, did not significantly differ from embryos originating from spikes receiving different treatment procedures. Plant regeneration frequencies averaged 75% across the three techniques. **Table 5.2** summarizes the percent embryo recovery for the different treatments and individual spikes per treatment. Embryo recovery of wheat polyhaploids ranged from 0 to 10.0%, 0 to 30.0%, and 13.63 to 41.67% for the floret spray, tiller injection, and detached tiller culture techniques, respectively. Embryo recovery using the detached tiller method was significantly higher ($x=28.70\%$, $p<0.05$) than from crosses that received 2,4-D tiller injections ($x=12.83\%$). In turn, tillers treated with an injection of 2,4-D in the uppermost internode had significantly higher ($p<0.05$) embryo recovery frequencies than plants that received 2,4-D sprays made 24 hours prior to maize pollination ($x=2.83\%$).

Cytological analysis confirmed that the wheat plants possessed the expected polyhaploid complement of $n=3x=21$ chromosomes. Chromosomes 1B and 6B were consistently identified because of their secondary constriction; occasionally, a 5D chromosome with its secondary constriction was also identified.

Table 5.1. Number of florets pollinated, embryos rescued, and wheat polyhaploid plants regenerated from wheat x maize crosses using three 2,4-D treatment techniques.

2,4-D Treatments								
Floret spray			Tiller injection			Detached tillers		
Florets pollinated	Embryos rescued	Plants regenerated (%)	Florets pollinated	Embryos rescued	Plants regenerated (%)	Florets pollinated	Embryos rescued	Plants regenerated (%)
24	1	0	24	3	67	24	8	100
24	0	-	24	0	-	24	6	100
20	0	-	18	0	-	22	7	100
20	1	100	22	5	23	21	5	80
20	2	100	20	4	75	20	6	50
20	0	-	20	6	83	22	3	100
20	1	0	22	4	50	20	3	100
20	0	-	22	5	80	22	7	29
24	0	-	22	0	-	22	9	78
24	1	100	20	2	100	24	10	90
-	-	-	20	1	100	-	-	-
Total:								
216	**6**	**67**	**234**	**30**	**77**	**221**	**64**	**81**

Laurie and Bennett (1988c) reported that embryos in caryopses, allowed to develop on the plants without growth regulator treatments, had poor viability, whereas spikelets cultured in solid MS medium with 2,4-D two days after pollination resulted in increased embryo recovery from 0.17 to 26.5%. Suenaga and Nakajima (1989) reported equal to better embryo recovery frequencies (18.0 to 31.9%) by injecting the uppermost stem internode with 100 mg 2,4-D/L. Exogenous treatments with 2,4-D appear to enhance embryo viability, although the mechanisms are not clear.

In our study, embryo recovery was unexpectedly low when we applied 2,4-D in the field with the tiller injection and floret spraying methods. Recovery was consistently high when we applied 2,4-D in the greenhouse with the detached tiller method (Table 5.1). The field environment was exceedingly wet and cold during the experiment, which might have negatively affected cross fertilization and seed development in the injected or sprayed spikes. Spraying of the florets was particularly ineffective, probably due to the exposure of unfertilized ovaries to 2,4-D 24 hours prior to pollination. Effective embryo recovery has been reported when 2,4-D spray applications were made 24 hours post pollination (Rines et al. 1990). Also, reasonably good frequencies of embryos were recovered (0.6 to 26.8%) when the spray procedure was used 24 hours post-pollination in wheat x *Tripsacum* crosses described later.

Table 5.2. Wheat polyhaploid embryo recovery frequencies (%) from *Triticum aestivum* L. x *Zea mays* L. crosses under three 2,4-D treatment techniques.

	2,4-D Treatments		
	Floret spray	Tiller injection	Detached tillers
	4.17	12.50	33.33
	0.00	0.00	25.00
	0.00	22.73	23.81
	5.00	20.00	31.82
	10.00	30.00	41.67
	0.00	0.00	31.82
	5.00	18.18	30.00
	0.00	22.73	13.64
	0.00	0.00	15.00
	4.17	10.00	40.91
		5.00	
X[a]	2.83c	12.83b	28.70a
Range	(0-10.00)	(0-30.00)	(13.6-41.67)
S_x[b]	1.07	3.21	3.00

[a] Column means followed by the same letter are not significantly different ($p<0.05$).

[b] Standard error of the mean for treatment averages.

Suenaga and Nakajima (1989) also observed a reduction in embryo recovery when tillers were injected one to two days before pollination. They speculated that 2,4-D treatments prior to pollination induced morphological and physiological changes in unfertilized florets that were detrimental to cross fertilization (see Marshall et al. 1983). On the other hand, 2,4-D treatments prior to pollination in wide crosses have been shown to improve embryo recovery frequencies (Kruse 1974a, Riera-Lizarazu and Dewey 1988). Thus, other factors besides 2,4-D applications prior to pollination may have affected the differences in embryo recovery.

In the greenhouse, detached tillers were drier and the caryopses larger than those obtained from the field material. Translocation and seed development were probably better under the greenhouse conditions as well. In another controlled experiment, we found the detached tiller technique to be significantly better than tiller injection across several wheat genotypes (unpublished data). So, we conclude that the use of detached tillers offers the most practical and versatile alternative for wheat polyhaploid production when crossing wheat x maize. However, as we point out later in this chapter, some modifications may be in order to improve the detached tiller system, especially when crossing other *Triticum* and Triticeae species with maize.

Although plant production frequencies from recovered embryos did not dramatically vary among the different treatments (frequencies ranged from 67 to 81%), we found embryo germination could be increased with improved embryo culture procedures or by enhancing embryo development on the crossed spikes. We rescued embryos 14 days after pollination. Allowing embryos to remain on the spikes longer might be appropriate if differentiated embryos are desired. Although polyhaploid frequency using detached tillers averaged about 23% (average embryo recovery frequency of 28.70% x average plant regeneration frequency of 81%), it could potentially be as high as 42% if we consistently obtained 100% differentiation and high embryo recovery.

In summary, the embryo excision/plantlet regeneration/polyhaploid production frequencies (all percentages) obtained so far are:

- 28.7/81.3/23.3 with detached tillers;
- 12.8/76.6/9.8 with tiller injection.

The recent success rates of two other laboratories not using the detached tiller procedure have been:

- 25.1/83.6/20.9 (Suenaga and Nakajima 1989);
- 21.7/43.7/9.5 (Inagaki and Tahir 1990).

Triticeae species x diverse pollen mixtures of *Z. mays*

As reported by Inagaki and Tahir (1990) and Laurie and Reymondie (1991), we have also recovered polyhaploid embryos using an assortment of wheat genotypes (**Table 5.3**). In addition, our results suggest that using detached tillers in the maize system (as described above) can be extended to recover polyhaploids in durum wheats and *T. turgidum* x *T. tauschii* derived amphiploids (Table 5.3).

In this study, we obtained a wide range of embryo recovery frequencies among hexaploid wheats, tetraploid wheats, and the synthetic hexaploids, averaging 15.6, 16.9, and 19.8%, respectively (Table 5.3). We recovered no embryos from *S. cereale* x maize crosses although Laurie et al. (1990) have reported embryo initiation in such crosses. Mean plant regeneration frequencies for bread wheats, durum wheats, and the synthetic hexaploids were 68.5, 73.9, and 74.5%, respectively. Successful chromosome doubling (**Figure 5.1**) with colchicine averaged 60.7% for *T. aestivum* cultivars, 69.5% for *T. turgidum* cultivars, and 63.6% for the synthetic hexaploids (Table 5.3).

Production frequencies of 1 to 4% have been considered to be acceptable for the economic production of polyhaploids (Comeau et al. 1988). In our study, the average doubled polyhaploid recovery for *T. aestivum*, *T. turgidum*, and the synthetic hexaploids (based on florets pollinated) ranged from 6.5 to 9.4%, with average embryo recovery frequencies of 14.7 to 19.4%, mean plant regeneration frequencies of 68.5 to 74.5%, and successful doubling frequencies of 60.7 to 69.5% (Table 5.3). Although the polyhaploid plant frequencies we obtained for wheat in this study more than adequately meet economic threshold levels, Suenaga and Nakajima (1989), Inagaki and Tahir (1990), and Riera-Lizarazu and Mujeeb-Kazi (1990) have reported higher frequencies across genotypes.

We attribute our lower recovery—compared to earlier results of Riera-Lizarazu and Mujeeb-Kazi (1990)—to continuous rainfall during tiller collection in the field. This led to a lack of complete tiller sterilization, which resulted in progressive decay of the spike culm base in the culture medium, in turn affecting normal nutrient translocation and seed development.

For such situations in the future, it may be best to use intact spikes (Suenaga and Nakajima 1989) or to modify the detached tiller process.

The detached tiller system was specifically designed to study nutrient translocation and seed development physiology in wheat spikes (Jenner 1970, Donovan and Lee 1977, Singh and Jenner 1983), hence modifications may be needed before we can apply the procedure to other *Triticum* and Triticeae species. One modification involves the use of sulfurous acid to suppress contamination in the culture solution and culm decay as reported by Kato et al. (1990). Also, we can avoid humid/wet environments by making crosses in environmentally controlled greenhouses.

Table 5.3. Embryos produced, recovery percentage, plant regeneration, and colchicine–induced doubling frequencies of *Triticum aestivum* L., *T. turgidum* L., and *T. turgidum* x *T. tauschii* lines following crosses with *Zea mays*.

Cultivars and lines	Embryos produced	Percent embryo recovery	Plants regenerated	Plants doubled
***Triticum aestivum*[a]**				
AgaA/6*Yecora 70	32	16.4	28	19
Alondra'S'/Pavon F 76'S'	44	19.6	34	21
Bagula'S'	55	21.2	38	25
Bobwhite'S'/Pavon F 76'S'	48	25.3	30	20
F12.71/Coc//GenN	17	11.7	9	4
Fukuhokomugi	35	16.7	29	17
Genaro T 81	26	14.1	22	14
Glennson M 81	16	9.6	10	6
Gov/Az//Mus'S'	16	11.2	10	7
Kauz'S'	40	18.6	27	15
Mirlo'S'/Buckbuck'S'	31	22.3	19	7
Opata M 85	16	8.8	9	4
Papago'S'	11	6.5	8	4
Pavon F 76'S'/Buckbuck'S'	4	6.7	1	1
Seri M 82	22	15.8	13	9
Tesia 79	7	7.4	6	4
Thornbird'S'/Kea'S'	22	18.3	10	7
Total/Average	**442**	**14.7**	**303**	**184**
Percentage				**60.7**
***Triticum turgidum*[a]**				
Cando/Ente//Arlequin	27	18.0	22	17
Altar 84	44	15.4	37	27
Laru'S'	18	11.8	13	6
Crocethia_1'S'	21	19.4	12	9
Arlequin	32	22.2	21	14
Total/Average	**142**	**17.4**	**105**	**73**
Percentage				**69.5**
***T. turgidum x T. tauschii* lines[b]**				
Duergand_2/*T. tauschii*	23	16.4	19	11
Ruff/*T. tauschii*	43	24.2	35	27
Yarmouk/*T. tauschii*	20	17.4	13	6
Gan'S'/*T. tauschii*	20	22.5	12	7
Decoy1/*T. tauschii*	12	16.4	9	5
Total/Average	**118**	**19.4**	**88**	**56**
Percentage				**63.6**

[a] *Triticum aestivum* cv. "Fukuhokomugi" was obtained from G. Fedak, Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. All other cultivars are part of CIMMYT's breeding germplasm.

[b] Obtained from CIMMYT's Wheat Wide Crosses Section.

In another example, we observed severely reduced tiller viability when detached tillers of *Secale cereale* cv. "Prolific" and "Sardev" were used. No embryos were recovered in this rye x maize combination. It appears that, in this particular case, an *in vivo* approach may hold more promise. If the detached tiller method prevails, then the constitution of the nutrient solution and the place of tiller detachment plus the constraints expressed earlier will have to be addressed.

In this study, seeds produced from crosses between the Triticeae species and maize lacked normal endosperm. In addition, the embryos were found floating in a watery solution inside the seeds. Generally, any embryo recovered from seed lacking normal solid endosperm is a polyhaploid. This could serve as a morphological diagnostic tool for screening selfed versus cross-pollinated products.

Cytological analysis of plants recovered from wheat x maize crosses showed them to possess the expected polyhaploid complement of n=3x=21 chromosomes for *T. aestivum* (**Figure 5.2b**) and n=2x=14 chromosomes for *T. turgidum* (**Figure 5.3b**), where each wheat parent had the euploid number of 2n=6x=42 (**Figure 5.2a**) or 2n=4x=28 (**Figure 5.3a**), respectively. Two

Figure 5.1. Spikes of wheat polyhaploids (n=3x=21, ABD): a) side and frontal views of a sterile polyhaploid (n=3x=21) spike without seeds; b) side and frontal views of a fertile spike as a consequence of colchicine treatment of a n=3x=21 sterile polyhaploid derived from wheat x maize hybridization.

T. aestivum polyhaploids were aneuploids with 20 chromosomes (**Figure 5.3c**) of which one died at the seedling stage. Another anomaly was a *T. aestivum* polyhaploid that possessed 21 chromosomes (**Figure 5.3d**) including a telocentric.

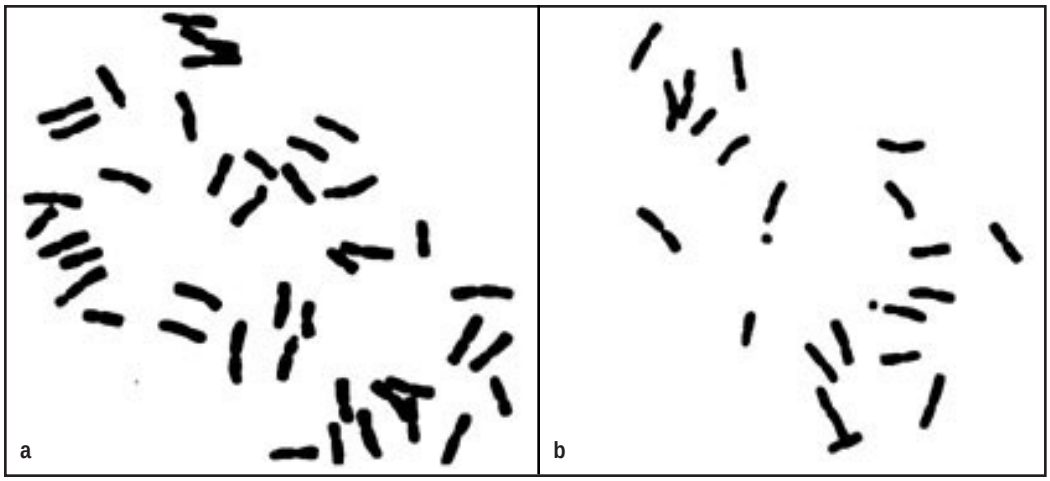


Figure 5.2. a) mitotic metaphase spread of *Triticum aestivum* L. (2n=6x=42, AABBDD); b) mitotic spread of a *T. aestivum* L. polyhaploid (n=3x=21, ABD).

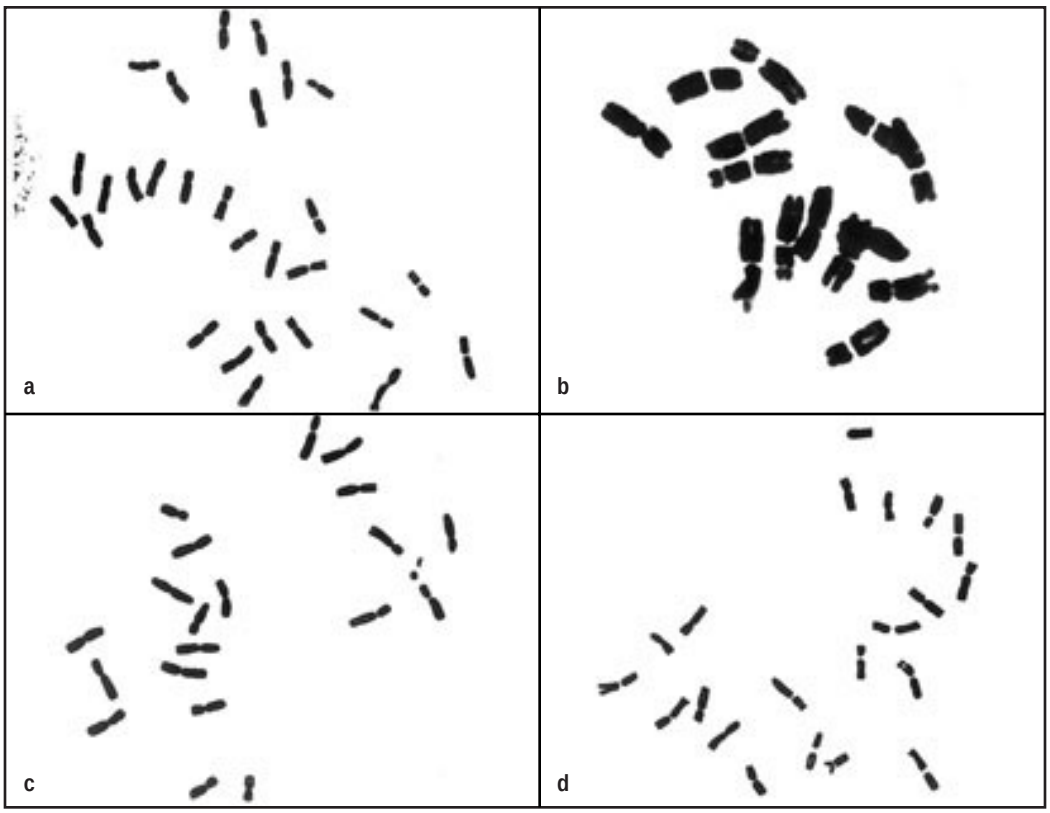


Figure 5.3. a) mitotic spread of *Triticum turgidum* cv."Altar 84" (2n=4x=28, AABB); b) mitotic spread of a *T. turgidum* cv. "Altar 84" polyhaploid (n=2x=14, AB); c) a polyhaploid of *Triticum aestivum* with 20 chromosomes; d) a *Triticum aestivum* polyhaploid with 21 chromosomes including a telocentric (t).

Polyhaploids of *T. aestivum* cultivars and the synthetic hexaploids showed very low A, B, and D genome association, i.e., allosyndetic pairing (**Table 5.4** and **Figure 5.4**). Ring bivalents were rare; the chiasmata ranged from 0.44 to 1.72/ meiocyte (Table 5.4). Riley and Chapman (1958) reported chromosome associations of wheat polyhaploids (n=3x=21) to be 18.05 univalents + 1.38 bivalents + 0.07 trivalents. Subsequently, Kimber and Riley (1963) reported a mean frequency for bread wheat of 19.18 univalents + 0.90 bivalents + 0.008 trivalents from analyses of eight euhaploids—mean chromosome pairing values indicating very low allosyndetic pairing. These chromosome pairing relationships are consistent with our data where the *T. aestivum* polyhaploids of several cultivars gave a mean metaphase I chromosome association frequency of 18.6 univalents + 0.01 ring bivalents + 1.24 rod bivalents + 0.06 trivalents (extracted from Table 5.4). Values for the synthetic (*T. turgidum* x *T. tauschii*) polyhaploids were 20.1 univalents + 0.44 bivalents. This low pairing occurred because the wheat cultivars and the synthetic hexaploids used had the dominant *Ph* locus (one that remains intact over the polyhaploid induction process), which restricts homoeologous pairing.

Genes for high molecular weight glutenins have been located on the long arms of homoeologous group 1 chromosomes (Payne and Lawrence 1983); grain Esterase genes are on the long arms of homoeologous group 3 chromosomes (Ainsworth et al. 1984); and those for ß-Amylase are on group 4 and 5 chromosomes (Ainsworth et al. 1983). Extensive allelic variations have also been reported for all three systems. **Figures 5.5-5.7** show banding profiles of high molecular weight glutenins, isozymes of seed esterase, and isozymes of ß-Amylase. We observed extensive variations in the banding profiles for all the above three systems among different cultivar families—probably as a consequence of allelic variation. Parental banding profiles of HMW glutenin and esterase were identical to those present in the doubled polyhaploid progenies. For ß-Amylase, there were some minor differences in the banding profiles within some families (Figure 5.7). This isozyme variation may

Table 5.4. Mean chromosome pairing with ranges in parentheses at metaphase I in some polyhaploids of *Triticum aestivum* L. and *T. turgidum* x *T. tauschii* synthetic hexaploids.

Polyhaploid cultivars	Metaphase I chromosomal associations (25 meiocytes)							
		Bivalents (II)			Trivalents (III)			Chiasmata per meiocyte
	I	Rings	Rods	Total II	Chain	Pan	Total III	
Fukuhokomugi	**18.8** (15-21)	**0**	**1.12** (0-3)	**1.12**	**0**	**0**	**0**	**1.12**
Bagula'S'	**18.4** (16-21)	**0.08** (0-1)	**0.96** (0-2)	**1.04** (0-2)	**0.08** (0-1)	**0.08** (0-1)	**0.16** (0-1)	**1.52**
Bobwhite'S'/Pavon F 76'S'	**17.7** (15-21)	**0.04** (0-1)	**1.48** (0-3)	**1.52** (0-3)	**0.08** (0-1)	**0** (0-1)	**0.08**	**1.72**
Genaro T 81	**19.0** (17-21)	**0**	**1** (0-2)	**1** (0-2)	**0**	**0**	**0**	**1**
Kauz'S'	**18.7** (16-21)	**0**	**0.96** (0-2)	**0.96** (0-2)	**0.12** (0-1)	**0** (0-1)	**0.12**	**1.2**
Mirlo'S'/Buckbuck'S'	**19.0** (17-21)	**0.04** (0-1)	**0.96** (0-2)	**1** (0-2)	**0**	**0**	**0**	**1.04**
Duergand_2/*T. tauschii*	**20.1** (17-21)	**0**	**0.44** (0-2)	**0.44** (0-2)	**0**	**0**	**0**	**0.44**

be partially attributed to post translational modifications (Ainsworth et al. 1983), whereas some of the band intensity differences may also be accounted for by variation in endosperm protein concentration. The close similarity in the banding profiles of the doubled polyhaploid progenies and their parents suggests stable transmission of genetic information by this procedure. It also indicates that the parental genetic information for the evaluated enzyme systems is fixed in the doubled polyhaploid progeny without alteration.

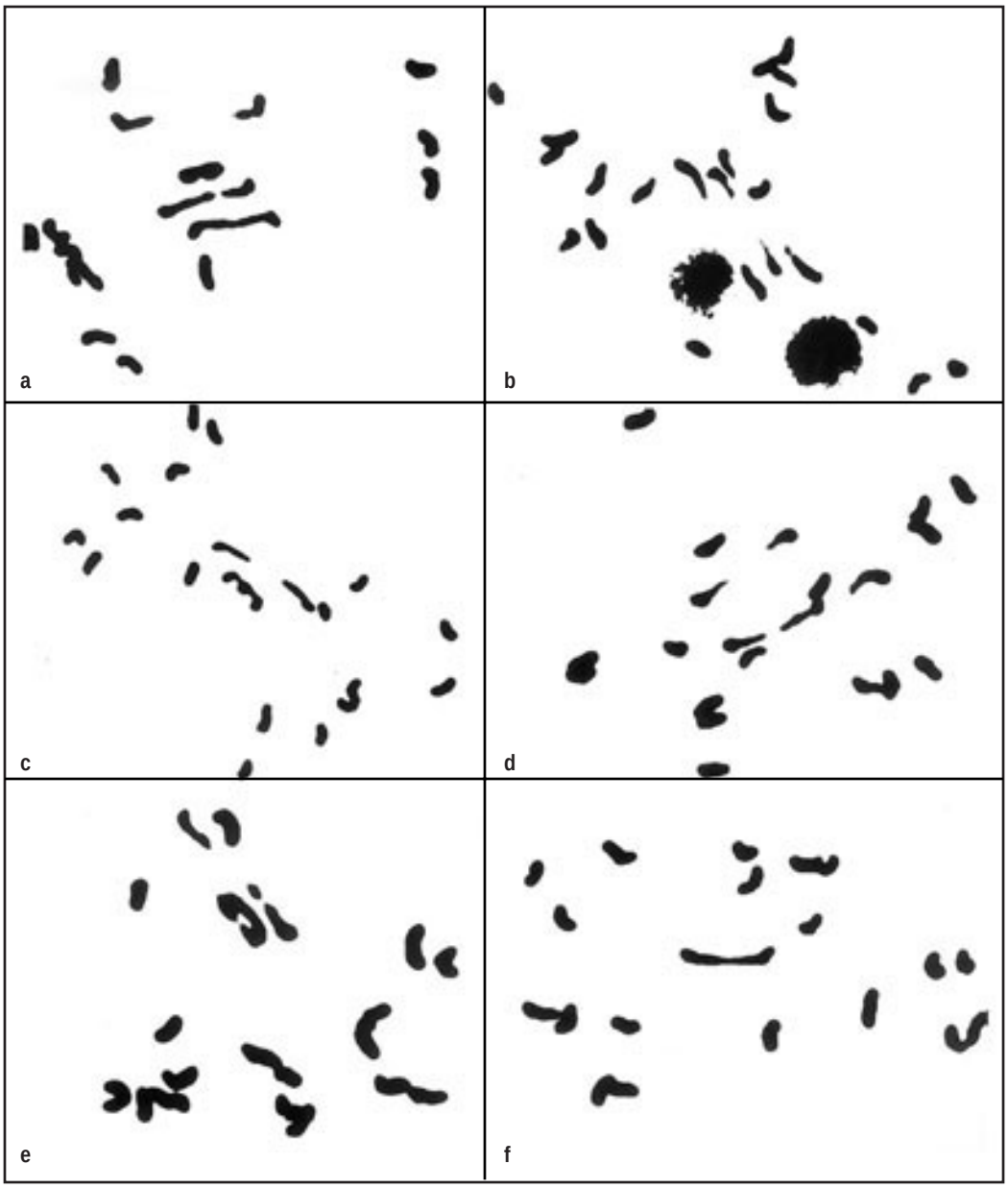


Figure 5.4. Polyhaploid chromosome configurations of *Triticum aestivum* L. at Metaphase I of meiosis showing variable univalents and bivalents as in: a) 13 univalents + 3 rod bivalents + 1 ring bivalent; b) 15 univalents + 3 rod bivalents; c) 17 univalents + 2 rod bivalents; d) 15 univalents + 3 rod bivalents (1 separated); e) 17 univalents + 1 rod bivalent + 1 ring bivalent; and f) 19 univalents + 1 rod bivalent.

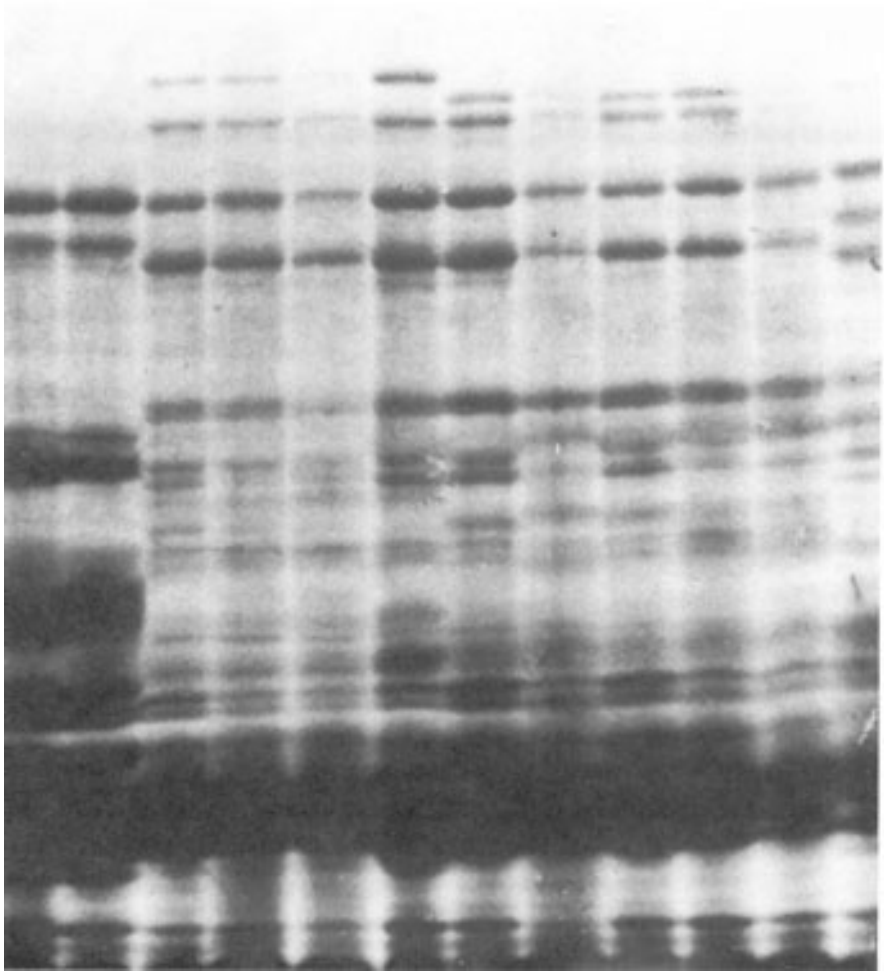


Figure 5.5. SDS-PAGE separation of seed proteins from durum and bread wheat cultivars and their extracted doubled haploids. From left to right: Altar 84 (A) and a doubled haploid (1); Seri M 82 (S) and three doubled haploids (1, 2, 3); Kauz (K) and four doubled haploids (1, 2, 3, and 4); and Chinese Spring (CS).

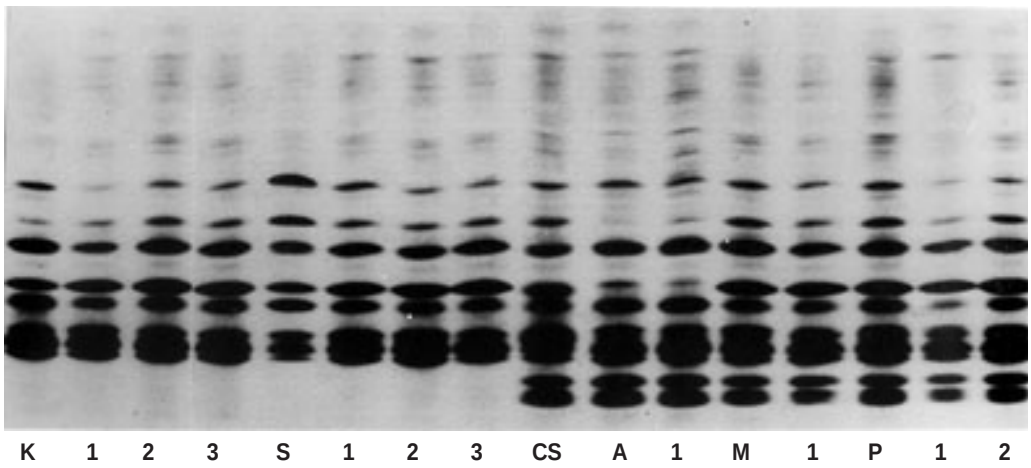


Figure 5.6. Grain esterase profiles of durum and bread wheat cultivars and their extracted doubled haploids. From left to right: Kauz'S' (K) and three doubled haploids (1, 2, and 3); Seri M 82 (S) and three doubled haploids (1, 2, and 3); Chinese Spring (CS); Altar 84 (A) and its doubled haploid (1); Mirlo'S'/Buckbuck'S' (M) and its doubled haploid (1); and Papago'S' (P) with two doubled haploids (1, 2).

Conclusions

The use of the maize system for polyhaploid production in the Triticeae is very encouraging since genotype specificity does not exist. Reaching homozygosity in earlier generations will certainly accelerate work in cereal breeding programs. Despite the current, presumably site-specific, contamination problem we encountered with the detached tiller method, the potential for its application in polyhaploid production research in cereals looks promising. Laurie and Reymondie (1991) corroborate this contention where high frequency polyhaploid production has been reported in spring and winter wheat x maize crosses. More durum wheat and rye genotypes need to be tested to further evaluate the detached tiller method.

Wheat x *Tripsacum dactyloides* Hybridization

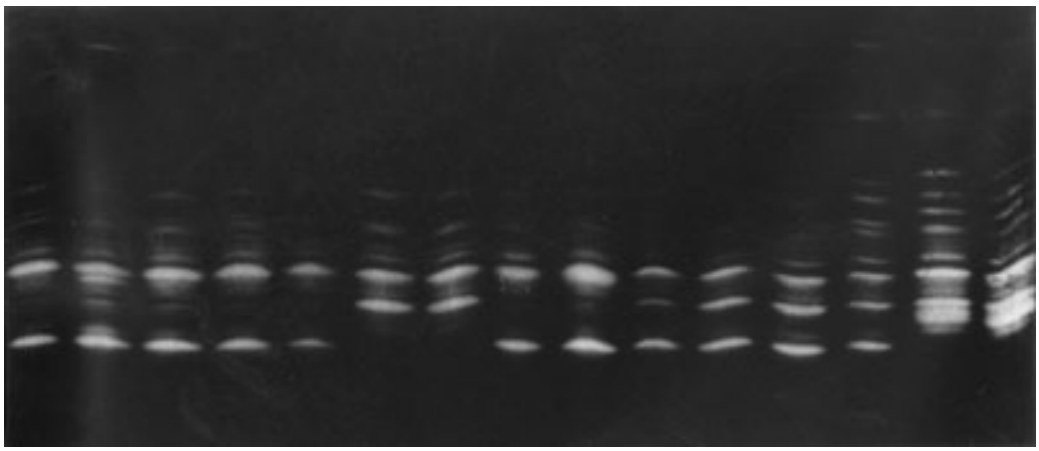
The taxonomic proximity of eastern gamagrass (*Tripsacum dactyloides* L.) to maize (Doebley 1983) has encouraged us to evaluate cross combinations involving wheat (*T. aestivum* and *T. turgidum*) and *T. turgidum* x *T. tauschii* amphiploids with *Tripsacum* as a novel and alternate sexual route for the production of cereal polyhaploids. It may also facilitate extending the crossing cycle in Mexico by at least eight weeks.

Plant materials

Cultivars of *T. aestivum*, *T. turgidum*, and amphiploids derived from *T. turgidum*/*T. tauschii* were grown in outdoor pots at El Batan, CIMMYT, Mexico, and used as female parents in crosses with *Tripsacum dactyloides* also grown outdoors (**Table 5.5**).

Crossing, embryo rescue, plant regeneration, and transplanting procedures

Spikes were hand-emasculated before anthesis and covered with glassine bags. When the stigmatic surface was receptive (three to four days after emasculation), the spikes were pollinated with fresh *Tripsacum* pollen. One day after pollination, the emasculated floral cups were flooded with an aqueous solution of 50 mg



S 1 2 3 CS A 1 M 1 P 1 2 3 SY 1

Figure 5.7. ß-Amylase profiles of durum and bread wheat cultivars and their extracted doubled haploids. From left to right: Seri M 82 (S) and three doubled haploids (1, 2, and 3); Chinese Spring (CS); Altar 84 (A) and its doubled haploid (1); Mirlo'S'/Buckbuck'S' (M) and its doubled haploid (1); Papago'S' (P) and three doubled haploids (1, 2, and 3); and the synthetic hexaploid Ruff/*T. tauschii* (SY) and its doubled haploid (1).

2,4-D/L and 150 mg gibberellic acid (GA_3)/L. To evaluate the effect of 2,4-D on embryo recovery, crosses involving the hexaploid wheat cultivar Ciano T 79 and the tetraploid wheat cultivar Altar 84 were given three treatments:

- Some spikes did not receive 2,4-D;
- Some spikes received 2,4-D, but were not pollinated;
- Other spikes were pollinated and treated with 2,4-D (**Table 5.6**).

Embryo rescue, plantlet regeneration, and transplanting procedures were similar to those reported in the section on wheat x maize hybrids. The cytological processes for mitosis and meiosis were also identical to those earlier reported with the exception that we integrated a modified procedure (Mujeeb-Kazi et al. 1994a).

Importance of 2,4-D treatment

As mentioned earlier, Suenaga and Nakajima (1989) and Inagaki and Tahir (1990) found that 2,4-D treatment of the spikes is critical to recovering seeds and embryos from wheat x maize crosses. Our preliminary trials show that 2,4-D is also important for embryo recovery in wheat x *Tripsacum* crosses (Table 5.6). In crosses involving *T. aestivum* cv. Ciano T 79 and *T. turgidum* cv. Altar 84, we recovered embryos only from pollinated florets treated with 2,4-D. We did not recover embryos from unpollinated

Table 5.5. Embryo recovery and plant regeneration from hybridization of some synthetic hexaploids (*Triticum turgidum* x *T. tauschii*) and *T. aestivum* and *T. turgidum* cultivars with *Tripsacum dactyloides*.

Cultivar or line	Florets pollinated	Embryos recovered	Plants regenerated
Synthetic hexaploids			
T. turgidum x *T. tauschii* 1	68	10	8
T. turgidum x *T. tauschii* 2	74	7	5
T. turgidum x *T. tauschii* 3	62	11	8
T. turgidum x *T. tauschii* 4	86	18	13
T. turgidum x *T. tauschii* 5	40	16	12
T. turgidum x *T. tauschii* 6	40	11	8
T. turgidum x *T. tauschii* 7	40	18	14
T. turgidum x *T. tauschii* 8	40	15	12
Total	**450**	**106**	**80**
Percentage		**23.5**	**75.5**
***T. aestivum* cultivars**			
Glennson M 81	40	12	10
Seri M 82	40	11	9
Opata M 85	40	9	7
Bacanora T 88	40	13	9
Alondra/Pavon F 76	40	12	10
Spinebill	156	20	16
Bagula	150	27	21
Bobwhite/Pavon F 76	148	31	24
Total	**654**	**135**	**106**
Percentage		**20.6**	**78.5**
***T. turgidum* cultivars**			
Altar 84	88	19	13
Arlequin	40	12	9
Crocethia_1'S'	40	14	8
Total	**168**	**45**	**30**
Percentage		**26.8**	**66.7**

pistils after 2,4-D treatment or from pollinated florets without a 2,4-D treatment (Table 5.6). Exogenous 2,4-D treatments may be important in early stages of embryo development in wheat x *Tripsacum* crosses.

Results

In all crosses receiving 2,4-D and GA_3 treatments 24 hours after pollination, we obtained a wide range of embryo recovery frequencies. The mean frequencies were 20.6% for *T. aestivum*, 26.8% for *T. turgidum*, and 23.5% for the synthetic hexaploids (Table 5.5). There was no apparent genotype specificity, implying that *Tripsacum*, like maize and other species of the Panicoideae, is also insensitive to the *Kr* crossability alleles of wheat. A more detailed study is needed to reveal the extent of this insensitivity in different *Tripsacum* accessions because Suenaga and Nakajima (1989) observed variation among maize cultivars.

Embryo recovery frequencies were slightly low in this experiment—perhaps due to variations in technique (Comeau et al. 1992). Embryos were smaller (averaging 0.5 mm long) than those resulting from wheat x maize crosses (averaging 1 mm). In order to reduce the number of daily applications, we doubled the GA_3 concentration—to 150 mg/L (Suenaga and Nakajima 1989, Furusho et al. 1991). This doubling might have been detrimental to normal embryo development. The GA_3 variable needs further evaluation to determine whether embryo size could be improved by using a lower GA_3 concentration or by omitting it altogether. We anticipate that with normal embryo development better germination frequencies will result.

Table 5.6. Embryo recovery frequencies in crosses between *Triticum aestivum* cv. Ciano T 79 and *T. turgidum* cv. Altar 84 with *Tripsacum dactyloides* after various treatments.

Cultivars	Florets pollinated	Pollination status	2,4-D* applied	Embryos recovered
Ciano T 79	144	Yes	No	No
	148	No	Yes	No
	126	Yes	Yes	Yes
Altar 84	102	Yes	No	No
	156	Yes	Yes	Yes

* 2,4-D = 2,4-dichlorophenoxyacetic acid.

As with the wheat x maize crosses, seeds produced from wheat x *Tripsacum* lacked a normal endosperm. Embryos were lodged at the micropylar end of shriveled seeds or were floating in a watery solution (probably translocated solutes) in well-developed seeds. In spikes treated with 2,4-D after pollination, the ovary tissues were enlarged as happens in normal seed development, turgid but filled with liquid (Suenaga and Nakajima 1989, Inagaki and Tahir 1990, Riera-Lizarazu and Mujeeb-Kazi 1990). Sometimes embryos were found, other times not.

Cytological analyses showed the *T. aestivum* polyhaploids to possess 21 chromosomes (**Figure 5.8a**), the *T. turgidum* polyhaploids to possess 14 chromosomes, and polyhaploids from the synthetic hexaploids to possess 21 chromosomes. The secondary constriction site resolution readily identified the 1B and 6B chromosomes (Figure 5.8a) in all samples.

Meiotic analyses of some ABD polyhaploids (n=3x=21) demonstrated negligible allosyndetic chromosome pairing at metaphase I (**Table 5.7, Figures 5.8b-d**). Riley and Chapman (1958) and Kimber and Riley (1963) reported similar low chromosome pairing relationships—data fairly consistent with our observations (Table 5.7). We detected no chromosome abnormalities.

Plant regeneration frequencies from recovered embryos were 66.7% for durum wheats, 78.5% for bread wheats, and 75.5% for the synthetic hexaploids—similar to the earlier regeneration frequencies of 73.9, 68.5, and 74.5%, respectively, of polyhaploids from maize crosses (Riera-Lizarazu et al. 1992). In the maize studies, we found colchicine doubling ranged between 63.6 and 69.5%—an aspect we did not incorporate into the *Tripsacum* investigation.

Because of our diversified research interests in the synthetic hexaploids, we placed their polyhaploids in a glasshouse where we bagged each spike in an operational maintenance procedure. We obtained spontaneous seed set on seven *T. turgidum* cv. Ruff 'S' x *T. tauschii* polyhaploids (**Table 5.8**) and somatic analyses supported the anticipated chromosome count of 2n=6x=42, AABBDD. As mentioned earlier, each polyhaploid possessed n=3x=21 chromosomes, hence a meiotic restitution-related process seems to have produced the doubled seed progeny—an event of frequent occurrence in intergeneric and interspecific hybrids.

Conclusions

Crosses between wheat and *Tripsacum* resulted in the production of wheat polyhaploids of various genotypes. Unlike wheat anther culture or sexual

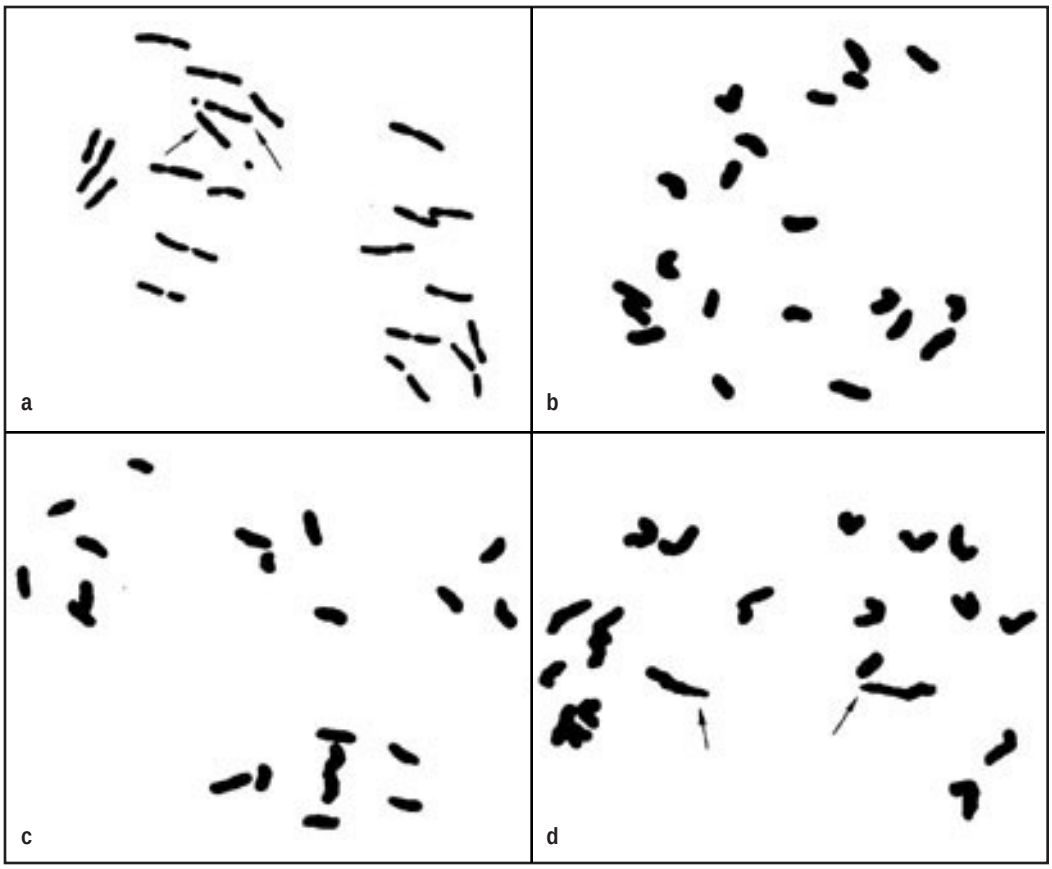


Figure 5.8. Somatic and meiotic cytology of a n=3x=21, ABD polyhaploid derived from a hexaploid wheat x *Tripsacum dactyloides* cross: a) 21 somatic chromosomes with 1B and 6B satellited chromosomes (arrows); b) a meiocyte at metaphase I with 21 univalents; c) metaphase I cell with 21 univalents; d) a metaphase I cell with 21 chromosomes associated as 19 univalents + 1 stretched rod bivalent (arrows).

hybridization of wheat with *H. bulbosum*, troublesome genotypic specificity and aneuploidy were absent. As with maize, this makes *Tripsacum*-mediated polyhaploid production a superior system for producing polyhaploids.

The merits of using *Tripsacum* instead of maize or a combination of both are worthy of consideration and further evaluation. In the field at El Batan, Mexico, *Tripsacum dactyloides* flowers six to eight weeks earlier than maize, which would allow a prolonged crossing cycle if both maize and *Tripsacum* are used as pollen donors. Regardless of which of these are used as male parents, polyhaploid production through such hybridizations will aid in accelerating progress in cereal breeding programs; other cytogenetic applications will be enhanced as well (Mujeeb-Kazi et al. 1991b). Easier production of doubled polyhaploid populations of different genotypes will facilitate genetic and genome mapping studies in cereals.

Finally, a long-term utility of *Triticum* x *Tripsacum* hybridizations is the possibility of transferring to wheat some of *Tripsacum*'s desirable traits, such as drought tolerance and insect resistance. Earlier, Laurie and Bennett (1986) theorized a similar concept for transferring the more efficient C-4 photosynthetic pathway from maize to wheat. Retention of the alien chromosomes in wheat will be a crucial step if such introgressions are ever to materialize.

Table 5.8. Spontaneous doubling in polyhaploids of *T. turgidum* cv. Ruff'S' x *T. tauschii*; spikes, seed number, and somatic chromosome counts of three seeds per doubled plant.

Polyhaploid identification	Spike number	Total seeds per plant	Somatic root tip counts	
			Polyhaploid	Doubled seed
B91-7086	6	29	n=3x=21	42
B91-7087	9	5	n=3x=21	42
B91-7088	7	7	n=3x=21	42
B91-7089	5	20	n=3x=21	42
B91-10327	8	40	n=3x=21	42
B91-10328	8	12	n=3x=21	42
B91-10329	6	20	n=3x=21	42

Table 5.7. Mean chromosome pairing with ranges in parentheses at metaphase I in some polyhaploids of *Triticum aestivum* L. and *T. turgidum* x *T. tauschii* synthetic hexaploids.

Polyhaploid n=3x=21, entries	Metaphase I Chromosomal Associations					Chiasmata per meiocyte
	I	Bivalents (II)		Total II	Trivalents	
		Rings	Rods			
Bobwhite/Pavon F 76	17.4 (15-19)	0	1.8 (1-3)	1.8	0	1.8
Opata M 85	17.6 (15-19)	0	1.7 (1-3)	1.7	0	1.8
Bacanora T 88	18.2 (17-19	0	1.4 (1-2)	1.4	0	1.4
Ruff/*T. tauschii* 1	20.8 (19-21)	0	0.1 (0-1)	0.1	0	0.1
Ruff/*T. tauschii* 2	21.0	0	0	0	0	0
Ruff/*T. tauschii* 3	21.0	0	0	0	0	0
Ruff/*T. tauschii* 4	21.0	0	0	0	0	0
Ruff/*T. tauschii* 5	21.0	0	0	0	0	0
T. aestivum [a]	18.05			1.38	0.07	-
T. aestivum [b]	19.18			0.90	0.008	-
T. aestivum [c]	8.6	0.01	1.24	1.25	0.06	-

[a] Riley and Chapman (1958). [b] Kimber and Riley (1963). [c] Riera-Lizarazu et al. (1992).

Applications of Polyhaploidy

RFLP genome mapping in wheat

F1 recombinants of inbred doubled polyhaploids can shorten the time it takes to obtain valuable homozygous lines. In the process commonly known as haplo-diploidization, a homozygous line is instantly obtained when the chromosomes of a polyhaploid plant are doubled. In wheat, where polymorphisms at the DNA level are relatively low, this system can be used to obtain polyhaploid plants from a cross that shows polymorphisms. Upon doubling the chromosomes of these polyhaploids using colchicine treatment, we can produce a population of homozygous plants that represents the variation in the initial cross. These progeny can then be used for RFLP mapping of the cereal genomes.

Since polymorphic loci in hexaploid wheat appear to be rare, RFLP linkage mapping can be achieved by using populations of wild progenitors where polymorphisms are more prevalent. Of these wheat relatives, *Triticum tauschii* accessions, which share complete homology with the D genome of hexaploid bread wheat, have been found to be highly polymorphic at the DNA level. RFLP mapping of hexaploid wheat is now feasible with the use of these synthetic hexaploids—the result of crossing *T. turgidum* (AABB) with *T. tauschii* (DD)—see Chapter 3. When the chromosomes are doubled, a reconstituted hexaploid wheat is produced (AABBDD). Our procedure is the following. First, we cross the durum cultivar Ruff with *T. tauschii* to produce a highly DNA-polymorphic synthetic hexaploid. We then cross this synthetic with hexaploid bread wheat cultivars such as Buckbuck, Opata M 85, and Ciano T 79. We cross the resulting F1 derivatives with maize to produce the polyhaploids. We then double the chromosomes of these polyhaploid plants to produce homozygous lines.

Our polyhaploid production procedure has been routinely effective, so we have not emphasized recording the number of embryos excised from pollinated florets. Typically, enough embryos can be excised to allow a regeneration frequency of between 70 and 80% and a doubling frequency of between 60 and 70% (**Table 5.9**). To date, we have produced at least 300 doubled polyhaploid plants for our collaborators at Cornell University, who are involved in the genome mapping project.

Production of alien chromosome addition lines

In wheat wide crosses, polyhaploidy can be further exploited for the production of alien chromosome addition lines from populations that have varying chromosome numbers. Preferably, plants with 22 chromosomes (21 chromosomes of wheat plus 1 alien chromosome) are recovered. The final product after colchicine treatment is a plant with 44 chromosomes (42 wheat plus an alien pair). This process not only simplifies our production of disomic addition lines, but also resolves the constraints of paternal transmission of alien

Table 5.9. Polyhaploid embryo production of three F1 DNA polymorphic crosses between *Triticum aestivum* (cvs. Buckbuck, Opata M 85, Ciano T 79) and a synthetic hexaploid (*T. turgidum*/*T. tauschii*) using the maize polyhaploid induction system. Also included are values for plants regenerated and doubled.

Characteristic observed	Buckbuck synthetic	Opata 85 synthetic	Ciano 79 synthetic
Number of embryos	245	260	207
Plants regenerated	172	180	154
Plants doubled	107	136	115

chromosomes. In addition, it reduces the analyses necessary for recovering 44-chromosome disomic derivatives following the selfing of a 43-chromosome plant containing 21 bivalents plus 1 univalent.

If a wide cross program were built exclusively around the wheat cultivar "Chinese Spring", the *Hordeum bulbosum* procedure (40 to 45% polyhaploid recovery) would be satisfactory. However, in our program where commercial wheat cultivars are used, the *H. bulbosum* technique is ineffective and we logically favor the wheat x maize methodology. We have initially applied the procedure to derivatives of *Thinopyrum elongatum* x *T. aestivum*. From 180 backcross derivatives, with somatic chromosome numbers of 43, 44, and 45, we have obtained seed set after colchicine treatment on 62 plants. Doubled haploids with 44 chromosomes have so far allowed diagnostics of four homoeologous groups through isozyme applications (**Table 5.10**). More 44-chromosome plants will be produced for completing the addition set. The wheat cultivar Goshawk'S'—involved in the hybrid, its amphiploid, and in its backcrosses—has poor crossability with *S. cereale*—indicative of a dominant crossability *Kr* locus.

Table 5.10. Disomic *Thinopyrum elongatum* additions to wheat variety Goshawk'S' developed by doubled haploidy and identified by isozyme analysis for homoeology.

Homoeologous group	Isozyme line marker	Identification numbers
1	HMW-Glu	INVO 92-6704 6721
2	EST	INVO 92-6870 6876
5	β-Amylase	INVO 92-6899 6911
7	α-Amylase	INVO 92-6840 6854

BCI-selfed derivatives

We are also applying the maize procedure to BCI-selfed derivatives of *T. aestivum* x *Th. bessarabicum* where more than one alien chromosome is present in a derivative. We anticipate this will allow us to fix multiple disomic additions. The procedure will simplify the introgression of complex genes (for traits such as salt tolerance) located on different *Th. bessarabicum* chromosomes.

Some Closing Impressions

- The potential of stored maize and *Tripsacum* pollen is being explored because it could be a significant factor in extending the use of the methodologies discussed in this chapter to countries where cropping cycles are separated or where adequate facilities are lacking for growing plants under controlled conditions.
- Simplification of genetic studies, pyramiding of simple genes (e.g., for leaf rust resistance), and applications in wide crosses to homozygosity and molecular mapping populations are just a few avenues that could be further exploited and diversified.
- Equally promising is the development of doubled polyhaploids from F1 combinations for traits like salt tolerance where the soil heterogeneity makes genetic studies almost prohibitive.
- Just as 2,4-D is unequivocally recognized as being an essential exogenous regulator in this methodology, we argue that the quality and quantity of maize or *Tripsacum* pollen are equally critical.
- Can maize and *Tripsacum* chromosomes be retained in a wheat background? If so, will any characters be expressed? Only future research will provide the answer.

Chapter 6

Applications of Tissue Culture in Wheat Wide Crosses

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Reagan Waskom, and Murray W. Nabors

The use of tissue culture technologies to grow wheat plants from somatic or polyhaploid cells has significant potential to aid plant breeders in their efforts to develop improved wheat cultivars. Callus induction from immature embryos—including its maintenance and eventual plantlet regeneration—is a way to tap into the heritable variability of *Triticum aestivum* for desired traits that are either simply inherited or under more complex polygenic control. Tissue culture applications in the Triticeae associated with embryo culture and hybrid plantlet differentiation have become routine in producing hybrids from intergeneric and interspecific crosses. X *Triticosecale* Wittmack (triticale) is a notable example.

In the 1980s, the United States Agency for International Development (USAID) provided funding that supported a tissue culture program at Colorado State University. This evolved into a larger internationally recognized effort known as the Tissue Culture for Crops Project (TCCP). In a five-year collaboration between CIMMYT and the Colorado effort, a TCCP researcher was stationed in our wheat wide crosses laboratory. The TCCP researcher in our laboratory focussed on long-term callusing and regeneration of certain wheat cultivars and their utility in facilitating alien genetic transfers from wild grasses to wheat. The collaboration terminated at the conclusion of USAID's long-term grant to the TCCP, but the impacts of the association are still being realized through the development of cytogenetic stocks and germplasm that is resistant or tolerant to *Tilletia indica* (Karnal bunt, KB), salt, and other stresses.

CIMMYT-Based Tissue Culture Activities

We have used long-term tissue (callus and embryo) culture and regeneration to demonstrate the potentials of inducing variability within various groups of the Triticeae for morphological, biochemical, and cytological characteristics.

Callus culture

Operational constraints in intergeneric hybridization are associated with alien gene introgression and obtaining hybrid plants with a doubled chromosome number (amphiploid). Callus culture has significantly helped us overcome these constraints by:

- Promoting chromosome pairing in wheat/alien species hybrids similar to the chromosome associations in wheats possessing the recessive *ph* locus on chromosome 5B.
- Inducing amphiploidy in two intergeneric hybrid combinations mediated by altered chromosome division in the regenerated plants.

The test systems we used for callus culture were *T. aestivum* or *T. turgidum* x *Aegilops variabilis*. In the following, we discuss the cytogenetical and practical implications of our observations.

Why use *Ae. variabilis*?—An *Ae. variabilis* accession (no. 13E in the CIMMYT Wheat Wide Crosses Working Collection) was reported to possess a remarkable level of resistance to KB—a quarantinable seedborne disease that can seriously restrict international movement of wheat seed. The accession showed 0% infection under the boot inoculation procedure (Warham et al. 1986). Several cytogeneticists (Sears 1977, Jewell 1983, Jewell and Mujeeb-Kazi 1982) concluded that the F1 hybrid—resulting from the cross *T. aestivum* cultivar Chinese Spring (CS) x *Ae. variabilis*—exhibits a low wheat/alien chromosome association frequency. This shows that there is a limitation in the genetic exchange between wheat and *Ae. variabilis* through normal chromosomal recombination. Conventional cytogenetic procedures provide some opportunity for homoeologous chromosome exchange by enhancing chromosomal pairing through chromosome 5B manipulation; like using the *ph* locus. As an alternative, we decided to apply the callus culture procedure in an attempt to induce random chromosomal exchanges in the crosses of wheat/*Ae. variabilis* (Vahidy et al. 1989).

Long-term callusing and regeneration—Cultivars of *T. aestivum* and *T. turgidum* (**Table 6.1**) were grown in pots in a 2:1:1 sterilized mixture of soil:sand:peat moss. We maintained the plants under greenhouse conditions of 24°C day/14°C night, 15 hours natural light, and approximately 65% relative humidity. Immature embryos excised at 15 days post-anthesis were cultured on LS medium (Linsmaeier and Skoog 1965) with 2,4-D for both callus induction and maintenance. The calli from both species were maintained until the seventh one-month passage. At each monthly passage, we regenerated some embryogenic (E) callus into

Table 6.1. Data from regenerated *Triticum aestivum* and *T. turgidum* plants from callus maintained up to seven months.

	Months in callus					
	3	4	5	6	7	
Wheat cultivars	Number of plants regenerated					**Total**
Triticum aestivum						
Mirlo'S'/Buckbuck'S'	21	33	26	6	0	86
Bobwhite'S'	1	17	12	0	0	30
Alondra'S'/Pavon F 76'S'	24	36	21	15	11	107
Goshawk'S'	30	68	33	6	7	144
Pavon F 76	0	0	3	0	0	3
Total						370
T. turgidum						
Crocethia_1'S'	0	8	0	0	0	8
Laru'S'	12	12	4	10	0	38
Duergand_2	9	0	0	0	0	9
Arlequin	22	3	0	0	0	25
Rokel'S'/Kamilario'S'	2	0	0	0	0	2
Total						82
Grand Total						**452**

plants. We placed the plants in pots and maintained them under the above greenhouse conditions. These regenerated plants were phenotypically observed, cytologically analyzed, and individually harvested to obtain R1 seed.

F1 hybrids of *Triticum* spp. x *Ae. variabilis*— Additional plantings of *T. aestivum* (including CS), *T. turgidum*, and *Ae. variabilis* (CIMMYT accession 13E) were maintained in the greenhouse. The *Triticum* spp. were crossed with *Ae. variabilis* (as the pollen parent); immature embryos were excised 15 days after pollination. We plated the embryos on MS medium (Murashige and Skoog 1962) for plantlet differentiation—these plantlets served as the cytogenetic control. We plated the remaining immature embryos on LS medium for up to 22 months with monthly transfers (or passages) of E callus/calli. At each monthly passage, a portion of the callus was regenerated into plants. The plants were transferred to greenhouse growing conditions, cytologically analyzed, and advanced by backcrossing with appropriate wheat cultivars. The procedures for hybridization, mitotic cytology, and meiotic analysis were similar to those described by Mujeeb-Kazi and Miranda (1985) and Mujeeb-Kazi et al. (1987, 1989).

Results with *Ae. variabilis*—The callus-mediated approach to introgress alien genes into *Triticum* spp. seems workable for crosses with *Ae. variabilis*. This alien accession crosses easily with *T. aestivum* and *T. turgidum*, is positive for N-banding, and has several characteristic biochemical markers. It is now unequivocally accepted as a cytogenetic standard where resulting hybrids derived from the *Ae. variabilis* cross with CS have 35 chromosomes and express a mean chromosomal association frequency of less than one open bivalent per meiocyte. Additionally, no fertile amphiploid derivatives have been obtained, whereas BCI derivatives obtained by pollinating the F1 hybrids with *T. aestivum* were highly aneuploid (Jewell 1980, 1983; Jewell and Mujeeb-Kazi 1982; Mujeeb-Kazi and Asiedu 1989) and a negligible number of normal 56-chromosome BCI derivatives were produced.

Intergeneric hybrids derived from CS and *Ae. variabilis* (13E) were produced at a high frequency. The homozygous *kr1*, *kr2*, and *kr3* genes on homoeologous group 5 promoted hybrid formation. The endosperm was well formed, but despite this all embryos were excised for direct regeneration (control) or plated in LS media for callus induction and maintenance for up to 22 months. Callus portions were being regenerated at each monthly passage (T_2 to Tn; **Figure 6.1** and **Table 6.2**). All control F1 hybrids possessed the normal 35-chromosome characteristic of the hybrid combination and had low pairing. The

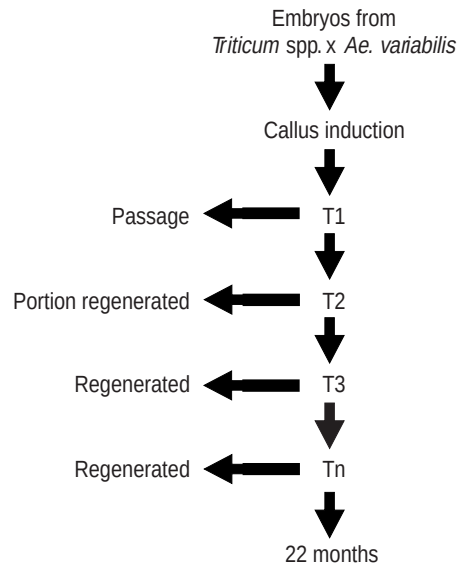

Figure 6.1. Schematic showing callus induction, transfer, and regeneration protocol from crosses of *Triticum* spp. x *Aegilops variabilis*.

regenerated F1 hybrids expressed a certain degree of aneuploidy (hyperploid or hypoploid coupled with inclusion of telocentric chromosomes), which could not be correlated with the length of time in callus. In the 35-chromosome regenerated plants, aneuploidy involving structural chromosomal changes was prevalent—and in one plant it influenced the 5B chromosome. When N-banded, both short and long arms of chromosome 5B had characteristic banding sites that were stable across different cells and had varied chromosome contraction stages (**Figure 6.2**). The regenerated plant with the 5B structural change indicates total absence of bands on the 5B short arm and serves as an ideal cytological marker for this critical chromosome where, by backcrossing and selection, it should be possible to obtain derivatives homozygous for the 5B chromosome marker. This then could be easily exploited in transfers of the CS *ph1b* mutant stock. So far, we have not obtained backcross derivatives from this 5B modified F1 hybrid.

Chromosome analysis of the regenerated plants revealed interesting variation from the standard low pairing characteristic (**Table 6.3**). Several plants had highly paired multivalent associations, which suggests that control mechanisms of chromosomal pairing have been influenced (Table 6.3)—some even up to the level of multivalency that prevails in CS *ph1b* hybrids with *Ae. variabilis* (Asiedu et al. 1989). Although the dominant *Ph* gene in chromosome 5B suppresses homoeologous chromosome pairing, there are other suppressors like those on

Table 6.2. Partial data for plants regenerated from *Triticum aestivum* x *Aegilops variabilis* callus during different passages (T).

Passages (T)	Plants regenerated
T2	54
T3	5
T4	15
T5	12
T6	64
T7	32
T8	9
T9	10
T11	12
T12	11
T13	5
T15	20
Total	249

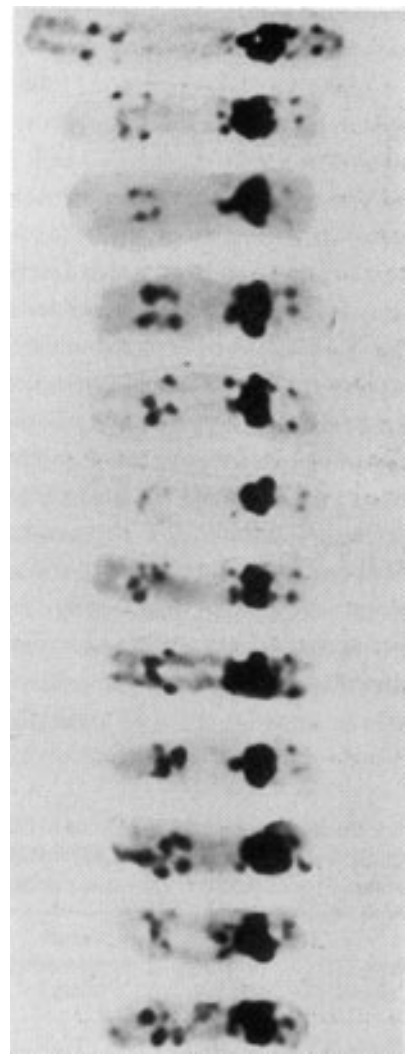

Figure 6.2. N-banded chromosome 5B from *Triticum aestivum* L. cv. Chinese Spring showing consistency of banding sites on both arms despite stage of contraction of the chromosomes and their extraction for the figure from different cells and root tips.

chromosomes 3AS, 3DS, and 4D (minor contribution) (Mello-Sampayo and Canas 1973, Driscoll 1973). Since the callus-induced influence is random, genetic changes in the suppressors could be similarly influenced—not discounting the fact that pairing promoter genes may also contribute positively to the observed meiotic associations of some regenerated plants (Table 6.3). These modifier gene(s) may also be present in the 13E accession—an aspect that we would have to critically analyze under optimum environmental conditions.

Amphiploids have significant advantages in germplasm distribution, maintenance, and cytogenetic manipulation. However, efforts over the last 30 years to produce an amphiploid from the CS x *Ae. variabilis* combination have not been successful. In our studies, this seems important to achieve because of the practical significance of *Ae. variabilis*' important traits for KB resistance and aluminum tolerance and also because the BCI progeny—produced by pollinating with wheat the F1 derived from CS x *Ae. variabilis*—was highly aneuploid (Jewell and Mujeeb-Kazi 1982). As a consequence, it was probable that all 14 alien disomic additions would not be obtained, thus decreasing our chances to transfer the alien chromosome(s), which confer KB resistance or aluminum tolerance. Jewell (1980, 1983), who used F1-based backcrossing, did manage to create an incomplete set of alien disomic chromosome addition lines. This accomplishment encouraged us to attempt:

- Obtaining an amphiploid from CS x *Ae. variabilis* with 70 chromosomes; and then
- Deriving the alien additions by making backcrosses onto the amphiploid, which may complete the set of alien disomic addition lines.

We were unsuccessful at inducing chromosome doubling via direct colchicine treatment of the F1 hybrids. However, we observed occasional seed set on a few plants in advanced growth stages of the callus-regenerated F1 hybrids of CS x *Ae. variabilis* and *T. turgidum* x *Ae. variabilis* (**Table 6.4**). Apparently, seed set is a random event. After germination, cytological analysis revealed these seeds to possess the anticipated 70 or 56 chromosomes with meiotic regularity; they were also self-fertile as evident from the C-1 plus C-2 derivatives produced (Table 6.4). We attribute the initial seed set on F1 regenerants to meiotic restitution since the plants that set seeds possessed either 35 chromosomes (*T. aestivum* x *Ae. variabilis*) or 28 chromosomes (*T. turgidum* x *Ae. variabilis*). One meiotic division

Table 6.3. Meiotic associations in hybrids of Chinese Spring (CS) *Ph* x *Aegilops variabilis* (13E) with low pairing; of CS *ph1b* x *Ae. variabilis* (13E) with high pairing; in a CS *Ph* x *Ae. variabilis* (13E) callus–regenerated F1 with modified increased pairing.

	Mean Meiotic Metaphase I Chromosomal Association						
	I	II Rings	II Rods	III	IV	V	VI
CS (Normal)	34.68		0.16				
CS (*ph1b*)	9.50	1.6	7.2	2.1	0.25	0.08	0.04
CS (Regenerated)	13.20	0.70	6.60	1.60	0.40	0.20	

Source: Asiedu et al. (1989).

would lead to chromatid separation in the 35- or 28-chromosome hybrids. This, if coupled with meiotic restitution, would subsequently produce male and female gametes of 35 or 28 chromosomes that, upon fusion, are capable of forming progeny with 70 or 56 chromosomes. A chimerically doubled sector (shoot tip) may be another explanation. The 70-chromosome progenies of the *T. aestivum* x *Ae. variabilis* combination should serve as the base for developing normal BCI derivatives, which should lead to a more complete set of alien disomic addition lines.

Applications of callus culture—In callus culture, the variation that emanates from long-term callusing and regeneration can have significance for wheat breeding programs. However, we are not exactly sure how callus-induced variability (Scowcroft 1989) differs in quality and quantity from that obtained through applications of ionizing, non-ionizing, and chemical mutagenic sources. Nevertheless, the findings of Larkin and Scowcroft (1981), Lorz et al. (1988), and Scowcroft (1989) demonstrated callus culture-induced variation, which has been called "somaclonal variation". This aspect was exploited in the first phase of our study with several tetraploid and hexaploid wheat cultivars where we measured callusing and regeneration responses (Table 6.1). We observed the resulting progenies from each cultivar for morphological, cytological, and biochemical responses.

As shown in Table 6.1, bread wheats were more prolific in producing E callus (**Figure 6.3a**) and possessed a higher number of regenerated plants (370) than durum wheats (82). **Figures 6.3b** and **6.3c** show the early and advanced regeneration stages for *T. aestivum*. There were several abnormalities in spike development (**Figures 6.4a-d**) that at the R-0 stage may be transient changes. Any heritable changes remain to be determined from study of the advanced generations.

Somatic root tip cytology was done on 255 bread wheats and 46 durum wheats. We also performed meiotic analyses on a random sample of spikes. Somatic root-tip count data predominantly expressed counts of 42 for *T. aestivum* and 28 for *T. turgidum* (**Figures 6.5a-b**). The chromosome numbers for *T. aestivum* ranged from 39 to 45 and for *T. turgidum* from 28 to 56; there were one or two telocentric chromosomes in the *T. turgidum*-based germplasm. There was a single *T. turgidum* plant with 56 chromosomes (**Figure 6.3d**), attributed to spontaneous doubling, which was male-sterile but female-fertile; it set seed after backcrossing to *T. turgidum*. The meiotic analyses of a few 42- and 28-chromosome plants provided evidence of

Table 6.4. Regenerated plants of F1 hybrid with *Ae. variabilis* (13E) of *Triticum aestivum* and *T. turgidum* showing cytologically doubled progeny.

Female parent	Somatic chromosome number	Passages	C-0 seed number	Somatic chromosome number	C-1 seed number	C-2 seed number
T. aestivum	35	12	1	70	14	Not advanced
T. turgidum	28	7	23	56[a]	57	Not advanced
T. turgidum	28	8	4	56	51	69
T. turgidum	28	10	9	56	56	633

[a] Some aneuploidy (mixoploidy).

cytological variations where—apart from the normal bivalent formation—meiocytes possessed several univalents, trivalents, and quadrivalents. We advanced all R-0 derivatives since the intrinsic R-0 meiotic changes implied a great potential for selecting stable variants in advanced generations. So far, we have partially selected in advanced generations for plant height, days to anthesis, maturity, solid stem, and isozymic electrophoretic banding differences. These materials are being evaluated further in yield trials by the respective CIMMYT wheat breeding sections.

In addition to inducing variability, we are exploiting callus induction procedures to achieve alien gene transfers in intergeneric hybridization. Generally, these divergent hybrids

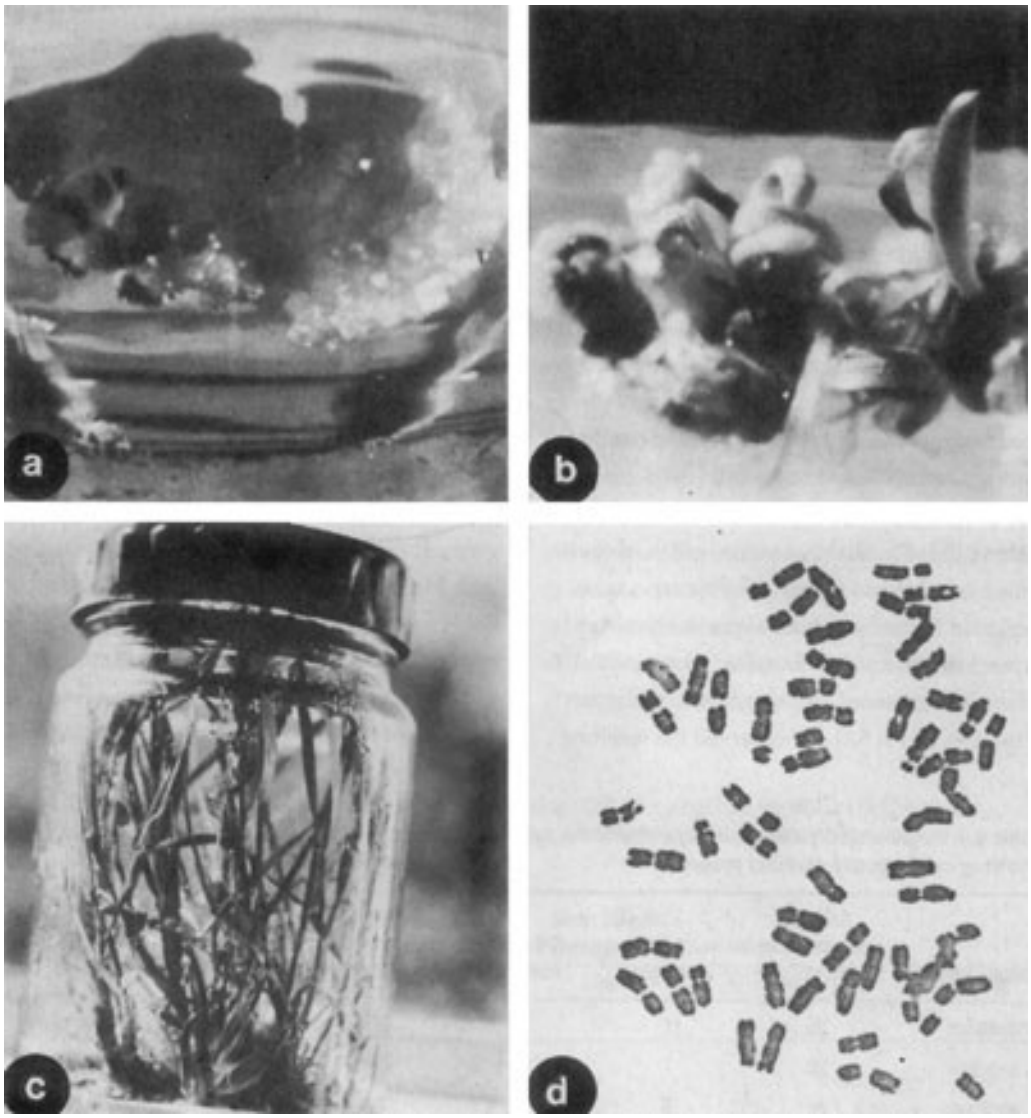

Figure 6.3. a) Embryogenic callus formation in *Triticum aestivum* cultures; b) Early regeneration in *T. aestivum* cultures; c) Advanced regeneration in *T. aestivum*; d) A spontaneously doubled (2n=8x=56) *T. turgidum* somatic cell.

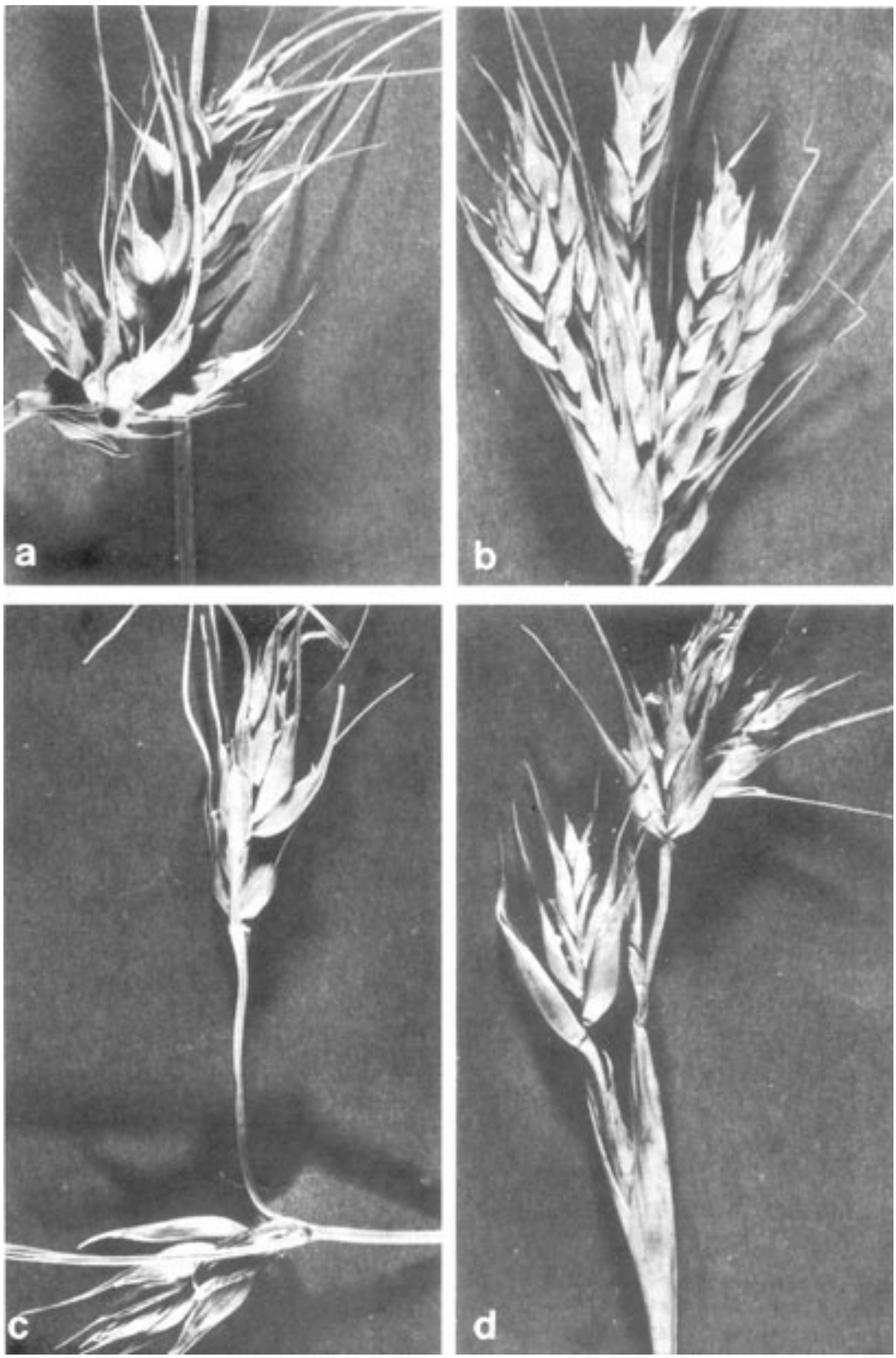

Figure 6.4. Spike morphology variations (a-d) in R-0 plants (callus regenerated) of *Triticum aestivum*.

express low chromosome recombination frequencies (Sharma and Gill 1983a, Mujeeb-Kazi et al. 1987, 1989); several hybrids did not respond positively to amphiploid induction procedures (Islam et al. 1981). Although there are cytogenetic means to facilitate wheat/alien genetic recombinations, callus culture offers a convenient alternative, as demonstrated with wheat x rye combinations (Lapitan et al. 1984, 1986, 1988).

Embryo culture

We routinely use embryo culture to speed up seed increase. We also use it to aid in producing distant hybrids derived from interspecific and intergeneric crosses where the hybrids require nutrient support because their interploid endosperm is either absent or rudimentary. Embryo culture's utility in the production of primary hexaploid and octoploid triticales has ultimately led to its use in obtaining more diverse hybrid combinations.

Although there is a wide array of media available, Taira and Larter (1978) and MS media appear to be the most promising. This can be gauged by the successful range of complex intergeneric hybrids we have produced so far (Mujeeb-Kazi and Bernard 1985b, Mujeeb-Kazi et al. 1987, 1989). Other media should also be considered because embryos have been excised from some crosses of wheat and alien species that did not differentiate into hybrid plants (**Table 6.5**). We recommend critical analyses to determine if we should explore the use of other media or novel methods.

There have been numerous successes in wide crosses in recent years:

- Polyhaploid plants derived from *T. aestivum* x *Zea mays* crosses (See Chapter 5 and Laurie and Bennett 1986, 1987, 1988c; Laurie and Reymondie 1991; Suenaga and Nakajima 1989; Inagaki and Tahir 1990; Riera-Lizarazu and Mujeeb-Kazi 1990);
- The high frequency of fertilization in *T. aestivum* x *Sorghum bicolor* crosses (Laurie and Bennett 1988b) and generation of polyhaploid plants (Ohkawa et al. 1992);

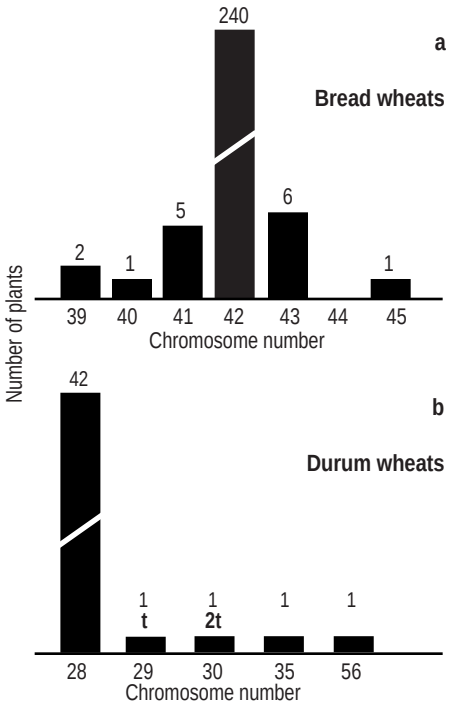

Figure 6.5. a) Number of *Triticum aestivum* regenerated plants and their somatic chromosome numbers; b) Number of *T. turgidum* regenerated plants and their somatic chromosome number (t = telocentric chromosome).

Table 6.5. Some *Triticum aestivum* x alien species combinations where embryos were excised but could not be differentiated into hybrid plants.

Alien species (Male parent)	Percentage seed set	Embryos excised
Agropyron cristatum	1.35	1
A. smithii	3.85	1
A. strigosum	8.33	3
A. tauri	9.43	4
Elymus salina	11.11	2
E. junceus	6.67	1

Source: Mujeeb-Kazi et al. (1987).

- Other successful crosses between *T. aestivum* and *Pennisetum americanum* (Ahmad and Comeau 1990), *Z. mays* ssp. *mexicana* (Ushiyama et al. 1991), and *Tripsacum dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993, Chapter 5);
- Recovery of hybrid plants (Ahmad and Comeau 1991) from the unique cross *T. aestivum* x *Elymus scabrus* (source of accessions with apomictic genes).

These various successes may change future applications of embryo culture quite dramatically from its present simplistic form. However, we must be aware that hybridization successes can vary greatly across diverse locations for a multitude of ill-defined reasons. For example, a simple cross in one location (CS bread wheat x *A. intermedium*) yielded hybrid progeny at a frequency level of 60% under field crossing without embryo culture (Mujeeb-Kazi et al. 1987); however, special inputs were necessary when the cross was made elsewhere and the success rate was still less than 1% (Gu et al. 1984).

Conclusions

Tissue culture applications have been important in the production of complex hybrids within the Triticeae. Their use will even widen the existing range of hybridization possibilities. Embodied in such research applications is the callus culture methodology. This not only provides us with advantages in inducing variability in euploid wheat cultivars or in facilitating *in vitro* screening for resistance or tolerance to stress- or toxin-producing pathogens, but also provides us with the capacity to structurally alter chromosomes. Additionally, the process may modify recombination frequencies in otherwise low-pairing, complex hybrids as well as facilitate recovery of hybrid derivatives with double the number of chromosomes that could be significant in cytogenetic inputs for practical crop improvement (Mujeeb-Kazi et al. 1993b).

Chapter 7

Applications of Biochemical Markers in Wheat Wide Crosses

Manilal D.H.M. William and
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The introduction of alien genetic material into bread wheat (*Triticum aestivum*) can be greatly facilitated by developing efficient techniques that rapidly identify this material in a wheat background. At CIMMYT, we are gaining experience in the use of biochemical markers, involving seed storage proteins and isozymes, and molecular markers involving DNA/*in situ* hybridization and assays based on polyerase chain reactions (PCRs). This chapter details our work with biochemical markers. Chapter 8 describes our use of molecular markers, which show great potential in tracking alien chromatin (Mujeeb-Kazi et al. 1993a).

Types of Biochemical Markers

Seed storage proteins

Storage proteins—components of which are glutenins and gliadins—provide the energy source that enables a seed to germinate. They are commonly used in characterizing and identifying alien genetic material. High-molecular-weight (HMW) glutenins are composed of HMW protein aggregates formed by the association of a number of constituent polypeptide chains referred to as glutenin subunits. The HMW glutenin aggregates can be reduced to their component subunits with a reducing agent such as 2-mercaptoethanol, which breaks the disulfide bonds, and an ionic detergent such as sodium dodecyl sulfate (Beitz and Wall 1972, Hamazu et al. 1972). The technique using SDS-PAGE can then separate the component subunits. The allelic nature of different banding patterns of HMW glutenins has also been well established (Payne et al. 1981a,b). Gliadins, the other component of seed storage proteins, are coded by genes on the short arms of homoeolgous group 1 and group 6 chromosomes (Wrigley and Shepherd 1973, Payne et al. 1982). Knowledge of seed storage proteins has provided a basis for studies relating different allelic combinations to flour quality as well as being used as a tool in cytogenetics since storage protein genes can serve as effective markers for identifying the presence or absence of particular chromosomes or chromosome arms.

Isozymes

Isozymes are different forms of proteins that have the same basic functions involving plant metabolism. More than 100 structural genes for isozyme markers have been identified and located on different chromosomal segments in wheat (Asiedu et al. 1989). The main advantage in using isozyme markers to detect alien genetic material is the speed with which the material can be screened because they have adequate polymorphisms resulting from their co-dominant and nonepistatic inheritances (Tanksley 1983). Information regarding the homoeology of the alien chromosomes in the addition lines can be ascertained by identifying

the genes they possess that are orthologous to sets of *T. aestivum* genes of which the chromosomal locations are known (McIntosh 1983). This can also be done by studying the ability of the alien chromosomes to substitute for and pair with specific wheat chromosomes.

Electrophoretic Techniques for Identifying Biochemical Markers

Electrophoresis is a method in which an electric field is used to cause the migration of charged molecules, mainly proteins and nucleic acids in solution or gel matrices. The rate of migration of the proteins depends on the strength of the electric field, the net charge, the size and shape of the protein molecules. It also depends on the ionic strength and the viscosity of the medium in which the molecules are moving. Enzyme-specific stains are used to develop banding profiles for the different enzyme systems. Most stains contain a substrate specific to the enzyme that allows the catalytic reactions of the enzyme to take place. A dye, used to visualize the enzyme reactions, allows identification of proteins with the same substrate specificity from a tissue extract that may contain hundreds of different enzymes. Vallejos (1983) provides basic recipes for commonly analyzed enzymes.

Chromosome Locations of Genes that Control Biochemical Markers

The availability of compensating nullisomic-tetrasomic stocks has greatly facilitated finding the chromosomal locations of genes that control the isozymes in *T. aestivum*. Once the gene location for a particular enzyme is assigned to a particular homoeologous group, ditelosomics for that particular group have been used to localize the genes to specific chromosome arms (Hart 1979). Alien disomic addition lines and ditelosomic addition lines have been used to determine the chromosomal and chromosome arm location of genes for isozymes in the Triticeae relatives of *T. aestivum* (Hart 1979, Brown 1983).

Analysis of the two parental species and the amphiploid is important in characterizing alien genetic material when using isozymes as diagnostic markers. For most monomeric enzyme systems, the first step is to observe a combination of banding profiles in the amphiploid and/or the addition line carrying the homoeologous alien chromosome (Hart and Tuleen 1983). Sometimes, the alien species may show a certain degree of polymorphism for a particular enzyme system resulting from allelic differences and/or the mutually incompatible nature of the alien species. In such cases, it is necessary to study the polymorphism within and among different accessions of the alien species. We can identify markers when the two parental species show remarkably different banding profiles. In the cases of multimeric (oligomeric) enzyme systems, the procedure is more complicated since it is possible that the protomers from the two parental species can randomly combine to give rise to active oligomeric proteins. In such cases, evaluation of multiple bands as well as their staining intensities becomes necessary (Hart and Tuleen 1983). **Table 7.1** lists some isozyme markers and the alien species that we currently use at CIMMYT.

Limitations to Using Biochemical Markers

Limits to the detection of segregating alleles

Activity bands on a gel after enzyme-specific staining represent enzyme phenotypes and do not necessarily explain all underlying allelic

variation. The resolving power of the electrophoretic procedures may not be sufficient to separate the total allelic variation associated with a particular enzyme. Hence, this aspect of hidden heterogeneity cannot be exclusively resolved by any single method of protein separation.

Null alleles

Although isozymes are said to be codominantly inherited, there are occasions when deviations may occur as a consequence of codominant expression. The phenomenon of null alleles thus gains importance. A null allele under a homozygous condition may result in complete absence of enzyme activity. It then becomes necessary to conduct genetic studies to confirm or deny the involvement of null alleles.

Table 7.1. Some isozyme markers identified with isoelectric focusing for alien species with potential of being applied to wide hybridization in the Triticeae.

Seed GPI	Leaf GPI	Seed EST	Leaf EST	Grain ADH	Grain PGM
L. racemosus	*Ps. juncea*	*E. triticoides*	*Ps. juncea*	*Ps. juncea*	*Ha. villosa*
L. mollis	*L. racemosus*	*L. mollis*	*L. mollis*	*L. mollis*	*L. mollis*
E. triticoides	*Th. curvifolium*	*Ps. juncea*	*L. racemosus*	*H. vulgare*	*Th. scythicum*
Th. acutum	*Th. trichophorum*	*Ha. villosa*	*E. triticoides*	*Ae. umbellulata*	
Th. intermedium	*Th. gentryi*	*Th. curvifolium*	*Th. curvifolium*	*Ae. ovata*	
Th. gentryi	*Th. intermedium*	*Th. acutum*	*Th. elongatum*	*Ae. triaristata*	
Ae. umbellulata		*Th. scirpeum*	*Th. acutum*	*Ae. columnaris*	
Ae. variabilis		*Th. bessarabicum*	*Th. trichophorum*	*Ae. biuncialis*	
Ae. crassa		*Th. elongatum (10x)*	*Th. scirpeum*	*Ae. variabilis*	
Ae. searsii		*Th. intermedium*	*Th. bessarabicum*	*Ae. triuncialis*	
Ae. bicornis		*Th. trichophorum*	*Th. intermedium*	*Ae. cylindrica*	
Ae. caudata		*Ae. umbellulata*	*Th. gentryi*	*Ae. comosa*	
Ae. triuncialis		*Ae. uniaristata*	*Ae. uniaristata*	*Ae. uniaristata*	
Ae. biuncialis		*Ae. variabilis*	*Ae. variabilis*	*Ae. crassa*	
Ae. triaristata		*Ae. ventricosa*	*Ae. umbellulata*	*Ae. vavilovi*	
Ae. ovata		*Ae. ovata*	*Ae. ventricosa*	*Ae. ventricosa*	
Ae. squarrosa		*Ae. triaristata*		*Ae. juvenalis*	
Th. distichum		*Ae. columnaris*		*Th. acutum*	
Th. junceiforme		*Ae. biuncialis*		*Th. gentryi*	
Th. junceum		*Ae. triuncialis*		*Th. intermedium*	
A. cristatum		*Ae. caudata*		*Th. varnense*	
Th. varnense		*Ae. cylindrica*			
Th. scythicum		*Ae. comosa*			
		Ae. mutica			
		Ae. speltoides			
		Ae. longissima			
		Ae. sharonensis			
		Ae. bicornis			
		Ae. searsii			
		Ae. crassa			
		Ae. vavilovi			
		Ae. squarrosa			
		Th. distichum			
		Th. junceiforme			
		Th. junceum			
		Th. varnense			
		Th. scythicum			

GPI = Glucose phosphate isomerase; EST = Esterase; ADH = Alcohol dehydrogenase; PGM = Phosphoglucomutase.

Post-translational modifications

Polypeptide synthesis is the result of several independent events such as: 1) transcription, 2) translation, 3) processing, and 4) transport of the translated protein product. The first two steps involve the actual coding of the nucleotide sequences into a primary protein structure and the last agglomerated step is post-translational in nature, which may also be important in giving the final structure to a protein product. In addition, degradation of proteins by proteolytic action, repeated freezing, thawing, and aging of samples may also result in modifying the nature of the native enzyme product. These modifications are classified as post-translational modifications and adequate attention to this aspect must be given when biochemical markers are used in genetic studies.

Tracking Chromosomes in Intergeneric Hybrids

Psathyrostachys juncea

Psathyrostachys is a genus of the Eurasian interior where its species grow on rocky open slopes from the Middle East and European Russia across Central Asia to North China (Tzvelev 1976). The genus contains about 10 species, all of which contain the basic N genome (Jensen et al. 1990; Table 2.1). The potential importance of this grass includes its tolerance to salinity and drought (Dewey 1984) and its resistance to barley yellow dwarf virus (Plourde et al. 1990). These biotic and abiotic attributes of *Ps. juncea* (2n=2x=14, NN) make the species an invaluable germplasm source for these important traits. We used eight different accessions from geographically diverse regions to develop isozyme markers for tracking *Ps. juncea* chromosomes in a wheat background.

We evaluated eight individual seeds and a composite of 15 seeds from each accession for the following isozymes: malate dehydrogenase (MDH), seed esterase (EST-5), shikimate dehydrogenase (SKDH), phosphoglucomutase (PGM), ß-amylase (ß-AMY), and glucose-phosphate isomerase (GPI). We then compared the banding profiles of *Ps. juncea* with those of the hexaploid wheat varieties *T. aestivum* cv. Chinese Spring (CS) and Seri M 82, used as the bread wheats in the intergeneric crosses of *T. aestivum*/*Ps. juncea* (William and Mujeeb-Kazi 1992). We selected five of the isozymes to determine their usefulness as markers for tracking the *Ps. juncea* chromosomes in a wheat background. GPI was not a positive marker.

MDH—The MDH genes were first located on the long arms of homoeologous group 1 chromosomes using starch and polyacrylamide gel electrophoresis (Benito and Salinas 1983). Using isoelectric focusing (IEF), Liu and Gale (1989) located another set of MDH genes on the short arms of homoeologous group 5 chromosomes. The MDH banding profiles are in the range of isoelectric points (pI) between pH 3.99 to 6.89. CS and Seri M 82 have identical banding patterns with 13 bands. The banding resolution is better at the basic side of the gel. Individual *Ps. juncea* accessions give slightly polymorphic banding profiles, but the composite seed samples of these accessions contain all the bands that each of the individual seeds analyzed possess. CS and Seri M 82 have two bands, with pIs 6.21 and 5.06, which are not present in any of the *Ps. juncea* accessions. *Ps. juncea* has a unique band with a pI of 6.89 that is not present in the two wheat cultivars. This can serve as a biochemical marker to identify the *Ps. juncea* chromosome(s) homoeologous to group 1 or group 5 of wheat in a wheat background.

EST-5—CS and Seri M 82 have banding profiles with an array of EST-5 bands with pI values between 4.18 and 7.34. The smear of bands at the

acidic end has been previously excluded in analyzing EST-5 isozymes in bread wheat (Ainsworth et al. 1984). There are differences in the banding profiles of CS and Seri M 82, with the two most basic bands of CS encoded by chromosome 3A (Ainsworth et al. 1984) being absent in Seri M 82.

All eight *Ps. juncea* accessions show a certain degree of polymorphism in the banding profiles for esterases, a likely consequence of its self-incompatibility nature. The polymorphism may be due further to allelic variation present in *Ps. juncea* as the material evaluated was not sufficient for conforming to the allelic nature as earlier reported for hexaploid wheat (Ainsworth et al. 1984). The induced autotetraploid accession (PI 531828) with improved self-fertility also shows some polymorphism. The *Ps. juncea* accessions analyzed have two bands in the acidic range with pI values of 4.18 and 4.29, bands that are not present in CS and Seri M 82. Consequently, these bands can be effectively used as markers to track the presence of *Ps. juncea* chromosomes that are homoeologous to group 3.

SKDH—Koebner and Shepherd (1983) investigated SKDH-1 of *T. aestivum* using polyacrylamide gel electrophoresis and found genes located on the short arms of homoeologous group 5 wheat chromosomes. Benedettelli and Hart (1988) expanded the study of SKDH by using IEF and leaf tissue to identify eight activity bands. Seeds of CS and Seri M 82 have identical six-band profiles. All bands in CS and Seri M 82 are in the pI range of 4.71 to 6.14. The banding profiles of the composites of *Ps. juncea* accessions, having a pI range of 5.56 to 7.09, also contain five to six bands, of which two (pI 6.47 and 7.09) are distinct and always present in the individual seed analyses. These two bands (pI 6.47 and 7.09) may serve as markers for detecting and tracking alien *Ps. juncea* chromosomes that are homoeologous to group 5.

PGM—CS has a three-band profile with pIs between 4.67 and 5.20. All single seeds and respective composites of *Ps. juncea* accessions have a single diagnostic pI 4.47 band anodal to the banding profile of CS. This band could serve as a marker for the *Ps. juncea* chromosomes that are homoeologous to group 4. Some *Ps. juncea* accessions express a few faint bands that overlap with CS bands in the more basic range and hence cannot be easily utilized as markers.

ß-AMY—Gene loci controlling ß-AMY are located on two sets of homoeologous chromosomes. Ainsworth et al. (1983) suggested that the genetic control of ß-AMY is on both group 4 and group 5 chromosomes, i.e., 4A, 5A (possible translocation from 4B), and 4D. In addition, another set of genes is located on group 2 chromosomes, designated as ß-*Amy*-2 (Sharp et al. 1988). In wheat, on the basis of IEF, 33 activity bands were identified (Ainsworth et al. 1983). Similarly, bands with pIs in the range between 4.41 and 5.93 were identified in this study for cultivars CS and Seri M 82, with the banding profiles of both cultivars being quite similar.

Individual seeds of different *Ps. juncea* accessions have banding patterns with two to four distinct bands in a pI range between 4.33 and 4.50, with an array of faint bands of higher pI values but a resolution inadequate for differentiation from the wheat cultivars. Some individual seed samples among all eight accessions of *Ps. juncea* have only two sharp bands that overlap the low pI bands of CS and Seri M 82. Each accession exhibits considerable polymorphism, which

complicates the possibility of identifying *Ps. juncea* chromosomes in a wheat background if this marker is used.

Native polyacrylamide gel electrophoresis shows better differences between *Ps. juncea* and *T. aestivum* than those observed in IEF. Isoelectric focusing separates proteins according to their net charge whereas native polyacrylamide gel electrophoresis separates proteins according to the charge/mass ratio. *Ps. juncea* has two to four bands at different migratory positions compared to the *T. aestivum* cultivars. Thus, these marker bands could serve as diagnostic markers in a *T. aestivum* x *Ps. juncea* hybridization program to identify and track *Ps. juncea* chromosomes that are homoeologous to group 4 or group 5.

Thinopyrum bessarabicum

Thinopyrum, a genus of Europe, the Middle East, and Central Asia, consists of diploids 2n=14 (*Th. bessarabicum*), segmental allotetraploids, 2n=28 (*Th. junceiforme*), segmental allohexaploids, 2n=42 (*Th. junceum*), and decaploids, 2n=70 (*Th. ponticum*). The genus *Thinopyrum* was initially erected with only six species (Löve 1980) of the J genome composition. Due to the similarities of the J and E genomes, Dvorak (1981), Dewey (1984), McGuire (1984), Wang (1985), Pienaar et al. (1988), and Wang and Hsiao (1989) suggested combining the J genome of *Thinopyrum* and the E genome of *Lophopyrum* into one genus while retaining the J genome designation (see Chapter 2, Table 2.1). We are looking at *Th. bessarabicum* (2n=2x=14, JJ), a self-fertilizing maritime grass, as a possible source of salinity tolerance for transferring to wheat.

The biochemical characterization of *Th. bessarabicum* involved procedures similar to those adopted for *Ps. juncea* except that we also evaluated the amphiploid of CS x *Th. bessarabicum* (2n=8x=56, AABBDDJJ) and disomic addition lines with 44 chromosomes. We evaluated HMW glutenins and five isozymes for their potential as markers.

HMW glutenins—There was no polymorphism within different seeds of a given accession of *Th. bessarabicum* or between accessions. *Th. bessarabicum* has a single unique band with an HMW glutenin region and faster mobility compared to the banding profile of CS. This marker band is also present in the amphiploid. The HMW glutenins are located in the long arms of homoeologous chromosomes of group 1 in wheat (Beitz et al. 1975, Lawrence and Shepherd 1980). Using this marker band, we have been able to identify the addition lines carrying *Th. bessarabicum* chromosomes that are homoeologous to group 1 (i.e., 1JJ).

Superoxide dismutase (SOD)—Genes for SOD are located in wheat chromosomes that are homoeologous to group 2 (Neuman and Hart 1986). CS has a banding pattern with three bands. *Th. bessarabicum* has a sharp band cathodal to the banding pattern of bread wheat, which can serve as a marker to identify the presence of *Th. bessarabicum* chromosomes that are homoeologous to group 2. The amphiploid also shows the marker band clearly, which can serve as a marker to identify the 2J alien disomic chromosome addition.

EST-5—Numerous bands were observed for CS, with slight polymorphism prevalent in the banding patterns of different accessions of *Th. bessarabicum*. All individual seeds analyzed in different accessions of *Th. bessarabicum* had one band in the acidic region of the gel that did not show much polymorphism. This band was not present in bread wheat, but was clearly expressed in the amphiploid. Genes for EST-5 in

wheat are located in the long arms of chromosomes that are homoeologous to group 3 (Ainsworth et al. 1984), so, using this marker, we have been able to identify the addition lines of *Th. bessarabicum* that have chromosomes homoeologous to this group.

ß-AMY—CS had a banding pattern with three or four bands when ß-AMY was separated on 8.5% acrylamide native PAGE. Individual seeds of *Th. bessarabicum* had a banding profile with two to four bands with faster mobilities compared to CS. The variability expressed by individual seeds of *Th. bessarabicum* does not cause a limitation in using ß-AMY as a marker. The banding patterns of individual seeds of *Th. bessarabicum*—whether they had two or four bands—had distinctly faster mobilities compared to the banding patterns of CS. **Figure 7.1** shows the banding patterns of the amphiploid together with that of CS and a composite sample of *Th. bessarabicum*. In addition to the bands present in CS, the amphiploid possessed two faster moving bands—a characteristic of the banding pattern expressed by some individual *Th. bessarabicum* seeds. Since genes controlling ß-AMY are located on 4Aß, 4DL, and 5AL (Ainsworth et al. 1984), it has been possible to identify the 5J addition line using this marker.

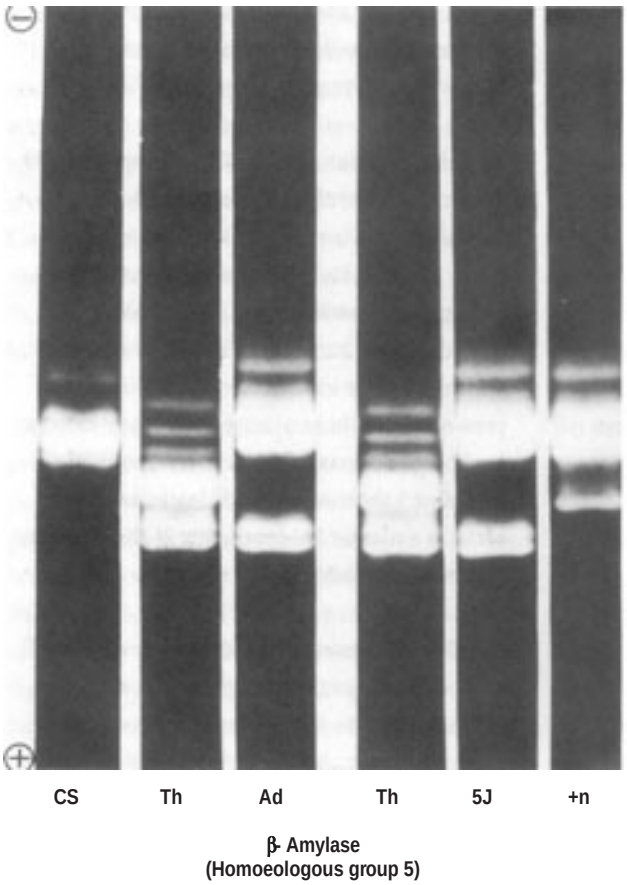

Figure 7.1. Separation of Beta-Amylase (ß-AMY) isozymes on native-PAGE (8.5% gels) for *Thinopyrum bessarabicum* (Th), Chinese Spring (CS), the amphiploid (Ad), and addition lines (5J, +n).

Glutamate oxaloacetate transaminase (GOT)—There are three zones of enzyme activity where GOT isozymes are separated on native-PAGE (Hart 1975). In the region of GOT-2, three activity bands have been observed (Hart 1975). Genetic analysis of GOT-2 isozymes has established that genes responsible for band 1 are located on chromosome 6DL; band 2 on 6AL, 6BL, and 6DL; and band 3 on 6AL and 6BL (Hart 1975).

Analysis of leaf extracts from individual seedlings of different *Th. bessarabicum* accessions showed no polymorphism for the GOT-2 banding pattern. *Th. bessarabicum* expressed a simple band in the GOT-2 zone that coincided with that of band 1 in the GOT-2 of CS. The amphiploid showed a clear increase in staining intensity of band 1 in GOT-2 with no change in intensities in the other two

bands. This band with increased activity has been used as a marker for the tracking of *Th. bessarabicum* chromosomes that are homoeologous to group 6.

Alpha-Amylase (α-AMY)—α-AMY isozymes have two activity zones when separated on IEF-polyacylamide gels—the genes of which are located on the long arm of chromosomes that are homoeologous to group 6 (α-AMY1) and group 7 (α-AMY2) in wheat (Gale et al. 1983). α-AMY2 isozymes, which have acidic isoelectric points (pIs), are controlled by genes located on group 7 chromosomes in wheat (Gale et al. 1983). Individual seeds of different accessions showed the presence of one strong band and two more of lesser activity in α-AMY2 regions. The most acidic band of *Th. bessarabicum* was not present in CS. The amphiploid, in addition to having the banding profile of CS, clearly expresses the marker band specific to *Th. bessarabicum*. Using this marker band, we have been able to identify the addition lines with chromosomes that are homoeologous to group 7.

Results—Using the above markers, we have identified six disomic chromosome additions of *Th. bessarabicum* in bread wheat (William and Mujeeb-Kazi 1993b).

Genomic status of tetraploid *Leymus racemosus*

Leymus Hochst., a genus naturally occurring in Eurasia and North America, contains about 30 polyploid species. The genus has been based on variations of two basic genomes, the N genome of *Psathyrostachys* and the J genome of *Thinopyrum* (Dewey 1984). Based on genome analysis, the *Leymus* genome was designated JJNN by Dewey (1984); but since has also been designated NNXX (Chapter 2, Table 2.1). Morphological similarities among *Leymus* and *Psathyrostachys* spp. include more than one spikelet per spike node, a large number of florets per spike, and a perennial nature.

Leymus spp., which can grow on saline or alkaline soils, may owe their salinity tolerance to the J genome of *Thinopyrum* (Dewey 1984). Their tolerance to drought (and perhaps salinity as well) may come from the N genome of *Psathyrostachys* (Dewey 1984).

On the basis of molecular markers, Zhang and Dvorak (1991) suggested a genomic designation of $N_1N_1N_2N_2$ for the tetraploid *Leymus* species rather than JJNN. Since we are actively using species of *Leymus*, *Psathyrostachys*, and *Thinopyrum*, we initiated a basic study using isozyme zymograms to further analyze the genomic relationships among these three genera. Using at least two different accessions each of *Ps. juncea*, *Th. bessarabicum*, and *L. racemosus*, we evaluated the HMW-glutenins and the isozymes ß-AMY, GOT, 6-phospho gluconate dehydrogenase (6-PGD), Endopeptidase (EP), GPI, and SOD. As a result, we selected five markers that would cover different chromosomes.

Glutenins—The banding patterns of the HMW-glutenins show that *L. racemosus* and *Ps. juncea* possess HMW-glutenins much more similar to each other than to the HMW-glutenins of *Th. bessarabicum*, indicating that there is more similarity between the HMW-glutenin genes of *Ps. juncea* and *L. racemosus* compared to that of *Th. bessarabicum*.

ß-AMY—The IEF banding pattern of ß-AMY indicates some polymorphism within accessions for all the species we evaluated. *Th. bessarabicum* has a banding pattern with 11 bands. *L. racemosus* does not have any bands that coincide with the

multitude of bands of *Th. bessarabicum* on the more basic side of the gel. The most acidic bands in *L. racemosus* are not present in *Th. bessarabicum*. The two accessions of *Ps. juncea* also give three or four bands, with the more basic bands of *Th. bessarabicum* being absent. The more acidic bands of *Ps. juncea* correspond with those of *L. racemosus*. Due to the polymorphism present within and among different accessions, a band-to-band comparison is seemingly difficult. There is, however, more similarity between the banding patterns of *Ps. juncea* and *L. racemosus* as compared to the banding pattern of *Th. bessarabicum*, further indicating a greater similarity between the *ß-Amy* genes of *Ps. juncea* and *L. racemosus* than between *Th. bessarabicum* and *L. racemosus*. Banding patterns of ß-AMY on native-PAGE also indicate more similarity between *Ps. juncea* and *L. racemosus* compared to that of *Th. bessarabicum* and *L. racemosus*.

GOT—The banding profile of GOT has three activity zones characterized as GOT-1, GOT-2, and GOT-3 (Hart 1975). *Th. bessarabicum* has a single band in the GOT-2 region with R_f of 0.74, coinciding with the faster moving GOT-2 band in CS. *Ps. juncea* also has a single GOT-2 band with R_f of 0.68. *L. racemosus* has three bands, one of which coincides with the single band of *Ps. juncea*. The other two bands of *L. racemosus* have slower mobility. No similarity exists between the GOT-2 banding patterns of *L. racemosus* and *Th. bessarabicum*, however, there are similarities among GOT banding patterns of *Ps. juncea* and *L. racemosus*.

EP—*Th. bessarabicum* has a banding pattern with two bands. *L. racemosus* and *Ps. juncea* also have two bands with identical isoelectric points. The two bands of *Th. bessarabicum* have more basic pI values compared to those of *L. racemosus*, suggesting that the endopeptidase genes of *L. racemosus* and *Ps. juncea* are more similar than are the EP genes of *Th. bessarabicum*.

GPI—*Ps. juncea* has a seven-band pattern with some polymorphism among accessions. *Th. bessarabicum* has five bands and *L. racemosus* has some bands that are common to *Ps. juncea* as well as *Th. bessarabicum*. Hence, *L. racemosus* has GPI genes common to both *Ps. juncea* and *Th. bessarabicum*.

Results—Preliminary results of this ongoing study indicate that *L. racemosus* has more isozyme/protein genes similar to those of *Ps. juncea* with some isozyme systems showing similarity to the genes of *Th. bessarabicum* and *L. racemosus*.

Rye isozymes

We are looking to develop isozyme markers for both arms of *Secale cereale* chromosomes using CS/Imperial rye disomic chromosome addition lines (source: late E.R. Sears, Missouri, USA). There are several projects underway in the CIMMYT Wide Crosses Section that would directly benefit by establishing rye markers. These include:

- Introduction of 1AL/1RS, 1BL/1RS, and 5AS/5RL into CIMMYT wheats;
- Characterization and classification of backcross derivatives from crosses involving the 1BL/1RS chromosome translocation with the *ph1b* mutant to determine the translocation points;
- The amount of rye introgressions;
- Development of rye addition lines in the background of Pavon F 76 and Altar 84 and production of rye telocentric stocks.

Table 7.2 lists biochemical markers that we have identified using CS/Imperial rye addition lines.

Tracking Chromosomes in Interspecific Hybrids

As discussed in Chapter 3, *Triticum tauschii* (*Aegilops squarrosa*; 2n=2x=14, DD) has been generally accepted as the D genome donor to *Triticum aestivum* (2n=6x=42) (Kimber and Feldman 1987). The importance of the D genome for quality parameters has also been well established (Orth et al. 1973, Kerber and Tipples 1969). Lagudah et al. (1991) reported that the evolutionary origin of hexaploid wheat (AABBDD) occurred with a small number of *T. tauschii* genotypes of restricted geographic origin, implying a narrow genetic diversity for the D genome. If primitive or wild forms have played a role in the evolution of a species, other diverse wild form accessions may be of further use in crop improvement. Thus, *T. tauschii* accessions can serve as a source of new genes for bread wheat improvement (Appels and Lagudah 1990). The wide ecological adaptability of *T. tauschii* would help not only to introgress genetic material from *T. tauschii* into *T. aestivum*, but may also enhance the adaptability of the cultivated bread wheats into areas considered marginal or currently unprofitable for wheat cultivation.

Table 7.2. Biochemical markers that have been identified at CIMMYT using Chinese Spring/Imperial rye addition lines.

Marker	Chromosomal location
Seed storage proteins	
Glutenin	1RL
Gliadins	1RS
Isozymes	
Superoxide dismutase	2R
ß-glucosidase	2R
Diaphorase	3R
Phospho gluco mutase	4RS
Alcohol dehydrogenase	4R
Shikimate dehydrogenase	5RS
6-phospho gluconate dehydrogenase	4RL, 6RS
Glutamate oxaloacetate transaminase (GOT-2)	6R
Esterase (leaf)	6R
Acid phosphatase	7RS

Evaluation of variability in *Triticum tauschii*

We conducted a study to evaluate the variability associated with some *T. tauschii* accessions for seed storage proteins (HMW subunits of glutenins and gliadins) and three isozymes (EST-5, ß-Amy, and GPI). We have utilized these *T. tauschii* accessions in producing numerous synthetic hexaploids at CIMMYT (Chapter 3, Mujeeb-Kazi et al. 1994b). A sample of synthetic hexaploids also helped us determine that the variability for seed proteins and isozymes observed in *T. tauschii* could be incorporated into the synthetics.

We further compared the wide variability of *T. tauschii* accessions with the variability of the synthetic hexaploids. This variability, if expressed in the synthetics, could provide new genetic diversity for incorporation into bread wheats and also serve as additional evidence of the phylogenetic relationship between *T. tauschii* and hexaploid wheat. In 60 different accessions, we observed polymorphisms for the storage proteins and isozymes mentioned above. Due to the importance of HMW glutenins for quality parameters, we identified the allelic combinations present for the *Glu-D1* locus by comparing *T. tauschii* accessions with bread wheat cultivars of known allelic combinations. We also evaluated a sample of synthetic hexaploids together with the *T. turgidum* and *T. tauschii* accessions used in producing the synthetics to show that the polymorphisms associated with *T. tauschii* are present in the synthetic hexaploids.

HMW glutenin subunits—A wide variation of HMW subunit combinations exists among the different *T. tauschii* accessions (**Figure 7.2**). Each subunit was assigned a number (Payne et al. 1984) by running bread wheat standards with known allelic combinations along with the *T. tauschii* accessions in the same gel. The *Glu-D1* locus is characterized by the presence of a pair of subunits, one with faster mobility (Payne et al. 1981a). To maintain consistency with previous work on HMW glutenins (Lagudah and Halloran 1988), we use the superscript "t" when

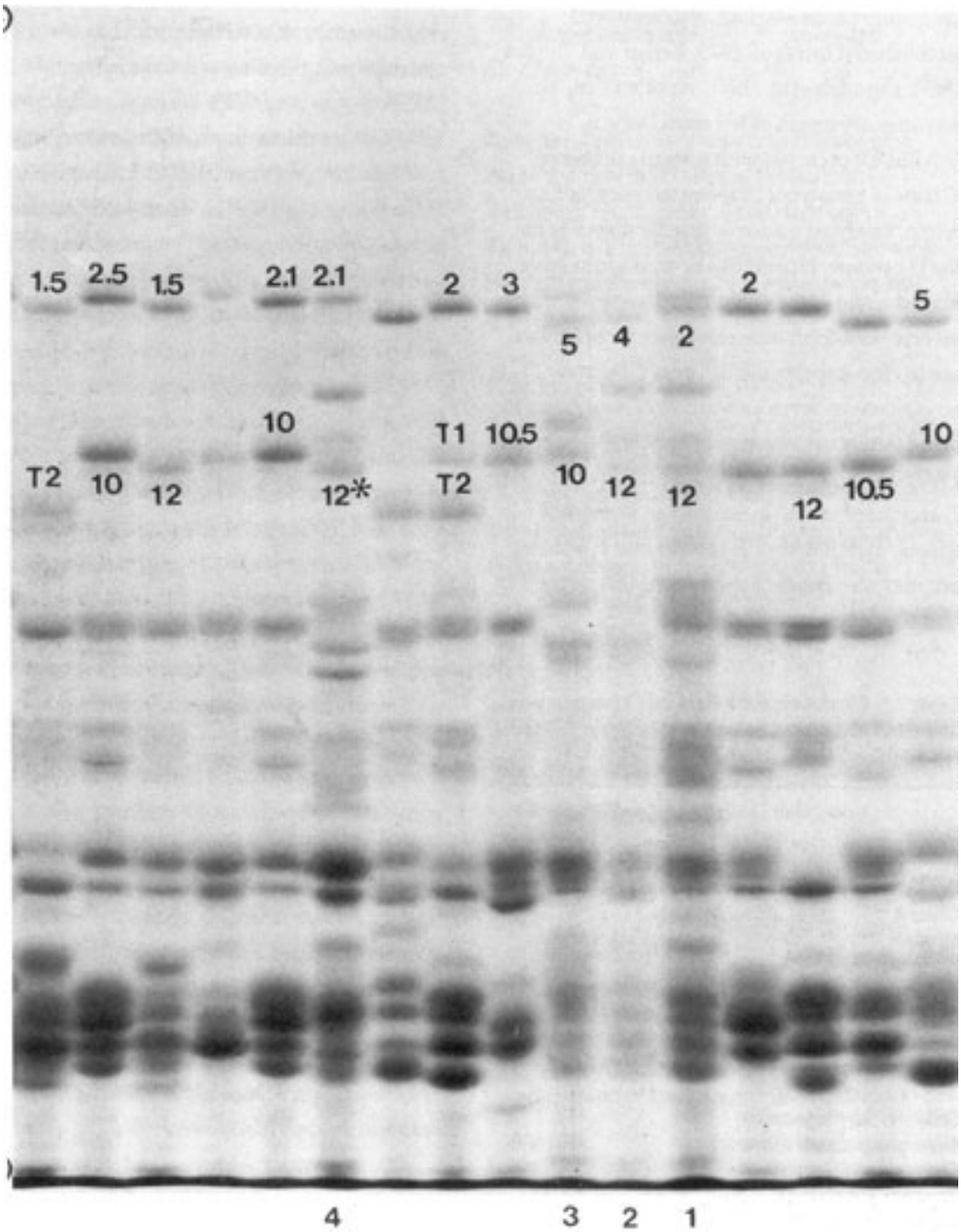

Figure 7.2. SDS-PAGE of high molecular weight glutenin subunits of *Triticum tauschii* accessions.

assigning gene symbols to the D genome of *T. tauschii* to distinguish them from their analogous gene loci in the D genome of hexaploid wheat. We identified 15 *Glu-D^{t}1* subunit combinations among the different *T. tauschii* accessions (Figure 7.2) (William et al. 1993).

Our research indicates the presence of more variability for HMW glutenin subunits among different *T. tauschii* accessions in the *Glu-D^{t}1* locus than previously described (Lagudah and Halloran 1988). The bread making qualities of our synthetic hexaploids with different subunit combinations from *T. tauschii* have been briefly examined (Peña et al. 1991). Identification of some desirable allelic combinations in the synthetic wheats shall inevitably lead to their introduction into *T. aestivum* by conventional breeding programs.

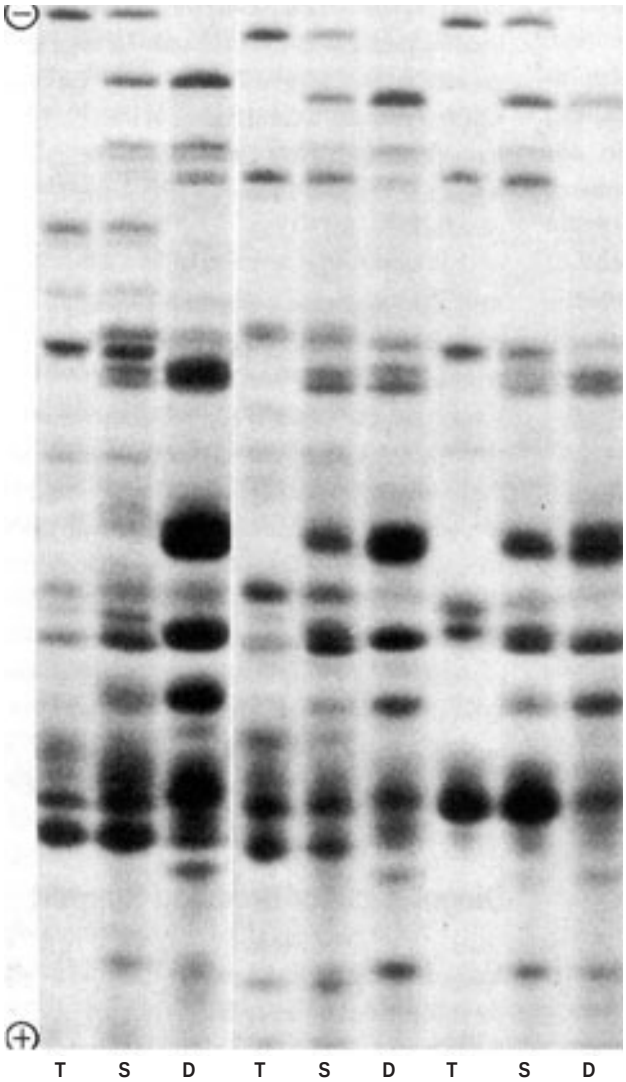

Figure 7.3. SDS-PAGE of high molecular weight glutenin subunits of synthetics (S) with the durum (D) and *Triticum tauschii* (T) parent combinations.

Lagudah et al. (1991) have suggested that the evolutionary origin of bread wheat occurred with a restricted number of *T. tauschii* genotypes. The banding patterns in **Figure 7.3**, covering a sample of synthetics evaluated for the HMW glutenin subunits in combination with durum and *T. tauschii* parents, clearly demonstrate that the allelic combinations in *T. tauschii* are present in the synthetic hexaploids together with the allelic combinations of the durum parents. Although we have not made crosses between different accessions to establish the allelic nature of the subunit combinations present in *T. tauschii*, the fact that the subunits are expressed in the synthetic hexaploids is an adequate indication of the allelic nature of the variants of the *Glu-D^{t}1* locus. This analysis can also validate the authenticity of the hexaploids.

Gliadins—In bread wheat all ω-gliadins, most γ-gliadins, and some ß-gliadins are coded by genes on short arms of chromosomes that are homoeologous to group 1 (Wrigley

and Shepherd 1973, Payne et al. 1982). Some gliadins are also coded by genes on chromosomes that are homoeologous to group 6 (Zehatschek et al. 1981). Similar to the situation of *Glu-D1*, recombination among allelic forms has not been frequently observed (Sozinove and Poperelya 1980). Kasarda (1980) suggested a prevalence of tightly linked genes in the gliadin coding loci causing them to be inherited as blocks (Mecham et al. 1978). Multiple allelism of gliadin coding loci in homoeologous group 1 and group 6 chromosomes of wheat has been established (Sozinove and Poperelya 1980, Metakovsky et al. 1984). We have observed at least 56 different gliadin patterns among the different accessions of *T. tauschii*. The polymorphisms for *Gli-D^{t}1* appear to be greater than those for the *Glu-D^{t}1* locus. Quality parameters associated with gliadins are not as well defined as for glutenins, yet their possible impact on quality aspects should not be discounted.

EST-5—We found a total of five phenotypic classes in the *T. tauschii* accessions and these results indicate that the D genome of *T. tauschii* possesses more variability for EST-5 than does the D genome of bread wheat. The different phenotypic classes we observed in *T. tauschii* accessions are also expressed in the synthetic hexaploids derived from their combinations with *T. turgidum* cultivars.

ß-AMY—Mainly, we found two different phenotypic classes of ß-Amy among the different accessions of *T. tauschii*. The more prevalent phenotype has a profile of 14 bands while the other possesses 12 bands with two more acidic bands of high intensity. Both phenotypes of *T. tauschii* readily express in the synthetic hexaploids.

GPI—There are no polymorphisms among different accessions of *T. tauschii* for GPI banding phenotypes, which is indicative of GPI's molecular nature. Chojecki and Gale (1982) also reported very little polymorphism among different bread wheats in seed GPI. Since GPI is important in the glucose metabolic pathway, it is possible that a selection against mutations has occurred. Durum wheat has a banding profile with four or five bands. When GPI from the D genome of *T. tauschii* was added to the durums to form synthetic hexaploids, we observed a banding profile with 10 or 11 bands—similar to the banding profiles of cultivated bread wheats, which is probably a consequence of the formation of active GPI dimers due to the interaction of *Gpi-D^{t}1* subunits with A and B genome subunits.

Although bread wheats may have naturally evolved from a small number of *T. tauschii* accessions (Lagudah et al. 1991), *Gpi-D^{t}1* subunits of any *T. tauschii* accession can interact with the A and B genome subunits to give rise to the bread wheat banding profile. The possibility of interaction of subunits from *Gpi-D^{t}1* with the subunits of A and B genomes further indicates close homoeology between the GPI subunits of different genomes. A synthetic hexaploid's expression of the same banding pattern present in bread wheat is compatible with the already established phylogenetic relationship between the ancestral D genome donor and *T. tauschii* accessions.

Diagnostics for Breeding Support

Use of isozyme markers in the development of 1BL/1RS isolines

Bread wheat—The short arm of chromosome 1R of *Secale cereale* L. carries genes for resistance to leaf rust (*Lr19*), stem rust (*Sr26*), stripe rust (*Yr9*),

and powdery mildew (*Pm8*). The highly successful Veery'S' lines, developed at CIMMYT (Rajaram et al. 1983) in crosses involving the winter wheat cultivar Kavkaz, possess the 1RS chromosome arm in the form of a 1BL/1RS translocation (Zeller 1973). These 1BL/1RS wheats have demonstrated high yield and stability (Rajaram et al. 1983). Despite the desirable agronomic traits, the 1BL/1RS wheats generally produce flour with inferior dough quality (Dhaliwal et al. 1987), although recent findings do not confine this deleterious quality defect (Peña et al. 1990) exclusively to the 1BL/1RS translocation. To evaluate critically these effects of the 1BL/1RS translocation, we plan to produce and analyze substitution lines of such translocation stocks.

Developing a 1B,1B isoline of a 1BL/1RS,1BL/1RS wheat cultivar involves crossing the 1BL/1RS wheat with a 1B donor, thus generating the F1 translocation heterozygote 1BL/1RS,1B. The F1 is advanced by backcrossing (BC) to the 1BL/1RS,1BL/1RS female parent for eight generations (until BCVIII). In each BC generation, it is necessary to identify the 1BL/1RS,1B translocation heterozygote for subsequent backcrossing, culminating at BCVIII when the heterozygote 1BL/1RS,1B is selfed and the 1B,1B substitution homozygous derivative is selected. The homozygous 1BL/1RS recovered at the BCVIII selfing stage is the extracted control. We follow a similar procedure to develop 1BL/1RS,1BL/1RS isolines from a 1B,1B parent except that the female parent in the original cross and subsequent backcrosses will be of the 1B homozygous type.

Identification of the translocation heterozygote 1B,1BL/1RS is possible by applying the diagnostic C and/or N banding techniques (Jahan et al. 1990; ter Kuile et al. 1990, 1991). Since these techniques are time consuming and cumbersome, we developed a complementary efficient means of tracking the 1BL/1RS,1B heterozygotes in segregating backcross populations by using a combination of protein separation techniques. GPI isoelectric focusing and polyacrylamide gel electrophoresis in acid medium (Acid-PAGE) of the gliadins were used.

In this study, we used several 1B or 1BL/1RS homozygous *T. aestivum* cultivars and their heterozygote (1B,1BL/1RS) F1 progenies. We crossed the cultivars homozygous for 1B with Glennson M 81 or Seri M 82 (homozygous for 1BL/1RS,1BL/1RS) and the cultivars homozygous for 1BL/1RS,1BL/1RS with Pavon F 76'S' or Ciano T 79, which are homozygous for 1B. Glennson M 81 and Ciano T 79 are red-grained cultivars. Five randomly chosen seeds from each parent and their F1 hybrids were analyzed for GPI and gliadin electrophoretic patterns. Cut endosperms were directly laid over the gel in the IEF.

The same endosperm halves first used for GPI analysis were subsequently utilized for gliadin extraction. Our gliadin extraction procedures, gel composition, and running conditions were similar to those of Ng et al. (1988).

We could readily differentiate the F1 hybrid cross combinations heterozygous for the 1B,1BL/1RS chromosomal constitution from their homozygous parents (1B,1B or 1BL/1RS,1BL/1RS) by using a combination of GPI-IEF and gliadin A-PAGE. The most cathodal band of GPI zymograms of wheat seed extracts is the product of the *Gpi-B1* gene located on the short arm of chromosome 1B (Chojecki and Gale 1982). We distinguished 9 or 10 bands in the GPI banding

patterns of 1B homozygous or 1B,1BL/1RS heterozygous wheats. Homozygous 1BL/1RS wheat lines expressed six to eight bands with the most cathodal band being absent (**Figure 7.4**), serving as a distinct marker in the differentiation of the homozygous 1BL/1RS types from the homozygous 1B or the 1B,1BL/1RS heterozygote genotypes. This initial screening has the advantage of rapidly eliminating the 1BL/1RS homozygotes, and then we only have to do a subsequent A-PAGE analysis on those endosperm halves that exhibit the 1B banding characteristic. The GPI banding profiles of CS and the 1R disomic addition line were similar (Figure 7.4). This may be due to cofocusing of the rye GPI bands with those of CS as suggested earlier (Chojecki and Gale 1982). A single GPI IEF gel enables rapid screening of at least 48 cut endosperms.

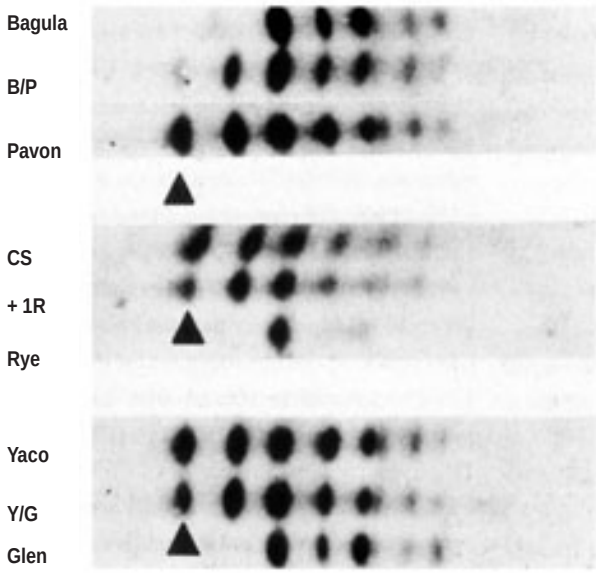

Figure 7.4. Grain glucose phosphate isomerase banding profiles of two different combinations of parents and their F1 progeny. Banding profiles of Chinese Spring (CS), the 1R disomic addition line (+1R), and Imperial rye (Rye) are also shown. The marker band from the short arm of chromosome 1B is indicated by the arrows.

The ω-gliadin region in the banding patterns resolved by A-PAGE electrophoresis is characterized by two distinct gliadin marker bands specific for Imperial rye (**Figure 7.5**). Genes for these two distinct marker bands are located in the short arm of *S. cereale* chromosome 1R (Friebe et al. 1989) and have been diagnosed as products of the rye *Sec-1* locus (Lawrence and Shepherd 1980). These rye-specific bands are clearly present in the CS+1R disomic addition line as well as in the 1BL/1RS homozygous parents (Figure 7.5). Homozygous 1B wheats (e.g., CS and Yaco) show some bands in the ω-gliadin region, controlling genes that apparently belong to the short arm of 1B, but these bands are distinctly different from the two bands of rye (Figure 7.5). The translocation heterozygote 1B,1BL/1RS shows the two rye-specific bands but, in addition, it also has the bands specific to the homozygous 1B parent. This group of bands resulting from the genes located in 1RS + 1BS unequivocally diagnoses the translocation heterozygotes (Figure 7.5), hence enhancing the efficiency of substitution line development in homozygous 1BL/1RS or 1B *T. aestivum* cultivars (William et al. 1992).

Both techniques are nondestructive so their embryos can still germinate after their endosperms are analyzed. The procedures also allow us to analyze a large number of samples with a high degree of accuracy. As a consequence, we can extend the development of the 1B or 1BL/1RS substitution lines in

T. aestivum over a wider range of cultivars than we did earlier (ter Kuile et al. 1990). Once these stocks are developed, they will form a valid basis of evaluating the desirable agronomic as well as quality traits associated with the 1RS chromosome arm in bread wheats with the translocation, or where the translocation has been incorporated in 1B,1B wheat cultivars.

Durum wheat—When developing isozyme markers for identifying the translocation heterozygote 1BL/1RS,1B in durum wheat substitution lines, we could identify the heterozygote by using the GPI banding profile alone (**Figure 7.6**). GPI subunits of chromosome 1D behave similarly to GPI-R1 subunits (Chojecki and Gale 1982). Durum wheat cultivars have a banding profile with four bands, whereas bread wheats have 9 or 10 bands with the additional bands falling within the

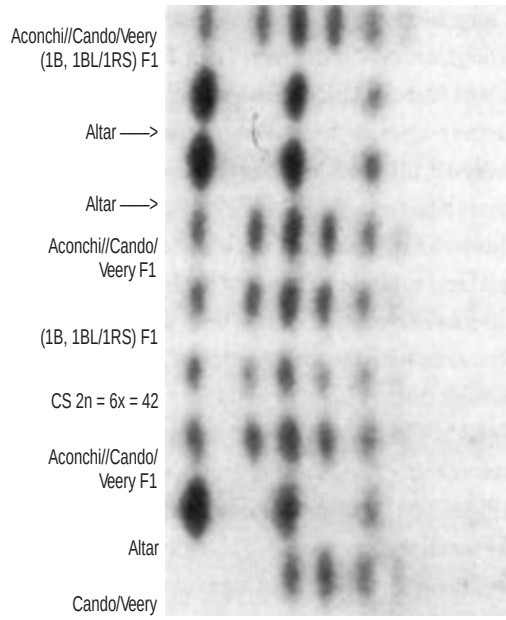

Figure 7.6. Grain glucose phosphate isomerase (GPI) banding patterns of 1B/1B durums, 1BL/1RS,1BL/1RS durums, and the heterozygote 1B,1BL/1RS durums on IEF (pH 5-8.5).

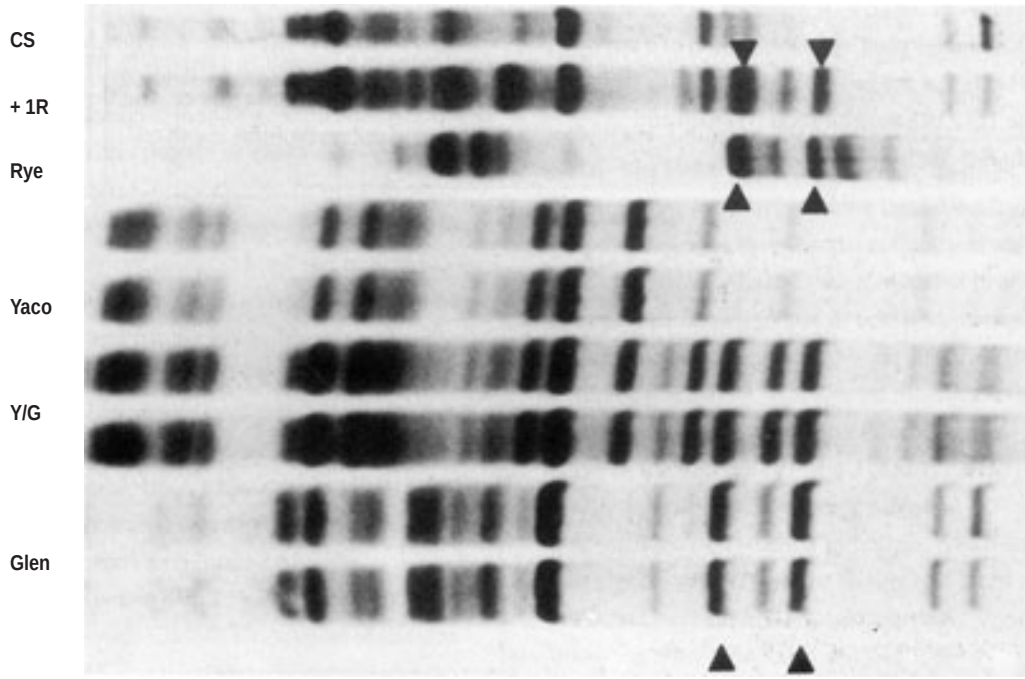

Figure 7.5. Gliadin banding profiles of two parents and their F1 progeny. Banding profiles of Chinese Spring (CS), the 1R disomic addition line (+1R), and Imperial rye (rye) are also shown. The marker bands of rye are indicated by arrows.

T. turgidum banding profile (Figure 7.6). *T. turgidum* cv. Cando/Veery and *T. aestivum* cv. Seri M 82, both 1BL/1RS homozygous, exhibited 8 bands whereas the F1 of the Aconchi//Cando/Veery 1B,1BL/1RS heterozygote showed 9-10 bands similar to *T. aestivum* cv. CS (Figure 7.6). This was expected since *Gpi-A1* and *Gpi-B1* gene products exhibited a banding profile specific to durums homozygous for 1B with 4 bands. However, with the introduction of the GPI-R1 subunit from rye, the F1 heterozygote produced Gpi-A1, GPI-B1, and GPI-R1 subunits. Upon recombining the GPI-R1 subunits with those of GPI-A1 and GPI-B1, the durum heterozygous for the translocation gave a GPI banding pattern essentially similar to CS. Comparison of GPI banding patterns among the Altar 84 (homozygous for 1B), Cando/Veery (homozygous for 1BL/1RS), the F1 heterozygote of Altar//Cando/Veery, and CS reconfirms that the GPI-R1 subunits behaved and interacted with GPI-A1 and GPI-B1 subunits in a similar manner to the GPI-D1 subunits. This comparison also enables positive identification of durum wheats that are heterozygous (1B,1BL/1RS) for the 1BL/1RS translocation (William and Mujeeb-Kazi 1993a).

Other Detection Techniques

Other techniques we have used to detect alien chromosomes in wheat, but not described here, include chromosome morphology as shown by differential banding techniques, chromosome pairing relationships, and plant phenotypic differences. Differential C and N bandings can be reliable and useful techniques for alien chromosome characterization and identification. Other available cytological markers include chromosomal size/arm ratios and secondary constrictions (Mujeeb-Kazi and Kimber 1985, Mujeeb-Kazi and Miranda 1985). Some of the most commonly used phenotypic features include spike morphology, presence of anthocyanin pigmentation, grain color, grain surface texture, and stem morphology. These morphological traits are subject to modification upon introgression of alien genetic material and therefore can be used in the characterization of alien chromatin. However, there are difficulties in efficient use of these traits due to their modification because of environmental conditions (e.g., moisture availability, fertility), dominant/recessive inheritance, and the low amount of polymorphism involved.

Chapter 8

Applications of Molecular Markers in Wheat Wide Crosses

Manilal D.H.M. William and Abdul Mujeeb-Kazi

Detecting alien introgressions and chromosomal interchanges is becoming increasingly dependent on the use of molecular markers. Initial work of this kind depended on the presence of repetitive DNA sequences that make up between 70 and 80% of the cereal genomes. About 75% of the three wheat genomes are made up of repeated DNA sequences; about 20-25% of the genomes are made up of nonrepeated sequences (Flavell and Smith 1976). Most of the nonrepeated sequences of the wheat genomes are about 1000 nucleotides long and interspersed between the repeated sequences (Moore et al. 1993).

Repeated sequences undergo changes such as amplification, deletion, and divergence (Flavell 1982)—changes that have resulted in their use as probes with genome- or chromosome-specificity. In this regard, heterochromatic rye sequences were among the first to be used as DNA probes to identify rye addition lines and translocation lines in wheat through southern hybridizations (Appels and Moran 1984). The molecular techniques we use in the CIMMYT Wheat Wide Crosses Laboratory currently include *in situ* hybridization and randomly amplified polymorphic DNA sequences (RAPDs) based on polymerase chain reaction (PCR). We also plan to explore restriction fragment length polymorphism (RFLP) techniques through linkages with CIMMYT's Applied Molecular Genetics Laboratory and other molecular laboratories that may have use for our germplasm.

Molecular Marker Techniques

In situ hybridization

The *in situ* hybridization technique involves hybridization of labeled DNA and subsequent detection on a cytological chromosomal preparation on a microscope slide. Radioactive labeling and autoradiographic detection techniques were used earlier (Gall and Pardue 1969, Gerlach and Peacock 1980). The major limitations with radioactive labeling are poor resolution due to increased background dispersion because of radioactivity and the longer exposure time required—not to mention the safety hazards of handling radioactive material. Wide adaptability of *in situ* hybridization procedures was made possible by the development of nonradioactive labeling techniques. Generally, these techniques depend on labeling DNA with a hapten—like biotin or digoxigenin—for which a strong ligand, such as streptavidin or another specific antibody, is well characterized. The ligands are conjugated to an enzyme such as peroxidase or acid phosphatase, which converts an enzyme-specific substrate into a colored precipitate. More recent modifications of the technique involve using fluorochromes either as the haptens or instead of enzymes to enable detection. Besides being safer and quicker, the biotin labeling technique, which was first used in animal systems (Singer and Ward 1982) and later applied to plant material involving wheat (Rayburn and Gill 1985b), increases the resolution of *in situ* hybridizations.

When *in situ* hybridization techniques are used to detect alien introgressions, the use of total genomic DNA as a probe is sometimes more advantageous than a cloned repetitive DNA sequence. With probes, hybridizations usually occur in limited areas of chromosomes unless the probe used is dispersed and highly repetitive. When using a repeated sequence probe from rye in *in situ* hybridizations, Lapitan et al. (1986) observed unequal hybridizations in different regions of the rye chromosome segments. Bedrook et al. (1980) and Jones and Flavell (1982) reported major interstitial sites and the presence of some cross hybridization to wheat when they used a repeated rye sequence as a probe. Rayburn and Gill (1985b) observed 24 hybridization sites in 11 chromosomes of wheat when they used a rye sequence probe, pSc 119.

Schwarzacher et al. (1989) developed a technique in which they used a labeled total genomic DNA as a probe, which resulted in uniform labeling along the whole chromosome length. This allowed tracking of the chromosome at interphase and prophase and enabled the identification of chromosomal interchanges involving small chromosomal segments. This technique was later refined by using labeled genomic DNA from one species in a cross as a probe and higher quantities of sheared total genomic DNA from the other species of the same cross as a blocking agent to avoid cross hybridization between homoeologous sequences of the two species. It has been used in the identification of 1BL/1RS wheats (Le et al. 1989, Heslop-Harrison et al. 1990), triticale (Le et al. 1989), and other alien chromosomes in wheat backgrounds (Anamthawat-Jonsson et al. 1990; Schwarzacher et al. 1992; Friebe et al. 1992, 1993; Anamthawat-Jonsson and Heslop-Harrison 1993; Mukai et al. 1993).

In situ hybridization techniques have been further used in genomic studies (Lapitan et al. 1987), evolutionary relationships (Rayburn and Gill 1985a), and physical mapping of plant chromosomes (Leitch et al. 1991). Mouras et al. (1989) have used the technique to identify single-copy DNA sequences in tobacco.

Polymerase chain reaction (PCR)

PCR is an *in vitro* method of synthesizing nucleic acid by which a particular DNA segment can be specifically replicated (Mullis and Faloona 1987). It involves two oligonucleotide primers that flank the DNA fragment to be amplified, repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequence, and extending the annealed primers with thermo-stable *Taq* DNA polymerase. In a typical PCR amplification, two oligonucleotide primers hybridize to specific sequences in opposite strands of duplex DNA. The orientation of the primers is such that elongation occurs inward when the DNA is amplified using *Taq* polymerase. Therefore, typical PCR amplifications result in an exponential increase in the target sequence of DNA flanked by the two primers.

Another potential set of molecular markers involves use of randomly amplified polymorphic DNA sequences (RAPDs), based on the PCR. In utilization of RAPDs, randomly synthesized short sequences of oligonucleotide primers are used to generate amplified DNA sequences. The polymorphisms generated using PCR are based upon the distance between the two sequences in the genomic DNA complementary to the oligonucleotide primers. These have the potential to diagnose alien chromatin presence in a wheat background.

RFLPs

RFLP methodology depends on the use of DNA probes for detecting alien introduction sources at the molecular level. Most work in this regard has centered on cloning of repetitive DNA sequences for developing species-specific probes and ascertaining the presence or absence of polymorphisms between species (Appels and Moran 1984, Metzlaff et al. 1986). Although there are advantages, such as the availability of a multitude of repeated sequences in the cereal genomes and presence of adequate species divergence correlated with the formation of new repeat families, large homoeologies also exist among the repetitive DNA fractions of most Triticeae species, which requires screening of a large number of clones. Another possible disadvantage (e.g., rye heterochromatic sequences) is that repeated DNA sequences isolated as probes may be localized to specific regions of the chromosome (Bedbrook et al. 1980). This makes it difficult to detect introgressions involving small chromosome segments. However, with rye, dispersed repeated sequences have also been identified that can detect each rye chromosome in a wheat background (Appels et al. 1986). RFLP-based methods have also been used to characterize wheat-rye recombinant lines (Koebner et al. 1986), wheat-barley addition lines (Heun et al. 1991), and studies of genome relationships (Zhang and Dvorak 1991).

Applications

Since *Thinopyrum bessarabicum* is an important source of salinity tolerance (see Chapters 4 and 7), we have crossed it with bread wheat at CIMMYT to produce disomic addition lines. Using this cytogenetically advanced combination has allowed us to characterize a large number of biochemical markers (see Chapter 7). With the objective to develop molecular markers for tracking *Th. bessarabicum* chromatin in wheat backgrounds and subsequently detect subtle introgressions, we have conducted genomic *in situ* hybridizations using the amphiploids of *T. aestivum* cv. Chinese Spring (CS) x *Th. bessarabicum* as well as disomic addition lines of *Th. bessarabicum* in wheat. Total genomic DNA from *Th. bessarabicum* was used as the probe. We have also developed RAPD-based markers for the same hybrid combination to enhance diagnostic capabilities.

In situ hybridization

Genomic applications—For chromosome preparations, we germinated and treated seeds of the amphiploid and the wheat parent as described by Rayburn and Gill (1985b). The seeds were placed on wet blotting paper and allowed to imbibe at room temperature for the first 24 hours, then the temperature was decreased to 4°C for the next 24-48 hours, followed by another 24-48 hours at room temperature. Seedling root tips were harvested and pretreated for 24 hours in ice water in a refrigerator. After fixation in a 3:1 solution of ethanol:acetic acid for two-four days, the root tips were stained in 1% acetocarmine for 15-30 minutes and squashes on slides were made. Slides were stored in a freezer at -70°C to await hybridization.

We used the procedure of Hoisington at al. (1992) to isolate total genomic DNA from CS and *Th. bessarabicum*. The CS DNA that was used as the blocking DNA was sheared to 500-2000 bp by passing it several times through a 1-ml tuberculin needle with a 24G needle attached. The *Th. bessarabicum* DNA was nick-translated with biotinylated-11dUTP using a kit obtained from Enzo Diagnostics, Inc.

For *in situ* hybridization, slides were taken from the freezer and their coverslips were removed. The chromosomes were denatured using 70% Formamide at 70°C and rapidly dehydrated (Rayburn and Gill 1985b). The genomic *in situ* hybridization and detection procedures were similar to those of Mukai and Gill (1991). We used the Detek-Hrp kit (Enzo Diagnostics, Inc.) to detect the biotinylated probe using the substrate diaminobenzidine tetrahydrochloride (DAB) from Sigma.

After genomic *in situ* hybridization of the CS/*Th. bessarabicum* amphiploid, 14 of the 56 chromosomes were brown in color. The remaining 42 chromosomes were all light blue (**Plate 1**). The unlabeled CS DNA effectively kept the labeled *Th. bessarabicum* DNA from hybridizing to the 42 wheat chromosomes. When CS DNA was not used as a block, all 56 chromosomes of the amphiploid appeared brown.

We believe these results indicate that enough homoeology exists between *Th. bessarabicum* and wheat so that, unless wheat DNA is used as a blocking agent, *Th. bessarabicum* DNA can cross-hybridize with wheat DNA. Another indication of this residual homoeology is the stringency of the post-hybridization washing. When post-hybridization washes were performed at 37°C (Mukai and Gill 1991), the 42 wheat chromosomes did not appear blue, but were slightly brown. Differentiation between the light brown wheat and dark brown *Th. bessarabicum* chromosomes was still possible, but it was not easy. Upon raising the post-hybridization washing temperature to 45°C, the 42 wheat chromosomes appeared blue with very little, if any, brown color. These results also show that there was enough homoeology between wheat and *Th. bessarabicum* to warrant more rigid washing conditions. The conditions we used were more rigorous than those of Heslop-Harrison et al. (1990); however, they used 20 to 30 times more blocking DNA than we did. This difference in blocking DNA concentration could be a factor in obtaining cross hybridization between alien and wheat DNA. Obtaining optimum differentiation between wheat and alien chromosomes with genomic *in situ* hybridization may be the function of a proper balance between blocking DNA amount and the rigor of post-hybridization washing conditions.

We found the genomic *in situ* hybridization to be a rapid method to detect *Th. bessarabicum* chromosomes in a wheat background. The stage is now set for us to apply genomic *in situ* hybridization to analyze advanced derivatives of this cross. The significance of this technique is that we will now be able to detect subtle alien DNA introgressions in wheat backgrounds that are difficult to detect with conventional methods. Using this technique, we have also been able to detect the presence of rye chromatin in the rye disomic addition 1R and the rye translocations 1BL/1RS, 5AS/5RL, 1AL/1RS, and 6BS/6RL (Mujeeb-Kazi et al. 1993a). However, the ratios of blocking DNA and the stringency of the washes were different from those used for *Th. bessarabicum*.

Fluorescent *in situ* hybridization (FISH) applications—Through the assistance of Dr. M.N. Islam-Faridi (CIMMYT Maize Program), we have applied fluorescent *in situ* hybridization (FISH) applications for diagnosing the presence of entire alien chromosomes, translocated chromosome arms, or smaller introgressed chromosome segments in wheat backgrounds. The procedures are similar to those described above (Rayburn and Gill 1985b) except that fluorescence was used as the detection diagnostic (Schwarzacher et al. 1989). **Tables 8.1** and **8.2** provide the germplasm and mixture details. Our

Table 8.1. Germplasm used for FISH analyses with cytological and origin details.

Germplasm	Cytology	Origin
X *Triticosecale*	2n=6x=42, AABBRR	CIMMYT
T. aestivum cv. CS+1R1R	2n=6x=42+2=AABBDD+1R1R	E.R. Sears
T. aestivum cv. CS+6R6R	2n=6x=42+2=AABBDD+6R6R	E.R. Sears
T. aestivum cv. Tam 200	2n=6x=42;1AL/1RS,1AL/1RS	Texas A & M
T. aestivum cv. Seri M 82	2n=6x=42;1BL/1RS,1BL/1RS	CIMMYT
T. aestivum	2n=6x=42;1BL/1RS, 1BL/1RS and 5AS/5RL, 5AS/5RL	CIMMYT & IPSR
T. aestivum	2n=6x=42;6BS/6RL, 6BS/6RL	Univ. of Illinois
T. turgidum cv. Altar 84	2n=4x=28;1BL/1RS, 1BL/1RS	CIMMYT
T. aestivum/Th. bessarabicum//S. cereale	2n=5x=35;ABDJR	CIMMYT

IPSR = Institute of Plant Science Research, Cambridge, U.K.

Table 8.2. Specific details of the FISH mixture used for different germplasm comprising of *Triticum aestivum* (CS), *T. turgidum* (A), *Secale cereale* (R), *Thinopyrum bessarabicum* (J), *Th. elongatum* (E), and *T. tauschii* (D).

Germplasm	De-ionized formamide (%)	Dextran sulfate (%)	SSC (%)	Labeled probe		Block DNA[a]	Volume used (µl/slide)
				Biotin	Digoxigenin		
X *Triticosecale*	44.01	8.80	3.52	60ng R	—	18x A	28.40
T. aestivum cv. CS+1R1R	46.99	9.40	3.76	40ng R	—	20x CS	26.60
T. aestivum cv. CS+6R/6R	47.17	9.43	3.77	40ng R	—	20x CS	26.50
T. aestivum cv. TAM 200	41.00	8.25	1.47	40ng R	—	20x CS	27.25
T. aestivum cv. Seri M 82	41.00	8.25	1.47	40ng R	—	20x CS	27.25
T. aestivum	41.00	8.60	1.72	40ng R	—	20x CS	29.00
T. aestivum	41.00	8.25	1.47	40ng R	—	20x CS	27.25
T. turgidum cv. Altar 84	48.45	9.69	3.88	35ng R	—	25x A	25.80
T. aestivum/Th. bessarabicum//S. cereale	38.09	7.94	3.17	40ng J	40ng R	20x CS	31.50
T. aestivum Lr19	48.58	9.71	3.88	40ng E	—	20x CS	25.75
	36.23	7.25	2.90	40ng E	50ng D	20x CS	34.50
T. aestivum Lr24	58.54	9.71	3.88	40ng E	—	20x CS	25.75
T. aestivum Lr25	44.91	8.98	3.59	50ng R	—	20x CS	27.80
T. aestivum Lr29	48.54	9.71	3.88	40ng E	—	20x CS	25.75
T. aestivum Lr37	44.56	8.91	3.57	70ng D	—	18x A	28.00

[a] 20x = 20 times amount of probe DNA as an example of values in the column.
SSC = Saline solution concentration (Sodium citrate + Sodium chloride).

program also tested for alien DNA introgressions of *Lr19,24,25,29,* and *37* in leaf rust isogenic lines of *T. aestivum* cv. Thatcher (Mujeeb-Kazi et al. 1993c).

Using total genomic DNA of *S. cereale* as a probe enabled us to identify complete rye chromosomes (**Plate 2a**), rye chromosome additions (**Plate 2b**), and wheat/rye translocations in metaphase cells (**Plates 2c-d**; **3a,c-d**; **4a-b**) or in interphase (**Plate 3b**). Distortion of chromosome morphology was not prevalent. The translocation break-points were exceptionally clear as centric break-fusion products. The *in situ* hybridization signals with fluorescein-avidin and propidium iodide were distinctly identified by their yellow and orange to red fluorescien, respectively, under Zeiss filter 9. The DNA-specific dye DAPI used earlier gave uniform blue fluorescence (Schwarzacher et al. 1992) with Zeiss filter 2 and the alien chromosomes could not be distinguished from those of wheat. We have been able to observe the hybridization signals with DAPI using Zeiss filter 2 repeatedly and obtained exceptional clarity in contrast (Plates 2 and 3). Double labeling also enabled the detection of *Th. bessarabicum* and *S. cereale* chromosomes (**Plate 4c**), thus opening the possibility of using alien species amphiploids as bridge parents in crosses with wheat. Alien DNA introgression in all *Lr* lines was possible; *Lr25* is included in the composite of **Plate 4d**.

The rye translocations have characteristics that contribute to resistances or tolerances to biotic or abiotic stresses. These are specifically:

- 1BL/1RS: resistance genes for leaf, stem, and stripe rusts and mildew.
- 1AL/1RS: greenbug resistance.
- 5AS/5RL: copper up-take efficiency.
- 6BS/6RL: cereal cyst nematode resistance.

Other rye chromosomes not yet involved in translocations have, as disomic additions to wheat, been associated with resistance to Karnal bunt. As discussed, *Th. bessarabicum* contributes to salinity tolerance.

Conclusions—It has been fortunate that whole arm translocations (1BL/1RS or 1AL/1RS) have yielded superior cultivars that are grown on more than 5 million hectares around the world. However, when utilizing wild relatives in crop improvement, the ideal alien transfer should involve a minimal amount of alien DNA since it brings in less undesired alien genetic material. We anticipate that, when such transfers—engineered through genetic manipulation strategies—are detected, the power of this diagnostic tool will be fully realized. The *in situ* hybridization technique is rapid, sensitive, and gives superior resolution provided that somatic preparations possess quality chromosome separation with minimum cytoplasmic debris and have a desirable mitotic index.

PCR-based RAPD markers

We developed PCR-based RAPD markers to identify the amphiploid of *T. aestivum* x *Th. bessarabicum* (2n=8x=56; AABBDDJJ). These markers will be used on disomic addition lines of *Th. bessarabicum* to evaluate their chromosome specificities.

We evaluated 26 short oligonucleotide primers (Operon, Inc., California, USA). The PCR reactions were carried out in a 25-µl reaction mixture consisting of 20 ng genomic DNA; 0.1 mM each of dATP, dCTP, dGTP, and dTTP (Perkin-Elmer Co., Connecticut, USA); 0.001% gelatin (Sigma Chemical Co.); 2.0 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100; 0.75 units of *Taq* polymerase ($MgCl_2$ and a

10x dilution buffer, Promega Chemicals Co., Wisconsin, USA), and 15 ng of the respective primers. The themocycler cycles were: one cycle for 2 min. at 93°C and 35 cycles for 1 min. at 92°C, 1 min. at 36°C, and 1 min. at 71°C. At the end of 35 cycles, a temperature of 72°C was maintained for 5 min. prior to terminating the reaction. The reaction mixtures were overlaid with 30 ml of mineral oil before the differential temperature treatments. Approximately 10 ml of the reactions were separated on 1.1% agarose gel in a BRL Horizon 11.14 gel apparatus at 60V for 3.5 hours and viewed under ultraviolet light.

Genomic DNA can be amplified and polymorphisms detected with single primers of arbitrary sequences using PCR (Williams et al. 1990, Welsh and McClelland 1990). Chromosome-specific RAPD markers have also been developed in tomato (Klein-Lankhorst et al. 1991). Our results indicate that genomic DNA in complex hexaploid wheats can be amplified using arbitrary primers of 10 nucleotides in length. Of the 26 primers we evaluated using CS, *Th. bessarabicum*, and their amphiploid, seven primers showed easily detectable polymorphisms among these three species. These seven primers and their sequences are:

- L 5—'5ACGCAGGCAC3';
- L 16—'5AGGTTGCAGG3';
- L 18—'5ACCACCCACC3';
- L 20—'5TGGTGGACCA3';
- N 1—'5CTCACGTTGG3';
- N 3—'5GGTACTCCCC3';
- N 5—'5ACTGAACGCC3'.

The use of PCR-based RAPD markers is still a novel technique for identifying alien genetic material compared to morphological and biochemical markers. Therefore, it may not yet be feasible to allocate marker bands to specific chromosomes or chromosome arms unless the materials used for PCR have already been characterized using the conventional techniques. However, if a given addition or a substitution line is identified with the conventional techniques—and since polymorphisms generated by PCR are most probably based on short repetitive DNA sequences—it may be possible to track the minute chromosomal alien introgressions more effectively using the PCR technique, provided that it is capable of detecting and amplifying the alien-specific sequences in the introgressed material.

D'Ovidio et al. (1990) reported amplification of wheat genomic DNA using primers specific to the gamma gliadin gene. Wheat genomic DNA has also been amplified by using random primers coupled with intron-specific primers (Weining and Langridge 1991).The results of our study show that genomic DNA can also be amplified using random primers (Devos and Gale 1992). In an initial study using different concentrations of DNA and *Taq* polymerase, we found that under our conditions optimum results can be obtained with 20 ng DNA and 0.75 units of *Taq* polymerase in the 25-µl reaction.

Since sequence information in wheat is limited and not available in *Th. bessarabicum*, we propose the use of random primers in tracking alien chromatin in the wheat background. **Figure 8.1** shows the patterns of amplification products when different random primers were used to amplify the genomic DNA. For all seven primers, there were some amplification products in the amphiploid that were common to wheat and *Th. bessarabicum*. The marker amplification products present in the amphiploid specific to *Th. bessarabicum* are marked in Figure 8.1. To establish the repeatability of the patterns of amplification products, the same primer was

used at least three times with no observed differences in the patterns of the repeated runs. Further, to evaluate whether there was polymorphism in the patterns of amplification products among different plants, DNA was isolated from individual seedlings of CS and amplified using two different primers, i.e., primers N 1 and N3 (**Figure 8.2**). This demonstrates a lack of polymorphism in the DNA extracts from individual CS seedlings for the products that are amplified at high intensity. Although the DNA of the amphiploid contains the genomic DNA of both CS and *Th. bessarabicum*, in some cases the amphiploid did not show the complete profiles of the two parents. However, the amphiploid showed no additional amplification products not present in the two parents.

In RAPDs, primer binding to a complementary sequence is a random process. Due to the genome size increase in the case of the amphiploid, the availability of a particular sequence in 20-ng DNA is less compared to that of the two parents. Further, in RAPDs, primers may bind when there is incomplete homoeology. It is also possible that greater similarity between a primer and the template of one of the parental genomes results in preferential amplification of this sequence in the amphiploid. This may explain why the amphiploid did not have all the bands that were present in CS and *Th. bessarabicum*. However, the consistency in the patterns of amplification products for a given primer indicates that the markers common to the alien species could be effectively used, although the amphiploid does not contain all the amplification products specific to the alien species. The CIMMYT Wide Crosses Section has produced several disomic addition lines of *Th. bessarabicum* (2n=6x=42+2), which are in agronomically superior plant types, possess high fertility, and are cytologically stable with a high frequency of 22 bivalents at meiosis. We are now

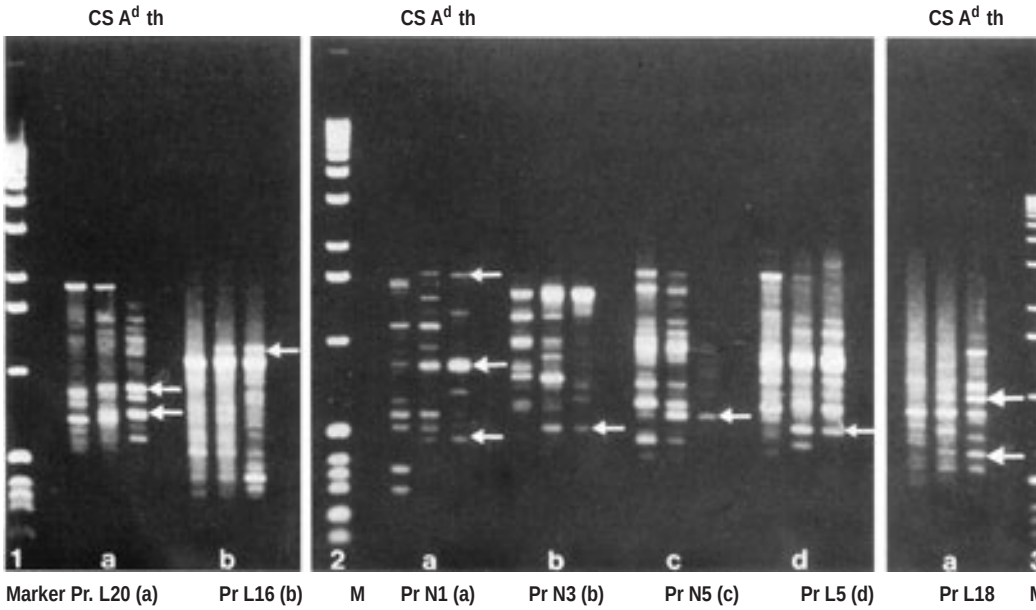

Figure 8.1. Amplification of genomic DNA (20 ng) of *Triticum aestivum* cv. Chinese Spring (CS), *Thinopyrum bessarabicum* (th), and the amphiploid of CS/*Th. bessarabicum* (A^d). Bands observed in the amphiploid specific to *Th. bessarabicum* are indicated by arrows. The standards (1,2,3) and the respective primer runs are indicated as 1, a, and b; 2, a to d; and 3 and a.

characterizing these additions with isozyme, cytological, and RAPD markers (Mujeeb-Kazi 1993, William et al. 1994).

Although the use of biochemical and cytological markers may be convenient in identifying disomic addition lines, they have a limited use in tracking small segments of alien chromatin in a wheat background. We propose that, once a disomic addition line has been characterized by other means, PCR-based RAPD markers may be used to identify these and subsequently extended to detect alien introgressions involving small segments of alien chromatin in wheat backgrounds. Our initial study shows the presence of the marker amplification products of *Th. bessarabicum* in the amphiploid. Locating the chromosomes of these markers would be subsequently undertaken using disomic addition lines.

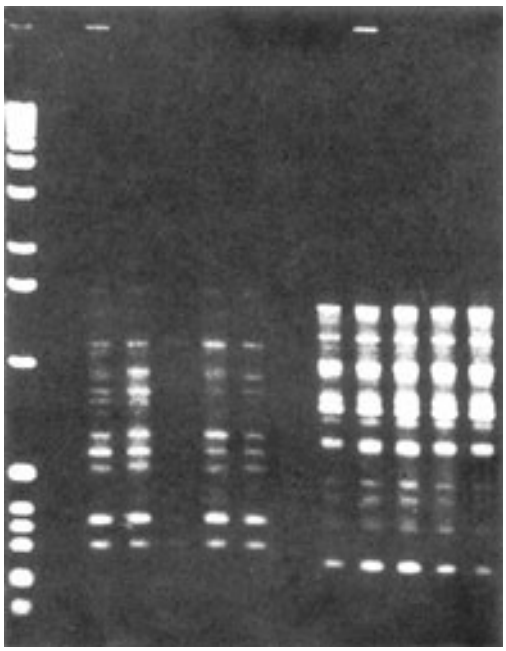

Figure 8.2. Amplification of genomic DNA of *Triticum aestivum* cv. Chinese Spring (20 ng) using the primers N 1 and N 3. Each track indicates amplification products from DNA extracts from different seedlings of CS. Track 3 with the first primer did not resolve due to lack of photographic quality.

RFLP markers

RFLP markers are another tool that could be applied in wheat wide crosses. Although we have not yet done any work with these, we anticipate that CIMMYT's Applied Molecular Genetics Laboratory will play a key role in applying RFLP methodology to wheat wide cross derivatives.

Conclusions

The molecular techniques discussed in this chapter may be useful in tracking introgressions involving small segments of alien chromatin in wheat backgrounds. Although it is important to develop molecular markers using less advanced material such as amphiploids and addition lines, initial identification and characterization can be done using the relatively inexpensive, more universal, and less complicated cytological techniques or biochemical markers (Chapter 7). Once the material is characterized, molecular markers could be established and subsequently used to detect the presence of minute chromosomal alien introgressions. It seems to us that researchers now attach emphasis to germplasm development suited to exploit the full diagnostic power of these molecular techniques, which apparently are far beyond identification of complete alien chromosomes that, at this stage, is receiving more attention. Now is the time to design effective applied studies that are based upon homoeologous exchanges where the use of the chromosome 5B *ph1b* system is one aspect.

CHAPTER 9

Conclusions and a Look to the Future

Abdul Mujeeb-Kazi

Wide crosses among species of the Triticeae are nothing new. A century has passed since the first triticale was produced (Rimpau 1891) and nearly 90 years since Farrer (1904) described the first wheat x barley cross. Over the decades of the 20th century, interspecific hybridizations have progressed rather quickly, but intergeneric hybridizations—due to their complex nature—have taken a slower and more involved route.

It is doubtful if there is a group within the Triticeae that cannot be hybridized with wheat, although we have not made serious efforts to utilize some annuals like *Eremopyrum*, *Henrardia*, *Heteranthelium*, and *Taeniantherum*. For practically oriented intergeneric hybrid programs, we believe that once a hybrid plant is produced, there are relatively few limitations to restrict development of the advanced derivatives. Manipulation procedures for hybrid production can enable any competent laboratory to produce viable plants. Enough information now exists to permit technicians to have success in terms of frequencies and novel combinations.

We are optimistic that most restrictions on crossability in the grass family can be overcome. As evidence, we cite some of the successful crosses between wheat and alien species outside the Triticeae including:

- *Zea mays* (Inagaki and Tahir 1990, Laurie and Bennett 1988c, Suenaga and Nakajima 1989, Riera-Lizarazu and Mujeeb-Kazi 1990, Riera-Lizarazu et al. 1992);
- *Pennisetum glaucum* (Ahmad and Comeau 1990);
- *Sorghum bicolor* (Laurie and Bennett 1988b, Ohkawa et al. 1992);
- *Z. mays* ssp. *mexicana* (Ushiyama et al. 1991);
- *Tripsacum dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993).

Additionally, there have been successes with some difficult crosses between wheat and alien species within the Triticeae including:

- *Psathyrostachys juncea* (Mujeeb-Kazi and Asiedu 1990, Plourde et al. 1990);
- *Elymus scabrus* (Ahmad and Comeau 1991);
- *Agropyron cristatum* (Chen et al. 1989; Limin and Fowler 1990).

More recently, in our efforts to make alien variability available for *T. turgidum* improvement, several hybrids and amphiploids derived from crosses of durum x alien species (**Appendix 5**) have been produced (Mujeeb-Kazi 1992).

The Radical Approach in Intergeneric Hybridizations

Beyond actual hybrid production, successful use of alien variability in wheat improvement is a slow process. We have attempted to hasten this aspect in our intergeneric program at CIMMYT in a somewhat radical approach that de-emphasizes confirmation of the presence of alien introgression. For example, elite germplasm developed from derivatives of *Thinopyrum distichum* has been: 1) used in CIMMYT's wheat

breeding programs; 2) distributed to national programs; and 3) released as varieties, such as Pasban-90 for irrigated and saline sodic soils and Rohtas-90 for rainfed areas in Pakistan's Punjab.

Most recently, the derivatives of a cross involving *Th. curvifolium* have led to selections that exhibit superb resistance to spot blotch caused by *Helminthosporium sativum* (Villareal et al. 1993; Mujeeb-Kazi et al. 1994d). These selections are superior to the material previously available to wheat breeders (e.g., cultivar BH 1146 and some *T. aestivum* cultivars of Chinese origin). The *Th. curvifolium* derivatives have also shown promise for resistance to serious wheat diseases caused by *H. tritici-repentis*, *Fusarium graminearum*, and *Septoria tritici*. We have widely distributed this germplasm and it is being extensively utilized by CIMMYT's wheat breeding program in Mexico.

We have yet to confirm conclusively the alien introgression(s) in these derivatives of *Th. distichum* and *Th. curvifolium*. However, initial investigations using the A600 *Th. elongatum* probe (courtesy R. Appels, CSIRO, Australia), applied to *Th. curvifolium* derivatives, do show the presence of an alien insert band. Further repetitious and elaborate testing is forthcoming. Unless unequivocal data emerge to confirm alien introgression, we hypothesize these significant salt tolerance and spot blotch resistances to be a consequence of transgressive segregation, involving Inia/Genaro and Chinese Spring//Glennson 81/3/Alondra/Pavon, respectively. In any event, we are pleased with our fortuitous success because our ultimate goal is to contribute to wheat improvement. Perhaps forthcoming diagnostic techniques (e.g., the A600 probe) will further unravel the presence of alien introgressions. In the mean time, the stable status of the derivatives permits their exploitation by breeding programs. Since little is known about the genetics of *H. sativum* resistance, we plan to conduct simultaneous genetic analyses on the *Th. curvifolium*-resistant material. Since the susceptible cultivar Ciano T 79 is highly polymorphic (RFLP), the mapping protocols are available for us and others to pursue this avenue of research.

We consider this radical approach to be a rapid mechanism to get needed germplasm to CIMMYT breeders. Since most of our F1 hybrids were derived from crosses between alien species and the bread wheat Chinese Spring (a poor agronomic variety that is highly crossable), we were compelled to topcross these hybrids to bread wheat varieties with good agronomic backgrounds (Mujeeb-Kazi et al. 1987, 1989). We screened the agronomically superior derivatives and selected resistant/tolerant materials for increase and distribution. With this reverse strategy of leaving scientific questions unanswered, at least for the time being, we were able to distribute germplasm with needed attributes that had not been obtained by breeders in their conventional programs that used similar cultivar combinations.

To a considerable extent, basic research will resolve questions that we leave unanswered. However, over the last four or five years, we have embarked on a more meticulous methodology with our intergeneric hybridizations that provides an opportunity for more basic research. These basic activities involve: 1) systematically producing disomic alien chromosome additions, 2) screening these materials, 3) developing substitution lines from desirable selections, 4) inducing translocations, and 5) integrating, at appropriate stages, genetic

manipulation methodology based around the *Ph* locus of chromosome 5B. Cytological markers assist the process; morphological and biochemical markers aid in establishing wheat/alien chromosomal homoeology. Reciprocal crosses and production of amphiploids are also contributing to success in this area. This high precision program is likely to narrow the range of research investigations we undertake in the future.

Short-Term Returns with Interspecific Hybridization

As explained in Chapter 3, interspecific hybridization offers short-term payoffs in our quest to introgress alien variability into wheat. Our priority has been to exploit variability in the D genome of *Triticum tauschii* through production of synthetic hexaploid wheats (*T. turgidum* x *T. tauschii*) and via direct crosses of *T. aestivum* x *T. tauschii*; in the latter crosses, the *T. aestivum* cultivars are susceptible to such diseases as helminthosporium, fusarium, septoria, and Karnal bunt (see Chapter 3). Questions linger about the interactions of the A, B, and D genomes in synthetic hexaploids derived from crosses between *T. turgidum* and *T. tauschii*. However, it is an area that we have given attention to—fully realizing that, in the past, simple resistance traits of *T. tauschii* accessions were suppressed or diluted in the synthetics derived from them. Despite this dilution aspect, if a synthetic hexaploid expresses resistance or tolerance when its durum parent expresses susceptibility, crossing the resistant or tolerant synthetic hexaploid onto susceptible *T. aestivum* cultivars seems to be a valid approach. Additional merits of using synthetic hexaploids in wheat improvement (i.e., contributions to wheat breeding, germplasm conservation, global distribution, and molecular mapping) are left to other researchers to study.

With nearly 525 synthetics already produced from *T. turgidum* x *T. tauschii* crosses at CIMMYT (see **Appendix 2**), we have now turned our attention to crossing *T. aestivum* directly with *T. tauschii* as described in Chapter 3 and will be intensively pursuing this avenue over the next five to seven years. We anticipate obtaining more genetically diverse germplasm that is resistant or tolerant to Karnal bunt, *Helminthosporium sativum*, *Fusarium graminearum*, and salt toxicity. A new objective is to transfer Russian wheat aphid resistance from *T. tauschii* and *T. dicoccum* accessions to *T. aestivum* by developing their amphiploid (*T. dicoccum* x *T. tauschii*). Enriching wheat with A genome variability will surely follow; we already have 155 AA genome hexaploids (2n=6x=42; AAAABB) among our genetic stocks (see **Appendix 3**). Exploiting *T. tauschii*'s D genome variation for durum wheat improvement through A/D homoeologous exchange will be another challenge.

What of the Future?

There seems to be no major impediment in wheat wide crosses to restrict genetic advances at the plant level. The germplasm derived from cytogenetic manipulation forms the backbone of our diagnostic applications, comprising of diagnostic markers involving RAPDs, isozymes, and *in situ* hybridization. However, use of this germplasm enables breeders to continue their work without critical time lapses as we advance from one developmental phase to the next in our wide crosses program. Collaborative research in areas of novel system applications and diagnostic procedures is, and will continue to be, a major avenue towards accomplishing effective alien transfers. Such collaboration will enable us to reach new scientific understandings and at the same enable the distribution of new germplasm to farmers in an efficient manner. We anticipate

our short-term efforts (interspecific hybridization) and long-term efforts (intergeneric hybridization, stretched over 7 to 12 years) will provide quality returns for CIMMYT's crop improvement mandate.

The structure of the CIMMYT wide crosses section is designed to link plant level manipulation with cellular and molecular approaches—two aspects that are essential to the section's function and effectiveness. We anticipate that a number of very advantageous approaches will subsequently emerge to aid in cereal crop improvement. When these breakthroughs are refined and applied, they will find complementary use in wheat improvement and may even have the potential to replace several conventional stages of genetic manipulation. Although we are receptive to and cognizant of these futuristic applications, we have not discussed them in any detail in this research report because progress has been limited to date.

We anticipate that the successful use of wheat polyhaploids will receive greater application in our program as well as in the breeding/molecular areas. Viable stored pollen may provide an additional boost to the application of the wheat x maize or wheat x *Tripsacum* techniques of polyhaploid induction described in Chapter 5. Additional diversification is expected from sexual crosses with sorghum to produce wheat polyhaploids since wheat x sorghum fertilization frequencies are the highest so far recorded (Laurie and Bennett 1988b). In conjunction with colleagues in the USDA/ARS laboratory in Logan, Utah, USA, we also anticipate extending the polyhaploid procedure to range grasses where analysis of such polyhaploids should help us to clarify further genomic relationships within the perennial Triticeae.

In 1992, we began using molecular marker techniques (Chapter 8) for enhancing our program's efficiency (Rayburn et al. 1993, William et al. 1993a). We cannot predict how soon these techniques (e.g., PCRs and FISH) will become routine for reaching our goals and assisting breeders. However, applications anticipated from these techniques should be somewhat similar to our effective use of biochemical markers (Chapter 7).

In our 15 years of investigations, we have progressed to a stage that allows us to project a prosperous future—not too distantly placed early in the next century. Historically, wide crosses were never anticipated to yield on-the-farm products in a short time frame and provide answers for each and every aspect of development. However, we have concentrated on doing just that and are optimistic about upcoming varietal releases and registration of new genetic stocks (see **Appendices 2-6** for some genetic stocks currently available).

Uncategorized resistances to helminthosporium and fusarium are already in elite plant types for interested breeders to exploit. *T. tauschii*-derived resistances to helminthosporium, fusarium, *Septoria tritici*, and Karnal bunt and tolerance to salt have been identified and are available to breeders. The polyhaploid systems for wheat, which utilize crosses of wheat x maize and wheat x *Tripsacum*, are superior to anther culture and to crossing wheat with *H. bulbosum*. Although the above stand as our major technical accomplishments, we also want to include as our contributions: service to programs, maintenance of alien species, routine use of tissue culture, and the incorporation of molecular techniques (particularly FISH) that may increase the scope of the overall program.

Through the germplasm we have developed, we have been directly or indirectly linked to other CIMMYT base programs (i.e., breeding, pathology, soils, physiology, agronomy, baking quality, and training) and national agricultural research programs. In the future, these linkages will inevitably develop further and place a heavy demand on our wide crosses staff. With the existing personnel, we are operating with a modest budget. If alien germplasm is to continue playing a role in reaching objectives within the CIMMYT Wheat Program's current agenda, it will soon have to pass the test of making practical gains measured through advances in wheat crop productivity. To achieve this, we require prudent financial support to maintain a critical mass of personnel and a balanced vision. This is critically needed now at a time that we view as being a "watershed" juncture of the Wide Crosses Program.

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APPENDIX 1

Durum wheat cultivars and lines used by CIMMYT in crosses with *Triticum tauschii* (*Aegilops squarrosa*), *T. boeoticum*, *T. monococcum*, and *T. urartu* to develop D genome synthetics and A genome hexaploids.

Entry no.[a]	Cultivar or line
1	CROCETHIA_1
2	ARLEQUIN_1
3	ROKEL/KAMILARIO
4	ALTAR 84
5	DUERGAND_2
6	LARU
7	68.111/RUGBY//WARD RESELECTION/3/STIFTAIL
8	68.111/RUGBY//WARD
9	68.111/RUGBY//WARD/3/FLAMINGO/4/RABICORNO
10	6973/WARD 7463//74110
11	CAPEITI/GEDIZ/3/GOOSE//JORI C69/CRANE
12	D67.2/P66.270
13	CERCETA
14	STERNA
15	RABICORNO//GANSO/CRANE
16	SORA
17	SCAUP
18	SNIPE/YAVAROS79//DACKIYE/TEAL
19	TK SN1081
20	YAVAROS_2/TEZONTLE
21	YARMOUK
22	DECOY1
23	GARZA/BOYERUS
24	68.111/RUGBY//WARD
25	ARAOS
26	GAN
27	SCOOP_1
28	DUSTY-US/CELTA//PALUSTRIS/3/STERNA_5
29	YAVAROS79//DACKIYE/RABICORNO/3/SNIPE
30	YAVAROS_3/SCOTER//JORI C69/CRANR/3/YAVAROS79
31	YAR
32	68112/WARD
33	FLAMING0/USA21111
34	ALGERIAN86/4/FLAMINGO/PALES//MEXICALI_1/3/RUFF/FLAMINGO/5/ENTE
35	BONITO
36	COCORIT71/CAPEITI
37	LCK59.61
38	TRINAKRIA
39	RASCON
40	AJAIA
41	SKARV_2
42	SCOT/MEXICALI_1
43	FALCINELLUS
44	GREENSHANK
45	SHAG_22
46	KAPUDE
47	ARLEQUIN
48	CHEN_7
49	ACO89
50	ALCATRAZ_3
51	LOCAL RED
52	PBW 114
53	RUFF

[a] Numbering system within the Wide Crosses Section.

APPENDIX 2

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii*.

Synthetic I.D. No.[a]	Cross
1	68.111/RGB-U//WARD RESEL/3/STIL/4/T.TAUSCHII (164)[b]CIGM88.1161[c]
2	CROC_1/T.TAUSCHII (168) CIGM87.2755
3	ALTAR 84/T.TAUSCHII (178) CIGM88.1168
4	ACO89//T.TAUSCHII (178) CIGM90.527
5	ALTAR 84/T.TAUSCHII (188) CIGM87.2765
6	DOY1/T.TAUSCHII (188) CIGM88.1175
7	RABI//GS/CRA/3/T.TAUSCHII (190) CIGM88.1178
8	**ALTAR 84/T.TAUSCHII (191)**[d] CIGM87.2766
9	**ALTAR 84/T.TAUSCHII (191)** CIGM87.2766
10	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (191) CIGM88.1179
11	SORA/T.TAUSCHII (191) CIGM88.1180
12	ALTAR 84/T.TAUSCHII (192) CIGM87.2767
13	**SORA/T.TAUSCHII (192)** CIGM88.1182
14	**SORA/T.TAUSCHII (192)** CIGM88.1182
15	**SORA/T.TAUSCHII (192)** CIGM90.540
16	**SORA/T.TAUSCHII (192)** CIGM90.540
17	ALTAR 84/T.TAUSCHII (193) CIGM87.2775
18	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (193) CIGM88.1183
19	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (196) CIGM88.1186
20	ALTAR 84/T.TAUSCHII (198) CIGM87.2768
21	GAN/T.TAUSCHII (201) CIGM88.1191
22	CROC_1/T.TAUSCHII (205) CIGM86.946
23	ALTAR 84/T.TAUSCHII (205) CIGM87.2770
24	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (205) CIGM88.1192
25	SORA/T.TAUSCHII (207) CIGM88.1193
26	**CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (208)** CIGM88.1194
27	**CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (208)** CIGM88.1194
28	**SORA/T.TAUSCHII (208)** CIGM88.1195
29	**SORA/T.TAUSCHII (208)** CIGM88.1195
30	**CROC_1/T.TAUSCHII (210)** CIGM87.2754
31	**CROC_1/T.TAUSCHII (210)** CIGM87.2754
32	**CROC_1/T.TAUSCHII (210)** CIGM87.2754
33	ALTAR 84/T.TAUSCHII (211) CIGM87.2771
34	D67.2/P66.270//T.TAUSCHII (211) CIGM87.1197
35	SORA/T.TAUSCHII (211) CIGM90.541
36	CROC_1/T.TAUSCHII (213) CIGM86.947
37	D67.2/P66.270//T.TAUSCHII (213) CIGM88.1200
38	DUERGAND_2/T.TAUSCHII (214) CIGM86.951
39	ROK/KML//T.TAUSCHII (214) CIGM86.959
40	CROC_1/T.TAUSCHII (215) CIGM86.948
41	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (215) CIGM88.1204
42	SORA/T.TAUSCHII (215) CIGM90.542
43	DOY1/T.TAUSCHII (216) CIGM88.1208
44	D67.2/P66.270//T.TAUSCHII (217) CIGM88.1209
45	YUK/T.TAUSCHII (217) CIGM90.561
46	ARLIN_1/T.TAUSCHII (218) CIGM86.955
47	D67.2/P66.270//T.TAUSCHII (218) CIGM88.1211
48	ALTAR 84/T.TAUSCHII (219) CIGM86.940
49	ALTAR 84/T.TAUSCHII (220) CIGM87.2760
50	D67.2/P66.270//T.TAUSCHII (220) CIGM86.1212
51	DUERGAND_2/T.TAUSCHII (221) CIGM86.953
52	ALTAR 84/T.TAUSCHII (221) CIGM87.2761
53	D67.2/P66.270//T.TAUSCHII (221) CIGM88.1214
54	**TK SN1081/T.TAUSCHII (222)** CIGM88.1217
55	D67.2/P66.270//T.TAUSCHII (222) CIGM88.1216
56	**TK SN1081/T.TAUSCHII (222)** CIGM88.1217
57	ALTAR 84/T.TAUSCHII (223) CIGM87.2762
58	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (223) CIGM88.1218
59	D67.2/P66.270//T.TAUSCHII (223) CIGM88.1219
60	**CROC_1/T.TAUSCHII (224)** CIGM86.950
61	**CROC_1/T.TAUSCHII (224)** CIGM86.950
62	**CROC_1/T.TAUSCHII (224)** CIGM86.949
63	**CROC_1/T.TAUSCHII (224)** CIGM86.949
64	**ALTAR 84/T.TAUSCHII (224)** CIGM86.941
65	**ALTAR 84/T.TAUSCHII (224)** CIGM86.941
66	**ALTAR 84/T.TAUSCHII (224)** CIGM86.942
67	ARLIN_1/T.TAUSCHII (225) CIGM86.956
68	CETA/T.TAUSCHII (230) CIGM88.1223
69	GAN/T.TAUSCHII (236) CIGM88.1228
70	YAV_2/TEZ//T.TAUSCHII (243) CIGM88.1231
71	**DUERGAND_2/T.TAUSCHII (247)** CIGM88.1237
72	**DUERGAND_2/T.TAUSCHII (247)** CIGM88.1237
73	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FG/5/ENTE/6/T.TAUSCHII (254) CIGM89.393
74	AOS/T.TAUSCHII (269) CIGM88.1249
75	GARZA/BOY//T.TAUSCHII (271) CIGM88.1250
76	SCA/T.TAUSCHII (279) CIGM88.1252
77	ACO89/T.TAUSCHII (282) CIGM90.528
78	GARZA/BOY//T.TAUSCHII (286) CIGM88.1254
79	ACO89/T.TAUSCHII (290) CIGM90.524
80	ALTAR 84/T.TAUSCHII (291) CIGM87.2781
81	GARZA/BOY//T.TAUSCHII (307) CIGM88.1266
82	**LARU/T.TAUSCHII (309)** CIGM87.2783
83	**LARU/T.TAUSCHII (309)** CIGM87.2783
84	**ACO89/T.TAUSCHII (309)** CIGM90.525
85	**ACO89/T.TAUSCHII (309)** CIGM90.525
86	GARZA/BOY//T.TAUSCHII (311) CIGM88.1270
87	ACO89/T.TAUSCHII (315) CIGM90.526
88	68.111/RGB-U//WARD/3/T.TAUSCHII (316) CIGM88.1273
89	68.111/RGB-U//WARD/3/T.TAUSCHII (321) CIGM88.1277

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii* (cont'd).

Synthetic I.D. No.[a]	Cross
90	68.111/RGB-U//WARD/3/T.TAUSCHII (322) CIGM88.1279
91	**SORA/T.TAUSCHII (323)** CIGM88.1240
92	**SORA/T.TAUSCHII (323)** CIGM88.1240
93	68.111/RGB-U//WARD/3/T.TAUSCHII (325) CIGM88.1285
94	68.111/RGB-U//WARD/3/T.TAUSCHII (326) CIGM88.1288
95	68.111/RGB-U//WARD/3/T.TAUSCHII (328) CIGM88.1292
96	**ALTAR 84/T.TAUSCHII (328)** CIGM88.1289
97	**ALTAR 84/T.TAUSCHII (328)** CIGM88.1289
98	68111/RUGBY//WARD/3/T.TAUSCHII (329) CIGM88.1293
99	68.111/RGB-U//WARD RESEL/3/STIL/4/T.TAUSCHII (332) CIGM88.1297
100	ALTAR 84/T.TAUSCHII (332) CIGM88.1299
101	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
102	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
103	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
104	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
105	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
106	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
107	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (411) CIGM88.1323
108	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (412) CIGM88.1324
109	CHEN_7/T.TAUSCHII (429) CIGM89.438
110	YUK/T.TAUSCHII (434) CIGM88.1334
111	SCOOP_1/T.TAUSCHII (434) CIGM88.1335
112	GAN/T.TAUSCHII (437) CIGM90.583
113	SRN/T.TAUSCHII (446) CIGM88.1342
114	**DOY1/T.TAUSCHII (446)** CIGM88.1343
115	**DOY1/T.TAUSCHII (446)** CIGM88.1343
116	GAN/T.TAUSCHII (446) CIGM90.586
117	DOY1/T.TAUSCHII (447) CIGM88.1344
118	68.111/RGB-U//WARD RESEL/3/STIL/4/T.TAUSCHII (449) CIGM89.447
119	**YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (460)** CIGM88.1348
120	**YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (460)** CIGM88.1348
121	**YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (477)** CIGM88.1351
122	**YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (477)** CIGM88.1351
123	DOY1/T.TAUSCHII (488) CIGM88.1353
124	YAV_3/SCO//JO69/CR/3/YAV79/4/T.TAUSCHII (498) CIGM88.1356
125	**YAR/T.TAUSCHII (493)** CIGM89.463
126	SCA/T.TAUSCHII (493) CIGM90.557
127	**YAR/T.TAUSCHII (493)** CIGM89.463
128	DOY1/T.TAUSCHII (510) CIGM88.1360
129	DOY1/T.TAUSCHII (511) CIGM88.1363
130	68.111/RGB-U//WARD/3/T.TAUSCHII (511) CIGM88.1362
131	DOY1/T.TAUSCHII (515) CIGM90.566
132	ALTAR 84/AOS//T.TAUSCHII (521) CIGM89.473
133	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (521) CIGM90.529
134	GAN/T.TAUSCHII (522) CIGM88.1370
135	YAR/T.TAUSCHII (524) CIGM89.474
136	6973/WARD 7463//74110/3/T.TAUSCHII (35A) CIGM88.1377
137	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (629) CIGM90.590
138	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (629) CIGM90.534
139	**CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (633)** CIGM89.501
140	**CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (633)** CIGM89.501
141	**CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (633)** CIGM89.501
142	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (637) CIGM90.535
143	FGO/USA2111//T.TAUSCHII (658) CIGM89.506
144	CROC_1/T.TAUSCHII (662) CIGM89.510
145	CROC_1/T.TAUSCHII (725) CIGM89.525
146	CETA/T.TAUSCHII (742) CIGM89.527
147	68.111/RGB-U//WARD RESEL/3/STIL/4/T.TAUSCHII (781) CIGM89.537
148	68.111/RGB-U//WARD RESEL/3/STIL/4/T.TAUSCHII (783) CIGM89.538
149	CETA/T.TAUSCHII (783) CIGM90.531
150	YAR/T.TAUSCHII (783) CIGM90.686
151	CROC_1/T.TAUSCHII (784) CIGM89.539
152	YUK/T.TAUSCHII (784) CIGM90.661
153	YAR/T.TAUSCHII (809) CIGM90.767
154	CETA/T.TAUSCHII (819) CIGM89.545
155	CROC_1/T.TAUSCHII (826) CIGM89.546
156	CETA/T.TAUSCHII (850) CIGM89.552
157	YUK/T.TAUSCHII (864) CIGM90.760
158	CETA/T.TAUSCHII (872) CIGM89.555
159	**68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
160	**68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
161	CROC_1/T.TAUSCHII (879) CIGM89.479
162	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (882) CIGM89.561
163	SORA/T.TAUSCHII (884) CIGM90.543
164	CROC_1/T.TAUSCHII (886) CIGM89.563
165	68.111//RGB/WARD/3/FGO/4/RABI/5/T.TAUSCHII (890) CIGM89.564
166	**RABI//GS/CRA/3/T.TAUSCHII (891)** CIGM90.602
167	**RABI//GS/CRA/3/T.TAUSCHII (891)** CIGM90.602
168	**RABI//GS/CRA/3/T.TAUSCHII (895)** CIGM90.603
169	**RABI//GS/CRA/3/T.TAUSCHII (895)** CIGM90.603

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii* (cont'd).

Synthetic I.D. No.[a]	Cross
170	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (900) CIGM89.569
171	RABI//GS/CRA/3/T.TAUSCHII (904) CIGM90.605
172	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (904) CIGM90.682
173	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (905) CIGM89.571
174	RABI//GS/CRA/3/T.TAUSCHII (914) CIGM90.606
175	SORA/T.TAUSCHII (939) CIGM90.544
176	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (948) CIGM89.575
177	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (949) CIGM89.576
178	CETA/T.TAUSCHII (954) CIGM89.578
179	YAV_2/TEZ//T.TAUSCHII (963) CIGM90.610
180	CETA/T.TAUSCHII (976) CIGM89.579
181	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FG/5/ENTE/6/T.TAUSCHII (518) CIGM90.545
182	CROC_1/T.TAUSCHII (518) CIGM86.944
183	PBW114/T.TAUSCHII (DHALIWAL, INDIA)[e]
184	RUFF/T.TAUSCHII (DHALIWAL, INDIA)[e]
185	LARU/T.TAUSCHII (TA2459) CIGM87.2784
186	ALTAR 84/T.TAUSCHII (Y86-87S401) CIGM87.2779
187	ALTAR 84/T.TAUSCHII (JBANGOR) CIGM86.3277
188	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
189	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
190	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
191	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
192	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
193	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
194	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
195	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
196	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
197	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
198	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
199	**YAV_2/TEZ//T.TAUSCHII (249)** CIGM88.1239
200	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
201	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
202	**68112/WARD//T.TAUSCHII (369)** CIGM88.1313
203	**68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (809)** CIGM89.543
204	**68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (809)** CIGM89.543
205	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (809)** CIGM89.543
206	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (809)** CIGM89.543
207	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (809)** CIGM89.543
208	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
209	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
210	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
211	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
212	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
213	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
214	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
215	**68.111/RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (878)** CIGM89.559
216	**CETA/T.TAUSCHII (895)** CIGM89.567
217	**CETA/T.TAUSCHII (895)** CIGM89.567
218	**CETA/T.TAUSCHII (895)** CIGM89.567
219	**CETA/T.TAUSCHII (895)** CIGM89.567
220	LCK59.61/T.TAUSCHII (173) CIGM90.798
221	GAN/T.TAUSCHII (180) CIGM90.799
222	GAN/T.TAUSCHII (257) CIGM90.807
223	D67.2/P66.270//T.TAUSCHII (257) CIGM90.808
224	D67.2/P66.270//T.TAUSCHII (308) CIGM90.809
225	LCK59.61/T.TAUSCHII (308) CIGM90.810
226	ARLIN/T.TAUSCHII (308) CIGM90.811
227	LCK59.61/T.TAUSCHII (313) CIGM90.812
228	LCK59.61/T.TAUSCHII (324) CIGM90.815
229	LCK59.61/T.TAUSCHII (344) CIGM90.816
230	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (358) CIGM90.817
231	SRN/T.TAUSCHII (358) CIGM90.818
232	SCOOP_1/T.TAUSCHII (358) CIGM90.820
233	YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (381) CIGM90.821
234	YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (397) CIGM90.822
235	SCOOP_1/T.TAUSCHII (407) CIGM90.823
236	GAN/T.TAUSCHII (408) CIGM90.824
237	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (431) CIGM90.826
238	YAV_2/TEZ//T.TAUSCHII (435) CIGM90.827
239	YAV_2/TEZ//T.TAUSCHII (437) CIGM90.828
240	YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (443) CIGM90.830
241	RABI//GS/CRA/3/T.TAUSCHII (457) CIGM90.832
242	YAV_2/TEZ//T.TAUSCHII (457) CIGM90.833
243	YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (457) CIGM90.834
244	YAV79//DACK/RABI/3/SNIPE/4/T.TAUSCHII (490) CIGM90.841
245	YAR/T.TAUSCHII (513) CIGM90.842
246	SCA/T.TAUSCHII (518) CIGM90.845
247	YAR/T.TAUSCHII (518) CIGM90.846
248	TK SN1081/T.TAUSCHII (519) CIGM90.847
249	SCA/T.TAUSCHII (523) CIGM90.849
250	SNIPE/YAV7979//DACK/TEAL/3/T.TAUSCHII (528) CIGM90.854
251	BOTNO/T.TAUSCHII (617) CIGM90.863
252	BOTNO/T.TAUSCHII (620) CIGM90.864

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii* (cont'd).

Synthetic I.D. No.[a]	Cross
253	BOTNO/T.TAUSCHII (625) CIGM90.865
254	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (628) CIGM90.866
255	CIT71/CPI//T.TAUSCHII (629) CIGM90.868
256	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (629) CIGM90.869
257	D67.2/P66.270//T.TAUSCHII (633) CIGM90.871
258	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (633) CIGM90.872
259	SCOOP_1/T.TAUSCHII (634) CIGM90.873
260	D67.2/P66.270//T.TAUSCHII (646) CIGM90.876
261	D67.2/P66.270//T.TAUSCHII (659) CIGM90.878
262	SCOOP_1/T.TAUSCHII (659) CIGM90.879
263	CETA/T.TAUSCHII (661) CIGM90.881
264	SCOOP_1/T.TAUSCHII (662) CIGM90.883
265	6973/WARD 7463//74110/3/T.TAUSCHII (665) CIGM90.886
266	CETA/T.TAUSCHII (665) CIGM90.887
267	ARLIN/T.TAUSCHII (665) CIGM90.888
268	BOTNO/T.TAUSCHII (666) CIGM90.889
269	LCK59.61/T.TAUSCHII (689) CIGM90.892
270	LCK59.61/T.TAUSCHII (690) CIGM90.894
271	TK SN1081/T.TAUSCHII (690) CIGM90.895
272	LCK59.61/T.TAUSCHII (693) CIGM90.896
273	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (700) CIGM90.897
274	TRN/T.TAUSCHII (700) CIGM90.898
275	LCK59.61/T.TAUSCHII (783) CIGM90.900
276	CETA/T.TAUSCHII (796) CIGM90.901
277	SNIPE/YAV79//DACK/TEAL/3/T.TAUSCHII (877) CIGM90.906
278	YAV_2/TEZ//T.TAUSCHII (882) CIGM90.907
279	GAN/T.TAUSCHII (890) CIGM90.909
280	GAN/T.TAUSCHII (897) CIGM90.911
281	YAV7_2/TEZ//T.TAUSCHII (895) CIGM90.910
282	GARZA/BOY//T.TAUSCHII (165) CIGM92.1611
283	GARZA/BOY//T.TAUSCHII (171) CIGM92.1614
284	SRN/3/CHUR/HUI//POC/4/MOEWE/5/T.TAUSCHII (175) CIGM92.1842
285	SCOT/MEXI_1//T.TAUSCHII (186) CIGM92.1621
286	GARZA/BOY//T.TAUSCHII (195) CIGM92.1623
287	GARZA/BOY//T.TAUSCHII (232) CIGM92.1845
288	GARZA/BOY//T.TAUSCHII (233) CIGM92.1627
289	GARZA/BOY//T.TAUSCHII (240) CIGM92.1629
290	GARZA/BOY//T.TAUSCHII (241) CIGM92.1631
291	GARZA/BOY//T.TAUSCHII (265) CIGM92.1637
292	GARZA/BOY//T.TAUSCHII (270) CIGM92.1638
293	GARZA/BOY//T.TAUSCHII (276) CIGM92.1640
294	GARZA/BOY//T.TAUSCHII (278) CIGM92.1642
295	GARZA/BOY//T.TAUSCHII (280) CIGM92.1643
296	GARZA/BOY//T.TAUSCHII (281) CIGM92.1644
297	GARZA/BOY//T.TAUSCHII (283) CIGM92.1646
298	ARLIN/T.TAUSCHII (283) CIGM92.1647
299	GARZA/BOY//T.TAUSCHII (284) CIGM92.1649
300	GARZA/BOY//T.TAUSCHII (287) CIGM92.1651
301	6973/WARD 7463//74110/3/T.TAUSCHII (289) CIGM92.473
302	DOY1/T.TAUSCHII (293) CIGM92.1849
303	GARZA/BOY//T.TAUSCHII (294) CIGM92.1652
304	ARLIN/T.TAUSCHII (295) CIGM92.1657
305	DUERGAND_2/T.TAUSCHII (295) CIGM92.1850
306	ROK/KML//T.TAUSCHII (295) CIGM92.1655
307	CROC_1/T.TAUSCHII (298) CIGM92.1659
308	CROC_1/T.TAUSCHII (299) CIGM92.1660
309	GARZA/BOY//T.TAUSCHII (300) CIGM92.1662
310	FALCIN/T.TAUSCHII (312) CIGM92.1665
311	RASCON/T.TAUSCHII (312) CIGM92.1666
312	RASCON/T.TAUSCHII (314) CIGM92.1669
313	KAPUDE/T.TAUSCHII (314) CIGM92.1668
314	SCOT/MEXI_1//T.TAUSCHII (314) CIGM92.1667
315	ARLIN/T.TAUSCHII (317) CIGM92.1851
316	AJAIA/T.TAUSCHII (330) CIGM92.1675
317	ARLIN_1/T.TAUSCHII (333) CIGM92.1680
318	ALTAR 84/T.TAUSCHII (333) CIGM92.1676
319	CROC_1/T.TAUSCHII (333) CIGM92.1677
320	LARU/T.TAUSCHII (333) CIGM92.1678
321	DUERGAND_2/T.TAUSCHII (333) CIGM92.1679
322	ROK/KML//T.TAUSCHII (333) CIGM92.1681
323	DOY1/T.TAUSCHII (333) CIGM92.1682
324	KAPUDE/T.TAUSCHII (341) CIGM92.1684
325	RASCON/T.TAUSCHII (343) CIGM92.1686
326	DOY1/T.TAUSCHII (349) CIGM92.1687
327	GARZA/BOY//T.TAUSCHII (350) CIGM92.1689
328	GARZA/BOY//T.TAUSCHII (366) CIGM92.1692
329	RASCON/T.TAUSCHII (367) CIGM92.1695
330	DOY1/T.TAUSCHII (370) CIGM92.1696
331	GARZA/BOY//T.TAUSCHII (374) CIGM92.1698
332	GARZA/BOY//T.TAUSCHII (375) CIGM92.1699
333	RASCON/T.TAUSCHII (385) CIGM92.1701
334	KAPUDE/T.TAUSCHII (385) CIGM92.1700
335	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE /6/T.TAUSCHII (389) CIGM92.496
336	FALCIN/T.TAUSCHII (389) CIGM92.1702
337	ARLIN/T.TAUSCHII (410) CIGM92.1706
338	DOY1/T.TAUSCHII (415) CIGM92.1708
339	68.111/RGB-U//WARD/3/T.TAUSCHII (426) CIGM92.1711
340	GARZA/BOY//T.TAUSCHII (427) CIGM92.1712
341	DOY1/T.TAUSCHII (428) CIGM92.1713
342	GARZA/BOY//T.TAUSCHII (433) CIGM92.1715
343	GARZA/BOY//T.TAUSCHII (439) CIGM92.1717
344	68112/WARD//T.TAUSCHII (451) CIGM92.491
345	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE /6/T.TAUSCHII (451) CIGM92.497
346	68.111/RGB-U//WARD/3/T.TAUSCHII (452) CIGM92.1721
347	68.111/RGB-U//WARD/3/T.TAUSCHII (454) CIGM92.1723
348	68.111/RGB-U//WARD/3/T.TAUSCHII (456) CIGM92.1725

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii* (cont'd).

Synthetic I.D. No.[a]	Cross
349	DOY1/T.TAUSCHII (458) CIGM92.1727
350	68.111/RGB-U//WARD/3/T.TAUSCHII (458) CIGM92.1872
351	GREEN/T.TAUSCHII (458) CIGM92.1871
352	68.111/RGB-U//WARD/3/T.TAUSCHII (463) CIGM92.1731
353	GARZA/BOY//T.TAUSCHII (467) CIGM92.1733
354	GARZA/BOY//T.TAUSCHII (484) CIGM92.1742
355	GARZA/BOY//T.TAUSCHII (503) CIGM92.1745
356	ALTAR 84/T.TAUSCHII (507) CIGM92.1746
357	CROC_1/T.TAUSCHII (507) CIGM92.1747
358	LARU/T.TAUSCHII (507) CIGM92.1748
359	DUERGAND_2/T.TAUSCHII (507) CIGM92.1749
360	ROK/KML//T.TAUSCHII (507) CIGM92.1750
361	DOY1/T.TAUSCHII (507) CIGM92.1751
362	GARZA/BOY//T.TAUSCHII (520) CIGM92.1753
363	DOY1/T.TAUSCHII (532) CIGM92.1754
364	LCK59.61/T.TAUSCHII (536) CIGM92.481
365	GAN/T.TAUSCHII (163) CIGM93.177
366	CROC_1/T.TAUSCHII (170) CIGM93.178
367	CETA/T.TAUSCHII (170) CIGM93.179
368	YAV_2/TEZ//T.TAUSCHII (170) CIGM93.180
369	ALTAR 84/T.TAUSCHII (174) CIGM93.182
370	CETA/T.TAUSCHII (174) CIGM93.183
371	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (174) CIGM93.362
372	CROC_1/T.TAUSCHII (177) CIGM93.185
373	DOY1/T.TAUSCHII (177) CIGM93.187
374	GAN/T.TAUSCHII (182) CIGM93.192
375	CROC_1/T.TAUSCHII (231) CIGM93.199
376	SCA/T.TAUSCHII (248) CIGM93.200
377	DOY1/T.TAUSCHII (255) CIGM93.202
378	CROC_1/T.TAUSCHII (256)CIGM93.203
379	CETA/T.TAUSCHII (256) CIGM93.204
380	DOY1/T.TAUSCHII (256) CIGM93.205
381	DOY1/T.TAUSCHII (258) CIGM93.207
382	GAN/T.TAUSCHII (259) CIGM93.209
383	DOY1/T.TAUSCHII (264) CIGM93.211
384	GAN/T.TAUSCHII (264) CIGM93.212
385	DOY1/T.TAUSCHII (267) CIGM93.213
386	GAN/T.TAUSCHII (267) CIGM93.214
387	GAN/T.TAUSCHII (268) CIGM93.215
388	SCA/T.TAUSCHII (272) CIGM93.216
389	CROC_1/T.TAUSCHII (275) CIGM93.218
390	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (277) CIGM93.372
391	DOY1/T.TAUSCHII (285) CIGM93.219
392	GAN/T.TAUSCHII (285) CIGM93.374
393	ALTAR 84/T.TAUSCHII (304) CIGM93.376
394	SKARV_2/T.TAUSCHII (304) CIGM93.377
395	DOY1/T.TAUSCHII (318) CIGM93.223
396	DOY1/T.TAUSCHII (322) CIGM93.225
397	CETA/T.TAUSCHII (327) CIGM93.226
398	DOY1/T.TAUSCHII (334) CIGM93.227
399	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (334) CIGM93.379
400	CROC_1/T.TAUSCHII (362) CIGM93.382
401	DOY1/T.TAUSCHII (372) CIGM93.229
402	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (390) CIGM93.233
403	DOY1/T.TAUSCHII (390) CIGM93.385
404	AAZ_3/T.TAUSCHII (398) CIGM93.386
405	CROC_1/T.TAUSCHII (406) CIGM93.236
406	SCA/T.TAUSCHII (409) CIGM93.237
407	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (409) CIGM93.388
408	GAN/T.TAUSCHII (413) CIGM93.238
409	DOY1/T.TAUSCHII (418) CIGM93.239
410	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (418) CIGM93.240
411	CROC_1/T.TAUSCHII (436)CIGM93.242
412	CROC_1/T.TAUSCHII (444) CIGM92.244
413	GAN/T.TAUSCHII (459) CIGM93.245
414	CROC_1/T.TAUSCHII (466) CIGM93.247
415	CROC_1/T.TAUSCHII (481) CIGM93.254
416	DOY1/T.TAUSCHII (489) CIGM 93.257
417	CETA/T.TAUSCHII (499) CIGM93.259
418	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (502) CIGM93.261
419	ALTAR 84/T.TAUSCHII (502) CIGM93.395
420	STY-US/CELTA//PALES/3/SRN_5/4/T.TAUSCHII (502) CIGM93.396
421	CROC_1/T.TAUSCHII (516) CIGM93.264
422	DOY1/T.TAUSCHII (516) CIGM93.265
423	CROC_1/T.TAUSCHII (517) CIGM93.266
424	DOY1/T.TAUSCHII (517) CIGM93.267
425	CETA/T.TAUSCHII (525) CIGM93.268
426	DOY1/T.TAUSCHII (526) CIGM93.271
427	68.111//RGB-U/WARD/3/FGO/4/RABI/5/T.TAUSCHII (535) CIGM93.
428	ARLIN_1/T.TAUSCHII (536) CIGM93.275
429	CETA/T.TAUSCHII (540) CIGM93.399
430	CROC_1/T.TAUSCHII (1008) CIGM93.401
431	CETA/T.TAUSCHII (1011) CIGM93.290
432	DOY1/T.TAUSCHII (1011) CIGM93.291
433	ALTAR 84/T.TAUSCHII (1012) CIGM93.292
434	DOY1/T.TAUSCHII (1016) CIGM93.294
435	DUERGAND_2/T.TAUSCHII (1016) CIGM93.402
436	CETA/T.TAUSCHII (1016) CIGM93.403
437	DUERGAND_2/T.TAUSCHII (1022) CIGM93.295
438	CETA/T.TAUSCHII (1022) CIGM93.296
439	CROC_1/T. TAUSCHII (1023) CIGM93.404
440	CETA/T.TAUSCHII (1024) CIGM93.297
441	DOY1/T.TAUSCHII (1024) CIGM93.298
442	CETA/T.TAUSCHII (1025) CIGM93.299
443	DUERGAND_2/T.TAUSCHII (1027) CIGM93.300
444	DOY1/T.TAUSCHII (1027) CIGM93.302
445	CETA/T.TAUSCHII (1027) CIGM93.406
446	CETA/T.TAUSCHII (1030) CIGM93.305

D genome synthetic hexaploids (2n=6x=42; AABBDD) produced at CIMMYT by crossing durum x *Triticum tauschii* (cont'd).

Synthetic I.D. No.[a]	Cross
447	DOY1/T.TAUSCHII (1030) CIGM93.306
448	CETA/T.TAUSCHII (1042)[e]
449	CETA/T.TAUSCHII (166) L94.312[f]
450	CETA/T.TAUSCHII (172) L94.84
451	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (184) L94.119
452	CETA/T.TAUSCHII (194) L94.85
453	CETA/T.TAUSCHII (187) L94.90
454	CETA/T.TAUSCHII (200) L94.390
455	LOCAL RED/T. TAUSCHII (219) L94.176
456	LOCAL RED/T. TAUSCHII (220) L94.282
457	LOCAL RED/T. TAUSCHII (221) L94.283
458	LOCAL RED/T. TAUSCHII (222) L94.177
459	LOCAL RED/T. TAUSCHII (223) L94.178
460	CETA/T.TAUSCHII (235) L94.570
461	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (239) L94.106
462	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (244) L94.40
463	CETA/T.TAUSCHII (244) L94.52
464	ALTAR 84/T. TAUSCHII (244) L94.34
465	CETA/T.TAUSCHII (262) L94.27
466	CETA/T.TAUSCHII (263) L94.87
467	CETA/T.TAUSCHII (266) L94.28
468	CETA/T.TAUSCHII (271) L94.313
469	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (273) L94.21
470	CETA/T.TAUSCHII (273) L94.29
471	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (292) L94.39
472	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (296) L94.22
473	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (305) L94.309
474	CETA/T.TAUSCHII (306) L94.303
475	CETA/T.TAUSCHII (336) L94.121
476	CETA/T.TAUSCHII (345) L94.573
477	CETA/T.TAUSCHII (371) L94.313
478	CETA/T.TAUSCHII (386) L94.401
479	CETA/T.TAUSCHII (391) L94.31
480	CETA/T.TAUSCHII (392) L94.402
481	DUERGAND_2/T.TAUSCHII (402) L94.348
482	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (439) L94.46
483	CETA/T.TAUSCHII (445) L94.317
484	LOCAL RED/T.TAUSCHII (449) L94.287
485	CETA/T.TAUSCHII (450) L94.89
486	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (453) L94.54
487	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (461) L94.366
488	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (485) L94.48
489	CETA/T.TAUSCHII (485) L94.32
490	CETA/T.TAUSCHII (508) L94.123
491	CETA/T.TAUSCHII (530) L94.91
492	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (533) L94.80
493	CETA/T.TAUSCHII (533) L94.92
494	DUERGAND_2/T.TAUSCHII (545) L94.130
495	CETA/T.TAUSCHII (557) L94.320
496	CETA/T.TAUSCHII (1013) L94.94
497	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1018) L94.289
498	CETA/T.TAUSCHII (1018) L94.294
499	ARLIN_1/T.TAUSCHII (1018) L94.357
500	DOY1/T.TAUSCHII (1018) L94.359
501	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1021) L94.337
502	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1026) L94.338
503	CETA/T.TAUSCHII (1026) L94.295
504	DUERGAND_2/T.TAUSCHII (1026) L94.300
505	DOY1/T.TAUSCHII (1026) L94.360
506	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1029) L94.291
507	CETA/T.TAUSCHII (1029) L94.95
508	DUERGAND_2/T.TAUSCHII (1029) L94.301
509	DOY1/T.TAUSCHII (1029) L94.361
510	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1031) L94.29
511	CETA/T.TAUSCHII (1031) L94.297
512	DUERGAND_2/T.TAUSCHII (1031) L94.302
513	CETA/T.TAUSCHII (1036) L94.323
514	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1038) L94.342
515	CETA/T.TAUSCHII (1038) L94.325
516	CETA/T.TAUSCHII (1043) L94.298
517	CETA/T.TAUSCHII (1046) L94.129
518	CETA/T.TAUSCHII (1053) L94.328
519	CROC_1/T.TAUSCHII (212) L94.633
520	CROC_1/T.TAUSCHII (493) L94.636
521	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.TAUSCHII (1014) L94.345

[a] Numbering system within the Wide Crosses Section.
[b] Number in parentheses is the *T. tauschii* accession number in the wide cross working collection.
[c] Cross number to use when requesting germplasm.
[d] Boldface indicates independent hybrid of the same cross.
[e] Germplasm not currently available for distribution.
[f] Combinations with the "L94" cross number are still in the greenhouse/laboratory stage of development; these will eventually receive a CIGM number. The L94 number can also be used when requesting germplasm.

APPENDIX 3

A genome hexaploids (2n=6x=42; AAAABB) produced at CIMMYT by crossing durum x various accessions of A genome diploid species (T*riticum monococcum,= T. boeoticum,*T*. urartu)*.

Hexaploid I.D. no.[a]	Cross
1	YUK/T.BOEOTICUM (1)[b] CIGM90.769[c]
2	YUK/T.BOEOTICUM (2) CIGM90.770
3	STY-US/CELTA//PALS/3/SRN_5/4/T.BOEOTICUM (3) CIGM90.640
4	SCA/T.BOEOTICUM (3) CIGM90.667
5	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE/6/T.BOEOTICUM (3) CIGM90.771
6	SCA/T.BOEOTICUM (10) CIGM90.669
7	GARZA/BOY//T.BOEOTICUM (10) CIGM90.773
8	GARZA/BOY//T.BOEOTICUM (12) CIGM90.774
9	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE/6/T.BOEOTICUM (13) CIGM90.775
10	SCA/T.BOEOTICUM (14) CIGM90. 671
11	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE/6/T.BOEOTICUM (14) CIGM90.776
12	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE/6/T.BOEOTICUM (15) CIGM90.777
13	GARZA/BOY//T.BOEOTICUM (16) CIGM90.778
14	BOTNO/T.BOEOTICUM (20) CIGM92.440
15	GARZA/BOY//T.BOEOTICUM (21) CIGM90.780
16	SCA/T.BOEOTICUM (23) CIGM90.674
17	DOY1/T.BOEOTICUM (23) CIGM90.781
18	SHAG_22/T.BOEOTICUM (24) CIGM92.1593
19	DOY1/T.BOEOTICUM (26) CIGM90.782
20	DOY1/T.BOEOTICUM (27) CIGM90.783
21	SCA/T.BOEOTICUM (28) CIGM90.675
22	DOY1/T.BOEOTICUM (28) CIGM90.784
23	SCA/T.BOEOTICUM (31) CIGM90.676
24	SCA/T.BOEOTICUM (33) CIGM90.677
25	SCOOP_1/T.BOEOTICUM (33) CIGM90.697
26	SCA/T.BOEOTICUM (34) CIGM90.678
27	BOTNO/T.BOEOTICUM (35) CIGM92.443
28	D67.2/P66.270//T.BOEOTICUM (35) CIGM92.450
29	SCA/T.BOEOTICUM (36) CIGM90.679
30	SCA/T.BOEOTICUM (39) CIGM90.680
31	SCA/T.BOEOTICUM (40) CIGM90.681
32	SCOOP_1/T.BOEOTICUM (40) CIGM90.698
33	SCOOP_1/T.BOEOTICUM (46) CIGM90.699
34	SCOOP_1/T.BOEOTICUM (50) CIGM90.700
35	LCK59.61/T.BOEOTICUM (52) CIGM92.438
36	STY-US/CELTA//PALS/3/SRN_5/4/T.BOEOTICUM (54) CIGM90.642
37	SHAG_22/T.BOEOTICUM (55) CIGM92.1598
38	AJAIA/T.BOEOTICUM (55) CIGM92.1599
39	AJAIA/T.BOEOTICUM (56) CIGM92.1601
40	SHAG_22/T.BOEOTICUM (56) CIGM92.1600
41	SCOOP_1/T.BOEOTICUM (59) CIGM90.701
42	SCOOP_1/T.BOEOTICUM (60) CIGM90.702
43	68.111/RGB-U//WARD/3/T.BOEOTICUM (61) CIGM90.790
44	SHAG_22/T.BOEOTICUM (68) CIGM92.1602
45	SCOOP_1/T.BOEOTICUM (69) CIGM90.1703
46	SHAG_22/T.BOEOTICUM (70) CIGM92.1603
47	SCOOP_1/T.BOEOTICUM (71) CIGM90.704
48	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE /6/T.BOEOTICUM (74) CIGM92.455
49	BOTNO/T.BOEOTICUM (75) CIGM92.446
50	D67.2/P66.270//T.BOEOTICUM (75) CIGM92.452
51	SCOOP_1/T.BOEOTICUM (79) CIGM90.705
52	SCOOP_1/T.BOEOTICUM (80) CIGM90.706
53	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ ENTE/6/T.BOEOTICUM (83) CIGM92.456
54	SCOOP_1/T.BOEOTICUM (87) CIGM90.707
55	SHAG_22/T.BOEOTICUM (88) CIGM92.1605
56	SCOOP_1/T.BOEOTICUM (89) CIGM90.708
57	SCOOP_1/T.BOEOTICUM (90) CIGM90.709
58	SCOOP_1/T.BOEOTICUM (91) CIGM90.710
59	SCOOP_1/T.MONOCOCCUM (98) CIGM90.711
60	AOS/T.MONOCOCCUM (98) CIGM90.791
61	AOS/T.MONOCOCCUM (111) CIGM90.793
62	68.111/RGB-U//WARD/3/T.MONOCOCCUM (112) CIGM92.463
63	BOTNO/T.MONOCOCCUM (112) CIGM92.465
64	SCOOP_1/T.MONOCOCCUM (118) CIGM90.712
65	AOS/T.MONOCOCCUM (118) CIGM90.794
66	FGO/USA2111//T.MONOCOCCUM (119) CIGM90.795
67	FGO/USA2111//T.MONOCOCCUM (122) CIGM90.796
68	DOY1/T.URARTU (542) CIGM90.567
69	DOY1/T.URARTU (543) CIGM90.568
70	DOY1/T.URARTU (550) CIGM90.570
71	68.111/RGB-U//WARD/3/T.URARTU (550) CIGM90.570
72	68.111/RGB-U//WARD/3/T.URARTU (551) CIGM90.857
73	68.111/RGB-U//WARD/3/T.URARTU (553) CIGM90.858
74	68.111/RGB-U//WARD/3/FGO/4/RABI/5/T.URARTU (554) CIGM90.859
75	68.111/RGB-U//WARD/3/FGO/4/RABI/5/T.URARTU (555) CIGM90.860
76	DOY1/T.URARTU (560) CIGM90.573
77	DOY1/T.URARTU (563) CIGM90.574
78	DOY1/T.URARTU-55 (564) CIGM90.575
79	GAN/ T.BOEOTICUM (7) CIGM93.78
80	DUERGAND_2/T.BOEOTICUM (18) CIGM93.82
81	YAV_2/TEZ//T.BOEOTICUM (18) CIGM93.83
82	YAV_2/TEZ//T.BOEOTICUM (25) CIGM93.87
83	GAN/T.BOEOTICUM (29) CIGM93.89

A genome hexaploids (2n=6x=42; AAAABB) produced at CIMMYT by crossing durum x various accessions of A genome diploid species (T*riticum monococcum, = T. boeoticum,* T*. urartu)* (cont'd).

Hexaploid I.D. no.[a]	Cross
84	DUERGAND_2/T.BOEOTICUM (37) CIGM93.93
85	YAV_2/TEZ//T.BOEOTICUM (37) CIGM93.348
86	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (38) CIGM93.95
87	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (41) CIGM93.97
88	DUERGAND_2/T.BOEOTICUM (43) CIGM93.99
89	YAV_2/TEZ//T.BOEOTICUM (43) CIGM93.100
90	DUERGAND_2/T.BOEOTICUM (44) CIGM93.101
91	YAV_2/TEZ//T.BOEOTICUM (44) CIGM93.102
92	DUERGAND_2/T.BOEOTICUM (45) CIGM93.103
93	YAV_2/TEZ//T.BOEOTICUM (45) CIGM93.104
94	YAV_2/TEZ//T.BOEOTICUM (47) CIGM93.105
95	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (48) CIGM93.106
96	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (49) CIGM93.107
97	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (53) CIGM93.108
98	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (57) CIGM93.111
99	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (58) CIGM93.112,352
100	YAV_2/TEZ//T.BOEOTICUM (62) CIGM93.113
101	YAV_2/TEZ//T.BOEOTICUM (64) CIGM93.114
102	YAV_2/TEZ//T.BOEOTICUM (65) CIGM93.115
103	YAV_2/TEZ//T.BOEOTICUM (66)[d]
104	YAV_2/TEZ//T.BOEOTICUM (67) CIGM93.117
105	YAV_2/TEZ//T.BOEOTICUM (73) CIGM93.119
106	SCA/T.BOEOTICUM (75) CIGM93.120
107	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (76) CIGM93.121
108	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (77) CIGM93.122
109	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (78) CIGM93.123
110	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (81) CIGM93.124
111	YAV_2/TEZ//T.BOEOTICUM (82) CIGM93.125
112	YAV_2/TEZ//T.BOEOTICUM (83) CIGM93.126
113	SCA/T.BOEOTICUM (92) CIGM93.127
114	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (93) CIGM93.129
115	CPI/GEDIZ/3/GOO//JO69/CRA/4/ T.MONOCOCCUM (99) CIGM93.130
116	CPI/GEDIZ/3/GOO//JO69/CRA/4/ T.MONOCOCCUM (101) CIGM93.132
117	SRN/T.MONOCOCCUM (111) CIGM93.134
118	**SRN/T.MONOCOCCUM (112)**[e] CIGM93.136
119	**SRN/T.MONOCOCCUM (112)** CIGM93.137
120	YAV_2/TEZ//T.MONOCOCCUM (113) CIGM93.138
121	CPI/GEDIZ/3/GOO//JO69/CRA/4/ T.MONOCOCCUM (114) CIGM93.139
122	CPI/GEDIZ/3/GOO//JO69/CRA/4/ T.MONOCOCCUM (115) CIGM93.140
123	YAV_2/TEZ//T.MONOCOCCUM (121) CIGM93.143
124	CROC_1/T.URARTU (548) CIGM93.280
125	DOY1/T.URARTU (552) CIGM93.283
126	ALTAR84/T.URARTU (558) CIGM93.284
127	CETA/T.URARTU (558) CIGM93.285
128	DOY1/T.URARTU (559) CIGM93.286
129	DOY1/T.URARTU (561) CIGM93.288
130	CETA/T.URARTU (562) CIGM93.289
131	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (4) L94.519[f]
132	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (5) L94.520
133	ARLIN/T.BOEOTICUM (6) L94.637
134	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (9) L94.521
135	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (30) L94.523
136	ARLIN/T.BOEOTICUM (32) L94.638
137	CETA/T.BOEOTICUM (42) L94.388
138	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (51) L94.524
139	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (63) L94.525
140	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (72) L94.306
141	ARLIN/T.BOEOTICUM (84) L94.639
142	CPI/GEDIZ/3/GOO//JO69/CRA/4/T.BOEOTICUM (85) L94.526
143	ARLIN/T.BOEOTICUM (86) L94.640
144	ARLIN/T.MONOCOCCUM (94) L94.641
145	ARLIN/T.MONOCOCCUM (96) L94.642
146	CPI/GEDIZ/3/GOO//JO69/CRA/4/ T.MONOCOCCUM (100) L94.527
147	ARLIN/T.MONOCOCCUM (102) L94.643
148	ARLIN/T.MONOCOCCUM (103) L94.644
149	ARLIN/T.MONOCOCCUM (104) L94.645
150	ARLIN/T.MONOCOCCUM (105) L94.646
151	ARLIN/T.MONOCOCCUM (109) L94.647
152	ARLIN/T.MONOCOCCUM (116) L94.648
153	ARLIN/T.MONOCOCCUM (117) L94.649
154	ARLIN/T.MONOCOCCUM (120) L94.650

[a] Numbering system within the Wide Crosses Section.
[b] Number in parentheses is the diploid species accession number in the wide cross working collection.
[c] Cross number to use when requesting germplasm.
[d] Germplasm not currently available for distribution.
[e] Boldface indicates independent hybrid of the same cross.
[f] Combinations with the "L94" cross number are still in the greenhouse/laboratory stage of development; these will eventually receive a CIGM number. The L94 number can also be used when requesting germplasm.

APPENDIX 4

Partial (P) or complete (C) cross combinations of *Triticum aestivum* with some perennial Triticeae (each BCI has been selfed several times).

Cross	P or C	Chromosome status[a]
T.AESTIVUM/TH.INTERMEDIUM//T.AESTIVUM	P	2n=9x=63
T.AESTIVUM/TH.ACUTUM//T.AESTIVUM	P	2n=2x=63
T.AESTIVUM/TH.PULCHERRIMUM//T.AESTIVUM	P	2n=9x=63
T.AESTIVUM/TH.TRICHOPHORUM//T.AESTIVUM	P	2n=9x=63
T.AESTIVUM/TH.VARNENSE//T.AESTIVUM	P	2n=9x=63
T.AESTIVUM/TH.BESSARABICUM//T.AESTIVUM	P	2n=7x=49
T.AESTIVUM/TH.CURVIFOLIUM//T.AESTIVUM	C	2n=8x=56
T.AESTIVUM/TH.SCYTHICUM//T.AESTIVUM	C	2n=8x=56
T.AESTIVUM/TH.SCIRPEUM//T.AESTIVUM	C	2n=8x=56
T.AESTIVUM/TH.JUNCEIFORME//T.AESTIVUM	C	2n=8x=56
T.AESTIVUM//TH.REPENS/A.DESERTORUM/3/T. AESTIVUM	C	2n=8x=56
T.TURGIDUM//T.TURGIDUM/TH.DISTICHUM[b]	C	2n=8x=56

[a] Some variation is observed during maintenance.
[b] Seed source: R. Pienaar.

APPENDIX 5

Amphiploids resulting from combinations of *Triticum aestivum* and *T. turgidum* with some Triticeae species and their expected chromosome status.

Cross	Chromosome status[a]
T.TURGIDUM/TH.JUNCEIFORME	2n=8x=56
T.TURGIDUM/TH.INTERMEDIUM	2n=10x=70
T.TURGIDUM/TH.DISTICHUM[b]	2n=8x=56
T.TURGIDUM/TH.ACUTUM	2n=10x=70
T.TURGIDUM/AE.VARIABILIS	2n=8x=56
T.TURGIDUM/TH.JUNCEIFORME	2n=8x=56
T.TURGIDUM/TH.PULCHERRIMUM	2n=10x=70
T.TURGIDUM/AE.VAVILOVI	2n=10x=70
T.TURGIDUM/AE.OVATA	2n=8x=56
T.TURGIDUM/TH.TRICHOPHORUM	2n=10x=70
T.AESTIVUM/TH.BESSARABICUM	2n=8x=56
T.AESTIVUM/H.VILLOSA	2n=8x=56
T.AESTIVUM/AE.VARIABILIS	2n=10x=70
T.AESTIVUM/TH.SCYTHICUM	2n=10x=70
T.AESTIVUM/TH.TRICHOPHORUM	2n=12x=84
T.AESTIVUM/TH.INTERMEDIUM	2n=12x=84
T.AESTIVUM/TH.ACUTUM	2n=12x=84
T.AESTIVUM/TH.PODPERAE	2n=12x=84
T.AESTIVUM//TH.REPENS/A.DESERTORUM	2n=8x=56
T.AESTIVUM/TH.SCIRPEUM	2n=10x=70
T.AESTIVUM/TH.JUNCEIFORME	2n=12x=70
T.AESTIVUM/TH.ELONGATUM	2n=8x=56
T.AESTIVUM/TH.JUNCEUM	2n=12x=84
TH.ELONGATUM/T.AESTIVUM	2n=8x=56
TH.FIBROSUM/T.TURGIDUM	2n=8x=56

[a] Some variation is observed during maintenance.
[b] Seed source: R. Pienaar and E.R. Sears, respectively.

APPENDIX 6

Some intergeneric and trigeneric hybrids produced from crosses between Triticeae species and maintained at CIMMYT, El Batan, Mexico, under greenhouse conditions.

Cross number[a]	Hybrid combination	Chromosome status
	A. *T. aestivum*/*Thinopyrum* species	
B82-10824	CHINESE SPRING/TH.ACUTUM	2n=6x=42
B82-11004	FREMONT/TH.ACUTUM	2n=6x=42
B82-11058	NACOZARI 75/TH.ACUTUM	2n=6x=42
B82-10692	CHINESE SPRING/TH.CAESPITOSUM	2n=5x=35
B82-10759	CHINESE SPRING/TH.CAMPESTRE	2n=7x=49
B82-10939	CHINESE SPRING/TH.CURVIFOLIUM	2n=5x=35
B81-1084	CHINESE SPRING/TH.ELONGATUM (10x)	2n=8x=56
B81-1176	CHINESE SPRING-CNO (E)/TH.ELONGATUM (10x)	2n=8x=56
B81-1194	NYU BAY/TH.ELONGATUM (10x)	2n=8x=56
B81-1064	PAVON 76/TH.ELONGATUM (10x)	2n=8x=56
B81-1065	ZARAGOZA/TH.ELONGATUM (10x)	2n=8x=56
B82-10516	CHINESE SPRING/TH.INTERMEDIUM	2n=6x=42
B82-10848	GLENNSON 81/TH.INTERMEDIUM	2n=6x=42
B82-10656	NACOZARI 75/TH.INTERMEDIUM	2n=6x=42
B82-5063	CHINESE SPRING/TH.PODPERAE	2n=6x=42
B82-10643	CHINESE SPRING/TH.PULCHERRIMUM	2n=6x=42
B82-10840	CHINESE SPRING/TH.BESSARABICUM	2n=4x=28
B82-10780	CHINESE SPRING/TH.JUNCEIFORME	2n=5x=35
B82-10990	FIELDER/TH.JUNCEIFORME	2n=5x=35
B82-10910	FREMONT/TH.JUNCEIFORME	2n=5x=35
B82-10995	PAVON 76/TH.JUNCEIFORME	2n=5x=35
B82-11049	CHINESE SPRING/TH.JUNCEUM	2n=6x=42
B79-1006	TOBARI 66/TH.JUNCEUM	2n=6x=42
B82-10682	CHINESE SPRING/TH.RECHINGERI	2n=5x=35
B82-10669	PAVON 76/TH.RECHINGERI	2n=5x=35
B82-5049	CHINESE SPRING/TH.REPENS	2n=6x=42
B82-10559 to 10585	CHINESE SPRING//TH.REPENS/A.DESERTORUM (C3)	2n=35=57
B82-11042	CHINESE SPRING/TH.SCIRPEUM	2n=5x=35
B82-10865	CHINESE SPRING/TH.SCYTHICUM	2n=5x=36
B82-10861	FREMONT/TH.SCYTHICUM	2n=5x=35
B82-10602	CHINESE SPRING/TH.TRICHOPHORUM	2n=6x=42
B82-10899	CHINESE SPRING/TH.VARNENSE	2n=6x=42
B82-10948	NACOZARI 75/TH.VARNENSE	2n=6x=42
B82-10975	PAVON 76/TH.VARNENSE	2n=6x=42
B82-11008	FIELDER/TH.VARNENSE	2n=6x=42
B83-4630	CHINESE SPRING PH/TH.ACUTUM	2n=6x=42
B84-4614	CHINESE SPRING PH/TH.CAESPITOSUM	2n=5x=35
B84-5984	CHINESE SPRING PH/TH.ELONGATUM	2n=8x=56
B84-5995	CHINESE SPRING PH/TH.INTERMEDIUM	2n=6x=42
B86-6002	CHINESE SPRING PH/TH.TRICHOPHORUM	2n=6x=42
	B. *T. aestivum*/*Haynaldia* species	
B32-2528	CHINESE SPRING/H.VILLOSA	2n=4x=28

Some intergeneric and trigeneric hybrids produced from crosses between Triticeae species and maintained at CIMMYT, El Batan, Mexico, under greenhouse conditions (cont'd).

Cross number[a]	Hybrid combination	Chromosome status
	C. *T. aestivum*/*Leymus* species	
LOG82-135	FREMONT/L.ANGUSTUS	2n=9x=63
B82-10694	CHINESE SPRING/L.CINEREUS	2n=5x=35
B79-1002	CHINESE SPRING/L.RACEMOSUS	2n=5x=35
B82-10652	CHINESE SPRING/L.RACEMOSUS	2n=5x=35
B82-10653	CHINESE SPRING/L.TRITICOIDES	2n=5x=35
	D. *Thinopyron* and *Leymus* species/*T. aestivum*	
B81-1058	TH.ELONGATUM (10x)/BONZA	2n=8x=56
B80-1054	TH.ELONGATUM (10x)/JUPATECO 73	2n=8x=56
B81-1037	L.FIBROSUS/PAVON'S'	2n=5x=35
B81-1038	L.FIBROSUS/PAVON 76	2n=2x=14
	E. *Elymus* species/*T. aestivum*	
B81-1012A	E.AGROPYROIDES/CHINESE SPRING-CIANO (E)	2n=6x=42
B79-1018	E.AGROPYROIDES/NYU BAY	2n=6x=42
B79-1019	E.AGROPYROIDES/ZARAGOZA 75	2n=6x=42
B81-1009A	E.DAHURICUS/PITIC 66	2n=6x=42
	F. Other combinations	
B79-1009	TH.ELONGATUM (4x)/H.VULGARE	2n=3x=21
B84-5982	TH.BESSARABICUM/TH.JUNCEIFORME	2n=3x=21
B79-1008	TH.ELONGATUM (4x)/S.CEREALE	2n=3x=21
LOG 80-70	TH.TRACHYCAULUM/L.RACEMOSUS	2n=4x=28
LOG 80-22	E.AGROPYROIDES/L.RACEMOSUS	2n=5x=35
B79-1027	E.CANADENSIS/H.VULGARE	2n=3x=21
B81-1008	ELYMUS SP./S.CEREALE (4x)	2n=4x=28
B79-1031	H.VULGARE/E.CANADENSIS	2n=3x=21
B79-1015	H.VULGARE/E.PATAGONICUS	2n=4x=28
	G. *T. turgidum*/*Thinopyrum* (Th) species	
B83-4316	COCORIT71/TH.ACUTUM	2n=5x=35
B83-4604	G-803/TH.ACUTUM	2n=5x=35
B83-4297	YAVAROS79/TH.ACUTUM	2n=5x=35
B83-4337	COCORIT71/TH.LITTORALE-CAMPESTRE	2n=6x=42
B83-4346	COCORIT71/TH.INTERMEDIUM	2n=5x=35
B83-4438	MEXICALI75/TH.INTERMEDIUM	2n=5x=35
B83-4309	YAVAROS79/TH.INTERMEDIUM	2n=5x=35
B83-4373	COCORIT71/TH.JUNCEIFORME	2n=4x=28
B79-1004	COCORIT71/TH.JUNCEUM	2n=5x=35
B81-5036	COCORIT71/TH.PODPERAE	2n=5x=35
B83-4416	MEXICALI75/TH.PODPERAE	2n=5x=35
B83-4385	COCORIT71/TH.PULCHERRIMUM	2n=5x=35
B83-4470	MEXICALI75/TH.PULCHERRIMUM	2n=5x=35
B81-5040	MEXICALI75/TH.SCYTHICUM	2n=4x=28
B83-4524	MEXICALI75/TH.TRICHOPHORUM	2n=5x=35
B83-4536	MEXICALI75/TH.VARNENSE	2n=5x=35
B83-4572	G-803/TH.VARNENSE	2n=5x=35
B83-4552	CAPPELLI PH PH/TH.ACUTUM	2n=5x=35
B83-4545	CAPPELLI PH PH/TH.INTERMEDIUM	2n=5x=35
B83-4546	CAPPELLI PH PH/TH.VARNENSE	2n=5x=35

Some intergeneric and trigeneric hybrids produced from crosses between Triticeae species and maintained at CIMMYT, El Batan, Mexico, under greenhouse conditions (cont'd).

Cross number[a]	Hybrid combination	Chromosome status
	H. *T. timopheevii* / *Thinopyrum* species	
-	T. TIMOPHEEVII/TH. ELONGATUM (10x)	2n=7x=49
	I. *Agropyron* species/*T. turgidum*	
B79-1012	TH.ELONGATUM/COCORIT71	2n=3x=21
B81-1040	L.FIBROSUS/COCORIT71	2n=4x=28
B80-1046	L.FIBROSUS/MEXICALI75	2n=4x=28
B80-1050	L.FIBROSUS/QUILAFEN	2n=4x=28
L80-1765B	A.SCABRIFOLIUM/MEXICALI75	
L80-1062	A.SIBERICUM/COCORIT71	2n=4x=28
B80-1033	A.TRACHYCAULUM/COCORIT71	2n=4x=28
B80-1032	A.TRACHYCAULUM/MEXICALI75	2n=4x=28
	J. *Elymus* species/*T. turgidum*	
B81-5035	E.VIRGINICUS/COCORIT71	2n=4x=28
	K. Trigeneric hybrids	
L80-1758B	L.FIBROSUS/T.TURGIDUM//S.CEREALE	2n=5x=35
B84-6025	E.CANADENSIS/T.AESTIVUM//S.CEREALE	2n=6x=42
B83-6112	T.AESTIVUM/TH.CURVIFOLIUM//S.CEREALE	2n=6x=42
B83-6153	T.AESTIVUM/TH.INTERMEDIUM//S.CEREALE	2n=7x=49
B83-5269	T.AESTIVUM/TH.JUNCEIFORME//S.CEREALE	2n=6x=42
B84-6023	T.AESTIVUM/A.PULCHERRIMUM//S.CEREALE	2n=7x=49
B81-1255	T.AESTIVUM/L.RACEMOSUS//TH.ELONGATUM	2n=10x=70
B81-1259	T.AESTIVUM/L.RACEMOSUS//T.AESTIVUM/3/ TH.ELONGATUM	2n=59 -66
B81-1270	T.AESTIVUM/L.RACEMOSUS//S.CEREALE	2n=6x=42
B81-5109	T.TIMOPHEEVII/H.BOGDANII**//S.CEREALE	2n=4x=28

[a] Numbering system within the Wide Crosses Section.

GLOSSARY

Addition line—A line with an extra alien chromosome either in one (monosomic) or in two (disomic) doses to the euploid chromosome complement, e.g., in wheat as 2n=6x=42+1 or 2n=6x=42+2.

Allele—One, two, or more alternate forms of a gene occupying the same locus on a particular chromosome.

Allopolyploid—Individual plant arising from the crossing of two or more species/genera, each with different chromosome sets that are represented at least once or in a greater number, i.e., three (ABD) in bread wheat.

Allosyndetic—Term used to describe the chromosome association (pairing) at meiosis between complete or partially homologous chromosomes of the parental gametes.

Allozyme—Any of the multiple forms of a multimeric enzyme, the subunits of which are coded by alleles of the same gene.

Amino acid—The building block of a protein. There are 20 common amino acids that occur naturally. They all have the same basic structure.

Amphiplasty—A morphological change in chromosomes during wide hybridization where the secondary constrictions of one parent do not express in the hybrid.

Amphiploid—A plant derivative with the doubled chromosome composition of an F1 hybrid resulting from a wide cross. It either originates spontaneously or is induced by colchicine treatment of the F1 plant.

Aneuploidy—Cells having one or more whole chromosomes of a euploid complement absent from or in addition to that complement.

Anneal—The incubation of a mixture of DNAs in single-stranded form to make double-stranded DNA.

Annual—A plant requiring annual replanting, such as the major food crops: wheat, maize, rice, beans, etc.

Anodal—The positive electrode, abbreviated "+".

Anther culture—Part of a stamen (anther) placed on an artificial medium for eventual regeneration into haploid plants.

Anthocyanin pigmentation—Reddish/purple pigment distributed/localized in plant parts that can serve as a morphological marker.

Apomictic—The replacement of sexual reproduction by an asexual process that does not result in gametic fusion.

Autopolyploid—The presence of more than two chromosome sets that are characteristic of the species.

Autotetraploid—A species that possesses four homologous chromosome sets per cell.

Bivalent—Chromosome pairing configuration during the first meiotic division, which consists of two completely or partially homologous chromosomes associated in the form of a ring or a rod.

Bivalentization—Phenomenon of bivalent formation brought about in systems where reduced multivalents occur as a consequence of cytological and genetic control mechanisms.

Boot inoculation test—Disease screening test in which the pathogen inoculum is injected into the young wheat spike prior to its complete emergence. The void space is comprised of the foliage plus the embedded spike, which is called the boot.

Bridge cross—An indirect means of combining two incompatible species (A and C) by using a compatible third species (B) that hybridizes with both A and C.

Callus—A disorganized mass of undifferentiated plant cells that grow out from plant tissue placed in an artificial medium.

Cathodal—The negative electrode, abbreviated "-".

Chiasmata—Term used in association with possible exchange of homologous parts between nonsister chromatids in prophase I of the meiotic cell cycle.

Chimera—A plant composed of tissues of two or more idiotypes as a consequence of mutation or somatic segregation.

Chromatin—A complex ensemble of DNA, basic chromosomal protein, nonhistone chromosomal protein, and a small amount of RNA in the interphase nuclei of eukaryotes.

Colchicine—A chemical used to induce polyploidy, i.e., doubling the chromosome number.

Cytological marker—A diagnostic marker that relies upon cytological uniqueness (mitotic chromosome variations or differential staining) so as to identify wheat and alien contributions to a hybrid.

Desynaptic—The falling apart during late prophase I (diplotene or diakinesis) of chromosomes earlier paired during the zygotene and pachytene stages.

Digoxigenin—A chemical used for nonradioactive labelling; in CIMMYT's case, for *in situ* hybridization.

Disulfide bridge—Form of linkage between SH molecules that are associated with the structure of proteins.

Ditelosomics—Cells that lack two homologous chromosome arms.

Electrophoresis—A method whereby charged molecules in solution, mainly proteins and nucleic acids, migrate in response to an electric field.

Enzyme—Protein, which even in low concentrations, speeds up, enables, or controls chemical reactions in living organisms without itself being used up in the reactions.

Epistasis—Form of gene interaction whereby one gene interferes with the phenotypic expression of another nonallelic gene.

Eukaryote—A plant whose cells have nuclei with membranes, chromosomes, and well defined nuclear division like mitosis and meiosis.

Euploid—Term used for cells with one complete set or with whole multiples of the basic number of chromosomes characteristic of the species.

Gel—The base component upon which protein separations are realized, for example, during electrophoresis.

Gene—The chemical units of heredity located on a chromosome that, when expressed, determine an organism's characteristics.

Genome—The complete genetic code for any individual species.

Germplasm—The genetic material that forms the physical basis of heredity for a species and that is transmitted from one generation to the next by means of germ cells.

Gliadin—A storage protein component; used in characterizing and identifying alien genetic material in a wheat background.

Glutenin—A storage protein component; used in characterizing and identifying alien genetic material in a wheat background.

Haplo-diploidization—The instant creation of a homozygous line when the chromosomes of a haploid plant are doubled.

Haploid—Cell with a single genome.

Helical configuration—The natural conformation of many biological polymers (e.g., nucleic acid, proteins), characterized by a spiral structure with a repeating pattern.

Heterochromatic—Segments or entire chromosomes of eukaryotes that remain condensed during interphase and stain differently.

Homoeologous—Term used for the three chromosomes in each of the seven basic chromosome groups of wheat, meaning they are similar and considered to have a common evolutionary origin.

Homoeologous chromosome group—The chromosomal structure that places the wheat chromosomes (21 different chromosomes) in an orthogonal classification of seven groups of three chromosomes each.

Homologous—State in wheat where, under the influence of the dominant pairing regulator gene (Ph), the 42 chromosomes at meiosis pair to form 21 bivalents; pairing takes place between homologous chromosomes.

Homozygous—Term used to denote that the genetic stability of a plant is maximized.

Hybridity—The state of being heterozygous or a hybrid.

Hybridization—Any cross-mating of two genetically different individuals, which results in hybrid progeny.

Hyperploid—A plant containing cells with one or more added chromosomes or chromosome segments in their complement.

Hypoploid—A plant containing cells that lack one or more chromosomes or chromosome segments in their complement.

Idiotype—Total heredity determinant of an organism.

***In situ* hybridization—**Hybridization of labelled DNA or RNA probes with denatured cellular DNA or cells on microscopic slides.

Intergeneric—A cross mating between two species possessing dissimilar genomes.

Interspecific—A cross mating between two species possessing similar genomes.

Interstitial—Term used in meiotic analyses where successive chiasmata are present in a bivalent association between chromosomes; referred to as "interstitial chiasma".

Isoelectric focusing—A method in which proteins are separated in a pH gradient according to the isoelectric points of a protein mixture.

Isozyme—Multiple forms of an enzyme that occur within the same organism, which have similar or identical catalytic activities; used in characterizing and identifying alien genetic material in a wheat background.

***Kr* crossability—**Genetic loci (*Kr*1, 2, and 3) that influence the hybridization frequency between wheat and rye and reportedly extend to other combinations in cereals. Recessive loci promote crossability and are on homoeologous group 5.

Ligand—A small molecule (including activators, substrates, and inhibitors of enzyme activity) that is bound to a protein by noncovalent forces.

Meiocyte—Any cell in which the nucleus divides by meiosis.

Micropylar—Term associated with the structural position details of the embryo where one end is referred to as the "micropylar end", which is flanked by the integument layers.

Microsporogenesis—The formation of microspores in anthers of angiosperms.

Multimeric—An active aggregate resulting from the interaction of polypeptides (monomers).

Nucleolar organizer—The chromosome region that is active in nucleolus formation and contains ribosomal DNA from which ribosomal RNA is transcribed.

Nucleotide—Any of the monomeric units that are the building blocks of the polynucleotides, which are referred to as nucleic acids.

Oligomeric—A protein composed of identical subunits (polypeptide chains).

Oligonucleotide—Series of nucleotides where each molecule comprises a base, a sugar, and a phosphate group.

Ontogeny—The course of development of an organism.

Orthologous—Two genes (or proteins) that diverged at the same time when the species harboring them diverged.

Paralogous—Duplicate genes (or proteins).

Passage—Term used in tissue culture defined as the duration involved between transferring embryogenic callus over a certain period (four to six weeks for wheat).

Peptide bond—Chemical linkage between amino acids in which the carboxyl group of one amino acid is joined with the amino group of another amino acid.

Perennial—Plants that do not require annual replanting, such as coffee, cocao, rubber, etc.

Phylogenetic—The evolutionary history of an organism or taxonomic group.

Pivital-differential evolution—Associated with evolution in a group where a genome in a polyploid species has not been substantially modified (pivital), but the other genome(s) present have undergone extensive modification (differential).

Polyacrylamide—A chemical used in electrophoresis.

Polygenic—Traits or characters whose expression is controlled by many genes.

Polyhaploid—A haploid plant that originates from a polyploid species.

Polymorphism—The occurrence of one or several chromosomes in two or more alternate structural forms within a plant population as a consequence of mutational or evolutionary change.

Polypeptide—A polymeric-covalently bonded arrangement of amino acids joined together by peptide linkages.

Polypeptide chain—A chain of amino acids joined together by peptide bonds.

Polyploid—Plants with somatic cells having three or more complete chromosome sets instead of two, as in diploids; these either arise spontaneously or are induced.

Probe—A defined DNA or RNA sequence used to detect complementary sequences through hybridization techniques.

Pyramiding—Accumulation through hybridization of several genes in an individual plant so as to make a species less vulnerable to prevalent stresses.

Ring bivalent—Chromosome pairing configuration at meiosis of a ring shape emerging from association of homologous chromosomes.

Somaclonal variation—Variation that occurs in tissue culture-regenerated derivatives.

Somatic cell—Vegetative cellular tissues of an organism that multiply by the mitotic division.

Southern hybridization—Transfer of single-stranded, restricted DNA fragments, separated in an agarose gel, by a nitrocellulose filter, which is then analyzed by hybridization with radioactive or biotinylated single-stranded DNA or RNA probes.

Storage protein—Protein present in an ungerminated seed that provides energy for the developing embryo; used in characterizing and identifying alien genetic material in a wheat background.

Streptavidin—Immunochemical used for *in situ* hybridization.

Suppressor—A genetic mechanism that masks resistance genes in hybrids of either parent involved in the combination.

Synthetic—Artificially developed; in CIMMYT's case this relates to hybrids containing the wheat genomes donated by wheat's progenitors.

Synthetic hexaploid—The hexaploid of *Triticum turgidum* x *T. tauschii* with 42 chromosomes (2n=6x=42; AABBDD).

Telocentric—Chromosomes or chromatids with a terminal centromere (primary constriction).

Total genomic DNA—The nuclear DNA leaf extract utilized in genomic blocking/label application diagnostics for *in situ* hybridization.

Transgressive segregration—The appearance in a single generation (F2 backcross, etc.) of one or more genotypes that fall outside the limits of variation defined by the parents and the F1 of the cross, in respect to one or more characters.

Triticeae—A tribe of approximately 325 perennial and annual species that contains a vast genetic reservoir of germplasm for crop improvement; includes the forage grasses and three major cereals—wheat, barley, and rye.

Trivalent—A three-chromosome association at meiosis.

Type species—Species whose genomic constitution is the base for bringing all taxa with the same basic genome or combination of genomes into that genus.

Univalent—An unpaired chromosome at meiosis

Wide cross—A cross between two plants that do not normally hybridize, such as between two genera (e.g., whea and rye).

Zygote—A diploid cell formed by the fusion of two haploid gametes during fertilization.

Zymogram—Protein banding profile seen on an electrophoretic gel.

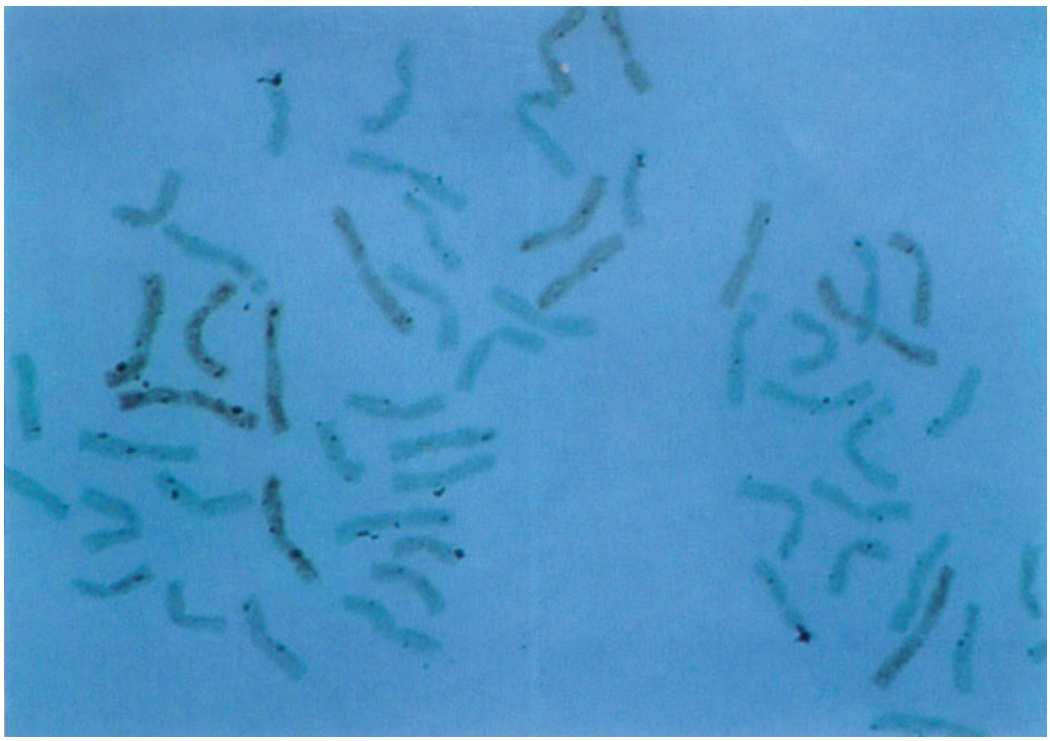

Plate 1. Detection of *Thinopyrum bessarabicum* chromosomes in the amphiploid of *Triticum aestivum* cv. Chinese Spring/*Th. bessarabicum* (2n=8x=56, AABBDDJJ) using genomic *in situ* hybridization.

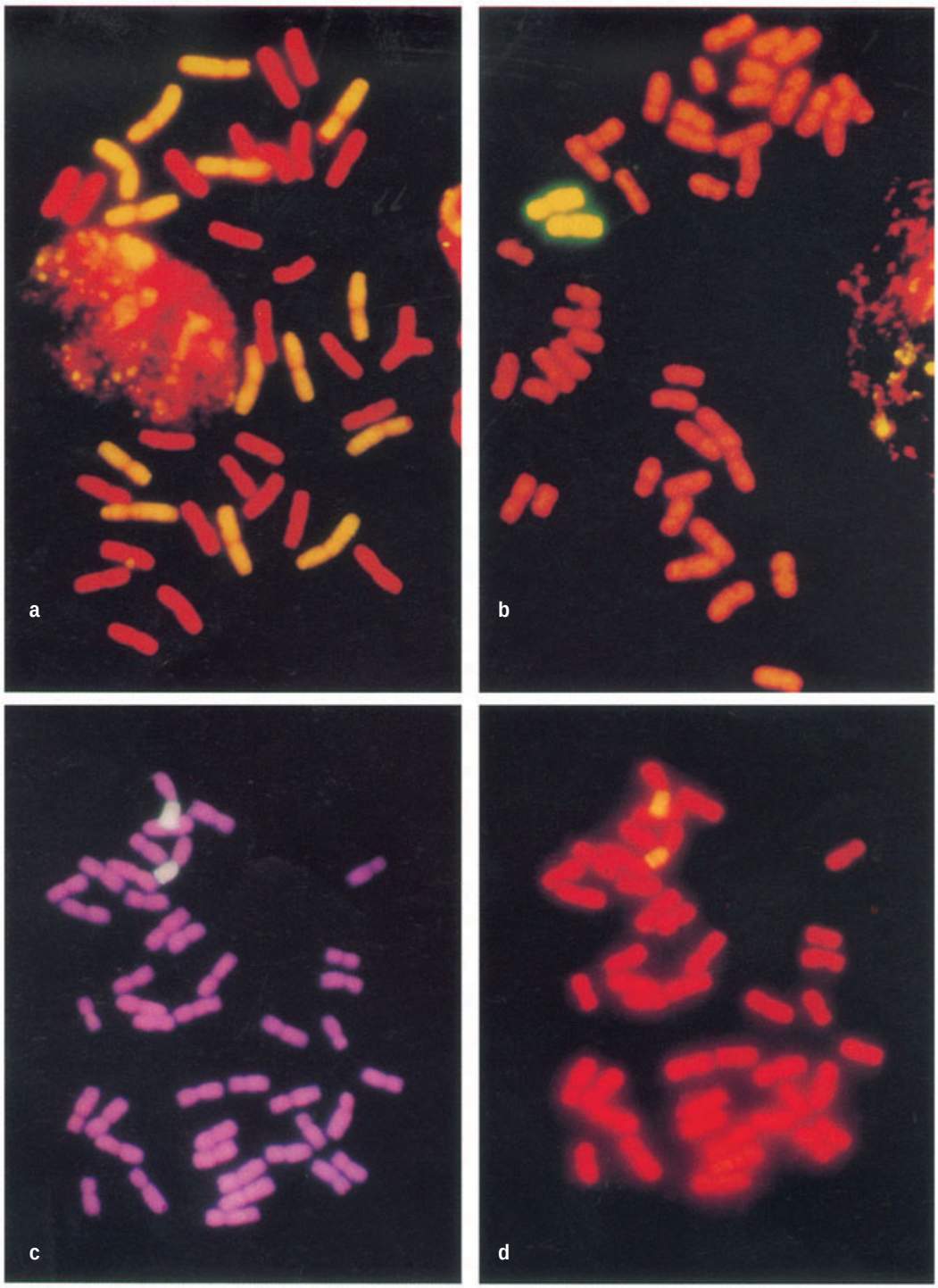

Plate 2. a) A fluorescence micrograph of X *Triticosecale* (2n=6x=42, AABBRR) with filter 9. The unlabeled wheat chromosomes are orange-red while the labeled yellow 14 chromosomes are of rye. b) Rye chromosome 1R disomic addition line to *T. aestivum* cv. Chinese Spring (2n=6x=42+1R1R). Filter 9 allowed detection of the two rye chromosomes. c) A translocated 1BL/1RS wheat cultivar with 42 chromosomes in which the two rye segments (1RS) are visible. The unlabeled wheat chromosomes are bluish-purple whereas with filter 2 (DAPI stain), the rye exchange was detectable. d) The same cell as in c) viewed under filter 23 showing the diagnostic for 1RS segments.

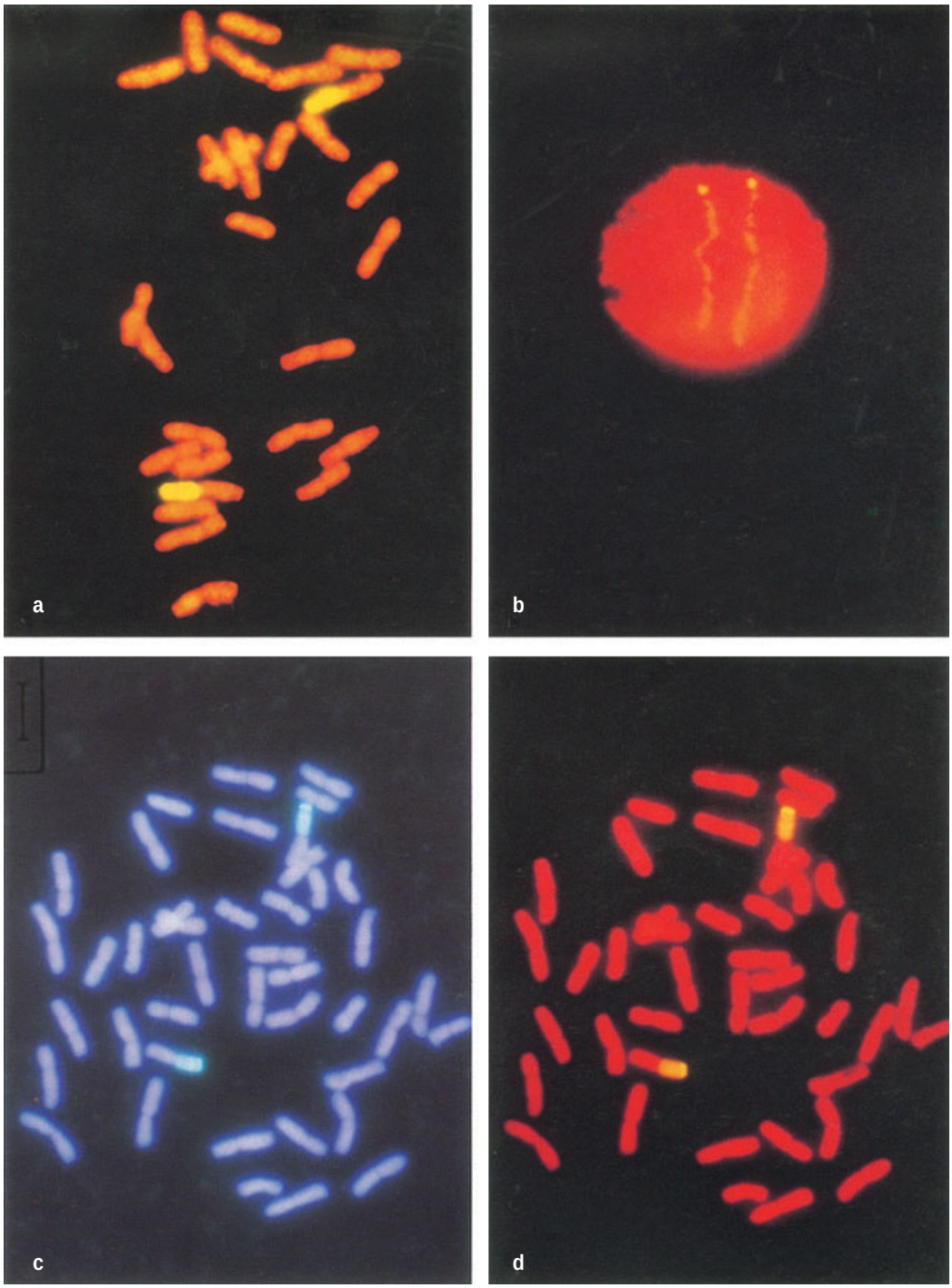

Plate 3. a) A 1BL/1RS homozygous substitution in a durum wheat (2n=4x=28) showing the bright yellow rye arm (1RS) when rye DNA was used as a probe and unlabeled wheat DNA was used for blocking with filter 9. b) An interphase mitotic cell of a homozygous 1AL/1RS segment using filter 23. c) A homozygous 1AL/1RS *T. aestivum* cultivar with the 1RS rye segment detected with filter 2. d) 1AL/1RS metaphase cell with 42 chromosomes. Filter 23 and labeled rye DNA discriminated the 1RS segment.

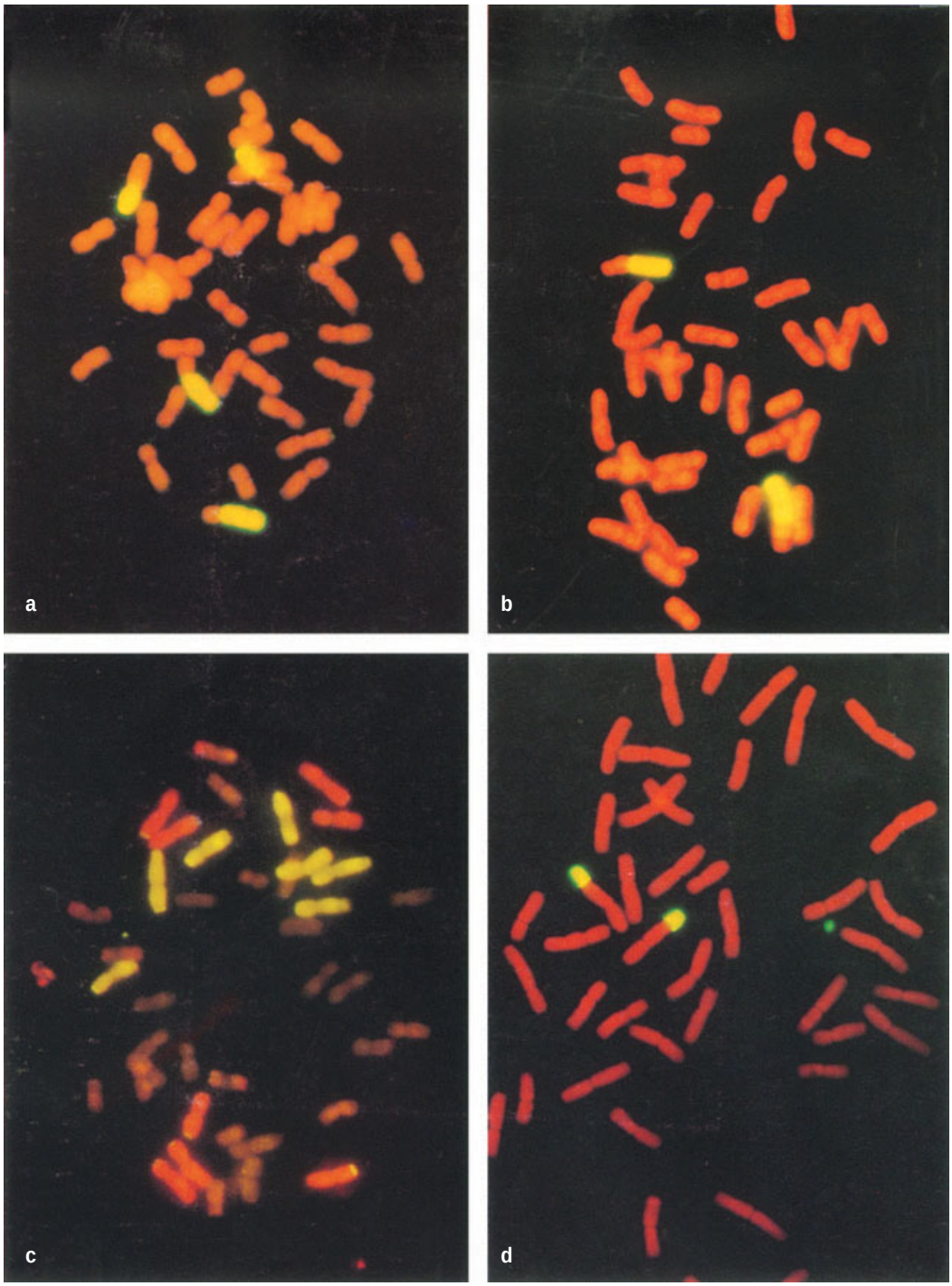

Plate 4. a) A homozygous double translocation in wheat involving rye segments 5RL and 1RS onto wheat chromosomes 5AS and 1BL, respectively, detected with filter 9 and rye DNA as a probe. b) A homozygous 6BS/6RL translocation wheat with 42 chromosomes showing the two rye (6RL) segments as detected under filter 9 with rye DNA as a probe. c) Double labeling on an aneuploid trigeneric hybrid of *T. aestivum*/ *Th. bessarabicum*//*S. cereale* with 40 chromosomes. Wheat DNA was unlabeled; *Th. bessarabicum* and *S. cereale* were labeled with biotin and digoxigenin, respectively. The eight reddish *Th. bessarabicum* and seven yellowish-green rye chromosomes were resolved under filter 23. d) A homozygous rye DNA insert associated with the leaf rust *Lr25* gene. Rye DNA was used as a probe and unlabeled wheat DNA was used for blocking. Detection was made under filter 2.