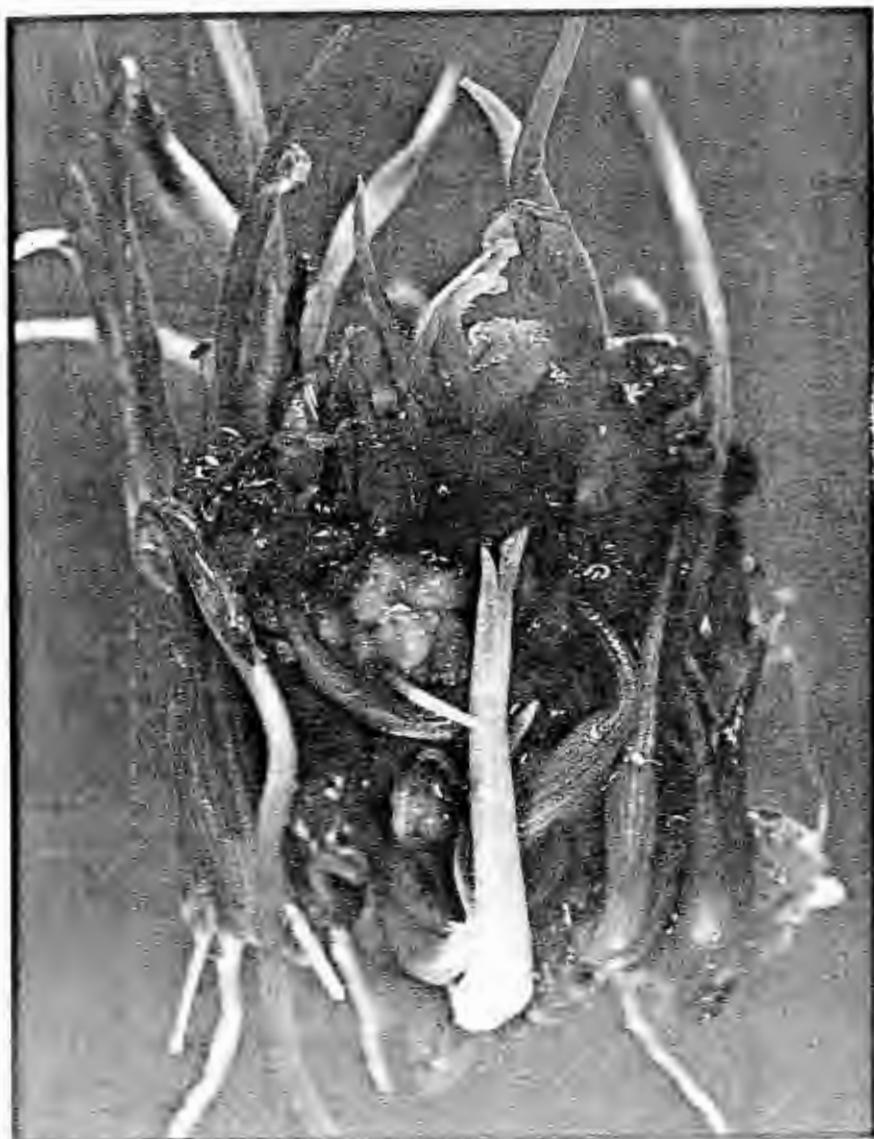


Review of Advances in Plant Biotechnology, 1985-88

A. Mujeeb-Kazi and L.A. Sitch, technical editors



International Maize and Wheat Improvement Center
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Preface

There is great potential for biotechnology as a tool in crop breeding. Despite the risk in such upstream research, many plant breeders are becoming more involved in this work to facilitate the rapid exploitation of new techniques. Genetic manipulation of plants involving tissue culture, restriction fragment length polymorphisms, isozymes, and incorporation of genes from wild species is creating a meteoric revolution for plant breeders.

To keep abreast of the rapid developments in this field, nearly 50 participants from developed and developing countries attended the Second International Symposium on Genetic Manipulation in Crops at the International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico, August 29-31, 1988. This meeting was a follow-up to the First International Symposium on Genetic Manipulation in Crops held Oct. 22-26, 1984, in Beijing, China.

The first symposium, which essentially reviewed genetic manipulation work up through 1984, was sponsored by Academia Sinica and the International Rice Research Institute (IRRI). The first meeting was so successful that it prompted CIMMYT to join Academia Sinica and IRRI in co-sponsoring the second symposium.

The second symposium provided the opportunity to analyze research achievements since 1984—hence the title of these proceedings, *Review of Advances in Plant Biotechnology, 1985-88*. The relatively small number of participants (compared to the first symposium) permitted conferees to focus more sharply on research needs and develop the collaborative mechanisms necessary for charting broad pathways for work in this field into the future.

These proceedings start off with a keynote address that looks into the scientific, social, economic, and ethical implication of genetic manipulation in crops. Following this are a selected collection of 27 papers and 6 posters presented during the symposium that update current work in the discipline and review the literature in four specific areas: 1) anther culture and haploid breeding, 2) protoplast culture, somatic hybridization, and transformation systems, 3) distant hybridization; and 4) somatic embryogenesis

and somaclonal variation. A fifth session addressed international collaboration in genetic manipulation of crop plants. Crops covered in these presentations include rice, wheat, maize, barley, triticale, citrus, sugar beet, brassicas, tropical forage legumes, cassava, and cotton.

It is the consensus of plant breeders, geneticists, and other biologists working in crop plant improvement that biotechnology holds the most hope for rapid improvement of crop plants, and for achieving the kinds of advances required for the sustained yield increases demanded in the face of an expanding world population and shrinking land resources. Both CIMMYT and IRRI, currently developing their research strategies towards the year 2000, found the ideas that emerged from this second symposium to be very useful in their program development.

It is now apparent that additional symposia on the subject of Genetic Manipulation in Crops will be held. At this meeting, an organizing committee was formed to start planning for the third symposium, tentatively set for 1991 in Africa.

We take this opportunity to recognize other members of the Second Symposium Organizing Committee, namely: Z.S. Li, H. Hu, and Q.Q. Shao from Academia Sinica; and G.S. Khush from IRRI. We express gratitude to the staff of CIMMYT for being our gracious hosts, and to the United Nations Development Programme, the Rockefeller Foundation, the U.S. Agency for International Development, and the Third World Academy of Sciences for their financial assistance. We thank Gene P. Hettel, science writer/editor for CIMMYT Information Services, for editing these proceedings and coordinating their publication.

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Marker-assisted introgression of alien chromatin into wheat

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The study involved assessment of some schemes for increasing the probability of transfers of alien chromatin into the wheat genome in wheat-alien hybrids. Backcross I (BCI) seed set, earlier a significant constraint, has been obtained from *ph1b* or N5BT5-/Alien sp. F₁ hybrids involving *Aegilops* species and rye cultivars making alien introgression through allosyndetic pairing at F₁ a viable procedure. Another scheme deals with topcrossing the *Triticum aestivum* or *T. turgidum* based F₁ hybrids with *T. turgidum* or *T. aestivum*, respectively, in order to induce centromeric breakage and fusion of chromosomes. It has been successfully implemented in attaining advanced derivatives from the initial cross. Modifications of these major schemes were also initiated. Monitoring of the alien chromosomes in the progenies was through application of cytological, morphological, and biochemical markers. These have assisted in the production of several alien chromosome additions of *Haynaldia villosa*, *Agropyron junceum*, and *Elymus giganteus* in a *T. aestivum* background.

The wild relatives of wheat and other Triticeae have long been recognized as valuable reservoirs of useful genes for the improvement of this important cereal (Feldman and Sears 1981, Dewey 1984). Some of the barriers to interspecific hybridization have been overcome, thus permitting exploitation of a small but significant part of this gene source (Sharma and Gill 1983, Mujeeb-Kazi and Kimber 1985, Brar and Khush 1987, Mujeeb-Kazi and Asiedu 1988a,b). Apart from the hybridization barriers, the major limitation to introgression of alien genes into wheat has been the lack of pairing and recombination between wheat and alien chromosomes (ter Kuile *et al.* 1987). While pairing in wheat is influenced by a number of suppressor and promoter genes on chromosomes 2, 3, 4, and 5 (Sears 1976, Kimber and Feldman 1987), the most potent of the genes is well documented as the suppressor of homoeologous pairing (*Ph*) on 5BL of *Triticum aestivum* and *T. turgidum* (Okamoto 1957, Riley and Chapman 1958) although its mode of action is rather controversial (Feldman 1966, Driscoll 1979).

In order to induce allosyndesis, it has been necessary to remove the 5B chromosome (Lacadena 1967, Koebner and Shepherd 1986), suppress the gene with an alien genome, or use mutant lines of wheat (Sears 1984). Accessions of *Aegilops speltoides*, *Ae. mutica*, and *Ae. longissima* have been reported to show variability for ability to suppress the *Ph* gene (Dover and Riley 1972, Chen and Dvorak 1984). Similar promotion of heterogenetic pairing in wheat backgrounds has been reported for barley (Fedak 1977) and rye (Lelley 1976, Dhaliwal *et al.* 1977, Dvorak 1977, Naranjo *et al.* 1979). Studies of a number of polyploid *Aegilops* species have not revealed *Ph*-like genes in these species (Abu Baker and Kimber 1982, McGuire and Dvorak 1982, Sharma and Gill 1986) even though the pairing systems in these species must be under some genetic control (Gupta and Fedak 1985). In the absence of the *Ph* genes in wheat/*Aegilops* hybrids, the *Aegilops* species may or may not influence homoeologous chromosome pairing (Abu Baker and Kimber 1982, McGuire and Dvorak 1982). Driscoll and Quinn (1970) reported variability among cultivars of *T. aestivum* for the level of heterogenetic pairing following hybridization to *Ae. variabilis*.

The methods used for wheat-alien transfers include irradiation to induce translocations (Driscoll and Jensen 1964, Sharma and Knott 1966) induction of centromeric breakage and fusion (May and Appels 1982, Lukaszewski and Gustafson 1983) and induction of allosyndesis (Riley *et al.* 1968, Joshi and Singh 1978, Koebner and Shepherd 1985, 1986). Driscoll (1968) proposed a combination of irradiation and meiotic control for increased rate and specificity of wheat-alien transfers. Darvey (1984) later suggested the use of the *ph* mutant in direct hybridization with alien species for the construction of an alien gene bank, but attempts by Sharma and Gill (1986) in this direction were unsuccessful, while those of ter Kuile *et al.* (1987) were promising.

Many of the important traits desired from the alien species are not easy or cheap to select for in the segregating populations, especially during early generations when the populations need to be screened to avoid carrying too many unwanted families later. Some of the complications are the lack of knowledge of the biochemical and genetic control of the characters, heterogeneity of the breeding materials, sporadicity of natural disease epiphytotics, environmental influence, the expense associated with artificial infestations, and the large number of samples or plants required for efficient screening. It is in this context that heritable and more easily identifiable characteristics, termed markers, associated with the desired agronomic traits become an important consideration (see Diagnostic Markers in Wheat Wide Crosses in the Poster Section of these Proceedings). Cusick and McIntosh (1987) and Hart and Gale (1987), respectively, have provided a linkage map of wheat and a listing of biochemical/molecular markers currently available for wheat research. Miller and Reader (1987) have compiled many of the morphological markers associated with specific homoeologous groups in the Triticeae. Furthermore, the use of differential chromosome staining for recognition of transferred alien segments has been demonstrated (Gill and Kimber 1977, Lukaszewski and Gustafson 1983).

Our objectives were to evaluate and test several hybridization schemes for the introduction of useful alien chromatin into wheat and to track such chromatin in the wheat background by using different markers in segregating populations.

MATERIALS AND METHODS

The alien species used are listed in Table 1. The *Aegilops* accessions were originally obtained from the Plant Breeding Institute (PBI), Cambridge. Seeds of *Haynaldia villosa* and *Thinopyrum distichum* were obtained from E.R. Sears (Missouri, USA) and R. Pienaar (South Africa), respectively. The *Leymus* and remaining *Thinopyrum* species were supplied by D.R. Dewey (Logan, Utah, USA). In addition to the foregoing, several cultivars of *T. aestivum* including 'Chinese Spring', its *ph1b* mutant, and nullisomic 5B tetrasomic 5A (or 5D) stocks and cultivars of *T. turgidum* var. *durum* were used. Seeds of the *ph1b* mutant were provided by K.W. Shepherd (Adelaide, Australia). All other cultivars used were from CIMMYT. Most of the alien species used are known to have useful traits for wheat improvement.

Four crossing schemes were employed:

- A. Hybrids were made between *ph1b* or CSN5BT5- with all the *Aegilops* species, the *S. cereale* cultivars, *Thinopyrum bessarabicum*, and *Thinopyrum junceiforme*. Embryo rescue onto standard artificial medium was performed for the hybrids involving the *Thinopyrum* species. The F₁ hybrids were backcrossed to CS, *ph1b*, and/or cultivars of *T. aestivum* and *T. turgidum* var. *durum*. All backcross I (BCI) plants obtained were backcrossed to cultivars of *T. aestivum*.

Table 1. Species of *Triticum* (*Aegilops*), *Haynaldia*, *Leymus*, *Secale*, and *Thinopyrum* used in this study.

Species	Acc. No. ^a	Genome ^b	Ploidy Level
<i>T. macrochaetum</i> (<i>Ae. biuncialis</i>)	10A	UM	4X
<i>T. dichasians</i> (<i>Ae. caudata</i>)	16A	C	2X
<i>T. cylindricum</i> (<i>Ae. cylindrica</i>)	17G	CD	4X
<i>T. cylindricum</i> (<i>Ae. cylindrica</i>)	18A	CD	4X
<i>T. juvenale</i> (<i>Ae. juvenalis</i>)	45A	DMU	6X
<i>T. tripsacoides</i> (<i>Ae. mutica</i>)	24D	Mt	2X
<i>T. ovatum</i> (<i>Ae. ovata</i>)	4E	UM	4X
<i>T. neglecta</i> (<i>Ae. triaristata</i>)	5F	UM	4X
<i>T. triunciale</i> (<i>Ae. triuncialis</i>)	15X	UC	4X
<i>T. peregrinum</i> (<i>Ae. variabilis</i>)	13E	US	4X
<i>T. syriacum</i> (<i>Ae. vavilovii</i>)	41A	DMS	6X
<i>H. villosa</i>	(ex Sears)	V	2X
<i>L. racemosus</i>	PI313965	JN	4X
<i>S. cereale</i> (cv. Elvon)	-	R	2X
<i>S. cereale</i> (cv. Prolific)	-	R	2X
<i>S. cereale</i> (cv. Semi-dwarf)	-	R	2X
<i>Thinopyrum distichum</i>	(ex Pienaar)	E ₁ E ₂	4X
<i>Thinopyrum bessarabicum</i>	Jaaska-11	J	2X
<i>Thinopyrum junceiforme</i>	PI414667	J ₁ J ₂	4X
<i>Thinopyrum scirpeum</i>	(ex Dewey)	E ₁ E ₂	4X

^a Accession number.

^b After Kimber and Feldman (1987) and Dewey (1984).

- B. F_1 hybrids of CS with *H. villosa* (Mujeeb-Kazi and Bernard 1985) and *Thinopyrum bessarabicum* (Mujeeb-Kazi *et al.* 1984, 1987) were backcrossed to *ph1b* and spikes of the progenies were bagged to ensure selfing (*T. aestivum*/Alien//*ph1b* Ⓣ).
- C. Cultivars of *T. turgidum* var. *durum* were used as pollen parents for crosses to F_1 hybrids of CS with *Th. bessarabicum* (Mujeeb-Kazi *et al.* 1984, 1987), *H. villosa* (Mujeeb-Kazi and Bernard 1985) and *L. racemosus* (Mujeeb-Kazi and Rodríguez 1981), respectively. The resulting BCI progenies were backcrossed to cultivars of *T. aestivum*. An F_1 hybrid, *T. turgidum* cv. Memo/Mexicali//*Th. junceiforme* (Mujeeb-Kazi and Bernard 1985), was also backcrossed to *T. aestivum* cv. Alondra/Pavon, i.e. *T. aestivum*/Alien//*T. turgidum*/3/*T. aestivum* and *T. turgidum*/Alien//*T. aestivum*.
- D. An F_1 hybrid, *T. turgidum* cv. Altar 84/*Th. scirpeum* (Mujeeb-Kazi, unpublished), was topcrossed with *ph1b* and the progeny was backcrossed to cultivars of *T. aestivum* directly or after one cycle of selfing, i.e. *T. turgidum*/Alien//*ph1b*/3/*T. aestivum*.

These approaches were designed to: 1) promote homozygosity for the *ph1b* locus after selfing the BCI in order to enhance recombination and 2) induce D genome and alien genome translocations (Mujeeb-Kazi 1984).

All plants were grown in the greenhouse and authenticity of hybrids was checked by root-tip counts (Mujeeb-Kazi and Miranda 1985) sometimes supported by Giemsa C-banding and isozyme analyses. Spikes were fixed in Carnoy's solution and the anthers were stained in alcoholic carmine and squashed in 2% acetocarmine for obtaining data on the meiotic configuration at metaphase I. For analyses of isozymes (Table 2), standard procedures for polyacrylamide gel isoelectric focussing (PAGE) and conventional electrophoresis on cellulose acetate or polyacrylamide gel were used. Esterase and malate dehydrogenase were stained according to Brown *et al.* (1978), aconitate hydratase according to Jung *et al.* (1986), and the remaining enzymes according to Vallejos (1983). All progenies were observed for characteristic morphological features.

Table 2. List of enzymes analyzed.

Enzyme	E.C. No.
Acid phosphatase	3.1.3.2
Aconitate hydratase	4.2.1.3
Alcohol dehydrogenase	1.1.1.1
Aspartate aminotransferase	2.6.1.1
Esterase	3.1.1.2
Glucosephosphate isomerase	5.3.1.9
Glucose-6-phosphate dehydrogenase	1.1.1.49
Malate dehydrogenase	1.1.1.37
Phosphoglucomutase	2.7.5.1
Phosphogluconate dehydrogenase	1.1.1.43
Shikimate dehydrogenase	1.1.1.25

RESULTS

The F_1 hybrids involving *Aegilops* species more resembled their *Aegilops* parents in spike morphology, although a phenotypic co-dominance was apparent. All the F_1 hybrids with the CS stocks were self-sterile, but many produced a few BCI seeds (Table 3). Backcross II (BCII) seed set was generally much higher than BCI seed set—3.6%, 10.6%, 20.5%, and 22.9% for *ph1b/Ae. variabilis*, N5BT5A/*Ae. biuncialis*, N5BT5/*Ae. variabilis*, and N5BT5A/*Ae. cylindrica*, respectively. Generally, chromosome pairing was higher in *ph1b* or N5BT5- x Alien hybrids than expected in the normal respective wheat x Alien hybrids (Table 4). The only direct comparison involving hybrids of *Ae. variabilis* with CS, *ph1b*, and N5BT5-, respectively, attests to this. The chromosome numbers of the BCI plants were generally lower than those expected when complete meiotic restitution of the female gametes is assumed (Table 5); a variation trend consistent with earlier observations of BCI derivatives among several intergeneric hybrids in the Triticeae (Jewell and Mujeeb-Kazi 1982; Mujeeb-Kazi and Bernard 1985). As shown for the rye hybrids (Table 6), the BCI plants may have different numbers of alien chromosomes. BCII or backcross III (BCIII) plants are currently available for all hybrids that were successfully backcrossed. Selfed progenies from CS/*H. villosa*/*ph1b* and CS/*Th. bessarabicum*/*ph1b* will soon be screened for selection of *ph1b* homozygotes.

BCI progenies from topcrossing F_1 hybrids of scheme C had the expected chromosome numbers assuming complete restitution of the female gametes. Those for *T. aestivum*/*T. bessarabicum*/*T. turgidum* had 42 chromosomes associated as 14

Table 3. Backcross I (BCI) seed set on F_1 hybrids.

F_1 hybrid	No. of florets pollinated	No. of BCI seeds	Percentage seed set
<i>ph1b/Ae. caudata</i>	250	0	0.0
<i>ph1b/Ae. juvenalis</i>	2736	68	2.5
<i>ph1b/Ae. mutica</i>	2954	11	0.4
<i>ph1b/Ae. ovata</i>	612	0	0.0
<i>ph1b/Ae. triuncialis</i>	414	4	1.0
<i>ph1b/Ae. vavilovii</i>	1918	134	7.0
<i>ph1b/Elvon rye</i>	608	2	0.3
<i>ph1b/Prolific rye</i>	302	2	0.7
<i>ph1b/Semidwarf rye</i>	476	0	0.0
<i>ph1b/Th. bessarabicum</i>	152	0	0.0
<i>ph1b/Ae. variabilis</i>	2058	18	0.8
CS/ <i>Ae. variabilis</i>	1112	22	2.0
N5BT5-/ <i>Ae. variabilis</i>	3452	10	0.3
N5BT5A/ <i>Ae. biuncialis</i>	582	2	0.3
N5BT5A/ <i>Ae. cylindrica</i> (18G)	1900	9	0.5
CS/ <i>Ae. cylindrica</i> (17A)	228	9	4.0
N5BT5A/Elvon rye	3560	18	0.5
N5BT5A/ <i>Ae. triaristata</i>	1628	8	0.5
N5BT5A/ <i>T. junceiforme</i>	608	0	0.0

Table 4. Mean meiotic metaphase I chromosome association.

Combination	Plant Chromosome		I	II ^a	II ^b	III	IV	V	VI
	Number	Number							
Nulli 5B Tetra 5A/Elvon	87-3290	28	8.5	3.7	2.7	1.75	0.25	0.25	
Nulli 5B Tetra 5D/ <i>Ae. variabilis</i>	87-3313	35	8.8	2.7	7.0	0.85	0.35		
<i>ph1b/Ae. variabilis</i>	87-77-8	35	9.5	1.6	7.2	2.1	0.25	0.08	0.04
<i>Ald/Pvn//Ae. variabilis</i>	87-247	35	27.6	0.1	3.3	0.2			
Nulli 5B Tetra 5A/ <i>Ae. triaristata</i>	87-274-1	35	11.2	0.7	5.6	2.8	0.5	0.13	0.04
<i>ph1b/Prolific rye</i>	87-3343	28	7.7	3.0	3.2	2.2	0.33		
<i>ph1b/Ae. vavilovii</i>	87-3868	42	17.9	0.05	7.4	1.83	0.38		
<i>ph1b/Ae. juvenalis</i>	87-3887	42	19.2	1.6	5.0	2.32	0.4	0.12	0.08
<i>ph1b/Ae. umbellulata</i>	87-3385	28	7.5	1.8	4.2	0.09	0.45	0.09	
<i>ph1b/Ae. mutica</i>	87-3352	28	6.7	1.9	3.0	1.81	0.90	0.36	0.09
<i>ph1b/Ae. ovata</i>	87-3349	35	10.0	1.9	4.8	2.22	0.90	0.18	0.04
Nulli 5B Tetra 5A/ <i>Ae. biuncialis</i>	87-3378	35	4.5	1.0	7.5	4.5			

^a II= Ring bivalent.^b II= Rod bivalent.**Table 5. Number of chromosomes of some backcross I (BCI) plants.**

Pedigree	Number of Plants	Chromosome number		
		Range	Mean	Expected
CS/ <i>Ae. variabilis</i> // <i>T. aestivum</i>	15	41-58	51.5	56
N5BT5A/ <i>Ae. variabilis</i> // <i>T. aestivum</i>	17	36-59	40.9	56
<i>ph1b/Ae. variabilis</i> // <i>T. aestivum</i>	9	37-42	39.0	56
<i>ph1b/Ae. vavilovii</i> // <i>T. aestivum</i>	56	40-48	42.8	63
<i>ph1b/Ae. juvenalis</i> // <i>T. aestivum</i>	25	41-47	43.2	63

Table 6. Number of rye chromosomes in backcross I (BCI) plants.

Pedigree	Chromosome number	
	Total	Rye
N5BT5A/Elvon//CNO	41	7
N5BT5A/Elvon//CS	49	7
<i>ph1b</i> /Elvon//CS	40	5

bivalents + 14 univalents (Mujeeb-Kazi 1984). Plants with different somatic chromosome numbers resulted from the subsequent backcross to *T. aestivum* cultivars; e.g., 95 plants of CS/*Th. bessarabicum*//*T. turgidum*/3/*T. aestivum* had a mean of 41.8 chromosomes with a range of 35-50. There was a significant level of necrosis among progenies of some of these crosses, the worst combinations of *T. turgidum*/*T. aestivum* for this being Altar 84/Yaco, Altar 84/Pavon, and Chen/Goshawk. Necrosis-free combinations included Altar 84/Ciano 79, Yavaros//Mirlo/Buckbuck, and Yavaros//Buckbuck/Bluejay. Progenies from another cycle of backcrossing to *T. aestivum* cultivars are being analyzed. Owing to low self-fertility only four plants resulted from selfing of the BCI plants of *T. turgidum* cv. Altar 84/*Th. scirpeum*//*T. aestivum* cv. Chinese Spring (*ph1b*). The BCII plants produced directly have been bagged for selfing.

Backcross derivatives from all schemes showed some segregation for characters such as spike morphology, pubescence, presence of anthocyanin, isozyme patterns, and the number of alien chromosomes when differential C-banding chromosome staining was used for derivatives involving *S. cereale*, *H. villosa*, and *L. racemosus*.

DISCUSSION

These preliminary results show some of the alternative ways in which introgression of alien genes into wheat may be accomplished. The demonstration of BCI seed set in F_1 interspecific and intergeneric hybrids involving the *ph1b* mutant means wheat alien recombination at F_1 is a viable route for genetic exchanges. Indeed, when coupled with embryo rescue techniques, more BCI progenies may be obtained as BCI seeds of hybrids with *Ae. ovata* and *Ae. caudata* shrivelled and died on the parent plants. The only accurate comparison of percent seed set involves *Ae. variabilis* where the CS/*Ae. variabilis* F_1 set more BCI seeds than *ph1b*/*Ae. variabilis*. This supports the hypothesis of Sharma and Gill (1986) that there may be an influence of the *ph* mutant status on restitution in female gametes. BCI seed set on N5BT5-/*Ae. variabilis* and a rather rough comparison for the situation of *Ae. cylindrica* where two different accessions were involved suggest a similar role of the nullisomic 5B status. However, the number of somatic chromosomes of some of the BCI plants seems to indicate that, for these plants, BCI seed setting resulted not from restitution but from a process akin to a reduction division in the female gametes possibly owing to the high level of chromosome association at metaphase I. In the case of *T. aestivum*/*Ae. juvenalis* and *T. aestivum*/*Ae. vavilovii*, this may have been coupled with some similarity of the D genomes of the alien species with that of *T. aestivum* (Chapman and Miller 1978).

The low number of chromosomes in these plants may result in reduction in the number of alien chromosomes at BCI. However, as shown in the BCI plants from N5BT5A/Elvon, the total complement of alien chromosomes could be present even when the total number of chromosomes for the hybrid is less than expected. Reduction in the number of alien chromosomes may or may not be disadvantageous depending on the number of plants obtained and whether or not the critical alien chromosome or its segment is transmitted. So long as the critical alien segment is present in some of the BCI progenies, the wheat aneuploidy can be corrected with backcrossing without having to worry about disposing of many alien chromosomes. In the case of the hybrids for which BCI seed set was unsuccessful, scheme B may be the alternative as proposed

by Sharma and Gill (1986). However, it may be helpful to backcross the selected *ph1b* homozygotes, as well as the current progenies available from scheme B, to a cultivar of *T. turgidum* var. *durum*, which would introduce univalency of at least the D genome chromosomes in order to enhance allosyndetic pairing before backcrossing again to *T. aestivum*, unless there is sufficient desynapsis already.

Naturally cultivated wheat would be the most accessible source of genes for improvement of existing cultivars. When alien sources become necessary, the ease of hybridization to wheat, possibilities for wheat-alien chromosome recombination, and ploidy level should be taken into account. Generally, these imply giving priority to species that have genome similarities with cultivated wheat. Lower ploidy levels would normally speed up the transfer as there would be less unwanted chromatin to dispose of. Two further considerations in the decision to use an alien source are the genetic control of the trait and its expression in a wheat background (Mujeeb-Kazi *et al.* 1987, Mujeeb-Kazi and Asiedu 1988a). Single genes are most suitable for transfer from alien sources since only a small fraction of the alien genome is involved, thus limiting inclusion of deleterious alien chromatin. Characters under complex or multigenic inheritance may however be transferred if some of the genes have significant major effects leading to a reasonable expression of the trait after introduction of a small section of the alien genome or by avenues of interspecific hybridization, i.e. *T. turgidum* (AABB) or *T. aestivum* (AABBDD) x *T. tauschii* (DD) (Mujeeb-Kazi and Asiedu 1988a,b).

CONCLUSIONS

The efficiency of introgressing alien genes could be increased with judicious use of markers. For this purpose correlations should preferably be made as soon as practicable between the presence of the various markers and the desired traits in the progenies. Some of these may reveal close linkages between trait and marker loci or trait loci flanked on both sides by marker loci. Once such reliable associations have been established, screening for the markers may be substituted for screening for the agronomic traits at some generations or may be used to reduce the populations to be screened. This is especially so with markers that can be identified using endosperm portions or sampling for analyses during early seedling stages. It must be remembered, however, that just as there is an inverse relationship between genetic distance and ease of hybridization, the application of markers is easier with wider hybrids.

REFERENCES CITED

- Abu Baker, M., and G. Kimber. 1982. Chromosome pairing regulators in the former genus *Aegilops*. *Z. Pflanzenzuchtg.* **89**:130-138.
- Brar, D.S., and G.S. Khush. 1987. Wide hybridization and chromosome manipulation in cereals. *In: Principles of Cultivar Development*. Vol. 2. Crop Species. W.R. Fehr, ed., pp. 221-263. MacMillan Publ. Co. N.Y.
- Brown, A.H.D., E. Nevo, D. Zohary, and O. Dagan. 1978. Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). *Genetica* **49**:97-108.
- Chapman, V., and T.E. Miller. 1978. The relationship of the D genomes of hexaploid *Ae crassa*, *Ae. vavilovii* and hexaploid wheat. *Wheat Inf. Serv.* **16**:17-20.
- Chen, K.C., and J. Dvorak. 1984. The inheritance of genetic variation in *Triticum speltoides* affecting heterogenetic chromosome pairing in hybrids with *Triticum aestivum*. *Can. J. Genet. Cytol.* **26**:279-287.
- Cusick, J.E., and R.A. McIntosh. 1987. Linkage map of wheat (*Triticum aestivum*: 2n=42). *In: Genetic Maps 1987*, S.J. O'Brien, ed., pp. 670-677. Cold Spring Harbor Laboratory.
- Darvey, N. 1984. Alien wheat bank. *Genetics* **107** (Suppl.):24.
- Dewey, D. 1984. The genome system of classification as a guide to intergeneric hybridization with the perennial Triticeae. *In: Gene Manipulation in Plant Improvement*, J.P. Gustafson, ed., pp. 219-271. Plenum Press. N.Y. and London.
- Dhaliwal, H.S., B.S. Gill, and J.G. Waines. 1977. Analysis of induced homoeologous pairing in a *Ph* mutant wheat x rye hybrid. *J. Hered.* **68**:206-209.
- Dover, G.A., and R. Riley. 1972. Variation at two loci affecting homoeologous meiotic chromosome pairing in *Triticum aestivum* x *Aegilops mutica* hybrids. *Nature* (London), *New Biol.* **235**:61-62.
- Driscoll, C.J. 1968. Alien transfer by irradiation and meiotic control. *In: Proc. 3rd Int. Wheat Genet. Symp.*, pp. 196-203. Canberra, Australia.
- Driscoll, C.J. 1979. Mathematical comparison of homologous and homoeologous chromosome configuration and the mode of action of the genes regulating pairing in wheat. *Genetics* **92**:947-951.
- Driscoll, C.J., and N.F. Jensen. 1964. Characteristics of leaf rust resistance transferred from rye to wheat. *Crop Sci.* **4**:372-374.
- Driscoll, C.H., and C.J. Quinn. 1970. Genetic variation in *Triticum aestivum* affecting the level of chromosome pairing in intergeneric hybrids. *Can. J. Genet. Cytol.* **12**:278-282.
- Dvorak, J. 1977. Effect of rye on homoeologous chromosome pairing in wheat x rye hybrids. *Can. J. Genet. Cytol.* **19**:549-556.
- Fedak, G. 1977. Increased homoeologous chromosome pairing in *Hordeum vulgare* and *Triticum aestivum* hybrids. *Nature* (London) **266**:529-530.
- Feldman, M. 1966. The effect of chromosome 5B, 5D, and 5A on chromosomal pairing in *Triticum aestivum*. *Proc. Nat. Sci. USA.* **55**:1447-1453.

- Feldman, M., and E.R. Sears. 1981. The wild gene resources of wheat. *Scientific Amer.* **244**:102-112.
- Gill, B.S., and G. Kimber. 1977. Recognition of translocations and alien chromosome transfers in wheat by the Giemsa C-banding technique. *Crop Sci.* **17**:264-266.
- Gupta, P.K., and G. Fedak. 1985. Variation in induction of homoeologous chromosome pairing in 6x *Aegilops crassa* by genomes of six different species of *Secale*. *Can. J. Genet. Cytol.* **27**:531-537.
- Hart, G.E., and M.D. Gale. 1987. Biochemical/molecular loci of hexaploid wheat (*Triticum aestivum*, 2n=42, Genomes AABBDD). In: Genetic Maps 1987, S.J. O'Brien, ed., pp. 678-684. Cold Spring Harbor Laboratory.
- Jewell, D., and A. Mujeeb-Kazi. 1982. Unexpected chromosome numbers in backcross I generations of F₁ hybrids between *Triticum aestivum* and related alien genera. *Wheat Inf. Service* **55**:5-9.
- Joshi, B.C., and D. Singh. 1978. Introduction of alien variation into wheat. In: Proc. 5th Int. Wheat Genet. Symp., pp. 342-348. Delhi, India.
- Jung, C., P. Wehling, and H. Loptien. 1986. Electrophoretic investigation on nematode-resistant sugar beets. *Z. Pflanzenzuchtg.* **97**:39-45.
- Kimber, G., and M. Feldman. 1987. Wild Wheat. An introduction. Special report 353, College of Agriculture, University of Missouri-Columbia.
- Koebner, R.M.D., and K.W. Shepherd. 1985. Induction of recombination between rye chromosome 1RL and wheat chromosomes. *Theor. Appl. Genet.* **71**:208-215.
- Koebner, R.M.D., and K.W. Shepherd. 1986. Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinants. *Theor. Appl. Genet.* **73**:197-208.
- Lacadena, J.R. 1967. Introduction of alien variation into wheat by gene recombination. I. Crosses between mono-V (5B) *Triticum aestivum* L. and *Secale cereale* L. and *Aegilops columnaris* Zhuk. *Euphytica* **16**:221-230.
- Lelley, T. 1976. Introduction of homoeologous pairing in wheat by genes of rye suppressing 5B effect. *Can. J. Genet. Cytol.* **18**:485-489.
- Lukaszewski, A.J., and J.P. Gustafson. 1983. Translocations and modifications of chromosomes in Triticale x Wheat hybrids. *Theor. Appl. Genet.* **64**:239-248.
- May, C.E., and R. Appels. 1982. The inheritance of rye chromosomes in early generations of Triticale x Wheat hybrids. *Can. J. Genet. Cytol.* **24**:285-291.
- McGuire, P.E., and J. Dvorak. 1982. Genetic regulation of heterogenetic chromosome pairing in polyploid species of the genus *Triticum sensu lato*. *Can. J. Genet. Cytol.* **14**:57-82.
- Miller, T.E., and S.M. Reader. 1987. A guide to the homoeology of chromosomes within the Triticeae. *Theor. Appl. Genet.* **74**:214-217.
- Mujeeb-Kazi, A. 1984. Wide Crosses. In: CIMMYT Report on Wheat Improvement, pp. 54-65.
- Mujeeb-Kazi, A., and R. Rodriguez. 1981. An intergeneric hybrid of *Triticum aestivum* x *Elymus giganteus*. *J. Hered.* **72**:253-256.
- Mujeeb-Kazi, A., and M. Bernard. 1985. Intergeneric hybridization to induce alien genetic transfers into *Triticum aestivum*. *Pak. J. Bot.* **17**:271-289.

- Mujeeb-Kazi, A., and G. Kimber. 1985. The production, cytology, and practicality of wide hybrids in the Triticeae. *Cer. Res. Comm.* **13**:111-124.
- Mujeeb-Kazi, A., and J.L. Miranda. 1985. Enhanced resolution of somatic chromosome constrictions as an aid to identifying intergeneric hybrids among some Triticeae. *Cytologia* **50**:701-709.
- Mujeeb-Kazi, A., and R. Asiedu. 1988a. Alien germplasm for wheat (*Triticum aestivum* L.) improvement facilitated by cytogenetic manipulation and use of novel techniques. In: Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector, pp. 211-231. USAID Biotech. Meeting, April 17-21, 1988, Virginia, USA.
- Mujeeb-Kazi, A., and R. Asiedu. 1988b. The potential of wide hybridization in wheat improvement. In: Biotechnology Series in Agriculture, Y.P.S. Bajaj, ed. Springer-Verlag (in press).
- Mujeeb-Kazi, A., S. Roldan, and J.L. Miranda. 1984. Intergeneric hybrids of *Triticum aestivum* L. with *Agropyron* and *Elymus* species. *Cer. Res. Comm.* **12**:75-79.
- Mujeeb-Kazi, A., S. Roldan, D.Y. Suh, L.A. Sitch, and S. Farooq. 1987. Production and cytogenetic analysis of hybrids between *Triticum aestivum* and some caespitose *Agropyron* species. *Genome* **29**:537-553.
- Naranjo, T., J.R. Lacadena, and R. Giraldez. 1979. Interaction between wheat and rye genomes on homologous and homoeologous pairing. *Z. Pflanzenzuchtg.* **82**:289-305.
- Okamoto, M. 1957. Asynaptic effect of chromosome V. *Wheat Inf. Serv.* **5**:6.
- Riley, R., and V. Chapman. 1958. Genetic control of the cytologically diploid behavior of hexaploid wheat. *Nature* **182**:713-715.
- Riley, R., V. Chapman, and R. Johnson. 1968. Introduction of yellow rust resistance of *Aegilops comosa* into wheat by genetically induced homoeologous recombination. *Nature* **217**:383-384.
- Sears, E.R. 1976. Genetic control of chromosome pairing in wheat. *Ann. Rev. Genet.* **10**:31-51.
- Sears, E.R. 1984. Mutations in wheat that raise the level of meiotic chromosome pairing. In: Gene manipulation in plant improvement, J.P. Gustafson, ed., Plenum Press.
- Sharma, H.C., and B.S. Gill. 1983. Current status of wide hybridization in wheat. *Euphytica* **32**:17-31.
- Sharma, H.C., and B.S. Gill. 1986. The use of *ph1* gene in direct genetic transfer and search for *Ph*-like genes in polyploid *Aegilops* species. *Z. Pflanzenzuchtg.* **96**:1-7.
- Sharma, D., and D.R. Knott. 1966. The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. *Can. J. Genet. Cytol.* **8**:137-143.
- ter Kuile, N., V. Rosas, R. Asiedu, and A. Mujeeb-Kazi. 1987. The role of some cytogenetic systems in effecting alien genetic transfers for *Triticum aestivum* improvement. *Agr. Abst.* p. 82.
- Vallejos, E. 1983. Enzyme activity staining. In: Isozymes in Plant Genetics and Breeding, Part A., S.D. Tanksley and T.J. Orton, eds., pp. 469-515. Elsevier Science Publishers, B.V., Amsterdam.

RESUMEN

El estudio incluyó la evaluación de algunos esquemas para incrementar la posibilidad de transferir cromatina extraña al genoma de trigo en híbridos de trigo y especies extrañas. La producción de semillas en la generación retrocruzada I (BCI), que antes constituía una limitación importante, se obtuvo de *phlb* o de los híbridos F_1 de N5BT5-/especies extrañas, que incluyen especies de *Aegilops* y variedades de centeno, con lo cual la incorporación de genes extraños mediante el apareamiento alosintético en la generación F_1 se convirtió en un procedimiento viable. Otro esquema se refiere al mestizaje de híbridos F_1 basados en *Triticum aestivum* o *T. turgidum* con *T. turgidum* o *T. aestivum*, respectivamente, con el fin de inducir la ruptura centromérica y la fusión de los cromosomas. Se tuvo éxito en la obtención de derivados avanzados del cruzamiento inicial; asimismo, se pusieron en marcha modificaciones necesarias de estos esquemas principales. La vigilancia de los cromosomas extraños en las progenies se llevó a cabo mediante la aplicación de marcadores citológicos, morfológicos y bioquímicos, que han participado en la producción de varias acumulaciones de cromosomas extraños de *Haynaldia villosa*, *Agropyron junceum* y *Elymus giganteus* en una base de *T. aestivum*.