Genetic Manipulation in Wheat (*Triticum aestivum* L.) Mediated by the Chromosome 5B *Ph* Locus and its Significance for the Transfer of Alien Genes

A. MUJEEB-KAZI

CIMMYT, Lisboa 27, Apartado Postal 6-641, Deleg. Cuauhtemoc, 06600 Mexico, D. F., MEXICO

ABSTRACT

Enhanced genetic recombination between chromosomes of *Triticum aestivum* and the alien species is crucial to effect alien gene introgression for practical agricultural goals. This apparently is the most logical and simplistic approach based upon genetic proximity and is characteristic of of interspecific hybridization. Consistent with the ever-generating natural variation prevalent for biotic/abiotic situations, genetic divergence based upon genomic dissimilarities becomes an asset, thus adding more significance to intergeneric hybridization.

Most of the wheat × alien species intergeneric hybrids are categorized as being low recombinant types and necessitate long-term research endeavors to accomplish alien gene incorporation. There is however, in *Triticcm*, a very potent chromosome pairing regulation gene (a suppressor of homoeologous pairing (Ph) on chromosome 5BL) whose manipulation has the capability of promoting allosyndesis in intergeneric hybrids and enhancing swifter alien transfers. This approach has been currently utilized for *T. aestivum × Aegilops variabilis* hybrids where the alien species is a karnal bunt resistant source.

In the normal *T. aestivum* cv. chinese Spring × *Ae. variabilis* hybrids the metaphase I mean chromosomal association is generally less than 1 bivalent per meiocyte. This can be manipulated to yield enhanced meiotic associations of 9.5 univalents + 1.6 ring bivalents + 7.2 rod bivalents + 2.1 trivalents + 0.25 quadrivalents + 0.08 pentavalents + 0.04 hexavalents when the Ph mutant stock (ph1b) is used in the cross with *Ae. variabilis*. Other chromosome 5B stocks (monosomic 5B, nullisomic 5B tetrasomic 5A or 5D) of alien species having Ph suppressors also contribute to enhanced F_1 wheat × alien recombination. Some of these events are discussed.

Key words : *Triticum aestivum* L., Wheat, Chromosome 5B, Ph locus, alien transfer.

INTRODUCTION

Invariably in all situations the early generations derived from wheat × alien species hybridizations tend to be poor agronomic plant types. However, by employing appropriate breeding methodologies plant types can be significantly improved phenotypically upto the state that alien disomic chromosome additions or derivatives with subtle alein introgression are produced (Mujeeb-Kazi and Asiedu, 1989). Through application of such methodologies advanced progenies with disease

resistance have been obtained from combinations of *Triticum aestivum/Agropyron curvifolium* (*Thinopyrum curvifolium*); derivatives that offer resistance to *Fusarium graminearum, Helminthosporium* sp. and have initially demonstrated a resistant response to *Septoria* sp. as well. Though the specifics of the alien transfer (Mujeeb-Kazi; Unpublished) are yet to be elucidated we believe that event/s similar to that elaborated for low-pairing F_1 hybrids (Mujeeb-Kazi *et al.*, 1987) is/are plausible.

Alien transfer methodologies are basically associated with "genetic recombination", a process of natural occurrence when there is genetic proximity or one that can be cytogenetically induced. In the current presentation these aspects are highlighed through "interspecific" and "intergeneric" hybridization applcations.

INTERSPECIFIC HYBRIDIZATION

Bread wheat (*Triticum aestivum* L.; 2n=6x—42; AABBDD) improvement based upon conventional procedures has essentially exploited intervarietal hybridization processes. These processes have limited constraints of crossing and invariably all associations of parental traits plus segregation are based upon genetic recombination. Consequently, if a varied gene pool is to be tapped, utilizing the innumerable alien accessions possessing reasonable proximity to the A, B or D genomes of wheat would occupy a very high priority for effecting alien transfers merely because of genomic similarity, ease of hybridization and the ensuing high frequency of enhanced recombination. By virtue of this inference wheat improvement can be targeted for either of the three genomes; A, B or D.

Several accessions of the diploid source are available and some of the potential candidates comprise of *T. urartu, T. monococcum* or *T. boeticum* for the A genome, *Aegilops speltoides* as a Sitopsis section diploid amongst others in that group for allowing recombinational transfers into the B genome. plus *T. tauschii* (*Ae. squarrosa*) for wheat improvement via the D genome. Other probable sources would be the researchers discretion and elaborate reference could be made at least to Kimber and Feldman (1987). The procedures of incorporation of such alien variability differs amongst researchers as also does the choice of the genomic source. We, as a starting point, have concentrated on exploiting the D genome acce.sions of *T. tauschii* (*Ae. squarrosa*) in a variety of ways that are elaborated below :

a) Hybridization of *T. turgidum* (2n=4x=28, AABB) cultivars (resistant or susceptible for an attribute) with *T. tauschii* resistant accessions (2n=2x=14, DD), doubling of the 21 chromosome F_1 hybrids (induced or spontaneous) to yield the 42 chromosome synthetic hexaploids (2n=6x=42, AABBDD); Figure 1. The synthetic hexaploids after appropriate disease or stress screening if found to be resistant/tolerant get incorporated in resistance transfers to other *T. aestivum* cultivars by conventional crossing methodology.
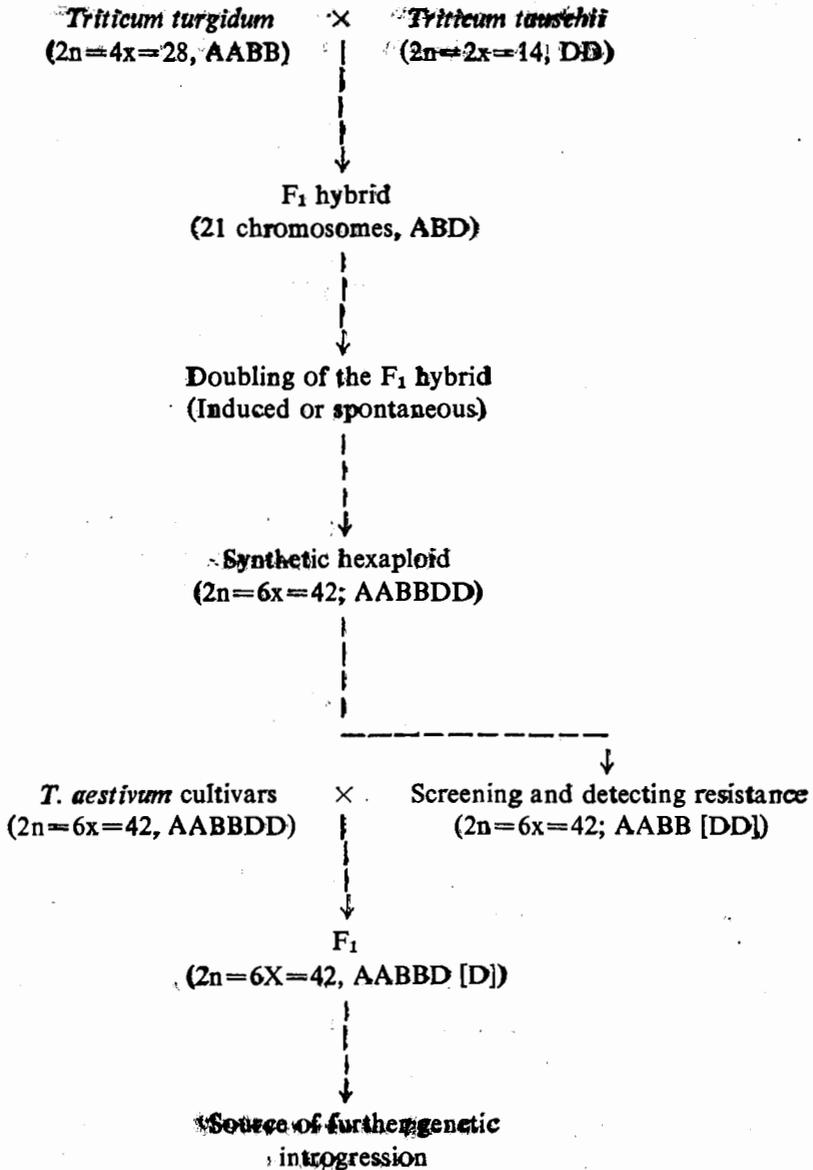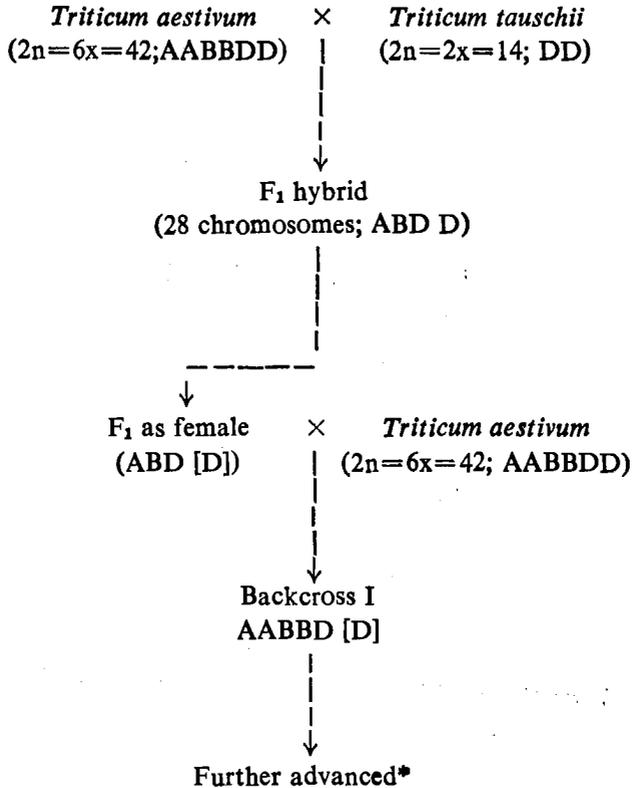
Triticum turgidum $\times$ *Triticum tauschii*
$(2n=4x=28,\ AABB)$ $(2n=2x=14,\ DD)$

$\downarrow$

F_1 hybrid
(21 chromosomes, ABD)

$\downarrow$

Doubling of the F_1 hybrid
(Induced or spontaneous)

$\downarrow$

Synthetic hexaploid
$(2n=6x=42;\ AABBDD)$

$\downarrow$

T. aestivum cultivars $\times$ Screening and detecting resistance
$(2n=6x=42,\ AABBDD)$ $(2n=6x=42;\ AABB\ [DD])$

$\downarrow$

F_1
$(2n=6X=42,\ AABBD\ [D])$

$\downarrow$

Source of further genetic
introgression

Fig. 1. A schematic showing the utilization of *Triticum tauschii* $(2n=2x=14,\ DD)$ in direct crosses with *T. turgidum* $(2n=4x=28,\ AABB)$ and the practical exploitation of the resultant synthetic hexaploid $(2n=6x=42,\ AABBDD)$.

b)   Hybridization of elite but susceptible *T. aestivum* cultivars with appropriate *T. tauschii* accessions and backcrossing the ABDD F_1 hybrid with the same elite *T. aestivum* cultivar as used in the initial cross to rapidly yield improved

BCI derivatives with AABBDD genomes, 5 genomes (AABBD) of which resemble the elite wheat cultivar used in the cross; (Figure 2).

$$
\begin{array}{ccc}
\textit{Triticum aestivum} & \times & \textit{Triticum tauschii} \\
(2n=6x=42; AABBDD) & & (2n=2x=14; DD)
\end{array}
$$

F₁ hybrid
(28 chromosomes; ABD D)

F₁ as female   ×   *Triticum aestivum*
(ABD [D])   (2n=6x=42; AABBDD)

Backcross I
AABBD [D]

Further advanced*

*Gill and Raupp 1987

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

c) Extracting the AABB genomes from commercial *T. aestivum* cultivars and then developing hexaploids by crossing with desired *T. tauschii* accessions. The procedure allows very stringent analysis of the genetic contribution of the alien D genome with negligible interference from the A and B genome recombinational segregation that is rampant from the above a and b procedures; (Figure 3).

It is imperative that the resistance of the D genome from *T. tauschii* be identified since genetic factors on the A plus B genomes may mask or modify (dilute) its expression. This however, is not a universal phenomenon and recent observations
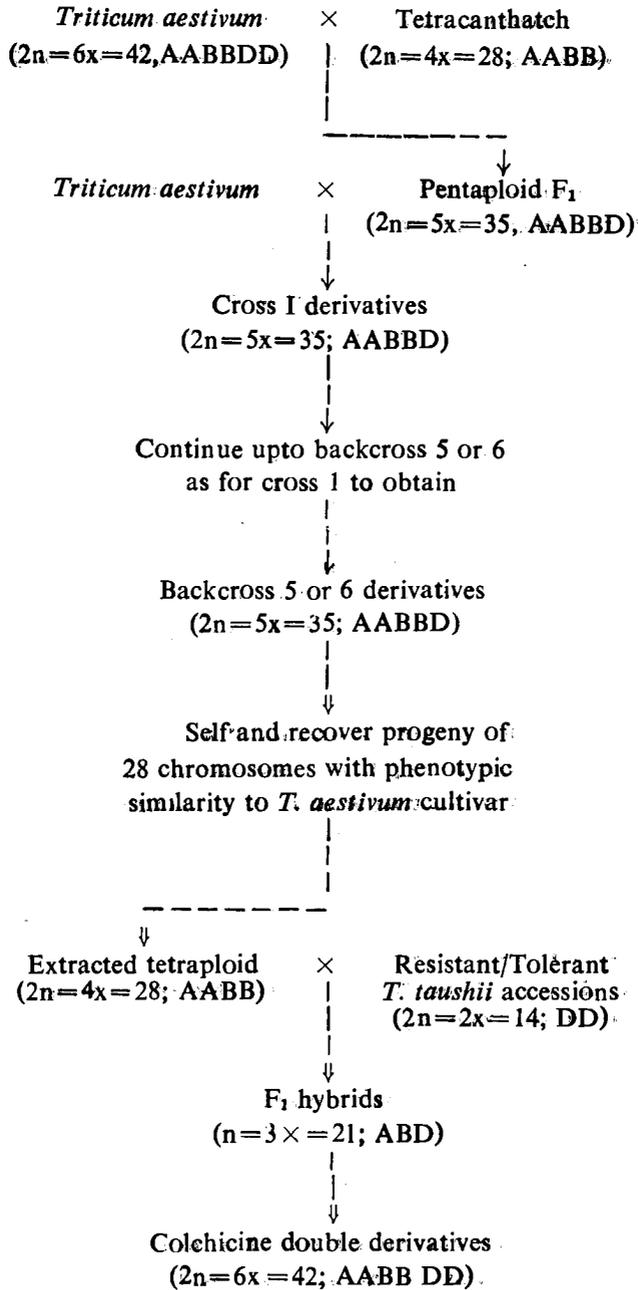
Triticum aestivum   ×   Tetracanthatch
(2n=6x=42,AABBDD) | (2n=4x=28; AABB)
                     |
                     |
            — — — — — — — —
                              ↓
Triticum aestivum   ×   Pentaploid F$_1$
                     | (2n=5x=35, AABBD)
                     |
                     ↓
        Cross I derivatives
        (2n=5x=35; AABBD)
                |
                |
                ↓
    Continue upto backcross 5 or 6
        as for cross 1 to obtain
                |
                |
                ↓
    Backcross 5 or 6 derivatives
        (2n=5x=35; AABBD)
                |
                |
                ⇓
    Self and recover progeny of
    28 chromosomes with phenotypic
    similarity to *T. aestivum* cultivar
                |
                |
                |
    — — — — — — — —
        ⇓
Extracted tetraploid   ×   Resistant/Tolerant
(2n=4x=28; AABB) | *T. taushii* accessions
                    | (2n=2x=14; DD)
                    |
                    ⇓
            F$_1$ hybrids
        (n=3×=21; ABD)
                |
                |
                ⇓
    Colchicine double derivatives
    (2n=6x=42; AABB DD)

Fig. 3. Schematic showing extraction of the AABB component from a 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).

of Multani *et al.*, (1988) indicate that the Karnal bunt disease resistance characteristics of *T. tauschii* were expressed in synthetic hexaploids where the *T. turgidum* base cultivar was susceptible.

INTERGENERIC HYBRIDIZATION

Biotic and abiotic aspects of crop production are beset with changing situations that fit the dynamic and static modus operandi (Mujeeb-Kazi and Kimber, 1985). Consequently, for added genetic variability and a different gene pool the annual/ perennial Triticeae provide tremendous genetic variability (Dewey, 1984). In contrast to interspecific hybridization these species are genomically quite diverse, rather difficult to hybridize with wheat and where successfully combined, the hybrids exhibit little or no allosyndetic meiotic association. Hence, beneficial alien transfers are invitably time consuming. Despite these limitations significent success and advancements have been made over the past two decades (Kruse, 1973; Islam *et al*, 1981; Sharma and Gill, 1983; Mujeeb-Kazi and Kimber, 1985; Mujeeb-Kazi *et al*, 1987, 1989; Mujeeb-Kazi and Asiedu, 1989, 1990; Gill, 1989). The evolution since the pioneering hybridization results of Kruse (1967, 1969, 1973) has been in the exploitation of the *ph*, or *Ph*, locus on the long arm of chromosome 5B; a locus that regulates pairing associations in euploid wheat. When its varied genetic stocks are utilized and hybridized with otherwise low pairing alien species the resultant F_1 hybrids have a desirable modification of chromosomal associations at metaphase I. This is exemplified by the *T. aestivum* × *Thinopyrum bessarabicum* F_1 hybrid combination. The hybrid has been produced by Alonso and Kimber (1980), Mujeeb-Kazi (1982), Mujeeb-Kazi *et al*, (1987), Sharma and Gill (1983) and Forster and Miller (1985) In all cases the alien species was hybridized with *T. aestiuum* cv. Chinese Spring yielding a metaphase I chromosomal association of less than or closer to 1 bivalent per cell in the 28 chromosome hybrid. By hybridizing *Th. bessarabicum* with a monosomic 5B stock of Chinese Spring (Forster and Miller, 1985), substantially higher meiotic pairing was observed but advanced derivatives from these high pairing 27 chromosome hybrids lacking chromosome 5B were not obtained. Another mechanism of inducing recombination appeared feasible through the use of the *phlb* system prevalent in Chinese Spring (Sears, 1977).

The system was exploited by Sharma and Gill (1986) where several F_1 hybrids were produced between *Aegilops* species and Chinese Spring *phlb* as a female parent. Cytogenetic analysis of the hybrid revealed enhanced meiotic associations at metaphase I as compared to the relative check hybrids of the alien species with Chinese Spring *PhPh*. Unfortunately however, none of these high pairing F_1 hybrids could be exploited since neither amphiploids nor backcross 1 (BCI) derivatives could be obtained; presumably an influence of the *phlb* genetic stocks maintenance. Subsequently, Mujeeb-Kaxi *et al.* (1988) produced an F_1 combination

between *T. aestivum phIb* × *Th. bessarabicum* that exhibited high pairing This could not be advanced by backcrossing with *phIb* but a BCI was obtained by pollinating the F_1 hybrid with Chinese Spring (*Ph Ph*). This allows the BCI derivative to be heterozygous for the *Ph* locus (*Ph, ph*) which upon selfing should yield segregates homozygous for the *ph* locus and be high pairing; a proposition that has yet to be tested. There is a high likelihood of detecting transfers from *Th. bessarabicum* by the above *phIb* exchange system since through addition line and biochemical studies several markers have been established. The various markers complement each other and range from cytological (C-banding positive), morpho-logical (slender or club-shaped spikes-group 2 and 5); solid stem (group 3) ; blue aleurone (group 4) plus a group of biochemical identifiers for other homoeolo-gous groups. For development of the above classifiers disomic additions have facilitated interpretations, with their stability greatly contributing to repeated confirmation and subsequent tracking in substitution line development. The stability of the disomic additions is expressed as 22 bivalents at metaphase I, a normal 22 + 22 split at anaphase I culminating in adequate self fertile progeny for the respective disomic line.

Asiedu *et al.* (1989) have more recently crossed several *Aegilops* species with Chinese Spring, Chinese Spring *phIb*, Chinese Spring nullisomic 5B tetrasomic 5A or 5D and monosomic 5B Chinese Spring stocks. In all these cases, using *Ae. variabilis* (a potent karnal bunt resistant source, Warham *et al*, 1986) as the alien species, high meiotic pairing was observed in the 35 and 34 *ph* locus or nullisomic 5B chromosome hybrids where the 35 chromosome F_1 hybrid with normal Chinese Spring possessed less than an average of 1 open bivalent per meiocyte. Contrary to the observations of Sharma and Gill (1986) using bud-pollinations, gibberellic acid applications and a slightly earlier embryo rescue timing (14 to 16 days instead of 18 to 20 days) BCI derivatives were obtained in each case when the *phIb/Ae. variabilis* and mono 5B/*Ae. variabilis* (34 chromosome) F_1 hybrids were pollinated by *phIb* and CS pollen respectively. This opens up an exciting mechanism of inducing enhanced wheat/alien recombination at F_1 and also exploiting it for practical gain through conventional breeding methodology. The above projection gains strength from the premise that alien genetic introgression is likely to occur in appropriate homoeologous sites and will not offset the genetic balance drastically. Such subtle exchanges are preferred but one must not discount the merits of larger chromosomal exchanges. This paper is concluded with the discussion of the 1B/1R chromosome translocation in *T. aestivum* and its influence in a national uniform wheat yield trial of advanced wheat lines where close to 70 percent of the breeders entries have the 1B/1R homozygote (Jahan *et al.* 1990). Globally over 5 million hectares are cultivated to 1B/1R translocated wheat despite the fact that the bread baking quality of such varieties is classified as poor. The rust and mildew

resistances on the rye arm have not held up in several countries but the elite germ-plasm still maintains its advantage through its wider adaptability, high yield potential, greater stability and some as yet uncategorized resistances or tolerances. Only near isogenic lines will hopefully establish this advantage of translocation; a research area that is actively in progress.

CONCLUSION

Since the pioneering work of Kruse (1967, 1969, 1973) signgificant advances have been made in exploiting alien genetic resources which are appropriately simplistic to utilize at the inerspecific level but highly complex at the stage of intergenerics. The basic theme that genetic variability for wheat improvement is essential shall persist. Breeders also will have to cope up with the inevitable fact that the dynamic/static systems for biotic/abiotic situations do exist and the former (dynamic) is liable to change more rapidly than the latter. There will not be a permanent advantage of an alien gene and presumably there may be negligible chances of a variety to emerge from alien transfer/s alone, since varietal release is a complex phenomenon and has to be coupled with various interactive aspects. The above statement is essential for the naive critics perspectives. The success however, of the 1B/1R cultivars, the 1A/1R Amigo plus other cultivars, Kite and its relatives, the double alien genetic inserts high performance in the mid-west USA variety Century and other alien genes in wheat (Sharma and Gill, 1983) provide unequivocal and unsurmountable evidence of direct success of varietal releases from alien introgression.

Optimistically we view developments of the not too distant a future somewhat comparable to the green revolution phase of Mexican wheats. The methodology of alien genetic transfers now seems adequately in place, the research constraints are well identified, diagnostic detectors (*In situ* hybridization, genomic blocking, polymerase chain reaction application) seemingly are quite appropriate and with the imminent availability of a wheat RFLP genetic linkage map, subtle transfers at the very DNA level should simplify gene tagging and exploitation with tremendous precision plus remarkable efficiency. The next decade offers the stage for realization of the above thoughts.

REFERENCES

Alonso, L. C. and Kimber, G. (1980). A hybrids between diploid *Agropyron junceum* and *Triticum aestivum. Cereal Res. Commun.* 8 : 355–358.

Asiedu, R., Mujeeb-Kazi, A. and Ter-Kuile, N. (1989). Markerassisted introgression of alien chromatin into wheat. In : *Mujeeb-kazi, A. and Sitch, L. A. (ed) Review of Advances in Plant Biotechnology,* 1985–88 *CIMMYT, Mexico, D. F., and IRRI, Manila, Philippines* : 133–144.

Dewey, D. R. (1984). The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In : *Gustafson, G. P. (ed) Gene manipulation in plant improvement. 16th Stadler Genetics Symp, Plenum Press, New York* : 209-279.

Forster, B. and Miller, T. E. (1985). A 5B deficient hybrid between *Triticum aestivum* and *Agropyron junceum. Cereal Res. Commun.* **13** : 93 95.

Gill, B. S. (1989). The use of chromosome banding and in situ hybridization for the study of alien introgression in plant breeding. In : *Mujeeb Kazi, A. and Sitch, L. A. (ed) Review of Advances in Plant Biotechnology, 1985-88 CIMMYT, Mexico, D. F. and IRRI, Manila, Philippines* : 157-163.

Gill, B. S. and Raupp, W. J. (1987). Direct gene transfers from *Aegilops squarrosa* L. to hexaploid wheat. *Crop. Sci.* **27** : 445 450.

Islam, A. K. M. R., Shepherd, K. W. and Sparrow, D. H. B. (1981). Isolation and characterization of euplasmic wheat barley chromosome addition lines. *Heredity* **46** : 161-174.

Jahan, Q., Ter-Kuile, N., Hashmi, N., Aslam, M., Vahidy, A. A. and Mujeeb Kazi, A. (1990). The status of the 1B/1R translocation chromosome in some released varieties and the 1989 candidate varieties of Pakistan. *Pakistan J. Botany* **22** : 1 10.

Kimber, G. and Feldman, M. (1987). Wild wheat. An introduction. *Special report 353, College of Agriculture, University of Missouri, Columbia* : 1 146.

Kruse, A. (1967). Intergeneric hybrids between *Hordeum vulgare* L. ssp. *distichum* (v Pallas 2n= 14) and *Secale cereale* L (v Petkus 2n = 14). In : *Royal Veterinary and Agricultural Collage Yearbook 1967, Copenhagen, Denmark* : 82-92.

Kruse, A. (1969). Intergeneric hybrids between *Triticum aestivum* L. (v Koga II 2n=42) and *Avena sativa* L. (v Stal 2n=42) with pseudogamous seed formation. In : *Royal Vet. and Agricultural Yearbook, Copenhagen, Denmark* : 188-200.

Kruse, A. (1973). *Hordeum*×*Triticum* hybrids. *Hereditas* **73** : 157-161.

Mujeeb-Kazi, A. (1982). Wide Crosses. *CIMMYT report on wheat improvement* : 78 -87.

Mujeeb-Kazi, A. and Asiedu, R. (1989). Alien germplasm for wheat (*Triticum aestivum* L.) improvement facilitated by cytogenetic manipulation and use of novel techniques. In: *Strengthening collaboration in biotechnology : International agriculture research and the private sector. USAID Conf, Virginia, USA* : 211-231.

Mujeeb-Kazi, A. and Asiedu, R. (1990). Wide hybridization potential of alien genetic transfers for *Triticum aestivum* improvement. In : *Bajaj, Y. P. S. (ed) Biotechnology in agriculture and forestry,* **13** : 111-127.

Mujeeb-Kazi, A. and Kimber, G. (1986). The production, cytology and practicality of wide hybrids in the Triticeae. *Cereal Res. Commun.* **13** : 111 124.

Mujeeb-Kazi, A., Roldan, S., Suh, D. Y., Sitch, L. A. and Faroog, S. (1986). Production and cytogenetic analysis of hybrids between *Triticum aestivum* and some caespitose *Agropyron* species. *Genome* **29** : 537 5 3.

Mujeeb Kazi, A., Ter Kuile, N. and Asiedu, R. (1988). Cytogenetic progress of advanced derivatives from *Triticum aestivum*× *Thinopyrum bessarabicum* F_1 hybrids. *Agronomy Abstracts* : 90.

Mujeed, Kazi, A., Roldan, S., Suh, D. Y., Ter-Kuile, N. and Farooq, S. (1989). Production and cytogenetics of *Triticum aestivum* L. hybrids with some rhizomatous *Agropyron* species *Theor. Appl. Genet.* **77** : 162 168.

Multani, D. S., Dhaliwal, H. S., Singh, P. and Gill, K. S. (1988). Synthetic amphiploids of wheat as a source of resistance to karnal bunt *(Neovossia indica)*. *Plant Breeding* **101** : 122-125.

Sears, E. R. (1977). An induced mutant with homoeologous pairing in wheat. *Can. J. Genet. Cytol.* **19** : 585-593.

Sharma, H. C. and Gill, B. S. (1983). Current status of wide hybridization in wheat. *Euphytica* **32** : 17-31.

Sharma, H. C. and Gill, B. S. (1986). The use of *ph1* gene in direct transfer and search for *Ph*-like genes in polyploid *Aegilops* species. *Zeit Pflanzenzuchtg* **96** : 1-7.

Warham, E. J., Mujeeb-Kazi, A. and Rosas, V. (1986). Karnal bunt *(Tilletia indica)* resistance screening of *Aegilops* species and their practical utilization for *Triticum aestivum* improvement. *Can. J. Pl. Pathol.* **8** : 65-70.