

INCORPORATION OF ALIEN GENETIC INFORMATION FROM *ELYMUS GIGANTEUS* INTO *TRITICUM AESTIVUM*

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SUMMARY

The F₁ hybrid of *Triticum aestivum*/*Elymus giganteus* had $n=5x=35$, ABDJX chromosomes with a mean chiasma frequency of 1.02 per cell. The backcross I (BC₁) progeny, *T.aestivum*/*E.giganteus*//*T.aestivum*, with $2n=8x=56$ (AABBDDJX) chromosomes, expressed variable meiotic associations. However, several meiocytes possessed 21 bivalents + 14 univalents. All fourteen *E. giganteus* chromosomes were identified by giemsa C-banding in the F₁ hybrid and the BC₁ plants. Repeated backcrossing to *T.aestivum* and/or selfing led to partially self-fertile plants after BC₁₁ and eventually resulted in the production of monosomic or disomic *E.giganteus* addition lines. These form 21 bivalents + 1 univalent or 22 bivalents at meiosis. Somatic cytological evidence indicated a preferential substitution for chromosome 6B of *T.aestivum* by an *E.giganteus* chromosome. Such plants only possessed one satellited chromosome pair of 1B.

Backcross progenies after BC₁₁ were tested for resistance to *Helminthosporium sativum* and *Fusarium graminearum*. Segregating lines have been tentatively identified that possess resistance to both pathogens.

INTRODUCTION

Intergeneric or wide hybridizations in the Triticeae, other than the production of *Tritico-Secale* Wittmack, have been both attempted and studied for some time. Wide hybridization has emerged as an important cytogenetic means of transferring alien genes into wheat (*Triticum aestivum* L.) hopefully to serve as additional source of genetic variability for breeding for resistance to insects, diseases and stress tolerances. Spin-offs from our practical, field-oriented wide hybridization program conform with generalized studies elsewhere on genome structure, genome relationships and phylogeny.

The overall focus of the program is to obtain for *T. aestivum* better resistance to diseases, such as those caused by *Helminthosporium sativum* or *Fusarium graminearum*, and greater tolerance to environmental stresses like salt, drought, aluminium, or copper. This paper reports on the *T. aestivum*/*Elymus giganteus* hybrid and discusses the agricultural potential of the derived progenies. The discussion digresses to encompass the limitations of wide hybridization research for those cases where homoeology of the alien genome(s) with wheat does not exist. Means and methodology for possibly overcoming these constraints are mentioned.

MATERIALS AND METHODS

The hybrid production, embryo culture, and the somatic/meiotic cytological, generation advance procedures have been described earlier (Mujeeb-Kazi and Miranda 1983; Mujeeb-Kazi and Rodriguez 1980, 1981, 1983).

Screening for *F. graminearum* on advanced lines derived from *T. aestivum*/*E. giganteus* F₁ was conducted at CIMMYT's field station in Toluca, and in the greenhouses at El Batan, Mexico. *Helminthosporium sativum* screening was done at CIMMYT's station in Poza Rica, Mexico. In both cases, a 0 to 9 scoring scale was adopted, 0 being immune and 9 highly susceptible. Detailed methodology to be reported elsewhere.

RESULTS AND DISCUSSION

Elymus giganteus (2n=4x=28; JJXX) was hybridized with *Triticum aestivum* L. cv. Chinese Spring in 1977 and 1979 (Mujeeb-Kazi and Rodriguez 1981a) under a controlled greenhouse environment. The hybrid was again produced in 1982, but under field conditions (Mujeeb-Kazi *et al.* 1983a, 1983b) and in a high frequency. Pollination of 234 florets of var. Chinese Spring with *E. giganteus* pollen set 7 seed. Embryo recovery was 1.7% and differentiation 0.9%. Presumably the excellent pollen supply from field grown plants contributed to the higher embryo recovery. Failure to obtain seed set with other wheat varieties apparently was a cause of the genetic superiority of Chinese Spring over the *T. aestivum* varieties that were additionally used in the study (Mujeeb-Kazi *et al.* 1983b, c). The higher success with Chinese Spring is attributed to kr₁kr₁, kr₂kr₂, kr₃kr₃, crossability genes on chromosomes of the homoeologous group 5 that probably function as complementary loci. The crossability and gene associations have been independently reported (Barclay 1975; Falk and Kasha 1981; Fedak 1977; Fedak and Jui 1982; Islam *et al.* 1975,

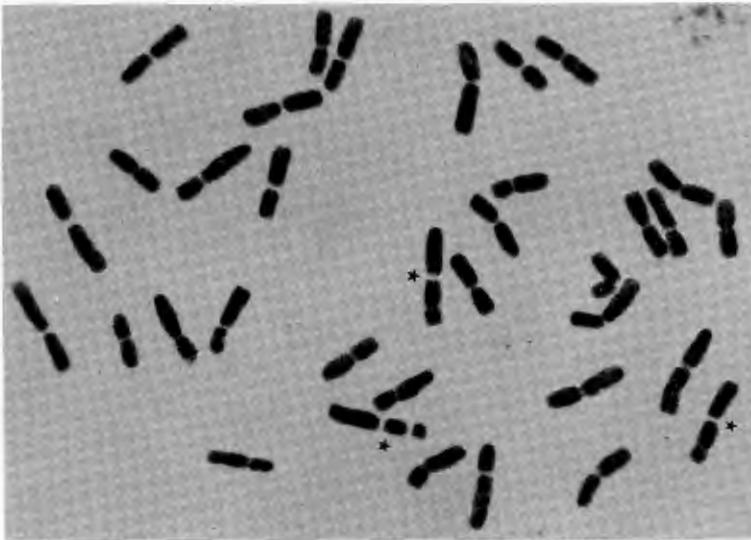


Fig. 1. An n=5x=35 somatic spread of the F₁ *T. aestivum*/*E. giganteus*. Note the 3 satellited chromosomes; 1B, 6B of *T. aestivum* and an *E. giganteus* satellited chromosome.



Fig. 2. A giemsa C-banded somatic preparation of the *T. aestivum*/*E. giganteus* F₁ with the *E. giganteus* banded chromosomes labelled from A to N.

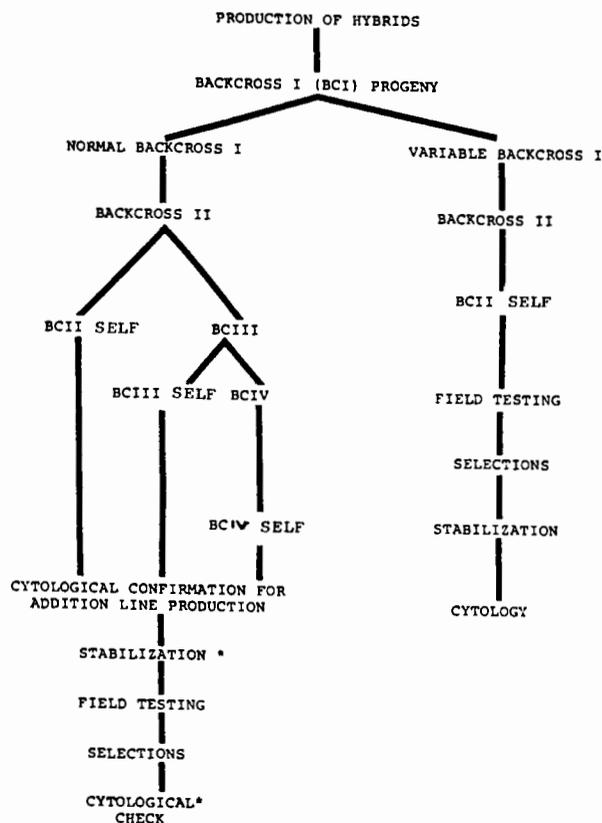


Fig. 3. A *T. aestivum*/*E. giganteus*/*T. aestivum* BC₁ plant somatic count of 56 chromosomes with a pair each of the satellited 1B and 6B chromosomes. Two chromosomes were not in the frame and were cropped in (*).

1978; Mujeeb-Kazi *et al.* 1983a, b; Riley and Chapman 1967).

The F₁ hybrid possessed a phenotype that differed from the Chinese Spring female parent and formed a selection sieve for advancing the hybrid for agricultural application. If the F₁ hybrid was to express wheat phenotype dominance, then chances of alien genetic expression would be negligible. Such seems to be the case in *T. aestivum*/*H. vulgare* hybrids (Islam *et al.* 1978, 1981; Mujeeb-Kazi and Rodriguez 1981b, 1983). There were 35 chromosomes in somatic cells ($n=5x=35$), 21 of wheat (ABD) and 14 of *E. giganteus* (JX). Three satellited chromosomes expressed (Fig. 1) i.e., 1B, 6B and an *E. giganteus* chromosome tentatively identified as chromosome F based on the C-banding pattern (Fig. 2) There was no intergenomic or autosyndetic chromosome pairing observed in the hybrid (Mujeeb-Kazi and Rodriguez 1981a).

Since fertile amphidiploids could not be obtained, BC₁ seeds were produced by fertilization of an unreduced egg cell in the F₁ with pollen from the BC *T. aestivum* parent. The BC₁ plants generally had 56 chromosomes (Fig. 3) though a few possessed 55, with the



* Somatic and/or meiotic cytology

Fig. 4. Schematic indicating advance of Backcross I progenies of normal or anomalous origin for practical agricultural utilization.

missing chromosome being that of *E. giganteus*. Univalency was present in the BC_I progeny but several meiocytes had chromosome associations of 21 bivalents+14 univalents at metaphase I. In the BC_I plants four satellited wheat chromosomes (Fig. 3) were always present (1B, 1B, 6B, 6B) and upon C-banding all of the *E. giganteus* chromosomes were readily identified. Such BC_I production is considered normal and occurs in a low frequency. Anomalous BC_I progenies occur frequently and are common to several hybrid combinations among some Triticeae (Mujeeb-Kazi and Bernard 1982; Jewell and Mujeeb-Kazi 1982; Rodriguez and Mujeeb-Kazi 1981). The advance of both cytologically normal and anomalous BC progenies (Fig. 4) indicates that for normal BC_I types, as for *T. aestivum*/*E. giganteus*||*T. aestivum*, direct field testing is feasible immediately upon restoring self-fertility after BC_{II}. There is, however, the option that sequential advance of the normal BC_I progenies could be carried out, thereby providing critical chromosomal information on a continuous basis. For those BC progenies derived from anomalous BC_I progenies, the advancement steps after BC_{II} self-fertility is achieved are oriented towards direct field testing, circumventing cytology until plant selections are stabilized (Fig. 4).

The BC_I plants were self-sterile and set from 2 to 3 BC_{II} seeds per spike when pollinated by *T. aestivum*. The BC_{II} progeny varied in their range of self-fertility and their chromosome numbers. The latter were from 44 to 52 with a majority of the plants possessing 49 chromosomes. Each BC_{II} plant was selfed and additionally backcrossed with *T. aestivum* for producing BC_{III} seed, which set in a high frequency. The progressive increase in chiasma formation and bivalent formation occurred as anticipated from F₁ to BC_{II}, reaching a maximum by BC_{III} or BC_{II} self. Partial data for F₁, BC_I and BC_{II} are included in Table 1.

Several advanced backcross progenies have been identified as segregating for resistance to *F. gramineum* and *H. sativum*. These selections are being subjected to another screen to confirm the resistance observations. The selected plants had desirable plant characteristics, a consequence of using commercial wheats as the BC_I parents and their continued use as recurrent parents in subsequent backcrosses. Partial cytological analyses of the advanced

Table 1. Mean and standard deviation of the chiasma frequency per pollen mother cell in the F₁ and some BC_I and BC_{II} plants of *T. aestivum*/*E. giganteus* combination.

Lab. number	Combination	Generation	Chromosome number	Mean chiasma frequency/cell	Standard deviation (±)
1002	<i>T. aestivum</i> / <i>E. giganteus</i>	F ₁	35	1.02	×
5121	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	56	36.40	0.55
5124	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	56	32.95	0.68
5126	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	55	34.65	0.69
5129	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	65	33.20	0.84
5132	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	65	35.20	0.66
5135	<i>T. aestivum</i> / <i>E. giganteus</i> <i>T. aestivum</i>	BC _I	65	32.29	0.73
5139	<i>T. aestivum</i> / <i>E. giganteus</i> 2 <i>T. aestivum</i>	BC _{II}	52	39.54	0.28
5141	<i>T. aestivum</i> / <i>E. giganteus</i> 2 <i>T. aestivum</i>	BC _{II}	45	39.40	0.30
5142	<i>T. aestivum</i> / <i>E. giganteus</i> 2 <i>T. aestivum</i>	BC _{II}	44	38.00	0.49

BC progenies indicates recovery of plants that are characterized as:

- (i) being euploid with 42 chromosomes forming 21 bivalents,
- (ii) possessing alien disomic chromosome substitutions,
- (iii) having alien monosomic or disomic chromosome additions, or
- (iv) possess an array of aneuploid compositions.

The euploids, if resistant to the stress or disease factors, will eventually be cytologically analyzed for events that may have culminated in spontaneous substitutions or chromosome translocations. One spontaneous substitution that was readily observed was for the satellited 6B chromosome. Though the mechanism needs more elucidation, presumably the substitution process comprises at least a partial-asynapsis in the early backcross generations, as well as univalent shift. The incorporation of the alien univalent as a disome may occur either as a result of mis-division of the univalent chromosome and transfer of the separation products to the same pole, or after selfing of the backcross plants. Fig. 5 presents somatic evidence from a BC_{III} selfed plant in which the 43 chromosomes, with the 6B satellited chromosome pair absent, were meiotically associated as 21 bivalents+1 univalent (see also Fig. 6). Several BC_{II} selfed or BC_{III} plants have, upon selfing, produced alien disomic chromosome addition lines. These have 44 chromosomes and form 22 bivalents (Fig. 7). The various addition lines are now being characterized using C-banding identification patterns established for the *E. giganteus* chromosomes (Fig. 2).

BC_{III} selfed plants also provide the possibility of obtaining univalency for other wheat chromosomes with the potential of additional spontaneous alien chromosome substitutions. In one such BC_{III} selfed plant, 42 chromosomes were associated as 19 bivalents+4 univalents (Fig. 8). One univalent appears to be the satellited 1B chromosome. It has a distinct

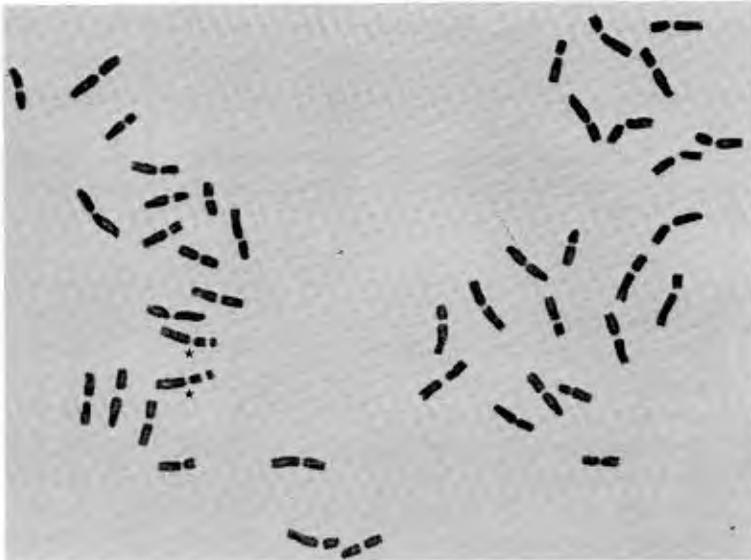


Fig. 5. A BC_{III} selfed plants somatic preparation with 43 chromosomes and presence of the 1B 1B satellite pair. Note absence of 6B 6B satellited chromosomes.

secondary constriction and a short arm: long arm ratio resembling the 1B 1B chromosomes shown earlier in Fig. 5. There is possibly a second satellited univalent (marked).

The progenies derived from *T. aestivum*/*E. giganteus* offer promise of practical utilization. Excellent plant types, with alien chromosomes present in different numbers, have resulted in segregating lines with potential disease resistance and with potential stress tolerances as yet unexplored. There are however, limitations to having entire alien chromosomes. These are well recognized, and mechanisms exist that would enable successful manipulations for obtaining subtle alien genetic transfers. Such manipulations involve the use of aneuploids of chromosome 5B, *PhPh* mutant, irradiation, crosses of advanced backcross progenies with *Aegilops speltoides*, tissue culture and pentaploid induced translocation. All procedures are being utilized at the present time, now that their absolute necessity and potential benefits have been determined. The choice of using some of these manipulative options rather than others is based upon the application of mathematical inputs on F₁ meiotic analyses (Kimber 1982).

Significant practical success from this wide hybridization program is expected within one decade. For this to occur a balance between basic and applied research is absolutely essential; the cytological input constitutes a prerequisite for achieving practical gains.

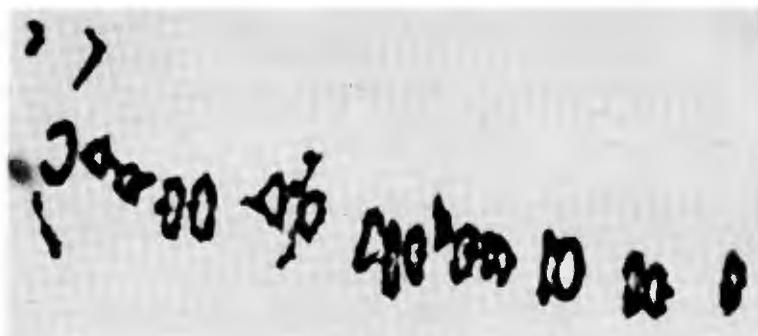


Fig. 6. Metaphase I association of 21 bivalents+1 univalent from a BC_{III} selfed plant of *T. aestivum*/*E. giganteus*//3*T. aestivum*, exemplifying an alien monosomic chromosome addition.

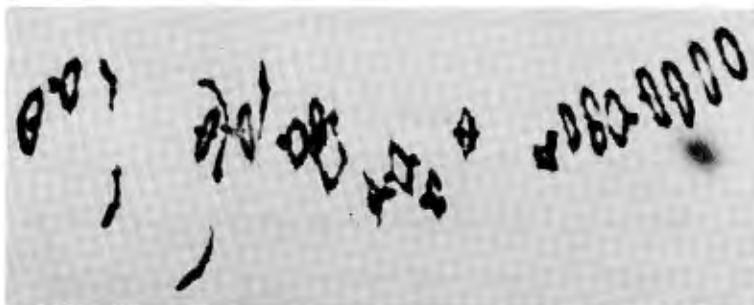


Fig. 7. A BC_{III} F₁ plant from *T. aestivum*/*E. giganteus*//3 *T. aestivum* with 44 chromosomes meiotically associated as 22II signifying a disomic alien chromosome addition.

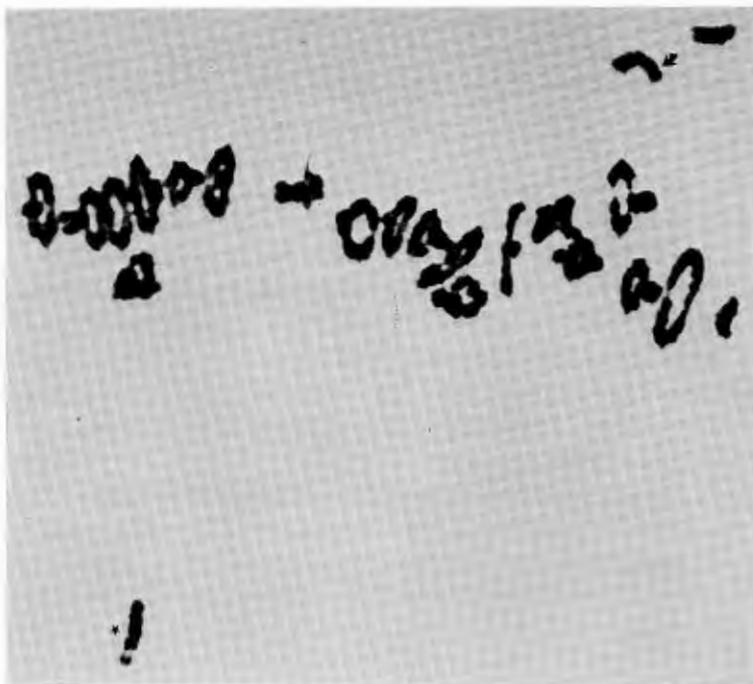


Fig. 8. *T. aestivum*/*E. giganteus*/3 *T. aestivum* (BC_{III} selfed) plant with 42 chromosomes forming 10 bivalents and 4 univalents. IB univalent is satellited and marked, as is another possible satellited univalent (↑).

The fact that genes introduced from alien species into cultivated varieties are not fundamentally different from those already present is well understood. They are exposed to the same limitations imposed by mutating pathogens that can break the incorporated alien resistance. The potential may be far greater for incorporation of genes for stress tolerance where, in the absence of a mutable pathogen, alien transfers may have longer lasting effects (Mujeeb-Kazi and Kimber, in preparation).

ACKNOWLEDGEMENT

The authors acknowledge the expert photographic assistance of Mr. Leobardo Terpan and his colleagues in CIMMYT's photography section.

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