



# An intergeneric hybrid of *Triticum aestivum* L. × *Elymus giganteus*

**ABSTRACT:** The intergeneric hybrid of *T. aestivum* L. cv. Chinese Spring ( $2n = 6x = 42$ , AABBDD) with *E. giganteus* ( $2n = 4x = 28$ , JJXX) expressed a biparental phenotype. The hybrid was  $2n = 5x = 35$ , with ABDJX somatic chromosomes that were meiotically represented as 32.8 univalents, 1.02 bivalents. The *Triticum* genomes maintained their specificity, and no autosyndetic pairing occurred between the J and X genomes. Meiotic restitution in the  $F_1$  and gibberellic acid treatment aided in production of *T. aestivum* – *E. giganteus* × *T. aestivum* backcross-1 seed. These were of the AABBDDJX ( $2n = 8x = 56$ ) genomic composition, although one  $BC_1$  plant possessed 55 chromosomes.  $BC_2$  seed have been produced and await cytological identification. The role of Chinese Spring in intergeneric crossability is discussed.

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THE PROJECTED pressures of world population growth on the future availability of food resources are of principal concern to the plant breeder. These pressures have led to several innovative approaches in breeding crop species<sup>7</sup>, and include the *Triticum* species. Other than *Secale*, researchers have concentrated in hybridizing various genera with *Triticum*, i.e., *Avena sativa*<sup>13</sup>; *Hordeum bulbosum*<sup>1</sup>; *H. distichum*<sup>2</sup>; *H. chilense*<sup>15</sup>; *H. vulgare*<sup>6,8,12,18,25</sup>, and *H. bogdani*<sup>10</sup>.

In earlier studies at this institution emphasis was on manipulating intergeneric crossability within the Triticeae using immunosuppressants, and concentrating on barley-wheat combinations. Currently the manipulative procedure has been modified<sup>17</sup>, and *Elymus* species are used as germplasm donors in the intergeneric hybridization program. Although literature is scarce on hybrids of *Triticum* × *Elymus*, the genus *Elymus* has been well characterized by D. R. Dewey (pers. comm.). The *E. giganteus* phenotype and xerophytic leaf texture made it an ideal choice for a paternal donor to *T. aestivum*.

This paper reports a *T. aestivum* × *E. giganteus* hybrid. Also described are the  $F_1$  phenotype, cytogenetics, backcross-1 and -2 progenies.

## Materials and Methods

The *Triticum aestivum* L. cv. Chinese

Spring ( $2n = 6x = 42$ , AABBDD) × *Elymus giganteus* L. ( $2n = 4x = 28$ , JJXX) hybrids were obtained by normal crossing and embryo culture procedures<sup>25</sup> in 1977 and 1979. The hybrids were vegetatively propagated under greenhouse conditions of 26.6°C/15.5°C. (14-hour day/10-hour night) and approximately 45 percent relative humidity.

From the clone progeny 18 spikes were sampled for meiotic analysis. All metaphases were examined for chromosome relationships using 2 percent propionic-orcein stain. Data were tabulated for mean chromosome relationships for 450 pollen mother cells (PMC's).

Spike morphology comparisons were made between *T. aestivum*, *T. aestivum* × *E. giganteus*, and *E. giganteus*.

## Results and Discussion

The increasing interest in intergeneric hybridization in Triticeae reflects the importance of cereals. Various crosses have been made with varying degrees of success: some are of pure biological interest, some are products of chance events, and others are a result of attempts aimed at justifying specific happenings or hypotheses. In the mid-1960's A. Kruse pioneered the role of growth hormones in facilitating intergeneric hybridization, and his contributions have been successfully utilized by several investigators<sup>1,2,6,8,15</sup>. An animal effective

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immunosuppressant, E-Amino-n-Caproic acid (EACA) assisted embryo development in *T. turgidum* × *S. cereale* crosses<sup>24</sup>, and supported embryo + endosperm development coupled with environment in *T. timopheevii* × *S. cereale* crossability investigations<sup>15</sup>. However, several intergeneric crosses were made without chemical manipulation<sup>9,10,17,24</sup> and were constantly repeated<sup>16</sup>. The *T. aestivum* L. cv. Chinese Spring and *E. giganteus* hybrid reported here is included in this latter category.

Of the *T. aestivum* cultivars, Chinese Spring appears to cross more readily with *H. vulgare*<sup>8,8,15</sup> either as a male or female parent. This, however, is not a rule considering the reports of Kruse<sup>12</sup>, and Thomas et al.<sup>25</sup>, but in absence of critical crossability data Chinese Spring was used in crosses with *E. giganteus*. *Elymus* species were incorporated in the Triticeae intergeneric hybridization investigations during 1976 (R. G. Anderson, pers. comm.) to give the cereal based program another practical agricultural impetus, in addition to the earlier emphasis of protein content/quality transfer from *H. vulgare* to *Triticum* species. The first hybrid obtained was from a cross between *T. aestivum* L. cv. Chinese Spring and *E. giganteus*; it was repeated in 1979.

*E. giganteus* is an exceptionally coarse, strongly rhizomatous, Asian grass that commonly grows on sandy sites along river banks and seashores<sup>4</sup>. It is an allotetraploid ( $2n = 4x = 28$ ), with two distinctly different genomes<sup>19</sup> and is genomically represented as JJXX<sup>5</sup>. The *E. giganteus* ( $2n = 4x = 28$ , JJXX) characteristics are presented in Table I, as are those for the *T. aestivum* parent, and *T. aestivum* × *E. giganteus* hybrid. The spike morphological details of the parents and the hybrid are illustrated in Figure 1. Distinct biparental morphology was observed in the hybrid. This was a unique situation because earlier reports in the literature, irrespective of the cross direction, indicated the *Triticum* morphology was dominant. Only recently was an explanation proposed for biparental morphology. A. Kruse (pers. comm.) noted that the hexaploid and tetraploid *Triticum* phenotype was always expressed in crosses with *Hordeum* irrespective of the cross direction; it was only at the diploid level that the maternal expression dominated. Thus, in the present hybrid, the characteristics of *T. aestivum* were masked by *E. giganteus* to such an extent that the biparental phenotype was expressed.

Only since the mid-1970's has meiotic data reflected the intergeneric hybrids obtained within the Triticeae, other than

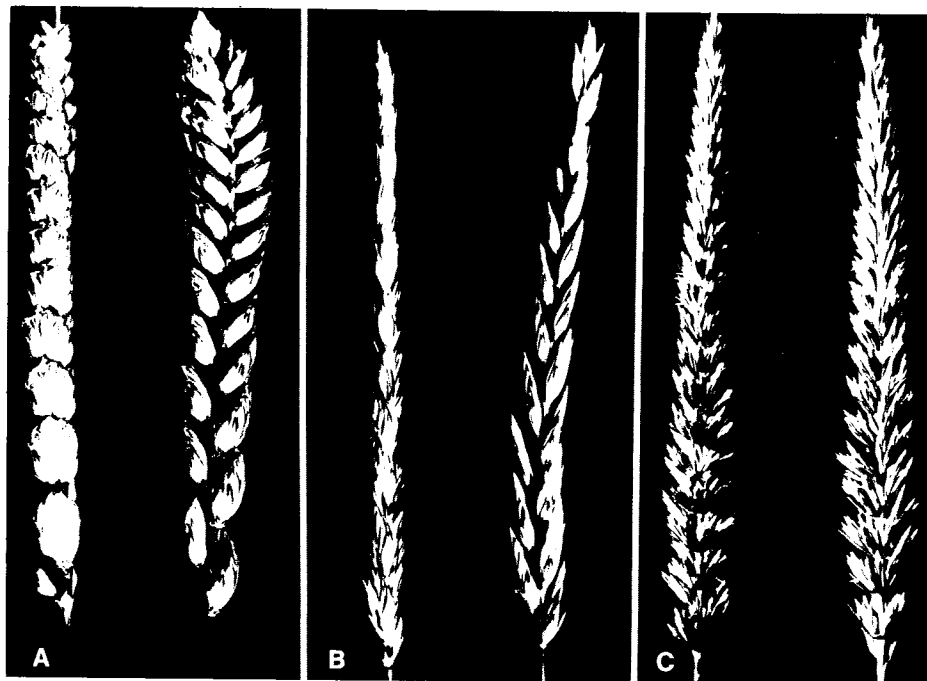


FIGURE 1 Dorsal and lateral views of spikes. A—*T. aestivum* cv. Chinese Spring. B—*T. aestivum* × *E. giganteus* hybrid. C—*E. giganteus*.

crosses between *Triticum* and *Secale*. The meiotic relationships of *T. timopheevii* × *H. bogdanii*<sup>10</sup> was the first published report that demonstrated a possible indication of some homology between the genomes of *H. bogdanii* and *T. timopheevii*. Mujeeb et al.<sup>18</sup> did not observe any homologous rela-

tionships between cultivars of *H. vulgare*, *T. turgidum*, and *T. aestivum*. The intraspecific differences in certain species of the genus *Triticum* must be borne in mind, as must also the idea of determining whether there are extensive intraspecific differences in *Hordeum*<sup>6</sup>. Data of Fedak<sup>6</sup>

Table I. Mean spike characteristics from 10 spikes of *T. aestivum* L. cv. Chinese Spring, *T. aestivum* L. cv. Chinese Spring × *E. giganteus* hybrid, and *E. giganteus*

Character	<i>T. aestivum</i>	<i>T. aestivum</i> × <i>E. giganteus</i>	
		<i>E. giganteus</i>	<i>E. giganteus</i> *
Spike:			
Length (cm)	8.30	11.63	26.6
Width (cm)	0.95	1.33	2.52
Rachis:			
Nodes/spike	27.00	18.33	35.3
Internode length (cm)	0.40	0.68	0.75
Spikelet:			
Length (cm)	1.00	1.68	1.93
Width (cm)	0.93	0.80	0.34
Spikelets/spike			
Florets/spikelet	119.00	93.5	136.30
	4.55	5.11	4.6
Glume:			
Body length (cm)	0.95	1.15	2.01
Awn length (cm)	0.05	0.32	0.47
Lemma:			
Body length (cm)	0.85	0.80	1.34
Awn length (cm)	0.05	0.08	0.05
Anther length (cm)	0.40	0.31	0.60

\* Data from Dewey<sup>5</sup>



FIGURE 2 Meiotic metaphase relationship of 33 univalents and one rod bivalent (arrow) in *T. aestivum* × *E. giganteus* hybrid ( $2n = 5x = 35$ , ABDJX).

and Martin and Chapman<sup>15</sup> illustrated homoeologous pairing in the hybrids of *H. vulgare* × *T. aestivum*, and *H. chilense* × *T. aestivum*. This led Fedak<sup>6</sup> to suggest that the high chiasmata frequency in the  $F_1$  hybrid may be a consequence of the suppression of the diploidization system of *T. aestivum* L. cv. Chinese Spring. Meiotic relationships for the *H. vulgare* × *T. aestivum* hybrid reported by Islam et al.<sup>8</sup> were not presented to indicate what changes in homologous pairing may have occurred. However, the authors did report that the

$F_1$ 's had "usually 28 univalents (Figure 2a), but some PMC's possessed up to three bivalents, including an occasional ring bivalent." Mujeeb and Rodriguez<sup>17</sup> observed homoeologous pairing in some *H. vulgare* × *T. aestivum* hybrids to give a chiasmata frequency/cell of 2.57, suggesting possible intergeneric homology from the bivalent relationships seen in PMC's, without enhanced homoeologous chromosome pairing.

The somatic chromosome number was  $2n = 5x = 35$  (ABDJX) in the *T. aestivum* ×

*E. giganteus* hybrid. It was slow tillering and had sparse foliage. Plants maintained under controlled greenhouse conditions allowed the production of clone progeny. Mean meiotic relationships at metaphase I in the hybrid were 32.8 univalents and 1.02 bivalents. The bivalents were always of the rod type (Figure 2). The data (Table II) suggest a maintenance of genome specificity in that the extent of 1.02-bivalent frequency falls close to 1.05 bivalents<sup>23</sup> for the *T. aestivum* polyhaploid. Further, the JX genomes (J from *E. junceus* and X from an unknown ancestor<sup>5</sup>, do not exhibit autsyndetic pairing or influence the *Ph* gene on 5BL. Pollen development beyond metaphase I followed a sequence that formed microspores exhibiting polymorphism of shape and size, with variations from the normal uniporate pollen. Attempts to induce amphiploidy have been in progress using colchicine and dimethylsulfoxide (0.2 percent + 2 percent for 7 hours as a root treatment or by capping for 4 hours) but without success. The meiotic analysis indicated that meiotic restitution may facilitate formation of backcross seed. This was earlier demonstrated<sup>14</sup> for *Aegilops helldreichii* × *T. durum*  $F_1$  hybrids where a high frequency of meiotic nonreduction resulted in  $F_1$  fertility. Consequently, spikes of *T. aestivum* × *E. giganteus* were backcrossed to either *T. aestivum* or *E. giganteus*, respectively. The first sequence of crosses did not set seed or exhibit stimulation. A second batch of spikes were pollinated on two successive mornings with *T. aestivum* L. cv. Chinese Spring and treated with gibberellic acid ( $GA_3$ ) at 75 ppm for two consecutive days. The procedure was successful. One seed was used for obtaining an embryo to be cultured in Taira's medium (T. Taira, pers. comm.) for small embryos. The endosperm was well developed, and remaining seed were left to mature on the hybrid. The backcross seed were expected to possess 56 chromosomes (AABBDDJX). It was presumed that meiosis would be regular for the AABBDD genomes, with JX maintaining univalency based on their meiotic expression in the ABDJX hybrid. The backcross progeny so obtained offered the potential of developing addition lines of *Elymus giganteus*, as conventionally expressed for other intergeneric hybrids within the Triticeae<sup>3,8</sup>.

It has since been verified that the backcross-1 ( $BC_1$ ) seed possess 56 chromosomes (AABBDDJX). One plant eliminated one chromosome after 5 weeks of sampling. All  $BC_1$  plants were vegetatively vigorous and produced spikes of intermediate phe-

Table II. Mean chromosome pairing at metaphase I in the *T. aestivum* L. ( $2n = 6x = 42$ ) × *E. giganteus* ( $2n = 4x = 28$ ) hybrid, compared with pairing in a *T. aestivum* polyhaploid ( $2n = 3x = 21$ , ABD)

Hybrids	Chromosomal configurations				No. cells
	I	II	III	IV	
<i>T. aestivum</i> × <i>E. giganteus</i>	32.8	1.02	—	—	450
<i>T. aestivum</i> *	18.84	1.05	0.02	—	750

\* Data from Riley & Law<sup>23</sup>

notype resembling the hybrid. Pistilloidy problems<sup>8</sup> were not expected here because of the maternal *Triticum* cytoplasm. Meiotic analysis of BC<sub>1</sub> plants was delayed in order to also include BC<sub>2</sub> material. The BC<sub>2</sub> seed set approached 30 percent, and was aided by 2 post-pollination applications of GA<sub>3</sub> at 75 ppm. Theoretically, this seed should possess all 42 *Triticum* chromosomes, and 0 to 14 *Elymus* chromosomes depending upon univalent separation at anaphase I or anaphase II. The BC<sub>1</sub> meiotic and BC<sub>2</sub> cytological investigations are in progress.

The *T. aestivum* × *E. giganteus* hybrid formation is a low-frequency cross as compared with barley × wheat or even wheat × barley. Thus, on such rudimentary data it may be presumptuous to attach any germplasm specificity to Chinese Spring. Islam, et al.<sup>8</sup> stated "It is not valid to attribute success in these crossing results to the particular parents involved, since effects due to changes in seasonal conditions and crossing procedure are confounded with parental differences." These same authors, however, attribute significant cross success to the use of Chinese Spring (15.9 percent when crossed with barley, Betzes) and parallel this with the earlier superior Chinese Spring crossability with rye<sup>22</sup>. Moreover, they compared their data with the maximum 3 percent value of Kruse<sup>12</sup>, who used different cultivars. Recently, however, Kruse (pers. comm.) obtained a high embryo recovery from *H. vulgare* L. cv. Bomi × *T. aestivum* crosses. Whether the *T. aestivum* used by Kruse carry the *kr<sub>1</sub>kr<sub>1</sub>kr<sub>2</sub>kr<sub>2</sub>* genes that influence the high crossability of Chinese Spring with rye is at this time a speculation. However, Thomas et al.<sup>25</sup> reported *H. vulgare* L. cv. Manker to cross better with *T. turgidum* L. cv. Cocorit 71 than its cultivars Apizaco or Dickson Hipoly, suggesting that the maternal barley germplasm may be the influencing factor, since Cocorit 71 has a crossability of less than 10 percent with *Secale cereale* (T. J. Killeen, pers. comm.). Another example of divergence from the germplasm specificity also may be obtained from the *T. timopheevii* × *H. bogdani* hybrid<sup>10</sup>. The first

cross gave one plump seed, but no hybrids were obtained from additional spikes of *T. timopheevii* similarly pollinated by *H. bogdani* (Kimber<sup>9</sup>, discussion after paper presentation). However, a trigeneric hybrid was obtained when the hybrid was pollinated with *S. cereale*<sup>11</sup>. The high frequency of *T. aestivum* polyhaploids as a consequence of Chinese Spring × *H. bulbosum* (2x, 4x) intergeneric hybridization is more an effect of the phylogenetic position of *H. bulbosum* than the *kr<sub>1</sub>kr<sub>1</sub>kr<sub>2</sub>kr<sub>2</sub>* Chinese Spring composition according to Rajendra et al.<sup>20,21</sup>.

Hybridization difficulties are generally overcome after an intergeneric hybrid is produced and its potential is exploited for practical agriculture. Nevertheless, germplasm specificity needs further investigation. The crosses of *T. aestivum* with *Elymus* species are of low frequency, and we are just now trying to determine if the embryo formation can be significantly enhanced following the observations of Kruse<sup>12</sup>, Mujeeb-Kazi<sup>17</sup>, and Taira and Larter<sup>24</sup>. Such crossability manipulation studies shall significantly aid us in combining genera for achieving genetic transfers.

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