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Hordeum vulgare × *Triticum aestivum* hybrids

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Intergeneric crossses involving *Triticum* and *Hordeum* have been attempted over the last decade. Significant success was achieved with plant growth hormones applied to the maternal parent, either as a pre- or post-pollination treatment (Kruse 1973, 1974). Hybridization seems easier with *Hordeum* as the female parent (Chapman and Miller 1978, Fedak 1977, Islam *et al.* 1975, Kruse 1973, Mujeeb *et al.* 1978b). In *Hordeum*, some genotypes combine better with wheat than others (Mujeeb *et al.* 1978b, Thomas *et al.* 1977), and in *T. aestivum* the cv. Chinese Spring appears to be outstanding in crosses with *Hordeum* species (Chapman and Miller 1978, Fedak 1977, Islam *et al.* 1975, 1978, Martin and Chapman 1977). Irrespective of the direction of the cross *Hordeum* combinations with *T. aestivum* are a potential source of i) amphiploid formation and addition line production (Chapman and Miller 1978, Islam and Shepherd 1981, Islam *et al.* 1975, 1978, 1981), ii) documenting the role of germplasm in intergeneric hybridization and cytogenetic instability (Mujeeb *et al.* 1978b, Thomas *et al.* 1977) and iii) illustrating chromosomal homoeology (Fedak 1977).

This paper presents information on the nature of homoeologous chromosome association in hybrids of *H. vulgare* cv. Manker × *T. aestivum* cvs. Bonza, Pavon and Chinese Spring. Furthermore, information is given on the phenotype of the hybrid plants, meiotic instability, non-reduction and on backcross (BC_1 and BC_2) seed formation.

Materials and methods

Hordeum vulgare cv. Manker plants were grown in pots and kept under field conditions at Ciudad Obregon, Mexico. Spikes were emasculated and pollinated with *Triticum aestivum* cvs. Bonza, Chinese Spring or Pavon two to three days after emasculation, depending upon stigma receptivity. Pollination was followed eight hours later by a gibberellic acid (GA_3) treatment, 75 ppm aqueous, into the floret cups as demonstrated by Kruse (1973). All efforts were devoted to obtaining intergeneric hybrids, and not toward compiling the basic crossability data. The embryos were excised 20 days after pollination, and cultured on a special medium for small embryos (Taira and Larter 1978). Upon differentiation the plantlets were transferred to peat pots, and maintained in the growth chamber under high humidity before ultimate transfer to pots with soil. The hybrids were identified on the basis of the somatic chromosome number using Mujeeb *et al's.*, schedule (1978a). Depending upon plant vigour the hybrids were multiplied by cloning and kept under growth

chamber conditions of 15°C day/10°C night, 14 h day/10 h night, and 45% relative humidity. The self-sterile hybrids were backcrossed with *T. aestivum* to obtain first backcross (BC_1) seeds. The florets were clipped and pollinated earlier than is done conventionally, i. e. before stigma receptivity is visible. Mostly two applications of gibberellic acid (75 ppm, aqueous) were made into each floret cup using Kruse's (1973) procedure, eight and 32 hours after pollination. BC_1 seeds were allowed to develop for 20 days. The embryos were then excised and cultured on a special medium for small embryos (Taira and Larter 1978). BC_1 plants were transferred to soil in pots, and grown in the greenhouse environment of 16 h day/8 h night, 22°C day/15°C night, and approximately 45% relative humidity. The BC_1 plants were similarly pollinated by *T. aestivum* and treated with gibberellic acid, as was done earlier to the self-sterile F_1 hybrids, to produce BC_2 progeny. No embryo culturing of the BC_2 seeds was necessary.

Counting of the somatic root-tip chromosomes of the hybrids and the BC_1 plants was done according to the procedure of Mujeeb *et al.* (1978a). F_1 and BC_1 spikes for meiotic analyses were fixed in Carnoy's solution (6 ethanol: 3 chloroform: 1 acetic acid) for 24 hours, then transferred to 70% ethanol and refrigerated until use. Anthers were hydrolyzed in 1 N HCl for 4 minutes at 58°C, rinsed in deionized distilled water and stained with Feulgen. Squashes were made in 45% acetic acid, or 2% propionic orcein, and chromosome relationships were observed at metaphase I.

Table 1. Mean chromosome associations in hybrids of *Hordeum vulgare* L. cv. Manker × *Triticum aestivum* L. cvs. Bonza, Pavon and Chinese Spring (in brackets: range)

Hybrid combination	Number of cells	I	Rod II	Ring II	III	IV	VI	Mean chiasma frequency per cell
Manker × Bonza	331	23.87 (0–28)	1.40 (0–11)	0.45 (0–13)	0.09 (0– 2)	0.03 (0–1)	0.01 (0–1)	2.60
Manker × Pavon	115	24.57 (2–28)	1.28 (0– 5)	0.46 (0–10)	0.10 (0– 2)	0.003 (0–1)		2.47
Manker × Chinese Spring	59	23.85 (11–28)	1.36 (0– 5)	0.24 (0– 6)	0.25 (0– 2)	0.05 (0–3)		2.63

Results and discussion

The hybrid spike phenotypes of *H. vulgare* × *T. aestivum* cvs. Bonza and Pavon appear to be influenced by the phenotype of the *T. aestivum* parent (Figs. 1, 2). The barley combination with the cv. Chinese Spring also had a similar phenotypic expression. Each hybrid was somatically stable for the somatic cells analyzed, and possessed n=4x=28 HABD chromosomes.

Homoeologous chromosome association was observed in each hybrid combination yielding mean chiasma frequencies per cell of 2.6, 2.5 and 2.6 respectively (Table 1). Surprisingly chromosome configurations of up to 14_{II} were expressed as

$13_{II(rings)}+1_{II(rod)}$, or $12_{II(rings)}+2_{II(rods)}$, but in a low frequency. Among other relationships, unique separation of up to five chromosomes was observed. The meiotic configurations are presented in Figs. 3 and 4.

Fedak (1977) observed enhanced homeologous chromosome association for *H. vulgare* cv. Betzes × *T. aestivum* cv. Chinese Spring hybrids with a chiasma frequency of 1.82/cell that was not autosyndetic. This was based on presumed barley-wheat chromosome associations expressed by the presence of heteromorphic bivalents, and was interpreted as an influence of the barley genome on the *Ph* locus. Mujeeb *et al.* (1978b) did not observe this relationship in *H. vulgare* cv. Manker ×

Fig. 1. Spike morphology of *H. vulgare* cv. Manker (left), *H. vulgare* × *T. aestivum* (center), and *T. aestivum* cv. Bonza (right). Note the phenotypic dominance of cv. Bonza in the hybrid.

T. turgidum cv. Cocorit 71 and *H. vulgare* cv. Manker × *T. aestivum* cv. Tobari hybrids, where the possible role of germplasm may be a factor.

We do not consider the mean chiasma frequency of 2.57/cell (Table 1) to be enhanced homoeologous association, because the random association of the HABD genomes would allow for six combining possibilities and higher frequencies. Additionally, the heteromorphic bivalents observed by us in the barley × wheat hybrids have not been interpreted exclusively as a consequence of a barley-wheat chromosome association. This is based upon the chromosome size differences within the *T. aestivum* ABD genomes, which would allow the observation of heteromorphic bivalents should two unequal sized chromosomes pair as a rod bivalent.

Meiotic data for barley × wheat, or the reciprocal cross, have appeared consistently in the literature (Chapman and Miller 1978, Fedak 1977, 1980, Islam *et al.* 1975, 1978, 1981, Kimber and Sallee 1976, Martin and Chapman 1977, Mujeeb-Kazi and Rodriguez 1980, 1981, and Mujeeb *et al.* 1978b). This provides an impetus to the intergeneric hybridization programme, since apart from biological curiosities there appears to be, through chromosome pairing and recombination (Fedak 1977, Islam *et al.* 1975, Kimber and Sallee 1976, Mujeeb-Kazi and Rodriguez 1980), the agricultural practicality of obtaining genetic transfers.

Fig. 2. Spike morphology of *H. vulgare* cv. Manker (left), *H. vulgare* × *T. aestivum* (center), and *T. aestivum* cv. Pavon (right). Note the phenotypic dominance of cv. Pavon in the hybrid.

Backcrossing the self-sterile F_1 plants to the respective *T. aestivum* parents prodcued 20 BC_1 seeds from which 19 embryos were excised and cultured. The endosperm was normal in all cases and the seeds may have matured on the spikes. However, caution was exercised so as to rapidly exploit the material produced and have a sizeable BC_1 population. Gibberellic acid treatment assisted seed setting for this backcross combination (Mujeeb-Kazi and Rodriguez 1980).

In barley × wheat hybrids, inducing amphidiploidy has not been possible when *H. vulgare* was the barley species. Thus, for the advance of a barley × wheat programme for practical agricultural gain, the alternate route would be to produce BC_1 progeny directly by pollinating the self-sterile F_1 hybrid with *T. aestivum*. The

procedure may be facilitated if a n=4x=28, HABD egg cell would be formed to be fertilized with n=3x=21, ABD pollen from *T. aestivum*, to produce a heptaploid 2n=7x=49, HAABBDD BC_1 progeny. The BC_1 progeny plants obtained have

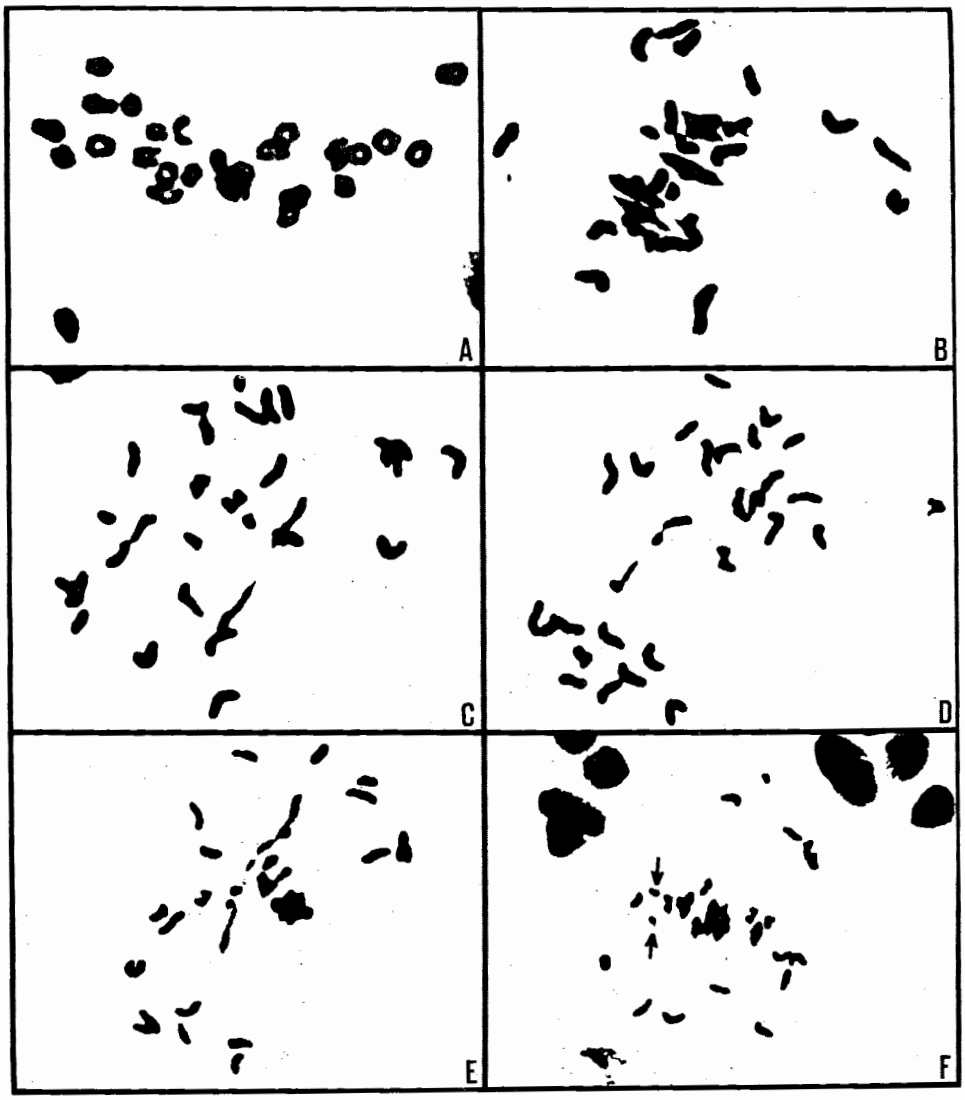

Fig. 3. Metaphase I chromosomes association in *Hordeum vulgare* × *Triticum aestivum* hybrids. A, 28_I chromatid separation. B, $4_{II(rings)}+20_I$. C, $1_{II(heteromorphic)}$. D, $1_{II(rod)}+26_I$. E, $2_{II(rings)}+2_{III}+18_I$. F, $4_{II(rings)}$. Five chromosomes express a unique separation. Arrowed mark shows complete separation of a such chromosome.

been somatically identified to range from 27 to 50 chromosomes. This variation seems a consequence of F_1 meiotic instability and meiotic non-reduction that allowed varied chromosome numbers to transgress to the egg cell. Some BC_1 plants did possess the heptaploid 2n=7x=49, HAABBDD composition with several meiocytes

expressing the $21_{II}+7_{I}$ chromosome association. The plants with 27 to 28 chromosomes had a mean meiotic relationship of $23.42_{I}+1.36_{II(rods)}+0.33_{II(rings)}+0.17_{III}+0.041_{IV}$ with a 2.60 chiasma frequency per cell. This was similar to the mean

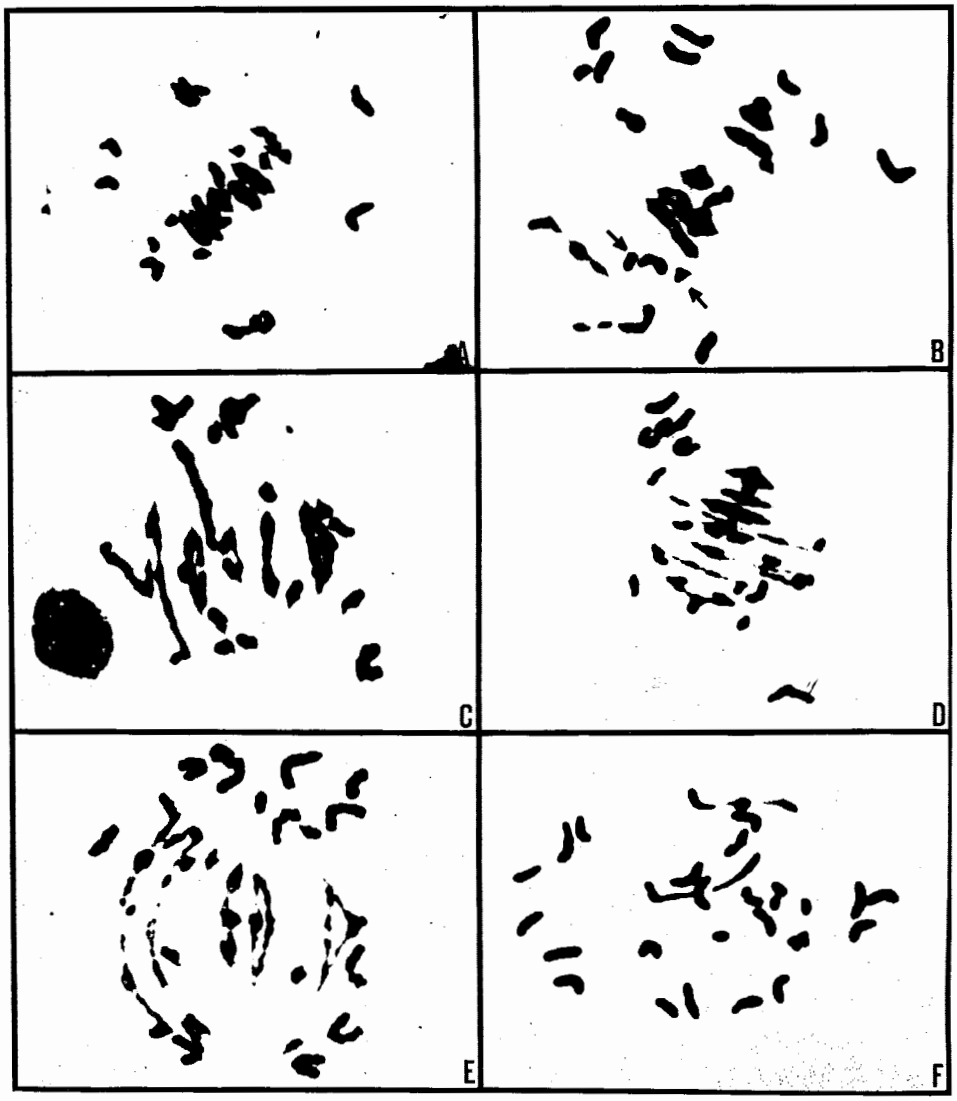

Fig. 4. Metaphase I chromosome association in *Hordeum vulgare* × *Triticum aestivum* hybrids. A, $5_{II(rings)}+1_{II(rod)}+16_{I}$, with unique separation. B, $6_{II(rings)}+1_{III}+13_{I}$, with unique separation of 1 chromosome arrowed. C, $3_{II(rings)}+2_{II(rods)}+1_{IV}+14_{I}$. D, $5_{II(rings)}+3_{II(rods)}+12_{I}$. E, $3_{II(rings)}+2_{II(rods)}+18_{I}$. F, $2_{II(rods)}$.

meiotic association observed in the F_1 hybrid (Table 1) and reported in detail by Mujeeb-Kazi (1981). These data may account for the recovery of cytologically normal euploids with the capacity of having subtle biochemical differences, as earlier observed by Kruse (1969) for *T. aestivum* euploids derived from *T. aestivum* ×

Avena sativa hybridization.

Islam *et al.* (1975) have obtained heptaploids similarly by backcrossing the

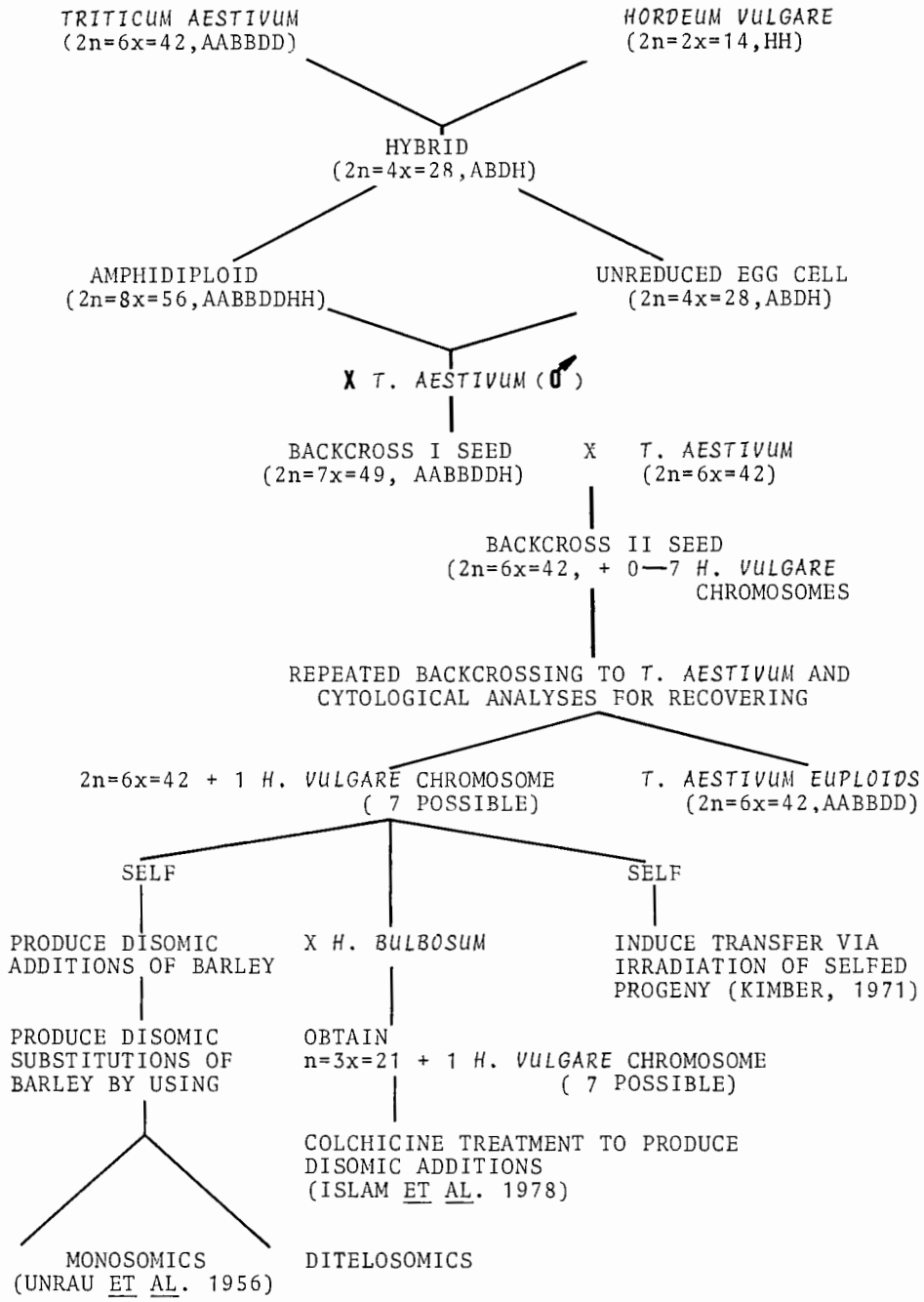

Fig. 5. Scheme of steps involved in synthesizing barley addition and substitution lines, obtaining genetic transfers, and for recovering *T. aestivum* euploids.

self-sterile F_1 hybrid with *T. aestivum*, for barley addition line production. They have since reported (1978) an efficient alternate approach for obtaining disomic addition lines utilizing the *T. aestivum* × *H. bulbosum* chromosome elimination technique (Barclay 1975). Chapman and Miller (1978) first developed the *H. chilense* × *T. aestivum* amphiploid. Then by reciprocal crosses of this amphiploid to *T. aestivum*, 49 chromosome plants (2n=7x=49, HAABBDD) were obtained for addition line production.

The exploitation of gene transfers between genera is the ultimate goal desired by plant breeders. Where intergenomic pairing and spontaneous transfers do not occur the only alternatives are to develop addition and substitution lines, or to irradiate the addition lines to induce alien transfers (Kimber 1971, Fig. 5). Based upon the meiotic relationships of the F_1 hybrid it is difficult to expect the *T. aestivum* euploids produced after backcrossing (Fig. 5) to carry much barley genetic information. If a genetic transfer occurred, this only can be seen if the barley genes show expressivity. We are now exploring the possibilities of producing new wheat × barley hybrids by using the *Ph* mutant as the maternal parent. The *Ph* mutant source is in *T. turgidum* cv. Capelli and *T. aestivum* cv. Chinese Spring.

Pistilloidy problems are commonly encountered when barley is the maternal parent in crosses with wheat (Islam *et al.* 1975). The pistilloidy problem encountered with BC_1 plants did not prevent BC_2 seed production when the BC_1 plants were further backcrossed by *T. aestivum*. Early pollinations and the GA_3 treatment aided in BC_2 seed-setting. Over 100 BC_2 seeds were obtained from the BC_1 plants pollinated by the respective *T. aestivum* cultivars. The BC_2 progeny is being cytologically analyzed, and further study was programmed to develop *T. aestivum* germplasm carrying barley genetic information. Since difficulties were encountered in obtaining self-fertile progeny from the BC_2 plants, emphasis was currently placed on the reciprocal combinations where the *T. aestivum* cultivars are other than Chinese Spring. A hybrid of *T. aestivum* cv. Tesia × *H. vulgare* has since been obtained with n=4x=28 HABD chromosomes. Pollinating this self-sterile F_1 by *T. aestivum* cultivars Veery "S" and Nacozari has produced BC_1 seed set. The schematic of Fig. 5 exemplifies the advance of this combination. These results shall soon be reported in detail.

Summary

Three *Hordeum vulgare* × *Triticum aestivum* hybrids are described. The phenotypic expression of the hybrids was similar to *T. aestivum*. The overall mean chromosome association in the hybrids was $24.3_{I}+1.33_{II(rods)}+0.38_{II(rings)}+0.15_{III}+0.03_{IV}+0.003_{VI}$ with a 2.57 mean chiasma frequency per cell. Early pollinations of the hybrids, before stigma receptivity was evident, with respective *T. aestivum* cultivars, followed eight and 32 hours later by gibberellic acid (75 ppm aqueous) treatment into the floret cups yielded first backcross (BC_1) seed set. The plants that resulted after embryo culture ranged in chromosome numbers from 27 to 50. Similar early pollinations of the BC_1 plants with *T. aestivum*, coupled with two post-pollination applications of gibberellic acid after eight and 32 hours produced over 100 BC_2 seeds. All BC_2 plants remained self-sterile.

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