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Production and identification of three 4Ag(4D) substitution lines of *Triticum aestivum – Agropyron*: relative transmission rate of alien chromosomes

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Summary. By crossing fertile nullisomics-4D of common wheat (nullisomics 72180 and 'Tianxuan' no. 15) with three octoploid *Agrotriticum* lines [partial amphiploids (2n=56) 78829 from the offsprings of wheat with *Agropyron intermedium* (=*Thinopyrum intermedium*, 2n=42) and 784 and 7631 from those of wheat with *Ag. elongatum* (*Thinopyrum elongatum*, 2n=70)], backcrossing the hybrid with 4D nullisomics as recurrent parent for one to two generations, and then selfing the BC individuals, we obtained three different alien substitution lines, 4Ei (4D), 4Ee(4D) blue grain, and 4Fe(4D) hairy leaf. The F_1 hybrids of 784 × 78829 had the basic chromosome pairing configuration of 27″ + 2′, indicating that 784 and 78829 differed by only one pair of chromosomes. This means that *Ag. intermedium* and *Ag. elongatum* have a genome (or genomes) in common. The formation of 22″ + 12′ in the F_1 of 784 × 7631 suggested that there was only one pair of homologous chromosomes of *Ag. elongatum* in 784 and 7631. Because F_1 plants of 7631 × 78829 generally formed 21″ + 14′ at metaphase I, the *Agropyron* genomes in the two octoploid *Agrotriticums* were presumed to differ from each other. Upon intercrossing the three alien substitution lines, we found that all of the pollen mother cells of the F_1 plants had 20″ + 2′ or more univalents at metaphase I. We concluded that the three *Agropyron* chromosomes were not homologous but belonged to the same homoeologous group. These results were confirmed by reciprocal crosses of the three octoploids. The relative transmission rates of 4Ee, 4Fe, 4Ei, 4R and 4D were calculated using blue grain and hairy leaf as genetic markers; these were 4D > 4Ee > 4Ei, 4Fe > 4R. The four alien chromosomes could compensate for the 4D of bread wheat, the substitution lines being vigorous and fertile.

Key words: Nullisomics – Octoploid *Agrotriticum* – Backcross – Alien substitution – Relative transmission rate

Introduction

In hybrids of wheat with its relatives, alien chromosomes are progressively eliminated in successive backcrosses to the wheat parent, followed by selfing. Thus, the hybrid derivatives generally have the full complement of wheat plus some alien segments. Several workers have attempted to transfer alien genes to wheat by addition, substitution and translocation (Johnson 1966, Wienhues 1974, Brar and Khush 1986). Sears (1972, 1981) modified the methods of O'Mara, Unrau and Jenkins (Unrau et al. 1956) and proposed a simple procedure for producing alien substitution lines. By crossing the 6B monotelosomic of 'Chinese Spring' with *Aegilops longissima* ($B^L B^L$, 2n=14), and then backcrossing the F_1 progeny with the monotelosomics, Kota and Dvorak (1985) obtained the $6B^L$ (6B) substitution. In China, Li Zhensheng et al. (1983, 1986) obtained stable and fertile 4D-nullisomics of wheat: they crossed one of the nullisomics, 'Tian Xuan' no. 15, with *Secale cereale* var 'Germany white grain' (RR, 2n=14), backcrossed the hybrids with the nullisomics and obtained the expected 4R(4D) substitution. Through crossing the nullisomics-4D with octoploid *Agrotriticum* lines and backcrossing the progeny with nullisomics, we produced three different wheat-wheatgrass substitution lines. This paper reports the cytogenetic identification and relative transmission rate of

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the chromosomes 4Ee, 4Fe, 4Ei, 4R, 4D in the genetic background of wheat.

Materials and method

The materials used in this study are: (1) 4D nullisomic wheat: nullisomic 72180, nullisomic 'Tianxuan' no. 15; (2) octoploid *Agrotriticum*: 784, 7631 (derived from cross and backcross progenies of wheat with *Ag. elongatum* (2n=70); 78829 from those of wheat with *Ag. intermedium*, 2n=42); (3) test-cross materials: 'Tianxuan' no. 15, 72180, 'Chinese Spring' (CS), 4R(4D) 'Tianxuan' no. 15 – 'Germany white grain' substitution.

The hybrids derived by crossing the nullisomics with the octoploids were backcrossed with the nullisomics as recurrent parent. Individuals with 2n=20″+1′ or 21″ in the BC1F1, BC1F2, and BC2F1 were selected and selfed, as well as test-crossed with CS or the original varieties of the nullisomics (Fig. 1). Meanwhile, the F_1 were also selfed, and individuals with 2n=41–43 chromosomes in the F_2 population were backcrossed with 4D-nullisomics. Individuals with 2n=41 and 42 were selected and again selfed as well as test-crossed (Fig. 2). The meiotic behavior of the test-crossed F_1 plants was studied to determine whether substitution had occurred in the test-crossed individuals (Figs. 1 and 2).

The three octoploids were hybridized with one another and meiosis in the F_1 hybrids was studied. Plump pollen grains and seed set on selfing were counted to identify whether the *Agropyron* genomes in the three octoploids were the same and if the three *Agropyron* chromosomes were the same in the three 4Ag(4D) substitutions. To ascertain the identity of the *Agropyron* chromosomes, the three stable alien substitutions were intercrossed and chromosome pairing in the F_1 progeny was analyzed. Meanwhile, the F_1 progeny was backcrossed to 'Chinese Spring' as female. Using the dominant traits blue grained and hairy leaf as genetic markers, the relative transmission rates of 4Ee, 4Fe and 4Ei were estimated in the backcrossed progeny. Similarly, crosses of 4Ee(4D) and 4Fe(4D) with CS and 4R(4D) were made, and progeny of these combinations was backcrossed with CS to determine the transmission rate of 4Ee and 4Fe to 4D and 4R.

For chromosome counts, excised root tips were pretreated at 0°–4°C for 24 h then fixed in 3:1 alcohol – acetic acid for 12–20 h and stored in the refrigerator until examination. Fixed root tips were hydrolyzed in 1 *N* HCl for 10 min at a constant temperature of about 60 °C, then stained with standard Haematoxylin staining procedure and squashed in 45% acetic acid solution. Young spikes at the appropriate developmental stage were fixed in 6:3:1 absolute alcohol – chloroform – acetic acid. Anthers were squashed in a modified carbol fuchsin solution or 1.5% acetocarmine.

Results

Production and primary identification of alien substitution

I. Fertility of the F_1 plants of nullisomics × octoploid *Agrotriticum*. It was relatively easy to cross nullisomics 72180 and 'Tianxuan' no. 15 with octoploid *Agrotriticum*, the average seed set being about 45%. The main chromo-

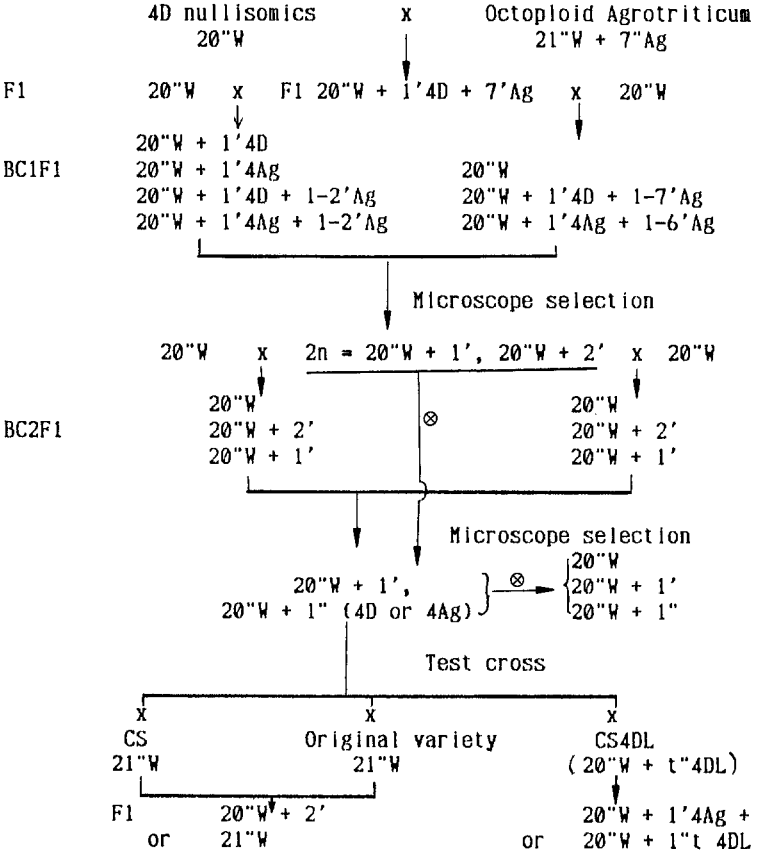

Fig. 1. Nullisomic backcrossing procedure 1 for producing alien substitutions

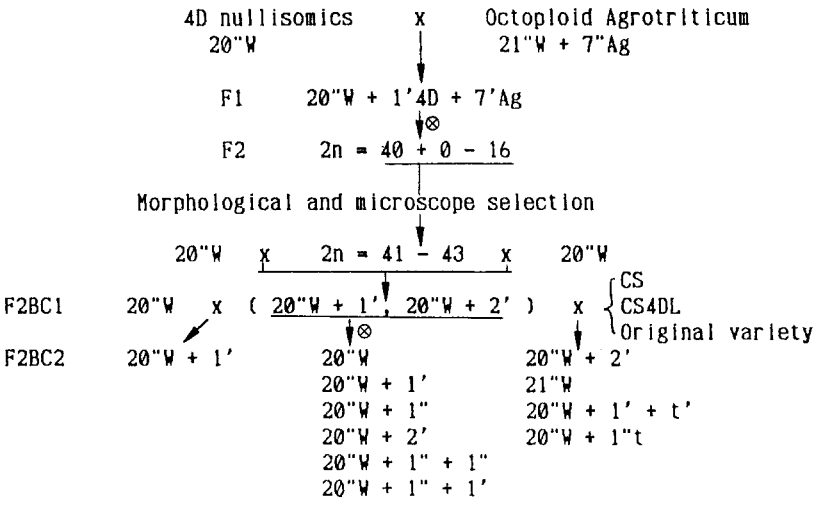

Fig. 2. Nullisomic backcrossing procedure 2 for producing alien substitutions

Table 1. The plump pollen percentage and seed set for selfing in F_1 hybrids and their parents

Materials	Number of accounted pollens	Plump pollen number	Plump pollen percentage (%)	Selfing seed frequency (%)
Nulli. 72180	784	727	92.7	90.0
Nulli. 'Tianxuan' no. 15	741	635	85.7	85.0
7631 (8x)	972	739	78.0	81.0
784 (8x)	1,302	1,048	80.5	61.2
78829 (8x)	1,231	807	65.6	80.6
(Nulli. 72180 × 78829) F_1	417	239	57.3	47.3
(Nulli. 72180 × 7631) F_1	663	325	49.0	13.8
(Nulli. 'Tianxuan' no. 15 × 7631) F_1	455	171	37.6	3.2
(Nulli. 'Tianxuan' no. 15 × 784) F_1	491	255	51.9	13.1

some pairing configuration of the F_1 generation was 20″+8′ (i.e., 20″W+1′4D+7′Ag). Pollen stainability with KI-I solution is given in Table 1. Clearly, there was no close relationship between the selfed seed set and plump pollen percentage of the F_1 individuals. This is because seed set is a function of both pollen fertility and female fertility. Some male and female fertility in the F_1 made it possible to backcross F_1 hybrids with nullisomics, the latter being the female recurrent parent. Also selected individuals were backcrossed with nullisomics in F_2 population (Fig. 2).

II. Production of alien substitutions. Monosomic alien substitutions MS4Ei(4D) were obtained from the cross nulli 72180 × 78829, regardless of the direction of the cross. When these were test-crossed with 72180, the chromosome pairing in the progeny was 20″+2′ in most of the PMCs at MI. By selfing MS4Ei(4D) individuals for two to three generations stable disomic alien substitution lines DS4Ei(4D) were developed (Table 2, Figs. 1 and 3). When F_1 hybrids of nulli 'Tianxuan' no. 15 × 784 were backcrossed with 4D-nullisomics as female recurrent parent, the BC1 progeny had light blue grains. Our continous backcrossing them with the nullisomics as male or female resulting in blue-grained monosomic substitution (MS) lines. After the blue-grained MS was selfed, blue-grained disomic substitution (DS) individuals were produced. When the MS were test-crossed (as male) with CS, all of the F_1 plants with 2n=42 had two or more univalents at MI (Table 2, Fig. 4). Because the blue-grain gene is located on 4E of *Ag. elongatum* (2n=70) (Li Zhensheng et al. 1983), the substitution lines produced in this program were probably 4Ee(4D).

When the F_1s of nulli 'Tianxuan' no. 15 × 7631 were backcrossed with the 4D nullisomics (female), a number of individuals with hairy leaves were observed in the $BC1F_2$ population; some regularly formed 21 bivalents at MI, and they were vigorous and fertile. Test-crossing them with CS proved that the hairy-leaf plants were alien substitution lines, and we tentatively designated them 4Fe(4D) (Table 2, Fig. 5). To determine differences between the three alian substitution lines, we crossed them with one another and proved that they could be discriminated.

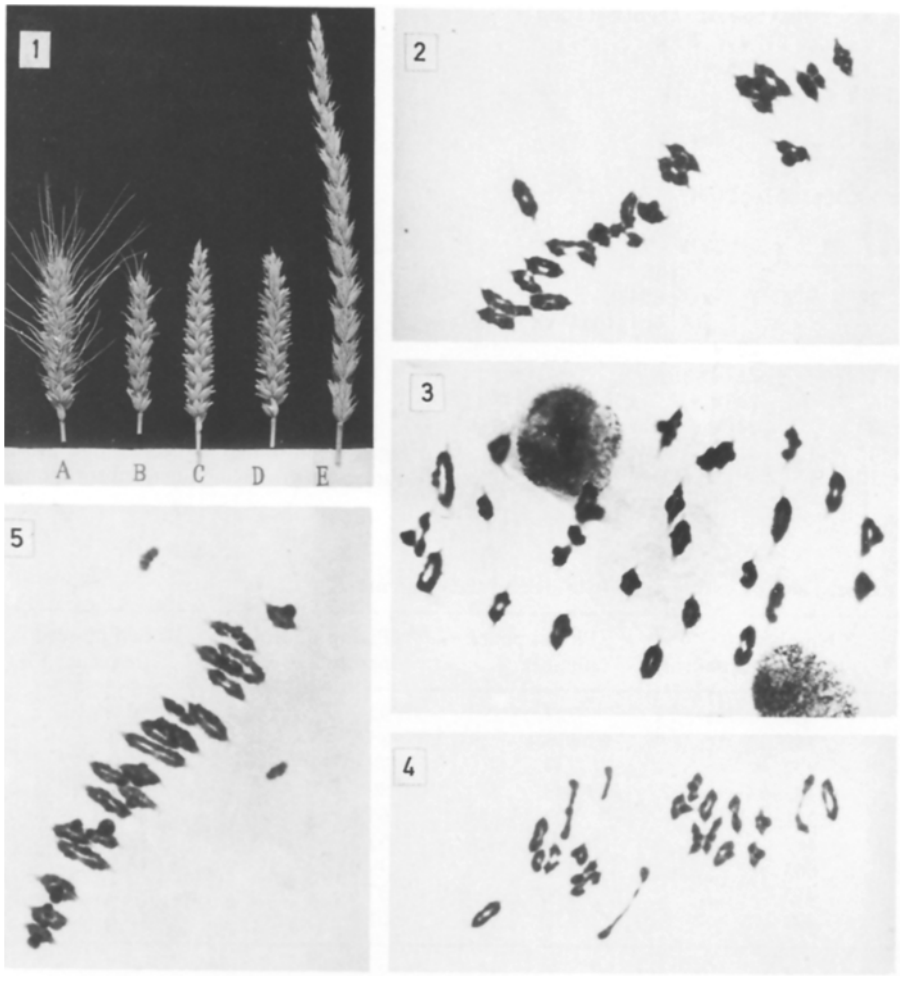

Fig. 3. Production and certification of the *T. aestivum–Ag. intermedium* 4Ei(4D) substitution. **1** *A* Common wheat 72180, *B* 4D nullisomic of 72180, *C* and *D* 4Ei(4D) substitution, *E* octoploid 78829. **2** 4D nullisomic of 72180, MI 20″. **3** 78829, MI 28″. **4** 4Ei(4D) substitution, MI 21″. **5** [72180 × MS4Ei(4D)] F_1, MI 20″ + 2′

Genome analysis of octoploid Agrotriticum

The F_1 individuals of (784 × 78829) had 24–28 bivalents at MI; 47% of the PMCs had 27″ + 2′. It appeared that there was one pair of non-homologous chromosomes between 784 and 78829, and it is probable that the chromosomes that remained unpaired in 4Ei(4D) and 4Ee(4D) are the two *Agropyron* chromosomes. It is also likely that the genome of 784 is almost the same as that of 78829 (Table 3). The most frequent chromosome pairing configuration was 22″ + 12′ (44.1%) in the F_1 hybrids of 784 × 7631, but that of the 7631 × 78829 F_1 was 21″ + 14′. Therefore, the genome of 7631 is different from that of 784 and 78829. The chromosome carrying the hairy-leaf gene is probably non-homologous with the 4Ag in 4Ee(4D)and 4Ei(4D). The seed set on selfing of the F_1 individuals gives an idea of the similarity and difference of the parental genomes (Table 4). For example, the F_1 average seed set of the 784 × 78829 F_1 was 74.4%, as against the parental average of 70.9%, but that of 784 × 7631 and 7631 × 78829 was 21.5% and 48.0%, respectively. This is probably because the genome of 784 is similar to that of 78829, but different from that of 7631.

4Ee, 4Fe and 4Ei as homoeologous chromosomes: their relative transmission rate (including 4D and 4R)

By crossing the three stable 4Ag(4D) substitution lines with each other, we found that all of the PMCs in the three combinations had two or more univalents at MI (Table 5). It appeared that the three *Agropyron* chromosomes belonged to the same homoeologous group, but were not homologous, the data being consistent with the reciprocal cross results of the three *Agrotriticums*. The substitution lines 4Ei(4D), 4Fe(4D), 4Ee(4D) and 4R(4D) were intercrossed and also hybridized with 'Chinese Spring', and the F_1s were test-crossed with CS as female. Data on grain color (blue versus white) and leaves (hairy versus non-hairy) in the BC1 and F_2 generations are summarized in Table 6.

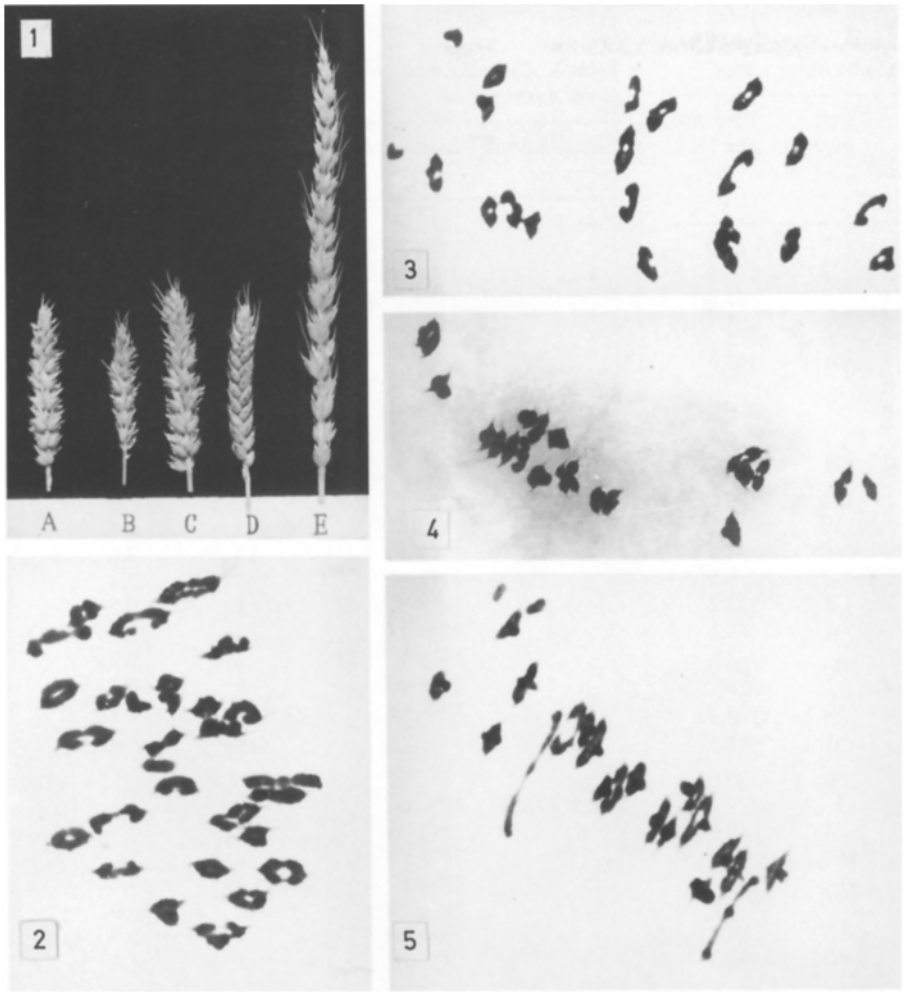

Fig. 4. Production and certification of *T. aestivum–Ag. elongatum* 4Ee(4D) blue-grained substitution. **1**: *A* and 'Tianxuan' no. 15, *B* 4D nullisomic of 'Tianxuan' no. 15, *C* and *D* 4Ee(4D) blue-grained substitution, *E* octoploid 784. **2** 784, MI 28″. **3** 4D nullisomic of 'Tianxuan' no. 15, MI 20″. **4** 4Ee(4D) blue-grained substitution, MI 21″. **5**. [CS × 4Ee(4D)] F_1, MI 20″ + 2′

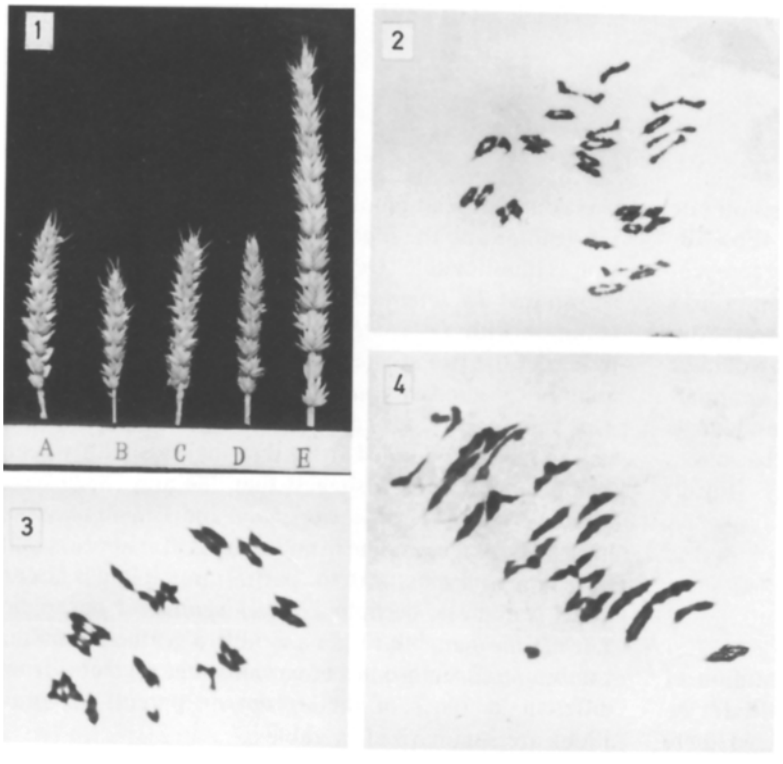

Fig. 5. Production and certification of *T. aestivum–Ag. elongatum* 4Fe(4D) hairy leaf substitution. **1**: *A* 'Tianxuan' no. 15, *B* 4D nullisomic of 'Tianxuan' no. 15, *C* and *D* 4Fe(4D) hairy leaf substitution, *E* octoploid 7631. *2* Octoploid, MI 28″. **3** 4Fe(4D) hairy leaf substitution, MI 21″. **4** [CS × 4Fe(4D)] F_1, MI 20″ + 2′

Table 2. Cytological verification of alien substitution lines

Alien substitution test crosses	Origin	Chromosome pairing behavior at MI	Cell number	Percentage (%)
MS4Ei(4D)	Nulli. 72180 × 78829 (8×)	19″+3′	12	11.9
		18″+1‴+2′	7	6.9
		18″+1⁗+1′	8	7.9
		20″+1′	74	73.3
			101	100.0
72180 × MS4Ei(4D)		19″+1‴+1′	1	0.8
		19″+4′	1	0.8
		20″+2′	121	98.4
			124	100.0
MS4Ee(4D)	Nulli. 'Tianxuan' no. 15 × 784 (8×)	18″+5′	1	2.2
		19″+3′	3	6.5
		18″+1‴+2′	1	2.2
		20″+1′	41	89.1
			46	100.0
CS × MS4Ee(4D)		19″+4′	5	8.3
		20″+2′	55	91.7
			60	100.0
DS4Fe(4D)	Nulli. 'Tianxuan' no. 15 × 7631 (8×)	19″+4′	4	2.6
		21″	153	97.4
			157	100.0
4Fe(4D) × CS		19″+4′	5	7.7
		20″+2′	60	92.3
			65	100.0

Table 3. Chromosome pairing behavior in F_1 among the octoploid *Agrotriticum*

Combinations	Pairing behavior	Cell number	Percentage (%)	
784 × 78829	28″	1	1.0	
	27″+2′	33	47.1	55.2%
	25″+1‴+2′	1	1.0	
	25″+1‴+3′	5	7.1	
	26″+4′	19	27.1	
	24″+1⁗+4′	1	1.0	
	24″+1‴+5′	1	1.0	
	25″+6′	8	11.4	
	24″+8′	1	1.0	
		70	100.0	
784 × 7631	24″+8′	1	0.7	
	23″+10′	13	9.0	
	22″+12′	63	43.4	
	20″+1⁗+12′	1	0.7	
	21″+14′	54	37.2	
	19″+1⁗+14′	1	0.7	
	20″+16′	6	4.1	
	19″+18′	3	2.1	
		145	100.0	
78829 × 7631	25″+6′	1	2.2	
	23″+10′	1	2.2	
	21″+1‴+11′	1	2.2	
	22″+12′	9	19.6	
	21″+14′	29	63.0	
	20″+16′	3	6.5	
	19″+18′	2	4.4	
		46	100.0	

The data indicated that the relative transmission rate of the five chromosomes was 4D > 4Ee > 4Ei, 4Fe > 4R. They also showed that 4Ee, 4Fe, 4Ei and 4R can compensate for 4D and be transmitted to the offspring in high frequency. Therefore, if the substituting chromosome donor species are closely related to *T. aestivum*, it would be more efficient to use nullisomics as female recurrent parent in procedure 1 (Fig. 1) and select vigorous individuals with chromosome number 41–43 in F_2 and backcross them for producing alien substitution lines (Fig. 2) (Zhang et al. 1989).

Discussion

There is no agreement on the genomic constitution of decaploid *Thinopyrum elongatum* and hexaploid *Th. intermedium*. Matsumura et al. (1958) believed that there was considerable homology between one *Th. intermedium* genome and the B genome of wheat, but this conclusion is questionable (Dewey 1984). Cauderon (1966) has shown that *Th. intermedium* has one or more genomes in common with *Th. elongatum*. Because of the polyploid nature of the two species it is difficult to cross them and analyze the similarity and differences of their genomes. This genome analysis could be attempted using the partial amphiploid derived from their hybrids with wheat. The results in Table 3 suggest that 784 and 78829 have similar genomes; i.e., *Th. elongatum* and *Th. intermedium* have at least one genome in common. It also appears that the *Agropyron* genome in the partial amphiploid is not an intact genome of decaploid *Th. elongatum* or hexaploid *Th. intermedium*, but rather a modified synthetic genome combining chromosomes or chromosome segments from different genomes of the *Agropyron* parents (Dvorak 1976).

Table 4. Plump pollen percentage and selfing seed setting frequency of octoploid hybrids

Combinations	Pollen number	Plump pollen number	Percentage (%)	Seed setting frequency (%)	Chromosome pairing basic configuration
78829 × 7631 F_1	1,377	881	64.0	48.0	21″+14′
7631 × 78829 F_1	993	557	56.1	48.3	21″+14′
784 × 78829 F_1	2,224	1,152	51.8	74.4	27″+2′
784 × 7631 F_1	1,269	446	35.1	21.5	22″+12′

Table 5. Chromosome pairing behavior in the combinations of the three 4Ag(4D) [including common wheat and 4R(4D)]

Combinations	Chromosome pairing behavior	Cell number	Percentage (%)
4Ee(4D) × 4Fe(4D)	18″+6′	2	4.0
	19″+4′	6	12.0
	20″+2′	41	82.0
	21″	1	2.0
4Ee(4D) × 4Ei(4D)	19″+4′	5	7.7
	20″+2′	60	92.3
4Fe(4D) × 4Ei(4D)	19″+4′	8	10.3
	20″+2′	70	89.7
4Ee(4D) × 4R(4D)	19″+4′	6	37.5
	20″+2′	10	62.5
4Fe(4D) × 4R(4D)	19″+4′	5	8.1
	20″+2′	50	90.9

Th. elongatum (2n = 70) and *Th. intermedium* (2n = 42) have several desirable characters needed for wheat improvement, such as rust disease resistance, drought tolerance, salt tolerance and resistance to barley yellow dwaf virus (Xin et al. 1988). Therefore, they are the perennial Triticeae species of greatest interest to wheat breeders. Moreover, they are crossable with wheat. In this study, we have found that some *Agropyron* chromosomes can be transmitted to the progeny at a high rate in the genetic background of wheat, making it possible to transfer useful alien genes into wheat through conventional hybridization. We hope this research will provide helpful information to wheat breeders in their attempts to transfer beneficial characters of the two perennial species to wheat.

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Table 6. The relative transmission rate of 4D, 4Ei, 4Ee, 4Fe and 4R

Combinations	F_1 dominant character	Separation of BC1 (CS as female)		Separation of F_2		Chromosome pairing major configuration
4Ee(4D) × CS	Blue grain	Blue	47, 49.5%	Blue	416, 44.2%	20″+2′
		White	48, 50.5%	White	526, 55.8%	
4Fe(4D) × CS	Hairy leaves			Hair	44, 54,8%	20″+2′
				Non-hair	37 45.2%	
4Ee(4D) × 4Fe(4D)	Blue grain	Blue	60, 61.7%	Blue	344, 60.2%	20″+2′
	Hairy leaves	Hair	38, 38,3%	White	227, 39.8%	
4Ee(4D) × 4Ei(4D)	Blue grain			Blue	509, 69.4%	20″+2′
				White	225, 30.6%	
4Fe(4D) × 4Ei(4D)	Hairy leaves	Hair	30, 46.9%			20″+2′
		Non-hair	34, 53.1%			
4Ee(4D) × 4R(4D)	Blue grain	Blue	50, 62.5%	Blue	319, 64.3%	20″+2′
		White	30, 37.5%	White	177, 35.7%	
4Fe(4D) × 4R(4D)	Hairy leaves			Hair	20, 60.6%	20″+2′
				Non-hair	11, 39.4%	

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