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Production and cytogenetic analysis of BC_1, BC_2, and BC_3 progenies of an intergeneric hybrid between *Triticum aestivum* (L.) Thell. and tetraploid *Agropyron cristatum* (L.) Gaertn.

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Summary. Intergeneric hybrids between *Triticum aestivum* cv 'Chinese Spring' and *Agropyron cristatum* 4*x* ($2n = 5x = 35$, ABDPP genomes) with a high level of homoeologous meiotic pairing between the wheat chromosomes were backcrossed 3 times to wheat. Pollination of the F_1 hybrid with 'Chinese Spring' resulted in 22 BC_1 seeds with an average seed set of 1.52%. Five BC_1 plants with 39–41 chromosomes were raised using embryo rescue techniques. Chromosome pairing in the BC_1 was characterized by a high frequency of multivalent associations, but in spite of this there was no evidence of homoeologous pairing between chromosomes of wheat and those of *Agropyron*. All of the plants were self sterile. The embryo rescue technique was again essential to produce 39 BC_2 plants with chromosome numbers ranging from 37 to 67. The phenomenon of meiotic non-reduction was also observed in the BC_3 progenies. In this generation male and female fertility greatly increased, and meiotic pairing was fairly regular. Some monosomic ($2n = 43$) and double monosomic ($2n = 44$) lines were produced. Analysis of these progenies should permit the extraction of the seven possible wheat-*Agropyron* disomic addition lines including those with the added chromosomes carrying the genes involved in meiotic non-reduction and in suppression of *Ph* activity.

Key words: *Triticum aestivum* – *Agropyron* – Intergeneric hybrids – Backcross derivatives – Chromosome pairing

Introduction

Interspecific and intergeneric hybridization in the tribe Triticeae is an important way of exploiting the genetic variability of the related wild species for wheat improvement and for the investigation of species' relationships (Sharma and Gill 1983; Sears 1983; Dewey 1984; Wang 1989). *Agropyron* Gaertn. is a perennial genus of the Triticeae consisting of 10–13 diploid ($2n = 14$), tetraploid ($2n = 28$) and hexaploid ($2n = 42$) species with tetraploids accounting for about 90% of the natural populations. All have the same basic P genome (Love 1984; Dewey 1984). In addition to being economically important forages, *Agropyron* species have been found to possess potentially valuable traits for wheat improvement, including tolerance to drought (Dewey 1984; Asay and Johnson 1990) and low temperature (Limin and Fowler 1987) and resistance to diseases such as barley yellow dwarf virus (BYDV) (Sharma et al. 1984) and *Septoria nodorum* (M. Trottet, personal communication). These traits could be useful for wheat improvement if they could be transferred.

Intergeneric hybrids of *Triticum aestivum* and some species of *Agropyron* Gaertn. have recently been produced (Chen et al. 1989, 1990; Limin and Fowler 1990; Li and Dong 1990, 1991; Ahmad and Comeau 1991). In most of these hybrids, a considerably high level of autosyndetic chromosome pairing at metaphase I (MI) has been observed, and backcrossing the hybrids to wheat has been found to be very difficult (Chen et al. 1989, 1991; Ahmad and Comeau 1991). A rare exception was observed in the study of Li and Dong (1991) in which the F_1 hybrid between *T. aestivum* cv 'Chinese Spring' × *A. michnoi* was partially self-fertile and BC_1 seeds were easily produced from the F_1 hybrid. However, the authors published very little morphological and cytogenetical data, and there is no further data on the later generations of these hybrids.

The paper presented here reports on the production and subsequent cytogenetical study of backcross deriva-

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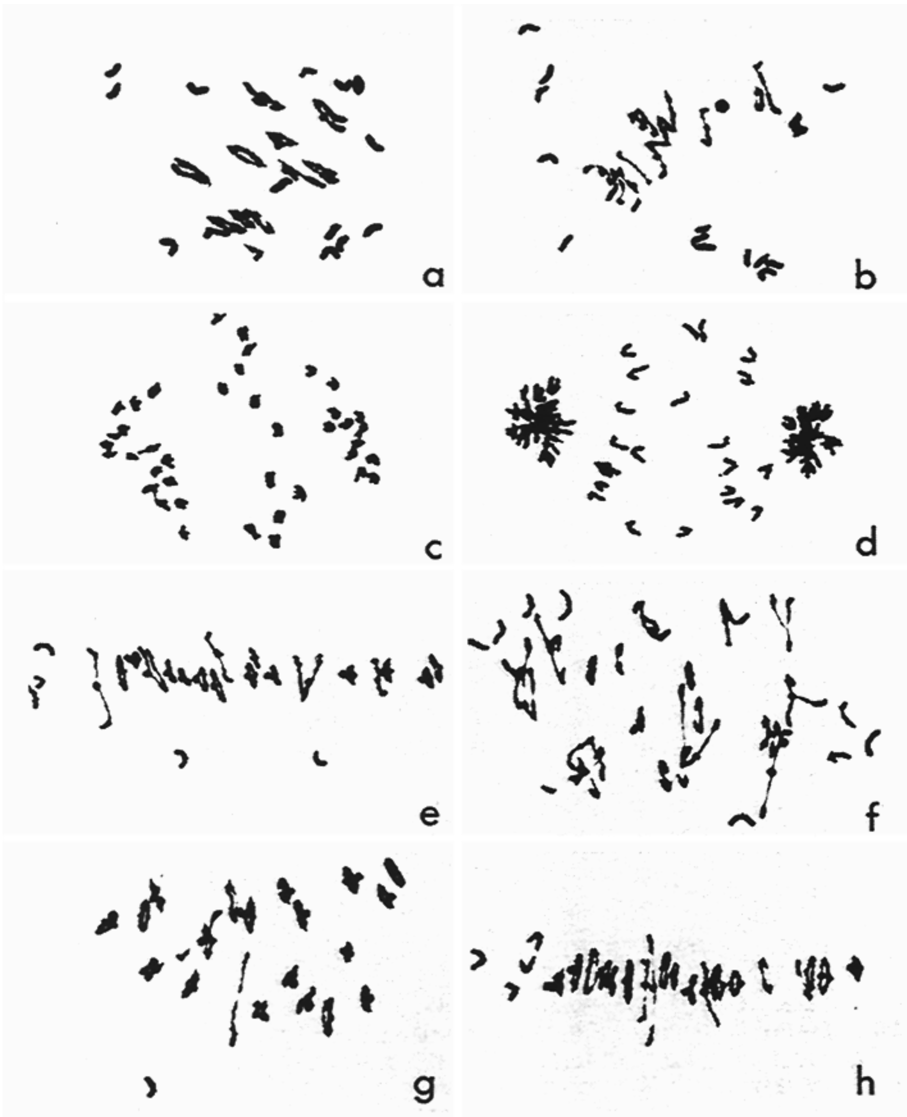

Fig. 1. a–d Meiosis in a BC_1 plant with $2n=40$. **a** MI showing 13I+6II+5III. **b** MI showing 14I+5II+2III+1IV+1VI. **c** AI showing a 12-12-16 segregation. **d** AI showing a precocious segregation of lagging chromatids. **e–h** Meiotic metaphase I in BC_2 and BC_3 plants. **e** BC_2 with $2n=43$, 5I+16II+2III. **f** BC_2 with $2n=61$, 9I+17II+6III. **g** BC_3 with $2n=43$, 1I+21II. **h** BC_3 with $2n=44$, 2I+21II

tives obtained from a F_1 hybrid of *T. aestivum* cv 'Chinese Spring' × *A. cristatum* 4*x*. The objective of this study was to produce a series of wheat-*Agropyron* disomic addition lines. As the *Agropyron* parent is resistant to important wheat diseases, including BYDV, it is our intention to use the resistant addition line(s) to introduce the resistance gene(s) into wheat.

Materials and methods

Intergeneric hybrids between *Triticum aestivum* cv 'Chinese Spring' (CS) ($2n=6x=42$, AABBDD) and *Agropyron cristatum* (accession 54, a tetraploid ecotype from Inner Mongolia, China, $2n=4x=28$, PPPP) had been produced and described by the authors prior to this investigation (Chen et al. 1989). Plant regeneration from immature influorescence callus cultures of these hybrids was carried out, and the original hybrid and some of the clones were then backcrossed using 'Chinese Spring' as the recurrent male parent. Embryos were dissected 15–20 days after pollination and subsequently cultured on an artificial medium to obtain, successively, BC_1, BC_2 and some BC_3 plants by means of the technique described by Chen et al. (1989). Most of the BC_3 derivatives and all of their selfed progenies were raised from mature seeds.

Observations were made on the morphology and fertility of each of the backcross derivatives. Chromosome counts in root-tip cells were made using a standard Feulgen technique. For meiotic studies, anthers at metaphase I were collected, fixed in a 3:1 ethanol-acetic acid solution, squashed in 1% acetocarmine and counterstained with carbol fuchsin.

Results

BC_1 generation

Pollination of 1511 florets of the F_1 hybrid plants by CS produced 23 grains, i.e. an average seed set of 1.52%. Only 5 BC_1 plants were obtained from the cultured embryos. The BC_1 plants were annual, like the recurrent

Table 1. Chromosome associations in four BC_1 and some of the BC_2 derivatives of cv 'Chinese Spring' × *A. cristatum* 4*x* hybrid (ranges in parentheses)

	2*n*	Number of cells observed	I	II Total	II Ring	III	IV	V	VI	Selfed seed set (%)
BC_1 plants										
A	41	51	11.04 (7–16)	9.55 (7–13)	4.63 (2–10)	3.12 (1–6)	0.29 (0–1)	0.02 (0–1)	0.04 (0–1)	0
B	40	50	12.01 (8–17)	7.40 (4–11)	4.88 (2–8)	3.76 (1–7)	0.20 (0–1)	0.15 (0–2)	0.06 (0–1)	0
D	40	50	11.40 (7–16)	8.48 (4–12)	5.64 (2–9)	3.54 (0–7)	0.18 (0–1)	0.06 (0–1)		0
E	39	50	13.52 (11–24)	9.14 (6–13)	7.24 (4–10)	2.12 (0–4)	0.10 (0–1)	0.04 (0–1)	0.04 (0–1)	0
BC_2 plants										
D-1	37	30	8.36 (6–12)	11.73 (8–14)	7.80 (4–10)	1.50 (0–4)	0.17 (0–1)			0
A-2	39	20	5.65 (4–9)	13.45 (11–17)	9.90 (8–14)	1.95 (0–3)	0.15 (0–1)			0
A-61	40	30	5.10 (4–10)	15.53 (13–17)	13.87 (11–16)	1.24 (0–2)	0.03 (0.1)			22.6
A-4	41	20	8.45 (5–13)	13.85 (11–18)	8.45 (5–11)	1.25 (0–4)	0.15 (0–1)	0.10 (0–1)		0
A-8	42	30	4.82 (3–6)	14.13 (10–16)	9.67 (7–14)	2.26 (1–4)	0.43 (0–2)	0.00 (0–0)	0.07 (0–1)	6.9
A-23	42	20	6.30 (5–10)	15.45 (13–17)	13.30 (10–16)	1.60 (0–3)				3.9
A-11	43	20	8.60 (5–13)	14.60 (12–17)	8.55 (6–11)	1.60 (0–3)	0.10 (0–1)			0
A-62	44	30	6.86 (4–10)	18.36 (16–20)	14.16 (10–17)	0.13 (0–2)				29.6
A-9	44	20	7.25 (4–12)	16.65 (13–19)	11.60 (3–16)	1.15 (0–2)				1.7
D-3	44	30	14.63 (11–20)	12.03 (8–14)	5.83 (4–9)	1.33 (0–5)	0.33 (0–1)			0
A-16	45	30	6.02 (3–9)	19.20 (16–21)	14.80 (12–18)	0.10 (0–2)	0.07 (0–1)			13.9
A-15	46	20	11.16 (6–16)	11.91 (10–16)	8.91 (6–11)	2.54 (0–4)	0.85 (0–2)			0
A-18	56	30	16.00 (9–24)	15.00 (9–18)	6.20 (3–12)	3.20 (2–6)	0.10 (0–1)			0
D-2	61	45	11.73 (7–19)	15.69 (8–20)	10.11 (6–15)	5.67 (0–9)	0.22 (0–2)			11.8

wheat parent, and morphologically intermediate between *Agropyron* and wheat although closer to wheat in several characteristics, such as plant height and spike length. The somatic chromosome counts of the BC_1 plants revealed 1 plant with $2n=39$, 3 with $2n=40$ and 1 with $2n=41$. The chromosome pairing behaviour observed in 4 of the BC_1 plants was similar, as shown in Table 1. All exhibited a high frequency of univalents and multivalents. The mean number of univalents ranged from 11.04 to 13.52, and the minimum number of univalents in a PMC was 7. More than 95% of the PMCs had trivalents (Fig. 1 a). Quadrivalents, quinquevalents and even hexavalents were also observed (Fig. 1 b). The high frequencies of univalents and multivalents resulted in only 37.0–46.6% of the chromosomes forming bivalents. At first anaphase (AI), 6–12 lagging chromosomes were observed in most of the cells of the 4 BC_1 plants (Fig. 1 c). Lagging chromatids often segregated precociously (Fig. 1 d). Other meiotic irregulaties also occurred in about 20% of the PMCs. For example, the failure of the first meiotic division resulted in chromosomes remaining at or near the equator and subsequently undergoing the second meiotic division. In some PMCs at metaphase I, all of the chromosomes had clearly visible chromatids like those at mitotic metaphase. At AI multipolar spindles and bridges were also observed.

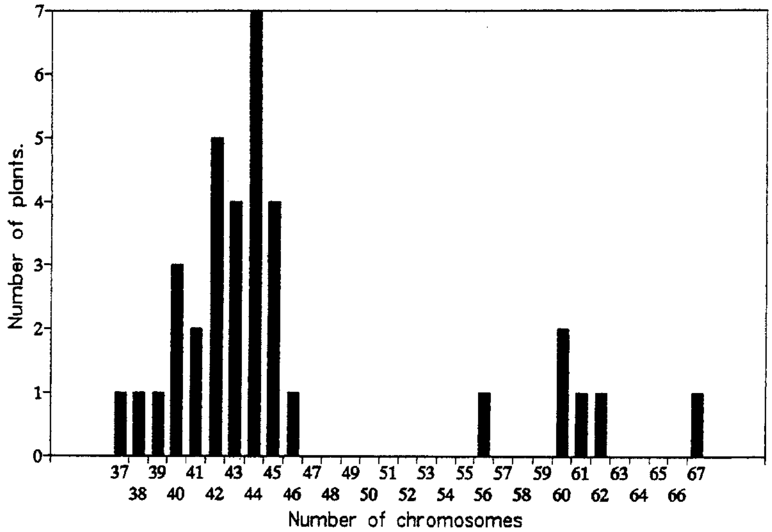

Fig. 2. Distribution of BC_2 plants according to their chromosome number

Table 2. Chromosome associations in some of the BC_3 derivatives of cv 'Chinese Spring' × *A. cristatum 4x* hybrid (ranges in parentheses)

BC_3 plants	$2n$	Number of cells observed	I	II Total	II Ring	III	IV	V	VI	Selfed seed set (%)	Number of *Agropyron* chromosomes[a]
D-1-1	42	30	2.13 (1–4)	19.27 (18–20)	16.67 (13–19)	0.27 (0–1)	0.13 (0–1)			63.2	1
A-61-4	42	20	2.40 (2–4)	19.65 (17–20)	18.25 (16–19)	0.10 (0–2)				70.6	1
A-8-5	43	20	1.35 (1–2)	19.25 (17–21)	17.55 (15–19)	0.45 (0–2)	0.45 (0–1)			90.1	1
A-4-2	43	30	3.63 (2–5)	18.05 (16–20)	16.52 (14–18)	0.33 (0–1)	0.40 (0–1)	0.10 (0–1)	0.03 (0–1)	29.2	2
A-9-19	43	20	3.6 (3–6)	15.2 (13–17)	12.00 (8–14)	1.80 (1–2)	0.90 (0–2)			6.1	2
A-4-5	44	20	4.25 (3–5)	17.85 (15–20)	14.55 (12–17)	0.40 (0–2)	0.50 (0–2)	0.05 (0–1)	0.10 (0–1)	0	3
A-8-1	44	20	2.10 (2–4)	18.95 (18–19)	17.65 (15–18)	0.00 (0–0)	1.00 (1–1)			68.1	2
A-23-1	44	21	3.09 (3–4)	19.09 (19–20)	18.09 (16–19)	0.91 (0–1)				9.4	2
A-23-12	44	20	3.40 (2–4)	20.30 (20–21)	18.95 (18–20)					34.8	2

[a] Possible *Agropyron* chromosome numbers

BC_2 generation

Backcrossing the 5 BC_1 plants gave 60 BC_2 seeds and 50 embryos. The seed set ranged from 1.3 to 10.5% (average 8.84%). The 39 BC_2 plants were obtained from 3 BC_1 plants – A: $2n=41$; D: $2n=40$; E: $2n=39$. BC_2 plants, particularly with respect to their spikes, were morphologically similar to wheat, but they varied greatly. Most of them were sterile, but some had a low seed set (1.7–29.6%) (Table 1). The chromosome numbers of 35 BC_2 plants ranged from 37 to 67 (Fig. 2), and these could be classified into two groups: 29 plants with 37–46 chromosomes and 6 plants with 56–67 chromosomes (Fig. 2).

Meiotic analyses were made on 21 BC_2 plants; 14 of these are reported in Table 1. Analyses on 4 plants having chromosome numbers similar to that of the BC_1 plants indicated fewer univalents and multivalents but a higher number of bivalents. The 8 BC_2 plants with $2n=42–46$ also had fewer univalents and more bivalents than their BC_1 parents (Fig. 1a, e). The BC_2 plants with $2n=56$ and 61 chromosomes had many univalents and multivanents (Fig. 1f).

BC_3 generation

Backcrossing BC_2 plants presumed to contain added *Agropyron* chromosomes yielded 116 BC_3 plants with a seed set ranging from 0% to 44%. The morphology of the BC_3 plants was very similar to that of 'Chinese Spring', and they were more vigorous than the BC_2 plants. In this generation, male and female fertility greatly increased and seed set by self pollination was higher than 60% in several plants (Table 2).

The numbers of somatic chromosomes were variable and depended upon the chromosome number of the parents. The backcross derivatives from BC_2 plants with $2n=37-44$ produced mainly $2n=40-45$, but there were also some plants with $2n=61$ and 64 chromosomes. The chromosome pairing configurations of some BC_3 plants were analysed, and meiotic behaviour was found to be significantly more regular than in the BC_2 generation. Some plants with 43 and 44 chromosomes resembled CS in appearance and showed good fertility (Table 2). By taking into account the minimum number of univalents observed in at least one cell, we were able to evaluate the number of P chromosomes present in any BC_3 derivative (Table 2). Of the 15 BC_3 plants analysed, 13 possessed 1–3 *Agropyron* chromosomes. It is interesting to note that some plants with $2n=43$ and $2n=44$ chromosomes which possessed 1 and 2 *Agropyron* chromosomes had 21 II + 1 I and 21 II + 2 I configurations in some PMCs; e.g. plants A-8-5 and A-23-12 (Fig. 1 g, h).

Discussion

Meiotic studies of F_1 intergeneric hybrids between common wheat × *Agropyron* species have revealed a high level of autosyndetic homoeologous pairing between the wheat chromosomes at MI as a result of the P genome of *Agropyron* suppressing the activity of the normal pairing system of wheat (Chen et al. 1989, 1990). Obtaining backcross derivatives from the hybrids has been very difficult (Chen et al. 1990; Chen 1991, Ahmad and Comeau 1991) and may be problematic, as discussed by Mujeeb-Kazi et al. (1987) and Sharma and Gill (1986). For example, Ahmad and Comeau (1991) obtained no backcross progenies after the pollination of 300 spikes of a wheat × *Agropyron fragile* ($2n=35$, ABDPP) hybrid. In the present study, despite homoeologous pairing between wheat chromosomes, BC_1 seeds were obtained at a rate of 1.52% which is similar to that of the F_1 hybrid (1.91%) (Chen et al. 1989). This relatively successful backcrossing rate could perhaps be explained by the use of *in vitro* immature embryo culture and by the fact that a part of the pairing observed in the F_1 hybrids consisted of homologous associations between the two P genomes of *Agropyron*.

BC_1 progenies having 39–41 chromosomes were probably produced from the fertilization of a reduced gamete of the F_1 hybrid by a normal 21-chromosome wheat gamete. Each gamete produced by the F_1 hybrid likely contained 7 *Agropyron* chromosomes. Consequently, the genomic constitution of the BC_1 plant with 41 chromosomes should be 7Ag + 34 W (Ag: *Agropyron* chromosomes, W: wheat chromosomes). Therefore, in the BC_1 plant chromosome pairing should theoretically be 13 II + 15 I. However, the meiotic study of 4 BC_1 plants with $2n=39-41$ chromosomes showed a higher frequency of multivalent associations than expected. The high percentage of multivalent associations could be due to the presence of 7 *Agropyron* P chromosomes inhibiting the effect of the *Ph1* gene and/or the occurrence of homeologous recombination or translocations in the F_1 hybrids that are likely to be of the wheat-wheat type (Chen et al. 1989, 1990).

The minimum number of univalents observed in BC_1 plants was 7, which should correspond to one P genome. If this is true, allosyndetic association or genetic recombination between wheat and *Agropyron* chromosomes never or, only rarely occurred in the BC_1 generation. Consequently, direct gene introgression by homoeologous recombination in early generations should be very difficult. Thus, gene transfer between wheat and *Agropyron* requires the production of addition lines and chromosome manipulation techniques as proposed by Sears (1981).

Non-reduced egg gametes occurred in the BC_1 and BC_2 generations at a relatively high frequency. Detailed analyses of subsequent generations should make it possible to demonstrate if this phenomenon is due to a pair(s) of *Agropyron* chromosomes or the entire P genome. If it is due to only one pair of chromosomes, this trait could be used as a chromosome marker. Islam and Shepherd (1980) demonstrated that a similar phenomenon in wheat × barley hybrids was controlled by the 5H chromosome of barley. Harlan and DeWet (1975) suggested that in most cases the production of restitution nuclei was due to failure of the first meiotic division. Our observations in the BC_1 and BC_2 generations seem to support their conclusion.

It is worthwhile noting that in the BC_3 generation some plants with 43 and 44 chromosomes were found having 21 II + 1 I and 21 II + 2 I chromosome associations, respectively. These were presumably monosomic and double monosomic addition lines. After self-pollination or/and backcrosses to common wheat, new monosomic or stable disomic addition lines can be produced. This would open the possibility of selecting a novel series – the seven possible wheat-*Agropyron* P genome addition lines.

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