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Hybrids and backcross progenies between wheat (*Triticum aestivum* L.) and apomictic Australian wheatgrass [*Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor]: karyotypic and genomic analyses

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Abstract Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) florets were emasculated and pollinated using two apomictic wheatgrass [*Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor, 2n = 6x = 42, SSYYWW] accessions, one of which produces 2n pollen. A 2n = 42 (B_{II}) hybrid and four 2n = 63 (B_{III}) hybrids were obtained. The spike morphology of the B_{II} hybrid was intermediate to that of its parents. The pollen mother cells (PMCs) of this hybrid contained on average 38.36 I and 1.62 II, which was consistent with its disparate genome composition (ABDSYW). Its pollen failed to stain and no BC_1 progeny was obtained. The B_{III} hybrids (reduced egg fertilized with unreduced sperm) were grasslike and had a full complement of *E. rectisetus* chromosomes, the synapsis of which was slightly impaired by wheat haplome and/or cytoplasm. Their PMCs contained on average 16.30 II, 25.72 I, and 1.54 multivalents (III plus IV). Pollen stainability in these hybrids was low (< 1%), and when they were used as females, one 54- and 60-chromosome BC_1 were obtained. A mean of 13.25 II was observed in PMCs of the 54-chromosome BC_1 and pollen stainability was 10%. Pollen stainability in the 60-chromosome BC_1 was only 5%. The use of 2n-pollen-producing *E. rectisetus* accession accelerated hybrid and BC_1 formation and may accelerate the ultimate transfer of apomixis to wheat.

Key words Apomixis · Asynapsis · B_{III} hybrids · Genome analysis · Wheat · Wide hybridization

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Introduction

The genus *Elymus* L. contains perennial grasses and is the largest and most widely distributed genus in the Triticeae (Dewey 1984). Through wide hybridization, this genus could enhance wheat (*Triticum aestivum* L.) with genes for disease resistance, stress tolerance (Dong et al. 1992), and apomixis (Crane and Carman 1987). Many appropriate intergeneric hybrids have already been obtained (Wang 1989; Lu and Bothmer 1992).

Elymus rectisetus (Nees in Lehm.) A. Löve & Connor is the only known apomict in the Triticeae (Dewey 1984). It is hexaploid (2n = 6x = 42) and morphologically resembles the sexual species *E. scabrus* (R. Br.) A. Löve. Both of these *Elymus* species are endemic to Australia and New Zealand and both share three genomes (Carman and Wang 1992), S, Y, and W (Torabinejad and Mueller 1993b). Near-obligate diplospory in *E. rectisetus* was first reported by Hair (1956) and was subsequently studied in detail by Crane and Carman (1987) and Carman et al. (1991). Sexual *E. scabrus* (Ahmad and Comeau 1991; Torabinejad and Mueller 1993a) and apomictic *E. rectisetus* (Wang et al. 1993) have been hybridized with wheat. In the present report the production and cytogenetic behavior of wheat by *E. rectisetus* hybrids and their BC_1 progenies are described in detail.

Materials and methods

Australian *E. rectisetus* from southeast Queensland (PI 533026), east central New South Wales (PI 533028), southeast New South Wales (PI 533090), the Flinders Range of South Australia (PI 533233), and central Tasmania (PI 533175) were used as male parents in wide hybridization experiments and were maintained in a greenhouse. Megasporogenesis in cleared ovules was examined by Normarski interference contrast microscopy (Crane and Carman 1987) to confirm apomixis in the pollen donors. The spring wheats 'Fukuhokomugi', 'Chinese Spring', and 'Anza' were used as female and recurrent parents.

Wheat spikes were emasculated 2 days before anthesis. A pretreatment of 75 ppm gibberellic acid (GA_3) plus 25 ppm 2,4-dichlorophenoxyacetic acid (2, 4-D) (Riera-Lizarazu et al. 1992) was applied after emasculation, and a post-treatment of 75 ppm GA_3 was applied 1 day after pollination. Hybrid seeds were harvested 2 weeks after pollination, surface sterilized for 10 min in 0.53% sodium hypochlorite and rinsed three times with sterile water. Embryos were aseptically excised and cultured on orchid agar medium (Difco) for germination. Hybrid progenies were multiplied and maintained by immature inflorescence culture (Wang et al. 1991).

Spikes of the hybrids and the following closely related forms of hexaploid Australian *Elymus* were fixed in Carnoy's fixative and stored in 70% ethanol at 4 °C: (1) sexual *E. scabrus* from southest Queensland (PI 533217 and 533221), (2) sexual *E. scabrus* ssp. *plurinervis* from northeast New South Wales (PI 533201) and from east central New South Wales (PI 533204), and (3) apomictic *E. rectisetus* (PI 533026 and 533028). The anthers were squashed in acetocarmine for studying meiosis in the pollen mother cells (PMCs). The aceto-orcein squash method (Mujeeb-Kazi and Miranda 1985) was used for mitotic preparations, and karyotypes of the hybrids were constructed from well-spread root-tip cells. The computer program Chrompac III (Green et al. 1984) was used to identify probable pairs of homologous mitotic chromosomes in the species and hybrids and to assign these pairs to their respective parents, i.e., wheat or *E. rectisetus*.

Results

Apomixis in the pollen donors

Megasporogenesis in *E. rectisetus* accessions used as pollen parents (PI 533026, 533028, and 533233) was analyzed to determine the mode of reproduction. The formation of megaspores in these accessions occurred primarily by apomeiosis (Fig. 1) in a manner typical of that observed in other facultative diplosporous *E. rectisetus* accessions (Crane and Carman 1987). Sexual development occurred facultatively at low frequencies (Table 1).

Production and fertility of F_1s and BC_1s

Wheat pollinated by *E. rectisetus* pollen failed to set seed when hormone treatments to the pistils were withheld. A

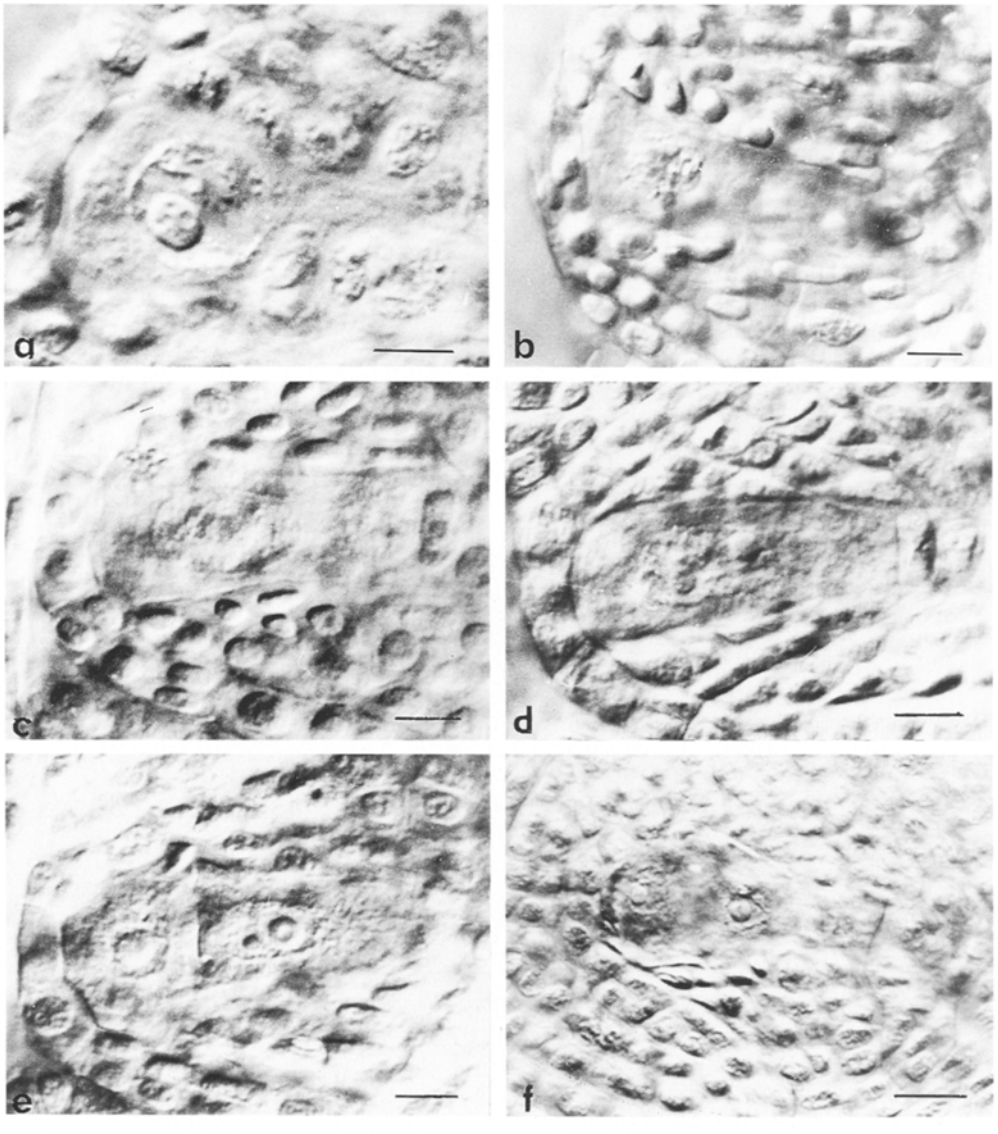

Fig. 1a–f Stages of apomictic megasporogenesis observed in *Elymus rectisetus* accession PI 533028. **a** Young megaspore mother cell (MMC), **b** vacuolate elongated MMC, **c, d** vacuolate elongated MMC with elongated nuclei, **e** ameiotic dyad, **f** young binucleate embryo sac. *Bars*: 10 μm

Table 1 Classification of female *E. rectisetus* meiocytes as to mode of reproduction. Cleared ovules were classified using interference contrast microscopy. Young megaspore mother cells (MMCs) and dyads were not classified as to mode of reproduction. Elongated MMCs were considered to be apomictic, and tetrads to be sexual

E. rectisetus accession	n	Young MMCs	Elongated MMCs	Dyads	Tetrads
PI 533026	69	15	34	17	3
PI 533028	74	15	37	20	2
PI 533233	31	7	14	8	2

few seeds were set when GA_3 and 2, 4-D were used as a pretreatment, and these contained a well-developed embryo floating in a watery endosperm. However, even with hormone treatments, most seeds were empty and shrivelled. Some of the rescued embryos produced only roots or shoots or became albino. Only five healthy F_1 hybrids were obtained. Four of these were 63-chromosome B_{III} hybrids (reduced egg fertilized with unreduced sperm) and were obtained in 1990 with the accession PI 533028 as the male parent. One of these, L9004, was made with 'Fukuhokomugi' and the remaining three, L9018, L9019, and L9020, were made with 'Chinese Spring'. Pollen stainability was less than 1% in these hybrids. The fifth hybrid, L9266, was a 42-chromosome B_{II} hybrid and was obtained in 1992 using 'Fukuhokomugi' and accession PI 533026 as the male parent. Pollen from this hybrid did not stain.

In 1991, a BC_1, L9105, with 54 chromosomes was obtained from the cross between L9004 and 'Fukuhokomugi'; pollen stainability in this BC_1 was 10%. Another BC_1, L9244, had 60 chromosomes and was obtained by pollinating L9018 with pollen from 'Anza'; pollen stainability in this BC_1 was 5%. No spontaneous seed set occurred in either of the BC_1s. The hybridization frequency for F_1s and BC_1s was less than 1% on a spike basis. The F_1s were vigorous short-lived perennials, while the BC_1s were more annual-like and could only be maintained for about a year. The F_1s and the BC_1s were multiplied and are being maintained by embryogenic tissue culture using immature inflorescences for callus induction. Seven morphologically variable BC_2 were produced from BC_1 L9015. Average seed set from the selfed BC_2 was 1.94 per spike and ranged from 0.40 to 4.25 per spike.

Our B_{III} hybrids involved the 2*n* pollen producer PI 533028 and were seen to resemble *E. rectisetus* more than wheat (Fig. 2). The B_{II} hybrid L9266 was intermediate to its parents (Fig. 3), and the BC_1 plants (54 and 60 chromosomes, respectively) obtained from the B_{III} hybrids resembled wheat more than *E. rectisetus* (Fig. 4).

Fig. 2 Spikes (*left to right*): 'Fukuhokomugi' wheat (♀ parent), B_{III} hybrid (2n = 63), and *Elymus rectisetus* accession PI 533028 (♂ parent)

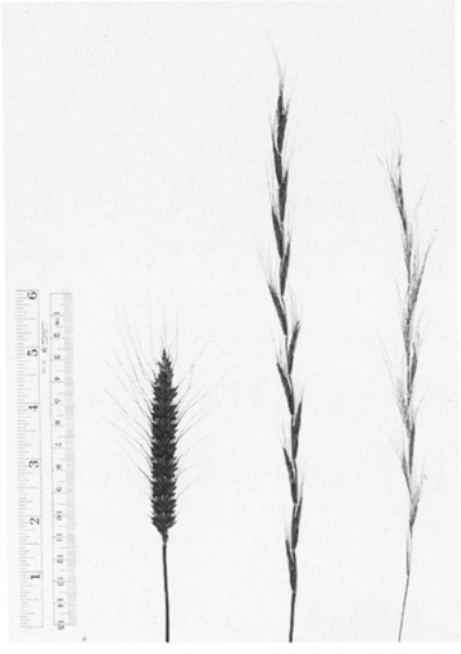

Fig. 3 Spikes (*left to right*): 'Fukuhokomugi' wheat (♀ parent), B_{II} hybrid (2n = 42), and *Elymus rectisetus* accession PI 533026 (♂ parent)

Fig. 4 Spike from 2n = 60 BC_1 ('Chinese Spring' wheat/*Elymus rectisetus*// 'Anza' wheat).

the standard wheat karyotype (Gill et al. 1991). The remaining 21 chromosomes (paired for the B_{III} hybrid, Fig. 5c, and single for the B_{II} hybrid, Fig. 5d) best matched those of *E. rectisetus*. Wheat chromosomes (Fig. 5a) were generally longer than those of *E. rectisetus* (Fig. 5b), although the lengths of a few chromosomes were similar.

Karyotypes of *E. scabrus* (PI 533221) and *E. rectisetus* (PI 533206) and the interspecific hybrid between these accessions were similar. Each had two pairs of large-satellited chromosomes, as predicted for *E. rectisetus*. Both of the satellited chromosomes of wheat, 1B and 6B, were visible in the cells of the B_{II} hybrid, the B_{III} hybrid, and the 54-chromosome BC_1. As expected, different numbers of satellited wheat chromosomes, e.g., two pairs in the BC_1 and two singles in the hybrids, were observed.

Karyotypes of the F_1s and their parents

The hexaploid Australian wheatgrasses, *E. rectisetus*, *E. scabrus*, and *E. scabrus* ssp. plurinervis, share three basic genomes (SSYYWW). Like wheat (Fig. 5a), each has two pairs of satellited chromosomes, as shown for *E. rectisetus* (Fig. 5b). In Fig. 5c and d chromosomes of the B_{III} and B_{II} hybrids, respectively, were arranged by parental genome donor and by length with the aid of the computer program Chrompac III (Green et al. 1984), which predicts homology based on an analysis of chromosome lengths, arm ratios, and presence or absence of satellites. The first 21 chromosomes are those that best matched

Meiotic pairing in *E. rectisetus*, the F_1s and the BC_1s

Meiotic data of *E. rectisetus*, the F_1 hybrids and the BC_1s are presented in Table 2 along with relevant data from other published works. The *E. rectisetus* accessions PI 533026 and PI 533233 are 1n pollen producers.

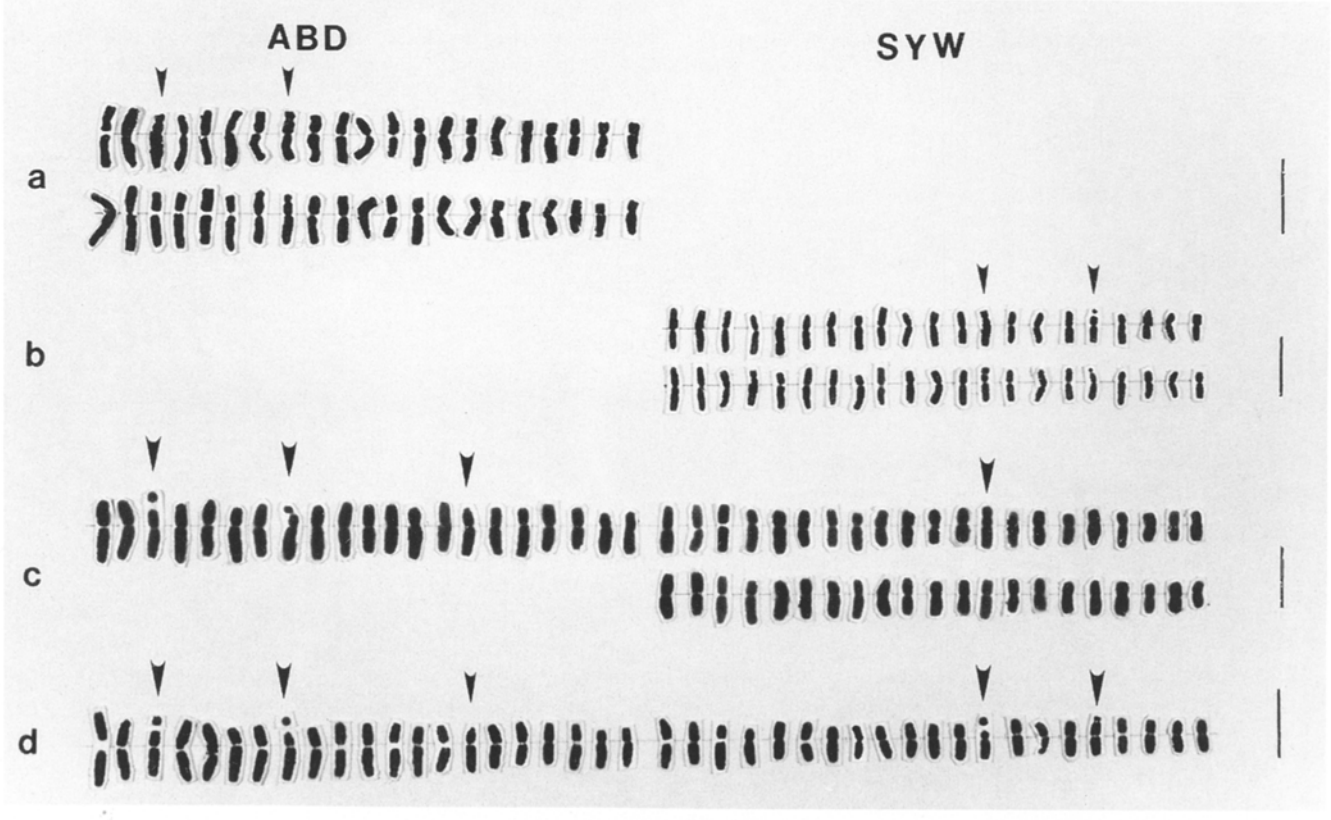

Fig. 5a–d Karyotypes. **a** 'Fukuhokomugi' wheat, **b** *Elymus rectisetus* accession PI 533028, **c** 2n = 63 B_{III} hybrid ('Fukuhokomugi' wheat/*E. rectisetus* accession PI 533028), **d** 2n = 42 B_{II} hybrid ('Fukuhokomugi' wheat/*E. rectisetus* accession PI 533026). *Arrowheads* denote satellited chromosomes. See text for details. *Bars*: 10 μm

Table 2 Mean MI chromosome associations of the species *E. rectisetus*, wheat × *E. rectisetus* F_1 hybrids, and a BC_1 plant

Species or hybrid	Plant code	2n	Number of cells	I	II			III	IV
					Rings	Rods	Total		
E. rectisetus	PI 533233	42	50	0.68 (0–4)	17.45 (13–21)	3.21 (0–8)	20.66		
E. rectisetus	PI 533026	42	50	0.56 (0–4)	17.88 (14–21)	2.84 (0–7)	20.72		
B_{III} hybrid[a]	L9004	63	36	27.68 (18–33)	3.76 (1–6)	11.86 (9–14)	15.62	1.28 (0–3)	0.06 (0–1)
B_{III} hybrid[b]	L9019	63	45	24.15 (19–29)	4.38 (1–7)	12.46 (9–17)	16.84	1.63 (0–3)	0.07 (0–1)
B_{II} hybrid[c]	L9266	42	80	38.36 (31–42)	0.10 (0–1)	1.52 (0–5)	1.62	0.08 (0–1)	0.04 (0–1)
BC_1[d]	L9105	54	35	27.25 (22–32)	9.39 (7–11)	3.87 (1–6)	13.26	0.05 (0–1)	0.02 (0–1)
B_{II} hybrid[e]	TSCAB	42	445	32.84 (26–40)	0.21 (0–2)	4.06 (0–8)	4.27	0.18 (0–2)	0.02 (0–1)
E. scabrus[f]	2887–8	42	82		20.35 (18–21)	0.65 (0–3)	21.00		
Haploid wheat[g]		21	155	19.10	0.89 (0–3)	0.04 (0–1)	0.93	0.02	
SYW hybrid[h]	119	21	100	20.10 (15–21)	0.45 (0–3)	0.45	0.45		

[a] 'Fukuhokomugi' × PI 533028, [b] 'Chinese Spring' × PI 533028, [c] 'Fukuhokomugi' × PI 533026, [d] L9004 × 'Fukuhokomugi', [e] 'Fukuhokomugi' × *E. scabrus* (Ahmad and Comeau 1991), [f] *E. scabus* (Torabinejad et al. 1987), [g] polyhaploid 'Fukuhokomugi' wheat (Ahmad and Comeau 1991), [h] *E. yezoensis* × *A. pectinatum* ssp. *pecinatum* (Torabinejad and Mueller 1993b)

Chromosome associations in these accessions were nearly normal with 21 bivalents at MI (Fig. 6a). Hybrids of *E. scabrus* (PI 533221) and *E. rectisetus* (PI 533026) formed from 16 to 21 bivalents with 0–10 univalents at MI. The *E. rectisetus* accession PI 533028 is a 2n pollen producer and was generally asynaptic.

Meiosis in the wheat-by-*E. rectisetus* F_1 hybrids generally involved two divisions with a near-normal chromosome synapsis. Pairing patterns in the two B_{III} hybrids analyzed were similar to each other (Table 2, Fig. 6b). The mean number of univalents in these hybrids (25.72 I, weighted mean across both F_1s) exceeded 21, which would only have resulted had the 21 wheat chromosomes behaved as univalents. Likewise, the mean number of bivalents was lower (16.30 II, weighted mean) than 21, which would only have resulted had the 21 pairs of *E. rectisetus* chromosomes behaved as bivalents. Trivalents and quadrivalents (1.54 III plus IV) do not account for this discrepancy. Meiotic associations in the B_{II} hybrid averaged 38.36 I + 1.62 II + 0.08 III + 0.04 IV. Most of these cells had from 1 to 5 bivalents that were mostly rod-shaped (Figs. 6c and d). Forty-two univalents (Fig. 6e) were observed in a few PMCs. The BC_1 plant, L9105, had a mean meiotic pairing of 27.25 I + 13.25 II + 0.05 III (Table 2, Fig. 6f).

Discussion

In previous hybridization attempts, we emasculated and pollinated over 10,000 pistils but failed to obtain wheat × *E. rectisetus* hybrids (Torabinejad et al. 1987). We attribute our recent success to the use of: (1) *E. rectisetus* accessions with better pollen viability, (2) hybridization in controlled environment greenhouses, and (3) improved procedures for pretreating emasculated florets with plant hormones 2,4-D and GA_3. Similar hormone pretreatments have been shown to increase wheat × barley hybrid formation (Riera-Lizarazu et al. 1992). The 2n-pollen-producing *E. rectisetus* accession PI 533028 was well suited to be a male parent in our experiments because it is vigorous and long-lived under greenhouse conditions, has a long flowering period, and has larger anthers with more abundant viable pollen than most other *E. rectisetus* accessions.

Chromosome doubling may be required prior to advancing wheat × *E. rectisetus* B_{II} hybrids to the BC_1 stage. All efforts to obtain BC_1s with the undoubled B_{II} hybrid failed. This, coupled with a complete absence of pollen stainability, is evidence of very high sterility in this B_{II} hybrid. Ahmad and Comeau (1991) also failed to obtain BC_1s after pollinating a wheat × *E. scabrus* B_{II} hybrid with wheat pollen. High sterility in these B_{II} hybrids is probably due to the divergent nature of the six genomes, S, Y, W, A, B, and D. Use of the 2n-pollen-producing *E. rectisetus* accession PI 533028 resulted in B_{III} hybrids with a full complement of *E. rectisetus* chromosomes. This conferred a small degree of pollen and egg fertility as evidence by the 1% pollen stainability and the production of two BC_1s (2n = 54 and 60), which formed presumably from reduced but genetically imbalanced eggs.

Synapsis among homologous chromosomes of the asynaptic *E. rectisetus* accession PI 533028 was largely restored in our B_{III} hybrids (Table 2). Thus, asynapsis in PI 533028 may be recessive, with the deficiency being compensated for in B_{III} hybrids by gene product(s) of the wheat nucleus and/or cytoplasm. Synapsis in the B_{III} hybrids indicates that the recombinational vacuum in this accession, which is introduced by apomixis and enforced by 2n-pollen formation, has not as yet permitted severe structural divergence of homologous chromosomes. Nevertheless, an average of only 16 bivalents was observed in these hybrids (Table 2), and the number of bivalents in only a few cells surpassed or even approached 21. Thus, homologous pairings of *E. rectisetus* chromosomes were slightly impaired by the wheat nucleus and/or cytoplasm. Additionally, product(s) of the wheat synapsis gene(s) may not completely compensate

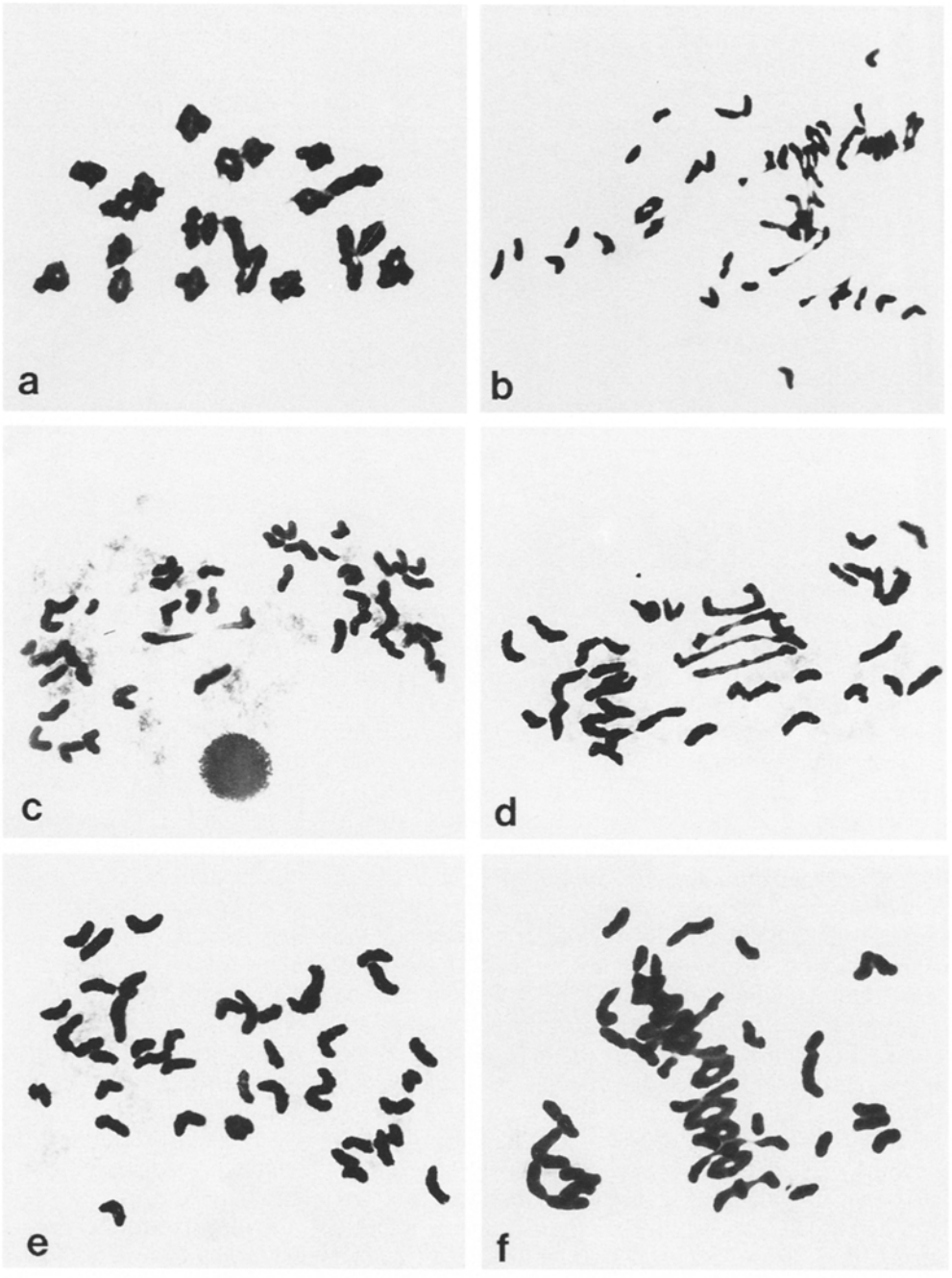

Fig. 6a–f MI preparations of PMCs from *Elymus rectisetus* and its hybrids with wheat. **a** 21 II in *E. rectisetus* accession PI 533026 (× 430), **b** B_{III} hybrid ('Fukuhokomugi' wheat/*E. rectisetus* accession PI 533028 (× 460), **c** B_{II}) hybrid ('Fukuhokomugi' wheat /*E. rectisetus* accession PI 533026) with 40 I + 1 II (× 425), **d** the same B_{II} hybrid with 36 I + 4 II (× 520), **e** the same B_{II} hybrid with 42 I (× 475), **f** 26 I + 14 II in the 54-chromosome BC_1 ('Fukuhokomugi'/*E. rectisetus*//'Fukuhokomugi' (× 450).

for the asynapsis tendency of the male parent. The latter hypothesis is supported by infrequent observations in our B_{III} hybrids of diplosporic megasporogenesis and chromosome restitution during microsporogenesis, both of which are typical events in PI 533028.

In the present study, fewer than 1% of the emasculated wheat spikes produced hybrids when pollinated with *E. rectisetus*. Nevertheless, these difficult-to-obtain hybrids may permit the transfer of apomixis to wheat, especially, if homoeologous chromosomes pair. Evidence of such pairing was observed in our B_{II} and B_{III} hybrids. The B_{II} hybrid L9266 had 1.62 bivalents per PMC at MI. While this is fewer than the 4.27 observed in wheat × *E. scabrus* (Table 2, Ahmad and Comeau 1991), it is more than the hypothetical sum (1.38) obtained by adding 0.93 from the 'Fukuhokomugi' wheat polyhaploid (ABD) and 0.45 from the SYW-genome hybrids of *E. yezoensis* Honda × *Australopyrum pectinatum* (Labillardiere) A Löve ssp. *pectinatum* (Table 2). It is also higher, with one exception, than the bivalent frequencies of the ABDSY-genome hybrids (Lu and Bothmer 1991).

Most PMCs in our B_{III} hybrids had one to three trivalents. Many of these were frying-pan trivalents with short chromosomes (probably from the S, Y, or W genome) in the ring and a longer chromosome (possibly from the A, B, or D genome) in the handle. The presence

of such bivalents and multivalents, observed in both B_{II} and B_{III} hybrids (Table 2), suggest that chromatin exchange occasionally occurs between wheat and *E. rectisetus* chromosomes.

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