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Production, morphology, and cytogenetics of *Triticum aestivum* (L.) Thell × *Elymus scabrus* (R. Br.) Love intergeneric hybrids obtained by in ovulo embryo culture *

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Summary. Intergeneric hybrids were produced between common wheat, *Triticum aestivum* (2n = 6x = 42, AABBDD), and an apomictic Triticeae species, *Elymus scabrus* (syn. *Agropyron scabrum*) (2n = 6x = 42, HHSSSS), the first successful report of this cross. Nine tiny, underdeveloped, and structureless embryos were obtained in vitro only by in ovulo embryo culture at 4 days after pollination, which gave rise to five mature hybrid plants. All the hybrid plants were vigorous and possessed a phenotype intermediate to the two parents. There were 2n = 6x = 42 (ABDHSS) somatic chromosomes in the hybrids. There was little or no homology between the parental genomes, as shown by an overall meiotic chromosome association of 32.83 I + 4.08 rod II + 0.21 ring II + 0.18 III + 0.02 IV. The hybrids were completely sterile and so far backcrosses to wheat parent have not been successful. Alternate approaches to induce gene transfer(s) from *E. scabrus* to wheat are being attempted.

Key words: Apomixis – Chromosome pairing – *Elymus* – Wheat – Wide hybridization

Introduction

Recently, increased emphasis has been placed on interspecific/intergeneric hybrids in plant breeding programs worldwide (Harlan 1976; Fatih 1983). Apart from their practical utilization in crop improvement, these hybrids offer cytological, evolutionary, or phylogenetic interpretation regarding the parental species involved in the hybridization (Mujeeb-Kazi and Kimber 1985). Additionally, they also show promise of becoming new crop species, through induced amphiploidy, in areas characterized by highly complex growth conditions (Mujeeb-Kazi et al. 1987).

Elymus is the largest genus in the Triticeae and consists of approximately 150 species (Dewey 1984). *Elymus* species occur naturally in Europe, Asia, North America, South America, and New Zealand-Australia. The genus is believed to encompass genomes "H" derived from *Critesion*, "S" derived from *Pseuderoegneria*, and "Y" of an unknown origin (Dewey 1984). The genomic organization is poorly understood, however, and this is especially true for the New Zealand-Australian species of *Elymus*.

Intergeneric hybrids between *Triticum aestivum* and genomically authentic *Elymus* species have been rather limited, despite the apparent genetic potential of disease resistance and/or stress tolerance that abounds in these alien species. A few intergeneric hybrids between wheat and *Elymus* species have been produced and cytogenetically characterized. These include *E. ciliaris*, *E. trachycaulus*, *E. yezoensis* (Sharma and Gill 1983); *E. canadensis* (Mujeeb-Kazi and Bernard 1985; Yen and Liu 1987); *E. caninus* (Sharma and Baenziger 1986); *E. caespitosus* (Mujeeb-Kazi et al. 1987); *E. cylindricus* and *E. dahuricus* (Yen and Liu 1987). Presumably, hybrids between wheat with *E. fibrosus* have also been produced, although cytogenetic data have not yet been reported (Mujeeb-Kazi and Bernard 1982). With the exception of hexaploid species *E. cylindricus* and *E. dahuricus* (unknown genome, possibly SSHH??), all the above-mentioned species are tetraploids (genomes SSHH or SSYY).

Elymus scabrus (R. Br.) Love is an especially important species, since it belongs to a small group of related

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species that contain apomictic genotypes, apomixis being very rare within the Triticeae (Dewey 1984). Several Triticeae cytogeneticists and wheat breeders around the world have tried to cross this species with *Triticum aestivum*, but no success has yet been reported (Dewey 1984).

We have been able to produce this interesting novel hybrid between wheat and *E. scabrus*. In this report we present aspects of crossability, phenotypic detail, cytogenetics, and genome composition of this new genetic combination.

Materials and methods

Four accessions of *E. scabrus* (2n=6x=42; D2911, D2883, J6C101, and J6C1059), kindly supplied by Dr. C. Crane (Utah State University, Logan, USA), and one *T. aestivum* accession cv "Fukuho" (2n=6x=42) were used in hybridization experiments. In all the crosses *T. aestivum* was used as the female parent. Seedlings of *E. scabrus* were vernalized for 6 weeks at 4 °C.

Crosses were made in the greenhouse by placing either mature, ready-to-dehisce anthers or fresh pollen collected on petri plates onto individual wheat pistils. Crossed pistils were sprayed with 75 mg/l gibberellic acid (GA_3) 1 and 2 days after pollination. The rest of the procedure, from in ovulo embryo culture to plant development, was the same as used by Ahmad and Comeau (1990). Root tips, for somatic chromosome counts, were prepared according to the method of Mujeeb-Kazi and Miranda (1985), with the exception that acetoorcein was replaced with acetocarmine. Spikes of the hybrid plants, as well as the parental species, at the appropriate stage of development, were fixed in Carnoy's fixative (6 parts 95% ethanol:3 parts chloroform:1 part glacial acetic acid, v/v). Anthers were squashed in acetocarmine for meiotic preparation. All photographs were taken on Tech Pan 2415 black and white film.

Results

Plant production and morphology

No seed set was observed when wheat was pollinated with *E. scabrus* pollen and the pistils were left on the mother plant. Therefore, a representative sample of crossed pistils from the four cross combinations (Table 1) was scored for prefertilization crossability barrier(s), using the fluorescence microscopy technique according to Ahmad and Comeau (1990). This preliminary experiment showed that *E. scabrus* pollen germinated on wheat stigma, albeit at very low frequency, and that pollen tubes occasionally penetrated the ovule. Apparently, a strong postfertilization crossability barrier was operating at a very early developmental stage of the hybrid embryos. Consequently, we decided to attempt in vitro embryo rescue using in ovulo embryo culture, and only these data are presented here.

Results obtained for the hybrid production between wheat and *E. scabrus* are presented in Table 1. A total of 1,799 wheat florets was pollinated, and 1,739 potentially fertilized ovules were cultured in vitro from the four cross combinations (Table 1). These resulted in nine (0.52% of ovules cultured), small, underdeveloped, and structureless embryos, all of which originated from the pollen parent, J6C1059, which seems significantly more crossable according to binomial confidence limits (Steel and Torrie 1960). Five embryos germinated directly to give five plantlets (0.29% of ovules cultured, or 55.56% of embryos cultured), whereas one embryo showed a tendency towards callus formation. The latter embryo was then induced to callus more profusely, and was later used for plant regeneration studies with the idea of obtaining intergeneric chromosomal exchanges. The remaining three embryos became necrotic and could not be rescued.

Table 1. Frequency of embryo and plantlet yield obtained from pollinating *Triticum aestivum* cv "Fukuho" with four accessions of *Elymus scabrus*

E. scabrus accession	Florets pollinated	Ovules cultured[a]	Embryos recovered (%)[b]	Plantlets obtained (%)[b]
D2883	416	399	0 (0.00)	0 (0.00)
D2911	524	508	0 (0.00)	0 (0.00)
J6C1001	228	217	0 (0.00)	0 (0.00)
J6C1059	631	615	9 (1.46)[c]	5 (0.81)[d]
Total	1,799	1,739	9 (0.52)	5 (0.29)

[a] These ovules potentially contained proembryos, but most of them were apparently not fertilized, judging from the poor pollen germination
[b] As percent of ovules cultured
[c] The embryo recovery for this genotype is very significantly better ($P<0.01$) according to binomial confidence limits
[d] The success for this genotype is significantly better ($P<0.05$) according to binomial confidence limits

Table 2. Spike and spikelet characteristics of *Triticum aestivum* cv "Fukuho", *Elymus scabrus* (J6C1059, and their F_1 hybrid. All measurements are in centimeters

Character	*Triticum aestivum*	F_1 hybrid	*Elymus scabrus*
Spike length	11.2±0.2*	20.3±1.1	22.0±1.0
Spike width	0.8±0.1	0.7±0.1	0.4±0.1
No. of nodes/spike	23.5±1.3	19.6±1.1	10.6±0.5
Internode length	0.5±0.0	1.1±0.1	1.8±0.1
Spikelet length	1.3±0.1	1.8±0.1	2.0±0.1
Spikelet width	0.7±0.1	0.7±0.1	0.4±0.1
No. of spikelets/spike	23.5±1.3	19.6±1.1	10.6±0.5
No. of florets/spikelets	4.5±0.6	6.2±0.4	7.2±1.1
Glume body length	0.9±0.1	0.9±0.1	1.0±0.1
Glume awn length	0.1±0.1	0.1±0.0	0.2±0.3
Lemma body length	1.0±0.0	1.1±0.1	1.2±0.1
Lemma awn length	4.9±0.4	3.4±0.8	1.8±0.1
Anther length	0.2±0.0	0.3±0.0	0.2±0.0

* Mean±standard deviation

Fig. 1 a, b. Spike (**a**) and spikelet (**b**) morphology of *Triticum aestivum* cv "Fukuho", F_1 hybrid, and *Elymus scabrus* J6C1059 (*left* to *right*, respectively)

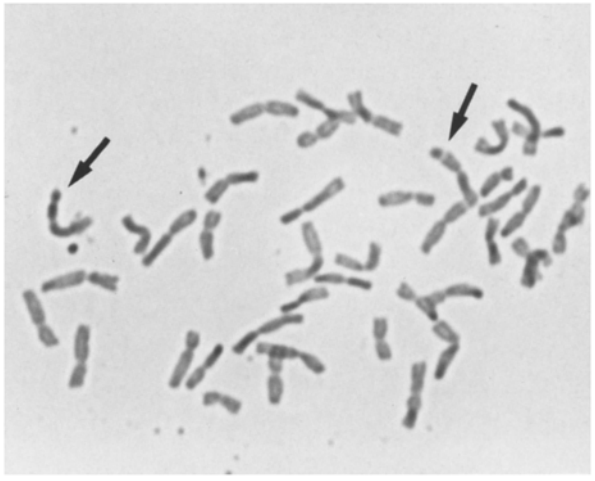

Fig. 2. Somatic chromosome spread of *Triticum aestivum* cv "Fukuho" × *Elymus scabrus* J6C1059 F_1 hybrid (2n = 6x = 42). Note the single dosage of satellited wheat chromosomes (*arrows*)

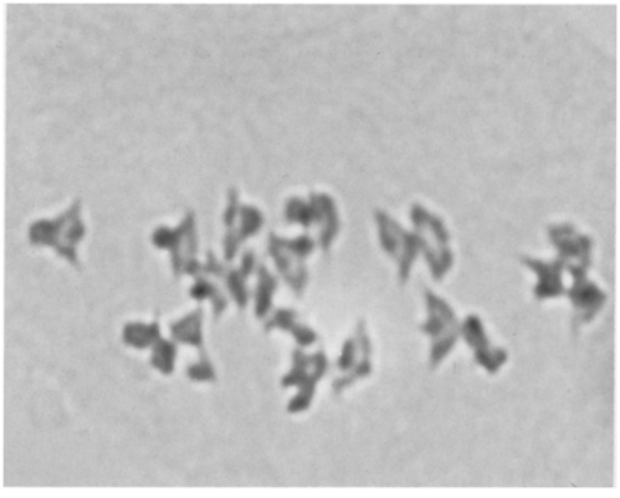

Fig. 3. A PMC of *Elymus scabrus* J6C1059 showing 21 ring IIs at metaphase I

All five F_1 plantlets grew to maturity and were labelled from TSCAB 1 to TSCAB 5. Juvenile plants were quite vigorous and tillered normally. Like the male parent, the hybrids had a rough leaf texture, pubescent leaf surface, and weakly serrated leaf margins. The leaves were quite wide, resembling the wheat parent. The spike characteristics of the F_1 hybrids were also indicative of definite morphological differences (Fig. 1 a, b), when compared with "Fukuho" wheat and *E. scabrus* J6C1059 (Table 2). The hybrids had an average pollen fertility of 1.17% and set no seed upon selfing, but could be vegetatively propagated although older plants tillered only with some difficulty.

Cytology

All the hybrids had the expected chromosome number of 2n = 6x = 42 and were karyotypically stable (Fig. 2). Since both parents had an identical number of chromosomes and differences in chromosome sizes of the two parents were not clear-cut, the hybrids were initially identified by virtue of amphiplasty, which resulted in the

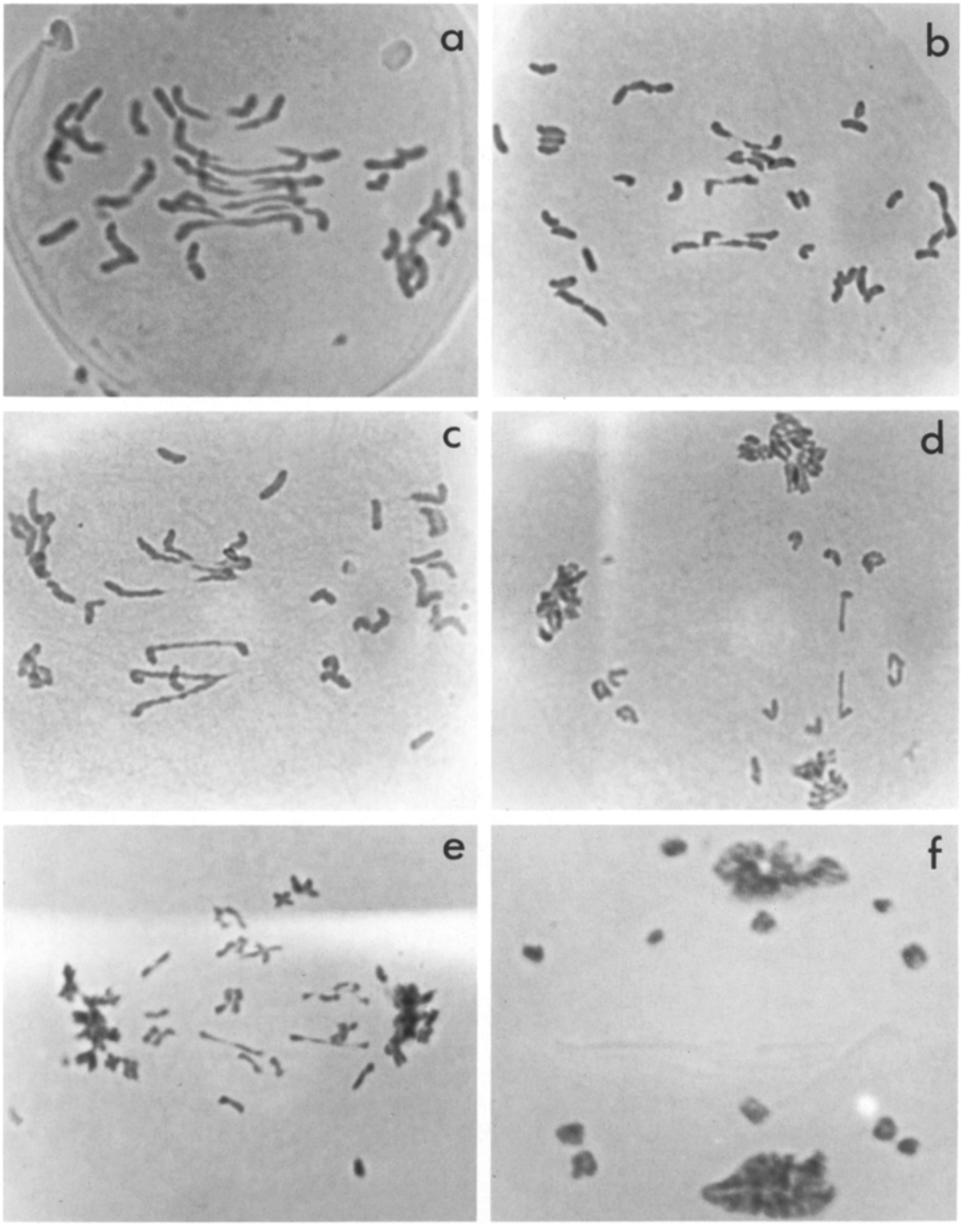

Fig. 4a–f. Meiotic configuration in PMCs of *Triticum aestivum* cv "Fukuho" × *Elymus scabrus* J6C1059 F_1 hybrids. **a** 8 rod IIs + 26 Is; **b** 4 rod IIs + 1 III + 31 Is; **c** 2 rod IIs + 2 IIIs + 32 Is; **d** tripolar anaphase I; **e** lagging chromosomes at anaphase I; and **f** micronuclei at the end of first meiotic division

presence of a single dosage of the satellited wheat chromosomes (*1B*, *6B* and, occasionally, *5D*; Fig. 2).

The chromosomes pairing in wheat and in *E. scabrus* accession J6C1059 (Fig. 3), the only accession from which hybrid plants were obtained, was similar and quite regular with 21 II. Meiosis in the hybrids was stable, and PMCs generally contained the expected somatic cell chromosome number of 2n = 6x = 42. Meiotic association data in the five hybrid plants were quite similar and are presented in Table 3. The overall average chromosome pairing at metaphase I in pollen mother cells (PMCs) of the hybrid plants was 32.83 I + 4.08 rod II + 0.21 ring II + 0.18 III + 0.02 IV. The number of chiasmata per PMC ranged from 4.23 to 5.63 in different

Table 3. Mean meiotic associations at metaphase I in *Triticum aestivum* cv "Fukuho" × *Elymus scabrus* J6C1059 intergeneric hybrids, polyhaploids "Fukuho" wheat, and an intergeneric hybrid genomically similar to polyhaploid hexaploid *Elymus* species

Plant designation	2n	No. of PMCs	II			I	III	IV	Chiasmata per PMC	Pollen fertility (%)
			Rod	Ring	Total					
TSCAB 1	42	84	3.96 (0–8)[a]	0.19 (0–2)	4.15 (1–8)	33.15 (26–40)	0.14 (0–2)	0.04 (0–1)	4.80 (1–8)	0.82
TSCAB 2	42	69	3.61 (0–6)	0.22 (0–1)	3.80 (0–6)	34.10 (30–38)	0.07 (0–1)	0.01 (0–1)	4.23 (2–7)	0.90
TSCAB 3	42	126	3.85 (1–8)	0.17 (0–2)	4.01 (1–8)	33.25 (26–40)	0.22 (0–2)	0.02 (0–1)	4.70 (1–9)	2.10
TSCAB 4	42	80	4.49 (1–8)	0.26 (0–2)	4.74 (1–8)	31.60 (26–37)	0.28 (0–2)	0.01 (0–1)	5.63 (3–9)	0.95
TSACB 5	42	86	4.49 (1–8)	0.19 (0–2)	4.67 (1–8)	32.06 (26–38)	0.20 (0–2)	–	5.26 (2–10)	1.08
Mean TSCAB	42	445	4.08 (0–8)	0.21 (0–2)	4.27 (0–8)	32.83 (26–40)	0.18 (0–2)	0.02 (0–1)	4.92 (1–10)	1.17
Polyhaploid "Fukuho"[b]	21	100	0.99 (0–3)	0.02 (0–1)	1.01 –	18.86 –	0.04 (0–1)	–	1.11 –	–
Polyhaploid "Fukuho"[c]	21	55	0.78 (0–3)	0.05 (0–1)	0.84 (0–3)	19.33 (15–21)	–	–	0.89 (0–4)	–
Mean polyhaploid "Fukuho"	21	155	0.89 (0–3)	0.04 (0–1)	0.93	19.10	0.02	–	1.00	–
Elymus canadensis × *Agropyron libanoticum*[d]	21	150	–	–	5.38	9.47 (3–7)	0.26 (7–15)	– (0–2)	–	–

[a] Figures in parenthesis represent range
[b] Data from Plourde et al. (1990)
[c] Data from Ahmad and Comeau (1990)
[d] Data from Dewey (1974). According to Dewey (1984), this hybrid of the genomic composition HSS is similar to polyhaploid of hexaploic *Elymus* species

Table 4. Frequency of PMCs showing multivalent formation in *Triticum aestivum* cv "Fukuho" × *Elymus scabrus* J6C1059 hybrids

Multivalent class	No. of PMCs[a]	Percent PMCs
1 III	69	15.51
1 IV	5	1.21
2 IIIs	7	1.57
1 III + 1 IV	2	0.45
Total PMCs with multivalents	83	18.65

[a] Based on a total of 445 PMCs from five F_1 hybrid plants

hybrid plants, with an average of 4.92. At the most, 8 II were observed in some PMCs (Fig. 4a), a majority of which were rod II. Of the 445 PMCs that were observed collectively of the five hybrids, 83 (or 18.65%) showed multivalents (III, IV, or their combination in different proportions), as shown in Figs. 4b, c, and Table 4. Collectively, 2.71% of the PMCs in the five F_1 hybrid plants were hypoploid/hyperploid. Meiotic abnormalities, such as multipolar anaphase (Fig. 4d), lagging chromosomes (Fig. 4e), and unequal/irregular cytokinesis, often encountered in wide hybrids, were also observed in the present study. This led to the formation of micronuclei (Fig. 4f) and variation in the number of cells in an otherwise tetrad. Spontaneous chromosome doubling and/or restitution nuclei formation were not observed in any PMC of the hybrid plants.

Attempts to backcross the hybrid plants with "Fukuho" wheat have so far been unsuccessful. The hybrids are being maintained vegetatively for amphiploid production, so as to maintain the *E. scabrus* and wheat genomes together for the production of aneuploid stocks.

Discussion

In the past, ever since the first report of apomixis in *E. scabrus* (Hair 1956), an enormous effort has gone into hybridizing wheat with *E. scabrus*, but with no success (Dewey 1984). Our crossability results show that it is indeed a difficult cross to obtain. The only crossable accession of *E. scabrus*, line J6C1059, gave a mere 1.46% embryo and 0.81% plantlet recovery. In this particular

cross, prezygotic barriers represent only part of the difficulty, and postzygotic abortion mechanisms are activated much earlier than in any previously reported wheat × *Elymus* species cross (see references in Introduction). Our success in hybridizing wheat with *E. scabrus* has been due to the fact that we alleviated the postfertilization bottleneck at a very early developmental stage, using a locally developed in ovulo embryo culture technique.

Although *E. scabrus* is an apomictic species, accessions and populations that reproduce sexually are also known (Love and Connor 1982). It is not known if the *E. scabrus* accession used in the present study, from which hybrids with wheat have been obtained, is apomictic or not. Even if it turns out that line J6C1059 is not apomictic, it does not in any way diminish the significance of the presently reported hybrid, which proves that wheat × *E. scabrus* hybrids are possible.

For the introduction of desirable alien variation into wheat, it becomes essential to know the genomic relationships of the alien species in the hybrids. This, however, is dependent on a clear understanding of the genomes present in the parental species involved. Wheat consists of genomes A, B, and D, but the genomic composition of *E. scabrus* populations is not clearly understood (Dewey 1984). Based on cytogenetic analysis in interspecific/intergeneric hybrids among New Zealand wheatgrasses and also those of *E. scabrus* with *Pseuderoegneria spicata* and *Critesion marinum*, it appears that *E. scabrus* has a genomic composition of HSS (Love and Connor 1982). However, we believe that the two S genomes present in *E. scabrus* are not identical and, at best, show only limited segmental homology. This is substantiated by the fact that mainly IIs (present study; Love and Connor 1982) and only occasionally IVs (Love and Connor 1982) are formed at meiosis in hexaploid *E. scabrus*. Due to the lack of cytogenetic analysis in appropriate hybrids, the two weakly differentiated S genomes of *E. scabrus* have not been given any further genomic classification. However, the two S genomes of *E. scabrus* should not be considered homologous, either to themselves or to the S_1 and S_2 genomes of *Elytrigia repens*, as shown by cytogenetic analysis of their intergeneric hybrids (Love and Connor 1982) and probably appropriately interpreted by Dewey (1984).

Hence, as logically deduced above, the HHSSSS genomes of *E. scabrus* do not have much homology with the AABBDD genomes of wheat. It will, therefore, be expected that only poor chromosome pairing, if at all, will be observed in the hybrids of the genomic composition ABDHSS. The observed mean meiotic association of 32.83 I + 4.08 rod II + 0.21 ring II + 0.18 III + 0.02 IV (and Figs. 4a–c) indeed attest to this expectation. We believe that the small number of IIs seen at meiosis have largely resulted from homoeologous chromosome pairing within the ABD genomes of wheat and HSS genomes of *E. scabrus*.

The nature of meiotic association in the hybrid plants could be better understood in the light of chromosome pairing behavior observed in parental species, polyhaploids, or the like. In polyhaploid "Fukuho" wheat, a mean of 0.94 II and 0.02 III per PMC have been reported [data pooled from Ahmad and Comeau (1990) and Plourde et al. (1990)]. Polyhaploids of *E. scabrus* J6C1059 are not yet available, but in *E. canadensis* (2n = 4x = 28; HHSS) × *Pseuderoegneria libanotica* (2n = 2x = 14; SS) intergeneric hybrids of the genomic composition HSS, a mean chromosome pairing frequency of 5.38 II and 0.26 III per PMC has been reported (Dewey 1974). Dewey (1974) also suggested that most, if not all, of the IIs observed in the HSS hybrids resulted from pairing of the two S genome chromosomes. In light of known genomic composition of some hexaploid *Elymus* species (Dewey 1984), we assume that the hybrid reported by Dewey (1974) is genomically similar, although not identical, to polyhaploid *E. scabrus*. Thus, in view of the above observations, the level of chromosome pairing (IIs and IIIs) frequency in the wheat × *E. scabrus* hybrids is well within limits expected due to intrageneric, intergenomic pairing.

In spite of our implication of a predominantly intrageneric, intergenomic pairing in the hybrids, it could not be ruled out that perhaps some pairing involves chromosomes from different species, especially since the parental species chromosomes of the two genera could not be distinguished beyound doubt at meiosis. The presence of a IV, either by itself or in conjunction with III, in the present hybrids has a high probability of involving at least one chromosome from either of the two parental species.

The apparent near absence of intergeneric homoeologous pairing in wheat × *E. scabrus* hybrids is of concern, if useful characteristics of *E. scabrus* are to be incorporated into wheat. If pairing, to any extent, in these hybrids represents a random pairing of only one of the wheat or *E. scabrus* chromosomes with corresponding homoeologues of wheat or *E. scabrus*, as happens in IV, recombinants may still be obtained but only by handling large populations. Alternative approaches to induce gene transfers from *E. scabrus* to wheat are being tried in our laboratory.

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