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Development of a complete set of *Triticum aestivum-Aegilops speltoides* chromosome addition lines

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Abstract *Aegilops speltoides* Tausch (2n = 2x = 14, SS) is considered as the closest living relative of the B and G genomes of polyploid wheats. A complete set of *Triticum aestivum* L. cv Chinese Spring-*Ae. speltoides* whole chromosomes and seven telosomic addition lines was established. A low pairing accession was selected for the isolation of the chromosome addition lines. Except for chromosomes 3S and 6S, which are presently only available as monosomic additions, all other lines were recovered as disomic or ditelosomic additions. The individual *Ae. speltoides* chromosomes isolated in the wheat background were assayed for their genetic effects on plant phenotype and cytologically characterized in terms of chromosome length, arm ratio, distribution of marker C-bands, and FISH sites using a *Ae. speltoides*-specific repetitive element, Gc1R-1, as a probe. The homoeology of the added *Ae. speltoides* chromosomes was established by using a standard set of RFLP probes. No chromosomal rearrangements relative to wheat were detected.

Key words *Triticum aestivum* · *Aegilops speltoides* · Chromosome addition · C-banding · In situ hybridization · RFLP

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Introduction

Aegilops speltoides Tausch (2n = 2x = 14, SS) has the highest genetic affinity to the B and G genomes of polyploid wheats (for a review see Friebe and Gill 1996; Tsunewaki 1996; Dvorak 1998). *Ae. speltoides* is the only outbreeding S-genome species belonging to the section *Sitopsis*, which also includes the diploid species *Aegilops sharonensis* Eig (2n = 2x = 14, $S^{sh}S^{sh}$), *Aegilops longissima* Schweinf. & Muschl. (2n = 2x = 14, $S^{l}S^{l}$), *Aegilops searsii* Feldman & Kislev ex Hammer (2n=2x=14, $S^{s}S^{s}$), and *Aegilops bicornis* (Forssk.) Jaub. & Spach (2n = 2x = 14, $S^{b}S^{b}$). *Ae. speltoides* is native to the eastern Mediterranean and Middle East region and exists as two varieties, *speltoides* (awnless lemma except for apical spikelet) and *ligustica* (awned lemma) (Kimber and Feldman 1987; van Slageren 1994). *Ae. speltoides* is a valuable reservoir for agronomically useful genes and is the source for the resistance genes *Lr28*, *Sr32*, *Lr35*/*Sr39*, *Lr36*, *Pm12*, and *Gb5*, which have been transferred to common wheat, *Triticum aestivum* L. (2n = 6x = 42, AABBDD) (Riley et al. 1968; Dvorak 1977; Dvorak and Knott 1980; McIntosh et al. 1982; Wells et al. 1982; Tyler et al. 1987; Dvorak and Knott 1990; Kerber and Dyck, 1990; McIntosh, 1991; Jia et al. 1996; for a review see Friebe et al. 1996).

The establishment of wheat-alien chromosome addition lines allows the study of the genetic effects of individual alien chromosomes in the background of hexaploid wheat. For the S-genome species, complete sets of wheat-alien chromosome additions were developed for *Ae. longissima* (Feldman 1975; Friebe et al. 1993), and *Ae. searsii* (Friebe et al. 1995), while a partial set was developed for *Ae. bicornis* (Shepherd and Islam 1988). So far, all accessions of *Ae. sharonensis* analyzed had a strong gametocidal gene located on chromosome $4S^{sh}$ that resulted in the preferential transmission of chromosome $4S^{sh}$ and prevented the development of a complete set of additions (Maan 1975; Miller et al. 1982; Miller 1983; our own unpublished results). Similar attempts to produce a complete set of chromosome addition lines

from *Ae. speltoides* failed because of extensive chromosomal rearrangements caused by chromosome 6S (Kota and Dvorak 1988). Recently, Lapochkina et al. (1998) attempted to produce a set of *T. aestivum-Ae. speltoides* chromosome addition lines; however, C-banding analysis revealed that only a few of the *Ae. speltoides* chromosomes were added to wheat. Here we report on the development, identification, and characterization of a complete set of *T. aestivum-Ae. speltoides* chromosome addition lines.

Materials and methods

Plant material

T. aestivum cv 'Chinese Spring' (CS) was crossed with the *Ae. speltoides* accessions #308 (9211, Nir Ezion, Israel), #322 (9213, Nir Ezion, Israel), #818 (9214, Technion, Israel), #829 (9212, Technion, Israel), and #2073 (9210, Kefar Yehoshua, Israel), which were provided by the Institute of Cereal Crops Improvement, Lieberman Germplasm Bank, Tel-Aviv University, Israel. Abbreviations: DA: disomic chromosome addition; MA: monosomic chromosome addition; DtA: ditelosomic addition; DS: disomic chromosome substitution; dDtS: double ditelosomic substitution; T: translocation; i: isochromosome.

Cytogenetic analysis

Chromosomal constitutions of the F_1 plants and backcross progenies were determined in root-tip meristems and their meiotic metaphase-I pairing behavior was analyzed in pollen mother cells (PMCs). The C-banding protocol and chromosome identification was after Gill et al. (1991). Microphotographs were taken with a Zeiss photomicroscope III using Kodak Imagelink HQ microfilm 1461.

Clone Gc1R-1 was used for fluorescence in situ hybridization (FISH) analysis. Probe Gc1R-1 is a 258-bp long, *Ae. speltoides*-specific repetitive element that was cloned from the wheat-*Ae. speltoides* translocation line T2B-2S and hybridizes to telomeric and subtelomeric regions of most *Ae. speltoides* chromosome arms (Nasuda 1999). Clone Gc1R-1 has 98% sequence homology to the 5'-end of the S-genome specific element pAesKB52 isolated by Anamthawat-Jónsson and Heslop-Harrison (1993). FISH was according to the protocol of Kynast et al. (2000). Clone Gc1R-1 was directly labeled with fluorescein-11-dUTP by nick-translation. Hybridization and detection conditions were as reported by Kynast et al. (1999). Chromosomes were counterstained with propidium iodide and signals visualized using a Zeiss Axioplan microscope equipped for phase contrast, and epifluorescence. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostic Instruments, Inc., Sterling Heights, Michigan, USA) and processed with Photoshop 4.0 software (Adobe Systems Inc., San Jose, California, USA). Images were printed on a Kodak ds 8650 PS Color Printer.

Restriction fragment length polymorphism (RFLP) analysis

Twenty two DNA probes were used, including BCD (barley cDNA) and CDO (oat cDNA) clones obtained from Dr. M. E. Sorrells, Ithaca, N.Y., USA, and PSR (wheat cDNA or genomic DNA) clones provided by Dr. M. D. Gale, Norwich, UK (Table 1). Genomic DNA of the addition-line plants was digested with four different restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, and *Dra*I) using the genomic DNAs of *Ae. speltoides* #829, CS, and the amphiploid CS-*Ae.speltoides* #829 as controls. DNA hybridization was as previously described by Qi et al. (1997).

Results

Development of chromosome addition lines

The F_1 plants involving CS and the *Ae. speltoides* accessions #308, #322, and #818 were of the high-pairing type, with 1–5 ring, and several rod bivalents per PMC. F_1 plants involving the accessions #829 and #2073 were low-pairing types usually with 1–3 rod bivalents per PMC (Fig. 1). The average chiasma frequency per PMC in the F_1 involving accession #829 was 2.2 (Table 2).

Table 1 Molecular probes mapped on the *T. aestivum* cv CS-*Ae.speltoides* #829 addition and ditelosomic addition lines. *no polymorphism detected by these markers

Constitution	Mapped probes/enzyme fragment	Constitution	Mapped probes/enzyme fragment
DA1S	BCD1434–1S/*Hin*dIII PSR596–1S/*Eco*RV* PSR544–1L/*Eco*RV BCD386–1L/*Eco*RV*	DA5 S	PSR945–5S/*Hin*dIII PSR628–5S/*Dra*I* PSR360–5L/*Eco*RI PSR580–5L/*Eco*RV
DtA1SS	BCD1434–1S/*Hin*dIII	T5SS·?	PSR945–5S/*Hin*dIII
DA2S	BCD433–2S/*Dra*I PSR388–2L/*Dra*I	DtA5SL	PSR360–5L/*Eco*RI PSR580–5L/*Eco*RV
DtA2SS	BCD433–2S/*Dra*I	MA6 S	PSR627–6S/*Dra*I PSR113–6S/*Hin*dIII* CDO497–6L/*Hin*dIII
DtA2SL	PSR388–2L/*Dra*I	DA7 S	CDO595–7S/*Eco*RV PSR129–7L/*Dra*I PSR311–7L/*Eco*RI
MA3S	PSR909–3S/*Hin*dIII PSR926–3S/*Dra*I PSR931–3L/*Hin*dIII	DtA7SS	CDO595–7S/*Eco*RV
DA4S	PSR144–4S/*Hin*dIII PSR163–4L/*Hin*dIII PSR920–4L/*Dra*I	DtA7SL	PSR129–7L/*Dra*I PSR311–7L/*Eco*RI
DtA4SL	PSR163–4L/*Hin*dIII PSR920–4L/*Dra*I		

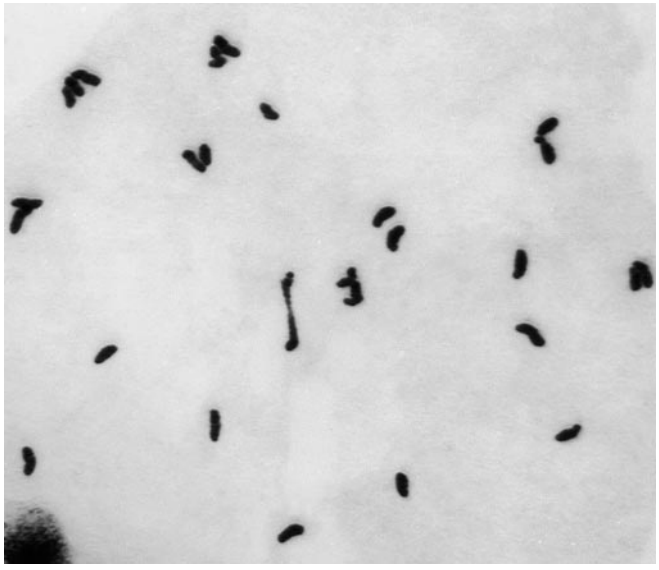

Fig. 1 Meiotic metaphase I pairing of the F_1 hybrid *T. aestivum* cv CS-*Ae. speltoides* accession #829 with one rod bivalent and 26 univalents

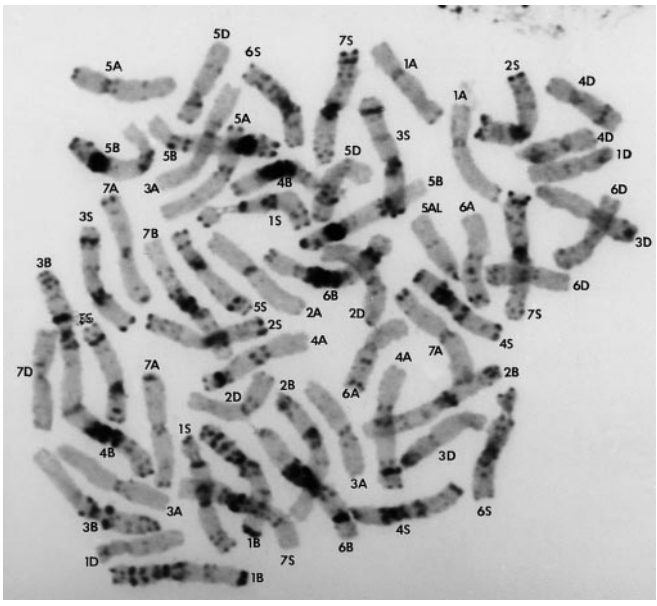

Fig. 2 C-banding pattern of the amphiploid *T. aestivum* cv CS-*Ae. speltoides* accession #829

Table 2 Meiotic metaphase I pairing in PMCs of the F_1 hybrid *T. aestivum* cv CS-*Ae. speltoides* #829

No. of PMCs	Univalents	Bivalents		Trivalents
		Rods	Rings	
14	28			
15	26	1		
8	26		1	
12	24	2		
3	24	1	1	
3	23	1		1
10	22	3		
11	22	2	1	
3	21	2		1
1	20	3	1	
3	18	5		
83	24.3	1.5	0.3	0.07
Retrads with micronuclei		217		
Tetrads without micronuclei		9		
Restitution dyads		25		

Most of the tetrads had micronuclei and about 10% restitution dyads were observed (Table 2). All F_1 plants involving the accession #2073 and about half of the F_1s involving the accession #829 had 2n = 29 chromosomes, indicating the presence of a B chromosome. No seed was set when 29-chromosome F_1 plants involving the accessions #829 and #2073 were pollinated with CS. However, 28-chromosome F_1 plants involving #829 produced an average of 10–15 seeds per head when pollinated with CS. Two types of seeds were obtained: small seeds with chromosome numbers in the upper 30s and 40s, and large seeds with chromosome numbers between 53 and 57. BC_1 plants with 2n = 57 chromosomes were selfed. Six of the BC_1 plants had 2n = 49 chromosomes. Two of the BC_1 plants were dwarfs and died as seedlings. The other four plants were healthy and produced numerous tillers. All plants exhibited a low level of homoeologous pairing with occasional trivalent and quadrivalent formation at meiotic metaphase-I. These plants were backcrossed as females with CS and produced about 50% seed set. BC_2 plants with 2n = 43 (21'' + 1'), 44 (21'' + 2'), and 45 (21'' + 3') chromosomes were pollinated again with CS. BC_3 plants with 43 chromosomes were selfed and disomic chromosome-addition plants were identified in the BC_3F_2.

C-banding and FISH patterns of *Ae. speltoides* chromosomes

All seven chromosome pairs of the *Ae. speltoides* accession #829 are identifiable by their diagnostic C-banding patterns (Figs. 2 and 3). The C-banding patterns of the *Ae. speltoides* chromosomes present in the amphiploid *T. aestivum* cv CS-*Ae. speltoides* accession #829 and in the derived chromosome and telosome addition lines are similar to those of the *Ae. speltoides* accession #829 (Figs. 2 and 3).

FISH analysis with the *Ae. speltoides* repetitive clone Gc1R-1 revealed mainly telomeric and subtelomeric hybridization sites on all seven chromosome pairs of the *Ae. speltoides* accession #829. The clone Gc1R-1 did not detect hybridization sites on any of the A-, B-, or D-genome chromosomes of wheat and, thus, allowed the detection of *Ae. speltoides* chromatin in a wheat background. The Gc1R-1 FISH patterns of the *Ae. speltoides* chromosomes were determined in the set of addition

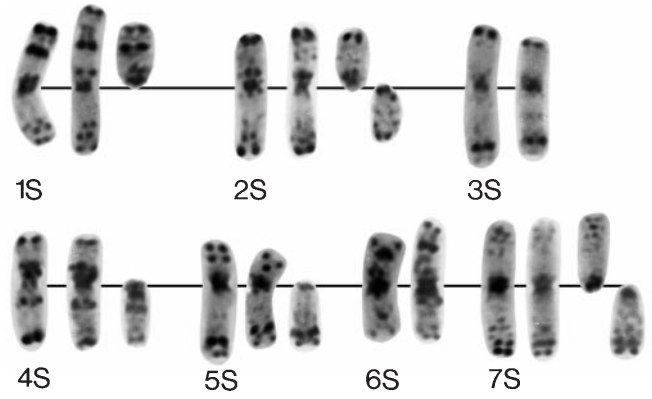

Fig. 3 C-banding patterns of the *Ae. speltoides* chromosomes present in accession #829 (*left*) and in the derived *T. aestivum* cv CS-*Ae. speltoides* accession #829 addition lines (*right*)

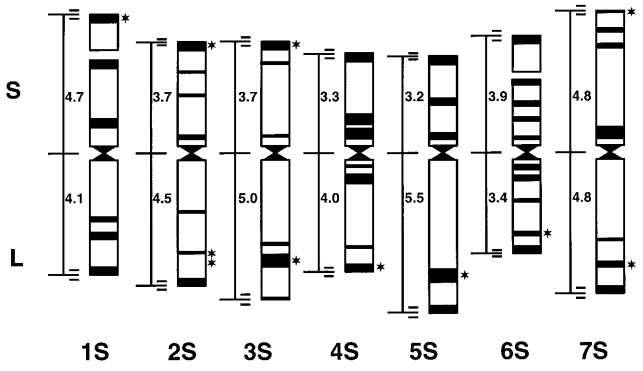

Fig. 5 Generalized idiogram of the *Ae. speltoides* accession #829 chromosomes present in the set of addition lines showing positions of C-bands and Gc1R-1 FISH sites (*asterisks*). Chromosome length data are given in micrometers

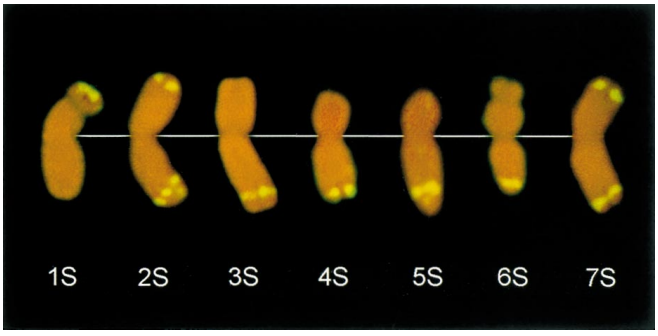

Fig. 4 Gc1R-1 FISH pattern of the *Ae. speltoides* accession #829 chromosomes present in the set of addition lines

Table 3 Chromosome lengths (S = short arm, L = long arm) and standard deviations given in µm, arm ratios (L/S), and relative lengths (S+L) given in percent of chromosome-3B lengths, 10.7 ± 1.1 µm, used as a standard (measurement data were based on ten chromosomes of each *Ae. speltoides* chromosome present in the amphiploid *T. aestivum-Ae. speltoides* accession #829)

Chromosome	S	L	S+L	L/S	% 3B length
1S	4.7 ± 0.3	4.1 ± 0.4	8.8 ± 0.5	0.9	0.82
2S	3.7 ± 0.2	4.5 ± 0.3	8.2 ± 0.4	1.2	0.77
3S	3.7 ± 0.3	5.0 ± 0.4	8.7 ± 0.6	1.4	0.81
4S	3.3 ± 0.3	4.0 ± 0.3	7.3 ± 0.5	1.2	0.68
5S	3.2 ± 0.2	5.5 ± 0.4	8.7 ± 0.5	1.7	0.81
6S	3.9 ± 0.4	3.4 ± 0.2	7.3 ± 0.5	0.9	0.68
7S	4.8 ± 0.3	4.8 ± 0.4	9.6 ± 0.6	1.0	0.90

lines and is shown in Fig. 4. Chromosome measurement data are summarized in Table 3 and a generalized idiogram of the *Ae. speltoides* chromosomes showing the position of C-bands in relation to Gc1R-1 FISH-sites is given in Fig. 5.

Identification of *Ae. speltoides* chromosome and telosome addition lines

C-banding analysis was used to identify a complete set of *Ae. speltoides* chromosome addition lines. Except for chromosomes 3S and 6S, which are presently only available in the form of monosomic addition lines, all the other *Ae. speltoides* chromosomes were recovered as disomic additions. Chromosome 6S spontaneously substituted for wheat chromosome 6A in a DS6S(6A) substitution line. Seven ditelosomic *Ae. speltoides* addition lines were identified including DtA1SS, DtA2SS, DtA2SL, DtA4SL, DtA5SL, DtA7SS and DtA7SL (Fig. 3). Furthermore, five whole-arm translocations (T2SS·7SS, T4SL·5SL, T5SS·?, T6BS·5SS and T6BS·7SL), four isochromosomes (i3SS, i4SL, i5SS and i5SL), and one terminal wheat-*Ae. speltoides* translocation (T5BS·5BL-5SL) were identified (Fig. 6). The C-banding patterns of all wheat chromosomes present in the set of chromosome, and telosome, addition lines is identical to those of the wheat parent cultivar CS.

Homoeology of the added *Ae. speltoides* chromosomes and telosomes

RFLP analysis confirmed the homoeology of the added *Ae. speltoides* chromosomes and telosomes present in the addition lines DA1S, DtA1SS, DA2S, DtA2SS, DtA2SL, MA3S, DA4S, DtA4SL, DA5S, DtA5SL, MA6S, DA7S, DtA7SS, and DtA7SL, and in the translocation lines T4SL·5SL, and T5SS·? (Table 1, Fig. 7).

Spike morphologies of the CS-*Ae. speltoides* addition lines

The overall spike morphologies of the *T. aestivum* cv CS-*Ae. speltoides* whole-chromosome and telosome

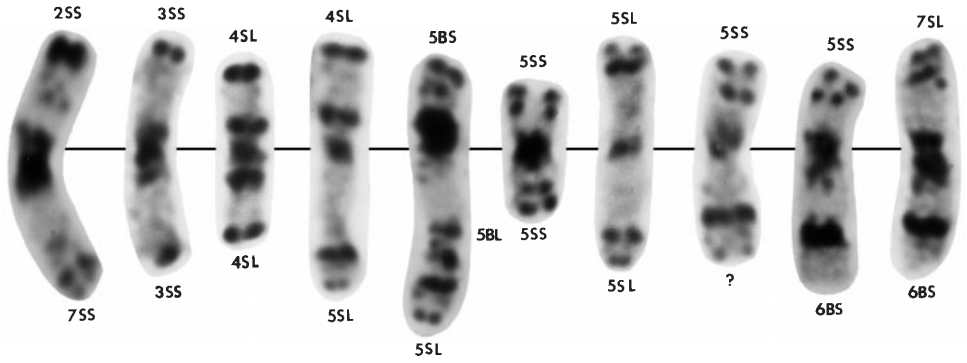

Fig. 6 Translocations and isochromosomes identified by C-banding; from left to right: T2SS·7SS, i3SS, i4SL, T4SL·5SL, T5BS·5BL-5SL, i5SS, i5SL,T5SS·?, T6BS·5SS, and T6BS·7SL

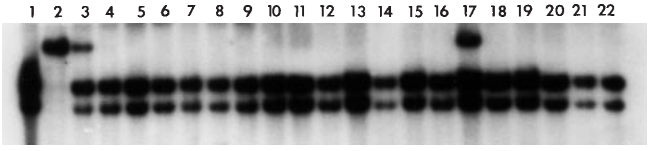

Fig. 7 Hybridization of homeologous group-1L probe PSR544 to *Eco*RV-digested genomic DNA of *T. aestivum* cv CS (lane 1), *Ae. speltoides* accession #829 (*lane 2*), the amphiploid *T. aestivum* cv CS-*Ae. speltoides* accession #829 (*lane 3*), and derived chromosome, telosome, and translocation lines (*lanes 4 to 22*). Polymorphic bands are present in *lanes 2, 3 and 17* (DA1S)

Fig. 8 Spike morphologies of *T. aestivum* cv CS-*Ae. speltoides* chromosome and telosome addition lines. *Left to right:* upper row *Ae. speltoides*, CS-*Ae. speltoides* amphiploid, CS, DA1S, DA2S, MA3S, DA4S, DA5S, DS6S(6A), DA7S; lower row DtA1SS, DtA2SS, DtA2SL, DtA4SL, DtA5SL, DtA7SS, DtA7SL

addition lines (Fig. 8) are similar to those of the CS-*Ae. longissima* and CS-*Ae. searsii* addition lines reported earlier (Friebe et al. 1993, 1995). Thus:

Spikes of DA1S and DtA1SS are similar in appearance to those of CS.

Spikes of DA2S are awned and have tenacious glumes. Spikes of DtA2SS have tenacious glumes and those of DtA2SL are awned.

Spikes of MA3S have a brittle rachis that tends to break at the base.

Spikes of DA4S are more lax at the base than those of CS and the upper spikelets tend to be sterile. Spikes of DtA4SL resemble those of CS.

Spikes of DA5S and DtA5SL are lax at the base and more compact at the top.

Spikes of MA6S and DS6S(6A) are smaller and more lax as compared to CS.

Spikes of DA7S and DtA7SL are more lax than those of CS, whereas those of DtA7SS are similar in appearance to those of CS. Seedlings of DA7S and DtA7SS have red coleoptiles.

Discussion

Several wild wheats including species belonging to the section *Sitopsis* have been considered as putative donor species for the G/B genomes of polyploid wheats (Kimber 1981). Of all the *Sitopsis* species the S genome of *Ae. speltoides* is the most closely related to the G- and B-genomes of *T. timopheevii* (Zhuk.) Zhuk., *T. turgidum* L., and *T. aestivum.* Close evolutionary relationship between the S and G/B genomes is indicated by similarities in repeated nucleotide sequences (Dvorak and Zhang 1990) and by similarities in C-banding and in situ hybridization patterns (Jiang and Gill 1994a, b; Badaeva et al. 1990, 1996a, b; Friebe and Gill 1996). RFLP analysis further identified *Ae. speltoides* as the plasmon donor of *T. timopheevii*, *T. turgidum*, and *T. aestivum* (Tsunewaki and Ogihara 1983; Ogihara and Tsunewaki 1988; Tsunewaki et al. 1990).

The C-banding pattern of the *Ae. speltoides* chromosomes present in the accession #829 is similar to those reported earlier for other accessions (Friebe and Gill 1996). However, in that study, chromosome identification was based only on similarities in morphologies and C-banding patterns with those of other S-genome species belonging to the section *Sitopsis*, which led to the miss-identification of chromosomes 2S and 3S. This also was indicated in a recent study by Maestra and Naranjo (1998) who used *ph1b*- and *ph2b*-induced meiotic meta-

phase-I pairing of wheat and *Ae. speltoides* chromosomes for determining their homoeologous relationships. The dissection of the S genome of *Ae. speltoides* in the form of chromosome and telosome addition lines allowed the unequivocal identification of the homoeologous relationships of all *Ae. speltoides* chromosomes.

The homoeologous relationships of *Ae. speltoides* chromosomes were also established by meiotic metaphase-I pairing analysis (Maestro and Naranjo 1998). The 14 S-genome chromosome arms showed normal metaphase-I pairing with their homoeologous wheat chromosome arms, indicating that the *Ae. speltoides* accession analyzed in this study did not have a translocation difference relative to CS wheat. Similarly, in the present study, RFLP analysis failed to detect chromosomal rearrangements relative to wheat. Maestro and Naranjo (1998) observed preferential pairing between the A-D and B-S genome chromosomes, supporting the close evolutionary relationship between the B- and S-genome chromosomes. Similarly, induced homoeologous metaphase-I pairing did not detect chromosomal rearrangements between the S^{sh}-genome chromosomes of *Ae. sharonensis* and those of wheat (Maestro and Naranjo 1997). *Ae. longissima* is the only S-genome species that differs from all other Sitopsis species by the presence of a species-specific translocation involving chromosome arms $4S^{l}L$ and $7S^{l}L$ (Hart and Tuleen 1983; Friebe et al. 1993; Naranjo 1995).

Meiotic metaphase-I pairing and RFLP analysis identified the presence of a cyclic translocation involving chromosomes 4A, 5A, and 7B in *T. turgidum* L. and *T. aestivum* (Naranjo et al. 1987, 1988a, b; Liu et al. 1992). King et al. (1994) suggested that the 4/5 translocation is ancient and predates the polyploidization of wheat. However, no 4/5 translocation is present in the B and D genomes of common wheat (Mickelson-Young et al. 1995). The group-5 long-arm probe PSR580 used in the present study maps distal to the breakpoint and, if present, allows the detection of the 4/5 translocation. However, this probe mapped on the long arm of chromosome 5S indicating absence of the 4/5 translocation in the accession used in the production of the addition lines.

Although a low level of homoeologous metaphase-I pairing was observed in the original *T. aestivum-Ae. speltoides* hybrid, no rearrangements detectable by C-banding were observed in the wheat chromosome complement of the addition lines, with the exception of one T5BS·5BL-5SL recombinant.

The overall arm ratios and sizes of *Ae. speltoides* chromosomes are similar to those reported for other S-genome species (Friebe et al. 1993, 1995; Badaeva et al. 1996a; Friebe and Gill 1996). However, in the present study an arm ratio of 0.9 was calculated for chromosome 1S, compared to 1.6, 1.7, and 1.7 estimated for chromosomes $1S^{s}$, $1S^{l}$, and 1B of *T. aestivum* (Gill et al. 1991), respectively. We presently do not know whether this discrepancy is caused by a measurement error or reflects an intrachromosomal rearrangement present in 1S.

Clone Gc1R-1 exclusively hybridized to telomeric and subtelomeric regions of all *Ae. speltoides* chromosomes, but neither to the closely related B-genome nor to the A- and D-genome chromosomes of wheat. Clone Gc1R-1 has 98% sequence homology to the S-genome-specific clone pAesKB52 isolated by Anamthawat-Jónsson and Heslop-Harrison (1993). FISH using pAesKB52 as a probe revealed hybridization sites on the S-, S^{sh}-, and S^{l}-genome chromosomes of *Ae. speltoides*, *Ae. sharonensis*, and *Ae. longissima*, respectively. The presence of clone pAesKB52 in the S genome of *Ae. speltoides* and the absence of the related clone Gc1R-1 in the B genome of wheat suggest that *Ae. speltoides* is not the direct B-genome progenitor. The close evolutionary relationship between the S and B genomes has so far prevented the cytological detection of *Ae. speltoides* chromatin in a wheat background using genomic in situ hybridization analysis. Although Gc1R-1 only tags the ends of S-genome chromosomes, it will be very useful for identifying and monitoring *Ae. speltoides* introgressions into wheat.

It is interesting to note that all 29-chromosome F_1 plants derived from the accessions # 829 and #2073, although both were of low pairing type, did not set seeds. Similarly, our attempts to transfer a supernumerary B chromosome from a different *Ae. speltoides* accession (#7717, provided by Dr. S. Ohta, Department of Bioscience, Fukui Prefectural University, Japan) were hampered because of extremely low seed set even after the second backcross with CS wheat. We do not know whether this effect is caused by the presence of the B chromosome or by genetic imbalance of the two parental genomes.

Interestingly, the 28-chromosome (ABDS) F_1 plants produced a number of large seeds when backcrossed with CS. Plants derived from these large seeds had chromosome numbers between 53 and 57 and were shown by C-banding to be amphiploids (AABBDDSS) (Fig. 2). Similarly, Chen and Dvorak (1984) reported that a low-pairing genotype of *Ae. speltoides* produced unreduced gametes leading to 48- and 49-chromosome BC_1 plants. For determining the mechanism involved in this chro-mosome-doubling, the F_1 plants were crossed as females with a double-ditelosomic substitution line dDtS $7S^{l}$ S $7S^{l}L$ (7D). Two types of seeds again were set on these plants. All plants derived from the small seeds had two telosomes indicating that the male parent had contributed a sperm nucleus to the zygote, whereas all plants derived from the large seeds were lacking the telosomes. In addition, several heads of the F_1 plants were bagged without pollination. No seeds were set on these heads, indicating that pollination is necessary for seed set. The exact mechanism involved in this chromosome-doubling is unknown. It is tempting to speculate that a similar chromosome-doubling mechanism may have acted in the ancient A/S-hybrid plants, which gave rise to the establishment of the tetraploid AABB and AAGG wheats *T. turgidum* and *T. timopheevii*.

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