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ORIGINAL ARTICLE

A set of *Triticum aestivum-Aegilops speltoides* Robertsonian translocation lines

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Abstract
Key message **Here we report the production of a set of wheat-Aegilops speltoides Robertsonian translocations covering all Ae. speltoides chromosome arms except the long arm of the homoeologous group 4 chromosome.**
Abstract Aegilops speltoides of the *Poaceae* family is the most probable donor of the B and G genomes of polyploid *Triticum* species and also an important source of resistance to diseases and pests of wheat. Previously, we reported the production of a complete set of *T aestivum-Ae. speltoides* chromosome addition lines and a set of disomic S(B/A)-genome chromosome substitution lines. The isolation of compensating Robertsonian translocations (RobTs) composed of alien chromosome arms translocated to homoeologous wheat chromosome arms is the important next step to exploit the genetic variation of a wild relative of wheat. Here, we report the development of molecular markers specific for the S-genome chromosomes and their use in the isolation of a set of 13 compensating wheat-*Ae. speltoides* RobTs covering the S genome of *Ae. speltoides* except for the long arm of chromosome 4S. Most of the RobTs were fully fertile and will facilitate mapping of genes to specific chromosome arms and also will accelerate the introgression of agronomically useful traits from *Ae. speltoides* into wheat by homologous recombination.

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Introduction

Common or bread wheat, *Triticum aestivum L.*, belongs to *Triticeae* tribe within the *Poaceae* family and is an allohexaploid species ($2n = 6x = 42$, AABBDD), where the A genome was derived from *T. urartu* Thumanian ex Gandilyan, the B genome was derived from a diploid species closely related to *Aegilops speltoides* Tausch, and the D genome was derived from *Ae. tauschii Coss* (Dvorak and Zhang 1990; Feldman et al. 1995; Huang et al. 2002). Wheat is a staple food for two-thirds of the human population. There are more than 300 species belonging to 20 genera of the *Triticeae* tribe including *Aegilops*, *Agropyron*, *Dasypyrum*, *Hordeum and Secale*, which are valuable sources for resistance to diseases, pests and abiotic stress for wheat improvement (Friebe et al. 1996).

Aegilops speltoides ($2n = 2x = 14$, SS), a diploid species belonging to the genus *Aegilops*, is native to the Fertile Crescent area and isolated areas in western Turkey and west-central Iran (van Slageren 1994). *Ae. speltoides* is the source of the leaf rust resistance genes *Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51,* and *Lr66*; stem rust resistance genes *Sr32*, *Sr39,* and *Sr4;* powdery mildew resistance genes *Pm12*, *Pm32,* and *Pm53;* tan spot-resistance gene *Tsn1*; and *Gb5* conferring resistance to greenbug (http://wheat.pw.usda.gov/GG3/. *Ae. speltoides* is considered to be the ancestor of the B and G genomes of tetraploid and hexaploid wheat (Kilian et al. 2007). However, as the only outcrossing species in the section *Sitopsis,* the differences between the S and B/G genomes are much greater than those between the

A and D genomes and their progenitor genomes (Dvorak 1976; Maestra and Naranjo 1998).

We previously developed a complete set of *T. aestivum-Ae. speltoides* chromosome addition lines (Friebe et al. 2000) and a set of disomic S(B/A)-genome chromosome substitution lines (Friebe et al. 2011). Here, we attempted the production of a set of wheat-*Ae. speltoides* Robertsonian translocations (RobTs) covering the complete *Ae. speltoides* genome. Although genetically closely related, the S- and B-genome chromosomes usually do not pair and recombine in the presence of the *Ph1* gene. Therefore, once this set of RobTs is established, gene transfer from *Ae. speltoides* can be achieved by crossing the appropriate wheat-*Ae. speltoides* RobT with the *Ae. speltoides* accession harboring the gene of interest. The RobTs also are pivotal material for finer genetic transfers by homoeologous recombination (Qi et al. 2007).

Materials and methods

Plant material

Seven *T. aestivum* cv. Chinese Spring-*Ae. speltoides* disomic chromosome substitution (DS) lines, DS1S#3(1B), DS2S#3(2B), DS3S#3(3A), DS4S#3(4B), DS5S#3(5B), DS6S#3(6A), DS6S#3(6B), and DS7S#3(7B), were used (Friebe et al. 2011). The nomenclature used for the description of chromosomes follows the guidelines suggested by Raupp et al. (1995), where the first number identifies the homoeologous group, followed by the genome designation and the letters S and L identify the short and long chromosome arm. The # sign is used to distinguish between the same homoeologous chromosome derived from different donor accessions. In the group-3 and group-6 substitution lines, *Ae. speltoides* chromosomes substitute for homoeologous A-genome chromosomes and line DS6S#3(6B) suffered from a terminal deletion in the short arm, resulting in the loss of the 6S#3S satellite. All plant material is maintained at the Wheat Genetics Resource Center at Kansas State University (http://www.k-state.edu/wgrc/). Each of the substitution lines was crossed to Chinese Spring to produce plants that were double monosomic for an S-genome and a homoeologous A- or B-genome chromosome. The chromosomal constitutions of the F_1 plants were determined in root-tip meristems, and the plants were allowed to self-pollinate to produce F_2 progenies that were screened for putative wheat-*Ae. speltoides* RobTs.

S-genome-specific PCR marker analysis

STS-PCR primers specific for S-genome chromosomes were designed on the basis of wheat expressed sequence tags (EST) mapped to wheat group 1–7 chromosomes by the wheat EST mapping project (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). STS-PCR amplification was according to Liu W et al. (2011b). PCR-amplified products were then divided into 10 µl aliquots and digested with six different four-base recognition restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, and *Mbo*I) for 2 h at 37 °C by adding 5 µl of enzyme mixture composed of 3.25 µl of ddH_2O, 1.5 µl of NEB buffer 2 or 4, 0.15 µl of 100× BSA and 0.1 µl of enzyme stock solution. PCR products were resolved on 1.5 % agarose gels and visualized by ethidium bromide staining under UV light.

The selection of the SSR markers was based on the SSR physical map of Sourdille et al. (2004). PCR was performed with 15 µl of reaction mixture containing 1× PCR buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM $MgCl_2$, 0.25 mM dNTPs, 5 pmol forward primer and reverse primer, respectively, 0.02 unit/µl of *T*aq DNA polymerase (Bioline USA Inc., Taunton, MA, USA) and 90 ng of genomic DNA. PCR amplification was according to Liu W et al. (2011b). Digestion products were resolved on 2.5 % agarose gels and visualized by ethidium bromide staining under UV light.

Mapped-FlcDNA-based markers used in this study were selected on the basis of the full-length cDNA map of Danilova et al. (2014). The sequences of FlcDNAs were blasted to the genomic DNA sequences of *Hordeum vulgare* L., *Brachypodium distachyon* L. and *Oryza sativa* L. to locate the sequences in the FlcDNA flanking putative introns larger than 300 bps in size. The PCR primers were designed based on the FlcDNA sequences flanking the introns by Primer3. PCR amplification and PCR product digestion followed STS-PCR protocols.

A total of 985 EST-based primers, 252 SSR primers and 211 FlcDNA-based primers were used to screen Chinese Spring wheat and the seven disomic wheat-*Ae. speltoides* substitution lines. We selected 16 EST-based primers (1.6 %), 4 SSR primers (1.6 %), and 12 FlcDNA-based primers (5.7 %) that were specific for S-genome chromosome arms (Table 1). The frequencies of informative markers were lower than reported previously for other *Aegilops* chromosomes (Liu W et al. 2011b). The frequency of informative markers derived from FlcDNA-based primers was 3.5 times higher than those derived from EST-based and SSR primers.

Identification of wheat-*Ae. speltoides* RobTs

Young leaves were collected from F_2 plants that were double monosomic for an S-genome and a homoeologous A- or B-genome chromosome. These plants were assayed with one distal marker for each of the short and long arm of the S-genome chromosome. Root tips were collected

Table 1 Polymorphic S-genome markers developed in this study

Marker type	EST-based			SSR			FlcDNA-based		
Chromosome	No. screened	No. polymorphic	Ratio (%)	No. screened	No. polymorphic	Ratio (%)	No. screened	No. polymorphic	Ratio (%)
1S#3	192	1	0.52	45	1	2.22	8	1	12.50
2S#3	96	1	1.04	44	0	0.00	95	4	4.21
3S#3	145	3	2.07	26	1	3.85	12	0	0.00
4S#3	96	0	0.00	53	1	1.89	31	3	9.68
5S#3	72	2	2.78	29	1	3.45	–	–	–
6S#3	192	5	2.60	25	0	0.00	37	0	0.00
7S#3	192	4	2.08	30	0	0.00	28	4	14.29
Total	985	16	1.62	252	4	1.59	211	12	5.69

from plants lacking one S-chromosome-specific marker to identify putative recombinants, which were then verified by genomic in situ hybridization (GISH) analysis. GISH was performed according to Liu W et al. (2011b) with minor modifications. Genomic DNA for probe labeling was extracted from *Ae. speltoides* using a DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). The ratio of *Ae. speltoides* probe to CS blocking DNA was 1:100–120 for GISH. In addition, two oligonucleotide probes, Cy-5(GAA)9 and 6-FAM-pAs1, painting tandem repeats, were used for fluorescence in situ hybridization (FISH) to identify the wheat chromosome arms involved in the translocated chromosomes. Post hybridization washes were in 2× SSC, twice at room temperature for 5 min each, twice at 42 °C for 10 and 5 min each, and once at room temperature for 5 min. Chromosomes were counterstained with propidium iodide (PI) or 4′,6-diamidino-2-phenylindole solution (DAPI) and mounted in Vectashield (Vector Laboratories, Burlingham, CA, USA, cat # H-1200, H-1300). Images were captured with a Zeiss Axioplan 2 microscope using a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics) and AxioVision 4.8 software (Zeiss). Images were processed with Adobe Photoshop CS3 (Version 10.0.1, Adobe Systems Incorporated, San Jose, CA, USA).

Results

Development of wheat-*Ae. speltoides* RobTs

Based on marker stabilities and deletion bin-locations on wheat chromosomes, we selected 17 markers to screen the progenies of double monosomic plants for marker dissociation indicating the presence of putative RobTs (Table 2). A total of 2050 plants from seven F_2 populations double monosomic for an S-genome and a homoeologous A- or B-genome chromosome were screened and 126 plants missing the short arm and 128 plants missing the long arm S-chromosome-specific markers were identified. Of the 254 plants showing marker dissociations, 53 plants with Robertsonian translocations and 4 plants with wheat-*Ae. speltoides* recombinant chromosomes were verified by GISH analysis. The remaining plants had either *Ae. speltoides* telosomes, isochromosomes, dicentric chromosomes, complete S-genome chromosomes or no GISH signals. The average frequency of recovered RobTs is 3.2 %, ranging from 0.7 % for chromosome 6S to 6.8 % for 5S. By combining S-chromosome-specific molecular marker analysis with GISH and FISH analysis, we were able to identify 13 compensating wheat-*Ae. speltoides* RobTs covering all *Ae. speltoides* chromosome arms except 4S#3L. Ten of the compensating wheat-*Ae. speltoides* RobTs involved homoeologous S- and B-genome chromosomes, three (T3S#3L·3AS, T3AS·3S#3L, T6S#3S·6AL) were between homoeologous S- and A-genome and one involved (T5DS.5S#3L) S- and D-genome chromosomes.

1S#3/1B RobTs

The FlcDNA-based marker *X1S2-1* located to the Chinese Spring chromosome deletion bin 1AS-0.86-1.0 and an EST-based marker *Xbe438469* mapped to the deletion bin 1BL3-0.85-1.0 were used as short and long arm markers to screen 181 F_2 progeny derived from plants double monosomic for chromosomes 1B and 1S#3. Ten plants were missing the short arm marker *X1S2-1* and five plants were missing the long arm marker *Xbe438469* (Table 3). GISH analysis of these 15 plants identified 3 RobTs (1S-76, 1S-160 and 1S-174) and 1 plant (1S-12) had a wheat-*Ae. speltoides* recombinant chromosome. The GISH/GAA-FISH pattern of plant 1S-174 showed that this plant was heterozygous for the compensating RobT T1BS·1S#3L (Figs. 1, 2a), that plant 1S-160 was heterozygous for the compensating RobT T1S#3S·1BL (Figs. 1, 2b), and plant 1S-76 had

Table 2 S-genome chromosome specific markers used in this study

Chromosome arm	Name	Forward primer sequence	Reverse primer sequence	Deletion bin	Enzyme	Source
1S#3S	*X1S2-1*	CTAGATGCTGCTGTGGGTGA	CTGTACTGCTGGCGTCGTTA	1AS-0.86–1.0	*Hae*III	FlcDNA-based
1S#3L	*Xbe438469*	GCTCGCTGCCACTTCTTTAC	ATCCAGAAGAACGCGACCAT	1BL3-0.85–1.0	*Msp*I	EST-based
2S#3S	*X2S4g9p4*	GAGGTCCGCATGAAGGCAAT	AATACACGCCGGAGAAGGGA	2AS5-0.78–1.0	*Hae*III	FlcDNA-based
2S#3L	*Xbe444521*	CCAATGACTGGCATGTGAAG	CTTCGGATCGAGACACTTCC	2BL6-0.89–1.0	*Mbo*I	EST-based
3S#3S	*Xbe426356*	CGTAACCTGTCACGAGCAGA	TGTGGACAGCATCAACAAGC	3DS3-0.24–0.55	*Mbo*I	EST-based
3S#3L	*Xbf484536*	GTTCCACCCCGCAGAAGA	CGCAGCTCGTCATCATAGAA	3DL3-0.81–1.0	*Mbo*I	EST-based
4S#3S	*X4S22*	CTCTCCCTGTTGAGCCTTTG	CGTTCAAGCTGATCCCTAGC	4AS1-0.26–0.63	*Alu*I	FlcDNA-based
4S#3L	*X4L22*	AAATCCTGCAATGGTGTTGG	TACTTCAGCGTTCGCAAGTG	C-4AL12–0.43	*Msp*I	FlcDNA-based
5S#3S	*Xgwm205*	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	5D43.7*	–	SSR
5S#3L	*Xbe607065*	CTCGATGCGCTGTATGAGAA	CAGCTTATCAGCTTGCTCCA	5BL16-0.79–1.0	*Hae*III	EST-based
6S#3S	*Xbe591786*	ATGGAGGAGATGGGGCTTAT	ATATGATCAGGGCGTGAAGC	C-6AS1-0.35	*Rsa*I	EST-based
	Xbe604119	GGACCCATGGCTTCTTAAAC	GGACCATGAAGGGGAGGTAG	C-6AS1-0.35	*Mbo*I	EST-based
6S#3L	*Xbe403154*	AATGCAGCTATGCCTTCTCA	GCACCTGCTACAGGTTCCTC	6AL8-0.9–1.0	*Mse*I	EST-based
7S#3S	*X7S45*	GGCTGCTGTACTTGGAGAGG	AATGGCAGCATTTCAAGGTC	7AS8-0.45–0.89	*Hae*III	FlcDNA-based
	X7S1-2	CCTTTATCTGCGGTGGACAT	TTAAGCATGGGTGGTCTTCC	7A(B,D), FL0.45**	*Hae*III	FlcDNA-based
7S#3L	*Xbf201318*	GGATTGGTCTGAGGGGAAAT	TGGACTCTTTGATCCGTTCC	7AL21-0.74–0.86	*Mse*I	EST-based

* Genetic map location, ** located by cDNA-FISH

Table 3 Molecular marker and GISH screening results of progenies derived from double monosomic S/(B/A) plants for homoeologous wheat and *Ae. speltoides* chromosomes

Target S chromosome	1S#3	2S#3	3S#3	4S#3	5S#3	6S#3	7S#3	Total
No. of plants screened	181	188	474	282	132	438	352	2047
No. of plants missing short arm marker	10	24	17	7	7	28	30	123
No. of plants missing long arm marker	5	7	40	14	20	13	29	128
No. of plants with a short arm RobTs (GISH)	1	3	5	1	6	2	6	24
No. of plants with a long arm RobTs (GISH)	2	9	4	2	3	1	3	24
No. of plants with a recombinant chromosome (GISH)	1	1			2			4
No. of plants with a dicentric chromosome (GISH)			1			1		2
No. of plants with a telochromosome (GISH)	3	12	10	6	4	6	18	59
No. of plants with a isochromosome (GISH)	3	2	8		1	8	13	35
No. of plants with a complete S chromosome (GISH)	3		7	2				12
No. of plants without GISH signals	2	4	22	10	11	23	19	91
Frequency of RobTS recovery (%)	1.7	6.4	1.9	1.1	6.8	0.7	2.6	3.0

a noncompensating RobTs identified as T1S#3L·1BL (not shown). The GISH/FISH-GAA analysis of plant 1S-12 identified the wheat-*Ae. speltoides* recombinant chromosome as T1S#3S·1S#3L-1BL (Fig. 1). Of the remaining 11 plants that showed marker dissociation, 3 plants had 1S#3 telosomes, 3 plants had isochromosomes, 3 plants had complete 1S#3 chromosome and 2 plants had no GISH signals (Table 3).

2S#3/2B RobTs

A total of 188 F_2 progeny derived from plants double monosomic for 2B and 2S#3 was screened with the group-2 short arm marker *X2S4g9p4* and the group-2 long arm marker *Xbe444521* (Table 2). Of these, 7 plants had only the short arm marker *X2S4g9p4* and 24 plants had only the long arm marker *Xbe444521*. GISH analysis of these plants revealed that three plants were heterozygous for 3S#3S RobTs, nine plants were heterozygous for 3S#3L RobTs and one plant was heterozygous for a recombinant chromosome. GISH/FISH-GAA analysis showed that plant 2S-103 (and plants 2S-5, 2S-49, 2S-143, 2S-150, 2S-151, 2S-164 and 2S-173) had the compensating RobT T2BS·2S#3L (Figs. 1, 2c) and that plant 2S-148 (and 2S-51) had the compensating RobT T2S#3S·2BL (Figs. 1, 2d). The GISH/FISH-GAA pattern identified the recombinant chromosome present in plant

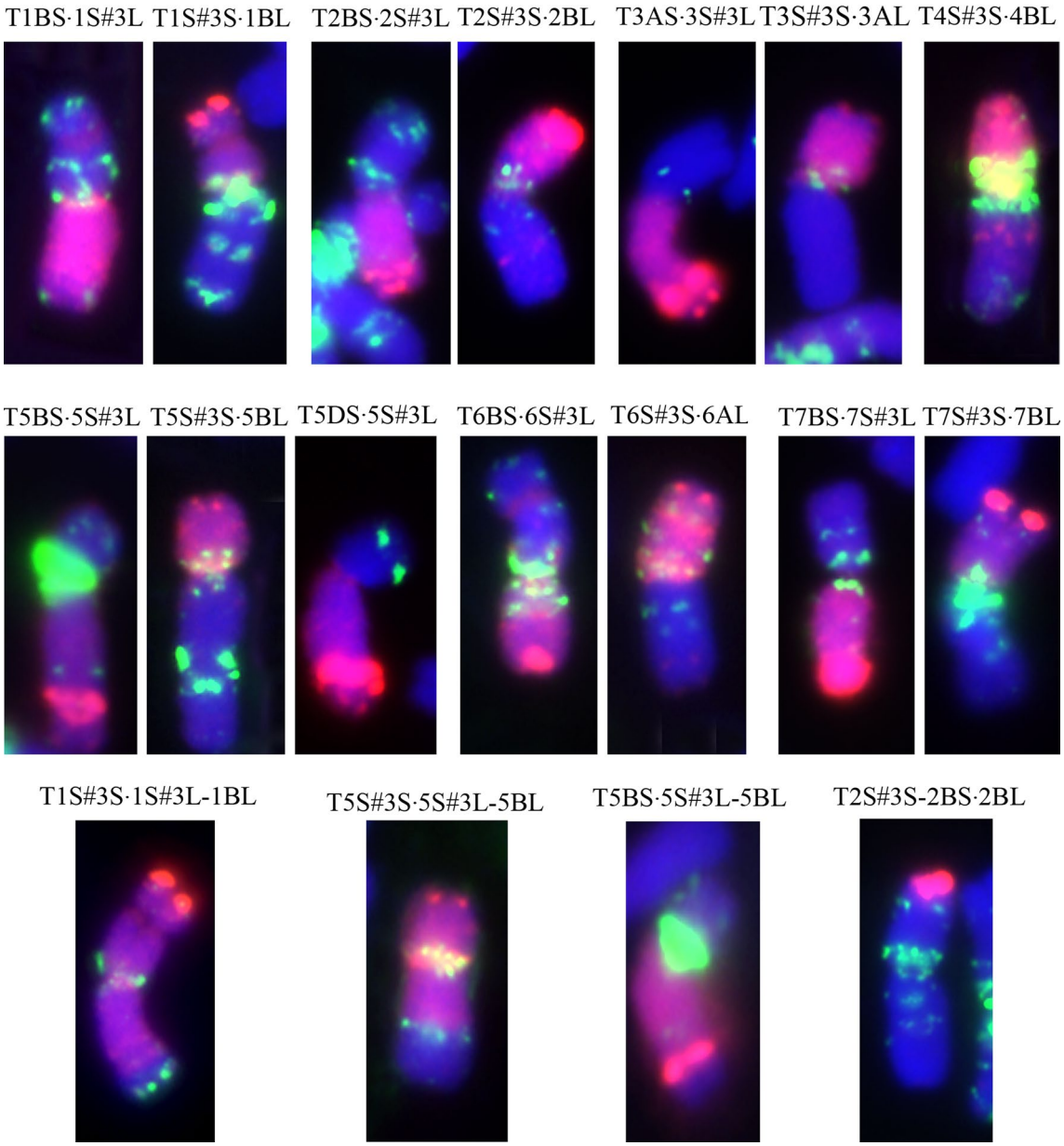

Fig. 1 GISH/GAA-FISH pattern of wheat-*Ae. speltoides* Robertsonian translocations, *Ae. speltoides* chromatin is visualized in *red* fluorescence, GAA hybridization sites are visualized by *green* fluorescence (for T5DS.5S#3L clone pAS1 was visualized with *green* fluorescence), and chromosomes are counterstained with DAPI and fluoresce *blue*

2S-38 as T2S#3S-2BS·2BL (Fig. 1). In addition, 2 plants had the noncompensating RobTs T2S#3L·2BL (2S-41) and T2S#3S·2BS (2S-81), 12 plants had telosomes, 2 plants had isochromosomes and 4 plants had no GISH signals (Table 3). The frequency of recovered RobTs for chromosome 2S#3 is much higher (6.4 %) than those of any other S-genome chromosome except 5S#3. However, the number of RobTs recovered also depends on the chromosome arm involved and 9 out of the 12 2S#3 RobTs involved the long arm of 2S#3.

3S#3/3A RobTs

A total of 474 F_2 progeny derived from plants double monosomic for 3A and 3S#3 was screened with the group-3 short arm marker *Xbe426356* and the group-3 long arm marker *Xbf484536* (Table 2). Seventeen plants were missing the short arm marker, whereas 40 plants were missing the long arm marker. Molecular marker and GISH analysis of these plants identified five RobTs involving the 3S#3 short arm (3S-211, 3S-244, 3S-262,

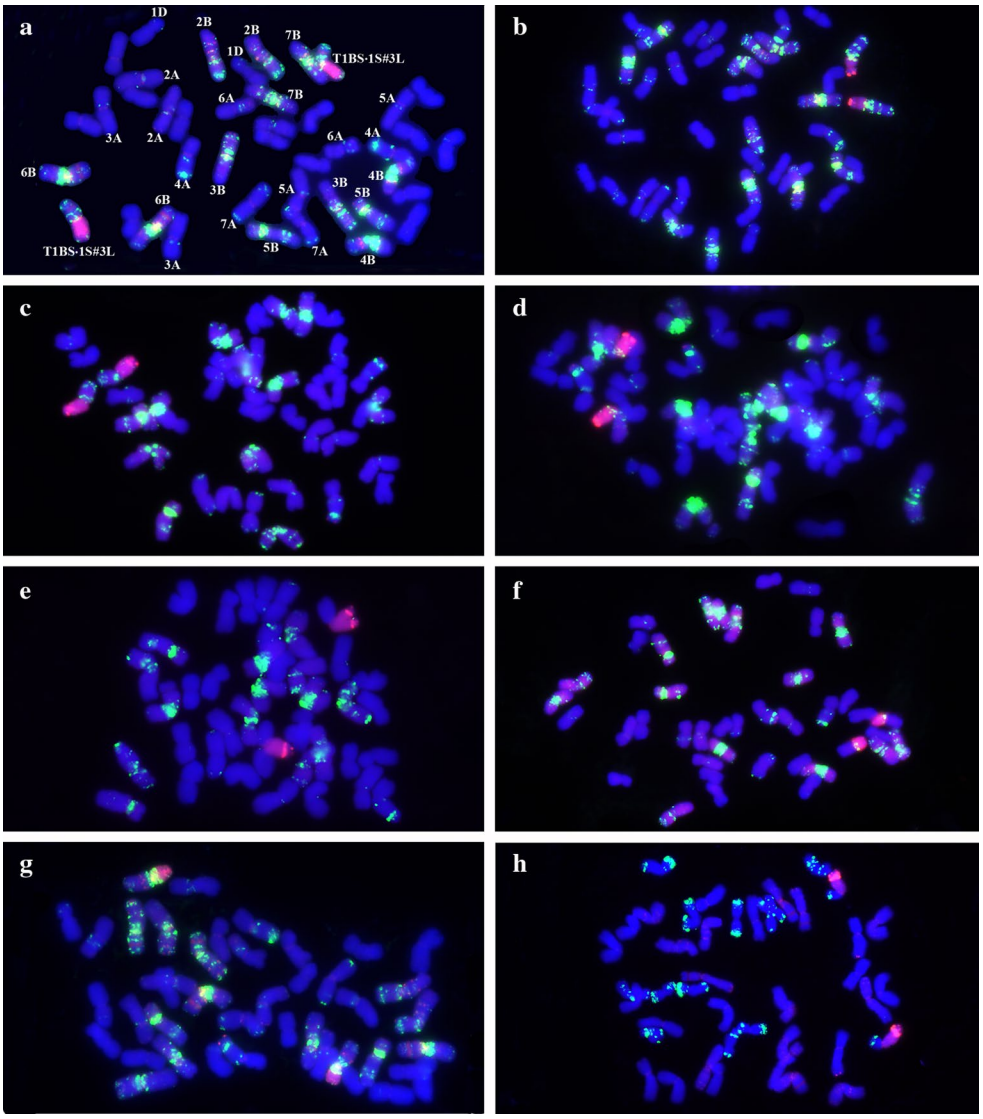

Fig. 2 GISH/GAA-FISH pattern of mitotic metaphase chromosomes homozygous for wheat-*Ae. speltoides* Robertsonian translocation: **a** T1BS.1S#3L, **b** T1S#3S.1BL, **c** T2BS.2S#3L, **d** T2S#3S.2BL, **e** T3AS.3S#3L, **f** T3S#3S.3AL, **g** T4S#3S.4BL, **h** T5DS.5S#3L; *Ae. speltoides* chromatin is visualized in *red* fluorescence, GAA hybridization sites are visualized by *green* fluorescence (for T5DS.5S#3L clone pAS1 was visualized with *green* fluorescence), and chromosomes are counterstained with DAPI and fluoresce *blue*

3S(4)24, and 3S(4)67) and four plants involving the 3S#3 long arm (3S-1, 3S-18, 3S-226, and 3S(4)86). GISH/FISH-GAA analysis showed that plant 3S(4)86 was heterozygous for the compensating RobT T3AS·3S#3L (Figs. 1, 2e) and that plant 3S(4)24 was heterozygous for the compensating RobT T3S#3S·3AL (Figs. 1, 2f). One plant (3S-202) was heterozygous dicentric chromosome involving the short arm of 3S#3. In addition, 10 plants had telosomes, 8 plants had an isochromosome, 7 plants had a complete 3S#3 chromosome, and 22 plants had no GISH signal (Table 3).

4S#3/4B RobTs

A total of 285 F_2 progeny derived from plants double monosomic for 4B and 4S#3 was screened with the group-4 short arm marker *X4S22* and the group-4 long arm marker *X4L22* (Table 2). Twenty-one progenies showed marker dissociation, with 7 plants missing the short arm marker and 14 plants missing the long arm marker. Molecular marker and GISH analysis revealed that one plant was heterozygous for a RobT involving the short arm of 4S#3 (4S-112) and that two plants (4S-4 and 4S-42) were heterozygous

for a RobT involving 4S#3L. Plants 4S-4 and 4S-42 were both sterile. The GISH/FISH-GAA of plant 4–112 revealed that the RobT present in this plant was a compensating type T4S#3S·4BL (Figs. 1, 2g). In addition, six plants had telosomes, two plants had complete 4S#3 chromosomes and ten plants had no GISH signals (Table 3).

5S#3/5B RobTs

A total of 132 F_2 progeny derived from plants double monosomic for 5B and 5S#3 was screened with the group-5 short arm marker *Xgwm205* and the group-5 long arm marker *Xbe607065* (Table 2). Seven plants were missing the short arm marker and 27 plants were missing the long arm marker. Molecular marker and GISH analysis identified six RobTs involving the 5S#3S arm and two plants involving the 5S#3L arm. GISH/FISH-GAA analysis revealed that plant 5S-25 was heterozygous for the compensating RobT T5BS·5S#3L and a wheat-*Ae. speltoides* recombinant chromosome T5BS·5S#3L-5BL (Figs. 1, 3a). The plant 5S-20 (and 5S-12 and 5S-63) were heterozygous for the compensating RobT T5S#3S·5BL (Figs. 1, 3b). Plant 5S-122 was heterozygous for the recombinant chromosome T5S#3S·5S#3L-5BL (Fig. 1). In addition, 4 plants had telosomes, 1 plant had an isochromosome and 11 plants had no GISH signals (Table 3). Because plants lacking the long arm of chromosome 5B are missing the *Ph1* locus that controls the diploid-like pairing of hexaploid wheat, we previously produced the compensating RobT T5DS.5S#3L, which is meiotically stable and fully fertile (Figs. 1, 2f) (Friebe et al. unpublished).

6S#3 RobTs

A total of 438 F_2 progeny derived from plants double monosomic 6A/6S#3 or 6B/6S#3 was screened with the group-6 short arm markers *Xbe591786* or *Xbe604119* and the group-6 long arm marker *Xbe403154* (Table 2). Twenty-eight plants were missing the short arm marker and 13 plants were missing the long arm marker. Molecular marker and GISH analysis identified one plant (6S-20) as heterozygous for a RobT involving the 6S#3L arm and two plants (6S-171 and 6S6A(5)137) were heterozygous for RobTs involving the 6S#3S arm. The GISH/FISH-GAA pattern of plant 6S-20 revealed that this plant had the compensating RobT T6BS.6S#3L (Figs. 1, 3c), whereas plant 6S6A(5)137 had the compensating RobT T6S#3S.6AL (Figs. 1, 3d). The plant 6S-171 had the compensating RobT T6S#3S.6AL but suffered from a deletion of the complete 6S#3S satellite. In addition, 1 plant had a dicentric chromosome, 6 plants had telosomes, 8 plants had isochromosomes and 23 plants had no GISH signal (Table 3).

7S#3/7B RobTs

A total of 352 F_2 progeny derived from plants double monosomic for 7B and 7S#3 was screened with the group-7 short arm markers *X7S45* or *X7S1-2* and the group-7 long arm marker *Xbf201318* (Table 2). Marker dissociation was observed in 59 of these progenies and molecular marker and GISH analyses identified 6 plants that were heterozygous for a RobT involving 7S#3S and 3 plants were heterozygous for RobTs involviong 7S#3L. GISH/FISH-GAA analysis showed that plant 7S-299 was heterozygous for the compensating RobT T7BS.7S#3L (Figs. 1, 3e) and that plant 7S-240 was heterozygous for the compensating RobT T7S#3S.7BL (Figs. 1, 3f). In addition, telosomes and isochromosomes were observed in 18 and 13 plants, respectively, and no GISH signal was observed in 19 plants.

Plants heterozygous for the compensating wheat-*Ae. speltoides* were allowed to self-pollinate and their progenies were screened by GISH to identify homozygous RobT plants, which were recovered for all RobTs except the one involving the long arm of chromosome 4S#3. The reason why we were unable to recover a fertile RobT for the 4S#3L arm is unknown. However, it is interesting to note that recently Nave et al. (2016) reported that the 4BL arm harbors a major domestication locus that affects seed dormancy and it is also possible that the loss of this locus might affect plant fitness. Progenies of the RobT T5BS·5S#3L and the lines with the recombinant chromosome T5S#3S·5S#3L-5BL and T5BS·5S#3L-5BL were missing the *Ph1* gene and as a result were cytologically unstable and produced new recombinant telosomes and rearranged complete 5S#3 chromosomes.

Sporophytic compensation data

Most of the wheat-*Ae. speltoides* RobTs had similar or slightly lower seed set compared with the parental wheat cultivar Chinese Spring (Table 4). One exception was the RobT T2S#3S.2BL. Plants of this stock were weak and set fewer seeds compared with Chinese Spring, which was also observed previously for the disomic substitution line DS2S#3(2B) (Friebe et al. 2011). Similarly, the RobT T5BS 5S#3L had lower seed set because this line was missing the major diploid pairing controlling gene *Ph1*, which leads to multivalent/univalent formation at meiotic metaphase I and results in partial fertility. This was expected and, thus, we also produced the RobT T5DS.5S#3L, which had normal seed set (Table 4).

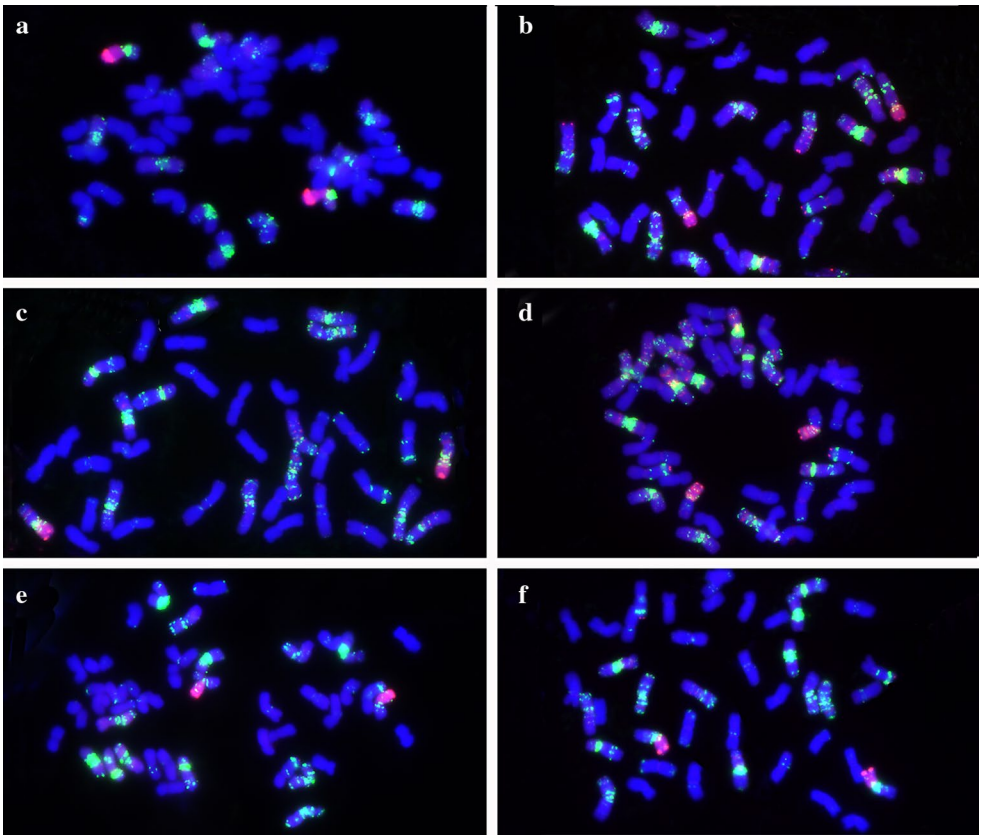

Fig. 3 GISH/GAA-FISH pattern of mitotic metaphase chromosomes homozygous for wheat-*Ae. speltoides* Robertsonian translocation: **a** T5BS.5S#3L, **b** T5S#3S.5BL, **c** T6BS.6S#3L, **d** T6S#3S.6AL, **e** T7BS.7S#3L, and **f** T7S#3S.7BL; *Ae. speltoides* chromatin is visualized in *red* fluorescence, GAA hybridization sites are visualized by *green* fluorescence, and chromosomes are counterstained with DAPI and fluoresce *blue*

Discussion

So far, chromosome deletion bin-mapped markers, including EST-based, SSR or KASP markers, are most often used for the development of wheat-alien translocations. A total of 985 EST-based markers were screened in this study, only 16 markers were S-genome-specific (1.6 %), ranging from 0 % for 4S to 2.78 % for 5S (Table 1). The mean frequency of S-genome-specific SSR markers was 1.59 %, which is even lower than that of EST-based markers (Table 1). No informative EST-based or SSR markers were identified for the short arm of chromosomes 1S, 2S or 4S, and both short and long arm of 7S. For obtaining chromosome arm-specific markers for these chromosomes, we used the Mapped-FlcDNA marker approach (Danilova et al. 2012, 2014). For this method, the FlcDNA-based primers were designed based on physically mapped full-length cDNA sequences flanking the introns. The PCR products were then further digested by four-base recognition restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, and *Mbo*I). This approach successfully identified S-genome specific markers for chromosome arms 1S#3S, 2S#3S, 4S#3S, 7S#3S, and 7S#3L with a mean frequency of 5.69 % (Table 1). The frequency of S-genome-specific markers observed in the present study is lower than those reported previously for *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey, *Leymus racemosus* (Lam.) Tzvelev, *Dasypyrum villosum* (L.) *P. Candargy* and *Ae. geniculata* Roth. (Liu C et al. 2011a, b).

The frequency of RobTs recovery depends on the chromosome and chromosome arms involved. In this study, chromosome 3S#3 and 5S#3 had the highest RobT recovery frequencies with 6.4 and 6.8 %, respectively. However, whereas three times more RobTs were recovered for the long arm of 2S#3, twice as many RobTs were observed for the short arm of chromosome 5S#3. The frequencies of recovered RobTs in progenies double monosomic for an alien and homoeologous wheat chromosome are similar to those reported previously ranging from a few up to almost 20 % (Lukaszewski 1993, 1994, 1997; Marais and Marais 1994; Friebe et al. 2005; Liu C et al. 2011a; Liu W et al. 2011b).

Table 4 Sporophytic compensation of *Aegilops speltoides* chromosome arms substituting for homoeologous B- and A-genome chromosomes of wheat (seed set was scored in three spikes per plant and five plants per line)

Line	Chromosomal constitution	Seed set per spikelet standard deviation
Chinese spring		2.9 ± 0.3
TA5598 L2	T1S#3S.1BL	3.0 ± 0.2
TA5678	T1BS.1S#3L	2.9 ± 0.5
TA5679	T2S#3S.2BL	2.1 ± 0.1*
TA5680L1	T2BS.2S#3L	2.7 ± 0.1*
TA5681	T3S#3S.3AL	2.5 ± 0.2
TA5682	T3AS.3S#3L	2.7 ± 0.1*
TA5683	T4S#3S.4BL	2.5 ± 0.3
TA5684 L3	T5S#3S.5BL	2.5 ± 0.4
TA5685	T5BS.5S#3L	1.0 ± 0.3*
TA5088	T5DS.5S#3L	2.5 ± 0.3
TA5686	T6S#3S.6AL	2.4 ± 0.2
TA5687	T6BS.6S#3L	2.9 ± 0.2
TA5688	T7S#3S.7BL	2.3 ± 0.4
TA5689	T7BS.7S#3L	2.5 ± 0.3*

* Seed set was scored on two plants

During the production of the wheat-*Ae. speltoides* chromosome addition lines, we identified one plant in which chromosome 6S#3 spontaneously substituted for wheat chromosome 6A resulting in the disomic substitution DS6S#3(6A) (Friebe et al. 2000). When we initiated the project to produce a complete set of disomic wheat-*Ae. speltoides* chromosome substitution lines, we targeted the B-genome chromosomes of wheat because they are supposed to be more closely related to the S-genome chromosomes compared to those of the A and D genomes. Thus, we crossed disomic S-genome chromosome addition plants ($2n = 44$) as males with the homoeologous B-genome monosomic ($2n = 41$) stocks and screened the resulting progenies for $2n = 42$ chromosome plants that were double monosomic for an S-genome and a homoeologous B-genome chromosome. GISH analysis of the self-pollinated offspring of these plants was then used to identify disomic S/B-genome substitutions, which were recovered for all S-genome chromosomes except for DS3S#3(3B) (Friebe et al. 2011, and this study). In this cross combination, we recovered a disomic substitution where 3S#3 substituted for the missing chromosome 3A of wheat DS3S#3(3A). We then used FISH analysis, using the GAA repeat as probe, to verify the chromosomal constitution of our 3B monosomic stock and confirmed that this line was indeed monosomic for chromosome 3B and not monosomic for 3A of wheat. These results suggest that at least chromosomes 3S#3 and 6S#3 have a close affinity to the corresponding A-genome chromosomes. Because we crossed the DS3S#3(3A) stock with euploid Chinese Spring wheat for the development of the compensating RobTs, both the group-3 short and long arm RobTs involve chromosome 3A of wheat.

The set of compensating wheat-*Ae. speltoides* RobTs will be very helpful for transferring agronomically useful genes from any *Ae. speltoides* accession into bread wheat. As reported previously (Friebe et al. 1994; Nagy et al. 2003; Cainong et al. 2010; Liu C et al. 2011a), once a target gene is identified in any *Ae. speltoides* accession and mapped to a specific chromosome arm, this accession can then be crossed with the appropriate wheat-*Ae. speltoides* RobT. In the resulting hybrid, the *Ae. speltoides* chromosome arm in the RobT will easily pair and recombine with the homologous chromosome arm of the *Ae. speltoides* accession and gene transfer can be achieved by homologous recombination and, after backcrossing, adapted lines can be obtained. These RobTs may harbor useful genes and will be the starting material for finer genetic transfers by homoeologous recombination (Qi et al. 2007).

Author contribution statement WL and D-HK performed the molecular marker and GISH analyses and WL, D-HK, BSG, and BF participated in writing the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have conflict of interest.

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