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Production of hexaploid triticale by a synthetic hexaploid wheat-rye hybrid method

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Abstract Hexaploid triticale, including its primary and secondary forms, is an important forage crop and a promising energy plant. Primary forms are usually developed by crossing *Triticum turgidum* L. with rye, with secondary forms obtained by crossing primary hexaploid triticale and/or hexaploid wheat with octoploid triticale. In this study, we developed an effective method for production of hexaploid triticale via hybridization of synthetic hexaploid wheat (SHW) with rye. The three employed SHW lines were derived from hybridization of *T. turgidum* with *Aegilops tauschii* Cosson, and inherited meiotic restitution genes, which can promote the formation of functional gametes in haploid status, from their *T. turgidum* parents. Although the resulting tetraploid F_1 hybrids with rye (genome ABDR) produced amphiploids (octoploid triticale) and partial amphiploids, the final hybrid products obtained through fertility selection over several generations were hexaploids. These hexaploids were the result of preferential elimination of D-genome chromosomes. In addition to complete hexaploid triticale with 28 intact A/B and 14 intact R chromosomes, we obtained hexaploid triticales with other chromosome constitutions, including monosomic, substitution, and translocation lines. Chromosomes 2D and 5D from the wild species *A. tauschii* were incorporated into the hexaploid triticales. Out of eight analyzed stable lines derived from three different SHW-L1/rye F_1 plants, we observed four lines with small-fragment translocations between wheat and rye chromosomes. Rapid production of hexaploid

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triticales using this method involves two factors: (1) hybridization between hexaploid wheat with a meiotic restitution gene(s) and rye and (2) selection for good fertility during F_3 and subsequent generations.

Keywords Chromosome elimination · Meiotic restitution · Translocation · Triticale

Introduction

Triticale (× *Triticosecale* Wittmack) is the first man-made crop derived from a cross of wheat (*Triticum*) and rye (*Secale*). It combines the high yield potential and grain quality of wheat with the growth vigor and tolerance to non-optimal growing environments possessed by rye (Varughese et al. 1997; Larter 2009). In 2009, 15.0 million tons of triticale grains were harvested from 29 countries (FAOSTAT Database 2010). Triticale is mainly used for forage or fodder, serving as a good source of protein, lysine, B vitamins, and readily digested starch; it is also used on a smaller scale as an ingredient in human food and in the brewing industry (Bird et al. 1999; McKevith 2004; Glatthar et al. 2005; Mcgoverin et al. 2011; Rakha et al. 2011). This hardy crop can be grown in marginal areas with poor soils and unsuitable climates, producing high yields where other cereal crops, such as wheat, perform unsatisfactorily (Griffith and Lang 2004). It thus has potential in fighting world hunger (Hansen 2010). Triticale has also recently been recognized as a promising energy crop because of its high biomass and grain yield (Oettler 2005; Davis-Knight and Weightman 2008; Bilgili et al. 2009; Bassu et al. 2011; Gowda et al. 2011; Estrada-Campuzano et al. 2012). In 2005, the Canadian Triticale Biorefinery Initiative (CTBI) was established to develop triticale as a dedicated industrial biofuel feedstock crop.

The first triticales to be developed were octoploids ($2n = 8x = 56$, AABBDDRR) generated by chromosome doubling of hybrids between common wheat (*Triticum aestivum* L.) and rye. Following the introduction of successful embryo culture techniques in the late 1940s, hexaploid triticales ($2n = 6x = 42$, AABBRR) were developed by crossing tetraploid wheat (*T. turgidum* L.) with rye. These primary hexaploid triticales are amphiploids containing all 28 wheat and 14 rye chromosomes. Because of their greater meiotic stability and fertility, hexaploid triticales are more successful crop plants than octoploid triticales (Lukaszewski and Gustafson 1987; Fox et al. 1990; Cheng and Murata 2002). Secondary hexaploids are primarily derived from crosses of primary hexaploid triticale and/or hexaploid wheat with octoploid triticale. In addition, hexaploid triticales can spontaneously appear in the selfing progenies of octoploid triticale (Nakata et al. 1984; Dou et al. 2006; Zhou et al. 2012). The majority of triticale grown worldwide today is secondary hexaploid triticale because of its desirable agronomic traits (Fox et al. 1990).

Aegilops tauschii Cosson ($2n = 2x = 14$, DD) is the source of the D-genome of common wheat. Although this wild species has been not used in triticale development, its D-genome may accelerate environmental adaptation (Gororo et al. 2001; Trethowan and Mujeeb-Kazi 2008) and enhance early plant vigor (Ter Steege et al. 2005). In addition, genetic variability within the D-genome of *A. tauschii* is much higher than within the D-genome of common wheat (Van Ginkel and Ogbonnaya 2007; Yang et al. 2009). The *A. tauschii* D-genome can be quickly incorporated into synthetic hexaploid wheat (SHW) using spontaneous chromosome doubling in haploid hybrids of *T. turgidum–A. tauschii* via unreduced gametes resulting from meiotic restitution (Zhang et al. 2010). Meiotic restitution also occurs in SHW-rye F_1 hybrids and results in the production of amphiploids or partial amphiploids (Zhang et al. 2007). While these observations suggest that SHW-rye hybridization may be useful for triticale development, its effectiveness needs to be further evaluated.

In this study, we identified the chromosome constitutions of some highly fertile derivatives of three SHW-rye hybrid combinations. These derivatives were usually found to be hexaploid triticales, and included monosomic, substitution, and translocation forms. The results of our study demonstrate that using meiotic restitution in hexaploid wheat-rye hybridization provides a simple method for hexaploid triticale development via the preferential elimination of D-genome chromosomes in the hybrid derivatives.

Materials and methods

Plant materials

Three synthetic hexaploid wheats, SHW-L1, Syn-SAU-8, and Syn-SAU-18, were used as female parents

in crosses with Chinese rye (*Secale cereale* L., $2n = 2x = 14$, RR) landrace Qinling (AS156). SHW-L1 was derived from a cross of *T. turgidum* ssp. *turgidum* line AS2255 with *A. tauschii* accession AS60, and inherited a gene(s) for meiotic restitution from AS2255 (Zhang et al. 2007). Syn-SAU-8 and Syn-SAU-18 were produced by spontaneous chromosome doubling via unreduced gametes resulting from meiotic restitution in *T. turgidum* ssp. *durum* 'Langdon' × *A. tauschii* AS2386 and *T. turgidum* ssp. *turgidum* AS2236-1 × *A. tauschii* AS77 hybrids (Zhang et al. 2010). These hexaploids also inherited the gene(s) for meiotic restitution from their *T. turgidum* parents Langdon and AS2236-1 (Zhang et al. 2010).

Emasculation and pollination were carried out as described by Liu et al. (1999). No embryo rescue or hormone treatment was applied for the production of F_1 seeds. F_2 and F_3 hybrid seeds were obtained by selfing individual F_1 or F_2 plants, respectively. F_1–F_3 hybrid seeds were germinated in petri dishes on filter paper, and the seedlings were transplanted into an experimental field using within-row spacing of 10 cm, with 30 cm between rows. F_3 plants having relatively good seed set of over 50 seeds were selected and then advanced to the F_4 generation. F_4 plants with high fertility, having at least 100 seeds, were advanced to the F_5 generation.

The crossability of the two species in each hybrid cross was calculated as the percentage of F_1 seeds obtained relative to the number of florets pollinated for that cross. The selfed seed set was calculated as the percentage of total seeds obtained relative to the total number of spikelets.

Cytological observations

Cytological observation of chromosome numbers in root-tip cells and chromosome pairing in pollen mother cells (PMC) in hybrid plants was according to procedures described previously (Zhang et al. 2007). For meiotic analysis, at least 20 PMCs were observed for each plant. Univalents (I), bivalents (II), trivalents (III), and quadrivalents (IV) were counted and their average numbers calculated.

For genomic in situ hybridization (GISH), tips of fixed roots were squashed in a drop of 45 % acetic acid. After freezing with liquid nitrogen, the squashed slides were uncovered, air-dried, and stored at −20 °C until use. GISH was performed as described previously (Hao et al. 2011). The GISH hybridization mixture included 100 % formamide, 20× SSC, probe DNA, sheared genomic DNA of common wheat cultivar Chinese Spring (as a blocker), sheared salmon sperm DNA, and 5 % dextran sulfate.

Clones pAs1 (Nagaki et al. 1995), pSc119.2 (Contento et al. 2005), and pTa71 (Fujisawa et al. 2006) were used as probes for fluorescence in situ hybridization (FISH). pAs1 is a D-genome-specific clone. Although weak hybridization signals are observed on some B and A-genome chromosomes, the FISH pattern obtained with this clone permits identification of the D-genome chromosomes (Rayburn and Gill 1986; Pedersen and Langridge 1997). pSc119.2 was used to identify B and R-genome chromosomes, and pTa71 was used to identify satellites. pAs1 and pSc119.2 were labeled with biotin-16-dUTP (Roche Diagnostics Gmbh, Mannheim, Germany REF 11745816910) or digoxigenin-11-dUTP (Roche Diagnostics Gmbh, Mannheim, Germany, REF 11745816910) according to the manufacturer's instructions. pTa71 was labeled with 50 % biotin-16-dUTP and 50 % digoxigenin-11-dUTP (Roche). Total genomic DNA of the rye cultivar Qinling was labeled with digoxigenin-11-dUTP (Roche) or biotin-16-dUTP (Roche). Unlabeled genomic DNA of the common wheat cultivar Chinese Spring was used as blocking DNA. Detection of the biotinylated probe was accomplished with streptavidin-Cy3 (SIGMA-ALDRICH CHEMIE Gmbh, steinheim, Germany, Pcod 1001238444) and digoxigenin using anti-digoxigenin-fluorescein (Roche Diagnostics Gmbh, Mannheim, Germany, REF 11207741910). The preparations for in situ hybridization were counterstained for analysis with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Inc., Burlingame, USA) or propidium iodide (PI) (Vector Laboratories, Inc., Burlingame, USA). Chromosome observations were made and documented using an Olympus BX-51 microscope coupled to a Photometric SenSys Olympus DP70 CCD camera. Raw images were processed using Photoshop ver. 7.1 (Adobe Systems Incorporated, San Jose, CA, USA).

Field evaluations

Field trial evaluations were conducted during the 2011–2012 growing season at the Triticeae Research Institute Experimental Station in Wenjiang, Sichuan

Table 1 Chromosomal constitutions and agronomic traits of triticale lines from SHW-L1/rye hybrids

Line	Chromosome constitutions[a]	No. of tillers	Plant height (cM)	Spikelet length (cM)	No. of spikelets on main spike	1000-grain weight (g)	Seed set[b] (seeds/spikelet)
186-1-4-18-8-8-27	42 (28AB + 14R)	9.3	139.7	14.3	31.3	26.8	1.1 (233.7/222.3)
186-3-5-26-14-14-31	42 (28AB + 14R)	6.6	157	13.3	34.8	27.5	1.4 (274.6/192.6)
186-4-2-31-17-17-33	42 (24AB + 14R + four T)	8.3	139.4	16.3	33.8	23.4	1.1 (224.3/222)
188-2-6-37-20-20-35	42 (26AB + two 2D + 12R + two T)	5.6	148.4	12.7	27	23	1.4 (204.1/144.5)
190-2-3-42-23-33-39	42 or 41 (27AB with one 6B + 14R)	6.1	167	13.6	35.6	28.6	1.5 (279.7/196.1)
10-1-1-57-28-28-43	41 (28AB + 13R) or (28AB + 12R + one T)	7.8	159	17.4	37.8	27.8	1.6 (387.5/242.4)
10-1-1-57-28-29-45	42 (28AB + 12R + two T)	5.3	168. 9	15.4	38.7	38.5	1.6 (257.6/171.4)
56-5-1-31-49	42 (28AB + 12R + two T)	3.8	171.7	14.2	36.4	40.2	1.7 (188.7/108.9)

[a] *AB* A and B chromosomes, *T* translocation

[b] Seed set was calculated as the ratio of the total number of seeds obtained relative to the total number of spikelets per plant

Province, China. Individual plants were spaced 10 cm apart within 2-m-long rows, with row spacings of 30 cm. For new triticale lines derived from SHW-L1/rye hybrids, experiments consisted of two replicates. At maturity, plant height, tiller number per plant, spike length, spikelet number, and 1000-grain weight were evaluated from 10 plants randomly selected from each plot. Plant height was calculated as height in centimeters measured from the soil surface to the tip of the spike (awns excluded). The main spikes of the selected plants were measured for spike length, spikelet number, and 1000-grain weight. The average value for each trait was then calculated.

Results

SHW-L1/rye, Syn-SAU-8/rye, and Syn-SAU-18/rye intergeneric hybrid combinations had high crossabilities, i.e., 32.6 % (140/430), 22.6 % (19/84), and 53.9 % (55/102), respectively. The 19 analyzed F_1 plants (10 from SHW-L1/rye, 2 from Syn-SAU-8/rye, and 7 from Syn-SAU-18/rye) all grew vigorously and were partially fertile. They produced a total of 112 F_2 seeds (6 seeds/plant) by selfing. F_2 plants showed a large range of variation with respect to seed set by selfing: of 18 plants investigated, 10 did not set F_3 seeds, while the remaining eight produced a total of 245 seeds. F_3 plant seed set also varied greatly, ranging from 0 to 510 seeds/plant. Vigorous F_3 plants with good seed-setting ability, derived from eight F_1 plants, were advanced to the next generation and used for further investigation.

SHW-L1/rye hybrid combinations

One F_5 and seven F_7 lines derived from SHW-L1/rye F_1 hybrid plants 10, 56, 186, 188, and 190 were analyzed. The plants grew vigorously and had a high level of seed set (Table 1). GISH and FISH were further used to identify their chromosome constitutions. By using PTa71, Psc119.2 and PAs1 as probes, we can identify the chromosomes of wheat parent SHW-L1 (Fig. 1). The two F_7 lines 186-1-4-18-8-8-27 (Fig. 2a) and 186-3-5-26-14-14-31 had the same chromosome constitution (28A/B and 14R chromosomes) as the primary hexaploid triticale between *T. turgidum* and rye. The F_7 line 190-2-3-42-23-33-39 was 6B-monosomic (Fig. 2b). Interestingly, five of the

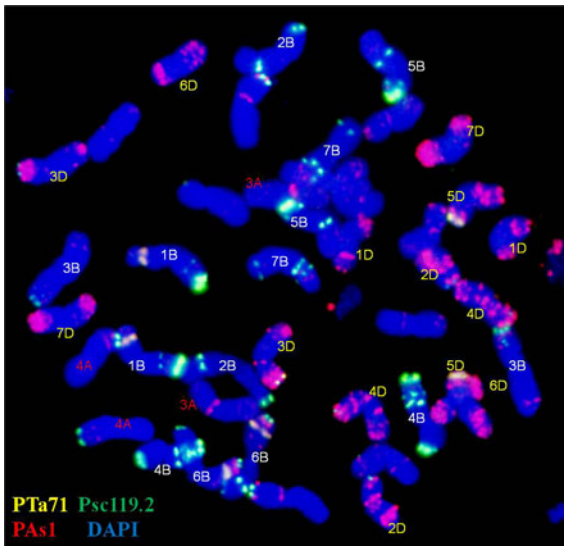

Fig. 1 Fluorescent in situ hybridization (FISH) using PAs1 (*red*), PSc119.2 (*green*), and PTa71 (*yellow*) as probes on mitotic chromosomes of wheat parent SHW-L1

eight lines carried translocations. Lines 10-1-1-57-28-29-45 (Fig. 2c) and 56-5-1-31-49 had a pair of terminal translocations involving large rye and small wheat fragments (Fig. 2d) in addition to possessing 28A/B and 12R chromosomes. The translocations in the two lines were of different origins, as they were derived from two different F_1 plants. The PMCs of lines 10-1-1-57-28-29-45 and 56-5-1-31-49 showed normal meiotic behavior, possessing approximately 21 bivalents, with chromosome pairing configurations of 0.71 I + 6.18 rod II + 14.47 ring II and 1.00 I + 7.47 rod II + 12.78 ring II + 0.13 III + 0.03 IV, respectively. Line 186-4-2-31-17-17-33 had 24A/B and 14R chromosomes and two pairs of centric translocations (Fig. 2e), i.e., 6BS-?RS/L and ?RS/L-6BL (Fig. 2f). A pair of 2D chromosomes and a pair of wheat-rye terminal translocations were identified in line 188-2-6-37-20-20-35 (Fig. 2g). We observed two cytotypes in F_7 line 10-1-1-57-28-28-43. One consisted of 28A/B and 13R chromosomes; the other comprised 28A/B and 12R chromosomes and one translocation chromosome (Table 1; Fig. 2h), indicating that this line was still cytologically unstable.

Syn-SAU-8/rye hybrid combinations

All material used for cytological observation of Syn-SAU-8/rye hybrid combinations was derived from F_2 plant 228(1)-1, which produced 51 F_3 seeds. Three F_4 plants were randomly analyzed. One of them, 228(1)-1-20-3, was a hexaploid triticale with 28A/B and 14R chromosomes (Table 2). Plant 228(1)-1-32-18 had 26A/B chromosomes and 15 rye chromosomes, including 3 1R chromosomes and 1 rye chromosome fragment (Table 2; Fig. 3a). Its F_5 seed 228(1)-1-32-18-3 possessed all the A/B chromosomes except for 1B and had 16 rye chromosomes including 4 1R chromosomes (Fig. 3b). Loss of D-genome chromosomes was also observed in six other analyzed F_5 seeds, as they showed no FISH signal specific for the D-genome. Of these six, plants 228(1)-1-32-4-2 (Fig. 3c), 228(1)-1-32-19-2, 228(1)-1-32-25-2, and 228(1)-1-32-26-1 were all hexaploid triticales. Plant 228(1)-1-32-4-1 was an aneuploid hexaploid with a monosomic 1B (Fig. 3d). Plant 228(1)-1-32-1-2 had 28A/B and 13R chromosomes plus 3 rye chromosome fragments (Table 2).

Syn-SAU-18/rye hybrid combinations

Two fertile F_1 plants, 233(1) and 233(2), produced a total of 22 F_2 seeds. The F_2 plants 233(1)-2 and 233(1)-6 produced 21 and 75 F_3 seeds, respectively. Three F_3 seeds 233(1)-6-2 (Fig. 4a), 233(1)-2-20, and 233(1)-6-12 were analyzed; they all had 14 intact rye chromosomes (Table 2). The seed 233(1)-2-20 also had one wheat-rye translocation chromosome and one rye chromosome fragment (Fig. 4b). The translocation chromosome formed a pair in the F_4 seed 233(1)-2-20-9 (Table 2). A pair of 5D chromosomes in F_4 seed 233(1)-2-20-4 (Fig. 4c) were inherited by its F_5 hybrid 233(1)-2-20-4-2, which had 26 A/B and 14 R chromosomes plus a pair of 5D chromosomes (Fig. 4d).

Discussion

Formation of functional gametes in F_1 haploid hybrids of synthetic hexaploid wheat and rye

Because they often produce non-functional reduced gametes, wheat-rye F_1 haploid hybrids are usually sterile or have very low fertility. In an earlier study (Zhang et al. 2007), we observed partially fertile tetraploid F_1 hybrids (genome ABDR) when synthetic hexaploid wheat line SHW-L1 was crossed with rye, and hypothesized that production of functional

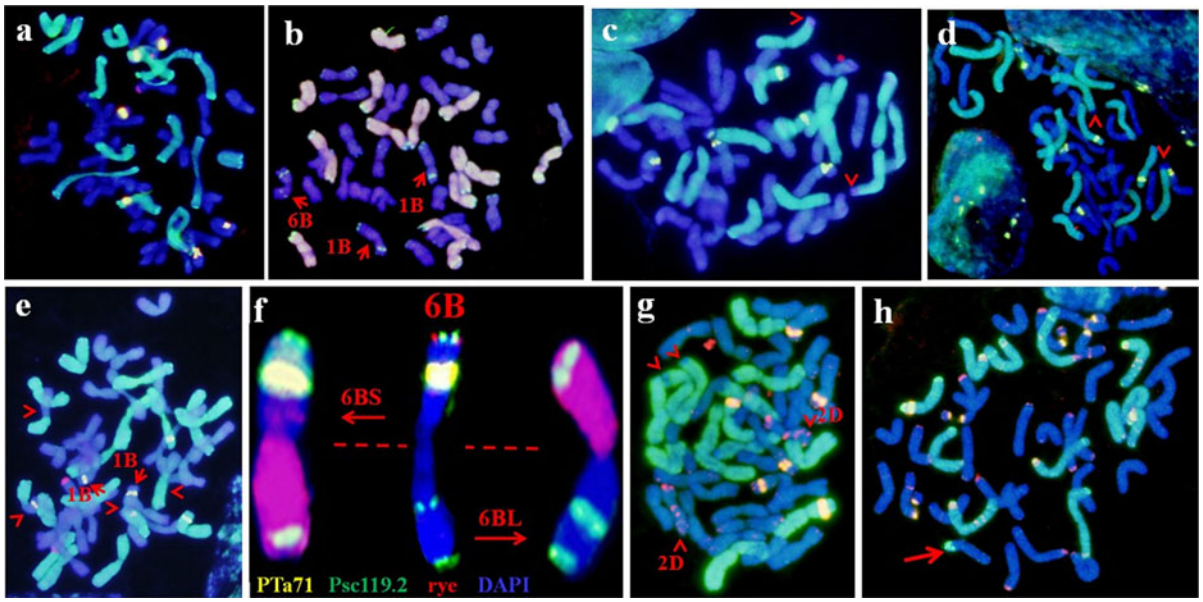

Fig. 2 Chromosome constitutions of SHW-L1/rye lines. In **a**, **c**, **d**, **e**, and **g**, chromosome constitutions were analyzed using R-genome (*green*), pTa71 (*yellow*), and pAs1 (*red*) probes, with unlabeled wheat genome DNA used as a blocker. **a** Line 186-1-4-18-8-8-27 had 28A/B and 14R chromosomes. **b** Line 190-2-3-42-23-33-39 had 27A/B and 14R chromosomes. It was first determined to have 27A/B and 14R chromosomes using R-genome, pTa71, and pAs1 probes. The slide was then re-analyzed with a pSc119.2 (*green*) probe, which revealed the absence of one 6B chromosome (*arrow*). **c** Line 10-1-1-57-28-29-45 had 28A/B and 12R chromosomes and a pair of wheat-rye translocation chromosomes (*arrows*). **d** Line 56-5-1-31-49 had 28A/B and 12R chromosomes and a pair of wheat-rye chromosomes (*arrows*). **e** Line 186-4-2-31-17-17-33 had 24A/B and 14 rye chromosomes and two pairs of centric wheat-rye chromosomes (*arrows*). **f** The two pairs of translocations in line 186-4-2-31-17-17-33 were reciprocal translocations between 6B and 2R or 3R based on analyses using pSc119.2 (*green*), pTa71 (*yellow*), and R-genome (*red*) probes, with unlabeled chinese spring genome DNA used as a blocker. **g** Line 188-2-6-37-20-20-35 exhibited 26A/B and 12R chromosomes, a pair of wheat-rye translocations (*arrows*), and a pair of 2D chromosomes (*arrows*). **h** Line 10-1-1-57-28-28-43 had 28A/B and 12R chromosomes and one translocation (*arrow*). It was first analyzed with R-genome (*green*), pTa71 (*yellow*), and pAs1 (*red*) probes, and then re-analyzed with rye (*green*), pTa71 (*yellow*), and pSc119.2 (*red*) probes. The analysis, however, was unable to identify which chromosomes were involved in translocations

gametes was induced by a meiotic restitution gene(s) from SHW-L1. This observation was further confirmed in the present study by the generation of partially fertile Syn-SAU-8/rye and Syn-SAU-18/rye F_1 hybrids. Both Syn-SAU-8/rye and Syn-SAU-18/rye have inherited meiotic restitution genes from their *T. turgidum* parents (Zhang et al. 2010). We therefore infer that the production of functional gametes in the present study was also promoted by meiotic restitution genes. When 10 SHW-L1/rye F_2 plants were randomly selected in the earlier study (Zhang et al. 2007), three were found to be amphiploids (octoploid, $2n = 56$), with the remaining plants determined to be partial amphiploids. This observation indicates that a large fraction of the F_2 seeds produced were derived from aneuploid gametes. A high ratio of functional aneuploid gametes to euploid gametes has also been reported in other haploid hybrids possessing meiotic restitution genes, including *T. turgidum–A. tauschii* (Zhang et al. 2008) and synthetic hexaploid wheat SHW-L1-*A. variabilis* with genome ABDUS[1] (Yang et al. 2010). It is thus obvious that meiotic restitution can result in aneuploid functional gametes in addition to euploid gametes.

Production of hexaploid triticale by preferential elimination of D-genome chromosomes

The original goal of this study was incorporation of the D-genome of the wild species *A. tauschii* into octoploid triticale ($2n = 8x = 56$, AABBDDRR) using synthetic hexaploid wheat, which has a meiotic restitution gene(s). When hybrid plants with high fertility were analyzed, however, no octoploid

Table 2 Chromosome constitutions of partial Syn-SAU-8/rye and Syn-SAU-18/rye hybrid plants

Plant code	No. of chromosomes (chromosome constitution) [a]
Syn-SAU-8/rye	
228(1)-1-20-3 F4	42 (28AB + 14 R)
228(1)-1-32-18 F4	41 (26AB absent two 1B + 15R with three 1R) + 1RF
228(1)-1-32-1-2 F5	41 (28AB + 13R) + 3RF
228(1)-1-32-4-1 F5	41 (27AB absent one 1B or 6B + 14R)
228(1)-1-32-4-2 F5	42 (28AB + 14 R)
228(1)-1-32-18-3 F5	42 (26AB absent two 1B + 16R with four 1R)
228(1)-1-32-19-2 F5	42 (28AB + 14 R)
228(1)-1-32-25-2 F5	42 (28AB + 14 R)
228(1)-1-32-26-1 F5	42 (28AB + 14 R)
Syn-SAU-18/rye	
233(1)-2-20 F3	? (14R + ?W + one w-r translocation) + 1RF
233(1)-6-2 F3	44 (30 W + 14R)
233(1)-6-12 F3	42 (28 W + 14R)
233(1)-2-20-9 F4	? (? + two 5D + 3WF + 1RF + two? w-r translocations)
233(1)-2-20-4 F4	? (? + one 2D + two 5D + one 7D + 14R)
233(1)-2-20-4-2 F5	42 (26AB + two 5D + 14R)

[a] *?* unknown, *W* wheat; *AB* A and B chromosomes, *RF* rye chromosome fragments, *WF* wheat chromosome fragment, *w-r* wheat-rye translocation

triticales were found; we instead obtained hexaploid triticales. Although we cannot exclude the possibility that some octoploid F_2 plants were formed, the typically low fertility of any such plants must have led to their elimination through fertility selection during production of the next generation (Lukaszewski and Gustafson 1987). Octoploid triticale is known to be unstable with respect to meiotic and mitotic processes, resulting in elimination of chromosomes from its progenies (Weimarck 1974; Lukaszewski and Gustafson 1987; Fu et al. 2010; Kalinka et al. 2010; Tang et al. 2012). Such chromosome elimination in octoploid triticale may result in hexaploid triticale (Nakata et al. 1984; Sasaki et al. 1985; Dou et al. 2006; Zhou et al. 2012). When we selected for high fertility, most of the products we obtained from hybrids between synthetic hexaploid wheat and rye were hexaploids. High fertility depends on cytologically stable conditions that may be achieved through chromosome elimination. Two chromosome elimination mechanisms have recently been reported in octoploid triticale. Kalinka et al. (2010) observed direct chromosome elimination in a cytomixis-like fashion from PMCs in newly synthesized octoploid triticales. Tang et al. (2012) detected unequal chromosome division in somatic cells and proposed that it may be a chromosome elimination pathway in octoploid triticales. In our study, we observed chromosome fragments without centromeres in some somatic cells of hybrid derivatives (Table 2). Centromere loss may thus be another mechanism for chromosome elimination, as centromere-less chromosomes cannot be passed along to future generations.

The FISH pattern due to pAs1-clone repeated sequences permits identification of D-genome chromosomes (Nagaki et al. 1995; Rayburn and Gill 1986; Pedersen and Langridge 1997; Dou et al. 2006). Our analysis using pAs1 as a probe indicated that most of the analyzed plants or lines contained chromosomes from A, B, and R genomes, with D-genome chromosomes being preferentially eliminated from hybrid progenies between synthetic hexaploid wheat and rye (Tables 1,2). Nakata et al. (1984), Dou et al. (2006), and Zhou et al. (2012) observed a similar phenomenon. When they analyzed hexaploid lines spontaneously derived from progenies of some primary octoploid triticales, they found that while all of them retained complete sets of A and B-genome chromosomes and most of the R-genome set, most of the D-genome chromosomes were eliminated. These studies all suggest that with respect to existence in

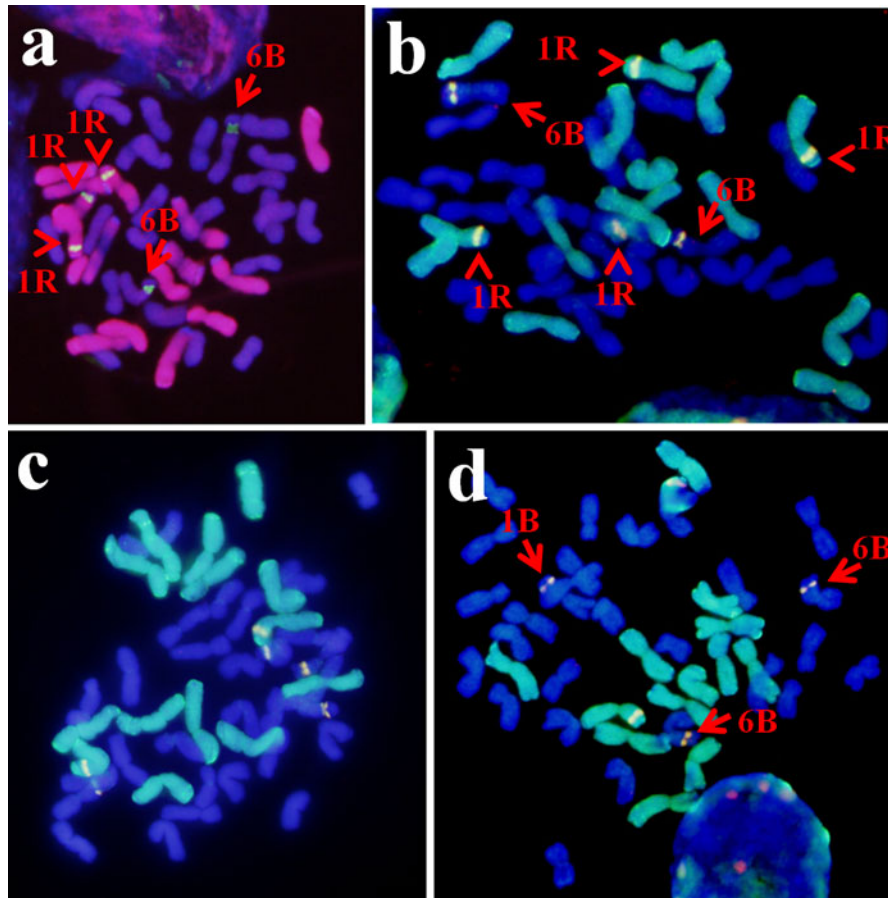

Fig. 3 Chromosome constitutions of Syn-SAU-8/rye hybrids. Chromosome constitutions were analyzed using R-genome, pTa71, and pAs1 probes, with unlabeled wheat genome DNA used as a blocker. **a** Plant 228(1)-1-32-18 had 15 rye chromosomes, including 3 1R chromosomes and 1R chromosome fragment (*red*). **b** Plant 228(1)-1-32-18-3 had 26A/B and 16R chromosomes, with 4 1R chromosomes but lacking 1B. **c** Plant 228(1)-1-32-4-2 had 28A/B and 14R chromosomes. **d** Plant 228(1)-1-32-4-1 had 27A/B chromosomes, with 1 1B and 14R chromosomes

cell nuclei, the D-genome is at a competitive disadvantage compared with A, B, and R genomes. Preferential elimination of the D-genome has also been reported in other hybrids, such as the amphiploid between hexaploid wheat and *A. kotschyi* (genome AABBDDU^{k}U^{k}S^{k}S^{k}) (Tiwari et al. 2010) and trigeneric hybrids between hexaploid wheat, rye, and *Psathyrostachys huashanica* (genome AABBDRNs) (Xie et al. 2012). It will consequently be interesting to further elucidate this phenomenon.

The utility of new hexaploid triticales derived from hexaploid wheat-rye hybrids

The results of our study indicate that complete and substituted hexaploid triticales can be produced using the hexaploid wheat-rye hybrid method. It is believed that many characteristics of hexaploid triticale can be improved by the introduction of D-genome chromosomes (Lukaszewski and Gustafson 1987; Dou et al. 2006). Previous studies focused on the incorporation of D-genome chromosomes from common wheat into hexaploid triticale. The D-genome of common wheat is derived from that of the diploid wild species *A. tauschii*. Direct incorporation of *A. tauschii* chromosomes into current triticale lines is valuable because of the high diversity (Van Ginkel and Ogbonnaya 2007; Yang et al. 2009), wide environmental adaptation (Gororo et al. 2001; Trethowan and Mujeeb-Kazi 2008; Mizuno et al. 2010), and high early plant vigor (Ter Steege et al. 2005) of this wild species. In this study, we obtained a F_7 line (188-2-6-37-20-20-35)

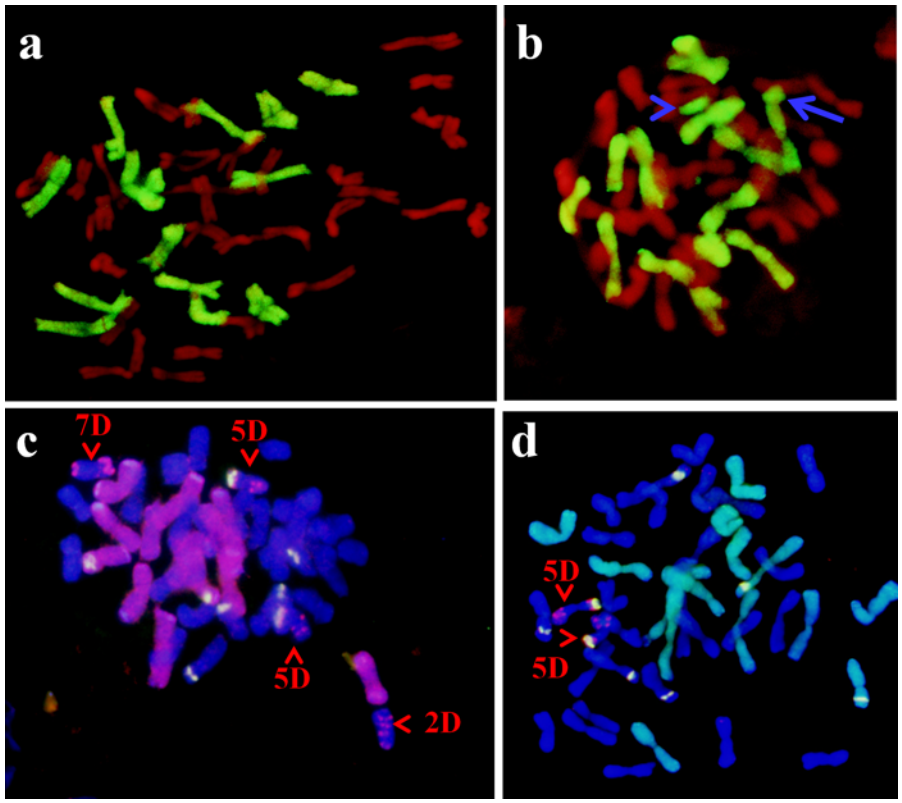

Fig. 4 Chromosome constitutions of some Syn-SAU-18/rye hybrids. **a** and **b** were analyzed with R-genome (*green*) probe and unlabeled chinese spring genome DNA as a blocker; **c** and **d** were analyzed with R-genome (*red* in **c** and *green* in **d**), pTa71 (*yellow*), and pAs1 (*red*) probes, with unlabeled chinese spring genome DNA used as a blocker. **a** Plant 233(1)-6-2 had 30A/B chromosomes and 14R chromosomes. **b** Plant 233(1)-2-20 had one wheat-rye translocation (*short arrow*) and one rye chromosome fragment (*long arrow*). **c** Plant 233(1)-2-20-4 had a pair of 5D chromosomes, 1 2D chromosome, and 1 7D chromosome. **d** Plant 233(1)-2-20-4-2 had 26A/B chromosomes, 14 rye chromosomes, and a pair of 5D chromosomes (*arrow*)

that carried a pair of 2D chromosomes from *A. tauschii* accession AS60 (Fig. 2g). The photoperiod response gene *Ppd-D1* on 2D is one of the critical genes for adaptation to growth habitats. Compared with its allele in common wheat variety Chinese Spring, *Ppd-D*^{t}*1* in *A. tauschii* AS60 shortens heading times (Xiang et al. 2009). We also obtained an F_5 line (233[1]-2-20-4-2) that carried a 5D chromosome pair from Chinese *A. tauschii* accession AS77 (Fig. 4d). The vernalization response gene *Vrn-D1*, another important gene for adaptation to growth habitats, is found on chromosome 5D. In addition, chromosome 2D of *A. tauschii* includes QTLs for leaf elongation rate and duration, cell production rate, and cell length, while 5D harbors QTLs for total leaf mass, total leaf area, and number of leaves and tillers (Ter Steege et al. 2005). These QTLs for early seedling growth are important for early establishment and eventual success of triticale plants.

Our observations also demonstrate that translocation lines of hexaploid triticale can be effectively produced using the hexaploid wheat-rye hybrid method. Translocation lines are important genetic materials for genetic improvement. Out of the eight SHW-L1/rye hybrid lines analyzed, five carried translocations. Because unpaired chromosomes in hybrids remain as univalents, they have a chance to misdivide and then fuse (Lukaszewski and Gustafson 1983). The centric translocations in line 186-4-2-31-17-17-33 (Fig. 2f) were probably produced in this way, i.e., by centric break-fusion. Terminal translocations involving small chromosome fragments were observed in four lines (Fig. 2c, d, g, h). Other studies

have demonstrated that small fragment translocations are rare, and only a few small fragment translocations between wheat and rye chromosomes have previously been reported (see review by Zhou et al. 2012). Although terminal translocations can arise from homoeologous pairing, the level of homoeologous chromosome pairing in wheat-rye F_1 hybrids is usually low. The observed translocations were thus more likely the result of non-centric breakage of chromosomes and subsequent fusion (Lukaszewski 1997; Oleszczuk et al. 2011) or arose from abnormal chromosome pairing in somatic cells (Fu et al. 2010; Tang et al. 2012). The exact mechanism of origin remains to be clarified, however.

"Genome shock" resulting from interspecific hybridization induces genomic changes (McClintock 1984). Interestingly, as reviewed by Ma and Gustafson (2008), wheat hybrids with rye have higher rates of genomic changes than wheat hybrids with other related species. Novel alleles can be induced in wheat-rye hybrids (Yuan et al. 2011). In this study, hexaploids, such as 186-1-4-18-8-8-27 and 186-3-5-26-14-14-31, with identical chromosome constitutions were derived from the same F_1 hybrid plant (Table 1). These are desirable materials for studying the evolutionary biology of distant hybridization: because an F_1 plant originates from the union of a female gamete with an ABD haploid genome and a male gamete with an R haploid genome, the variations in its derivatives can be attributed to the action of distant hybridization.

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