

Formation of unreduced gametes is impeded by homologous chromosome pairing in tetraploid *Triticum turgidum* × *Aegilops tauschii* hybrids

Chun-Jie Wang · Lian-Quan Zhang ·
Shou-Fen Dai · You-Liang Zheng ·
Huai-Gang Zhang · Deng-Cai Liu

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Abstract It is believed that unreduced gametes with somatic chromosome numbers play a predominant role in natural polyploidization. Allohexaploid bread wheat originated from spontaneous hybridization of *Triticum turgidum* L. with *Aegilops tauschii* Coss. Unreduced gametes originating via meiotic restitution, including first-division restitution (FDR) and single-division meiosis (SDM), are well documented in triploid F₁ hybrids of *T. turgidum* with diploid *Ae. tauschii* (genomic constitution ABD, usually with 21 univalents in meiotic metaphase I). In this study, two *T. turgidum* lines known to carry genes for meiotic restitution were crossed to tetraploid *Ae. tauschii*.

The resulting F₁ hybrids (genomes ABDD), had seven pairs of homologous chromosomes and regularly formed 14 univalents and seven bivalents at metaphase I. Neither FDR nor SDM were observed. The distribution of chromosome numbers among progeny obtained by self pollination and a backcross to *T. turgidum* showed the absence of unreduced gametes. These results suggest that high homologous pairing interfered with meiotic restitution and the formation of unreduced gametes. This may be related to asynchronous movement during meiosis between paired and unpaired chromosomes or to uneven distribution of chromosomes in anaphases, resulting in nonviable gametes.

Chun-Jie Wang and Lian-Quan Zhang have contributed equally to this research.

C.-J. Wang · L.-Q. Zhang · S.-F. Dai ·
Y.-L. Zheng · D.-C. Liu
Triticeae Research Institute, Sichuan Agricultural
University, Wenjiang of Chengdu city 611130, Sichuan,
People's Republic of China

H.-G. Zhang · D.-C. Liu
Key Laboratory of Adaptation and Evolution of Plateau
Biota, Northwest Institute of Plateau Biology,
Chinese Academy of Sciences, Xining 810001,
People's Republic of China

D.-C. Liu (✉)
Key Laboratory of Southwestern Crop Germplasm
Utilization, Ministry of Agriculture, Ya'an 625014,
Sichuan, People's Republic of China
e-mail: dcliu7@yahoo.com

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Introduction

Polyploidy is very common in nature. Many important crops such as wheat, potato, cotton, oat, sugarcane, banana, groundnut, tobacco and numerous horticultural species are polyploids. It is believed that unreduced gametes (2n gametes with the chromosome numbers of a somatic cell) play a predominant role in polyploidization (Harlan and De Wet 1975; Ramsey and Schemske 1998, 2002; Cai and Xu 2007; Jauhar 2007). Unreduced gametes can help in

crop improvement, not only in alien gene introgression into wheat, but also in the production of doubled haploids (Ramana and Jacobsen 2003; Zhang et al. 2007).

Meiotic abnormalities leading to $2n$ gamete formation mainly include abnormal cytokinesis and omission of the first or second meiotic division (Veilleux 1985; Bretagnolle and Thompson 1995; Peloquin et al. 1999; Ramana and Jacobsen 2003). Although environmental factors were shown to affect the frequency, $2n$ gamete production is under strong genetic control (Xu and Joppa 2000; Kato and Birchler 2006; Zhang et al. 2008a, 2010). Recently, d' Erfurth et al. (2008) isolated and characterized a mutation in *Arabidopsis thaliana* (*AtPSI*) that is responsible for the production of unreduced gametes through abnormal spindle orientation, with parallel or fused spindles, during meiosis II. By combining *AtPSI* with other mutants they selected a genotype that produced unreduced gametes, effectively turning meiosis into mitosis (d' Erfurth et al. 2009).

The Triticeae is a big tribe, including the important polyploid crops: common wheat (*Triticum aestivum* L., $2n = 6 \times = 42$, genome formula AABBDD), durum wheat (*T. turgidum* L. ssp. durum, $2n = 4 \times = 28$, AABB), triticale (\times *Triticosecale*, AABBRR) and some forage species. Various amphidiploids have been spontaneously produced within this tribe by the formation and union of unreduced gametes (Maan and Sasakuma 1977; Islam and Shepherd 1980; Blanco et al. 1983; Xu and Dong 1992; Li and Liu 1993; David et al. 2004; Tiwari et al. 2008; Loureiro et al. 2009). Common wheat originates from spontaneous hybridization of *T. turgidum* and *Ae. tauschii* Coss. ($2n = 2 \times = 14$, genome DD) followed by chromosome doubling of unreduced gametes (Kihara and Lilienfeld 1949; Cai and Xu 2007; Jauhar 2007). Unreduced gametes have been observed in triploid F_1 hybrid combinations of *Ae. tauschii* with six of the eight *T. turgidum* subspecies (Zhang et al. 2010) and in haploid plants of *T. turgidum* (Jauhar 2003) and *T. aestivum* (Jauhar 2007). Previous studies have indicated that unreduced gametogenesis is usually caused by two main pathways of meiotic restitution: FDR, when the first meiotic division is omitted and there is a normal second meiotic division (anaphase II) with production of dyads (Islam and Shepherd 1980; Fukuda and Sakamoto 1992; Xu and Joppa 1995), and

SDM, when a single equational division occurs at anaphase I with dyads as the final meiotic products (Aase 1930; Maan and Sasakuma 1977; Xu and Dong 1992; Matsuoka and Nasuda 2004). Genes for high frequencies of FDR were mapped on chromosome 4A and on chromosomes 3A and 6A of *T. turgidum* ssp. durum cultivar Langdon (Xu and Joppa 2000).

In this paper we report the absence of unreduced gametes in tetraploid hybrids (genomes ABDD) of *T. turgidum* and chromosome-doubled *Ae. tauschii*.

Materials and methods

Plant materials

Plant materials used in this study included Chinese *T. turgidum* L. ssp. *turgidum* lines Yuanzhumai (AS2255, accession number of the Triticeae Research Institute) and Ailanmai (AS313) and tetraploid *Ae. tauschii* (AS2410), which was produced by colchicine treatment of a diploid *Ae. tauschii* AS77 that was native to Henan, China. Previous work indicated that AS2255 and AS313 had genes for meiotic restitution in ABD hybrids (Zhang et al. 2007, 2010).

Production of hybrids

Emasculation and pollination techniques described by Zhang et al. (2008b) were followed. No embryo rescue technique or hormone treatment was applied when producing the F_1 hybrids. The F_1 seeds were germinated in Petri dishes before transplanting into a field at Triticeae Research Institute, Dujiangyan City, Sichuan. Some morphological parameters of the hybrid plants were recorded. Plant height was measured as the length of the longest tiller from the ground to the tip of the spike (awn being excluded). Tillers were counted as the number of spikes per plant. Spike length and number of spikelets were recorded for the longest tiller.

The F_1 plants were further selfed and backcrossed to their respective *T. turgidum* parents. Crossability was calculated as the percentage of the number of seeds obtained in relation to the number of pollinated florets. The selfed plant seedsets were calculated as percentages of the numbers of seeds obtained in relation to the numbers of florets in the spikes.

Cytological analysis

The procedures for preparation of plant materials and slides for determining chromosome numbers in root-tip cells and meiotic events in pollen-mother-cells (PMC) were described by Zhang et al. (2007). The observations were made and documented with an Olympus BX-51 microscope coupled with a Photometric SenSys CCD camera. Univalents (I), bivalents (II), trivalents (III), and quadrivalents (IV) were counted at metaphase I of meiosis.

Results

T. turgidum × tetraploid *Ae. tauschii* hybrids

Tetraploid hybrids involving both *T. turgidum* accessions (Table 1) were obtained without embryo rescue. Average crossabilities were 16.97 and 25.54% for AS2255 × AS2410 and AS313 × AS2410, respectively. Most crossed seeds germinated normally and produced vigorous plants (Table 2) with 28 chromosomes. They had tough, tenacious glumes and were obviously *Ae. tauschii* hybrids.

The F₁ hybrids showed very low fertilities (Table 2) with seedsets ranging from 0.09 to 0.29%. Germination rates of the F₂ seed ranged from 61 to 81%. If the formation and union of unreduced gametes was responsible for the fertility in the F₁

hybrid plants, their F₂ seeds should have 56 chromosomes with the genome formula AABBD₄. To test this hypothesis, somatic chromosome counts were made on root-tips of germinating seedlings. No octoploids were found (Table 3). Among seven F₂ seedlings from AS2255 × AS2410, two had 36 chromosomes and the others had 26, 27, 34, 35, and 48 chromosomes. Among six F₂ seedlings from AS313 × AS2410, two had 48 chromosomes and the others had 22, 32, 40, and 44. Some of the plants were partially fertile.

Metaphase I chromosome pairing in the ABDD hybrids was predominantly 14 univalents and seven bivalents (Table 4, Fig. 1A), the bivalents presumably being the D genome chromosomes. Neither FDR nor SDM was observed, meiosis and cytokinesis progressed more or less normally. Chromosomes moved to the opposite poles at anaphase I (Fig. 1B, C), but with some laggards remaining in the vicinity of the equatorial plane. Two daughter cells were usually formed at telophase I (Fig. 1D a), although a single daughter cell was occasionally observed (Fig. 1D b). The chromosomes aligned on the equatorial plates at metaphase II (Fig. 1E a) and anaphase II was characterized by lagging chromosomes and chromosome bridging (Fig. 1E b). Many of the tetrads contained one or more micronuclei (Fig. 1F b), evidence of lagging chromosomes that were not included in the daughter nuclei of the tetrads.

Table 1 Crossabilities of *T. turgidum* lines with *A. tauschii* accession AS2410

Hybrid	No. florets pollinated	No. kernels harvested	Crossability (%)	Germination (%) (seed number)
AS2255 × AS2410	1112	189	16.97	84.80 (58)
AS313 × AS2410	924	236	25.54	69.81 (53)

Table 2 The morphology and selfed seeds of *T. turgidum*, tetraploid *Ae. tauschii* and their F₁ hybrid

Plant types	No. plants	Plant height	No. tillers/plant	Spike length	No. spikelets/spike	No. selfed florets	No. seed	Seedset rate (%)	Germination (%) ^a
AS2255	5	112.85 b	4.15 c	10.98 b	26.34 a	–	–	–	–
AS313	5	105.48 b	4.30 c	10.22 b	25.12 a	–	–	–	–
AS2410	10	77.85 c	65.17 a	10.88 b	14.74 c	–	–	–	–
AS2255 × AS2410	49	135.99 a	10.57 b	19.05 a	20.82 b	19072	18	0.09	61.11 (18)
AS313 × AS2410	37	131.59 a	11.92 b	18.72 a	20.57 b	15632	45	0.29	81.25 (16)

^a Seed number used in germination is indicated in brackets. Mean columns followed by different letters are significantly different at $P < 0.05$, applying SSR (shortest significant ranges) multiple analysis

Table 3 Somatic chromosome numbers in F₂ and backcrossed plants

Hybrid combination	No. of plants counted (total plants)	The distribution of chromosome numbers (selfed seed set rate, %)
AS2255 × AS2410 F ₂	7 (10)	26 (3.68), 27 (0), 34 (2.69), 35 (80.53), 36 (2.05), 36 (36.73), 48 (11.16), N ^a (2.78), N (7.81), N (16.45)
AS313 × AS2410 F ₂	6 (11)	22 (–), 32 (8.05), 40 (32.78), 44 (22.22), 48 (14.37), 48 (1.79), N (0), N (0), N (2.04), N (5.26), N (12.79)
(AS2255 × AS2410) × AS2255 F ₁	3 (4)	30 (0), 34 (26.89), 34 (31.99), N (9.09)
(AS313 × AS2410) × AS313 F ₁	3 (4)	26 (2.34), 32 (0), 32 (–), N (68.75)

^a Failure to count for chromosome number

Table 4 Chromosome pairing configurations in tetraploid *Ae. tauschii* and its hybrids

Plant types	Genome	Chromosome no.	No. cell observed	I	Ring II	Rod II	III	IV
AS2410	DDDD	28	27	0.22 (0–2)	6.41 (1–14)	5.74 (0–13)	0.15 (0–1)	0.74 (0–3)
AS2255 × AS2410 F ₁	ABDD	28	50	14	5.34 (2–7)	1.66 (0–5)	0	0
AS313 × AS2410 F ₁	ABDD	28	50	14	6.44 (4–7)	0.56 (0–3)	0	0

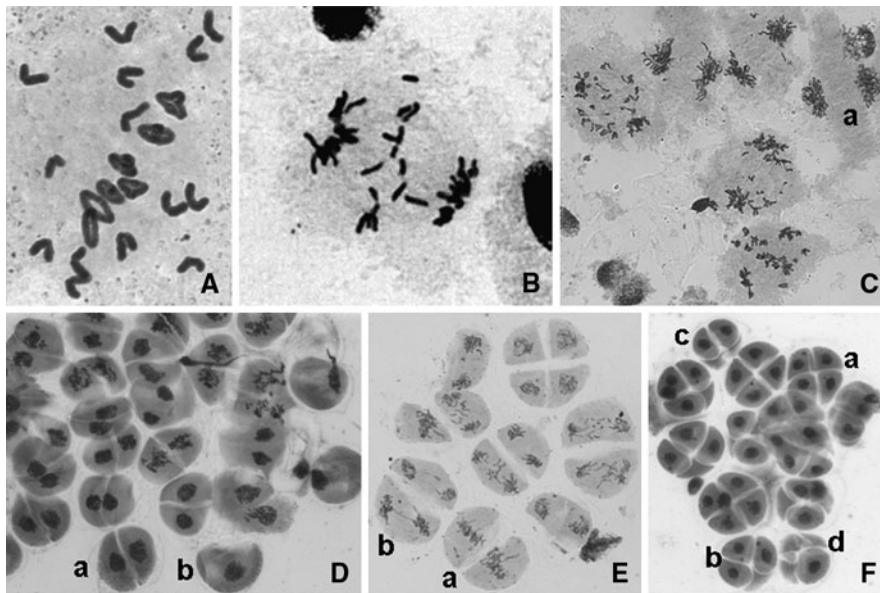


Fig. 1 Pollen-mother-cells of the *Triticum turgidum* ssp. *turgidum* AS2255 × *Aegilops tauschii* AS2410 F₁ hybrid. **A** Twenty eight chromosomes present as seven bivalents and 14 univalents at early metaphase I. **B** Some chromosomes move toward opposite poles at anaphase I, others lag in the centre of cells at anaphase I. **C** Overview of chromosome movement at

anaphase I, no laggards (*a*). **D** Pollen-mother-cells with two (*a*) and single nuclei (*b*) at telophase I. **E** Chromosomes aligned on the equatorial plate at metaphase II (*a*), second division with chromosome-bridges at anaphase II (*b*). **F** Tetrads with four (*a*), three (*b*), two (*c*), and one (*d*) nuclei

Table 5 Crossabilities of *T. turgidum* × tetraploid *Ae. tauschii* hybrids pollinated with the respective *T. turgidum* parents

Hybrid combinations	No. florets pollinated	No. seeds set	Crossability (%)	Germination (%) ^a
(AS2255 × AS2410) × AS2255	76	8	10.53	62.50 (8)
(AS313 × AS2410) × AS313	73	8	10.96	62.50 (8)

^a Seed number used in germination is indicated in brackets

Backcross [(*T. turgidum* × tetraploid *Ae. tauschii*) × *T. turgidum*] hybrids

Backcrossing of the tetraploid hybrids with the respective *T. turgidum* parents generated seedsets of about 11% (Table 5). The germination levels of these seeds were about 63%. If the *T. turgidum* × *Ae. tauschii* F₁ plants had produced unreduced female gametes (n = 28, ABDD), their union with the male gametes (n = 14, AB) should have produced seeds with 42 chromosomes (AABBDD). No plants with 42 chromosomes were observed among these progeny, some of which were partially fertile (Table 3).

Discussion

Previous observations on polyhaploid *T. turgidum* × *Ae. tauschii* hybrids that produced unreduced gametes indicated that almost all 21 chromosomes were unpaired at metaphase I (Aase 1930; Maan and Sasakuma 1977; Islam and Shepherd 1980; Fukuda and Sakamoto 1992; Xu and Dong 1992; Xu and Joppa 1995; Matsuoka and Nasuda 2004; Zhang et al. 2007). The presence of single paired D genome chromosomes did not affect the formation of unreduced gametes in the hybrids of *T. turgidum* ssp. durum Langdon D genome disomic substitution (LDN DS) lines with *Ae. tauschii* (Xu and Joppa 2000; Zhang et al. 2008a). Thus a single paired D genome chromosome was not sufficient to prevent the formation of unreduced gametes.

Our previous studies showed that unreduced gametes were formed through SDM or FDR in the triploid hybrids of *T. turgidum* lines AS2255 and AS313 with a range of diploid *Ae. tauschii* accessions including AS77 (Zhang et al. 2007, 2010). Due to the union of unreduced gametes, most F₂ plants from such hybrids had 2n = 42 (Zhang et al. 2010; our unpublished data). However, in the present study

neither FDR nor SDM was observed in corresponding hybrids of *T. turgidum* AS313 and AS2255 with tetraploid *Ae. tauschii* AS77 (AS2410). This tetraploid usually had seven bivalents at metaphase I. Two more or less normal divisions occurred in PMCs and usually resulted in tetrads (Fig. 1), rather than unreduced gametes. No plants with 56 chromosomes were found in the F₂ generation, and no plants with 2n = 42 were found among BC₁ plants.

A reasonable explanation for the above results is that homologous chromosome pairing of seven bivalents in the ABDD hybrids prevented meiotic restitution and formation of unreduced gametes. The cytological mechanism for homologous pairing preventing meiotic restitution may be related to different dynamic behaviors of paired and unpaired chromosomes. Cai et al. (2010) found that in the tetraploid wheat variety Langdon, homologous chromosomes paired as 14 bivalents and sister kinetochores for each of the chromosomes in a bivalent oriented syntelically (monopolar attachment). However, in hybrids of Langdon and *Ae. tauschii*, chromosomes appeared as univalents and sister kinetochores of the univalents oriented amphitelically (bipolar microtubule–kinetochore attachment) in 31.6% of meiocytes. Both bipolar and monopolar attachments were observed in other meiocytes. It was further suggested that the tension created by the amphitelic orientation of sister kinetochores and persistence of centromeric cohesion between sister chromatids at meiosis I contribute to the onset of non-reductional meiotic cell division (Cai et al. 2010). In our present study, asynchronous movement between univalents and bivalents during meiosis was suggested with some chromosomes (presumably previously paired chromosomes) moving to the opposite poles at anaphase I, whereas others (presumably unpaired chromosomes) stayed in the centre of the cells and were not included in the nuclei of the subsequent tetrad cells which presumably did not develop into viable gametes (Fig. 1B, C).

The pairing of chromosomes and subsequent movement to the poles at anaphase I seem necessary to prevent meiotic restitution, but a single bivalent is apparently not sufficient to prevent restitution (Xu and Joppa 2000; Zhang et al. 2008a). However, this study indicated that seven bivalents formed by two D genomes do prevent the restitution. Thus there may be a threshold somewhere between two and six pairs that leads to the difference. It is currently unclear as to how many paired chromosomes must go to the poles in order to prevent restitution.

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