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Frequent occurrence of unreduced gametes in *Triticum turgidum*–*Aegilops tauschii* hybrids

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Abstract Spontaneous chromosome doubling via union of unreduced (2n) gametes has been thought to be the way that common wheat (*Triticum aestivum* L.) was originated from the hybridization of *T. turgidum* L. with *Ae. tauschii* Cosson. Previous works have observed unreduced gametes in F₁ hybrids of *Ae. tauschii* with six of the eight *T. turgidum* subspecies. It is not clear, however, whether the formation of these unreduced gametes is a norm in the F₁ hybrids. In the present study, we tried to answer this question by assessing the occurrence frequency of unreduced gametes in 115 *T. turgidum*–*Ae. tauschii* hybrid combinations, involving 76 genotypes of seven

T. turgidum subspecies and 24 *Ae. tauschii* accessions. Our data show that these hybrid combinations differed significantly ($P \leq 0.01$, $F = 11.40$) in selfed seedset, an indicator for production of unreduced gametes. This study clearly showed that meiotic restitution genes are widely distributed within *T. turgidum*. However, significant differences were found between as well as within *T. turgidum* subspecies and in the interaction of the *T. turgidum* genotypes with those of *Ae. tauschii*. The possible application of the meiotic restitution genes from *T. turgidum* in production of double haploids is also discussed.

Keywords *Aegilops tauschii* · Amphidiploid · Fertility · *Triticum turgidum* · Unreduced gametes

Abbreviations

DHs	Double haploids
PMC	Pollen mother cell
<i>T. t. dicoccon</i>	<i>T. turgidum</i> ssp. <i>dicoccon</i>
<i>A. t.</i>	<i>Ae. tauschii</i>

Introduction

Polyploidy is very common in higher plants. It usually results from genome duplication within a species (autopolyploidy) or combination of genomes between species (allopolyploidy). The mechanism of polyploidization in nature is either by somatic chromosome doubling or through a union of two unreduced gametes

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(see reviews by Harlan and De Wet 1975 and Ramsey and Schemske 1998, 2002). It is believed that the latter is far more common than the former. Most important crops are polyploids. Understanding the mechanisms of their origin will lead to their utilities in crop improvement (Ramana and Jacobsen 2003).

Common wheat (*Triticum aestivum* L.) is an allohexaploid ($2n = 6x = 42$) with the A, B and D genomes. It was formed via a two-step hybridization process: hybridization of *T. monococcum* L. ($2n = 14$, AA) with *Aegilops speltoides* Tausch ($2n = 14$, SS—genetically the most similar to BB) resulting in *T. turgidum* L. ($2n = 28$, AABB) and then the hybridization of *T. turgidum* with *Ae. tauschii* Cosson ($2n = 14$, DD). Unreduced gametes are believed to play an important role in this process (Kihara and Lilienfeld 1949; Cai and Xu 2007; Jauhar 2007). In fact, unreduced gametes have been observed in the F_1 hybrids of *Ae. tauschii* with six of the eight *T. turgidum* subspecies (see review in Zhang et al. 2007) and in haploid plants of *T. turgidum* ssp. *durum* (Jauhar 2003). Cytological observations of male gametogenesis in the *T. turgidum*–*Ae. tauschii* hybrids have demonstrated that meiotic restitution is a major mechanism responsible for the formation of functional unreduced male gametes by the hybrids. Gene for high frequency of first-division restitution (FDR) or normal second-division of FDR cells have been mapped onto chromosome 4A or chromosomes 3A and 6A of durum wheat cultivar Langdon, respectively (Xu and Joppa 2000). However, it is not clear whether or not formation of unreduced gametes is a norm for *T. turgidum*–*Ae. tauschii* hybrids. Answer to this question is of significance both to our understanding of the origin of the common wheat and to the application of unreduced gametes in wheat improvement. In this study we tried to answer this question by assessing the frequency of occurrence of unreduced gametes in 115 *T. turgidum*–*Ae. tauschii* hybrid combinations, involving 76 genotypes of seven *T. turgidum* subspecies and 24 *Ae. tauschii* accessions.

Materials and methods

Plant materials

Seventy-six *T. turgidum* lines and 24 *Ae. tauschii* accessions used in this study came from diverse

geographic origins (Table 1). According to Van Slageren (1994), they belong to the following seven subspecies: *durum* (2 lines), *turgidum* (25 lines), *dicoccoides* (2 lines), *dicoccon* (34 lines), *turanicum* (11 lines), *carthlicum* (1 line), and *polonicum* (1 line), respectively. The 46 lines with a PI code were kindly provided by USDA-ARS, USA. The remaining 30 lines came from our own collection. The 25 *T. turgidum* ssp. *turgidum* lines are Chinese landraces. Of the 24 *Ae. tauschii* accessions, 14 (AS60, AS65, AS67, AS72, AS76, AS77, AS79, AS81, AS82, AS84, AS91, AS93, AS95, AS2395) belong to *Ae. tauschii* ssp. *tauschii*, and 10 (AS66, AS2386, AS2388, AS2393, AS2397, AS2399, AS2403, AS2404, AS2405, AS2407) belong to *Ae. tauschii* ssp. *strangulata*. AS72, AS76, AS77, AS79, AS81, and AS82 were Chinese accessions.

Production of wide hybrids

All wide hybridizations were made with *T. turgidum* as the female parent. Emasculation and pollination were done as previously described by Zhang et al. (2008a). No embryo rescue or hormone treatment was applied to the production of F_1 seeds. The F_1 seeds were germinated in Petri dishes before transplanted to the field at Triticeae Research Institute of Sichuan Agricultural University, located at Dujiangyan city of Sichuan province, P.R. China. The F_1 plants were selfed and the selfed seedset (percentage of selfed seeds over total selfed florets) of each plant was calculated. The selfed seedset rate of a hybrid combination was represented by the average percentage of seedset of all the plants for that combination. The Data Processing System 6.50 (<http://www.statforum.com/>) (Tang and Feng 2007) was used to do Student *t*-test and analysis of variance. Selfed seedset of each plant was first converted to an angle and the transformed data was then analyzed for difference between the hybrid combinations. To detect the difference between subspecies, the average selfed seedset of each combination was used.

Cytological observation

The procedures for cytological observation on chromosome number in root-tip cells of the F_1 and the F_2 plants and chromosome pairing in pollen-mother-cells (PMC) in the F_2 plants were previously described by Zhang et al. (2007). For meiotic

Table 1 Selfed seedset of F₁ hybrids of *Triticum turgidum* with *Aegilops tauschii*

Cross combinations ^a	Germination rate of F ₁ seeds ^b	No. F ₁ plants obtained	No. selfed florets	No. F ₂ seeds	Seed set rate (%)
<i>ssp. turgidum</i> AS2255 (China) × <i>ssp. tauschii</i> AS60	75.68 (74)	10	4520	407	9
<i>ssp. turgidum</i> AS313 (Sichuan, China) × <i>ssp. tauschii</i> AS60	73.86 (88)	10	5736	523	9.12
<i>ssp. dicoccoides</i> AS285 (Germany) × <i>ssp. tauschii</i> AS60	15.04 (113)	1	380	93	24.47
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. tauschii</i> AS60	17.5 (126)	5	824	40	4.85
<i>ssp. durum</i> Langdon × <i>ssp. tauschii</i> AS60	68.54 (89)	4	1238	456	36.83
<i>ssp. durum</i> Langdon × <i>ssp. tauschii</i> AS65 (Former Soviet Union)	43.48 (23)	6	838	217	25.89
<i>ssp. durum</i> Langdon × <i>ssp. tauschii</i> AS77 (Henan, China)	50 (4)	2	170	24	14.12
<i>ssp. durum</i> Langdon × <i>ssp. strangulata</i> AS2386 (Iran)	52.63 (19)	9	4850	920	18.97
<i>ssp. durum</i> Langdon × <i>ssp. strangulata</i> AS2399	10.26 (39)	4	1008	136	13.49
<i>ssp. durum</i> Langdon × <i>ssp. strangulata</i> AS2404	3.7 (27)	1	308	63	20.45
<i>ssp. durum</i> Langdon × <i>ssp. strangulata</i> AS2407	95.83 (24)	16	9806	1758	17.93
<i>ssp. durum</i> AS2262 (Syria) × <i>ssp. tauschii</i> AS77 (Henan, China)	100 (1)	1	554	5	0.9
<i>ssp. carthlicum</i> AS2268 (Germany) × <i>ssp. tauschii</i> AS65	100 (1)	1	536	5	0.93
<i>ssp. turgidum</i> AS2255 (China) × <i>ssp. tauschii</i> AS93	100 (1)	1	2322	6	0.26
<i>ssp. turgidum</i> AS2255 (China) × <i>ssp. tauschii</i> AS2395	50 (6)	3	1905	96	5.04
<i>ssp. turgidum</i> AS2255 (China) × <i>ssp. strangulata</i> AS2393	12.5 (8)	1	786	76	9.67
<i>ssp. turgidum</i> AS313 (Sichuan, China) × <i>ssp. tauschii</i> AS77 (Henan, China)	50 (16)	7	4754	31	0.65
<i>ssp. turgidum</i> AS2231-2 (Xinjiang,China) × <i>ssp. tauschii</i> AS77 (Henan, China)	68.57 (35)	23	14694	133	0.91
<i>ssp. turgidum</i> AS2236-1 (Sichuan, China) × <i>ssp. tauschii</i> AS77 (Henan, China)	92.31 (13)	12	8710	120	1.38
<i>ssp. turgidum</i> AS2236-1 (Sichuan, China) × <i>ssp. tauschii</i> AS91	100 (1)	1	2062	19	0.92
<i>ssp. turgidum</i> AS2236-2 (Sichuan, China) × <i>ssp. tauschii</i> AS82 (Henan, China)	100 (2)	2	2160	12	0.56
<i>ssp. turgidum</i> AS2238 (Sichuan, China) × <i>ssp. tauschii</i> AS77 (Henan, China)	100 (1)	1	1034	15	1.45
<i>ssp. turgidum</i> AS2239 (Sichuan, China) × <i>ssp. tauschii</i> AS2395	100 (1)	1	914	314	34.35
<i>ssp. turgidum</i> AS2240 (Sichuan,China) × <i>ssp. tauschii</i> AS77 (Henan, China)	81.82 (11)	6	4614	192	4.16
<i>ssp. turgidum</i> AS2240 (Sichuan, China) × <i>ssp. tauschii</i> AS84	80 (5)	4	2936	872	29.7
<i>ssp. turgidum</i> AS2285 (Sichuan, China) × <i>ssp. tauschii</i> AS77 (Henan, China)	100 (3)	3	3606	27	0.75
<i>ssp. turgidum</i> AS2291 (Shannxi, China) × <i>ssp. strangulata</i> AS2404	60 (10)	1	506	65	12.85
<i>ssp. turgidum</i> AS2295 (Sichuan, China) × <i>ssp. tauschii</i> AS76 (Shannxi, China)	50 (4)	2	3146	75	2.38
<i>ssp. turgidum</i> AS2296 (Sichuan, China) × <i>ssp. strangulata</i> AS2388 (Iran)	100 (1)	1	2654	261	9.83
<i>ssp. turgidum</i> AS2298 (Sichuan, China) × <i>ssp. tauschii</i> AS79 (Henan, China)	40 (5)	2	2718	87	3.2
<i>ssp. turgidum</i> AS2299 (Sichuan, China) × <i>ssp. tauschii</i> AS79 (Henan, China)	20 (10)	2	2054	33	1.61
<i>ssp. turgidum</i> AS2308 (Sichuan, China) × <i>ssp. tauschii</i> AS72 (Xinjiang, China)	50 (8)	4	3896	162	4.16
<i>ssp. turgidum</i> AS2308 (Sichuan, China) × <i>ssp. tauschii</i> AS81 (Henan, China)	50 (12)	6	3820	56	1.47

Table 1 continued

Cross combinations ^a	Germination rate of F ₁ seeds ^b	No. F ₁ plants obtained	No. selfed florets	No. F ₂ seeds	Seed set rate (%)
<i>ssp. turgidum</i> AS2310 (Sichuan, China) × <i>ssp. tauschii</i> AS60	100 (3)	3	3228	799	24.75
<i>ssp. turgidum</i> AS2312 (Sichuan, China) × <i>ssp. strangulata</i> AS2388 (Iran)	33.33 (3)	1	1710	1	0.06
<i>ssp. turgidum</i> AS2313 (Sichuan, China) × <i>ssp. strangulata</i> AS2388 (Iran)	27.27 (11)	3	3296	70	2.12
<i>ssp. turgidum</i> AS2326 (Gansu, China) × <i>ssp. strangulata</i> AS2388 (Iran)	81.82 (11)	9	7110	40	0.56
<i>ssp. turgidum</i> AS2334 (Gansu, China) × <i>ssp. strangulata</i> AS2388 (Iran)	14.29 (14)	2	1480	4	0.27
<i>ssp. turgidum</i> AS2351 (Henan, China) × <i>ssp. tauschii</i> AS67 (Iran)	100 (1)	1	560	83	14.82
<i>ssp. turgidum</i> AS2378 (Shannxi, China) × <i>ssp. tauschii</i> AS82 (Henan, China)	33.33 (3)	1	688	13	1.89
<i>ssp. turgidum</i> AS2380 (Shannxi, China) × <i>ssp. tauschii</i> AS77 (Henan, China)	50 (4)	2	2614	57	2.18
<i>ssp. turgidum</i> AS2381(Shannxi, China) × <i>ssp. tauschii</i> AS65 (Former Soviet Union)	25 (4)	1	492	29	5.89
<i>ssp. turgidum</i> AS2382 (Shannxi, China) × <i>ssp. strangulata</i> AS2388 (Iran)	11.11 (9)	1	1490	38	2.55
<i>ssp. dicoccoides</i> AS285 (Germany) × <i>ssp. strangulata</i> AS66 (Former Soviet Union)	61.11 (18)	4	3246	100	3.08
<i>ssp. dicoccoides</i> AS285 (Germany) × <i>ssp. strangulata</i> AS2386 (Iran)	50 (10)	2	2572	48	1.87
<i>ssp. dicoccoides</i> AS285 (Germany) × <i>ssp. strangulata</i> AS2404	14.29 (21)	3	2292	122	5.32
<i>ssp. dicoccoides</i> AS285 (Germany) × <i>ssp. strangulata</i> AS2405	33.33 (27)	6	7068	211	2.99
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. strangulata</i> AS66 (Former Soviet Union)	33.33 (15)	3	5484	17	0.31
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. strangulata</i> AS2386 (Iran)	80 (5)	4	2620	239	9.12
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. strangulata</i> AS2399	17.65 (17)	1	1778	73	4.11
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. strangulata</i> AS2404	95.45 (22)	18	16382	440	2.69
<i>ssp. dicoccoides</i> AS286 (France) × <i>ssp. strangulata</i> AS2407	55.17 (29)	9	6866	195	2.84
<i>ssp. dicoccon</i> PI94614 (Ukraine) × <i>ssp. strangulata</i> AS2405	14.29 (7)	1	2136	95	4.45
<i>ssp. dicoccon</i> PI94627 (Asia Minor) × <i>ssp. strangulata</i> AS2386 (Iran)	85 (20)	12	14870	70	0.47
<i>ssp. dicoccon</i> PI94650 (Czechoslovakia) × <i>ssp. strangulata</i> AS2404	44.44 (9)	4	3858	364	9.43
<i>ssp. dicoccon</i> PI94655 (Bulgaria) × <i>ssp. strangulata</i> AS2404	25 (8)	1	1682	101	6
<i>ssp. dicoccon</i> PI94655 (Bulgaria) × <i>ssp. strangulata</i> AS2407	81.25 (16)	10	12828	1156	9.01
<i>ssp. dicoccon</i> PI94666 (Dagestan) × <i>ssp. strangulata</i> AS2407	100 (1)	1	1348	7	0.52
<i>ssp. dicoccon</i> PI94670 (Iran) × <i>ssp. strangulata</i> AS2386 (Iran)	62.5 (40)	9	15024	794	5.28
<i>ssp. dicoccon</i> PI94670 (Iran) × <i>ssp. strangulata</i> AS2404	9.62 (13)	2	674	23	3.41
<i>ssp. dicoccon</i> PI94675 (Georgia) × <i>ssp. strangulata</i> AS2405	10.87 (46)	4	7104	207	2.91
<i>ssp. dicoccon</i> PI113961 (Georgia) × <i>ssp. strangulata</i> AS2404	86.96 (23)	14	9888	294	2.97
<i>ssp. dicoccon</i> PI113963 (Georgia) × <i>ssp. strangulata</i> AS2386 (Iran)	80 (10)	2	1660	293	17.65
<i>ssp. dicoccon</i> PI154582 (Taiwan) × <i>ssp. tauschii</i> AS95	50 (2)	1	354	50	14.12
<i>ssp. dicoccon</i> PI154582 (Taiwan) × <i>ssp. tauschii</i> AS2395	100 (2)	2	1080	32	2.96

Table 1 continued

Cross combinations ^a	Germination rate of F ₁ seeds ^b	No. F ₁ plants obtained	No. selfed florets	No. F ₂ seeds	Seed set rate (%)
ssp. <i>dicoccon</i> PI191781 (Portugal) × ssp. <i>strangulata</i> AS2399	100 (5)	5	2030	13	0.64
ssp. <i>dicoccon</i> PI221401 (Yugoslavia) × ssp. <i>strangulata</i> AS2399	24.53 (53)	3	3332	15	0.45
ssp. <i>dicoccon</i> PI221403 (Yugoslavia) × ssp. <i>strangulata</i> AS2397	14.29 (14)	2	396	26	6.57
ssp. <i>dicoccon</i> PI221403 (Yugoslavia) × ssp. <i>strangulata</i> AS2399	44.44 (36)	3	1084	106	9.78
ssp. <i>dicoccon</i> PI221403 (Yugoslavia) × ssp. <i>strangulata</i> AS2404	52.78 (36)	14	5226	586	11.21
ssp. <i>dicoccon</i> PI306533 (Romania) × ssp. <i>strangulata</i> AS2405	22.73 (22)	1	100	11	11
ssp. <i>dicoccon</i> PI350001 (Yugoslavia) × ssp. <i>strangulata</i> AS2405	25 (4)	1	298	1	0.34
ssp. <i>dicoccon</i> PI350001 (Yugoslavia) × ssp. <i>strangulata</i> AS2386 (Iran)	24.24 (33)	7	4422	78	1.76
ssp. <i>dicoccon</i> PI352331 (Germany) × ssp. <i>strangulata</i> AS2386 (Iran)	8.7 (46)	2	322	9	2.8
ssp. <i>dicoccon</i> PI352335 (USA) × ssp. <i>strangulata</i> AS2386 (Iran)	2.13 (47)	1	862	6	0.7
ssp. <i>dicoccon</i> PI352358 (France) × ssp. <i>tauschii</i> AS65 (Former Soviet Union)	100 (5)	5	3590	5	0.14
ssp. <i>dicoccon</i> PI352359 (Germany) × ssp. <i>strangulata</i> AS2386 (Iran)	13.89 (36)	3	783	6	0.77
ssp. <i>dicoccon</i> PI352367 (Ancient Palestine) × ssp. <i>strangulata</i> AS2386 (Iran)	39.13 (23)	2	1338	131	9.79
ssp. <i>dicoccon</i> PI352369 (Czech Republic) × ssp. <i>tauschii</i> AS60	78.57 (14)	5	1886	12	0.64
ssp. <i>dicoccon</i> PI352369 (Czech Republic) × ssp. <i>strangulata</i> AS2393	33.33 (9)	3	1190	4	0.34
ssp. <i>dicoccon</i> PI355465 (Namur, Belgium) × ssp. <i>strangulata</i> AS2405	25 (4)	1	1522	19	1.25
ssp. <i>dicoccon</i> PI355476 (Namur, Belgium) × ssp. <i>strangulata</i> AS2404	38.10 (21)	5	6662	13	0.2
ssp. <i>dicoccon</i> PI355477 (Canada) × ssp. <i>strangulata</i> AS2405	70 (10)	6	1284	7	0.55
ssp. <i>dicoccon</i> PI355477 (Canada) × ssp. <i>strangulata</i> AS2399	50 (24)	10	3596	9	0.25
ssp. <i>dicoccon</i> PI355490 (Czech Republic) × ssp. <i>strangulata</i> AS2399	50 (4)	1	20	2	10
ssp. <i>dicoccon</i> PI355497 (Former Soviet Union) × ssp. <i>strangulata</i> AS2399	14.71 (34)	5	4540	6301	13.9
ssp. <i>dicoccon</i> PI355497 (Former Soviet Union) × ssp. <i>strangulata</i> AS2403	33.33 (9)	3	1548	33	2.13
ssp. <i>dicoccon</i> PI355507 (Turkey) × ssp. <i>strangulata</i> AS2386 (Iran)	100 (2)	2	1458	46	3.16
ssp. <i>dicoccon</i> PI355527 (Balkans) × ssp. <i>strangulata</i> AS2399	100 (5)	5	6040	329	5.45
ssp. <i>dicoccon</i> PI377655 (Former Yugoslavia) × ssp. <i>strangulata</i> AS2399	91.3 (23)	16	16002	3418	21.36
ssp. <i>dicoccon</i> PI377655 (Former Yugoslavia) × ssp. <i>strangulata</i> AS2386 (Iran)	81.25 (48)	38	30240	2419	8
ssp. <i>dicoccon</i> PI377655 (Former Yugoslavia) × ssp. <i>strangulata</i> AS2407	21.28 (47)	10	7548	1953	25.87
ssp. <i>dicoccon</i> PI415152 (Israel) × ssp. <i>tauschii</i> AS60	8.33 (24)	2	1128	63	5.59
ssp. <i>dicoccon</i> PI434998 (Bosnia and Herzegovina) × ssp. <i>strangulata</i> AS2386 (Iran)	6.48 (108)	5	5600	105	1.88
ssp. <i>turanicum</i> PI124494 (India) × ssp. <i>tauschii</i> AS77 (Henan, China)	100 (3)	2	488	4	0.82

Table 1 continued

Cross combinations ^a	Germination rate of F ₁ seeds ^b	No. F ₁ plants obtained	No. selfed florets	No. F ₂ seeds	Seed set rate (%)
<i>ssp. turanicum</i> PI184526 (Portugal) × <i>ssp. strangulata</i> AS2386 (Iran)	33.33 (6)	2	676	6	0.89
<i>ssp. turanicum</i> PI184543 (Portugal) × <i>ssp. strangulata</i> AS2386 (Iran)	63.64 (11)	6	7872	18	0.23
<i>ssp. turanicum</i> PI211691 (Turkey) × <i>ssp. strangulata</i> AS2386 (Iran)	20 (15)	2	534	1	0.19
<i>ssp. turanicum</i> PI256034 (Spain) × <i>ssp. strangulata</i> AS2386 (Iran)	8.33 (12)	1	74	1	1.35
<i>ssp. turanicum</i> PI306665 (France) × <i>ssp. strangulata</i> AS2386 (Iran)	33.33 (3)	1	450	2	0.44
<i>ssp. turanicum</i> PI352514 (Azerbaijan) × <i>ssp. strangulata</i> AS2399	18.18 (11)	2	214	39	18.22
<i>ssp. turanicum</i> PI532136 (Egypt) × <i>ssp. tauschii</i> AS65 (Former Soviet Union)	66.67 (3)	1	160	4	2.5
<i>ssp. dicoccon</i> PI113961 × <i>ssp. strangulata</i> AS2388	5 (20)	1	1198	0	0
<i>ssp. dicoccon</i> PI306534 × <i>ssp. tauschii</i> AS66	11.11 (9)	1	86	0	0
<i>ssp. dicoccon</i> PI355465 × <i>ssp. strangulata</i> AS2404	100 (1)	1	280	0	0
<i>ssp. dicoccon</i> PI355477 × <i>ssp. strangulata</i> AS2386	75 (32)	23	10944	0	0
<i>ssp. dicoccon</i> PI377653 × <i>ssp. strangulata</i> AS2386	20.99 (88)	9	1900	0	0
<i>ssp. polonicum</i> PI14892 × <i>ssp. tauschii</i> AS82	100 (1)	1	1086	0	0
<i>ssp. turanicum</i> PI166450 × <i>ssp. strangulata</i> AS2386	30 (10)	3	728	0	0
<i>ssp. turanicum</i> PI184526 × <i>ssp. strangulata</i> AS2403	8.33 (12)	1	76	0	0
<i>ssp. turanicum</i> PI256034 × <i>ssp. strangulata</i> AS2404	44.44 (9)	1	170	0	0
<i>ssp. turanicum</i> PI306665 × <i>ssp. strangulata</i> AS2399	100 (1)	1	1042	0	0
<i>ssp. turanicum</i> PI337643 × <i>ssp. strangulata</i> AS2404	100 (1)	1	156	0	0
<i>ssp. turanicum</i> PI337643 × <i>ssp. strangulata</i> AS2386	100 (1)	1	136	0	0
<i>ssp. turanicum</i> PI347132 × <i>ssp. strangulata</i> AS2404	100 (1)	1	70	0	0

These hybrids were produced by crossing *T. turgidum* lines (female) with *Ae. tauschii* accessions without embryo rescue and hormone treatment

^a The known origins of *T. turgidum* or *Ae. tauschii* are indicated in brackets

^b The number of F₁ hybrid seeds of *T. turgidum* with *Ae. tauschii* used for germination are indicated in brackets

analysis, at least 22 PMCs were observed for each of synthetic hexaploid wheats (SHWs). The univalents (I), bivalents (II), trivalents (III), and quadrivalents (IV) were counted and their average numbers were calculated.

Results

Selfed seedset by F₁ hybrids

As Table 1 shows, F₁ hybrid seeds were obtained from 115 crosses involving 76 *T. turgidum* lines and 24 *Ae. tauschii* accessions without embryo rescue and

hormone treatment. Randomly selected hybrid seeds were germinated for the production of F₁ hybrid plants. Some F₁ plants were dead during seedling stage. However, F₁ plants grew vigorously and sprouted were obtained from the 115 cross combinations. All of the F₁ plants had tough tenacious glumes, a trait obviously inherited from their male parent *Ae. tauschii*. As expected, cytological observation of the root-tip cells confirmed that all of the F₁ plants were triploids with 21 chromosomes, indicating that they all were true hybrids. Our previous study has shown that selfed seedset (percentage of selfed seeds over total selfed florets) is a good indication for the formation of unreduced gametes (Zhang et al.

2007). Therefore, selfed seedset of each hybrid combination was investigated (Table 1). Clearly, the 115 hybrid combinations differed significantly ($P \leq 0.01$, $F = 11.40$) in the selfed seedset. The distribution frequency of selfed seedset rates was summarized in Table 2. About 11% of the combinations failed to set any seed by selfing. For example, we checked 10944 selfed florets in 23 F_1 plants between *T. turgidum* ssp. *dicoccon* (abbreviated as *T. t. dicoccon*, hereafter) line PI355477 and *Ae. tauschii* (abbreviated as *A. t*) accession AS2386 and found no seed at all. On the other hand, nine combinations (i.e. *T. t. durum* Langdon with *A. t* AS60, AS65 and AS2404; *T. t. dicoccon* PI377655 with *A. t* AS2399 and AS2407; *T. t. dicoccoides* AS285 with *A. t* AS60; *T. t. turgidum* AS2239 with *A. t* AS2395, *T. t. turgidum* AS2240 with *A. t* AS84 and *T. t. turgidum* AS2310 with *A. t* AS60) had selfed seedset higher than 20%.

The results of analysis of variance to detect the difference between subspecies were shown in Table 3. Analysis of variance was not done for the hybrids involving *T. turgidum* ssp. *dicoccoides*, *durum*, *polonicum*, and *carthlicum* because only a few lines of each were used. Our analysis suggested that, as a whole, *T. t. dicoccon* and *T. t. turgidum* were able to promote setting of significantly more

selfed seeds on F_1 s than *T. t. turanicum* could do (Table 3). The 76 *T. turgidum*–ssp. *strangulata* and 39 *T. turgidum*–ssp. *tauschii* combinations showed selfed seedset of 4.93 and 7.97%, respectively (Table 3). There were no significant differences on selfed seedset between *Ae. tauschii* subspecies *tauschii* and *strangulata* at the level of 5% ($t = 1.47$, $df = 52.67$, $P = 0.15$).

To investigate the impact of parental genotype on F_1 selfed seedset, we crossed different *T. turgidum* genotypes with the same *Ae. tauschii* accession and vice versa (Table 4). Analyzing our data revealed that *T. turgidum* lines differ significantly in their influence on the F_1 selfed seedset (Table 4). Parental genotype effect by *Ae. tauschii* accessions was very significant when *T. t. dicoccoides* AS285 or AS286 was used as the female parent but was not significant when *T. t. durum* Langdon or *T. t. turgidum* AS2255 was the female parent (Table 4). It seems that *T. turgidum* genotypes complemented or suppressed the *Ae. tauschii* genotypes in these cases.

Cytological observation of chromosomes in F_2 plants

Cytological observation was carried out to check the somatic chromosome number in the root-tip cells of

Table 2 Seedset rate distribution for 115 F_1 hybridization combinations

Seedset rate distribution	0	0–1%	1–5%	5–10%	10–20%	>20%
No. of cross combinations (%)	13 (11.3%)	30 (26.09%)	32 (27.83%)	19 (16.52%)	12 (10.43%)	9 (7.83)

Table 3 The summarized seedset rates based on seven *T. turgidum* and two *Ae. tauschii* subspecies

Taxon	No. total combinations	No. fertile combinations	Mean of selfed seedset rates
<i>T. turgidum</i> ssp. <i>dicoccon</i>	47	42	5.01 ^a
<i>turgidum</i>	32	32	6.20 ^a
<i>turanicum</i>	15	8	1.64 ^b
<i>dicoccoides</i>	11	11	5.60
<i>durum</i>	8	8	18.57
<i>carthlicum</i>	1	1	0.93
<i>polonicum</i>	1	0	0
Total	115	102	5.83
<i>Ae. tauschii</i> ssp. <i>strangulata</i>	76	12	4.93
<i>tauschii</i>	39	1	7.97
Total	115	102	5.83

The means of selfed seedset rates are based on all combinations of a subspecies

^a, ^b are significantly different at $P \leq 0.01$

Table 4 Summarized results of variance analysis on seedset rates for the combinations with same *T. turgidum* or *Ae. tauschii*

Items	F-value	P-value
The combinations with different <i>T. turgidum</i> line and same <i>Ae. tauschii</i> accessions		
Eight <i>T. turgidum</i> with ssp. <i>tauschii</i> AS60	12.08	0.0001
Five <i>T. turgidum</i> with ssp. <i>tauschii</i> AS65	3.97	0.04
Twenty-two <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2386	9.97	0.0001
Seven <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2388	53.54	0.0001
Eleven <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2399	11.11	0.0001
Fourteen <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2404	7.83	0.0001
Six <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2405	9.49	0.003
Five <i>T. turgidum</i> with ssp. <i>tauschii</i> AS2407	17.78	0.0001
The combinations with same <i>T. turgidum</i> line and different <i>Ae. tauschii</i> accessions		
ssp. <i>durum</i> Langdon with seven <i>Ae. tauschii</i>	1.07	0.40
ssp. <i>turgidum</i> AS2255 with four <i>Ae. tauschii</i>	2.23	0.14
ssp. <i>dicoccoides</i> AS285 with five <i>Ae. tauschii</i>	50.68	0.0001
ssp. <i>dicoccoides</i> AS286 with six <i>Ae. tauschii</i>	5.01	0.002

the F_2 plants (Fig. 1a) and the chromosome pairing in their pollen-mother-cells (Fig. 1b). About 80% F_2 plants observed were found to have $2n = 42$ chromosomes. As Table 5 shows, the examined F_2 plants with 42 chromosomes also had their chromosomes paired almost normally during meiosis, proving that they were amphidiploids.

Discussion

In this study, F_1 haploid hybrid plants with 21 chromosomes were obtained from 115 cross combinations without embryo rescue and hormone treatment. About 89% of the combinations produced F_2 seeds by selfing. Cytological analysis found that most of the F_2 plants were amphidiploids with 42 chromosomes (euploids), indicating that the genomes in the F_2 plants had been spontaneously duplicated by the union of two unreduced gametes. Both meiotic restitution and doubled somatic sectors can result in unreduced gametes. However, no fully fertile plant was observed in this study, thus excluding the idea of doubled somatic sectors for the production of unreduced gametes. Our previous cytological observations have clearly shown that the pairing of 21 chromosomes in the F_1 *T. turgidum*–*Ae. tauschii* hybrids was rare in the meiotic metaphase I and meiotic restitution is responsible for the production of functionally unreduced gametes in these hybrids (see review in Zhang et al. 2007).

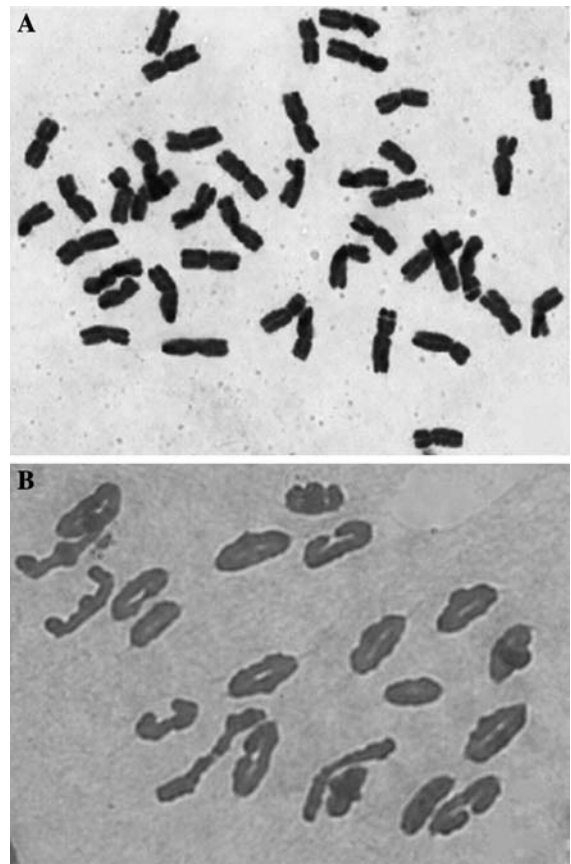


Fig. 1 Chromosomes of root-tip (a) and pollen-mother-cell (b) in new synthetic hexaploid wheats. a 42 chromosomes in the F_2 plants of AS2255 \times AS60. b Chromosome pairing with 21 bivalents, including 4 rod and 17 ring bivalents, at meiotic metaphase I in a cell of F_2 plants of Langdon \times AS60

This study suggested that *T. turgidum* genotypes play more important role than *Ae. tauschii* genotypes in producing F_1 selfed seeds and that some *T. turgidum* genotypes are more capable in inducing unreduced gamete production in the F_1 s than others. *T. turgidum* was originated from the cross of *Ae. speltoides* with *T. monococcum* (Kihara and Lilienfeld 1949). It seems that unreduced gametes most likely played a key role in this process. Obviously, every original *T. turgidum* plant should inherit the meiotic restitution genes. As David et al. (2004) suggested, maintaining a genetic basis for unreduced gamete production in progenitor species remains a paradox because genes inducing the production of such gametes are expected to diminish the individual fitness at the ploidy level of progenitors. *T. turgidum* genotypes that carry genes for unreduced gametes are favorable to producing interspecific hybrids with alien species. The significant difference in selfed seedset we observed among the hybrids involving different *T. turgidum* genotypes suggested that evolution is working towards reduction of the ability to form unreduced gametes in *T. turgidum* by accumulation of mutations decreasing activity of the meiotic

restitution genes and thus increasing the fitness of *T. turgidum* as a species.

This study clearly showed that meiotic restitution genes are widely distributed within *T. turgidum*. Of the eight subspecies proposed by Van Slageren (1994), all but *T. t. paleocolchicum* and *T. t. polonicum* showed ability to produce spontaneous amphidiploids in their hybrids with other species. Our data suggested that this ability is not limited to a few genotypes within each subspecies. The genotypes we tested in this study were collected from a large geographic area (Table 1). *T. turgidum* is known to grow together with *Ae. tauschii* on the farmland in some areas and thus has chance to make hybrids in multiple places. The wide spread of spontaneous amphidiploidization in the *T. turgidum*–*Ae. tauschii* F_1 hybrids found in our study, therefore, supports the multi-origin hypothesis for the hexaploid wheat proposed by many wheat scientists (Dvorak et al. 1998; Talbert et al. 1998; Lelley et al. 2000; Caldwell et al. 2004; Giles and Brown 2006; Zhang et al. 2008b).

Previous studies have shown that the meiotic restitution genes of *T. turgidum* were functional in

Table 5 Chromosome pairing of F_2 plants from synthetic hexaploid wheats

Combinations	Means of chromosome pairing configuration
AS2255 × AS60	3.88 I + 5.00 rod II + 14.00 ring II ^a
AS313 × AS60	1.27 I + 5.33 rod II + 15.07 ring II
AS285 × AS60	6.27 I + 6.80 rod II + 11.03 ring II
AS286 × AS60	1.08 I + 6.59 rod II + 13.86 ring II
Langdon × AS60	1.69 I + 6.94 rod II + 13.22 ring II
Langdon × AS77	1.54 I + 6.49 rod II + 13.74 ring II
Langdon × AS2386	1.15 I + 4.98 rod II + 15.44 ring II
Langdon × AS2399	1.61 I + 6.68 rod II + 13.52 ring II
AS2255 × AS2395	2.13 I + 5.80 rod II + 14.03 ring II + 0.07 III
AS2255 × AS2393	0.86 I + 5.60 rod II + 15.49 ring II
AS285 × AS2386	5.77 I + 9.07 rod II + 8.93 ring II + 0.03 III + 0.03 IV
AS285 × AS2404	5.10 I + 8.31 rod II + 10.14 ring II
AS286 × AS2386	0.59 I + 3.90 rod II + 16.80 ring II
AS286 × AS2407	0.58 I + 3.64 rod II + 17.06 ring II
AS2239 × AS2395	3.14 I + 6.34 rod II + 13.09 ring II
AS2240 × AS77	3.62 I + 5.37 rod II + 13.60 ring II
AS2285 × AS77	0.34 I + 4.06 rod II + 16.78 ring II
AS2291 × AS2404	2.60 I + 8.98 rod II + 10.68 ring II
PI94655 × AS2407	3.95 I + 4.05 rod II + 14.97 ring II
PI94675 × AS2405	1.00 I + 4.00 rod II + 16.50 ring II

^a I, univalent; II, bivalent; III, trivalent; IV, quadrivalent; chromosome pairing configuration for the first 14 synthetic hexaploid wheats were extracted from Li et al. (2008)

hybrid derivatives of *T. turgidum* with many relative species (see review in Zhang et al. 2007 and Tiwari et al. 2008). Here we prospect wide use of these *T. turgidum* meiotic restitution genes in wheat improvement. They can greatly help not only alien gene introgression into wheat, but also aid our efforts in doubled haploids (DH) production. The DHs of a true-breeding crop like wheat can quickly fix genetic recombination, and thus enhance breeding efficiency or facilitate genetic analysis.

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