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Cytological Evidence on Meiotic Restitution in Pentaploid F_1 Hybrids between Synthetic Hexaploid Wheat and *Aegilops variabilis*

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Abstract — Meiotic restitution responsible for the production of unreduced gametes is significant in both allopolyploid origin and plant breeding. There are lots of reports on meiotic restitution in hybrids of tetraploid *Triticum turgidum* wheat with alien species. Hexaploid wheat SHW-L1 was newly synthesized from the F_1 plants between a *T. turgidum* ssp. *turgidum* line with meiotic restitution gene(s) and *Aegilops tauschii*. Based on the male gametogenesis observation, the present study provided the experimental evidence on the occurring of meiotic restitution in the F_1 pentaploid ($2n=5x=35$, ABDUS^l) hybrids of synthetic hexaploid wheat SHW-L1 with *Ae. variabilis*. Meiotic restitution resulted in unreduced gametes that in turn spontaneously produced neopolyploids with genome AABBDDUUS^{l}S^l. The implications of these findings for gene flow between hexaploid wheat and alien species were discussed.

Key Words: *Aegilops variabilis*, gene flow, meiotic restitution, neopolyploid, synthetic hexaploid wheat, unreduced gametes.

INTRODUCTION

Tetraploid durum (*Triticum turgidum* L., $2n=4x=28$, AABB) and hexaploid bread wheat (*T. aestivum* L., $2n=6x=42$, AABBDD) are important cereals sustaining mankind. Bread wheat was formed by spontaneous hexaploidization after the intercrossing between cultivated *T. turgidum* and *Aegilops tauschii* (KIHARA 1944). The formation and union of unreduced gametes might have played a key role in the origin of bread wheat, which produced a fertile triploid F_1 hybrid between *T. turgidum* and *Ae. tauschii* and resulted in spontaneous production of amphidiploids that set hexaploid seeds (KIHARA and LILIENFELD 1949; RAMSEY and SCHEMSKE 2002; CAI and XU 2007; JAUHAR 2007; MATSUOKA *et al.* 2007).

Unreduced gametes derived from meiotic restitution via first-division restitution (FDR) or single-division meiosis (SDM) have been observed in F_1 hybrids of *T. turgidum* subspecies *dicoccoides*, *dicoccon*, *carthlicum*, *turanicum*, *durum* and *turgidum* with *Ae. tauschii* (KIHARA and LILIENFELD 1949; TANAKA 1959, 1961; XU and DONG 1992; FUKUDA and SAKAMOTO 1992a, b; XU and JOPPA 1995, 2000; MATSUOKA and NASUDA 2004; CAI and XU 2007; MATSUOKA *et al.* 2007; ZHANG *et al.* 2007). FDR produces dyads as final products through a process including the failure of chromosomes to move to the poles at anaphase I, the formation of restitution nuclei at telophase I and the equational division at anaphase II. SDM also produces dyads as final

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products through single equational division at anaphase I in the first division. Furthermore, unreduced gametogenesis has been suggested in F_1 hybrids of *T. turgidum* with other species *Ae. ovata*, *Ae. umbellulata*, *Ae. comosa*, *Ae. longissima*, *Ae. speltoides*, *Ae. ventricosa*, *Ae. crassa*, *Ae. triuncialis*, *Hynaldia villosa* and rye (*Secale cereale* L.) as well as in haploid plants of tetraploid durum wheat (MAAN and SASAKUMA 1977; BLANCO *et al.* 1983; STEFANI *et al.* 1983; LIU *et al.* 1986; XU and DONG 1992; PIGNONE 1993; XU and JOPPA 2000; JAUHAR *et al.* 2000; DAVID *et al.* 2004). Meiotic restitution usually takes place in a plant with a haploid genome, which is controlled by gene(s) in *T. turgidum* (XU and JOPPA 2000; JAUHAR *et al.* 2000).

Meiotic restitution gene(s) in *T. turgidum* can be transferred into synthetic hexaploid wheat through a process of amphiploidization between *T. turgidum* and *Ae. tauschii*. Whether or not meiotic restitution gene derived from *T. turgidum* still works in the haploid hybrids of synthetic hexaploid wheat with other species is an important issue on both practical application to promote the production for amphidiploids of inter-specific hybrids and doubled haploids, and theoretical studies to better understand the allopolyploid origin and DNA introgression. Hexaploid wheat SHW-L1 was newly synthesized from the F_1 plants between *T. turgidum* ssp. *turgidum* line AS2255 and *Ae. tauschii* AS60 (ZHANG *et al.* 2004). AS2255 has gene(s) for meiotic restitution (ZHANG *et al.* 2007). The pentaploid (ABDUS^l) F_1 hybrids of SHW-L1 with *Ae. variabilis* (Syn. *Ae. peregrine*, 2n=4x=28, UUS^{l}S^l) showed high fertility and spontaneously produced decaploid F_2 plants with 2n=70 (amphiploid value) or close to this amphiploid value. These data suggested that the re-synthesized hexaploid wheat, like its parent AS2255, is able to induce the formation of unreduced gametes in its F_1 hybrids with other species (ZHANG *et al.* 2007). However, there were not direct observations on meiotic process. Hitherto, the final link in the chain of evidence on the occurring of meiotic restitution in hybrids between newly synthetic hexaploid wheat and other species is still lack.

This study firstly provided the empirical verification of meiotic restitution in F_1 hybrids between synthetic hexaploid wheat and alien species (*Ae. variabilis*) by meiotic observation. The implications of this finding for DNA introgression between wheat-alien species were discussed.

MATERIALS AND METHODS

Plant materials - Plant materials used in this study included newly synthesized hexaploid wheat SHW-L1 (2n=6x=42, AABBDD), an amphiploid between *T. turgidum* ssp. *turgidum* line Yuanzhuimai (AS2255) and *Ae. tauschii* accession AS60 (ZHANG *et al.* 2004), and *Ae. variabilis* (2n=4x=28, UUS^{l}S^l) accession AS24 from France.

Production of hybrids - Synthetic hexaploid wheat SHW-L1 was pollinated with *Ae. variabilis* accession AS24 according to previous methods (LIU *et al.* 1999; ZHANG *et al.* 2007). No embryo rescue technique or hormone treatment was applied when producing the F_1 hybrids. In order to obtain selfed F_2 seeds, spikes of the F_1 were randomly selected and bagged before anthesis to prevent cross-contamination.

Cytological observation - The procedures of somatic chromosome and meiotic observation were the same as those described by ZHANG *et al.* (2007).

RESULTS

In crosses between synthetic hexaploid wheat SHW-L1 and *Ae. variabilis* AS24, hybrid F_1 seeds were obtained, from which 15 polyhaploid F_1 plants (2n=5x=35, genome ABDUS^l) were grown in 2006-2007 crop seasons. These plants were vigorously grown and showed an average height of 122.07 cm and 13.07 tillers. These F_1 hybrid plants were selfed to produce F_2 seeds. The selfed seedset rate was 3.41% in 2007, which was significantly lower ($p \leq 0.0001$) than 12.7% in 2004 and 19.4% in 2006 previously reported by us (ZHANG *et al.* 2007). The variation in the seedset over the years reflected a strong environmental influence. The lower seedset rate may be caused by the atypical temperature with warm winter and cold spring in 2006-2007 crop seasons, which might lead to a reduced fertility.

Root tips of random 15 F_2 plants were observed. Chromosome number range of F_2 was 59 to 70 with an average of 66.13 (TABLE 1), which was agreed with previous report that the pentaploid F_1 hybrids spontaneously produced decaploid F_2 plants with 2n=70 (amphiploid value) or close to this amphiploid value (ZHANG *et al.* 2007). Among the 15 F_2 hybrid plants observed (TABLE 1), 3 with chromosome number 2n=70 were euploids, the remainder 12 plants were aneuploids.

To elucidate the cytological mechanism of fertility in F_1 hybrids and spontaneously doubled chromosomes in F_2 plants, male gametogenesis was observed in the SHW-L1 × AS24 F_1 plants. Although 1 or 2 bivalents were observed in about 50% pollen-mother-cells (PMCs), most of 35 chromosomes were univalent at early metaphase I (Fig. 1-A). The chromosomes of some cells aligned on the equator at metaphase I (Fig. 1-B, a). Separation of sister chromatids occurred at anaphase I (Figs. 1-B, b; 1-C, c-e) via single-division meiosis (SDM). Meanwhile, first-division restitution (FDR) nuclei was also observed (Fig. 1 C, f), which then underwent a normal equational division at anaphase II (Fig. 1-D). Both of the two meiotic pathways could lead to formation of dyads (Fig. 1-E). Similar meiotic phenomena were observed in hybrids of *T. turgidum* AS2255 with *Ae. tauschii* AS60 (ZHANG *et al.* 2007). AS2255 is the female parent of SHW-L1, which carrys meiotic restitution gene(s).

Symmetric dyad daughter cells might have resulted in unreduced euhaploid gametes. The union of euhaploid female and male gametes leaded to the spontaneous formation of euploidy amphiploid F_2s (TABLE 1). Meanwhile, division abnormalities such as laggards (Fig. 1-C, c-d), multipolar separations (Fig. 1-C, e), and micronuclei (Fig. 1-E) occurred with a frequency of about 60%. These abnormal divisions may have resulted in the production of aneuhaploidy sporocytes responsible for the aneuploidy amphiploid F_2s (TABLE 1). In addition to dyads, triads (Fig. 1-E) and tetrads were also observed, probably indicating occurrence of two meiotic divisions in some pollen-mother-cells.

DISCUSSION

It was established that unreduced gametes could have evolutionary significance for the origin of polyploidy species (HUSBAND 2004). The origin of bread wheat provides an excellent example. Bread wheat was resulted from a recent spontaneous amphiploidization between *T. turgidum* and *Ae. tauschii*. It has been clearly indicated that meiotic restitution pathways, FDR or SDM, can lead to the formation of unreduced gametes that in turn spontaneously produced amphiploids in the hybrids of *T. turgidum* with *Ae. tauschii.*

Besides for the origin of polyploidy, spontaneously neopolyploids provide a likely route for gene flow, the incorporation of DNA from one species into the gene pool of another species (For examples, SHARMA and GILL 1983; CARPUTO 2003; DAVID *et al.* 2004). DAVID *et al.* (2004) found that fertile *T. turgidum-Ae. ovata* amphiploids were produced through the unreduced gametes, and that some of them carried *T. turgidum-Ae. ovata* recombinant chromosomes. Present study indicated that meiotic restitution occurs in the F_1 hybrids of synthetic hexaploid wheat with *Ae. variabilis* and results in unreduced gametes and then spontaneous amphiploids. During meiotic process in the F_1 hybrids, about 50% pollen-mother-cells showed 1 or 2 bivalents, which might lead to the production of SHW-L1-*Ae. variabilis* recombinant chromosomes. The recombinant chromosomes could be then transmitted to some of amphiploids via unreduced gametes. These amphiploids with recombinant chromosomes provide the 'bridge' for gene flow, which can be transmitted to its derivatives by backcrossing to parents.

T. turgidum having genes for meiotic restitution might have participated in the origin of bread wheat (KIHARA and LILIENFELD 1949; RAMSEY and SCHEMSKE 2002; CAI and XU 2007; JAUHAR 2007; MATSUOKA *et al.* 2007). It was resonably speculated that the original bread wheat carried meiotic restitution genes, which was then spread into different areas and sympatrically grown with some of wild relatives over large areas for many years. The spontaneous hybridization between sympatrically grown wheat and alien species is known to occur frequently (VAN SLAGEREN 1994; ZAHARIEVA and MONNEVEUX 2006). WEISSMANN *et al.* (2005) proved spontaneous hybridization between wheat and *Ae. variabilis* occurred in nature and found spontaneous DNA introgression from domesticated polyploid wheat into *Ae. variabilis* and the stabilization of this sequence in naturally wild populations despite not having

TABLE 1 — Chromosome distribution of observed F_2 plants.

No. chromosome	59	61	62	64	65	66	68	69	70
No. plants	1	1	1	2	1	1	4	1	3

homologous chromosomes between the two species. Our present study suggests that the neopolyploids between wheat and *Ae. variabilis* may be one of the routes for DNA introgression from wheat to *Ae. variabilis.* Further works on more cases for gene flow between wheat and other alien species and the distributions of meiotic restitution genes in *T. turgidum* and bread wheat are important to further understand hybrid speciation by introgression, and to better evaluate the ecological risks of transgenic wheats.

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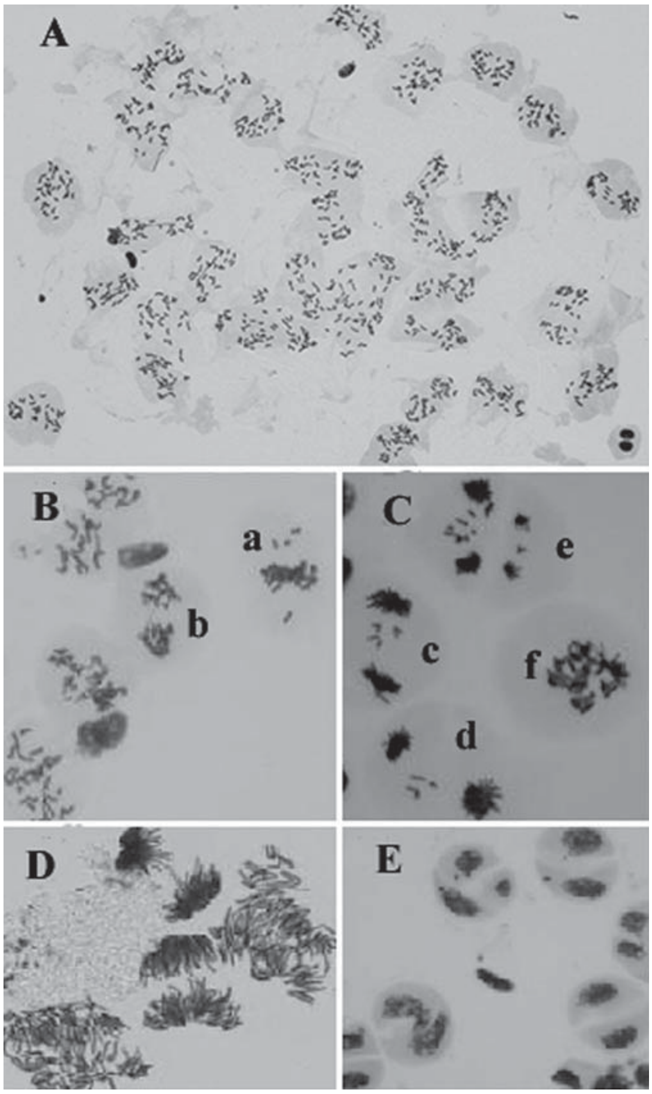

Fig. 1 — Meiotic observations of pollen-mother cells of newly synthetic hexaploid wheat SHW-L1 × *Aegilops variabilis* AS24 F_1 hybrids. (A) Asynaptic chromosomes presented as univalents at early metaphase I. (B) Univalents aligned at equator at metaphase I (a), separation of sister chromatids moved toward opposite poles with at anaphase I (b). (C) Abnormal separation of sister chromatids moved toward opposite poles with laggards (c, d) or multipolar separations (e), or chromosome restitution at telophase I (f). (D) Sister chromatids moved toward opposite poles at anaphase II. (E) Dyad with or without micronucei and triad.

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