

RESEARCH NOTE

Amphitelic orientation of centromeres at metaphase I is an important feature for univalent-dependent meiotic nonreduction

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Introduction

Univalent-dependent meiotic nonreduction, which leads to the production of unreduced gametes, is thought to be the predominant mechanism underlying allopolyploid plant formation. However, little is known about the underlying cytological mechanism. In the present study, we observed male sporogenesis in F₁ amphihaploid hybrids of wheat–rye by FISH with the help of diagnostic PrCEN-1 specific for the rye centromere. Our observations indicated that at meiotic metaphase I, the chromosomes were accumulated on the equatorial plate. At this stage, the elongated centromeres were amphitelicly oriented perpendicular to the equatorial plate, indicating tension from opposite poles. At late metaphase, the centromeres and sister chromatids started separating. Subsequently, the sister chromatids and centromeres split finally resulting in dyads. Our observations indicate that bipolar orientation of the sister kinetochores of univalents at the equatorial plate in metaphase I is important for the subsequent bipolar separation of sister chromatids in the first meiotic division.

Allopolyploids are common in plants. Wide hybridization, the first step for the origination of allopolyploids, brings divergent genomes from different species together into an amphihaploid hybrid. Because only one set of homologous chromosomes is present, amphihaploids (analogous to haploid plants) are usually sterile due to reduced meiosis. Meiotic nonreduction (meiotic restitution), however, can lead to production of functionally unreduced gametes, and their union immediately generates an amphidiploid (allopolyploid). Meiotic nonreduction is thought to be the

predominant mechanism underlying allopolyploid plant formation (Matsuoka 2011). Despite its evolutionary significance, however, little is known about the underlying cytological mechanism (De Storme and Geelen 2013).

Meiotic nonreduction has been largely reported in amphihaploid hybrids of the big tribe *Triticeae* (Cai and Xu 2007; Matsuoka 2011; Silkova *et al.* 2011). This tribe includes important allopolyploid crops such as common wheat, durum wheat, triticale (\times *Triticosecale* Wittmack) and many forage species. First division restitution (FDR) and second division restitution (SDR) have been reported as the two main cytological processes for meiotic nonreduction in *Triticeae* amphihaploids. FDR lacks chromosome segregation at anaphase I (AI) following nuclear restitution and has a normal second meiotic division (Xu and Joppa 2000; Jauhar 2007). SDR is characterized by a mitosis-like equatorial division of sister chromatids at AI without the second meiotic division (Matsuoka and Nasuda 2004; Zhang *et al.* 2007, 2008; Yang *et al.* 2010; Silkova *et al.* 2013). Since both of them are characterized by a single meiotic division leading to the formation of dyads with unreduced chromosome number, they are also called as single-division meiosis (SDM) (Matsuoka and Nasuda 2004) or mitotic-like division (Zhang *et al.* 2007, 2008). However, previous studies have been mainly based on the observation of meiotic chromosome behaviours in pollen mother cells (PMCs) by conventional staining techniques. The detail process for the occurrence of meiotic nonreduction in *Triticeae* amphihaploids need to be further illustrated using new techniques.

The centromere is the region on a chromosome that joins two sister chromatids and it provides a foundation for the assembly of a kinetochore on each sister chromatid. During a meiotic division, spindle fibres, formed by microtubules,

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attach to the kinetochores and pull two paired chromosomes or the sister chromatids of a chromosome apart and towards one of the poles. Observation of centromere behaviour, therefore, is important to reveal the cytological mechanism for meiotic nonreduction. In this study, we used fluorescence *in situ* hybridization (FISH) with a probe specific for rye centromeres to trace their positions and movements during meiosis in PMCs of a wheat–rye hybrid. Our observations suggest that amphitelic orientation of centromeres at metaphase I (MI) is an important feature of univalent-dependent meiotic nonreduction.

Materials and methods

Plant materials

The synthetic hexaploid wheat line Syn-SAU-6 ($2n = 6x = 42$, AABBDD) was used as the female parent in crossing with Chinese rye (*Secale cereale* L., $2n = 2x = 14$, RR) landrace Qinling (AS156). Syn-SAU-6 was produced by spontaneous chromosome doubling via unreduced gametes resulting from meiotic nonreduction in hybrids of *T. turgidum* ssp. *durum* line Langdon ($2n = 4x = 28$, AABB) with *Aegilops tauschii* accession AS65 ($2n = 2x = 14$, DD) (Zhang et al. 2010). Young leaves of the hexaploid wheat cultivar Chinese Spring were also used for genomic DNA isolation.

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 seeds were obtained by selfing individual F_1 plants. F_1 and F_2 seeds were germinated in Petri dishes on filter paper, and the seedlings were transplanted into an experimental field with spacing of 10 cm within rows and 30 cm between rows.

Cytological observations

Anthers of emerging spikes containing PMCs from F_1 hybrids and root tips from F_2 hybrids were fixed in 1:3 (v/v) acetic acid–ethanol and stored at 4°C. The fixed materials were squashed in a drop of 45% acetic acid. The squashed slides were uncovered after being frozen in liquid nitrogen, air-dried, and stored at –20°C until use. Genomic *in situ* hybridization (GISH) and FISH were done as described previously (Hao et al. 2013).

To identify rye chromosomes, the clone PrCEN-1 was used as the probe for FISH (Tang and Ren 2006). The sequence pAWRC.1 is a tandem repeat specific for the rye centromere (Francki 2001). PrCEN-1 contains part of pAWRC.1 (from 24 bp to 1.12 kb). The following primer set was used to prepare PrCEN-1: 5'-AAGATGCCGAGGCCGC-3', 5'-GAAGGACTTGTGTCCACGGC-3' (Tang and Ren 2006). PrCEN-1 was labelled with digoxigenin-11-dUTP (Roche Diagnostics GmbH, Mannheim, Germany) by nick translation following the manufacturer's instructions. Chromosome observations were made and documented using

an Olympus BX-51 microscope coupled to a Photometric SenSys Olympus DP70 CCD camera (Tokyo, Japan).

Results

Synthetic hexaploid wheat line Syn-SAU-6 was successfully crossed with rye cultivar Qinling with conventional crossing technology without additional treatment. A total of 23 F_1 hybrid seeds were obtained from 92 pollinated florets with 25% crossability. Seven F_1 seedlings were transplanted into an experimental field and two of them died at the seedling stage. The other five, although were amphihaploid (genome ABDR), all grew vigorously and were partially fertile, producing a total of 45 F_2 seeds (average 9 seeds per plant). The root-tips of five F_2 plants were investigated with GISH for their chromosome constitutions. Two were found to be amphiploids (octoploid, $2n = 56$), indicating the union of unreduced gametes with 21 wheat and seven rye chromosomes. The remaining three were partial amphiploids with chromosome numbers less than 56 (table 1).

Male sporogenesis was further observed in the F_1 haploid hybrids using the diagnostic PrCEN-1 FISH probe. Our observations showed that the chromosomes formed coiled chromatids joined by centromeres at prophase I. The PrCEN-1 signals for the seven rye centromeres were clearly visible (figure 1a). Condensed chromosomes were scattered at early MI (figure 1b) and then gradually moved to equatorial plate as metaphase progressed (figure 1c). The paired chromosomes in figure 1c indicate that the PMCs were at MI. A low level of chromosome pairing with 0.28 bivalents/PMC was seen in 20% of the 102 PMCs observed at MI (table 2). The ratios of chromosome pairing for wheat–wheat (figure 1c), wheat–rye, and rye–rye were 68.97%, 24.24%, and 3.45%, respectively.

With the help of the PrCEN-1 probe, we observed that rye centromere regions convened along the equatorial plate at metaphase and gradually elongated perpendicularly to the equatorial plate (figure 1, b&c). In most PMCs observed, as the sister chromatids began to separate at late metaphase, the amphitelicly oriented centromeres also began to separate into two parts, implying the beginning of anaphase (figure 1, d&e). Subsequently, the sister chromatids were equatorially divided at anaphase (figure 1, f&g). At this stage, the sister chromatids remained relatively condensed during their move towards the poles. The abnormal meiosis ended by forming dyads (figure 1h). The symmetric dyads

Table 1. Chromosome constitution of five F_2 seeds.

No. of plant	No. of chromosome	Chromosome constitution	
		Wheat	Rye
1	45	40	5
1	49	42	7
1	50	42	8
2	56	42	14

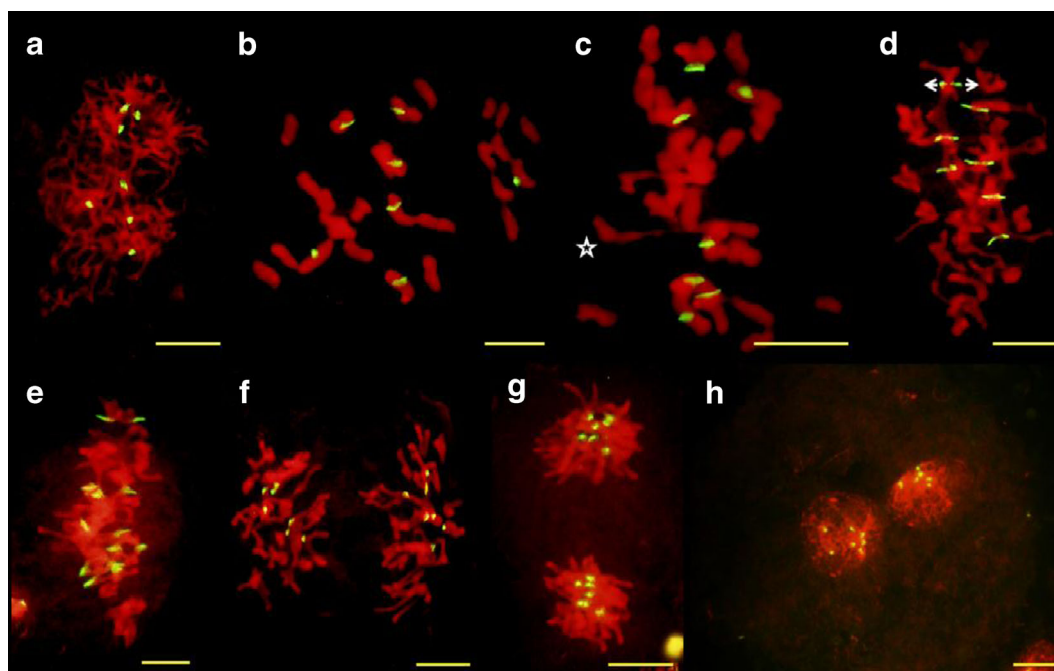


Figure 1. Meiotic observation of hybrids between synthetic hexaploid wheat line Syn-SAU-6 and rye. Rye centromeres were detected with the probe PrCEN-1 (green). a, prophase; b, early metaphase; c, metaphase; d & e, late metaphase; f & g, anaphase; h, dyad. Note that as MI progressed, the chromosomes gradually moved near to the equatorial plate (b, c). Metaphase was confirmed by wheat–wheat chromosome pairing (c). Sister chromatids and elongated centromeres began to separate at late metaphase (d & e). Scale bar, 10 μm .

Table 2. Chromosome pairing in F₁ hybrids.

Hybrid combination	No. of cells observed	Bivalents (range)		
		Rod	Ring	Multivalent
Syn-SAU-6 \times rye	102	0.28 (0–4)	0.00	0.00

were responsible for the production of euploid unreduced gametes. The union of two such unreduced games could result in the observed amphiploids.

Discussion

Unreduced gametes resulting from meiotic nonreduction have been reported in *T. turgidum*–rye (Xu and Joppa 2000) and hexaploid wheat–rye F₁ amphihaploid hybrids (Zhang *et al.* 2007; Silkova *et al.* 2011, 2013; Hao *et al.* 2013). In this study, the union of unreduced gametes from F₁ hybrids between synthetic hexaploid wheat line Syn-SAU-6 and rye immediately generated octaploid triticale. The hexaploid wheat Syn-SAU-6 inherited gene(s) for meiotic nonreduction from its *T. turgidum* parent Langdon (Xu and Joppa 2000; Matsuoka and Nasuda 2004; Zhang *et al.* 2007, 2008, 2010). We therefore inferred that the production of unreduced gametes in the present study was promoted by meiotic nonreduction gene(s) derived from Langdon.

Two cytological processes, FDR and SDR, were thought to be responsible for the production of unreduced gametes

in amphihaploid hybrids of *T. turgidum* Langdon–*A. tauschii* (Xu and Joppa 2000; Matsuoka and Nasuda 2004; Zhang *et al.* 2007, 2008). The division for wheat–rye hybrids in this study can be regarded as SDR since univalents separate sister chromatids in the first division, and the second division is abandoned, i.e., the nucleus is restituted (Silkova *et al.* 2013). Evidences for equatorial division with sister chromatid division at AI were shown. The chromosomes in figure 1c were clearly at MI. By comparing them with those shown in figure 1, d&e, particularly the appearance of rye centromeres, we can probably say that the sister chromatids in figure 1, d&e separated at AI. On the other hand, we did not see any evidence of FDR occurrence in the PMCs observed. Therefore, SDR was probably the predominant, if not, only mechanism for production of unreduced gametes in our wheat–rye F₁ hybrids.

The cytological mechanism for equatorial division with sister chromatid division at AI remains to be elucidated. Our data shows that almost all of the metaphase chromosomes are univalents, staying on the equatorial plate, with the centromeres (at least the rye centromeres) amphitelicly oriented (figure 1c). Then, the centromere regions elongate perpendicularly to the equatorial plate, indicating tension from opposite poles (figure 1, d&e). The microtubules that form the spindle fibre are responsible for this tension (Nogales and Ramey 2009). Cai *et al.* (2010) observed that sister kinetochores of univalents were attached by microtubules emanating from opposite poles, i.e.

bipolar attachment in Langdon-*A. tauschii* hybrid amphihaploids. Orientation of the sister kinetochores of univalents seems to be necessary for bipolar separation of sister chromatids in the first meiotic division (Lukaszewski 2010). Once chromosomes are amphitelicly oriented, they can undergo an equatorial division (separation of sister chromatids) at MI (Nogales and Ramey 2009). During the anaphase that follows, the spindle fibres split the centromeres and bring the sister chromatids to opposite poles (figure 1, f&g).

Based on the similar behaviours of unpaired chromosomes and their centromeres in both SDR and mitosis, we speculate that the forces applied on univalents during SDR are probably similar to those during mitosis. However, the behaviours of chromosomes and centromeres during meiosis for the paired chromosomes remain to be elucidated. Previous studies clearly indicated that a high level of homologous (Wang et al. 2010) or homoeologous (Xu and Joppa 2000) pairing interfered with meiotic restitution and the formation of unreduced gametes. However, this study and the previous studies suggested that a low pairing is not sufficient to prevent the occurrence of SDR (Xu and Joppa 2000; Zhang et al. 2008). The paired and unpaired chromosomes may have a different rhythm during meiosis. Nonsynchronous chromosome behaviours may result in SDR not effectively organizing all of the chromosomes together. Loss or gain of unorganized chromosomes can result in aneuploid or nonviable gametes.

It should be pointed out that meiotic nonreduction is affected by genotypes (Zhang et al. 2010; Silkova et al. 2011). *T. turgidum* line Langdon has a strong promoting action on meiotic nonreduction (Xu and Joppa 2000; Matsuoka and Nasuda 2004; Zhang et al. 2008). In this study, we used the synthetic hexaploid wheat line SAU-6 as the material to demonstrate the centromere behaviours. However, we are unclear how a genotype with weak promoting action on meiotic nonreduction affects centromere behaviours.

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