

# Occurrence and cytological mechanism of numerically unreduced pollen in diploid *Populus euphratica*

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## Abstract

Unreduced gametes are the driving force for the polyploidization of plants in nature, and are also an important tool for breeding of triploid individuals. The final heterozygosity of a 2n pollen grain depends on the cytological mechanism behind 2n pollen formation. In this study, meiotic abnormalities were analysed using fluorescent chromosome staining and indirect immunofluorescence during the microsporogenesis of 18 genotypes of diploid *P. euphratica* Oliv. ( $2n = 2x = 38$ ). Among the 18 genotypes, 16 genotypes produce 2n pollen and two genotypes produce only normal n pollen. In all 2n pollen producers, we found that the first meiotic division was normal but that the second division was characterized by frequent abnormal spindle orientation (parallel, tripolar, and fused spindles) and premature cytokinesis. The parallel, fused spindles and premature cytokinesis were considered to be leading dyad formation, and tripolar spindles seemed to be causing triad formation at the tetrad stage. There was a higher frequency of parallel spindles than other spindle forms, but no significant correlations between parallel spindles and dyads were observed. However, a significant association ( $r = 0.68$ ,  $P < 0.05$ ) between the tripolar spindles and dyads was found. In some Microspore mother cells (MMCs), an indirect immunofluorescence examination of meiosis II revealed that the parallel spindles led to the gathering of one or two non-sister groups of chromosomes, causing an incorporation of RMSs from two daughter nuclei. Therefore, the incorporated RMSs established two nuclear cytoplasmic domains for the control of division plane, resulting in either triad or dyad formation.

**Key words:** *Populus euphratica*, Unreduced pollen, Spindle orientation, Cytokinesis, Triploid breeding.

## Introduction

In plant species and hybrids, normal diploid ( $2n = 2x$ ) plants produce genetically four different male gametes (n, n, n, n) through complex pathways of microsporogenesis. Numerically unreduced (2n) gametes occur occasionally in stead of the expected haploid (n) gametes and likely played a key role in formation of polyploid series (HARLAN and DE WET, 1975; DEN NIS and PELOQUIN, 1977; VEILLEUX, 1985; RAMSEY and SCHEMSKE, 1998; RAMSEY, 2007; RIESEBERG and WILLIS, 2007). The induction of sexual polyploidization via controlled crosses

between naturally occurring and artificially induced-2n gametes have also been widely used in the breeding of forest trees (SEITZ, 1954; ZHANG et al., 2004; ZHU, 2006), potato (MENDIBURU et al., 1974; RAMANNA, 1979; CARPUTO and BARONE, 2005), grain crops (VEILLEUX, 1985; JAUHAR, 2003), fruit-bearing trees (SUGIURA et al., 2000), and ornamental plants (VAN TUYL and LIM, 2003; AKUTSU et al., 2007).

The mechanisms of 2n gamete formation have been studied in numerous plant species (VEILLEUX, 1985; BRETAGNOLLE and THOMPSON, 1995; RAMSEY and SCHEMSKE, 1998), and various possible pathways causing the formation of 2n gametes have been revealed: chromosome pairing, centromere division, spindle orientation, abnormal cytokinesis, post-meiotic doubling, and apomeiotic cells of the ovule. However, the most accepted currently held opinion is that 2n gamete formation is the result of irregular orientations of spindles and abnormal cytokinesis at the second meiotic division (VEILLEUX, 1985; BRETAGNOLLE and THOMPSON, 1995; RAMSEY and SCHEMSKE, 1998; ZHANG et al., 2009). Disturbed spindles and cytokinesis related to 2n pollen formation have been reported to be associated with changes in the configurations of the microtubules (GENUALDO et al., 1998; CONICELLE et al., 2003; ZHANG et al., 2009; ZHANG and KANG, 2010). In addition, D'ERFURTH et al. (2008) recently isolated and characterized a gene (*AtPSI*) involved in the abnormal orientation of spindles at meiosis II that apparently controlled diploid (2n) gamete formation in *Arabidopsis thaliana*.

The genetic constitution of 2n gametes generally divided into three categories, first division restitution (FDR), second division restitution (SDR) and indeterminate meiotic restitution (IMR). A FDR 2n gamete contains non-sister chromatids, while a SDR 2n gamete contains two sister chromatids (TANG, 2002). However, the third (IMR) type, numerically disproportionate numbers of chromosomes occur due to a restitution mechanism which cannot be categorised as either FDR or SDR. This restitution mechanism was detected in interspecific lily hybrids (LIM et al., 2001), and showed characteristics similar to both SDR and FDR. In this intermediate type, both univalents and bivalents are formed during metaphase I.

In the genus *Populus*, unreduced pollen has been documented in *P. canescens* Moench. ( $2n = 2x = 38$ ) (Seitz, 1954), *P. nigra* L. ( $2n = 2x = 38$ ) (BRADSHAW and STETTLER, 1993), *P. tomentosa* Carr. ( $2n = 2x = 38$ ) (ZHU et al., 1998; ZHANG et al., 2007; ZHANG and KANG, 2010), *P. tremula* L. ( $2n = 2x = 38$ ) (MUNTZING, 1936), *P. × euramericana* (Dode) Guinier and *P. × popularis*

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(ZHANG et al., 2009). However, little is known about the rates and cytological mechanism of numerically unreduced pollen in diploid *Populus euphratica* in China. Understanding of the cytological mechanism of 2n gamete formation is vital in their utilization in breeding because genetic consequences are different in terms of the transmission of parental heterozygosity and epistasis retained in the two types of 2n gametes. It has been calculated that a 2n gamete derived from FDR with crossing over will transmit roughly 80% of parental heterozygosity to its progeny while a SDR 2n gamete will transmit about 40% in potato (HERMSEN, 1984; PELOQUIN, 1982).

The objective of this study was to investigate the rate of natural numerically unreduced pollen and to elucidate the cytological mechanisms that lead to unreduced pollen formation in *P. euphratica*. Spindle orientation and other cytological characteristics were examined during the meiosis of Microspore mother cells (MMCs) in squashed anthers by indirect immunofluorescence. Based on our results on the observed spindle configurations, sporad types, and diameters of pollen grains, we propose the cytological mechanisms involved in the formation of numerically unreduced pollen and discuss the role of 2n pollen in triploid synthesis and polyploidy formation.

## Materials and Methods

### Plant material

In the spring of 2010, 18 individual male trees of diploid *P. euphratica* ( $2n = 2x = 38$ ) were sampled from a natural stand grown in Ejina County, Inner Mongolia, China. 6 floral branches were collected from each sampled tree and transported to Beijing. Floral branches were detached and forced, under greenhouse conditions, at Beijing Forestry University.

### Pollen grain diameter analysis

Separate pollen samples were collected from mature catkins of each clone and stored in glass bottles containing allochronic silica gel. Pollen grain were taken from the glass bottles with a dissecting needle, then mounted on a microscope slide, and stained with a 2% aceto-carmin solution. The diameter of each pollen grain was assessed using an eyepiece micrometer. A total of 1150 pollen grains were measured per individual tree.

### Cytological analysis of male meiosis and $\chi^2$ test

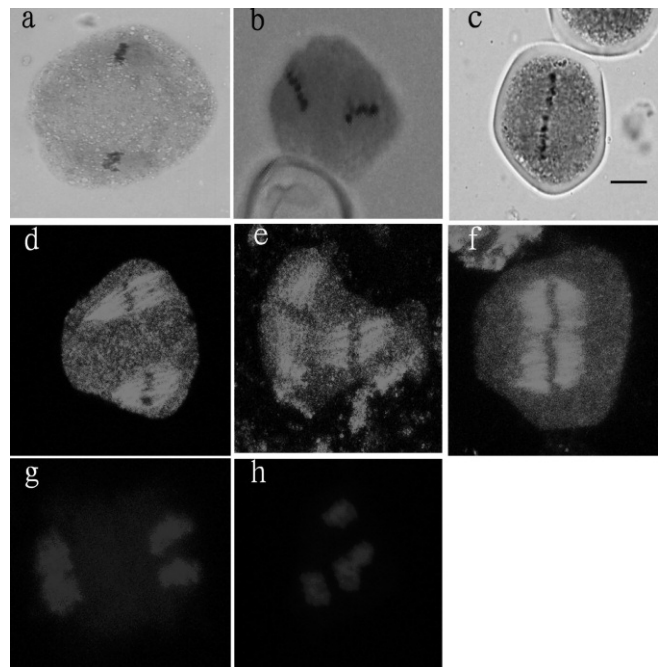
After the floral branches had been forced, flower buds were collected every 2–4 h and fixed in Carnoy's fluid (ethanol:acetic acid, 3:1) at 4°C until mature pollen grains appeared. For the meiosis analysis, anthers were dissected from the buds and squashed in a droplet of aceto-carmin solution (2%) onto a microscope slide using forceps. Developing microsporocytes were characterized in detail for their spindle orientations at metaphase II and anaphase II; dyads, triads, and tetrads were determined at the sporad stage. The frequency of cells with various types of spindle arrangements at metaphase and anaphase II was determined in 558–831 MMCs. More than 500 sporads were observed

to determine the proportion of tetrads, triads, and dyads at this stage.

Based on the assumption that one MMC has a certain volume and can divide into four or two parts, we can calculate a factor of appropriate 1.28 for the diameter of a diploid pollen grain compared with a haploid pollen grain (double volume of a globe), provided that the volume has not changed during the cleavage process. The largest amount of pollen grains was, in the present study, at 30  $\mu\text{m}$  in *P. euphratica* provided that it was the main average haploid diameter. Therefore, the observed 2n pollen rate was the number of large pollen grains ( $d > 30 \mu\text{m} \times 1.28 = 38.4 \mu\text{m}$ ) / 400 observed pollen grains and the expected 2n pollen rate was calculated as:  $(2 \times \text{dyads} + 1 \times \text{triads}) / (2 \times \text{dyads} + 3 \times \text{triads} + 4 \times \text{tetrads})$  under the microscope, replicated 5 times. A total of about 2000 pollen grains were used for the calculation. To assess the divergence between expected and observed 2n pollen frequencies, a Chi-square ( $\chi^2$ ) test was performed.

### Indirect immunofluorescence

The protocol for microtubule localization during microsporogenesis was adapted from Brown and Lemmon (1992). Accordingly, the anthers were fixed for 45 min in a 4% solution of paraformaldehyde, freshly prepared in a PEM buffer (50 mM PIPES, 5 mM EGTA, 1 mM  $\text{MgSO}_4$ ; pH 6.8), then permeabilized for 30 min in the buffer with 10% (v/v) dimethyl sulfoxide (DMSO), and finally extracted in 1% (v/v) Triton X-100 for 15 min. After three washings in phosphate-buffered saline



**Figure 1.** – Aspects of meiotic spindles at the second meiosis in microspore mother cells (MMCs) of diploid *Populus euphratica*. Bar: 10  $\mu\text{m}$ . **a** Parallel spindle, **b** tripolar spindle, **c** fused spindle. **d–f** Indirect immunofluorescence study of microtubule cytoskeleton patterns at metaphase II corresponding to **a–c**, **g–h** DAPI staining of chromosome at anaphase II, which form **a–b**.

Table 1. – Frequency of different types of spindles and sporads, expected and observed rates of 2n pollen grains in diploid *Populus euphratica*.

Trees	Different spindles at second meiotic division					Number of different sporads				Expected rate of 2n pollen (%)	Observed rate of 2n pollen (%)	$\chi^2$
	Total	MMCs	Parallel	Tripolar	Fused	Total sporads	Tetrads	Triads	Dyads			
	analyzed	(%)	(%)	(%)	analyzed	(%)	(%)	(%)	pollen (%)			
HY1	701		91.01	8.56	0.43	809	98.00	0.99	0.99	0.75	0.99	
HY2	558		85.67	10.75	3.58	806	96.15	0.75	3.10	1.77	0.97	
HY6	764		93.46	6.54	0	799	97.37	2.25	0.38	0.76	1.43	
HY7	723		94.19	4.15	1.66	863	97.33	1.74	0.93	0.91	0.49	
HY8	662		96.52	1.21	2.27	809	98.27	1.36	0.37	0.53	1.79	
HY14	831		87.01	12.03	0.96	781	90.79	3.58	5.63	3.85	4.35	
HY15	779		78.94	19.26	1.80	798	95.46	1.41	3.13	1.95	2.93	
HY16	741		93.93	0.67	5.40	533	98.31	1.31	0.38	0.52	0.97	
HY17	694		99.14	0	0.86	778	99.36	0.51	0.13	0.19	0	
HY20	797		84.31	10.04	5.65	812	93.10	4.56	2.34	2.36	3.81	18.71
HY23	599		98.00	0.33	1.67	813	96.80	1.23	1.97	1.31	4.33	
HY24	806		98.01	0.50	1.49	783	97.57	1.40	1.03	0.87	0.48	
HY25	773		96.24	2.85	0.91	812	95.07	3.20	1.73	1.69	4.37	
HY27	681		97.21	1.91	0.88	651	98.62	0.77	0.61	0.50	0.95	
HY28	718		98.88	0.28	0.84	787	98.09	1.14	0.77	0.67	0.49	
HY31	685		99.41	0.44	0.15	869	98.16	1.27	0.57	0.61	0	
HY33	759		82.87	3.95	13.18	796	94.10	2.63	3.27	2.35	2.34	
HY34	645		99.07	0.93	0	768	96.22	3.13	0.65	1.12	0.99	
Average	717.6		93.19	4.50	2.32	781.5	96.60	1.85	1.55	1.25		

MMC, Microspore mother cell.

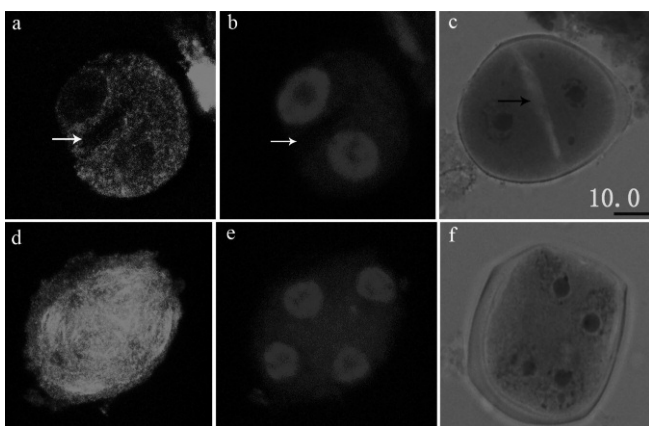


(PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM Na<sup>2</sup>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>; pH 7.4), the anthers were dissected with forceps to release the meiocytes, tetrads, or young microspores. Cells were then affixed to a slide coated with 0.1% (w/v) poly-L-lysine (P1274; Sigma, St. Louis, MO) and incubated in a moist chamber with an anti- $\alpha$ -tubulin antibody (T9026; Sigma) diluted 1:200 in PBS for 1 h at 37°C. Following further washing in PBS, the samples were labeled with a 1:50 (v/v) dilution of fluorescein isothiocyanate (FITC) conjugated anti-mouse immunoglobulin (Ig)G antibody (F0219; Sigma) for at least 1 h at 37°C in a moist chamber. For correlation with nuclear stages, samples were finally washed in PBS, counterstained with DAPI (40, 6-diamidino-2-phenylindole) for 5 min and mounted in a Prolong Gold antifade reagent (P36934; Invitrogen, Carlsbad, CA). The preparations were observed using a fluorescence microscope (model BX51; Olympus, Japan) and photographed with a CCD camera (model DP70; Olympus).

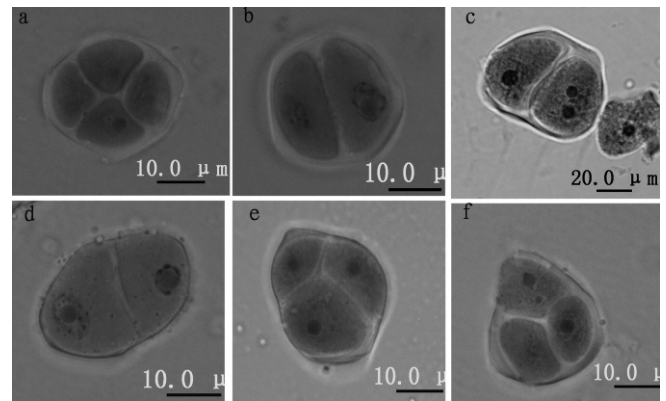
## Results

### Cytological characteristics of male meiosis

The first meiotic division of all 18 individual trees was found to progress normally, but a number of cytological events occurred during the second meiotic division that resulted in 2n pollen formation. At metaphase and anaphase II, the orientations of the spindles of homologous chromosomes were parallel, tripolar, and fused, as observed in pollen specimens that had been stained in the aceto-carmin solution (Fig. 1a-c). There was a predominance of parallel spindles in all 18 individual trees of *P. euphratica*. Tripolar and fused spindles were observed frequently in trees HY14 and HY20, respectively, whereas the tree HY33 was the highest total producer of these two abnormal spindles (see Table 1). The angle of the meiotic spindles at anaphase II was also arranged from parallel to tripolar, which was illustrated by the position of the four-sister chromosome



**Figure 2.** – Microtubules arrays, chromatin location, and cytokinesis at meiosis of MMCs in diploid *P. euphratica*. **a, d** Microtubules, **b, e** DAPI staining of chromosome, **c, f** Microtubules, acetocarmine staining of chromosome. Bar: 10  $\mu$ m. **a-c** The beginning of the furrow (arrow), showing the occurrence of simultaneous cytokinesis. **d-f** Cytokinesis after meiosis, with radial microtubule systems (RMSs) surrounding each nucleus.



**Figure 3.** – Different types of sporads at tetrad stage of diploid *P. euphratica*. **a** A tetragonal tetrad with four reduced microspores. **b** A dyad with two nucleoli of nearly the same size being included in each of the two restitution nuclei. **c** A dyad with two restitution nuclei, but one of them containing two nucleoli. **d** A dyad with one larger microspore and one smaller microspore, each of the two dyad microspores containing one restitution nucleus. **e** A triad with the large microspore containing one restitution nucleus and two smaller microspores each containing one nucleus. **f** A triad with one restitution nucleus containing two nucleoli.

complement (Fig. 1g, h). Indirect immunofluorescence microscopy of microtubule cytoskeleton patterns during meiosis revealed that the poles of the tripolar and fused spindles in some MMCs were joined at one or two axes of the cell (Fig. 1e, f).

### Cytokinesis and abnormal sporads formation

Premature cytokinesis after the first meiotic division was observed (Fig. 2a-c). The formation of cleavage furrows pinpointed the beginning of the simultaneous cytokinesis, and this cleavage formation progressed from the center to the periphery of the dividing cells (Fig. 2c).

Phragmoplasts were not found at telophase II, but radial microtubule systems (RMSs), which expanded from the sister nuclei and non-sister nuclei, were observed (Fig. 2d-f) in MMCs with parallel spindles. Generally, meiosis and cytokinesis resulted in the production of tetragonal tetrads with four reduced microspore (Fig. 3a), but different types of dyads (Fig. 3b-d) and triads (Fig. 3e, f) with one or two unreduced microspores were found at the tetrad stage. These unreduced microspores had a restitution nucleus with one (Fig. 3b, d, e) or two nucleoli (Fig. 3c, f). The triads presented asymmetric cytokinesis (Fig. 3e, f), but an unbalanced division of cytoplasm also occurred in the dyads. The frequency of triads, dyads, and tetrads was calculated for 18 clones of *P. euphratica* with staminate buds at the end of telophase II (Table 1). Of the more than 530 spores counted in randomly selected anthers from each sampled tree, the frequency of triads, dyads, and tetrads ranged from 0.51% to 4.56%, 0.37% to 5.63%, and 90.79% to 99.36%, respectively. The correlation analysis showed a significant association ( $r = 0.68$ ,  $P < 0.05$ ) only between the tripolar spindles and dyads (data not show), but the association of parallel and fused spindles between dyads and triads were not significant.

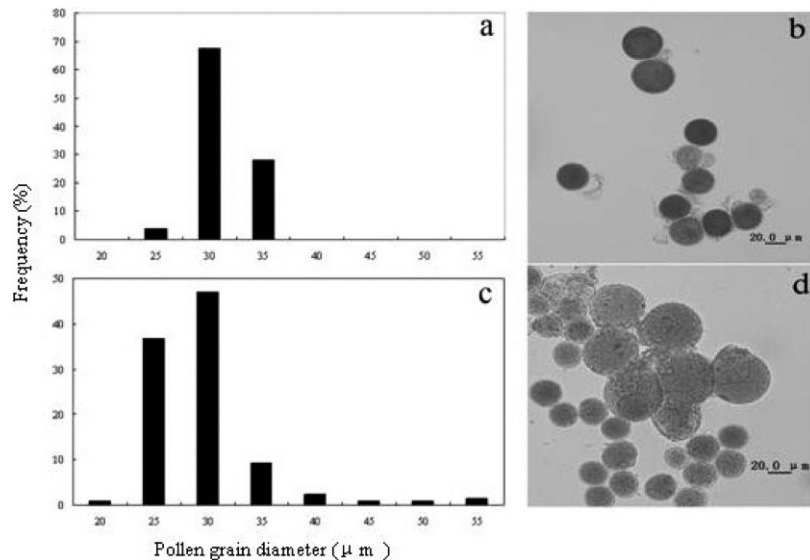


Figure 4. – Frequency distribution of pollen diameter in tree HY17 (a, b) and tree HY25 (c, d) of diploid *P. euphratica*. Bar. 20 µm.

#### Rates and its cytological determination of 2n pollen formation

The rates of 2n pollen of 18 sampled trees in diploid *P. euphratica* were presented in Table 1. Among the 18 genotypes, 16 genotypes produced 2n pollen and two genotypes (trees HY17 and HY31) produced only normal n pollen. In all 2n pollen producers, the rates of 2n pollen varied from 0 to 4.37%, and the rates of 2n pollen of trees HY25, HY14, HY23 and HY20 are higher than that of other producers.

Normal microsporogenesis in *P. euphratica* is simultaneous cytokinesis, and formed a tetrad in a tetrahedral arrangement (Fig. 3a). However, parallel spindles, tripolar and fused spindles (Fig. 1a–c), and premature cytokinesis II (Fig. 2a–c) were observed during the meiotic division. Tripolar and fused spindles were the variations of parallel spindles and could result in triads and dyads, respectively. Premature cytokinesis II could result in dyads.

The abnormal rates of meiosis in *P. euphratica* were examined among more than 500 meiotic cells. The expected 2n pollen rates were accordant with the observed 2n pollen rates by  $\chi^2$  test (Table 1).

#### Pollen grain size distribution

The fertile pollen grains were stained deeply by the 2% aceto-carmin solution, while the aborted ones could either not be stained or could only be stained lightly. Therefore, it was very easy to measure the pollen grains under a light microscope. The size of the pollen grains ranged from 18.4 µm to 53.5 µm among the 18 individual trees of diploid *P. euphratica*. However, the pollen grain-size distribution of the 18 individual trees was different. The diameters of the pollen grains of tree HY17 from 22.6 µm to 34.8 µm (Fig. 4a, b), but the largest range was found for tree HY25, from 19.6 µm to 53.5 µm (Fig. 4c, d).

## Discussion

#### Pollen grain size and ploidy level

In many plants, a positive correlation between pollen size and their ploidy level had often been seen (JACOB and PIERRET, 2000). Because the diameter of pollen grains may become larger due to the doubling of chromosome numbers, pollen size has been widely used as an indicator of 2n male gametes (VEILLEUX et al., 1982). ORJEDA et al. (1990) found that the 2n pollen of the sweet potato (*Ipomoea trifida*) was about 30% larger than normal pollen.

SEITZ (1954) observed the presence of giant pollen grains in the anomalous androgynous flowers of the grey poplar (*P. canescens*) and, subsequently the formation of triploids after self-pollination. The pollen grain of the triploid aspen (*P. tremuloides*) was found to be between 37 and 44 µm in diameter, and tetraploid hybrids were obtained by crossing pollen of the triploid tree with a diploid female (WINTON and EINSPAHR, 1970). The induced unreduced pollen of *P. tomentosa* was distinctly larger than normal pollen, which can be beyond 37 µm in diameter (ZHU et al., 1998). Based on these studies, the diameter of unreduced pollen grains in the poplar genus is at least 37 µm in diameter. In the present study, pollen grains with diameters >38.4 µm were frequently observed at different frequencies among all 18 trees (see Table 1), and giant pollen grains were also observed in some trees. These giant pollen grains are considered to be either 3n or 4n pollen grains (TAVOLETTI et al., 2000; CAMADRO et al., 2008), which would indicate that over-diploid pollen grains do occur in these trees of *P. euphratica*.

#### Cytological mechanism of 2n pollen formation

Abnormal meiotic division led to 2n pollen formation. These abnormal meiotic phenomena were mainly owing to the following: (1) Synaptic mutant: in potato, a synap-

tic mutant resulted in the homologous chromosomes failing to pair and cross-over rate reduction (DOUCHES, 1988). This mechanism can form FDR 2n gametes finally. (2) Abnormal spindles orientation: Parallel, fused, and tripolar spindles are the three main types of spindle configurations that have been showed in plants that produce 2n pollen (MOK and PELOQUIN, 1975; VEILLEUX, 1985; BRETAGNOLLE and THOMPSON, 1995; RAMSEY and SCHEMSKE, 1998; ZHANG et al., 2009; ZHANG and KANG, 2010; SILVA et al., 2011). The parallel spindles resulted in two FDR 2n pollens. The fused spindles led a mother cell to form a dyad and then two FDR 2n pollens were produced, and the tripolar spindles formed one FDR 2n pollen and two 1n pollen. (3) Nuclear fusion: at anaphase II and telophase II, two adjacent daughter nuclei fused together to form two FDR 2n pollen (CHEN, 1997). (4) Premature cytokinesis: cytoplasm divided early after meiosis I; however, sister chromatids divided normally. The result of this mechanism is the formation of two SDR 2n pollens (MOK and PELOQUIN, 1975). Many plants have more than one of the above mechanisms to form 2n pollen. Potato has parallel spindle, fused spindle, premature cytokinesis-1 and premature cytokinesis-2 (OLIVEIRA, 1995). Persimmon (*Diospyros kaki* L.) has parallel spindle, fused spindle and tripolar spindle (TANG, 2002). The parallel spindle, fused spindle, tripolar spindle and premature cytokinesis-2 were observed in *P. tomentosa* (ZHANG and KANG, 2010), *P. × euramericana* and *P. × popularis* (ZHANG et al., 2009).

In this study, we observed four types of abnormalities: parallel spindle, fused spindle, tripolar spindle and premature cytokinesis in *P. euphratica*. These abnormalities can be classified as having abnormal spindle fiber orientation during meiosis II and early division of cytoplasm after meiosis I. The statistical analysis of abnormal spindles and sporads indicated that the frequent parallel spindles may lead to triads and dyads formation at the tetrad stage. This could be owing to the gathering of one or two non-sister sets of chromosomes at telophase II, which results in two nuclear cytoplasmic domains in late-telophase cells and the division of MMCs into either triads or dyads. The dyad developed into two 2n pollens and the triad grew into one 2n pollen and two 1n pollens. The 2n pollen formed in these ways was genetically equivalent to FDR. Premature cytokinesis II could result in dyads. The 2n pollen grains formed via this abnormal approach were genetically equivalent to SDR.

Due to simultaneous cytokinesis, the RMSs emanating from daughter nuclei define the spore domains and determine the placement of intersporal walls (BROWN and LEMMON, 1992). The establishment of spore domains via RMSs can account for both the regular and irregular patterns of tetrad formation (BROWN and LEMMON, 1991). In *P. euphratica*, the position of the sister nuclei confined the RMSs as well as the plane of division.

#### *Implications for polyploidy breeding*

As a genetic consequence, two types of 2n gametes can be obtained, corresponding to FDR and SDR. Due to the equational division of sister chromatids, FDR gametes contain an equal number of parental chromosomes. On the other hand, in the case of SDR, sister chromosomes

move to the same daughter cell. Therefore, FDR gametes contain maximum heterozygosity and SDR gametes display maximum homozygosity in the absence of crossing-over (HERMSEN, 1984; VEILLEUX, 1985; PELOQUIN et al., 1999). Consequently, FDR gametes are particularly valuable in plant sexual polyploidization breeding programs (BRETAGNOLLE and THOMPSON, 1995). The unreduced pollen resulting from abnormal spindles is genetically equivalent to the FDR mechanism, while that from premature cytokinesis is genetically equivalent to SDR (RAMANNA, 1979). In the present study, the unreduced pollen of *P. euphratica* caused by abnormal spindles is genetically equivalent to the FDR mechanism, while that from premature cytokinesis is genetically equivalent to SDR. Because our cytological analysis was performed only to determine the expected heterozygosity of unreduced gametes, further investigations are required to reveal the actual level of heterozygosity of the unreduced gametes and their contribution to the progeny.

Natural triploids often occur in the genus *Populus*, and have been widely used in forestation and forestry production (ZHANG et al., 2004). These natural triploid hybrids are presumably due to the production of numerically unreduced pollen (ZHU et al., 1998; ZHANG et al., 2004). Studies on the genetic control of unreduced pollen and the techniques for discovering producers of a higher frequency of unreduced pollen will provide a platform for the genetic improvement and triploid breeding of *P. euphratica*.

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