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Full Length Research Paper

Meiotic behavior and pollen fertility of five species in the genus *Epimedium*

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Meiotic behavior and pollen fertility were analysed in five *Epimedium* species: *Epimedium chlorandrum*, *Epimedium acuminatum*, *Epimedium davidii*, *Epimedium ecalcaratum* and *Epimedium pubescens*. Chromosome numbers for five species were 2n = 2x = 12. All examined species displayed stable meiotic process and high pollen fertility (>76.67%). Meiotic abnormality partially affected pollen fertility. At metaphase I, the predominant chromosome configuration was 6II, and occasionally, 5II + 2I. A low frequency of meiotic abnormalities was recorded in five species. Chromosome bridges, laggards and micronuclei were the main abnormalities observed in *Epimedium*. "Diagonal bridge" was first found in *E. chlorandrum* due to the altered spindle axis. Polyad was only presented in *E. davidii* and might have resulted from abnormal cytokinesis. Pollen fertility was correlated with meiotic abnormality.

Keywords: *Epimedium*, meiosis, chromosomal abnormality, pollen fertility.

INTRODUCTION

The genus *Epimedium*, with more than 60 species, is common in the Mediterranean region and the western Asia (Stearn, 2002). China is the center of genus diversity, and has approximately 52 taxa of *Epimedium* (Ying, 2002; Guo et al., 2008). The major bioactive components in *Epimedium* are flavonoids and more than 60 flavonoids have been identified (Wu et al., 2008; Chen et al., 2008). It is effective in strengthening kidneys, enhancing sexual performance, treating cardiovascular diseases and improving immunity (Kovačević et al., 2006; Xu et al., 2007).

Cytogenetics on the genus *Epimedium* has mainly been devoted to chromosome counts. The chromosome numbers have been determined for 18 species from China and 11 species from Japan (Sheng et al., 2010; Kuroki, 1970, 1967). In *Epimedium*, most species are diploid with 2n = 2x = 12, except *E. yingjiangense* with 2n = 4x =24 (Sheng et al., 2010). Meiotic analyses in the genus *Epimedium* are few and only available in hybrid populations (Sheng et al., 2011). Pollen fertility was high in hybrids (>76.10%) and meiotic abnormalities occurred in a minority, including chromosome bridges in anaphase I/anaphase II, laggards in anaphase I, irregular chromosome segregation in anaphase I/anaphase II and micronuclei in telophase II/telophase II (Sheng et al., 2011). The regular meiosis ensures gamete viability and meiotic irregularities would generate sterile gamete and decrease the pollen viability (Pagliarini, 2000). Basic data on chromosome number, meiosis and pollen fertility estimations are important to study biodiversity, for germplasm characterization and for applications to plant breeding.

This study presents the first reports on the meiotic behavior in pollen mother cells (PMCs) and pollen fertility of five species of *Epimedium*: *Epimedium chlorandrum*, *Epimedium acuminatum*, *Epimedium davidii*, *Epimedium ecalcaratum* and *Epimedium pubescens*.

MATERIALS AND METHODS

Five species examined in the present study are listed in Table 1. Voucher specimens have been preserved in the Herbarium of Sichuan Agricultural University (SAU). All plants were cultivated in the Botanical Experimental Area of the College of Biology and Science at SAU.

Flower buds in the ideal stage for meiotic analysis were collected, fixed in acetic alcohol (1:3) for 24 h, transferred to 70% ethanol and

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Table 1. Sample numbers, chromosome numbers, voucher and localities of *Epimedium* analysed.

Species	Number of plants observed	Chromosome number (2n)	Voucher	Locality
E. acuminatum	5	12	Yu E.M. 4106	Emei, Sichuan
E. pubescens	5	12	Yu D.J. 2015	Dujiangyan, Sichuan
E. chlorandrum	3	12	Yu Y.A. 1635	Baoxing, Yaan, Sichuan
E. davidii	2	12	Yu Y. A. 6101	Baoxing, Yaan, Sichuan
E. ecalcaratum	1	12	Yu Y.A.1401	Baoxing, Yaan, Sichuan

stored at 4°C. Slide preparations were made by the squash technique and stained with 1% propionic carmine. Almost 300 PMCs were analysed for each plant.

Pollen grains were fixed in acetic alcohol (1:3) for 24 h at room temperature and stored in 70% alcohol at -18°C. Pollen fertility was estimated by examining the percentage of stained pollen grains with 0.5% 2,3,5-triphenyltetrazolium chloride (TTC). At least, 300 pollen grains per plant were evaluated. Photomicrographs were taken with the Olympus microscope BX51.

RESULTS

A total of sixteen individuals belonging to five species of *Epimedium* were examined for meiotic behavior of PMCs.

During the prophase I, the chromosomes condensed and then the homologous pairing was initiated. At diakinesis, 6 discrete bivalents linked by one or two chiasmata were visible. Rod and ring bivalents (Figure 1A and B) were dominant, while v-form bivalent (Figure 1B) was also observed. As meiosis proceeded into metaphase I, the highly condensed bivalents became aligned on the meiotic spindle and clustered in the equatorial plate of the cell. Frequent abnormality observed in metaphase I was precocious migrations or asynapsis with two univalents. In *Epimedium*, the predominant chromosome configuration was 6II and occasionally, 5II + 2I (Figure 1C). High irregularities were observed in anaphase I/telophase I. The percentage of bridge was the highest (26.67%) in *E. chlorandrum* (Figure 1D) and the lowest (3.33%) in *E. ecalcaratum* (Table 2). One or two laggard chromosomes (Figure 1E) were detected in *E. chlorandrum*, *E. acuminatum* and *E. pubescens*. Some micronuclei (Figure 1F) were found in *E*. *acuminatum* and *E*. *davidii* at telophase I. The chromosomes despiralized, nucleoli and nuclear membranes reappeared and dyads were formed.

In the second division, the chromosome recondesed at prophase II. Spindles at metaphase II mainly appeared paralleled (Figure 1G) and `T´ shaped (Figure 1H). At this point, cohesion at centromeres had broken down and sister chromatids separated at anaphase II. The chromosome bridge was widely observed in anaphase II /telophase II. Unexpectedly, “diagonal bridge” was found in *E. chlorandrum* (Figure 1I). Cytokinesis generated symmetric (Figure 1J) or tetrahedral (Figure 1K) tetrad containing haploid microspores. The minority of cells presented tetrahedral tetrad with micronuclei (Figure 1L). Polyad was observed frequently (16.00%) in *E. davidii* (Figure 1M).

The pollen fertility in five species is listed in Table 2. Pollen fertility was about 80%; the highest was 90% in *E. ecalcaratum* and the lowest was 76.67% in *E. davidii*.

DISCUSSION

We have investigated the meiotic process of PMCs of five *Epimedium* species. Chromosome counts for 5 species indicate diploidy with 2n = 2x = 12, which is consistent with the previous reports (Sheng et al., 2010).

The meiosis is basically normal with few abnormalities, involving univalents, chromosome bridges, unexpectedly “diagonal bridge”, laggards, micronuclei and polyploid.

Univalents were observed at metaphase I in *E*. *chlorandrum* and *E*. *davidii*. Precocious migration of univalents may have resulted from precocious chiasma terminalization at diakinesis or metaphase I or from synaptic mutants (Bione et al., 2000). These meiotic abnormalities may produce micronuclei in telophase I and meiosis II.

The most frequent abnormalities were bridges and laggards at anaphase I / II (Figure 1D, E and I). The highest percentage of bridges and laggards was observed in *E*. *chlorandrum* (26.67 and 5.00%). When cell division occurs, a broken chromosome with two centromeres is pulled to the opposite poles of the cell, forming a long chromosome bridge called chromatid bridge (Zhang et al., 1997). Rothfels (1975) demonstrated that the bridge may originate from chiasma formation in heterozygous inversions. “Diagonal bridge” at anaphase II was caused by the altered spindle axis and reduced the pollen fertility (Zhang et al., 1997). The laggards observed in *Epimedium* may lead to micronucleus formation. The meiotic abnormalities found in interspecific hybrids and haploid plants accounted for low percentage of pollen fertility (Bione et al., 2000).

Micronuclei and polyad with variable numbers of

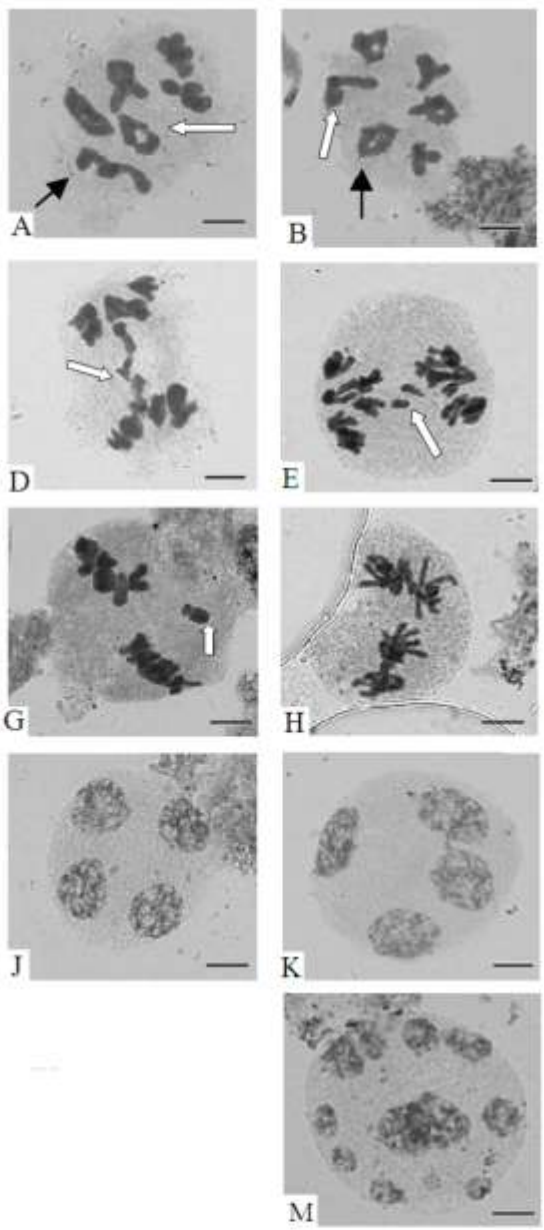

Figure 1. (A) Diakinesis in *E. chlorandrum*, n = 6, with rod bivalent (black arrow) and ring bivalent (white arrow); (B) Diakinesis in *E. ecalcaratum*, n = 6, with v-form (white arrow) and ring bivalent (black arrow); (C) Metaphase I in *E. davidii*, with two univalents (arrow); (D) Anaphase I in *E. chlorandrum*, with bridge (arrow); (E) Anaphase I in *E. chlorandrum*, with laggards (arrow); (F) Telophase I in *E. davidii*, with micronulei (arrow); (G) Metaphase II in *E. davidii*, with micronuleus (arrow); (H) Metaphase II in *E. chlorandrum*, with spindles appearing in `T´ shape; (I) Metaphase II in *E. chlorandrum*, with "diagonal bridge" (arrow); (J) Symmetrical tetrad in *E. pubescens*; (K) Tetrahedral tetrad in *E. acuminatum*; (L) Tetrahedral tetrad with micronulei (arrow) in *E. davidii*; (M) Polyad in *E. davidii*. Scale bar = 5 μm.

microspores were found in *E. davidii* (Figure 1F, G, L and M). The origin of polyad was diverse. In some species, micronuclei remained in the tetrad stage, affected the final product and formed ployads (Mendes-Bonato et al., 2001). Caetano-Pereira and Pagliarini (2001) reported that polyad were produced as a result of abnormal cytokinesis during meiosis, which generated sterile pollen grains. In *E. davidii*, the percentage of PMCs with polyad

Table 2. Frequency of abnormal PMCs and pollen fertility in *Epimedium*.

Taxon	Number of investigated PMCs	Number of PMCs with meiotic abnormality (%)				Total meiotic abnormality (%)	Pollen		
		Bridges	Laggards	Micronuclei	Polyads		Number of pollen grains analysed	Number of pollen grains stained	Pollen fertility (%)
E. acuminatum	300	6.67	2.00	2.33	0.00	11.00	300	246	82.00
E. pubescens	300	7.00	1.67	0.00	0.00	8.67	300	262	87.30
E. chlorandrum	300	26.67	5.00	0.00	0.00	31.67	300	242	80.67
E. davidii	300	9.11	1.62	11.6	16.00	38.33	300	230	76.67
E. ecalcaratum	300	3.33	0.00	0.00	0.00	3.33	300	270	90.00

(16.00%) and micronuclei (11.60%) was the highest (Table 2).

The meiotic abnormalities found in the *Epimedium* analyzed here have accounted for pollen sterility. These meiotic abnormalities hinder the normal cell division and partially affected the pollen fertility. In *E. davidii*, the percentage of cells with meiotic abnormality was the highest (38.33%), while pollen fertility was the lowest (76.67%). High meiotic stability ensures high pollen fertility. For *Epimedium* hybrids, PMCs meiosis revealed minor abnormal chromosomal behaviors and high pollen fertility (Sheng et al., 2011). Table 2 shows that *E. ecalcaratum* had a low frequency (3.33%) of meiotic abnormality, as a consequence, a high pollen fertility (90.00%). Generally, the meiosis in *Epimedium* was stable (<38.33% in meiotic abnormality) and pollen fertility was high (>76.67%). The data available suggested that pollen fertility was significantly correlated with meiotic abnormality.

In this paper, we provided a basic outline of meiosis in the genus *Epimedium*. Based on the data obtained, the PMCs split into four microspores and the whole meiosis was basically normal. The high pollen fertility was related to low level of meitotic abnormalities. The present paper enriches the database of cytology and can be useful for breeding program hybridization.

REFERENCES

Bione NCP, Pagliarini MS, Toledo JFF (2000). Meiotic behavior of several Brazilian soybean varieties. Genet. Mol. Biol. 23: 623-631.

Caetano-Pereira CM, Pagliarini MS (2001). A new meiotic abnormality in *Zea mays*: multiple spindles associated with abnormal cytokinesis in both divisions. Genome, 44: 865-871.

Chen XJ, Ji H, Zhang QW, Tu PF, Wang YT, Guo BL, Li SP (2008). A rapid method for simultaneous determination of 15 flavonoids in *Epimedium* using pressurized liquid extraction and ultra-performance liquid chromatography. J. Pharm. Biomed. Anal. 46: 226-235.

Guo BL, Pei LK, Xiao PG (2008). Further research on taxonomic significance of flavonoids in *Epimedium* (Berberidaceae). J. Syst. Evol. 46: 874-885.

Kovačević N, Čolić M, Backović A, Došlov-Kokoruš Z (2006). Immunomodulatory effects of the methanolic extract of *Epimedium alpinum in vitro*. Fitoterapia, 77: 561-567.

Kuroki Y (1967). Chromosome study in seven species of *Berberdaceae*. Memoir Ehime Univer sity Sectction II, Series B, 5: 175-181.

Kuroki Y (1970). Chromosome study in four species of *Berberdaceae*. Memoir Ehime University Sectction II, Series B, 6: 215-221.

Mendes-Bonato AB, Pagliarini MS, Silva N, Valle CB (2001). Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). Acta Scientiarum, 23: 619-625.

Pagliarini MS (2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. Genet. Mol. Biol. 23: 997-1002.

Rothfels K, Nambiar R (1975). The origin of meiotic bridges by chiasma formation in heterozygous inversions in *Prosimulium multidentatum* (Diptera: Simuliidae). Chromosoma (Berl.) 52: 283-292.

Sheng MY, Wang LJ, Tian XJ (2010). Karyomorphology of eighteen species of genus *Epimedium* (Berberidaceae) and its phylogenetic implications. Genet. Resour. Crop Evol. 57: 1165-1176.

Sheng MY, Chen QF, Wang Lj, Tian XJ (2011). Hybridization among *Epimedium* (Berberidaceae) species native to China. Sci. Hortic. 128: 342-351.

Stearn WT (2002). The genus *Epimedium* and other herbaceous Berberidaceae. Timber Press, Portland

Wu CS, Guo BL, Sheng YX, Zhang JL (2008). Simultaneous determination of seven flavonoids in *Epimedium* by liquid chromatographytandem mass spectrometry method. Chinese Chem. Lett. 19: 329-332.

Xu YQ, Li ZZ, Wang Y, Huang HW (2007). Allozyme diversity and population genetic structure of three medicinal *Epimedium*species from Hubei. J. Genet. Genomics 34: 56-71.

Ying TS (2002). Petal evolution and distribution patterns of *Epimedium L*., (Berberidaceae). Acta Phytotax. Sin. 40: 481-489.

Zhang SZ, Pan KY, Zhang DM, Hong DY (1997). Observation on abnormal meiosis of pollen mother cells in *Paeonia suffruticacosa* subsp. spontacea. Acta. Bot. Sin. 39: 397-404.