A Cytological Study of *Magnolia championii* Benth. (Magnoliaceae)

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Summary An analysis of the karyotype and meiotic behavior of *Magnolia championii* Benth. is presented. Its chromosome number is confirmed to be $2n=2x=38$. The ratio of the longest chromosome to the shortest chromosome is 2.31 and the karyotype is categorized to be 2B type. The index of karyotype asymmetry is about 56.47%, which is similar to other Magnolias. The meiosis process is normal except for one or two univalent pairs caused by the advanced disjunction of few bivalents. Single chromatid bridges and double chromatid bridges are observed at meiotic metaphase I in pollen mother cells and microspore tetrads. The low ratio of the abnormality, as shown in the research, does not constitute the main cause of the aborted pollen grains.

Key words Karyotype, *Magnolia championii*, Meiosis.

Magnolia championii Benth., a Magnoliaceae Jussieu. genus *Magnolia* Linn. subgenus *Magnolia* Dandy section *Gwillimia* DC., grows in Guizhou, south Guangxi and south-east and south-west of Canton. Its population, as the population reproduction model shows, has been on the decline, either in terms of the total number or in terms of its locations. The plants are difficult to find in the areas where specimens were collected in the past. That explains the fact that *M. championii* has been listed in the Forestry Regulations of Hong Kong.

Chromosomes often provide the students of evolution and phylogeny with valuable signposts which reveal the nature of the evolutionary processes at work and the trends which evolution has taken (Stebbins 1971). The abnormal behavior in meiotic processes indicates the endangered mechanism of vulnerable plants.

With an investigation into the number of the chromosome, karyotype of *M. championii*, and the meiotic processes of its pollen mother cells, this dissertation aims at accumulating more cytological data for phylogenetic research of Magnoliaceae and assigning the cytological reason for the low activity pollens.

Materials and methods

The sample plant under investigation grows in Shenzhen FairyLake Garden, where voucher specimens (Wang YL009) of the studied material are deposited. Somatic chromosome number and karyotype analysis were carried out on meristematic cells of root tips obtained from seedlings. Roots were pretreated in 8-hydroxyquinoline (0.002% w/v) for 2 h, then fixed and stored in Carnoy I (3 : 1 ethanol-acetic acid) for 2 h. Eligible floral buds for meiotic studies, obtained merely from the tree growing at the garden, were fixed in Carnoy I for 8–24 h.

Flame-drying protocol after enzyme digestion of cell wall was applied in getting chromosome slides (Zhu 1982) and chromosomes were stained in Giemsa. Metaphase plates were observed and photographed under the microscope (Zeiss Axioplan 2 Imaging Microscope). More than 20

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Plate I

Plate I. 1–4. The mitosis and karyotype of *Magnolia championii* Benth. Bar=2 μ m: 1. Metaphase ($2n=2x=38$); 2. Metaphase, arrow showing satellites; 3. Metaphase, arrow showing st chromosomes; 4. Karyotype of *M. championii*; 5–11. The Meiotic chromosome configuration of PMCs of *Magnolia championii* Benth. Bar=5 μ m: 5. Leptotene; 6. Zygotene; 7. Diakinesis; 8. Metaphase I, arrows indicate the univalent pair; 9. Anaphase I, showing single chromatid bridge; 10. Metaphase II, showing nonsynchronizaton; 11. Microspore tetrads, showing double chromatid bridge.

Table 1. The karyomorphological parameters of *M. championii*

Pair No.	Relative length %	Arm ratio	Type	Pair No.	Relative length %	Arm ratio	Type
1	7.10	1.08	m	11	5.04	1.03	m
2	6.68	1.06	m	12	5.03	1.74	sm
3	6.50	1.29	m	13	4.98	1.45	m
4	5.83	1.11	m	14	4.92	1.21	m
5	5.77	1.22	m	15	4.51	1.16	m
6	5.73	1.05	m	16	4.28	2.67	sm
7	5.69	1.14	m	17	4.20	3.16	st
8	5.55	1.16	m	18	4.06	1.59	m
9	5.35	1.15	m	19	3.44	1.34	m
10	5.33	1.29	m				

Table 2. A comparison of karyotype characteristics between some species in section *Gwillimia* DC.

Species	Karyotype formula	Asymmetry index	Ratio of chromosome length (L/S)	Karyotype type	Author
<i>M. championii</i>	32 m (2SAT)+4 sm+2st	56.47	2.3	2B	Present author
<i>M. delavayii</i>	30 m+6 sm+2st (2SAT)	57.02	2.6	2B	Li <i>et al.</i> (1998)
<i>M. delavayi</i>	32 m+4 sm (2SAT)+2st (2SAT)	56.99	2.0	2B	Li <i>et al.</i> (1998)
<i>M. coco</i>	32 m+4 sm+2st (2SAT)	56.28	2.2	2B	Li <i>et al.</i> (1998)

metaphase cells were observed to determine the chromosome number and three metaphase cells were measured for karyotypical analysis. The chromosome classification followed Levan *et al.* (1964) and Stebbins (1971) standard, and the karyotype asymmetry index followed Arano (1963). The results are as follows (Plate I).

Results

Karyotypical analysis

All the somatic cells observed have $2n=2x=38$ chromosomes (Plate I: 1). The karyotype is $2n=2x=38=32m(2SAT)+4sm+2st$, and belongs to 2B (Plate I: 2, 3, 4). One pair of m chromosomes have satellite, which are sometimes indistinct and invisible (Plate I: 2). The mean chromosome length ranges between 1.63–3.76 μm , with a total chromosome length of 100.84 μm . The longest chromosome is 3.58 μm , and the shortest 1.73 μm . The ratio of the longest to the shortest is 2.31. The index of karyotype asymmetry is about 56.47%. Karyomorphological parameters are given in Table 1.

Meiotic observations

In the meiotic prophase of pollen mother cell (PMC), the chromosomes are thin, long and entangled with each other, and therefore could not be distinguished easily (Plate I: 5, 6). In metaphase I, 19 bivalents are formed, eight of which are the ring type, and eleven the rod type (Plate I: 7).

A few bivalents divided ahead of their due time into chromatids (Plate I: 8). During the division, the bivalents grouped in two move separately to the two poles of the cell in anaphase I. Single chromatid bridges are observed at anaphase I (Plate I: 9). A regular pattern of division is perceived in anaphase II except for slight non-synchronization division of two cells (Plate I: 10). The double chromatid bridges are observed in microspore tetrads (Plate I: 11). Of the 25 PMCs observed, only one cell carries the chromatid bridge. So the ratio of the abnormality is as low as 4%.

Discussion

Karyotype characterization of section Gwillimia DC

Section *Gwillimia* DC., composed of about 20 species, is a primitive group in Magnoliaceae. Results of the eight studied species so far show that their chromosome numbers are all $2n=38$. Because of the small size, their karyotype analysis has only been tried on a few species. The index of karyotype asymmetry varies between 53.7 and 59. Most of them are m-chromosome or sm-chromosome with one or three satellites. Resembling each other morphologically, the karyotype of *M. championii* is also similar to *M. coco* (Lour.) DC., except for the position of the satellites, which are generally very easy to be lost.

The karyotypes of most species in Magnoliaceae are of the symmetry type and have little diversification. Most of them are 2B type, except for the genus *Liriodendron* L. and *Michelia* L., which are the 2A type (Chen *et al.* 2000). According to the karyotype classification of Stebbins (1971), A type is more primitive than B. In the whole family, the genera *Liriodendron* and *Michelia*, have quite an advanced position. By contrast, the karyotype of *Manglietia* and *Magnolia* section *Gwillimia*, the primitive groups, are B type, the advanced one.

The meiotic behavior of Magnoliaceae

Up to now, only a little meiotic behavior information about the family Magnoliaceae (He *et al.* 2005, Li and He 2003, Wang *et al.* 2004) is available, as different varieties like diploid, tetraploid, hexaploid in Magnoliaceae differ from each other in their meiotic behaviors. Regular behavior is shared by most diploid, such as *M. championii*, *M. coco*, *M. paenetaulauma* Dandy, *M. delavayi* Franch. and *M. figo* (Lour.) Spreng. (Wang *et al.* 2004), with the exception of some lagging chromosomes which result in quinquefid spores (unpublished information). At meiotic anaphase I, single chromatid bridge of *M. championii*'s PMCs is formed due to once sister-chromatid exchange, and this in turn determines a fertility rate of 50% of the pollens. Meanwhile the formation of the double chromatid bridge is caused by twice chromatid exchange, which is responsible for the sterile pollens (Zhang *et al.* 1997). Since the ratio of the abnormality is only 4% and produces 2% abnormal pollens only, it follows that the abnormal meiotic does not constitute a main cause for the aborted pollen grains compared with a rate of 0–30% of pollen germination.

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