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Full Length Article

A Study on the Octad Pollen Development in *Disepalum plagioneurum* (Annonaceae)

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Abstract

Octad pollen can be an important taxonomic character. Knowledge about the development of octad pollen, though, is very limited, and few reports have some contradictions. *Disepalum* is known for having octad pollen, but until now there is no report on its octad pollen development. In present study, the octad pollen development of *D. plagioneurum* (Diels) D.M. Johnson was observed. The results show the anthers of *D. plagioneurum* are septate, having 10-15 sporangial chambers, each containing one sporogenous cell, which then develops into a pair of connected pollen mother cells partitioned by a cell plate. The paired pollen mother cells undergo meiosis synchronously, generating two tetragonal tetrads that remain connected to each other but partitioned by a cell plate, thus forming a single octad in each sporangial chamber. Each tetrad of the octad is coated by pollen mother cell wall, which is digested quickly, but incompletely. The incomplete digestion makes each microspore rotate 180°, which pulls the distal side towards the center of the tetrad to reestablish as the proximal side, while the former proximal side becomes the distal side. At maturity, the pollen octad is about 200 μm long and 100 μm wide. The tapetum and septa among the anther locules are almost wholly digested, leaving only tapetal residue or a space among the octads. Our study confirms the rotation of microspores in octad pollen development and finds the main process of octad pollen development has no significant difference between *D. plagioneurum* and *Cymbopetalum baillonii*.

Keywords: Octad pollen; Annonaceae; Microspore; Pollen mother cell

Introduction

Disepalum plagioneurum (Diels) D. M. Johnson belongs to *Enicosanthellum*, family Annonaceae (Li *et al*., 2015). It is a woody plant distributed in forested slopes and valleys in Guangdong, Guangxi, Guizhou and Hainan provinces, China. It is a tree about 15 meters tall, and its inflorescences are terminal or leaf-opposed and 1-flowered, its sepals broadly ovate, its petals are yellowish green and broadly obovate (Li and Gilbert, 2013), and its pollen grains are shed in octads (Walker, 1971; Gan *et al*., 2015).

Octad pollen can be an important taxonomic character. Knowledge about the development of octad pollen, though, is very limited. According to Walker (1971), eight genera in Annonaceae release octad pollen grains, but only three species – two from the genus *Cymbopetalum* and one from *Hornschuchia* – have been studied (Tsou and Johnson, 2003; Tsou and Fu, 2007), and their results have some contradictions. Earlier Tsou and Johnson (2003) discovered no rotation of microspores but later. Tsou and Fu (2007) found that all microspores rotated for 180° before the octad pollen formed.

In the present paper, we studied the octad pollen development of *D. plagioneurum* in order to determine the process of octad pollen development, which in turn may have further use in horticulture or pollination biology.

Materials and Methods

Flowers of *D. plagioneurum* were collected from Diaoluoshan, Hainan, China in June 2011 (The voucher No. is Yang 20130414 in the herbarium, South China Botanical Garden), and rapidly fixed in formalin acetic alcohol (FAA: 70% alcohol, formaldehyde and glacial acetic acid in a ratio of 90: 5: 5). Anthers were dissected under a dissecting microscope (ZEISS StemiSV II) and stained in Ehrlich's hematoxylin (2%), dehydrated in an ethyl alcohol series (30, 50, 75, 85, 95% and 100%), infiltrated and embedded in

paraffin, and sectioned at 9 μm thickness with a microtome (HY 1508). All sections were observed and photographed under a LeicaDFC550 (Leica Microsystem, German) microscope.

Results

Pollen Mother Cell

Stamens of *D. plagioneurum* are numerous; anthers are slender, four-lobed and polysporangiate. A column of 10–15 sporangial chambers develop in each anther lobe. The septa separating each chamber are transverse and 1–2 cell layers thick (Fig. 1A). Initially, each chamber contains one sporogenous cell (Fig. 1B, C). Subsequently, the sporogenous cell undergoes mitosis and produces two conjoined pollen mother cells separated by a cell plate (Fig. 1A, D-I). Tapetal cells at this time are vacuolated and mostly binucleate, although a few are mononucleate or tetranucleate (Fig. 1B-J).

Meiosis

The paired pollen mother cells undergo meiosis synchronously (simultaneous type), generating two tetragonal tetrads coated by pollen mother cell wall and separated only by a cell plate, thus forming an octad in a sporangial chamber (Fig. 1K-P). The primary microspore octad is about 50 μm long and 25 μm wide (Table 1), with a thick proexine at the proximal side and thin pronexine at the distal side (Fig. 2A, C).

Microspore Rotation

After the meiosis, the tapetum starts to digest, as does the pollen mother cell wall (Fig. 2A-D). However, the digestion was incomplete and left some remnants in the aperture zone of each microspore. At this stage, every microspore started to rotate for 180°, pulling the distal side of each microspore towards the center of the tetrad, finally becoming the proximal side while the former proximal side became the distal side. Tapetum cells at this stage are starting to lose their cell walls (Fig. 2E-I).

Free Microspore

The octad, newly released from the pollen mother cell wall, was about twice as large (100 μm long and 50 μm wide) as the initial microspore octad (Table 1). Due to pollen mother cell wall digestion, though, a large space remained evident in the sporangial chamber. The octad quickly expanded into this space, while the tapetum protoplasts form a periplasmodium that invaded the space, after which the plasmodium was rapidly digested (Fig. 2E-I). Locule septa were still evident at this time, but its cells became crushed and digested (Fig. 2I-J). At maturity, pollen octads were at their maximum size of about 200 μm long and 100 μm wide (Fig. 2J).

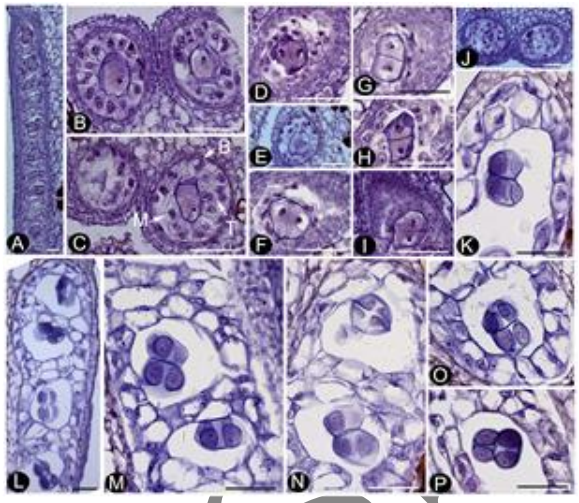

Fig. 1: Microsporegenesis of *D. plagioneura*-I. A. Longitudinal section of a stamen, show sporogenous cells divided by 1-2 cell layers of septa; B-C. Cross sections of stamens, show pollen mother cells with tapetum cells; D-I. Cross sections of stamens, show the division process of sporogenous cells, generating two daughter cells connecting with cell plates; J. Cross sections of stamens, show the tapetum septate cells; K-P. Longitudinal section of stamens, show the microspore octads and intact tapetum cells. Scale bars = 50 μm

The tapetum and septa cells became almost wholly digested, leaving only some tapetal residue or nothing but a space among the octads (Fig. 2K).

Discussion

The rotation of microspore was first reported in species of the *Poaceae* (Rowley, 1964), but received no academic attention. Tsou and Fu (2002) found the same rotation in Annonaceae. According to their descriptions on the microsporogenesis of *Annona glabra* and *A. montana*, the microspore mother cell develops a thin layer of the callosic mother cell wall material, which they called the "callosic envelope". In this study, after meiosis, a tetrad was generated within the callosic envelope. Soon after that, the meiotic tetrads built up a well-developed proexine at the proximal side, but only a thin proexine at the distal side. By the end of the tetrad stage, the microspore expands quickly and the callosic envelope started to digest. The distal side of microspore was intimately associated with the thin peripheral callose layer, and so the distal side was gradually pulled toward the center of the tetrad, whereas the proximal side becomes the distal side. Thus, each microspore of the tetrad had undergone an approxiamately 180° rotation, resulting in a tetrad with thick proexine at the distal side and thin proexine at the proximal side. Tsou and Johnson (2003) compared anther development in 13 genera and 15 species of Annonaceae. They described the microsporogenesis of most species but without mention on the rotation of microspore.

Table 1: The size of cells and tissues in each stage of octad development

No.	Stage	Cells/Tissues	Size (μm)
1	Pollen mother cell	Sporangial chambers	98 (80-101)
2	Pollen mother cell	Pollen mother cells	45 (38-53)
3	Pollen mother cell	Tapetal cells	21 (18-24)
4	Meiosis	Sporangial chambers	105 (90-115)
	Meiosis	Tapetal cells	18 (14-22)
5	Meiosis	Primary octad (long axis)	50 (48-66)
6	Meiosis	Octad released from pollen mother cell walls (long axis)	100 (80-105)
7	Microspore Rotation	Sporangial chambers	150 (140-165)
8	Microspore Rotation	Octad (long axis)	120 (115-128)
9	Microspore Rotation	Tapetal cells	15 (10-19)
10	Free microspore	Sporangial chambers	230 (220-265)
11	Free microspore	Octad (long axis)	200 (191-215)

Tsou and Fu (2007) compared the octad pollen formation of *Cymbopetalum baillonii* with tetrad pollen formation of *Annona*, finding they both had incomplete callose digestion and microspore rotation.

Lora *et al*. (2009) described pollen development in *A. cherimola*, observing a delay in the digestion of the pollen mother cell wall as well as in the tapetal chamber holding the four microspores together; nonetheless, the digestion did occur, and the microspores rotated until the pollen aperture sites faced each other. Lora *et al*. (2014) studied the main difference between the development of tetrad and monad pollen, and also found a delayed digestion of the callose and cellulose at the pollen aperture, resulting in an unlayered exine and the apertures and microspore rotation. However, In Li and Xu (2018)'s observation on five types of tetrad pollen formation process in *Pseuduvaria trimera*, microspore rotation was not described. In present study, the microspores were obviously rotated, with the rotation apparently driven by the digestion of the pollen mother cell wall. Here we have provided further evidence for the mechanism by which rotation occurs; namely, microspore connection to the disintegration and retreat of the pollen mother cell wall materials. Such materials are likely composed of callose and cellulose, and have been named "the callosic envelope" by Tsou and Fu (2002), "callose and cellulose" by Tsou and Fu (2007), "PMC (pollen mother cell) wall" by Lora *et al*. (2009), and "callose and cellulose remnants" by Lora *et al*. (2014).

According to Walker (1971), compound pollen is very common in Annonaceae, and eight genera release octad pollen grains. However, studies on their pollen development are very limited. Tsou and Johnson (2003) have described the octad development of *Cymbopetalum brasiliense* and *Hornschuchia polyantha*. According to their descriptions, the anthers were separated by many sporangial chambers; each chamber contained two homologous sporogenous cells, which were then developed into two pollen mother cells that ultimately underwent synchronous meiosis to generate two meiotic tetrads in contact (but not fused) with each other; each tetrad was enclosed by a callose envelope.

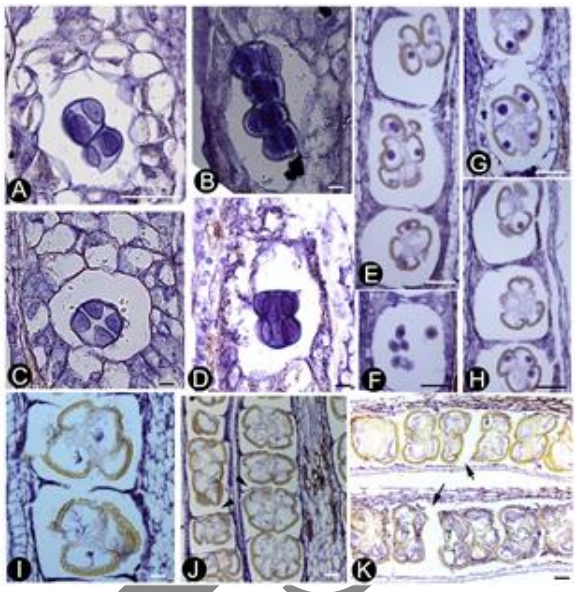

Fig. 2: Microsporegenesis of *D. plagioneura*-II. A-D. The tapetum starts to digest, so does the pollen cell wall; The primary microspore octads have thick proexine at the proximal side and thin pronexine at the distal side. E-I. Tapetum are degenerating, with degradants adhere to microspore octads; Microspores starts to rotate for 180°, pulling the distal side of each microspore towards the center of the tetrad, finally becoming the proximal side while the former proximal side becomes the distal side. Tapetum cells at this stage are starting to lose their cell walls. J. Locule septa cells become crushed and digested; Pollen octads are at their maximum size. K. The tapetum and septa cells become almost wholly digested, leaving only some tapetal residue or nothing but a space among the octads. Scale bars = 50 μm

After meiosis, the callose walls were digested and the octad pollen formed. Tsou and Fu (2007) found the pre-meiotic octad pollen development stages in *C. baillonii* were mainly consistent with that of *C. brasiliense* and *H. polyantha*. However, after meiosis, a cushion-like structure developed from the callose envelope and connected to the distal wall of each microspore. During callose digestion, the microspore rotated so that the proximal side and distal side exchanged positions. The callose-cellulose envelopes hold the eight microspores together and formed an octad. In the present study, the microsporogenesis of *D. plagioneurum* mainly agreed to *C. baillonii* (Tsou and Fu, 2007).

Conclusion

The anthers of *D. plagioneurum* are septate. The pollen mother cells are paired and grow from the sporogenous cells in sporangial chambers. Octad pollen is generated by a synchronous meiosis of the paired pollen mother cells. Early octad is coated by pollen mother cell wall, which is digested quickly, but incompletely, leads to a 180° rotation of each microspore. Then the octad expands quickly and become mature. Our study confirms the rotation of microspores in

octad pollen development and finds the main process of octad pollen development has no significant difference between *D. plagioneurum* and *C. baillonii*.

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