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Endosperm Development and Its Relationship to Embryo development in Blotched Tree Peony (*Paeonia rockii*)

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Abstract Endosperm in blotched tree peony (*P. rockii* cvs.) was nuclear type and of three successive development stages: free nuclear stage lasting from 4 to 29 days after anthesis (d.a.a.), cellularization stage from 29 to 48 d.a.a. and growth and mature stage finished before 85 d.a.a. Free endosperm nuclei multiplied to 200-500 or more and the cellularization of endosperm proceeded from the micropyle to chalazal end. As endosperm developed, the cells of outer integument adjacent to endosperm degenerated to form an evident degenerated zone through which nutrients were transferred into endosperm from integument. Firstly reserved substances were mostly starch gains in the cells, and, however, were gradually reserved into the cell walls so that matured endosperm was oleaginous. Endosperm and embryo corresponded with each other in certain stages and the relationship between them was helpful for the identification of embryo development by endosperm.

Key Words: development, endosperm, tree peony.

Introduction

Embryo and endosperm are equally the products of double fertilization. In *Paeonia* (*Paeoniaceae*), more attentions have been paid to embryogenesis with the peculiar free nuclear proembryo (Yakovlev and Yoffe, 1957 ; Murgai, 1959; Cave et al., 1962; Mattiessen, 1962; Carniel, 1967; Mu and Wang, 1985). These studies of embryo also got some information about the endosperm, especially in California peonies (*P. californica* and *P. brownii*) (Cave et al., 1961) and common herbaceous peony (*P. lactiflora*) (Mu and Wang, 1985). It is confirmed that the endosperm in *Paeonia* is of the nuclear type normally (Johri et al., 1992), but the entire process of its development is still left no observation in detail in any species, especially tree peonies. In this study, the endosperm development from secondary nucleus to mature was dealt with in one of tree peonies, blotched tree peony (*P. rockii*), and the time proceeding of various stages was supplied to show its relationship with the development of embryo.

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Materials and Methods

P. rockii 'Mei Qui Sa Jin' (Rose Sprinkling Gold) and 'Hui Die' (Grey Butterfly) growing in the garden of Northwest Normal University in Lanzhou of China were used in this study. The former is a purple double cultivar and the latter is a blended single one. Seeds can be produced normally in both of them. In open pollinated flowers, ovules, collected at 1, 2, 3, 4, 5, 6, 7 and 10 d.a.a., respectively, were fixed firstly in Carnoy's fluid (90% ethanol: acetic acid, 3:1, v/v) for 2-4 hours and then kept in FAA solution (formalin: acetic acid: 50% ethanol, 1:1:18, v/v/v). After 10 d.a.a., ovules were collected at an interval of every 7 days until 126 d.a.a. and fixed directly in FAA. Each fixation at least collected 50 ovules, which were hydrated in ethanol series, embedded in paraffin (melting point, 58-60 °C) and cut at 8-15 μ thick. Sections were mainly stained with iron or Herlich's hematoxylin

and some with Periodic acid-Schiff (PAS) reaction for showing starch grains. Observation and photograph were performed under Olympus Vanox microscope.

Results and Discussions

1. The development of endosperm

As summarized about existed literatures of *Paeinia* by Johri et al (1992), the endosperm of blotched tree peony is of the nuclear type. Its development from primary endosperm nucleus to mature could be described as three successive stages as reported in *Panicum miliaceum* (Cheng, 1993).

1) Free nuclear stage

This stage began with the division of primary endosperm nucleus and ended with the commencement of the cellularization of endosperm coenocyte. In blotched tree peony, it lasted about 25 days and could be observed in the ovules of from 4 to 29 d.a.a.

Primary endosperm nucleus, produced from the fertilization of secondary nucleus, was formed in a minority of ovules at 3 d.a.a. and in most cases at 4 d.a.a.. It began the first division (Fig. 1, 1) to form free nuclei at 4 d.a.a., which was a bit earlier than the first division of zygote occurring at 5 d.a.a. With repeatedly synchronous divisions, free nuclei multiplied increasingly to about 50-100 at 12 d.a.a. and distributed evenly in the peripheral cytoplasm around embryo sac. When free nuclei lay between 200 and 500 at 23 d.a.a., their divisions became asynchronous from the micropyle to chalazal regions. Meanwhile the giant nucleus, resulted from nuclear polyploidy or fusion, and amitosis occurred in some cases, which might be random in any location of endosperm, unlike common herbaceous peony in which they often appeared near to endosperm haustrum and in the margins adjacent to proembryo chalazal end (Mu and Wang, 1985). When cellularization was initiating in the micropyle end of endosperm at 29 d.a.a., free nuclei had risen to 200-500 or more in number, even more than 1,000 in a few cases. They still distributed equally but more densely in the peripheral cytoplasm of endosperm. At the chalazal end with fairly rich cytoplasm, however, there were several layers of free nuclei constituting a special endosperm haustrum. Similar structure was reported in *P. officinals* (Tiagi, 1970) and *P. lactiflora* (Mu and Wang, 1985).

With the multiplication of free nuclei in endosperm, the same event happened to proembryo coenocyte as well. But both multiplying rate and free nucleus number were more eminent in endosperm than in embryo (Table 1).

Table 1 Multiplication of free endosperm and embryo nuclei in *P. rockii.*

Days after anthesis	Number of free nuclei	
	Endosperm	Embryo
5	4-30	2-4
12	50-100	8-12
23	200-500	100-140
29	400-500	150-200

During the early nuclear stage, nucellear tissue still existed partially and the radial walls of its epidermal cells extended apparently and showed an extensive positive PAS reaction. But as nucellus disappeared in the late nuclear stage after 23 d.a.a., endosperm was directly adjacent to inner integument that would soon degenerate (Fig. 1, 2). And then endosperm continued enlarging and came directly close to outer integument (Fig. 1, 3), which, before long, started to withdraw for the degeneration from its inner side and to form a degenerated zone (Fig. 1, 4). By sections stained with PAS reaction, nucellus and inner integuement before the disappearance and endosperm coenocyte never contained evidently starch gains, which were rich only in the cells of outer integument.

2) Cellularization stage

Endosperm cellularization, the transition of endosperm from the coenocyte condition to a tissue consisted of cells, initiated from the micropyle end and proceeded towards the chalazal end. It had the duration of about 20 days. The first cell walls appeared in the endosperm layer around the proembryo at 29 d.a.a. and the morphological establishment of endosperm tissue was completed at 48 d.a.a.

Cellularization or wall formation in the endosperm was commonly initiated at about the same time as that in the proembryo. With the formation of the first

anticlinal wall between the intervals of every two nuclei at 29 d.a.a., free nuclei in the peripheral cytoplasm of endosperm coenocyte were partitioned off one by one to form a layer of so-called "open cells" without inner tangential walls (Fig. 1, 5). There were two distinct ways associated with the production of the first anticlinal walls. One was through the phragmoplast and cell plate of last nuclear division and the other was by the wall ingrowth of embryo sac. As reported in other plants (Cheng, 1993), the walls with two origins exist alternately in the first layer of "open cells". They occur so rapidly and the time to form is so close to each other that no difference between them can be distinguished under light

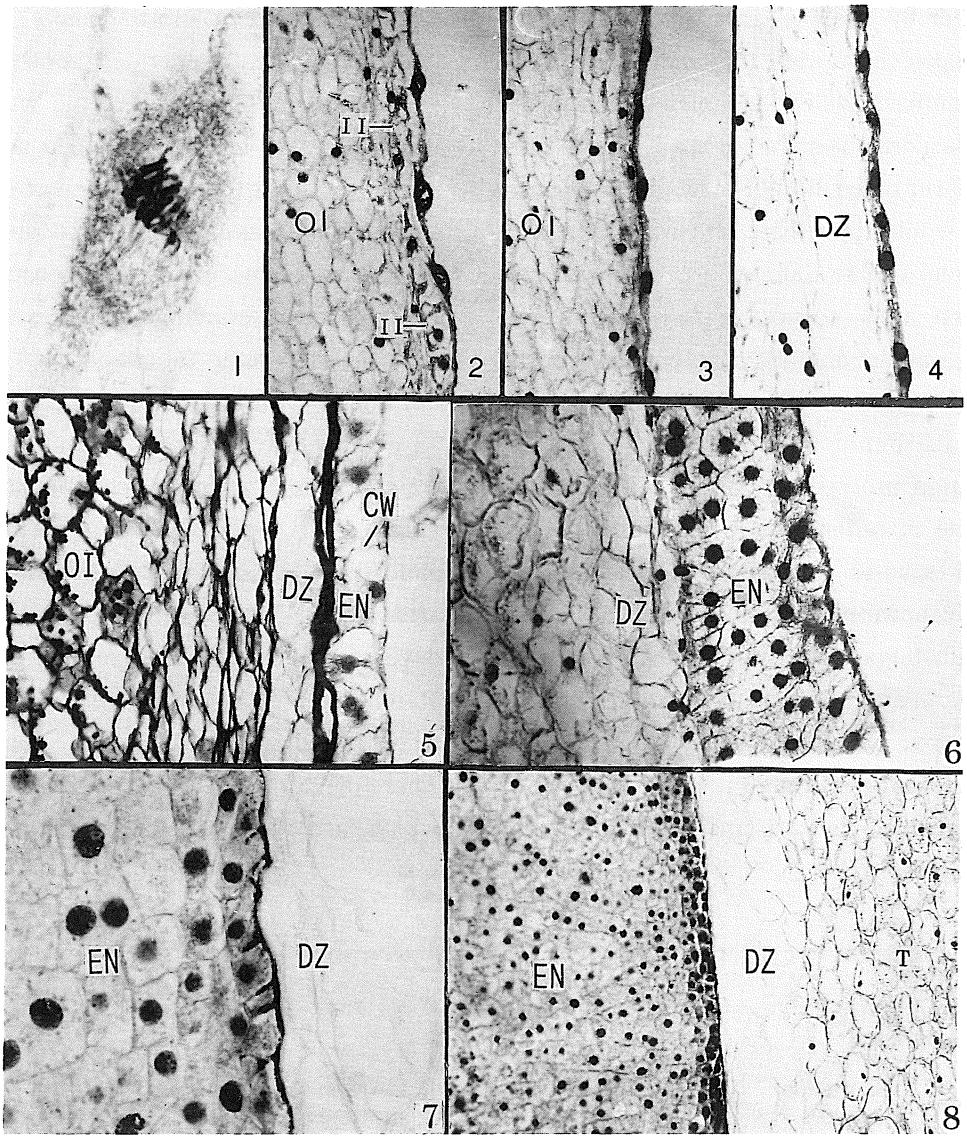

Fig. 1 The development of endosperm in *P. rockii*.

1: The metaphase of primary endosperm nucleus division. 2-4: Free nuclear stage [2. Inner integument (II) is in degenerating; 3: II disappeared and endosperm is adjacent to outer integument (OI) directly; 4: The degenerated zone (DZ) of OI is formed]. 5 and 6: Cellularization stage [5: First layer of "open cel" is formed, showing the first anticlinal cell walls (CW), the DZ and the amorphous substances with intensive positive PAS reaction between DZ and endosperm (EN). Note starch gains in normal cells of OI; 6: Endosperm cells is increasing layer by layer]. 7 and 8: Growth and mature stage [7: Showing amorphous substances stained deeply by hematoxylin and deposited in the outer tangential and radial walls of the outermost layer cells in the EN; 8: Showing the maturing EN, DZ and developing testa(T)]. 1 is magnified 750X, 8 is 75X and others are 150X; Except 5 is stained with PAS reaction and anti-stained with Herlich's hematoxylin solution, all others only with Herlich's hematoxylin solution.

microscope as soon as they have appeared.

Having been formed, the first layer of "open cells" soon underwent periclinal divisions to produce the first periclinal walls through phragmoplast and cell plate. As a result, a layer of complete cells formed around endosperm periphery and, in the inner of them to the central vacuole, there was still a layer of "open cells". Endosperm cells kept increasing centripetally layer by layer (Fig. 1, 6), always with the innermost "open cells", until they met each other in the center of embryo sac and completed the establishment of endosperm tissue at 48 d.a.a. When the lateral cells in fewer cases did not meet at last in the center, a cavity would form there and was occupied by a large vacuole. Unlike California peonies whose endosperms keep as a coenocyte forever in the chalazal region (Cave et al., 1961), the endosperm of blotched tree peony was cellularized throughout, including the haustrum in chalazal region.

As cellularization proceeded, starch gains, in the degenerated zone of outer integument adjacent to endosperm and several associated cell layers in degenerating, disappeared to transfer into the mass of amorphous substances with positive PAS reaction between endosperm and this zone as a form by which endosperm got nutrients (Fig. 1, 5).

3) Growth and mature stage

This stage ended at 85 d.a.a. and took about 40 days, during which endosperm grew to mature both in morphology and in physiology chiefly with much accumulation of nutrients.

The growth and mature of endosperm was accompanied with the development of testa in seed. Endosperm got a limited growth in the cellularization stage as it mainly underwent its tissue establishment, but it was at that time that ovule or seed got a rapid growth to come close to the size in mature. As the degenerated zone moved outward constantly, outer integument was thinner and thinner and, at last, its remained portion developed into a testa. With the translation of nutrients such as starch from integument into endosperm through the degenerated zone, endosperm became dry and hard and meanwhile starch gains appeared in endosperm cells. In the outer tangential and radial walls of outermost layer cells in endosperm, there was often an evident accumulation of amorphous substances stained deeply with Herlich's hematoxylin solution (Fig. 1, 7). It indicated that nutrients were entering endosperm through these cell wall passages. Thereafter, very thick cell walls with positive PAS reaction developed in cells with the mature of endosperm, but starch gains became obscure to PAS reaction while some no-color lipoid bodies appeared in cells. Therefore, a matured endosperm was oleaginous, in which carbonhydrates were mostly reserved into cell

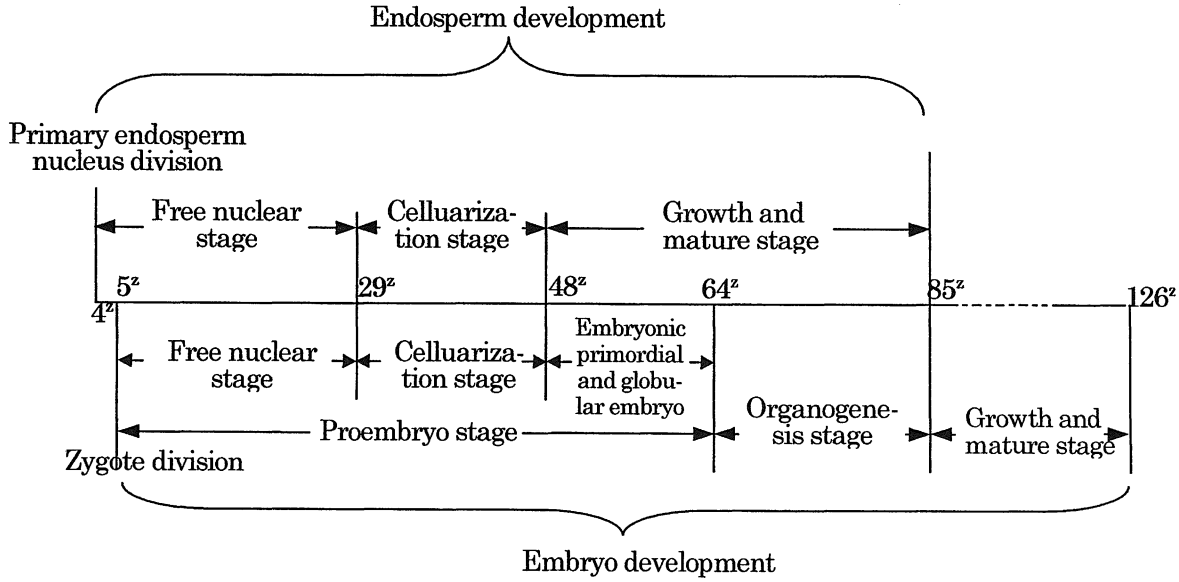

Fig. 2 Time proceeding and relationship of endosperm and embryo development in *P. rockii*.
[z]Days after anthesis.

walls but lipoids in cells.

Similar to many other plants, several layers of cells in the periphery of endosperm in blotched tree peony acted like a cambium. Their periclinally repeated divisions were most significant to the increase of cell number and led to the tissue heterogeneity of endosperm in morphology. Cells, smaller with much denser cytoplasm, were arranged more regularly in the peripheral regions. The closer to the inner center, the larger cells became with more vacuoles in cytoplasm and more irregular arrangement (Fig. 1, 8).

2. Relationship of endosperm and embryo development

Embryo development in *Paeonia* is more complicated for the early special embryogenesis that the proembryo develops from a coenocyte through cellularization to embryonical primordium and globular embryo (Yakovlev and Yoffe, 1957; Cave et al., 1962; Mattiessen, 1962; Carniel, 1967). Blotch tree peony had the same embryogenesis as other peonies, but we noticed further that its embryo development always corresponded with certain stage of endosperm (Fig. 2). Such a relationship would be very helpful for the identification of embryo development by endosperm. Special poly-embryonic primordium or temporal "poly-embryo" in the development of embryo of peonies (Yakovlev and Yoffe, 1957; Cave et al., 1962; Mattiessen, 1962; Carniel, 1967) is very attracted to botanists as well as breeders. To study this phenomenon or explore its utilization in breeding by bio-techniques like in vitro embryo culture, it is more important to determine and capture the correct developmental stage. In blotched tree peony, "poly-embryo" occurred as soon as endosperm had completed cellularization, which was not difficult to decide in morphology. Now that the time of cellularization in proembryo coenocyte varies much with species (Yakovlev and Yoffe, 1957; Cave et al., 1962; Mattiessen, 1962; Carniel, 1967; Mu and Wang, 1985) and there is no knowledge about if the change occurs synchronously in endosperm, whether such a relationship of endosperm and embryo as in blotched tree peony also fits to other peonies needs more investigations.

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