



## Cytoembryological studies on *Paeonia peregrina* L.

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Received 29 April 2003; Accepted 18 June 2003

### Abstract

The anther wall of *Paeonia peregrina* consists of epidermis, endothecium, 1-2 middle layers and a single-layered tapetum. In the secretory tapetum, multinucleate cells are resulted from acytokinetic mitosis and restitution nuclei are frequently produced in these cells. Meiotic division is regular in the pollen mother cells (PMC) of diploid and tetraploid forms. The ovules are anatropous, bitegmic and crassinucellate. The female gametophyte is of Polygonum type. In the mature embryo sac the egg cell, two synergids a secondary nucleus and three antipodal cells were observed. Leucoplasts were seen in all developmental phases up to mature embryo sac.

**Key words:** *Paeonia peregrina*, tapetum, microsporogenesis, megasporogenesis, megagametogenesis

### *Paeonia peregrina*'da sitoembriyolojik çalışmalar

#### Özet

*Paeonia peregrina*'nın anter çeperi epidermis, endotesyum, ara tabaka ve tapetumdan oluşur. Salgı tipi tapetumda çok nukleuslu hücreler asitokinetic mitoz bölünmeler sonucunda oluşur ve bu hücrelerde sık sık restitüsyon nukleuslarına rastlanır. Diploid ve tetraploid bitkilerin polen ana hücrelerinde mayoz bölünme düzenlidir. Tohum taslakları anatrop, bitegmic ve krassinusellat tiptedir. Dişi gametofit gelişimi Polygonum tiptedir. Olgun embriyo kesesinde bir yumurta hücresi, iki sinerjit, sekonder nukleus ve üç antipot hücresi bulunur. Olgun embriyo kesesine kadar tüm gelişim evrelerinde nişasta tanelerine rastlanmıştır.

**Anahtar sözcükler:** *Paeonia peregrina*, tapetum, mikrosporogenez, megasporogenez, megagametogenez

#### Introduction

According to the classical systems of angiosperm classification the genus *Paeonia* belongs to the family Ranunculaceae. However, many authors have pointed out that *Paeonia* is distinct from Ranunculaceae in respect to their vascular anatomy, floral anatomy, basic chromosome number and size and morphology of the chromosomes (Bhojwani and Bhatnagar, 1979).

Embryological data also support the view of removing *Paeonia* from Ranunculaceae to a separate

family, Paeoniaceae. Bhojwani and Bhatnagar (1979) made a table showing the embryological differences between the two families. The most peculiar embryological feature of this genus is its embryogeny (Yakovlev and Yoffe, 1957, 1965; Murgai, 1959; Cave et al., 1961; Wang, 1985, Dane and Olgun, 1997).

The aim of this paper is to study the embryological features of *Paeonia peregrina* L.

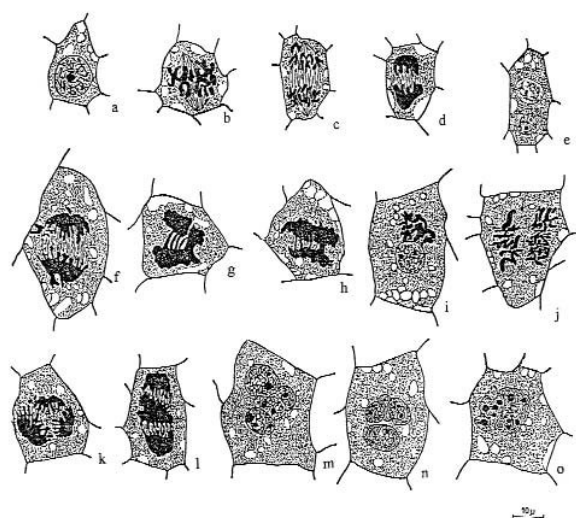
## Material and Methods

The flower buds were collected from the natural population at İspartakule and were fixed in acetic-alcohol (1:3). Sections were cut at 7-10  $\mu$ m and stained with Regaud's haematoxylin. The aceto-orsein squash method was also used in the study of microsporogenesis. KI+I was applied to the sections to identify starch grains in the embryo sacs.

## Results

### *The anther wall and the divisions in the tapetal cells*

The anther of *Paeonia peregrina* is tetrasporangiate. The anther wall consists of epidermis, endothecium, middle layers and tapetum. The single-layered epidermal cells become stretched during the maturation of anther. Endothecial cells do not show any thickenings. The cells of middle layers are crushed during the development. The cells of secretory tapetum are large and have dense cytoplasm. They are uninucleate in the early stage of development but the mitotic division is not followed by cytokinesis (Figure 1 a-e). As a result, two-nucleated tapetal cells are



**Figure 1:** Nuclear divisions in the tapetal cells of *Paeonia peregrina*. a. Prophase; b. Metaphase; c. Anaphase; d. Telophase; e. Binucleate cell; f-h. Irregular anaphase; i. Prophase in binucleate cell; j. Metaphase in binucleate cell; k,l. Anaphase in binucleate cell; m,n. Fournucleate cell; o. Nuclear fusion.

produced. Diploid chromosome number is 10 but tapetal cells with 20 chromosomes were also seen. The second mitotic division was observed resulting four-nucleated cells (Figure 1 i-n). Very often, due to the fusion of nuclei become polyploid (Figure 1 o). The following irregularities in the first and second mitoses were noticed: lagging chromosomes, chromosome bridges, fragments, unequal distribution of chromosomes (Figure 1 f-h, k,l). Nuclear fusions were frequently occurred resulting to the occurrence of large, irregular shaped giant nuclei with many nucleoli. Multinucleate tapetum degenerates at the time of separation of the microspores from each other.

### *Microsporogenesis*

In the majority of pollen mother cells (PMC) the meiotic division (microsporogenesis) undergoes normally (Figure 2 a-p). 5 bivalents were observed at diakinesis (Figure 2 g). Cytokinesis is of simultaneous type. The microspore tetrads are of isobilateral and tetrahedral type. The microspores in tetrad are surrounded by a thick callose wall.

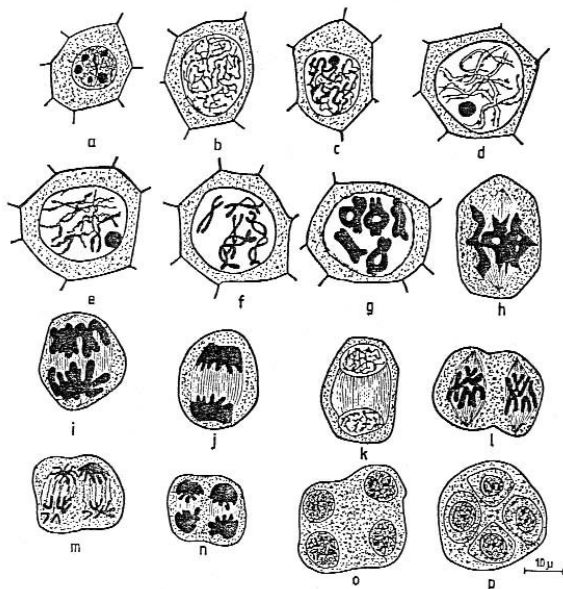
In some PMCs 10 bivalents were seen at diakinesis (Figure 3 a). Thus, both diploid and tetraploid chromosome number were counted. Some meiotic irregularities such as chromosome bridges, univalents and multivalents, lagging chromosomes, fragments were recorded in less number of PMCs of the diploid and tetraploid forms (Figure 3 b,c ) As a result of irregularities pentads and hexads were detected.

### *Megasporogenesis and Megagametogenesis*

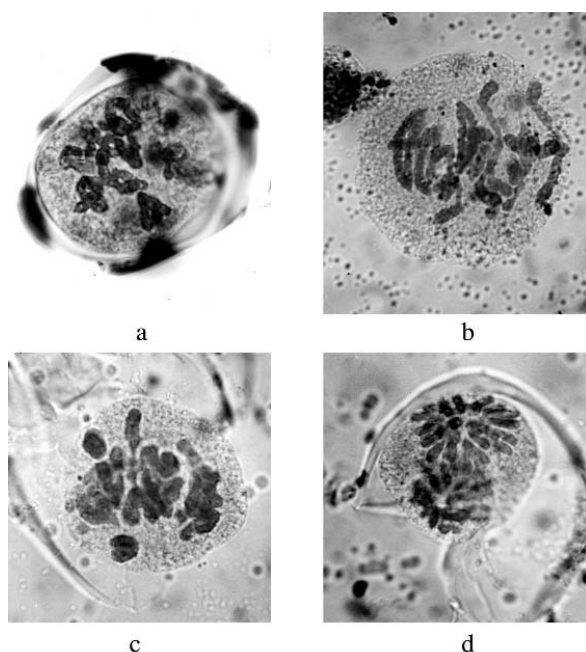
The ovary has 16 anatropous ovules placed along the ventral wall. The ovules are bitegmic and crassinucellate.

The archesporium is multicellular. The 3 or 4 archesporial cells differentiate in nucellus and become prominent with their dense cytoplasm and large nuclei (Figure 4 a). One of them functions as a megaspore mother cell (MMC) and undergoes meiosis (megasporogenesis). Before meiosis the size of MMC increases. 5 bivalents were counted at metaphase I ( $n=5$ ) (Figure 1 b). Cytokinesis is of the successive type. As a result of first meiosis, the two dyad cells are produced; the chalazal one is larger than the other (Figure 4 c) The megaspore tetrad is linear or T-shaped (Figure 4 d)

The chalazal megaspore is functional while the



**Figure 2:** Meiosis in the pollen mother cells of diploid *Paeonia peregrina*. a; Interphase; b,c. Leptotene; d. Zygotene; e. Pachytene; f. Diplotene; g. Diakinesis; h. Metaphase I; i. Anaphase I; j. Telophase I; k. Interkinesis; l. Metaphase II; m. Anaphase II; n,o. Telophase II; p. Microspore tetrad.



**Figure 3:** Meiosis in the pollen mother cells of tetraploid *Paeonia peregrina*. a. Diakinesis with multivalents; b,c. Metaphase I with univalents and multivalents; d. Anaphase I.

other three degenerate (Figure 4 e). 2, 4 and 8 nucleated embryo sacs are formed by three successive mitotic divisions (megagametogenesis) (Figure 4 f-h). In mature embryo sac the egg apparatus consists of the egg cell and the two synergids. These three cells show a triangular arrangement.

The two polar nuclei fuse in the early stage to form a secondary nucleus. In mature embryo sac the secondary nucleus lies near the egg apparatus (Figure 4 i). There are 3 antipodals on the chalazal pole. They are equal in size and have dense cytoplasm. The antipodals are the smallest cells of the embryo sac.

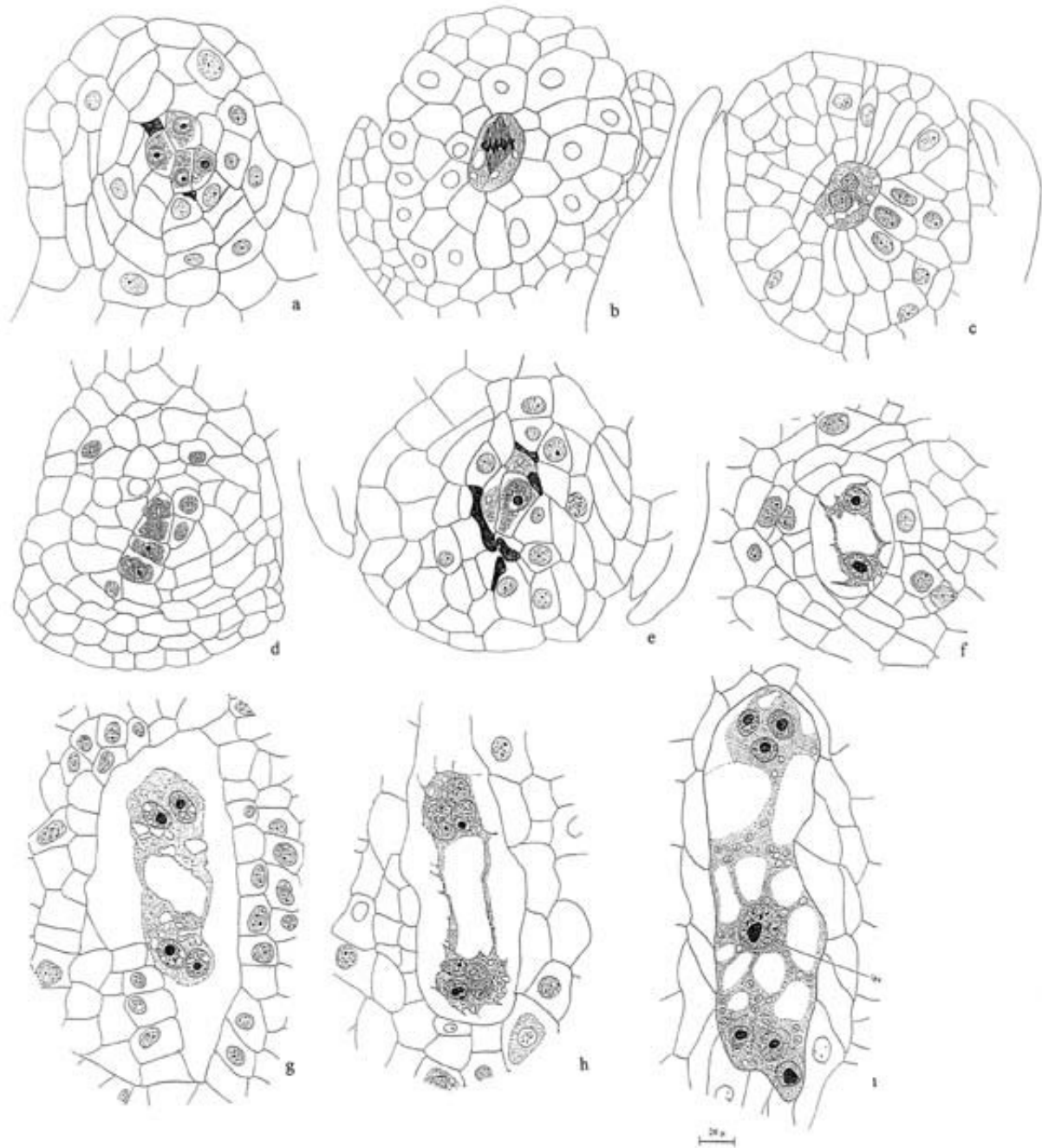
Vascular bundles were observed in nucellus and integuments in *P. peregrina*. The presence of vascular bundles in integuments is a primitive character. Starch grains were also noticed during the developmental stages, more conspicuous in mature embryo sac.

## Discussion

In the tapetal cells one or two mitotic divisions resulting in two to four nuclei which may fuse to form large polyploid nucleus were recorded in the anthers of *P. peregrina*. Acytokinetic mitosis and restitutional mitosis are the responsible mechanisms in the formation of polyploid tapetal cells in this species. Marquardt et al. (1968) reported  $16n$  ploidy level resulting from three mitoses in the tapetum of *P. tenuifolia*.

In the present study, both diploid ( $2n=2x=10$ ) and tetraploid ( $2n=4x=20$ ) PMCs were observed. Meiosis is regular in most of PMCs but irregular in some others. The regular meiosis results in the formation of isobilateral microspore tetrads. The regular meiotic division in PMCs were observed in some species of *Paeonia*; *P. anomala*, *P. wittmanniana*, *P. californica*, *P. brownii* (Stebbins and Ellerton, 1939, Walters, 1956). Meiotic irregularities were recorded in the PMCs of the species *P. suffruticosa*, *P. mlokosewitschi*, *P. tenuifolia* (Hicks and Stebbins, 1934; Dane, 1997). Dane (1997) observed some meiotic irregularities such as univalents, tetravalents, chromosome bridges, laggards in diploid and tetraploid forms of *P. tenuifolia*.

The ovules are anatropous, bitegmic and crassinucellate in *P. peregrina*. Archegonium is multicellular, but only one of them differentiates as a megaspore mother cell. Megasporogenesis is regular. The development of embryo sac conforms to the



**Figure 4:** Megasporogenesis and megagametogenesis in *Paonia peregrina*. a. Archesporial cells; b. Megaspore mother cell in Metaphase I; c. Dyad cells; d. 4 megaspores; e. Functional megaspore; f. 2-nucleate embryo sac.

Polygonum type. Multicellular archesporium is a family character of the Paoniaceae. Dane (1997) observed the multicellular archesporium developed into multiple megaspore mother cells giving rise to

banches of megaspore tetrads in *P. tenuifolia*. As a result, 2 or 3 embryo sacs were seen in one ovule in this species.

In all developmental stages up to mature embryo

sac leucoplasts were seen in *P. peregrina*. MMC, megaspore tetrad, functional megaspore contain starch grains. They were also found in 2-, 4-, 8-nucleated embryo sac and mature embryo sac. Starch grains were located in the outer integument cells, the integumentary tapetum, the nucellus, around secondary nucleus and in mature embryo sac of *P. tenuifolia* (Dane, 1997) but only in the integument tapetum of *P. anomala* (Yakovlev, 1957). Willemse and Went (1984) reported that the embryo sacs in the majority of angiosperms lack starch grains but it is abundant in some species. Vascular bundles which are rarely found were determined in the nucellar tissue in *P. peregrina* and these bundles were observed to be tracheids.

### Acknowledgement

We are greatly indebted and thankful to Prof. Dr. Jale Tören for providing the necessary research facilities, useful suggestions and encouragement and for her invaluable contribution to us.

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