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Formation of Embryo-Sac and Callose Deposition During Its Development in *Aletris luteoviridis*

By

Yoshihiko SATÔ* and Eimi KIRITO*

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Abstract. The developmental process of the embryo-sac in *Aletris luteoviridis* was investigated. Moreover, the detection of callose during this process was performed. The ovule was anatropous, and the crassinucellate or the tenuinucellate nucelli were invested with two integuments. The embryo-sac was formed according to the monosporic eight nucleate *Polygonum* type of development. Callose usually deposited in the wall separating two dyad cells and in three partition walls separating four tetrad cells. However, occasionally it deposited in the entire wall surrounding the dyad and the tetrad, besides in the partition walls.

Introduction

Aletris is in general treated as one of the genera of the large family Liliaceae. Dahlgren and Clifford (1982) propose the phylogenetic relationship among the monocotyledonous families. In their classification which is greatly influenced by Huber's one (1969), the monocotyledonous plants are segregated into small relatively homogenous families and the Liliaceae (s. l.) are, of course, segregated to many small families. They assign *Aletris* to the Melanthiaceae of them. There is a species with the bisporic *Endymion* type of embryo-sac formation in the Melanthiaceae (Dahlgren & Clifford, 1982), though a species with the monosporic *Polygonum* type also occurs in the family (Eunus, 1951). Unfortunately, the embryo-sac formation in *Aletris* remains to be investigated.

The deposition of callose during the embryo-sac formation in the angiospermous plants has come to be occasionally investigated (Kapil & Tiwari, 1978). In the Liliaceae (s. l.), the deposition has been investigated in *Allium* (Rodokiewicz, 1968), *Liriope* (Satô & Arima, 1984), *Ophiopogon* (Satô & Matsumoto, 1986b) and *Scilla* (Satô & Matsumoto, 1986a). However, the occurrence of callose during the embryo-sac formation has not been investigated in any genera of the Melanthiaceae in the sense of Dahlgren & Clifford (1982) yet. So, in *A. luteoviridis* it was investigated in addition to the investigation of the developmental pattern of embryo-sac.

* Department of Biology, Faculty of Education, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama, 240, Japan

Material and Methods

Many flowers at various stages of development were collected from several individuals of *Aletris luteoviridis* growing wild at Kiyosato in Yamanashi Prefecture at appropriate intervals extending from May to June in 1987. They were fixed in FAA (formalin-acetic-alcohol). After they were dehydrated in ethyl alcohol-*tert.* butyl alcohol series, they were embedded in paraffin (m. p. 57–60 C). These embedded materials serially sectioned at 6–10 μm thick. To investigate the developmental pattern of embryo-sac the sections were stained with Heidenhain's iron alum hematoxylin and fast green FCF combination. The materials for detecting callose were also fixed and sectioned according to the same procedure as the above-mentioned one. The sections were stained with aqueous solution of aniline blue (Smith & McCully, 1978) and observed under the fluorescence microscope. Callose emits bright-yellow fluorescence when treated with the fluorochrome. Moreover, since it is said that aniline blue does not necessarily stain callose alone (Nakamura, 1987), the disappearance of callosic florescence was confirmed by treating with β-1, 3-glucanase or zymolyase, not that it was performed for all the sections.

Observation

(1) Ovule

The ovule was completely divided into three locules with three parietal placentae. Many ovular primordia initiated on each placenta. The nucellus was invested with two integuments, of which the inner initiated earlier than the outer. The former was invariably composed of two layers of cells except its apical portion and grew rapidly to form a micropylar canal, while the latter also elongated rather rapidly and, before fertilization, covered the opening of the micropyle which had been formed by the inner integument to the ovarian locule (Fig. 1O). The ovular primordia began to bend on one side and the ovule attained to nearly an anatropous condition during the megasporogenesis (Fig. 1I).

A cell situated at the subepidermis of the nucellar tip usually differentiated into an archesporial cell (Fig. 1A). But, occasionally, two archesporial cells or megasporocytes (Fig. 1D) arranged longitudinally, differentiated in the same nucellus. In some ovules (Fig. 1C), the archesporial cell divided mitotically to form a primary parietal cell and a sporogenous cell. The former underwent the mitotic division once or twice to form a parietal layer, while the latter became functional as a sporogenous cell. In others (Fig. 1B), however, the archesporial cell directly functioned as a sporogenous cell; it differentiated without any mitotic divisions into a megasporocyte. That is, the nucelli in some ovules had the parietal layer, but those in others had no parietal layer. Thus, the nucellus of *A. luteovirtidis* was either crassinucellate or tenuinucellate. The former type of nucellus was much more usuall than the latter,

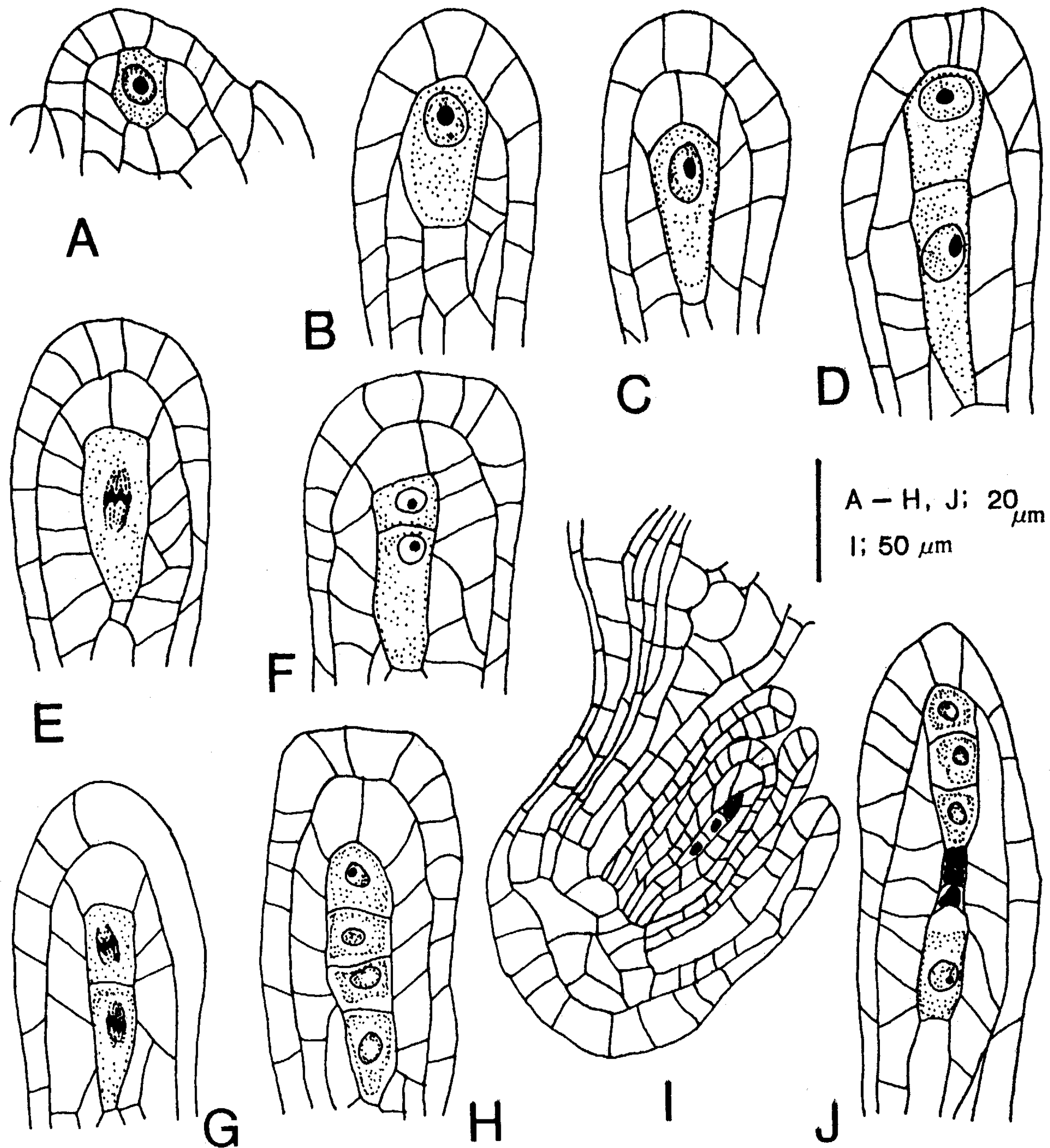

Fig. 1. Development of embryo-sac. A. Portion of ovule with archesporial cell. B. Tenuinucellate nucellus with megasporocyte. C. Crassinucellate nucellus with megasporocyte. D. Nucellus with two megasporocytes, arranged longitudinally. E. Nucellus with megasporocyte at metaphase of Meiosis I. F. Nucellus with dyad. G. Nucellus with two dyad cells, each at metaphase of Meiosis II. H. Nucellus with tetrad. I. Ovule with tetrad of which two micropylar cells are degenerating. J. Nucellus with triad and functional megaspore.

The parietal cells, if any, and other nucellar cells in contact with the micropylar half of the developing embryo-sac disappeared (Figs. 1N–Q), as the development of embryo-sac proceeded. The epidermis of nucellus remained to disappear at least until the end of the female gametogenesis (Fig. 1Q). The epidermal cells in principle underwent the anticlinal division, though they occasionally divided periclinally at the nucellar tip (Figs. 1N, P). The embryo-sac did not

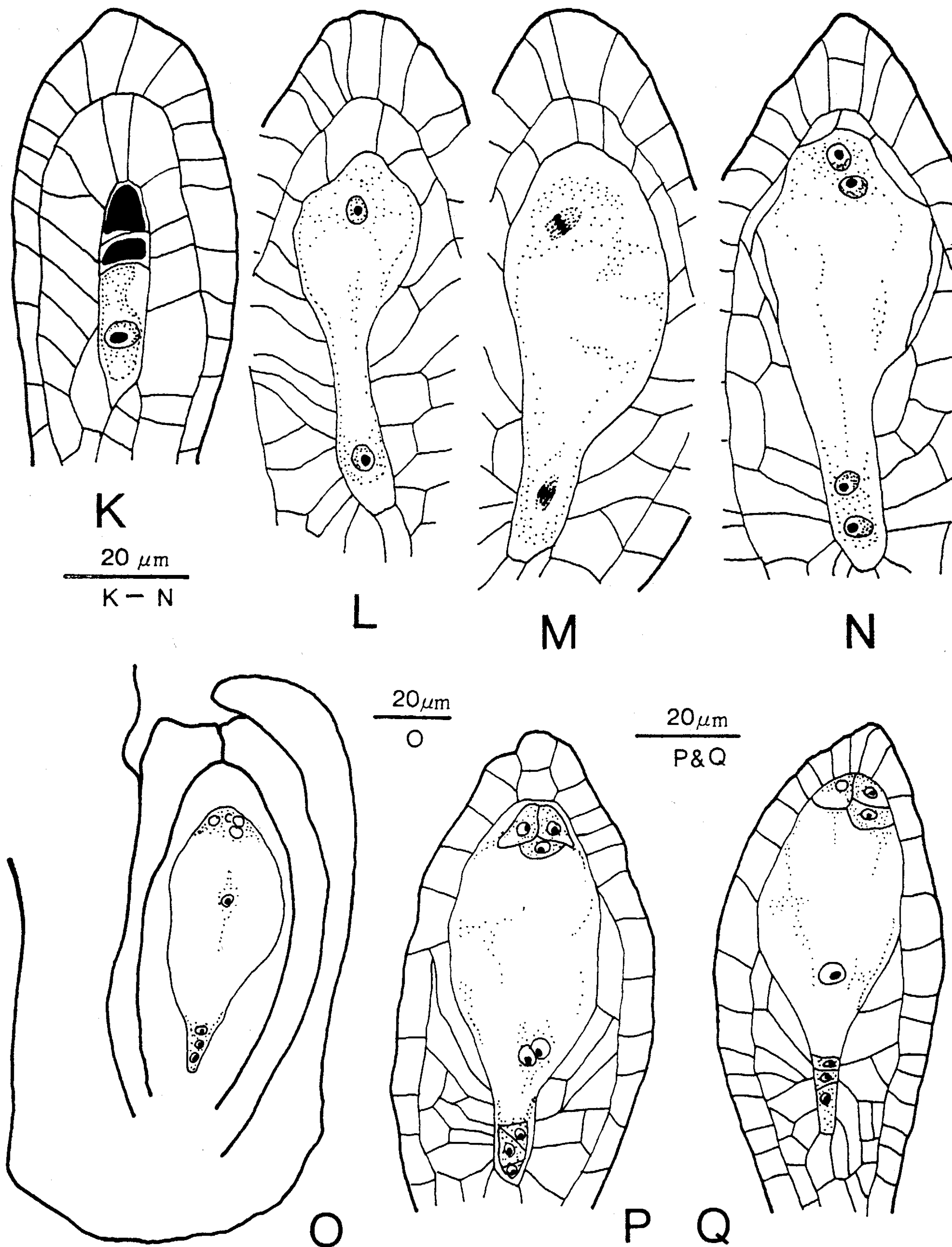

Fig. 1. Development of embryo-sac (continued). K. Nucellus with functional megaspore. L. Nucellus with two-nucleate embryo-sac. M. Nucellus with two-nucleate emrbyo-sac, each nucleus in division. N. Nucellus with four-nucleate embryo-sac. O. Ovule with eight-nucleate embryo sac. P. Nucellus with embryo sac in which two polar nuclei do not fuse yet. Q. Nucellus with mature embryo-sac in which two polar nuclei fuse already.

come in contact with the inner epidermis of the inner integument at least during the gametogenesis.

(2) Embryo-sac formation

The sporogenous cell in both the crassinucellate nucellus and the tenuinucellate one elongated along the axis of nucellus to differentiate as the megasporocyte (Figs. 1B–C). It underwent the first division of meiosis (Fig. 1E) to form two dyad cells (Fig. 1F), the chalazal cell being much larger than the micropylar. They successively underwent the second division of meiosis (Fig. 1G) in each cell, resulting in the formation of a four-celled tetrad (Fig. 1H). The four cells were usually separated with three transverse walls, though rarely the micrpylar wall of them lay down obliquely. The chalazal-most cell became larger and it entered the process of the embryo-sac formation, while the remaining three disappeared in order from the micropylar-most cell. Thus, the chalazal-most cell of the tetrad became a functional megaspore. Occasionally, two processes of megasporogenesis proceeded in the same nucellus (Fig. 1J).

The functional megaspore (Fig. 1K) underwent a mitotic division of its nucleus without cytokinesis to form a two-nucleate embryo-sac (Fig. 1L). The embryo-sac prominently elongated along the axis of nucellus. The two nuclei were situated at the poles of the embryo-sac and a large vacuole developed at its center. The two nuclei simultaneously divided (Fig. 1M) to form four nuclei, arranged in pairs, at the micropylar and chalazal ends of the embryo-sac (Fig. 1N). In the embryo-sac where the nuclear division was over, the central vacuole became larger rapidly and greatly. Together with this, the embryo-sac itself became larger, and the enlargement of the embryo-sac was accompanied by the disappearance of the nucellar cells. The four nuclei of the embryo-sac divided to form an eight-nucleate embryo-sac (Fig. 1O). The eight nuclei arranged in quartets at both the poles of the embryo-sac. The three nuclei of the micropylar quartet formed an egg apparatus and those of the chalazal quartet did an antipoldal apparatus. The two remaining nuclei migrated from the opposite ends of the embryo-sac into a central cell and they became polar nuclei (Fig. 1P). They fused with each other to become a diploid polar nucleus before fertilization (Fig. 1Q).

(3) Callose deposition during megasporogenesis

The fluorescence microphotographs in Fig. 2 were filmed the sections shown in Fig. 3 under the fluorescence microscope. There was no callosic fluorescence in any walls investing the archesporial cells and the sporogenous cells. Of course, callose was not detected in the wall of the parietal cells, if any. Moreover, it did not deposit even in the wall of the megasporocyte which was undergoing the first division of meiosis. The cell plate formed in the phragmoplast after the division attained to its telophase emitted the callosic bright-yellow fluorescence (Fig. 2A). The partition wall between two dyad cells, which was formed by the development of the cell plate, fluoresced more strongly than the cell plate did (Fig. 2B). The wall continued to fluoresce strongly till the end

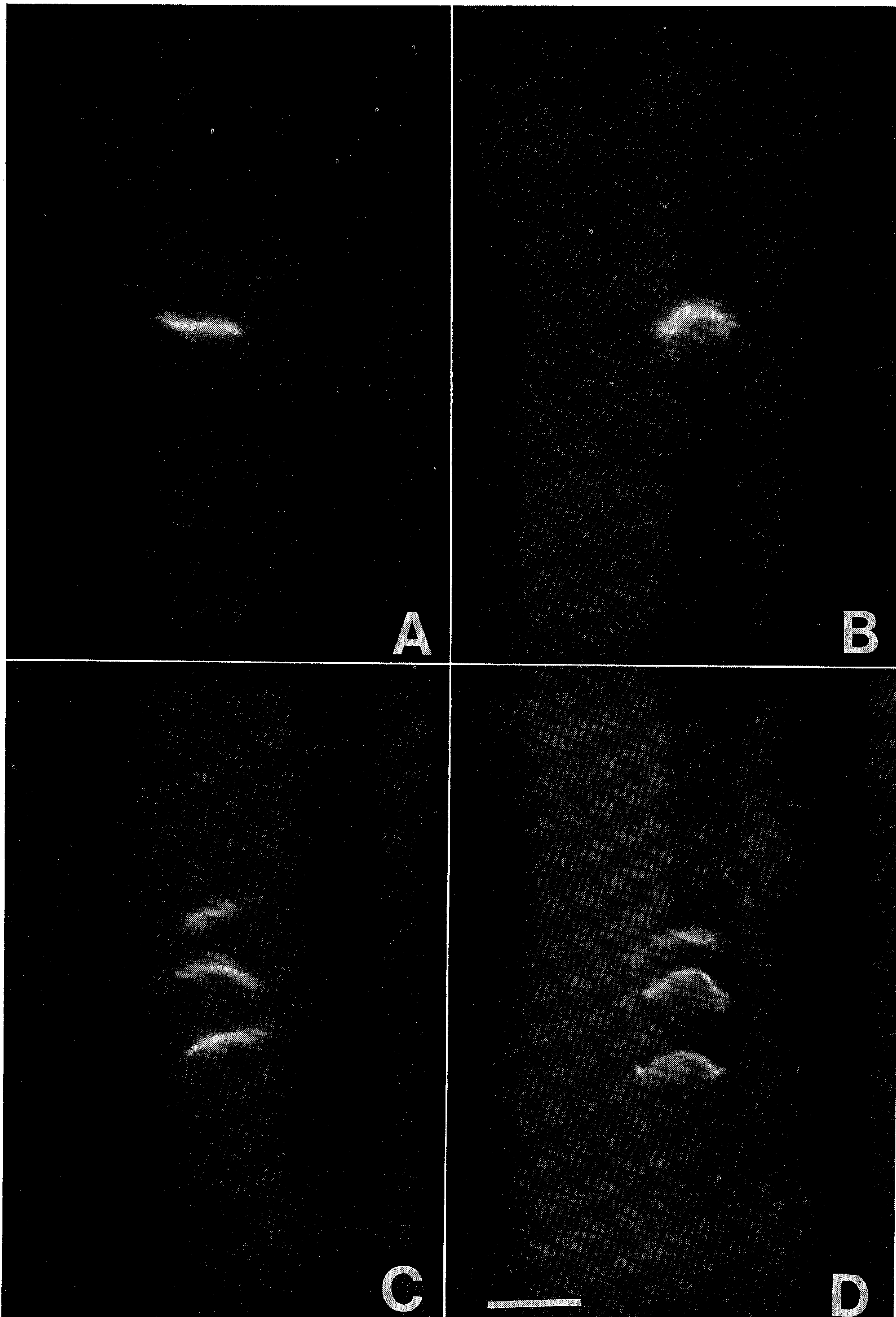

Fig. 2. Deposition of callose during megasporogenesis. A. Megasporocyte which is about to complete Meiosis I (see Fig. 3A). The cell plate is developing in the phragmoplast formed after the telophase. B. Two dyad cells, each cell at metaphase of Meiosis II (see Fig. 3B). C. Tetrad (see Fig. 3C). Callose deposits only in three partition walls. D. Tetrad (see Fig. 3D). Callose deposits the entire wall enclosing four tetrad cells besides the partition walls. (bar=10 μm)

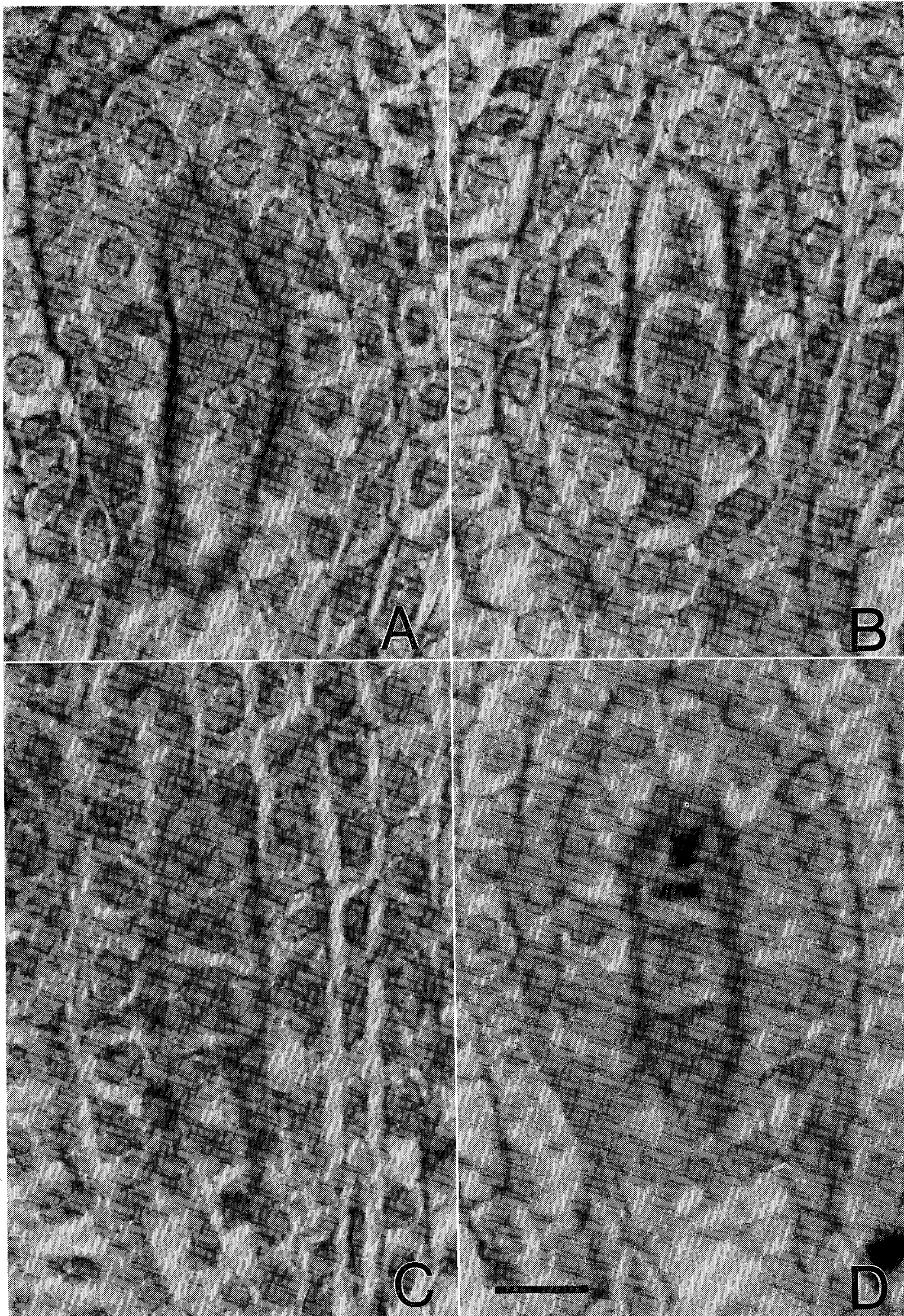

Fig. 3. Sections used for detection of callose. A. Megasporocyte which is about to complete Meiosis I (see Fig. 2A). B. Two dyad cells, each cell at metaphase of Meiosis II (see Fig. 3A). C. Tetrad (see Fig. 2C). D. Tetrad (see Fig. 2D). (bar =10 μm)

of the megasporogenesis. Two partition walls formed in both the dyad cells after the second division of meiosis, also, fluoresced. Accordingly callose deposited in three partition walls separating four tetrad cells (Fig. 2C). Usually, callose was not detected in the wall which invested entirely the dyad and tetrad. However, rarely the entire wall of the dyad and the tetrad (Fig. 2D) also fluoresced very weakly. Unfortunately, the fluorescence intensity of the entire wall of the dyad was too weak to take a microphotograph. As the degeneration of the non-functional cells of the tetrad proceeded, callose disappeared from the walls. After then, callose was not detected in the developing and the mature embryo-sacs at all.

Discussion

The ovule of *Aletris luteoviridis* is anatropous and its nucellus is invested by two integuments. The nucellus is either crassinucellate or tenuinucellate, the crassinucellate nucellus being more usual than the tenuinucellate. Two processes of the megasporogenesis occasionally proceed in the same nucellus. In the present investigation, however, two embryo-sacs which were developing in the same nucellus were not observed, though they exist in the nucellus of *Amianthium muscaetoxicum* which is a species of the Melanthiaceae in Dalgren & Clifford's sense (Eunus, 1951). The embryo-sac of *Aletris luteoviridis* is formed according to the monosporic eight nucleate *Polygonum* type of development. Although it is known that there is a species with the bisporic type of embryo-sac formation (Dahlgren & Clifford, 1982), no fact showing the existence of the bisporic type of embryo-sac formation was observed, as far as we observed the preparations stained with hematoxylin and fast green FCF. Although the antipodal cells of *Amianthium muscaetoxicum,* whose embryo sac is formed according to the *Polygonum* type of embryo-sac formation, increase in number of the nuclei and in number of the cells (Eunus, 1951), there was no such a fact in the antipodal apparatus of *Aletris luteoviridis.* Thus, the Melanthiaceae seems to have a wide variation in the embryological point of view.

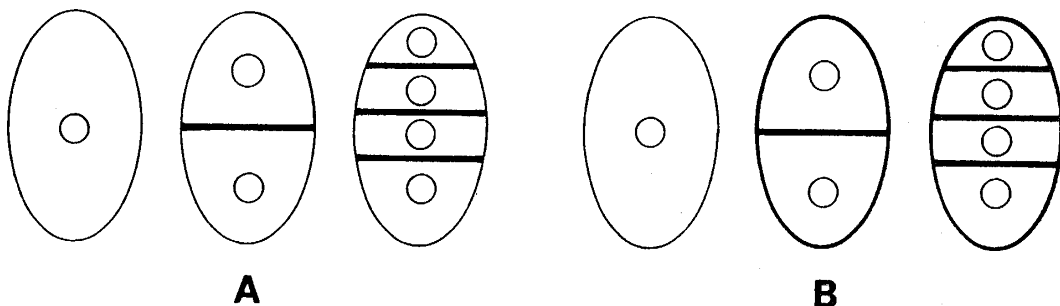

Fig. 4. Callose deposition during megasporogenesis. (Thick solid line indicates the wall in which callose deposits.) A. Callose deposits only in the partition walls of the dyad and the tetrad. B. Callose deposits in the entire walls of the dyad and the tetrad, besides in the partition walls.

Callose usually deposits only in a transverse wall of the dyad and in three partition walls of the tetrad, though occasionally it does in the wall enclosing the whole of two dyad cells and of four tetrad cells besides the partition walls. Although the latter pattern of callose deposition is well known in the process of the tetrad formation, the former is known only in the process of *Stachyurus praecox* (Satô & Takeda, 1985). It seems that, during the megasporogenesis of *Aletris luteoviridis,* there are two deposition patterns of callose as shown in Fig. 4. *Stachyurus* is a genus of the dicotyledonous Stachyuraceae and it was once assigned to the Theaceae (Lawrence, 1951). In many members of the Theaceae, their embryo-sacs are formed according to the bisporic type of development (Davis, 1966). Although callose deposition has been investigated in few species with the bisporic *Allium* type of embryo-sac development, callose deposits only in the wall separating two dyad cells in all these species (Kapil & Tiwari, 1978). In *Agraphis* which is closely related to *Aletris,* the embryo sac is formed according to the bisporic *Endyminon* type (Dahlgren & Clifford, 1982). In some of the species in which their embryo-sacs are formed according to the monosporic type of development, and which are taxonomically related to the species with the bisporic type of embryo-sac development, callose seems to deposit only in the wall separating respective cells of the dyad and the tetrad.

摘　要

ノギラン (*Aletris luteoviridis*) は一般的にはユリ科の一種として扱われている。しかし, DAHLGEN & CLIFFORD (1982) はこのユリ科を細分し, Melanthiaceae を設け, これにノギランを収容している。この Melanthiaceae の少数の種で胚嚢の形成過程が調査されているが, 単胞子性のほかに二胞子性の胚嚢形成様式を持つものがある (DAHLGEN & CLIFFORD, 1982)。大胞子形成過程に於ける減数分裂中の細胞の壁へのカロースの沈着様式に関する調査はまだ十分には行われていないが, 単胞子性と二胞子性の胚嚢形成過程ではその沈着様式にかなりの相違が, これまでの調査では認められている (KAPIL & TIWARI, 1978)。単胞子性の胚嚢形成過程では減数分裂を行っている大胞子母細胞及び二分子と四分子を構成している各細胞の壁には, ほぼ完全にカロースが沈着する。一方,二胞子性のものでは,二分子の隔壁にカロースの沈着がみられるだけである。しかし,キブシは, 双子葉植物のキブシ科の植物であるが,単胞子性の胚嚢形成様式を持つ。ところが,キブシの大胞子形成過程では二分子と四分子の隔壁にカロースが沈着するだけである (SATÔ & TAKEDA, 1985)。キブシはかつてツバキ科に含められており (LAWRENCE, 1951), ツバキ科と分類学的に近縁な関係にある。そのツバキ科の胚嚢形成様式はほとんどが二胞子性のものである。そこでノギランの胚嚢形成過程の調査に加えて, この過程でのカロースの沈着様式も調査した。胚珠は倒生で, 珠心を二枚の珠皮が被っている。胚嚢は単胞子性8核タデ型にしたがって形成される。カロースは通常は二分子と四分子のそれぞれの細胞を分けている隔壁にだけ沈着するが, 時には, 減数分裂に入った大胞子母細胞では確認できなかったが, 二分子や四分子の全体を囲んでいる壁にもわずかではあるが沈着する。このようにノギランでは, キブシと同じカロースの沈着が普通にみられ, これまでに調査されている単胞子性のカロースの沈着様式とほぼ同じ様式もときたま認められる。単胞子性の胚嚢形成過程でも二分子と四分子のそれぞれの細胞を分ける隔壁にしかカロースの沈着しない様式が,単子葉植物でもみられたことになり,単胞子性の胚嚢形成過程でみられるカロースの沈着様式は一様なものではなく, 種による変異があると思われる。ノギランやキブシでみられる隔壁にだけカロースが沈着する様式を, 二胞子性のカロースの沈着様式と安易に比較すること

はできないかも知れないが，ノギランもキブシも近縁な分類群に二胞子性の胚嚢形成を行うものがあることは注目されてよいと思われる。

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