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Pollen Development of *Aucuba japonica* Thunb.

By

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Abstract. The developmental pattern of pollen and anther wall in *Aucuba japonica* was investigated. In addition, during the pollen development, the detection of callose and PAS-positive substance was performed. The anther wall was formed according to the dicotyledonous type of development in the sense of Davis (1966). The tapetum was of the amoeboid type. The pollen was formed according to the simultaneous type of cytokinesis. The cells which were undergoing the meiosis were surrounded by the thick callose wall. The deposition of callose to the microsporocytes started from the walls which were in contact with the tapetum. The PAS-positive substance, which was observed in the form of granules, occurred in the pre-meiotic cells. The PAS-positive granules continued to exist in the cells which were undergoing the meiosis, but they disappeared from the cytoplasm of the microspores. However, they appeared again in the cytoplasm of the pollens with the vegetative and the generative nuclei. They accumulated around the vegetative nucleus, while they did not exist around the generative one. However, it was not confirmed under the light microscope and the fluorescence one whether there was the wall between the two nuclei or not.

Introduction

Many taxonomits (Lawrence, 1951; Engler, 1964; Hutchinson, 1973; Cronquist, 1981; and so on) have treated *Aucuba japonica* Thunb. as a species of the Cornaceae, but some ones (Airy Shaw, 1976; Takhtajan, 1980) have divided it into some families and have assigned the species to the Aucubaceae which were one of the families segregated by them. The embryological investigation of *A. japonica* has been occasionally performed. The embryo sac formation of the species was investigated by Palm & Rutgers (1917) and Satô (1971), and the histological and cytological studies of the embryo at several stages of its development also were performed by Satô & Yamaguchi (1987). Unfortunately, the pollen development has not been investigated yet. So, the writer intended to investigate the development of pollen of *Aucuba japonica.* The occurrence of polysaccharides during the spore development of the angiospermous plants has come to receive much attention (Kapil & Tiwari, 1978). So, the detection of callose and PAS-positive substance of polysaccharides was attempted in the cells during the pollen development. This paper reports on the development and structure of the anther wall, the development of the pollen and the occurrence

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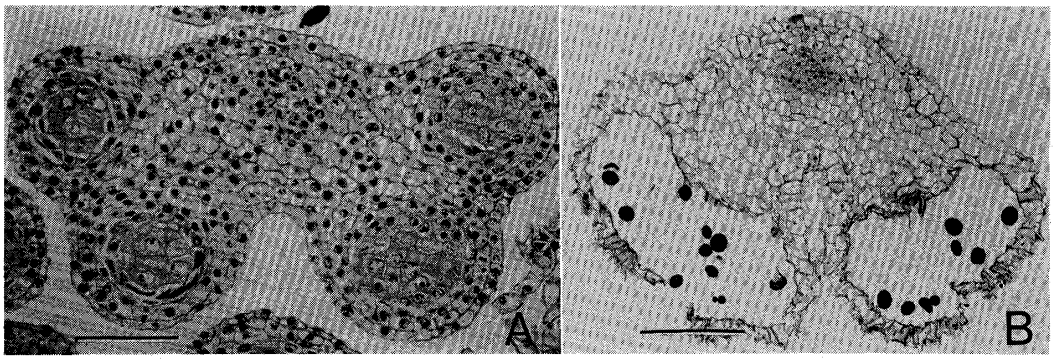

Fig. 1. Anther. A. Four sporangiate anther. B. Anther in which sterile partition plate has broken down. (bar; A=100 μm, B=150 μm)

of callose and the PAS-positive substance in the process of the pollen development in *A. japonica*.

Material and Methods

A. japonica is an evergreen dioecious shrub. Many flowers at various stages of development were collected from several male plants growing on the campus of the Yokohama National University at appropriate intervals extending from November in 1987 to March in 1988, and they were fixed in FAA (formalin-acetic-alcohol). After they were dehydrated in ethyl alcohol-*tert*. butyl alcohol series, they were embedded in paraffin or histosec (supplied by Merck and Co., Ltd.). These embedded materials serially sectioned at 6-10 μm thick. To investigate the pattern of the pollen development, the sections were stained with Heidenhain's iron alum hematoxylin and fast green 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 fluorescence was confirmed by treating with β-1, 3-glucanase or zymolyase, not that it was performed for all the sections. The materials for detecting PAS-positive substance were stained with Schiff's reagent (O'Brien & McCully, 1981). The stained matrerials also were observed under the fluorescence microscope. The PAS-positive substance emits bright-red fluorescence when treated with the Schiff's reagent.

Observation

(1) Anther wall

An anther contained four microsporangia arranged in pairs in two anther lobes which were separated by a connective. In a young anther (Fig. 1A),

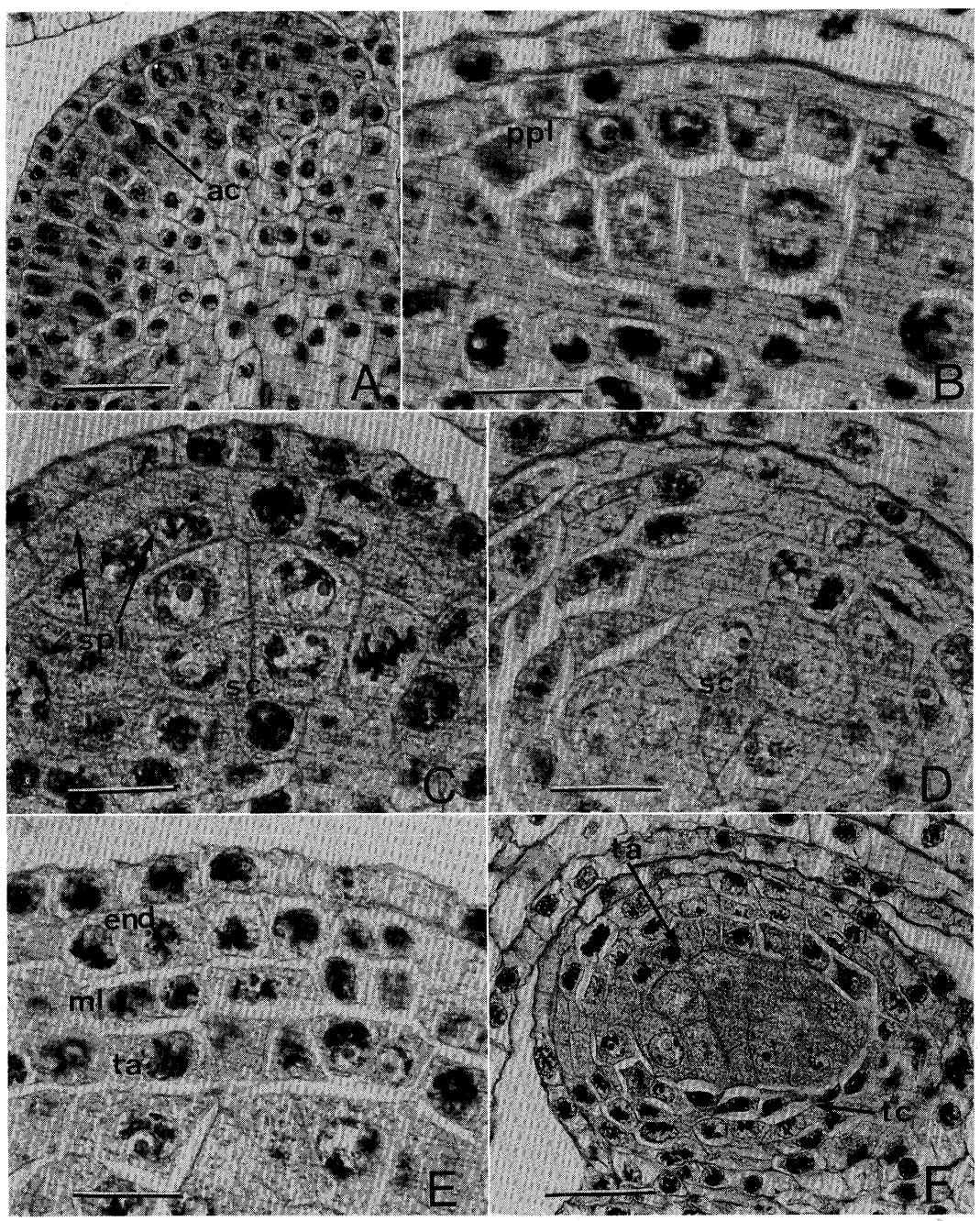

Fig. 2. Development of anther wall. A. Anther in which archesporial cells has been formed below epidermis. B. Anther in which primary parietal layer and sporogenous cells has been formed. C. Anther in which primary parietal cells are dividing periclinally to form two secondary parietal layers. D. Anther in which future endothecium and middle layer are being formed. E. Anther in which young endothecium, middle layer and tapetum has been arranged in good order. F. Anther locule in which tapetum encloses microsporocyte before meiosis. (ac; archesporial cell, end; endothecium, ml; middle layer, ppl; primary parietal layer, sc; sporogenous cell, spl; secondary parietal layer, ta; tapetum next to anther wall, tc; tapetum next to connective) (bar; A=50 μm, B—E=20 μm, F=80 μm)

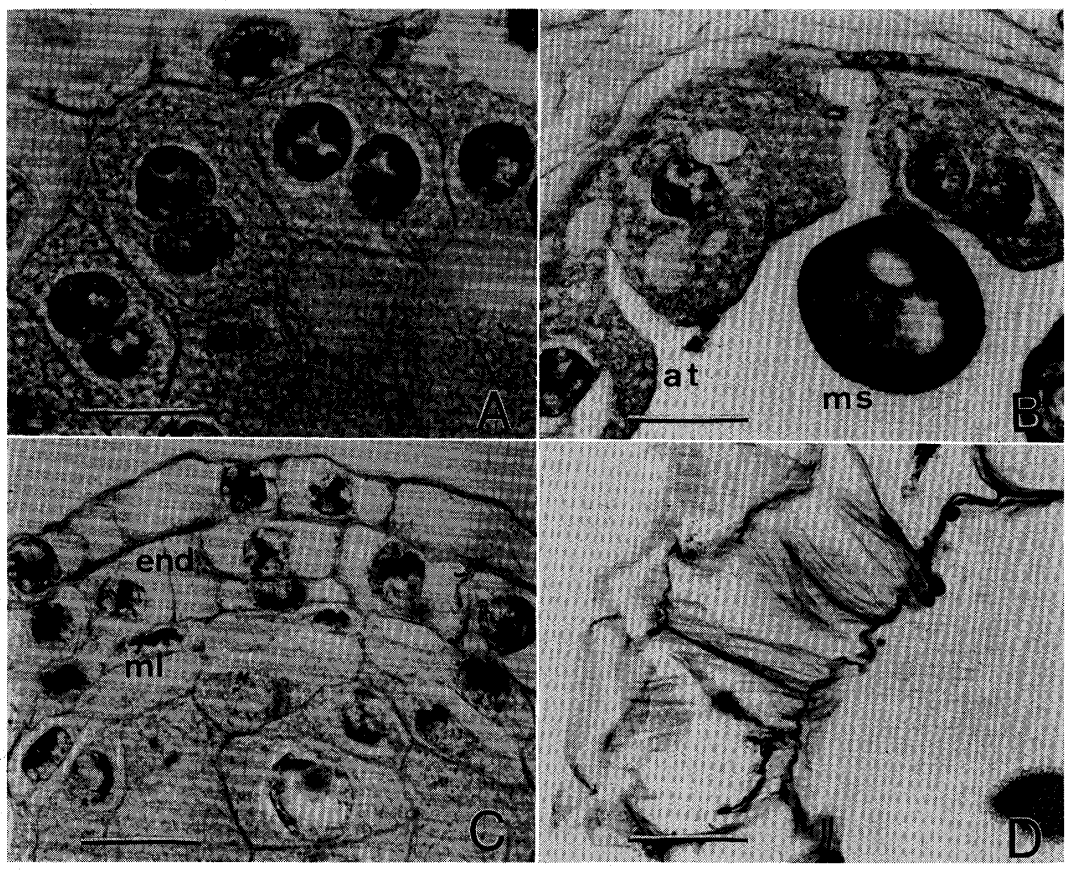

Fig. 3. Development of anther wall (continued). A. Two-nucleate tapetal cells. B. Anther with microspores. C. Anther with microsporocytes just before meiosis. Some cells of the middle layer occasionally divide, so that the layer becomes two partially. The vacuolarization in endothecial cells proceeds greatly. D. Tapetal cells with fibrous bands. (at; amoeboid tapetum, end; endothecium, ml; middle layer, ms; microspore) (bar; A—C=20 μm, D=30 μm)

members of each pair of the microsporangia were separated from each other by a partition plate of a sterile tissue. Before the anther released pollens, however, the sterile partition plate broke down and disappeared (Fig. 1B). As a result, a pollen sac was formed in each lobe of the anther.

In the descriptions of the development of anther wall in the angiospermous plants, there has been a lack of unity in the terminology. In this paper, the description is based on the terminology of Davis (1966). Anther walls originated by periclinal divisions of a group of hypodermal cells situated at the corners of the very young anther. Subepidermal cells situated at the corners of the anther differentiated as archesporial cells (Fig. 2A). They divided periclinally to form a primary parietal layer outwards and a primary sporogenous cells inwards (Fig. 2B). The former developed to form an anther wall, while the latter differentiated as microsporocytes after the several mitotic divisions. A primary parietal layer periclinally divided to form two secondary parietal layers (Fig. 2C). The outer of the two divided once more to form two parietal layers (Fig. 2D).

As a result, three parietal layers (Fig. 2E) were formed before the microsporocytes entered the first division of meiosis. These layers were the future endothecium beneath the epidermis, the middle layer and the innermost tapetum.

Externally to the microsporangium, the tapetum differentiated directly from the inner secondary parietal layer, whereas internally, metamorphosed cells of the connective tissue completed the tapetum around the circumference of the anther locule (Fig. 2F). However, the tapetal cells next to the anther wall usually differed from those next to the connective in shape and stain property. Many of these tapetal cells began to divide without cytokinesis to become 2-4 nucleate condition, usually 2-nucleate (Fig. 3A), before the microsporocytes entered the first division of meiosis. The contents of the tapetal cells were rich and large vacuole or vacuoles were absent, though small vacuoles were present throughout the cytoplasm. Therefore, they were strongly stained with hematine. From the end of the meiosis, the tapetal cells became irregular in shape and they got separated from each other (Fig. 3B). In the cells at this stage, the wall had been lysed. They gave rise to the periplasmodium along the periphery of the anther locule. Thus, the tapetum seems to be of the amoeboid type. However, the plasmodium which intruded among the developing microspores and pollen grains was not observed in the preparations used for the present investigation.

Some cells of the middle layer periclinally divided further and the layer became two partially (Fig. 3C). Almost all cells of the layer were compressed and became flat. As the differentiation proceeded, a large vacuole or vacuoles were formed in the cytoplasm. When the meiosis was over, they were absorbed and disappeared.

In the endothecial cells as in the cells of middle layer, a large vacuole or vacuoles developed as well (Fig. 3C). After the completion of meiosis, the endothecial cells increased prominently in radial extent. Together with their increase in size, the epidermal cells sloughed gradually. After the microspore completed the mitotic division, the sterile partition plate disappeared. In the endothecial cells, after then, the secondary wall thickenings were formed in the form of bands (Fig. 3D). The bands were located on the anticlinal and the inner tangential walls. The anther dehisced longitudinally along the furrow situated at each side of the anther between the members of a pair of sporangia.

(2) Pollen development

During the development of the anther wall, the sporogenous cells divided mitotically in various plane, the cells functioning as microsporocytes (Fig. 4A). The microsporocyte underwent the first division of meiosis (Fig. 4B) without the cytokinesis, though the phragmoplasts (Fig. 4C) appeared. After the first division of meiosis completed, the phragmoplasts disappeared. As a result, the microsporocyte (Fig. 4D) was two nucleate. The two nuclei underwent the second division of meiosis (Fig. 4E) to become four nuclei (Fig. 4F). During this division, also, the phragmoplast appeared between each two nuclei, though

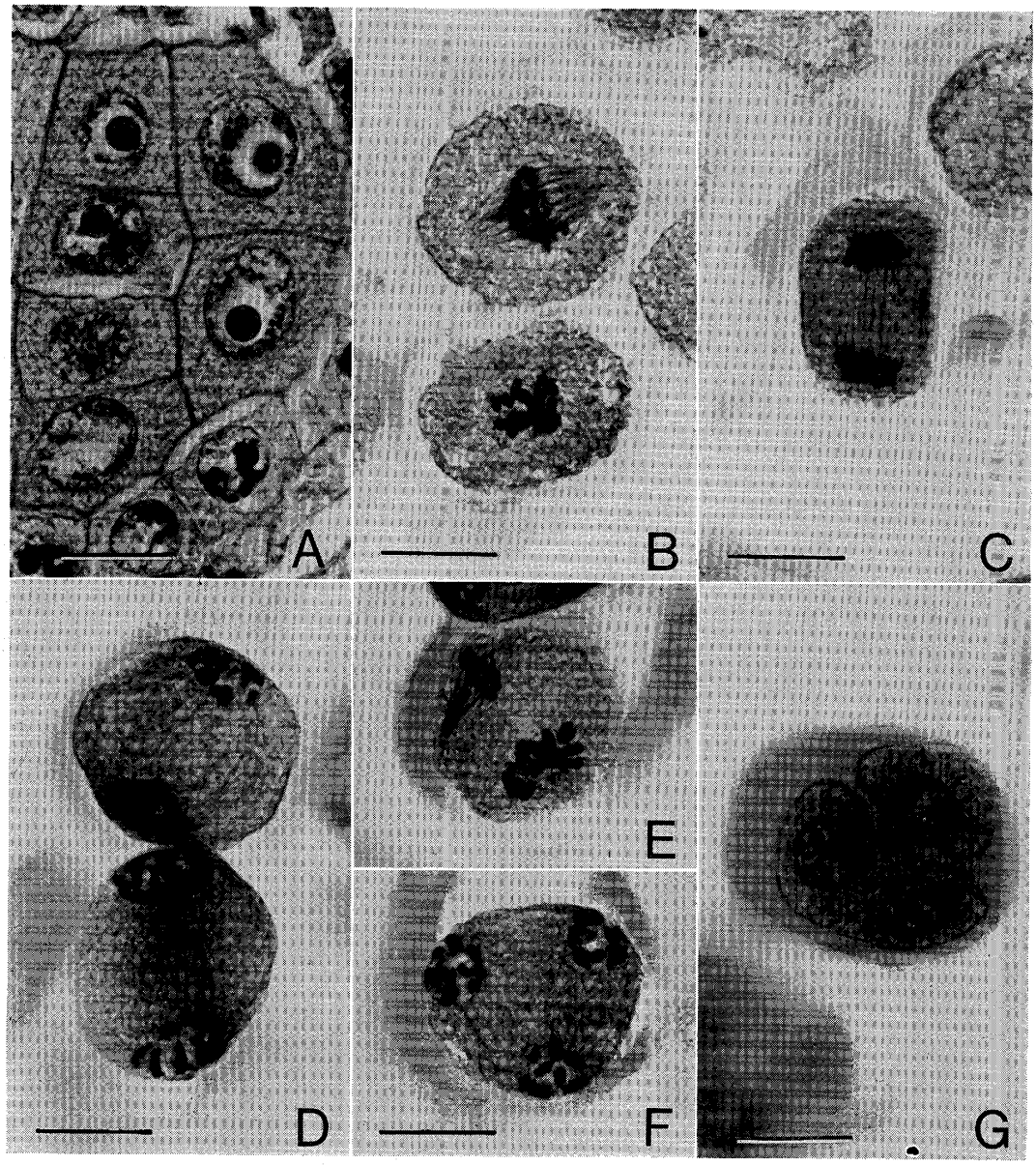

Fig. 4. Development of pollen. A. Microsporocytes arranged compactly in the sporangium. B. Microsporocytes at metaphase of the first division of meiosis. C. Microsporocytes at anaphase of the first division of meiosis. D. Two-nucleate cell. E. Cell in which the second division of meiosis is undergoing. F. Four-nucleate cell before furrowing. G. Tetrad after furrowing. (bar; A—G=20 μm)

no cell plate was formed. After the meiosis was over, the phragmoplast disappeared. The four nuclei usually arranged in a tetrahedral fashion. The quadripartition of the four-nucleate cell (Fig. 4G) took place by the formation of furrows. The furrows originated at the surface of the wall of cell in which the meiosis had already been over, and they developed inwardly until they joined in the center, resulting in the accompanying formation of walls which partitioned the original microsporocyte into four microspores. The four released

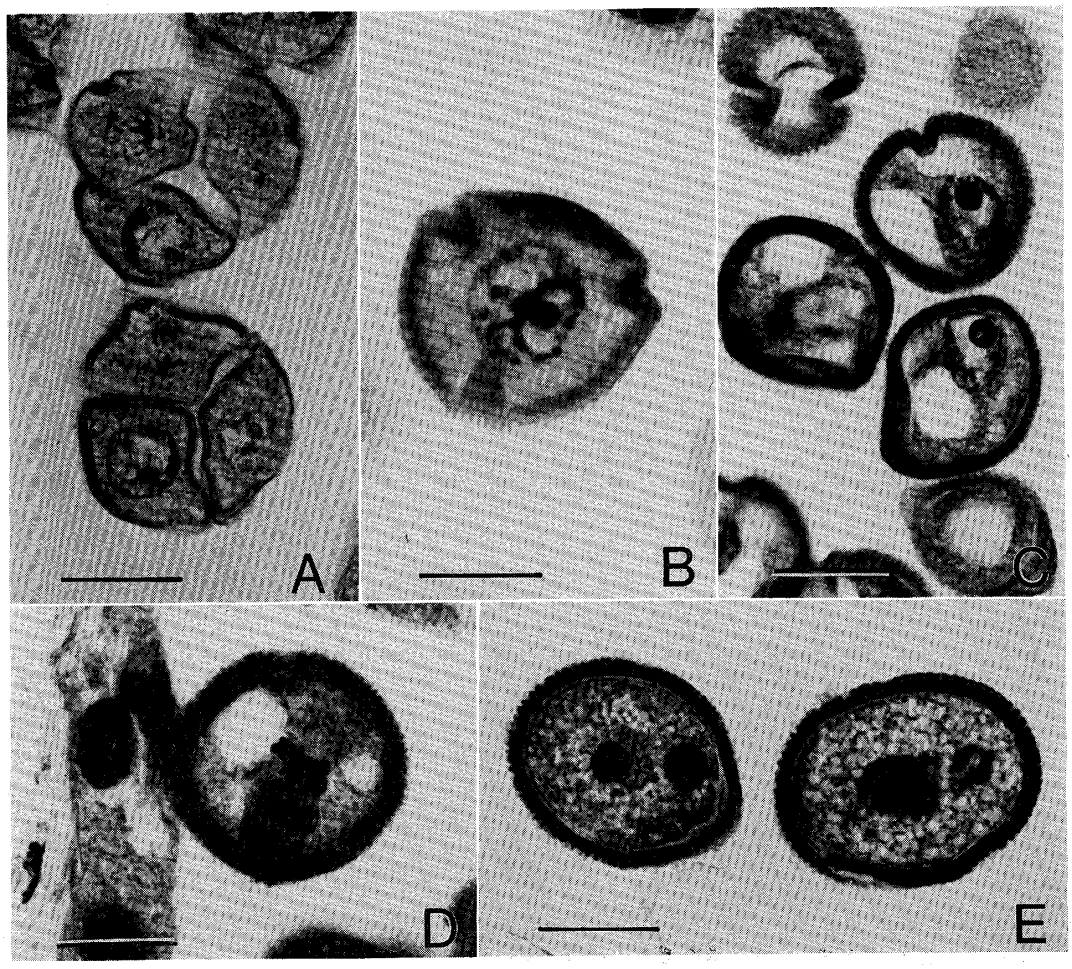

Fig. 5. Development of pollen (continued). A. Young microspores which begin to release. B. Microspore without vacuole. C. Microspores with large vacuole. D. Microspore at anaphase of the mitotic division of its nucleus. E. Pollens with vegetative nucleus and generative nucleus. (bar; A—E=20 μm)

from one another (Fig. 5A) to become free microspores (Fig. 5B). Immediately after the release, a large vacuole was absent in their cytoplasm and their nucleus occupied in their center. After then a large vacuole developed in the cytoplasm of microspores (Fig. 5C). The nuclei were pushed aside by the large vacuole to lie next to the intine. Meanwhile, a finely dotted exine enclosed the microspore and three slender furrows, each with a pore, were formed; the grain was of tricolporate type. The microspore (Fig. 5D) once divided mitotically to develop into a two-nucleate pollen (Fig. 5E). One of the two nuclei was larger than the other, and it had higher stain property than the other. The larger nucleus seemed to be vegetative, while the smaller seemed to be generative. However, it was very difficult to observe the cell wall by which the two nuclei were separated.

(3) Deposition of callose during pollen development

The microsporocytes which had just differentiated from the sporogenous

cells were polygonal and were compactly arranged (Fig. 4A). Many of them were in contact with the tapetal cells, though there were some microsporocytes surrounded by other microsporocytes only. As soon as the microsporocytes entered the first division of meiosis, callose began to deposite on the wall of microsporocytes. The deposition of callose onto the wall started from the wall which had been in contact with the tapetal cells of the anther wall (Fig. 6A). A little later, in the microsporocytes next to the connective, callose deposited onto the wall adjacent to the tapetal cells. As a result, callose wall enclosed the sporangium (Fig. 6B). After then callose deposited also in the corner where more than two microsporocytes were joined (Fig. 6C) and the deposition spread to other wall parts (Fig. 6D). By the metaphase of the first division of meiosis, the microsporocytes were separated not only from the tapetal cells but also from one another. The shape of the microsporocytes changed from polygon to sphere, and they became free. Meanwhile, callose wall rapidly and prominently increased in thickness (Fig. 7A), and the free microsporocytes invested with thick callose wall. The callose wall was retained till the time when the release of the microspores started after the meiosis (Figs. 7B-D). As the quardripartition in the 4-nucleate cell in which the meiosis had just been over was proceed-

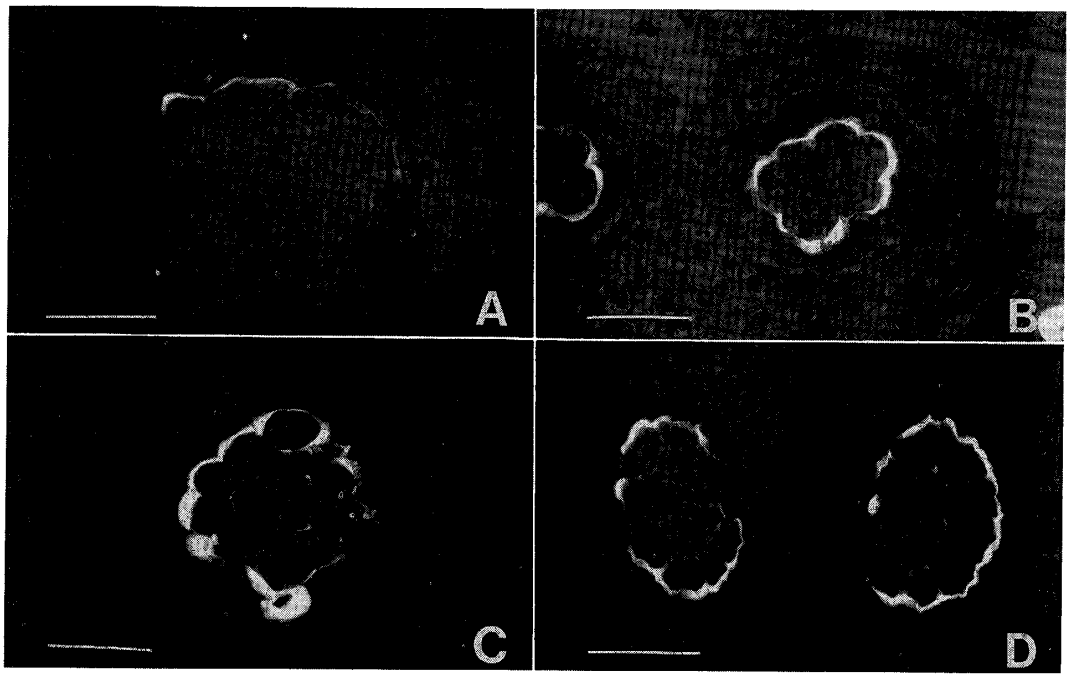

Fig. 6. Callose deposition during pollen development. A-D. Anther locules in which microsprocytes has just entered the first division of meiosis. A. Callose at first deposits on the walls of microsporocytes next to the tapetum of the anther wall. B. Callose then deposits on the walls of microsporocytes next to the tapetum of the connective. As a result, the callose walls are formed around the microsporangium. C. Callose deposits in the corner where microsporocytes are joined, after the deposition around the microsporangium. D. Callose then begins to deposit on the entire walls of the microsporocytes. (bar; A & C=40 μm, B & D=60 μm)

ing, callose walls developed centripetally along developing furrows. During the quardripartition, an exine and an intine began to be formed along the inside of callose wall. The callose wall around four microspores first disappeared and then the walls which separated four microspores disappeared (Fig. 7E), so that microspores were released and became free. In the free microspores and pollens, the intine (Fig. 7F) which lined the pores emitted the very weak fluorescence, though the other part of the intine did not emit the fluorescence. There was no fluorescent structure or substance within the microspores and pollen grains except for the intine which lined the pore.

(4) Occurrence of PAS-positive substance during pollen development

The PAS-positive substance was absent in the cytoplasm of the sporogenous cells which were dividing mitotically. The substance in the form of granules appeared in the microsporocytes (Fig. 8A) which did not enter the first division

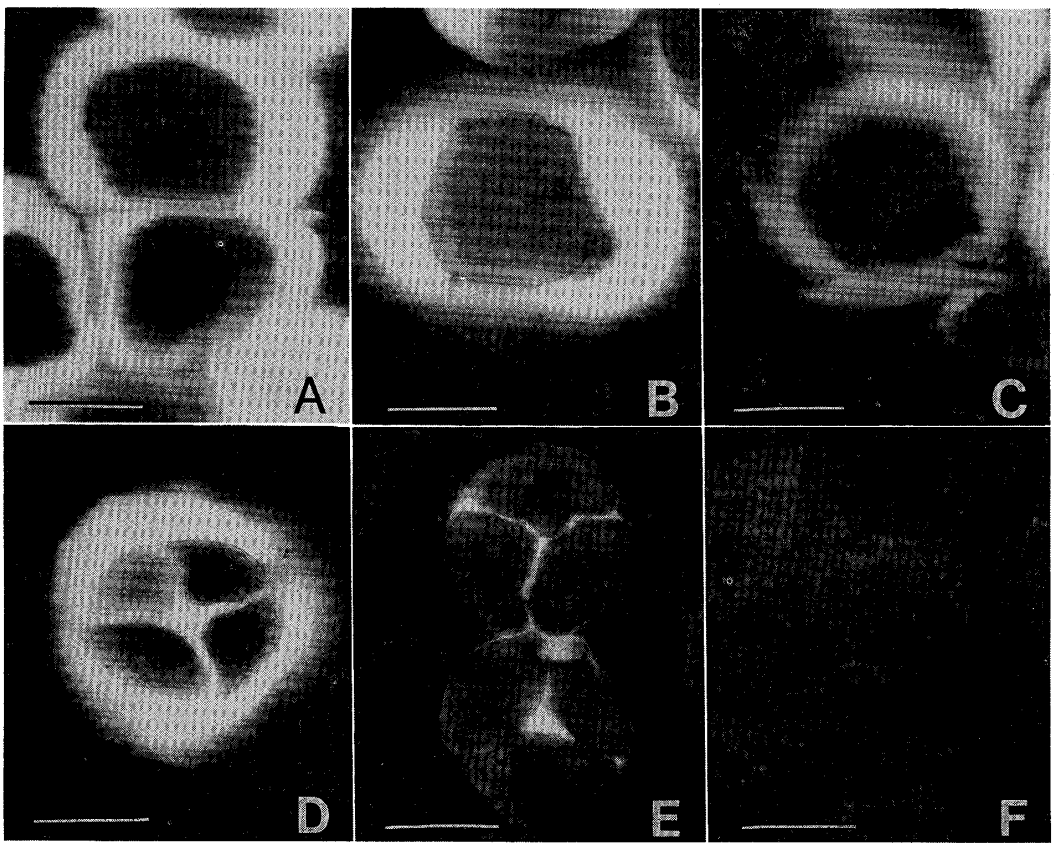

Fig. 7. Callose deposition during pollen development (continued). A. Microsporocytes at the metaphase of the first division of meiosis. Thick callose wall encloses every microsporocyte. B. Two-nucleate cells with thick callose wall. C. Four-nucleate cells before furrowing. D. Tetrad after furrowing. Callose deposits along the furrows, too. E. Outer callose wall which has enclosed entirely the tetrad at first disappear. Remaining callose walls separating four tetrad cells also disappear just later, so that the tetrad cells release to become microspores. F. Pollen with vegetative and generative nuclei. Callose wall lines every pore of the pollen. This callosic fluorescence invariably is very weak. (bar ; A—F=25 μm)

of meiosis yet. They were small, but prominent since the cytoplasm of the archesporial cells did not fluoresce except for the granules. They became larger gradually to attain to the maximum at the prophase of the first division of meiosis (Fig. 8B). As the meiosis proceeded further, the cytoplasm of the cells which were undergoing the meiosis emitted somewhat weaker fluorescence than that of the granules. Because of this cytoplasmic fluorescence, the outline of the granules became obscure, though they occurred in the cytoplasm (Figs. 8C-F). After the meiosis was over, the cytoplasmic fluorescence disappeared gradually. Because of this disappearance, again, the granules became prominent. They were situated along the wall of the tetrad cells (Fig. 8G). The fluorescence could not be observed in the cytoplasm of the free microspores. In the pollen grain in which the mitotic division had already been over, however, the PAS-positive granules accumulated around the large vegetative nucleus, but they were not observed around the small generative nucleus (Fig. 8H).

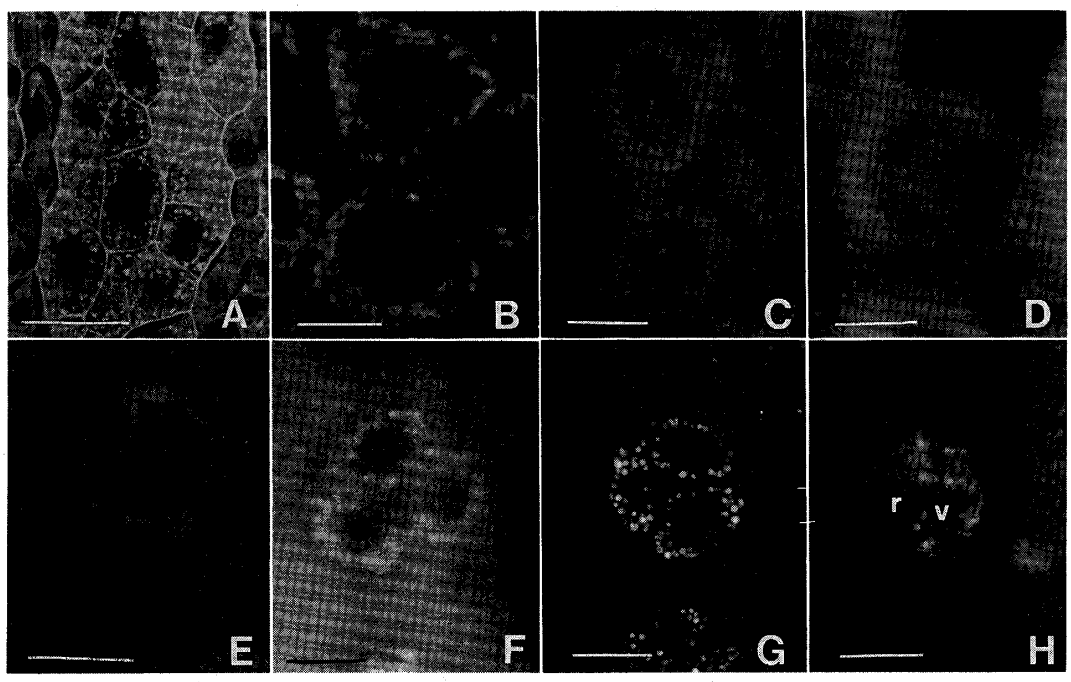

Fig. 8. PAS-positive substance during pollen development. A. Anther locule in which the microsporocytes do not enter the first division of meiosis yet. Many PAS-positive granules appear in microsporocytes long before meiosis. B. Microsporocytes shortly after meiosis. C. Microsporocytes at metaphase of the first division of meiosis. D. Two-nucleate cells. E. Four-nucleate cells in which the furrowing has just begun. F. Tetrad after the furrowing has just finished. G. Tetrads with the PAS-positive granules which become prominently due to the disappearance of the cytoplasmic fluorescence. H. Pollen with vegetative (v) and generative (r) nuclei. The PAS-positive granules accumulate around the vegetative nucleus. (bar; A=50 μm, B—H=20 μm)

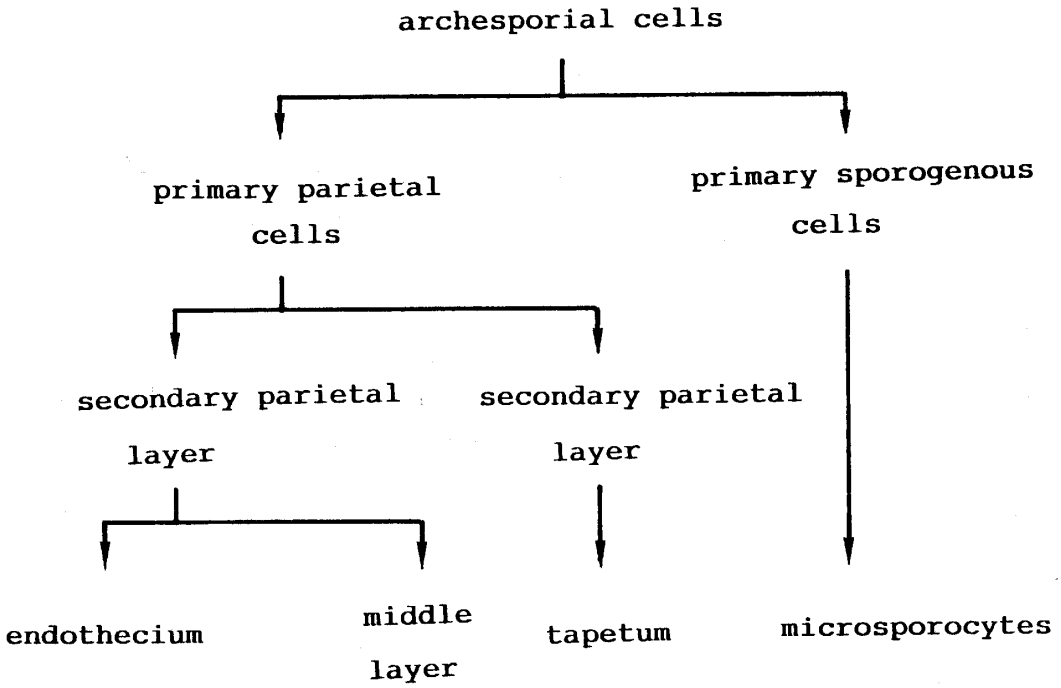

Fig. 9. Development of anther wall.

Discussion

(1) Development of anther wall

The developmental process of the anther wall in *Aucuba japonica* is summarized as in Fig. 9. Thus, the anther wall is formed according to the dicotyledonous type of its development in the sense of Davis (1966).

(2) Development of pollen

The development of pollen in *A. japonica* is of simultaneous type. As in the microsporocyte of almost all of the angiospermous plants, in *A. japonica*, callose walls enclose the microsporocytes which have just entered the first division of meiosis as well. The deposition of callose starts on the walls of microsporocytes next to the tapetum and rapidly extends to the cells in the center of the sporangium. Such a fairly regular sequence as this is also known in the process of the callose deposition in *Helleborus foetidus* (Waterkeyn, 1962). This may show that there is necessity of considering the tapetal contribution to the callose deposition. If so, callose deposition in *A. japonica* is very interesting, because callose starts to deposit earlier on the microsporocyte walls next to the tapetal cells of the anther wall than on those next to the connective. This seems to show that, in relation to the differentiation level and/or physiological maturity, there is a difference between the tapetal cells next to the anther wall and those next to the connective. It seems that, although Chapman (1987) has reviewed on four functions of the tapetum, the function in relation to the callose deposition will have to be added to the four functions.

In almost all of the angiospermous pollen, the cell plate is formed soon after the telophase of the mitotic division in the microspore. The wall is formed from the plate and becomes callosic. In the pollen of *A. japonica*, however, the occurrence of a rigid wall between the vegetative nucleus and the generative nucleus is very obscure, when it is stained with hematoxylin and fast green combination. In the pollen stained with aniline blue, moreover, there is no fluorescent wall between the two nuclei. But the PAS-positive granules accumulate around the vegetative nucleus and they are absent around the generative nucleus. Therefore, it seems that the wall separating the vegetative nucleus from the generative one is not formed, but the separation of the cytoplasm takes place between the two nuclei. Otherwise, it seems that the separation wall may be formed by a process different from the formation of a cell plate. The further investigation used the electron microscope is required in this respect.

摘　　要

アオキの花粉の形成過程と，この過程でのカロースと PAS 陽性物質の行動についての調査を行った。葯には葯隔をはさんで 2 個づつの小胞子嚢が作られる。しかし，半葯の 2 個の小胞子嚢を分けている壁は，葯の裂開前に消失する。葯壁は双子葉植物型に従って，また小胞子は同時膜形成型に従って形成される。小胞子の核は分裂し，2 核性の花粉が作られる。ヘマトキシリンとファストグリーンで染色した標本から，この生殖核と栄養核を分ける細胞壁が形成されるか否かについての判断は困難である。花粉は 3 溝孔粒で，この 2 核の状態で葯から柱頭に運ばれる。小胞子母細胞が減数分裂第一分裂にはいるとすぐに，小胞子母細胞の壁へのカロースの沈着が，絨毯細胞に接している壁から始まる。絨毯細胞に接している小胞子母細胞の他の壁や絨毯細胞に接していない小胞子嚢の内部の小胞子母細胞の壁にも，カロースの沈着が進む。ついにはどの小胞子母細胞もカロース性の壁で完全に被われる。減数分裂中の細胞は常に厚いカロース性の壁で被われている。4 核期の細胞が 4 個の四分子細胞に分けられるときに作られる溝にもカロースが沈着する。小胞子の外膜の形成が始まる頃に，4 個の四分子細胞を被う外側のカロース性の壁がまず消失し，続いて四分子細胞を分けていたカロース性の壁も消失する。こうして四分子細胞は互いにはなれ小胞子となる。被子植物の多くの花粉では，栄養核と生殖核を分ける壁に一時的にカロースが沈着する。しかし，アオキではこのカロース性の壁は観察できない。PAS 陽性物質は，減数分裂にはいる前の小胞子母細胞の細胞質に顆粒の形でみられ始める。減数分裂中の細胞では，この顆粒ばかりでなく，細胞質全体が PAS 陽性の性質を持つ。しかし，PAS 陽性物質は，減数分裂を終了した小胞子母細胞では，一時的に検出できなくなる。しかし，栄養核と生殖核を持った花粉では，生殖核の周辺には PAS 陽性物質はまったく検出できないが，栄養核の周りには PAS 陽性顆粒が多数存在している。この PAS 陽性顆粒の分布から，栄養核を含む部分と生殖核を含む部分に花粉の細胞質は分けられていると思われる。しかし，ヘマトキシリンとファストグリーンで染色した標本では，この 2 核の間の細胞壁の在否の判断は困難であり，花粉の細胞質内からカロースを検出することはできない等の理由から，この細胞質の分離は細胞壁によるものではないと思われる。電子顕微鏡を用いた今後の研究が望まれる。

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