Yoshiaki HAYASHI*: Embryology of *Magnolia salicifolia* Maxim. (Magnoliaceae)

林 義昭*: タムシバ（モクレン科）の胚発生学的研究

Embryological investigations in the genus *Magnolia* are not so many, including Guignard (1897, 1898) in *M. yulan* and *M. soulangeana*, Andrews (1902) in *M. obovata*, Maneval (1914) in *M. virginiana*, Farr (1918) in *M. tripetala*, Yamaha (1926) in *M. kobus*, Earle (1938) in *M. grandiflora*, Stoudt (1960) in *M. kobus*, Hayashi (1960, 1964) in *M. liliflora*, Kapil & Bhandari (1964) in *M. obovata*, Ly-Ti-Ba et al. (1970) in *M. grandiflora*, and Yamazaki (1982) in *M. kobus*. But up till now nothing has been known embryologically in *M. salicifolia*.

Recently some materials of *M. salicifolia* were obtained, thus giving an opportunity to study the remaining member of the genus and to compare it embryoiogically with the members already investigated.

Material and methods The material of *M. salicifolia* was collected in a field near the Takayu Spa in Fukushima Prefecture. Floral buds at different stages of development were fixed in FAA solution. Dehydration, infiltration, embedding and sectioning were carried out by the procedure outlined by Johansen (1940). Sections were exclusively stained with Heidenhain's iron-alum haematoxylin and safranin.

Observations

Microsporogenesis. Longitudinal sections of the young anther, which consists of a homogeneous mass of parenchymatous cells, collected on July 11th of the year prior to flowering, are rectangular in shape (Fig. 1A). By the end of the month it becomes two lobes, each with two microsporangia. The hypodermal layer of each lobe contains archesporial cells which divide periclinally to form the primary sporogenous tissue on the inside and the primary parietal cell layer on the outside (Figs. 1B, C, 3A).

The anther wall consists of an epidermis, endothecium of one celled layer, middle layer of 3 cells in thickness, and tapetum of 1-2 cells thick (Figs. 1D,

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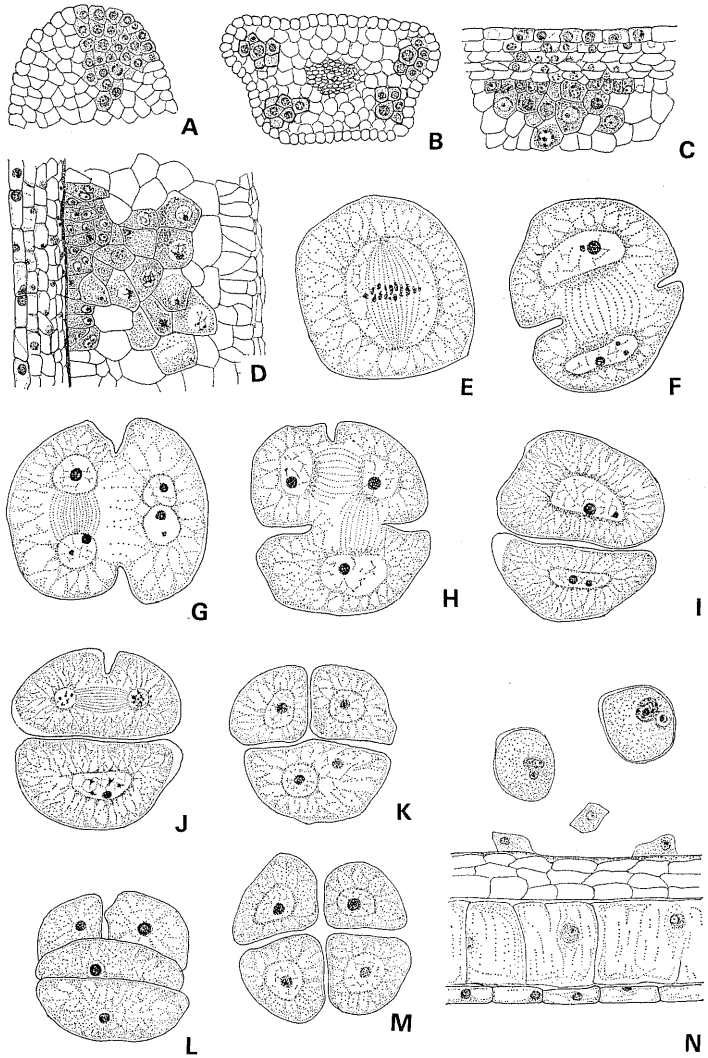

Fig. 1. Pollen formation of *Magnolia salicifolia*. A. A part of a very young anther showing a mass of meristematic cells. ×175. B. Transverse section of a young anther showing primary sporogenous cells and primary parietal cells. ×200. C. Massive sporogenous tissue. ×325. D. PMCs in synapsis stage. Anther wall consists of epidermis, endothecium, 3 or 4 middle layers and 1 or 2 tapetal layers. ×275. E. Metaphase in the first division of a PMC. ×1375. F. Heterotypic furrowing in the first division of a PMC. ×1375. G, H. The second division of PMCs showing homoeotypic spindle formation. ×1375. I. PMC in dyad stage. ×1375. J. Decussate type of division of a PMC showing heterotypic furrowing. ×1375. K. Decussate tetrad. ×1375. L. T-shaped tetrad. ×1375. M. Isobilateral tetrad. ×1375. N. Well-developed endothecium and binucleate pollen grains. ×325.

3B). The endothecial cells elongate tangentially, and are furnished with characteristic fibrillar thicknings. The middle layer tends to be compressed between the fibrous layer and the tapetum, and eventually collapses.

The cells of tapetum are at first uninucleate, but later become 2-3-nucleate by mitotic divisions. In the meantime the nucleus divides once or twice in a manner of cytokinesis forming a layer of 2-3 cells at the end of March (Figs. 1D, 3C). Some of the nuclei appear to be amoeboid and contain more than one nucleolus suggesting their polyploid nature. During the later stages of development the tapetal cells are conspicuously enlarged and remain in their original position for a while (Figs. 1D, 3C). On further development the tapetum begins to show signs of degeneration and eventually becomes disorganized at the 2-celled stage of the pollen grains (Figs. 1N, 3D).

On degeneration of the tapetal cells, minute globular oily bodies are seen on the inner side of the walls and are often well-defined during maturation of the pollen grains. These bodies which appear brownish yellow after treatment with haematoxylin and red with safranin, become deposited against the inner wall of the endothecial cells by the breakdown of the tapetal cells. Before the pollen grains are fully mature, the anther wall may consists of two sublayers, endothecium and compressed epidermis (Figs. 1N, 3D). At maturity two microsporangia in each theca become confluent by the breaking down of the intervening cell layers. Dehiscence of the anther is longitudinal.

The primary sporogenous cells divide to form the PMCs (Figs. 1D, 3C). The meiotic divisions of the PMCs are of the modified simultaneous type (Figs. 1E-M) as designated by Hayashi (1960). During meiosis II the spindles may lie parallel or at right angles to each other (Figs. 1G, H) and cytokinesis takes place by centripetal furrows (Figs. 1K-M). Thus the tetrads are mostly decussate (68.9%) but some may be isobilateral (28.6%) or even T-shaped (2.5%). The young microspores are liberated due to the breaking down of the original mother wall in the middle of April (Fig. 4D). The newly formed microspore has dense cytoplasm with a nucleus situated near the center (Fig. 3D). As the microspore increases in size, a large vacuole appears in the cytoplasm and the nucleus moves to one of the sides. The microspore after division gives rise to a large vegetative and a small generative cell (Fig. 1N). The mature pollen grains are oval or oblong, monocolpate, and shed at the 2-celled stage.

Ovule. The ovary, as characteristic of Magnoliaceae (s. str.), is mono-

carpellary apocarpous, unilocular and hypogynous with rarely one or most frequently two anatropous, bitegmic and crassinucellate ovule. The inner integument initiates as a rim-like outgrowth from the surface cells of the enlarging nucellus before the differentiation of the outer integument. At the stage when the archesporial cell becomes evident and functions as the megaspore mother cell, the integuments do not grow over the nucellar apex (Fig. 2A). The integuments completely surround the nucellus leaving a narrow micropyle while the megagametophyte becomes matured.

Megasporogenesis and megagametophyte. The megaspore mother cell undergoes meiosis leading to formation of a linear or T-shaped tetrad of megaspores (Figs. 2B, C). Generally the dyad produced after the first meiotic division consists of a larger chalazal cell and a smaller micropylar cell. The second division in the two dyad cells gives rise to four cells, in which the chalazal cell produces the functioning megaspore and the upper three degenerate (Fig. 2D). After three mitotic divisions the functioning megapore develops into an 8-nucleate gametophyte. The development of the embryosac thus conforms to the Polygonum type (Figs. 2E-G). An egg apparatus with an egg and two synergids at the micropylar end and three antipodals at the chalazal end have been observed. The polar nuclei usually meet in the middle of the embryo-sac and fuse before fertilization to form a secondary nucleus (Fig. 2H).

Fertilization and development of endosperm. The fertilization is porogamous. Germinating pollen grains on the stigma elongate pollen tubes which enter the micropyle to reach the embryo-sac. Syngamy and triple fusion take place more or less simultaneously. One male gamete fuses with the egg and the other with a secondary polar nucleus to form a primary endosperm nucleus. The pollen tube degenerates quickly. The antipodal cells generally degenerate, but rarely persist for a while after fertilization.

The primary endosperm nucleus divides earlier than division of the zygote, and a larger chalazal chamber and a smaller micropylar chamber are formed (Fig. 2I). These chambers by further division give rise to the cellular endosperm (Fig. 2J).

Embryogeny. The first division of the zygote is transverse resulting in a terminal cell and a basal cell (Figs. 2K, 4A). However, there is much variation in the subsequent behaviour of these two daughter cells. The divisions in the terminal cell may precede those of the basal cell or vice versa. Usually the

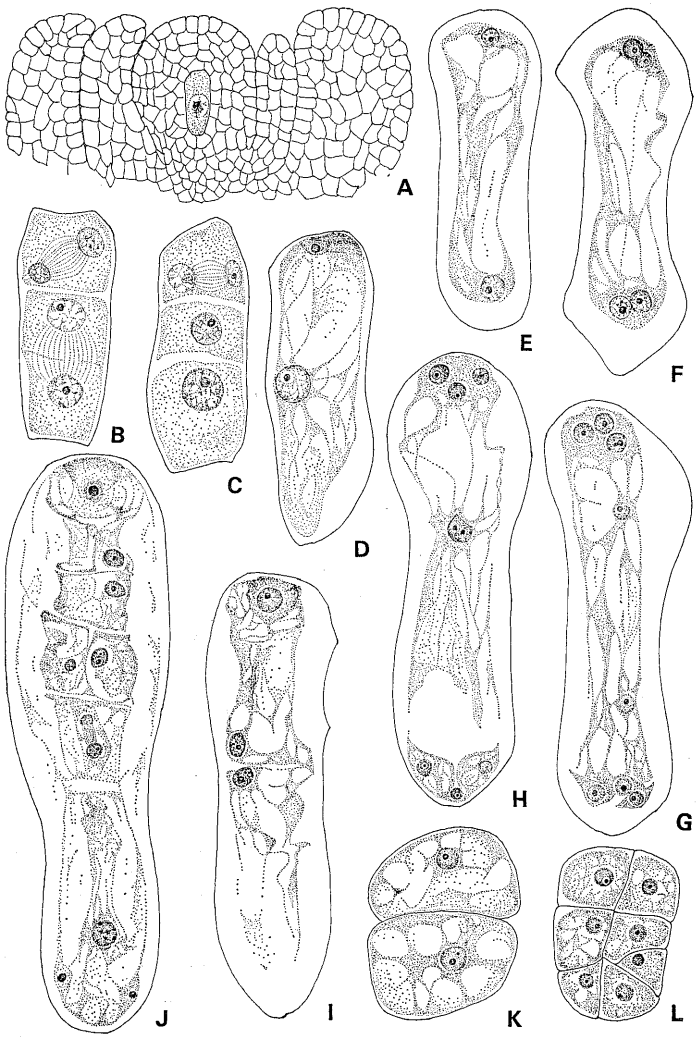

Fig. 2. Development of embryo sac and embryo in *Magnolia salicifolia*. A. Young ovule with a megaspore mother cell and two developing integuments. ×310. B. Dividing dyad cells. ×1000. C. Formation of T-shaped tetrad of megaspores. ×1000. D. Two of three micropylar nuclei already degenerated. ×950. E. Two-nucleate stage. 950. F. Four-nucleate stage. ×890. G. Eight-nucleate stage. ×680. H. Mature embryo sac showing an egg apparatus, a secondary polar nucleus and three antipodal cells. ×530. I. Two-celled stage of the cellular endosperm showing the undivided zygote. ×630. J. Slightly later stage in the development of endosperm showing multiplication on cells of endosperm, the zygote is still unicellular. ×630. K. Division of zygote into terminal and basal cells. ×1500. L. Octant stage. ×750.

basal cell then divides again transversely to form two superposed cells, while the terminal cell divides longitudinally resulting in two juxtaposed cells (Fig. 4B). Each of the two terminal cells divides vertically resulting in a quadrant stage, while the two basal cells remain undivided. The quadrant cells divide further transversely to form an octant (Fig. 2L). From this stage onwards the divisions are irregular and the resultant cells divide repeatedly to form a small globular proembryo (Fig. 4C). The proembryo gradually grows to form a heart-shaped young embryo (Fig. 4D). The mature embryo is straight and consists of two well-developed cotyledons, a short radicle and hypocotyl (Fig. 4E). The earlier division in the develepment of the proembryo are often irregular but the embryogeny conforms essentially to the Onagrad type (Johansen 1950).

Discussion There is a great deal of similarity in microsporogenesis and megasporogenesis between the present material and the members of the family (s. str.) already investigated by the several workers.

The arrangement of the microspore tetrad appears to be of some interest. The microspores are mostly arranged in a decussate fashion and some are isobilateral or T-shaped in the present material, while a tetrahedral arrangement has been recorded in *M. soulangeana* (Canright 1953) and *M. obovata* (Kapil & Bhandari 1964). The taxonomical significance of the variation in arrangement of the microspores is still obscure, although it is considered that the species referred to the same genus show more or less similar type in embryology.

It is obvious that in the present material the ovular archesporium consists of a single cell and the development of the embryo-sac conforms to the Polygonum type as is the case in *M. virginiana* (Maneval 1914), *M. grandiflora* (Earle 1938) and *M. liliflora* (Hayashi 1964). The feature may also be a common occurrence in many members of the Magnoliaceae (s. l.) : *Drimys winteri* (Strasburger 1905), *Liriodendron tulipifera* (Maneval 1914, Kaeiser & Boyce 1962), *Illicium anisatum, Schisandra repanda, Kadsura japonica* and *Michelia fuscata* (Hayashi 1963a, b, 1964). Therefore, it may de concluded that this type of the embryo sac development is a constant feature of the members of this family (s. l.).

The development of embryo in the present material falls to the Onagrad type described by Johansen (1950) as is the case in *M. virginiana* (Maneval 1914), *M. grandiflora* (Earle 1938) and *M. liliflora* (Hayashi 1964). This type seems to be a generic character, although the same feature has also been ob-

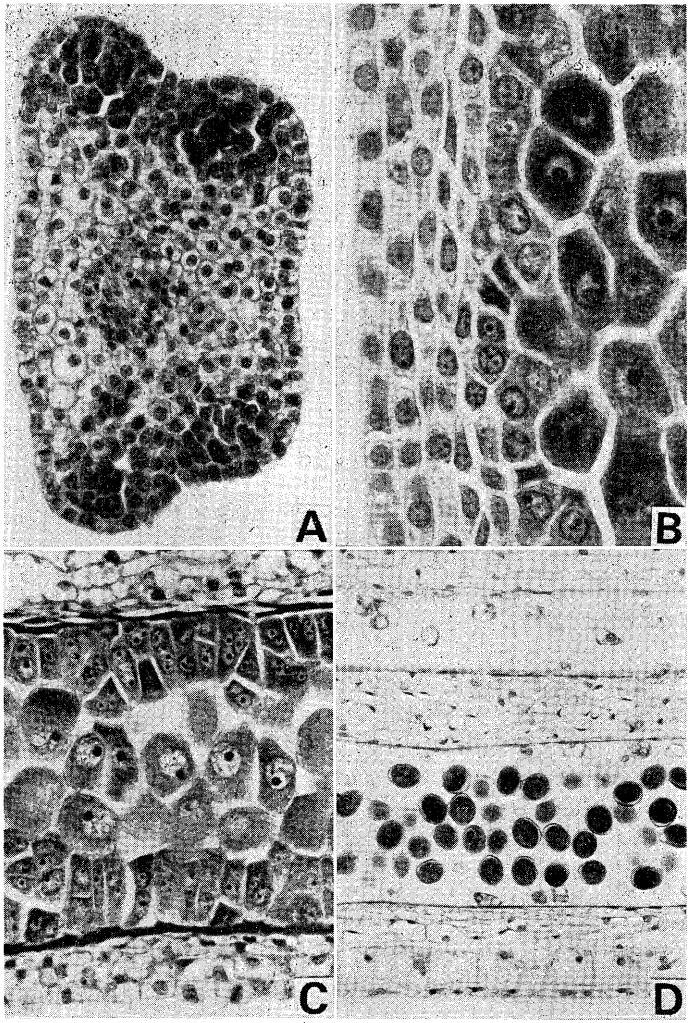

Fig. 3. Pollen formation of *Magnolia salicifolia*. A. Transection of a two-lobed four-loculed anther. ×500. B. Longisection of an anther showing the developing wall layers and the sporogenous cells surrounded by the tapetum. ×500. C. Binucleate tapetal cells and PMCs at synapsis. ×250. D. Longisection of an anther showing uninucleate young pollen grains and a well-developed endothecium. Note the glandular type of the tapetum. ×125.

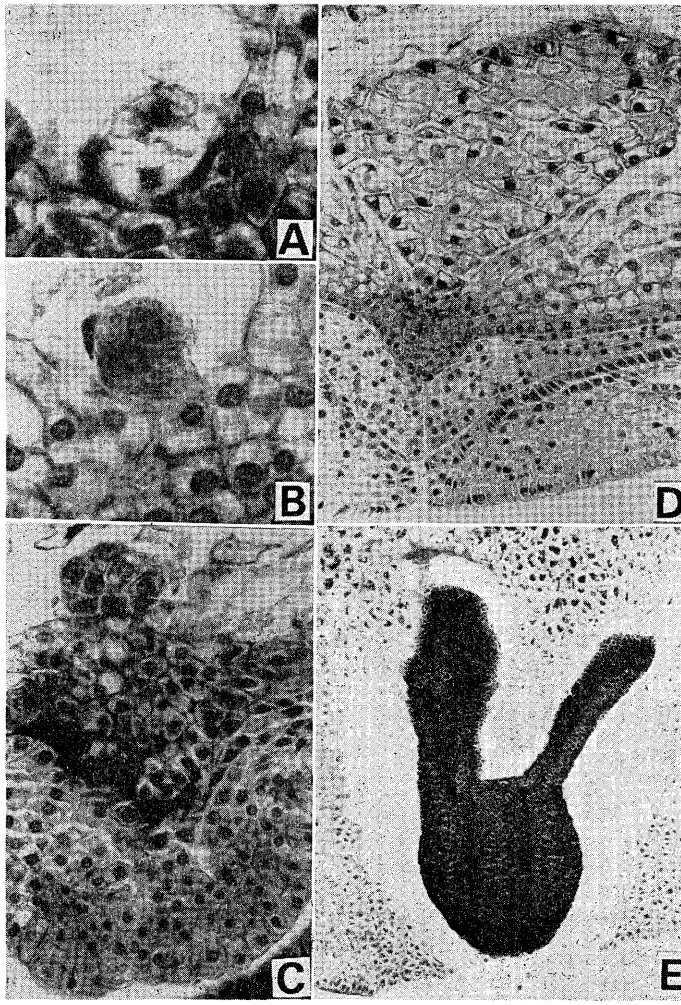

Fig. 4. Development of embryo in *Magnolia salicifolia*. A. Two-celled proembryo comprising terminal and basal cells. ×500. B. Four-celled proembryo. ×500. C. Micropylar half of a mature ovule showing an ovoid undifferentiated embryo. ×250. D. Heart-shaped embryo. ×125. E. Median longitudinal section of a ripe seed showing a dicotyledonous embryo surrounded by a mass of endosperm partially digested. ×50.

served in *Michelia champaca* (Padmanabham 1960) and *M. fuscata* (Hayashi 1964). In contrast to this, in *Illicium anisatum*, *Schisandra repanda* and *Kadsura japonica* (Hayashi 1963a, b) the Asterad type of the embryo development has been recorded. Furthermore, Ly-Ti-Ba et al. (1970) described irregularity in the embryo development in *Magnolia grandifolia* and they interpreted it as conforming to the Mégarchétype VI (Souèges 1939).

With regard to the embryo development in the Magnoliaceae, Johansen (1950) recorded the Onagrad type and Bhandari (1971) states that "the embryogeny is either irregular or follows the Onagrad type". Yamazaki (1982), based on the observation of the embryo development in *Magnolia kobus*, has sustained the Geraniad type.

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狭義のモクレン科，モクレン属に属するタムシバの小胞子形成，大胞子形成および胚発生について報告する。小胞子形成はくびれ込みにより十字対生型および双同側型の四分子を形成し，タペート細胞は腺型である。胚珠は2枚の珠皮をもち，倒生で，厚層珠心である。大胞子形成はタデ型で，胚乳形成は造膜型である。胚発生の初期は不規則であるが，本質的にはアカバナ型と考えられる。

□Uppeandra Dhar & P. Kachroo: **Alpine Flora of Kashmir Himalaya** 280 pp. 1983. Scientific Publishers, Jodhpur, India. 200ルピー (9,000円). カシミールヒマラヤの高山植物誌である。フロラ要素の分析に主体がおかれ，要素ごとの相対比や科単位での世界の分布との比較が行われ，約150種の分布図がしめされている。後ろ3分の1は種のリストで，属の検索表と著者らのコレクションによる標本があげられている。

(金井弘夫)