Megasporogenesis and development of female gametophyte in *Manglietia decidua* (Magnoliaceae)

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Manglietia decidua (Magnoliaceae) is the only deciduous species known in the genus. The ovules are anatropous, bitegmic and crassinucellar. The archesporium is one-celled and of hypodermal origin. The chalazal megaspore is functional and develops into the *Allium* type of female gametophyte. There is apparently a delay in development of the embryo while the endosperm is developing. As a cell wall partitioned the two nuclei into the first two endosperm cells, endosperm development was *ab initio* cellular. In the seed the outer integument became differentiated into an outer fleshy layer and an inner stony one, the inner integument persisting only at the micropylar region and forming a plugging tissue.

Key words: *Allium* type, cellular endosperm, female gametophyte, *Manglietia decidua*, megasporogenesis

Introduction

The family Magnoliaceae is a well-defined and horticulturally important angiosperm family consisting of fifteen genera and about 240 species (Law 1984) of trees and shrubs, characterized by large flowers. More than 80% of the species are distributed in subtropical and tropical regions of eastern Asia; the remainder occur in the Americas, indicating a relictual tropical disjunction (Azuma *et al.* 2001). The large flower typical of this family was once considered to represent the primitive floral type, based mainly on the existence of many fossil forms. However, recent improved understanding of phylogenetic relationships, together with new fossil discoveries, have demonstrated that small flowers with relatively few organs predominated in basal angiosperms, and the large flowers of Magnoliaceae are now regarded as relatively specialized within this grade (Crane *et al.* 1994, Endress 1994).

Manglietia is an Asian genus of about 25 species, very closely related to the genus *Magnolia*, from which it can be distinguished by evergreen leaves, greater number of ovules in each carpel, undifferentiated stamens, paracytic stomata, testa structure and pollen morphology (Dandy 1927, Vàn Tiêp 1980, Baranova 1998). Recent molecular research indicates that *Manglietia* is strongly supported as a monophyletic clade (Kim 2001, Li & Conran 2003).

Manglietia decidua, the revised name of *Sinomanglietia glauca* (Yu 1994) was found in Yichun city, Jiangxi province, China in the year of 1988 for the first time. Flower and fruit characters of this species are very similar to *Manglietia*, but the deciduous habit was unique (Zheng 1995). *Manglietia decidua* is the only deciduous species known so far in the genus and is listed as the national second-grade protected plant species in China due to its rarity and poor ability to regenerate naturally. These large endangered trees are scattered across a narrow range from 27°34´–27°36´N and 114°19´–114°22´E, and from sea level to 580–1100 m, where the annual average temperature is 17.2 °C, and the average rainfall is about 1606 mm. The total number of individuals in the wild is about 2000 (Yu *et al.* 1999, Zheng & Zheng 2000). Flowering occurs from April to May, and fruiting from September to October.

Embryological literature on Magnoliaceae is available for *Magnolia* (ten species), *Manglietia* (three species), *Michelia* (two species), *Pachylarnax* (one species), *Liriodendron* (two species) and *Tsoongiodendron* (one species) (Maneval 1914, Earle 1938a, Padmanabhan 1960, Kaeiser & Boyce 1962, Hayashi 1964, 1984, Kapil & Bhandari 1964, Ly-Ti-Ba *et al.* 1970, Melikian & Plisko 1988, Liao *et al.* 2000, Pan & Gong 2002, Pan *et al.* 2003, Tang *et al.* 2003). Unfortunately, few of the studies above provided comprehensive microphotographic evidence, and most of them were illustrated by camera lucida drawings. This report aims at increasing our basic understanding of megasporogenesis and female gametopyte development in the Magnoliaceae as well as providing important information for the conservation of an endangered species. Knowledge of reproductive biology is necessary for effective protection of endangered plants, especially for species with small populations. Studies on embryology are also important in the conservation and systematic analysis of this family.

Material and methods

Floral buds at successive developmental stages were collected from two individuals (one of them is the holotype individual) in Hongjiang country, Jiangxi province, China, from November 1999 to September 2000. Small samples were also collected in February–May 2001. Usually the samples were collected about every 3–5 days, but were collected every 1–2 days in March and April. The samples were fixed in FAA (formalin: glacial acetic acid: 50% ethyl alcohol = 5:5:90 v/v) then dehydrated in an ethyl alcohol series, embedded in paraffin, stained in Orange G and haematoxylin and sectioned (over 150 specimens) at 10–12 μm thickness. All sections were observed under an OLYMPUS BHS-2 microscope and photographed with OLYMPUS PM-10AD photomicrographic system.

Results

Megasporogenesis and megagametogenesis

The flowers of *Manglietia decidua* are large, solitary, terminal and bisexual. The perianth consists of 16–18 tepals, usually arranged spirally. The gynoecium is comprised of numerous apocarpous carpels arranged in a spiral manner on an elongate axis. Each carpel contains 6–8 ovules, which are anatropous, bitegmic and crassinucellar on the marginal placenta.

The ovule primordium begins to develop in November as a dome-shaped protuberance of cells at the base of the ventral surface of the carpel (Fig. 1A). The archesporial cell is of hypodermal origin and easily recognized by its large size as compared with that of the adjacent cells (Fig. 1B). It divides to form several parietal cells and an inner sporogenous cell, which enlarges and functions as the megaspore mother cell (Fig. 1C). The megaspore mother cell becomes deeply embedded in the tissue of the nucellus, hence the type of ovule is crassinucellate (Fig. 1D).

The first meiotic division of the megaspore mother cell results in two unequal dyad cells, of which the micropylar cell is smaller, while the bigger chalazal cell functions as megaspore (Fig. 1E). The uninucleate female gametophyte (Fig. 1F, G), coming directly from the functional megaspore, increases its volume and undergoes
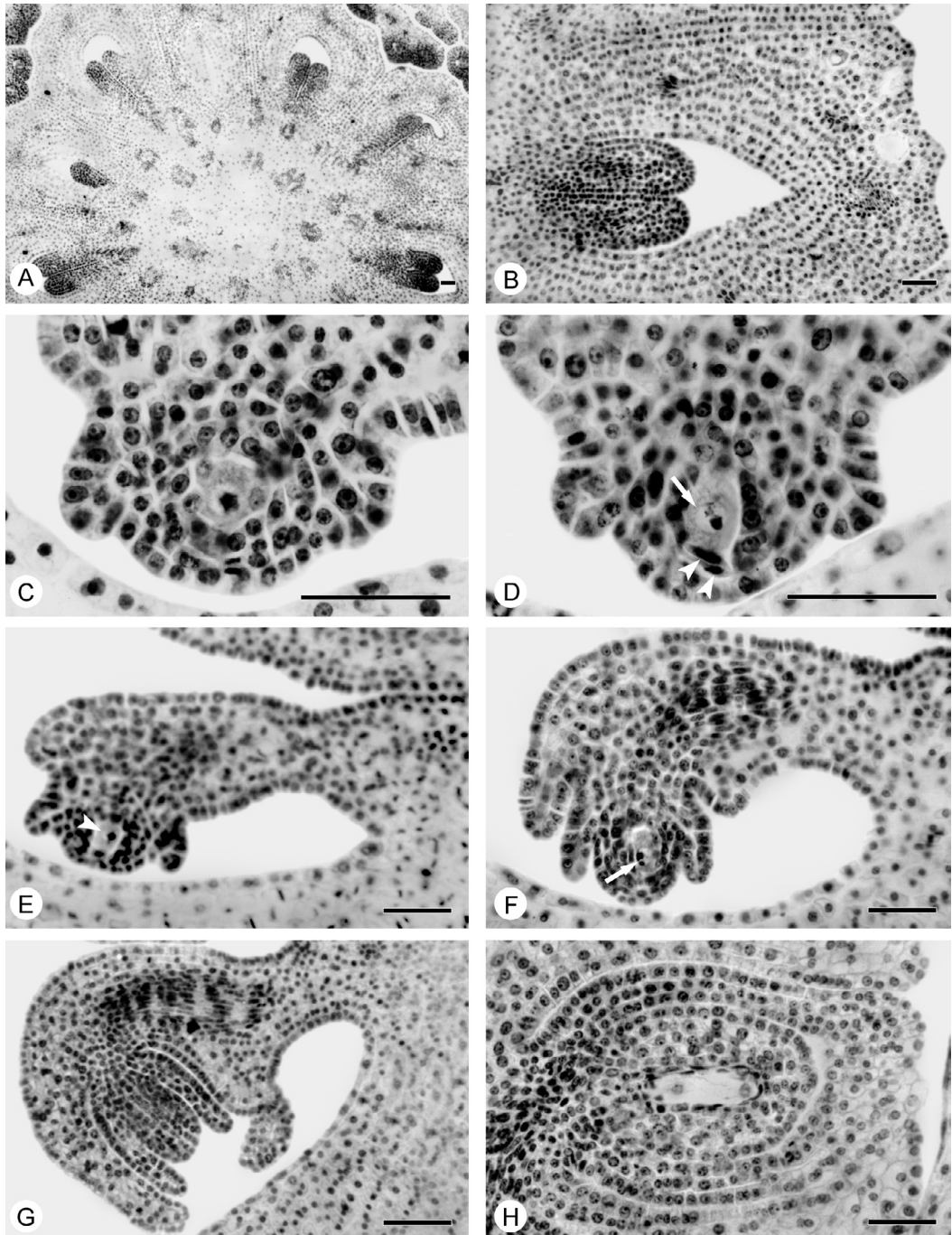
Fig. 1. Development of *Manglietia decidua* ovule. — **A**: Dome-like protuberances of ovule primordia on the ventral surface of carpellary wall (7 November). — **B**: Ovules, the archesporial cell is distinct. — **C** and **D**: Megasporogenesis, showing megaspore mother cell (arrow) and parietal cells (arrowheads) (7 March). — **E**: Megaspore mother cell dividing into two megaspores, the chalazal one (arrowhead) functioning, inner integument at an early stage of development. — **F**: Uninucleate female gametophyte (arrow) (22 March), two integuments developed. — **G**: Anatropous ovules, bitegmic, crassinucellar. — **H**: Two-nucleate stage of a female gametophyte (25 March). **A** and **B** are cross-sections of gynoecium, the others are cross-sections of carpels. Micropylar polarity is either on the right side or bottom. Scale bars = 50 µm.
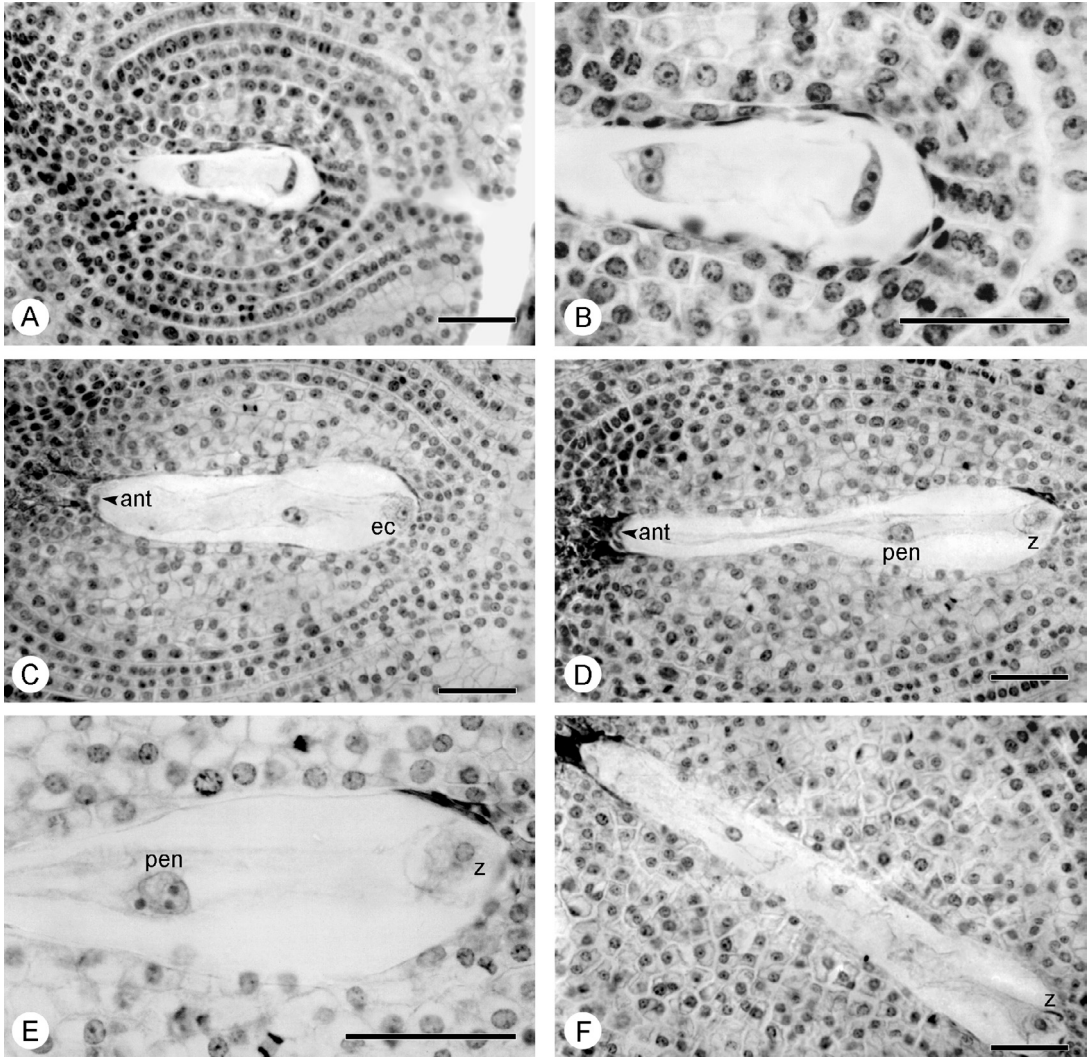
Fig. 2. Development and fertilization of *Manglietia decidua* female gametophyte. — **A**: Four-nucleate stage of development (28 March). — **B**: Detail of A. — **C**: Fusion of the two polar nuclei at the micropylar part of the female gametophyte, the egg cell and antipodal cells are distinct (9 May). — **D**: Fertilization of the secondary nucleus and zygote, the antipodal cells in process of degeneration (arrowhead) (20 May). — **E**: Detail of **D**. — **F**: Two-celled endosperm (28 May), zygote remains undivided and the hypostase has already developed (arrowhead). All are cross-sections of carpels. Micropylar polarity is on the right side. Scale bars = 50 μm. ant = antipodal cells, ec = egg cell, pen = primary endosperm nucleus, z = zygote.

the first nuclear division to give rise to two daughter nuclei, which move apart to the two poles of the female gametophyte (Fig. 1H). After three mitotic divisions the functional megaspore develops into an eight-nucleate female gametophyte with a two- and four-nucleate and an intermediate stage (Figs. 1H, 2A, B). The development of the female gametophyte thus conforms

to the monosporic 8-nucleate *Allium* type. Three of the nuclei at the micropylar pole constitute the egg apparatus consisting of an egg cell and two synergids, which degenerate very soon. Three cells at the chalazal end of the female gametophyte become the antipodals (Fig. 2C).

By maturity the ovule has elongated as much as two times its length at the four-nucleate

stage. The polar nuclei meet in the middle of the female gametophyte and fuse to form a secondary nucleus before fertilization (Fig. 2C). At fertilization, one male gamete fuses with the egg and the other with the secondary nucleus to form a zygote and primary endosperm nucleus, respectively (Fig. 2D, E). Fertilization of the egg cell was not observed, but was presumed to occur before that of the central cell, based on the shape, large size and the thick wall on the central-cell side (Fig. 2D, E). The antipodal cells are small, short-lived and generally degenerate soon after fertilization (Fig. 2D, F).

Embryogeny and endosperm development

The first division of the primary endosperm nucleus results in a two-celled endosperm with a chalazal chamber and a micropylar chamber (Fig. 2F). Repeated divisions accompanied by wall formation in the two chambers give rise to massive, oily, slightly ruminate cells rich in starch grains that at maturity fill almost the entire cavity of the seed (Fig. 3A, B, H). Therefore, the endosperm is cellular.

Division of the zygote is delayed until a well-developed endosperm tissue is formed. The first division of the zygote is transverse, resulting in the formation of an apical cell and a basal cell (Fig. 3B). The three-cell proembryo results from a longitudinal division of the apical cell (Fig. 3C). Repeated divisions of all cells result in an octant proembryo that becomes a globular embryo (Fig. 3D, E). The embryo then becomes spindle-shaped and later heart-shaped (Fig. 3F, G). The mature embryo is formed around the end of September. It consists of two well-developed cotyledons, surrounded by a mass of partially digested endosperm (Fig. 3H).

The nucellus has 2–3 layers when the female gametophyte is uninucleate (Fig. 1F). Its cell number peaks during megagametogenesis (Figs. 1H, 2A, C). It disorganizes gradually with the development of the embryo and disappears completely at the globular stage of development except for a crushed fragment that stained darkly near the left inner integument (Fig. 3E, F).

Initiation and development of the integument

The inner integument initiates as a rim-like outgrowth from the surface cells of the enlarging nucellus before the differentiation of the outer integument (Fig. 1C). It is entirely of dermal origin and forms when the archesporial cells have reached the megaspore mother cell stage (Fig. 1D). The outer integument is initiated a little later than the inner one, on the convex and lateral portions of the primordium by periclinal cell divisions in the subdermal layer (Fig. 1D). At a later stage, the elongating outer integument overgrows the inner integument. The unequal growth rates gradually bend the ovule over into its final anatropous position, forming a zigzag micropyle with the endostome and exostome (Fig. 1G). The completely anatropous condition is attained before the megagametophyte has become two-nucleate.

At the mature female gametophyte stage, the outer integument consists of 8–10 layers of parenchymatous cells, while the inner integument is comprised of 3 or 4 layers of tangentially compressed cells. After fertilization, considerable structural changes occur in the outer integument. At the globular stage of the proembryo the outer integument starts differentiating into an outer fleshy and an inner stony layer. The cells of the inner epidermis of the outer integument become greatly stretched and divide transversely giving rise to a multi-layered tissue that becomes lignified to form the hard stony zone (Fig. 3A, C). The remaining layers of the outer integument are parenchymatous and develop into the outer fleshy layer (Fig. 3F). The inner integument does not contribute substantially to the formation of the testa. It becomes crushed beginning at the globular proembryo stage of development (Fig. 3E, F), persisting only at the micropylar region, where it forms a tissue that plugs the micropyle and is still present in the mature seed (Fig. 3H).

Discussion

In the Magnoliaceae, all previously recorded species conform with the *Polygonum* type of
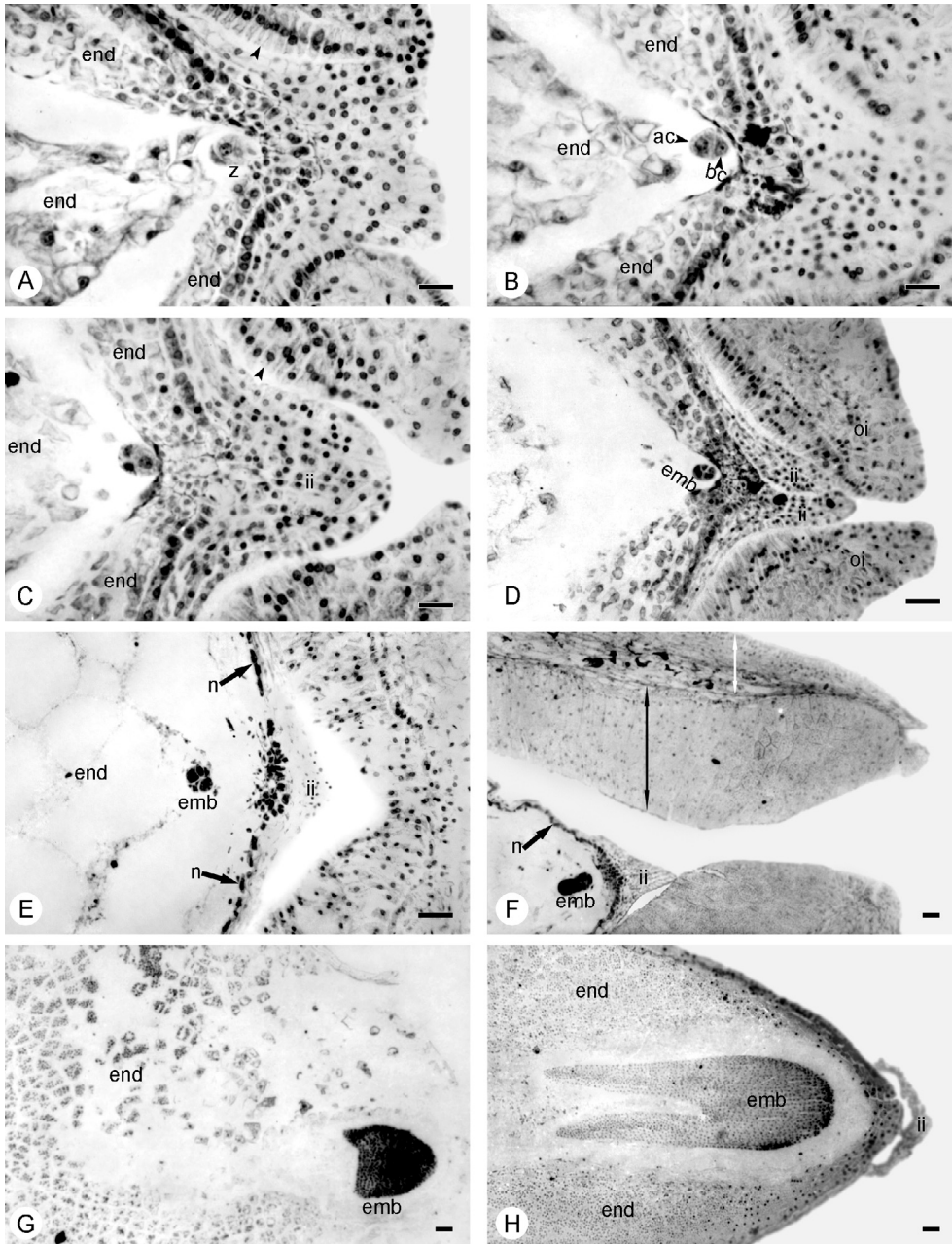
Fig. 3. Development of *Manglietia decidua* embryo. — **A**: Well-developed endosperm and the stretched cells of the inner dermal layer of the outer integument (arrowhead). — **B**: Two-celled proembryo, comprising apical and basal cells. — **C**: Three-celled proembryo, the stretched cells of inner epidermis of outer integument becoming additional layers (arrowhead) (10 June). — **D**: Octant stage of proembryo. — **E**: Globular proembryo, the arrows showing the remains of the nucellus, the inner integument becomes crushed. — **F**: Spindle-shaped embryo, the cap-shaped integument persisting (ii); the long black arrow shows the inner stony layer of the outer integument, the long white arrow shows the outer fleshy layer (21 July). — **G**: Heart-shaped embryo. — **H**: Dicotyledonous embryo surrounded by a mass of endosperm partially digested with the inner integument forming a plugging tissue (30 September). All are cross-sections of carpels. Micropylar polarity is on the right side. Scale bars = 50 μm. end = endosperm, z = zygote, ac = apical cell, bc = basal cell, ii = inner integument, emb = embryo, oi = outer integument, n = nucellus.

female gametophyte development, but the present study indicates the *Allium* type. The embryological characters in Magnoliaceae provide clearer factual basis for phylogenetic inferences of angiosperms.

While multicellular archesporia differentiate in the hypodermal layer at the apex of the nucellus in *Magnolia liliiflora* (Hayashi 1964), *M. obovata* (Kapil & Bhandari 1964), *M. stellata* and *Michelia fuscata* (Hayashi 1964), the present species indicates a one-celled ovular archesporium also found in *Magnolia salicifolia* (Hayashi 1984), *Manglietia glauca* and *Michelia guangxiensis* (Liao *et al*. 2000), as well as in *Manglietia insignis* (Pan & Gogn 2002) and *M. aromatica* (Pan *et al*. 2003).

In most of the examined species the division of the primary endosperm nucleus and subsequent divisions are followed by a transverse wall formation, dividing the female gametophyte into two chambers. Evidently, the endosperm formation of those species is of cellular type that exists in species of *Magnolia*, *Michelia* and *Liriodendron* (Maneval 1914, Padmanabhan 1960, Hayashi 1964, 1984, Kapil & Bhandari 1964). The present study identified cellular endosperm in *Manglietia decidua*. However, studies in *Magnolia grandiflora* (Earle 1938a), *Manglietia glauca* and *Michelia guangxiensis* (Liao *et al*. 2000) showed that 'early divisions of the endosperm nucleus are very rapid and the number of free nuclei formed is comparatively small' and 'wall formation occurs shortly after the appearance of these free nuclei' (Earle 1938a). These studies support the occurrence of both cellular and nuclear type endosperm development in this family.

The observations of Kapil and Bhandari (1964) of the seed coat fully confirm the reports of Gray (1857), Earle (1938b) and Maneval (1914) that the outer integument becomes differentiated into an outer fleshy layer and an inner stony one, while the inner integument is reduced to a membranous layer in *Magnolia obovata*, *M. stellata*, *M. umbrella* and *M. grandiflora* (Boer & Bouamn 1972, Mohana 1975, Umeda *et al*. 1994). The authors agree as regards the differentiation of the outer integument, but in this study the inner integument is represented by a mass of crushed cells and remaining nucellar cells that form a cap at the micropyle (Fig. 3F, H).

Liao *et al*. (2000) found that in *Manglietia glauca* abnormal development is very common in megasporogenesis, megagametogenesis, embryogeny and the development of endosperm. Abnormal development leads to abortion. Pan and Gong (2002) also stated that about 79% of egg cells and 87% of egg apparati (including eggs cells and synergids) of *Manglietia insignis* were degenerate resulting in a low rate of seed formation. Both of these studies were based on cultivated plant tissue. In this study, based on material collected from two individuals in the wild (one of them is the type tree), no abnormal phenomena at any stage of embryo development were observed. However, there are not very many young seedlings around the mature ones in the natural populations. We assume that the main reason for the species being endangered is possibly related to low seed germination rates and to ecological limitations, including temperature, humidity and shade.

Acknowledgements

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