

Ultrastructural Study on the Recalcitrant Seeds of *Machilus thunbergii* Sieb. & Zucc.

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(Manuscript received 21 April, 2001; accepted 28 May, 2001)

ABSTRACT: The recalcitrant (desiccation sensitive) seeds of *Machilus thunbergii* were histochemically and ultrastructurally investigated by using light and electron microscopy. The embryo of mature seed has 4-6 subopposite primary leaves in the plumule. We found two types of idioblastic secretory cells (oil cells and mucilage cells) in the cotyledons, but observed only the oil cells in the embryonic axes. Cells in shoot and radicle meristem of the fresh mature embryo have a large nucleus and small vacuoles. The cytoplasm is dense with organelles, and the plastids contain some starch grains and few oil droplets. Under 75% relative humidity storage condition, the desiccation processes set off the subcellular damages (vacuolation, withdrawal of plasmalemma) of the mature seeds. Such processes intensified with time and ultimately resulted in the loss of viability.

KEY WORDS: *Machilus thunbergii*, Recalcitrant seed, Oil cell, Mucilage cell, Seed storage, Ultrastructure.

INTRODUCTION

Orthodox (desiccation tolerant) seeds generally undergo maturation drying prior to shedding, and are shed from the parent plant at low moisture contents. Such seeds can be further dried to very low moisture contents (5-7%) without damage and are stored successfully under conditions of reduced temperature and low relative humidity (Farrant *et al.*, 1993). The recalcitrant (desiccation sensitive) seeds do not undergo maturation drying and are shed at relatively high moisture contents. They become increasingly desiccation-sensitive with the storage time (Berjak, *et al.*, 1992; Dodd *et al.*, 1989; Farrant *et al.*, 1988, 1992). There is no successful method for long-term storage of recalcitrant seeds, and the cause and processes of losing the viability is unclear.

None of species of Lauraceae in Taiwan are known as orthodox seeds. *Machilus thunbergii* Sieb. & Zucc. (Lauraceae), is a typical subtropical tree species and widely distributed in Taiwan from low altitude up to 2000 m (Lin and Chien, 1995; Lin and Chen, 1995). At maturity, the drupaceous berry is dark purple and contains only one seed. Mature seeds of *M. thunbergii* are desiccation sensitive and appear to be recalcitrant seed type (Lin and Chen, 1995). Unlike the cultivated orthodox seeds, the ultrastructure of recalcitrant tree seeds has rarely been studied. The physiological properties of recalcitrant seeds are closely related to moisture content. In this study, the effects of moisture content on the mature seeds of *M. thunbergii* were studied histochemically and ultrastructurally.

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MATERIALS AND METHODS

Mature fruits of *Machilus thunbergii* Sieb. & Zucc. were collected in May and June 1996 and 1997 from Yang-Min-Shan National Park, Taipei, at 300m elevation. The pericarp of freshly harvested fruits was immediately peeled to obtain the seeds. The seeds were then stored for one or two weeks in the sealed desiccators in which relative humidity (100 % and 75%) were maintained over saturated salt solutions (Lin and Chen, 1995). All storing treatments were performed at room temperature approximately 25°C. Seeds were also tested for germination. Visible protrusion of a radicle served as the criterion of germination (Lin and Chen, 1995).

The fresh seeds and the stored seeds were dissected into small pieces, fixed in 2.5% glutaraldehyde, postfixed in 1% OsO₄, dehydrated in an acetone series, then infiltrated and embedded in Spurr's resin under vacuum (Spurr, 1969). Thin sections for electron microscopy were cut on a Reichert-Jung Ultracut-E using a diamond knife. The sections were stained with uranyl acetate and lead citrate, and examined by a Hitachi H-600 TEM at 75 kV. For histochemical test, 1 -2 µm sections were made, and stained with Sudan black B for lipid and with Periodic Acid Schiff's (PAS) test for polysaccharides (Weng and Kuo-Huang, 1998).

RESULTS

The diameter of mature seeds of *Machilus thunbergii* ranges from 7 mm to 10 mm. The seed coat is chartaceous and ornate in sculpture. On the light-colored areas of seed coat (Fig. 1, A), 1-2 layers of dead cells with strip-like thickened wall were observed (Fig. 1, B). On the dark area of the seed surface, these dead cell layers were not found (Fig. 1, C). Right under the loose dead cell layers, there is one layer of tightly arranged macrosclereids. Beneath the macrosclereids, there are several layers of compressed and reduced cells (Figs. 1, B and C).

In the mature seed, the embryo has two large cotyledons (Figs. 1, D-F), and there is no endosperm. The length of the embryonic axis is about 2 mm and the plumule contains 4-6 subopposite primary leaves (Fig. 1, E). On the abaxial epidermis of the outer primary leaves, many young trichomes are located along the midrib; however, no stoma was observed.

In the mature embryo of *M. thunbergii*, except the areas of plumule and radicle apical meristems, two types of idioblastic secretory cells, i. e. mucilage cells and oil cells, were found (Figs. 2, A-D). The oil cells are more abundant than mucilage cells. In the cotyledons, these two kinds of secretory cells are randomly distributed in the parenchymatous tissue, however, along the marginal areas of the cotyledons, the mucilage cells are more abundant. It is interesting that in the embryonic axes (plumule, primary leaves, hypocotyle and radicle) only oil cells were found. Many starch grains were also found in the plastids of the parenchyma cells of ground tissues in the cotyledons and the embryonic axes.

In oil cell, the cell wall is suberized with cupule ingrowth at the inner wall, and the cell lumen is filled with oil substance (Figs. 3, C and D). The mucilage substance is accumulated between the plasmalemma and cell wall of the mucilage cells (Fig. 3, B). The cell walls between the mucilage and neighboring cells are transversed by many plasmodesmata. The cytoplasm is deeply stained, some starch grains are observed in the plastids, and tannin cells are found sporadically (Fig. 3, A).

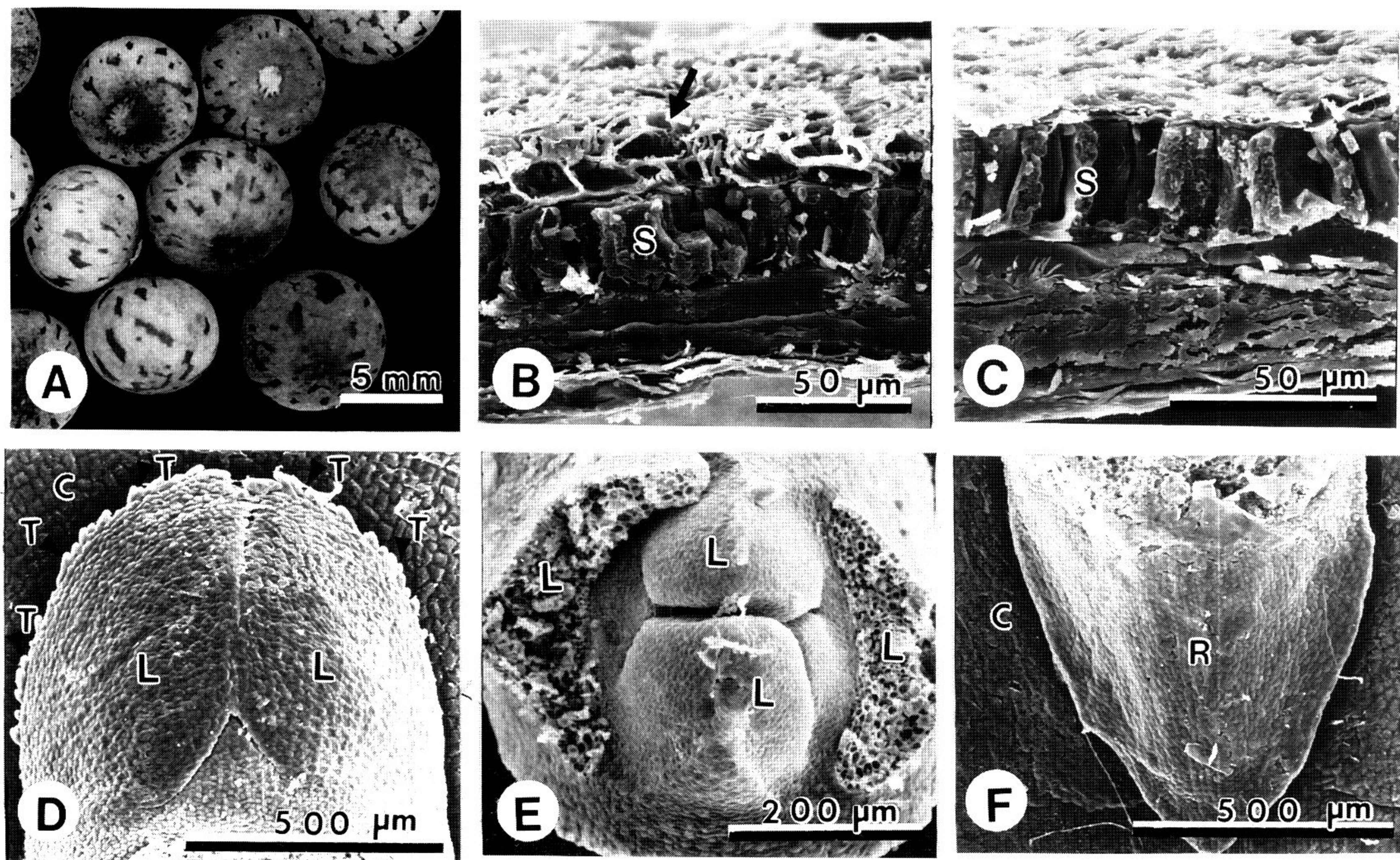


Fig. 1. A: Photograph of mature seeds showing the outer morphology of seed coat. B: SEM photograph of cross section of seed coat from the area corresponding to the light-colored areas shown in figure 1A. The seed coat consists of the outer 1-2 layers of dead cells with strip-like thickened wall (arrow), one layer of tight arranged macrosclereids, and the compressed cell layers of inner testa. C: SEM photograph of cross section of seed coat from the dark area, showing seed coat without the outer dead cell layers. D-F: SEM photographs of the embryo axis. D: Side view of the plumule of embryo showing the primary leaves. On the abaxial midrib of outer primary leaves there are some trichomes (arrows). E: Top view of the plumule of the embryo showing 4-6 primary leaves. F: Side view of the radicle. C: cotyledon; L: primary leaf; R: radicle.

The observed ultrastructure characteristics of cells in the apical meristem of the plumule (Figs. 4, A and B) and the radicle (Figs. 4, C and D) of a fresh mature embryo are typical for metabolically active tissues. Only small vacuoles were found, and the nucleus is large and generally has a distinct nucleolus. The cytoplasm is typically compact with Golgi activity or polysome formation. Mitochondria have well-defined cristae and relatively electron transparent matrices. Plastids contain some starch grains and few oil droplets; however, in the cytoplasm no oil body was found.

The germination percentage of the fresh seeds at the room temperature was about 94%. When the seeds stored in 100% relative humidity for one or two weeks, the germination percentage increased to 98% and then decreased to about 96%. But when the seeds stored in 75% relative humidity for one or two weeks, the germination percentage decreased to 93% or 85%, respectively. In comparison with the cells in the apical meristem of the fresh mature seed, there is no obviously ultrastructural change occurring in the apical meristem cells of the seeds stored in 100% relative humidity (Fig. 5, A). However the desiccation processes of mature seeds stored in 75% relative humidity cause the initiation of subcellular damages (vacuolation and withdrawal of plasmalemma), and the damage process intensified with the increasing storage days. After one week of storage, the mitochondria in the cells of plumule and radicle apical meristem are less organized, and there is a marked increase in vacuolation (Figs. 5, B and C). After two weeks of storage, the cellular ultrastructures are severely damaged (Fig. 5, D).

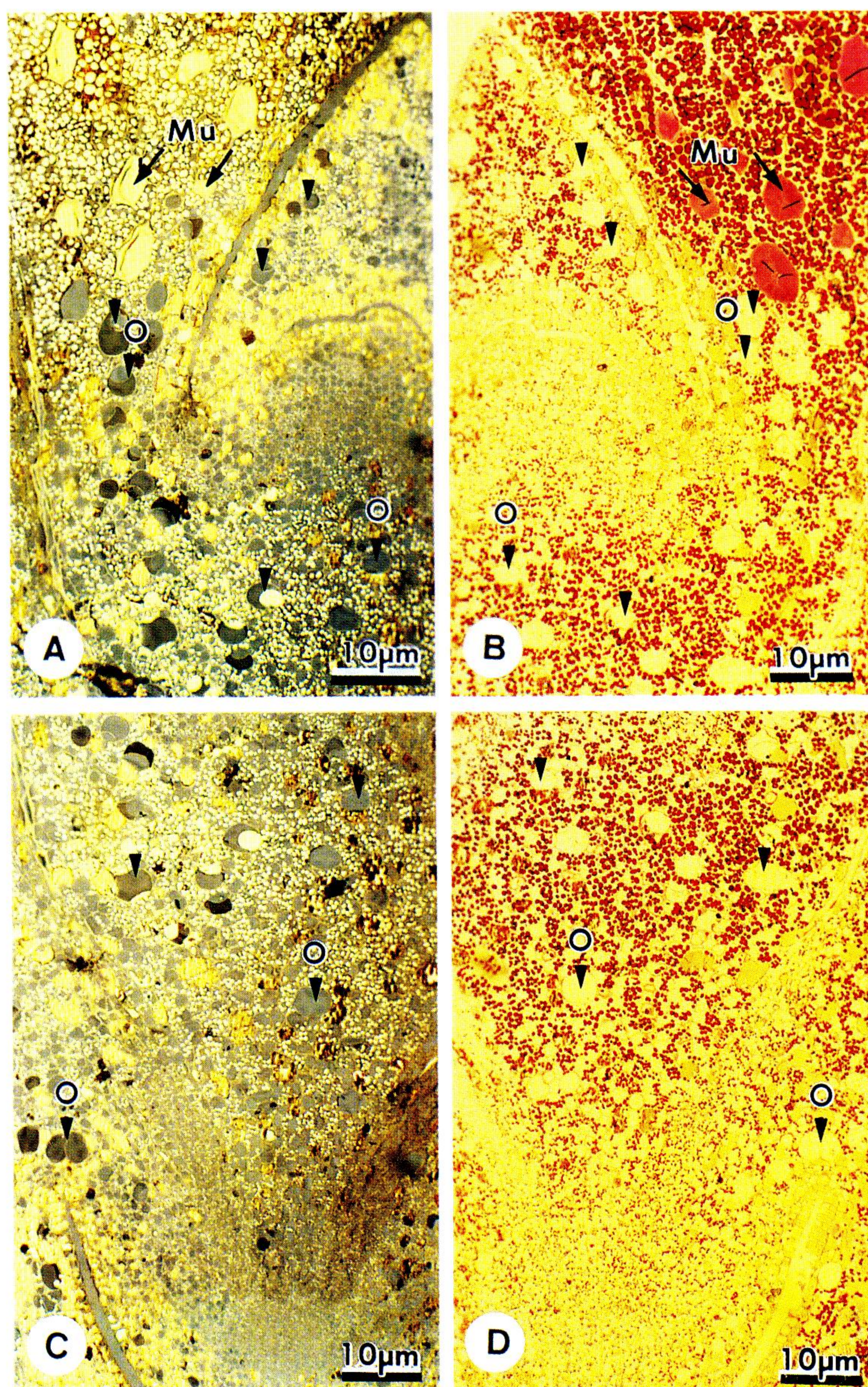


Fig. 2. Histochemical tests for demonstration of lipid and polysaccharide in the sections of embryo. A and C: Sudan black B staining. B and D: PAS staining. Two types of idioblastic secretory cells (oil cells and mucilage cells) randomly distributed in the cotyledons. Mucilage cells (arrows) are mostly located near the margin of the cotyledons, however, in the embryonic axes (plumule, young primary leaves and hypocotyle) only oil cells (arrowheads) were found. Mu: mucilage cell; O: oil cell.

DISCUSSION

The coloring and texture of seed coats are distinguishing features of seeds (Corner, 1976). In *Machilus thunbergii*, the mature seed coat has a layer of tight arranged macrosclereids and a few layers of compressed cells. In the light-colored areas of the seed surface, there are a few loose layers of dead cells with strip-like thickened wall, and the dead cells resulting in the surface sculpture of the seed. The characteristics of the seed coat may be used for taxonomy (Barthlott, 1981).

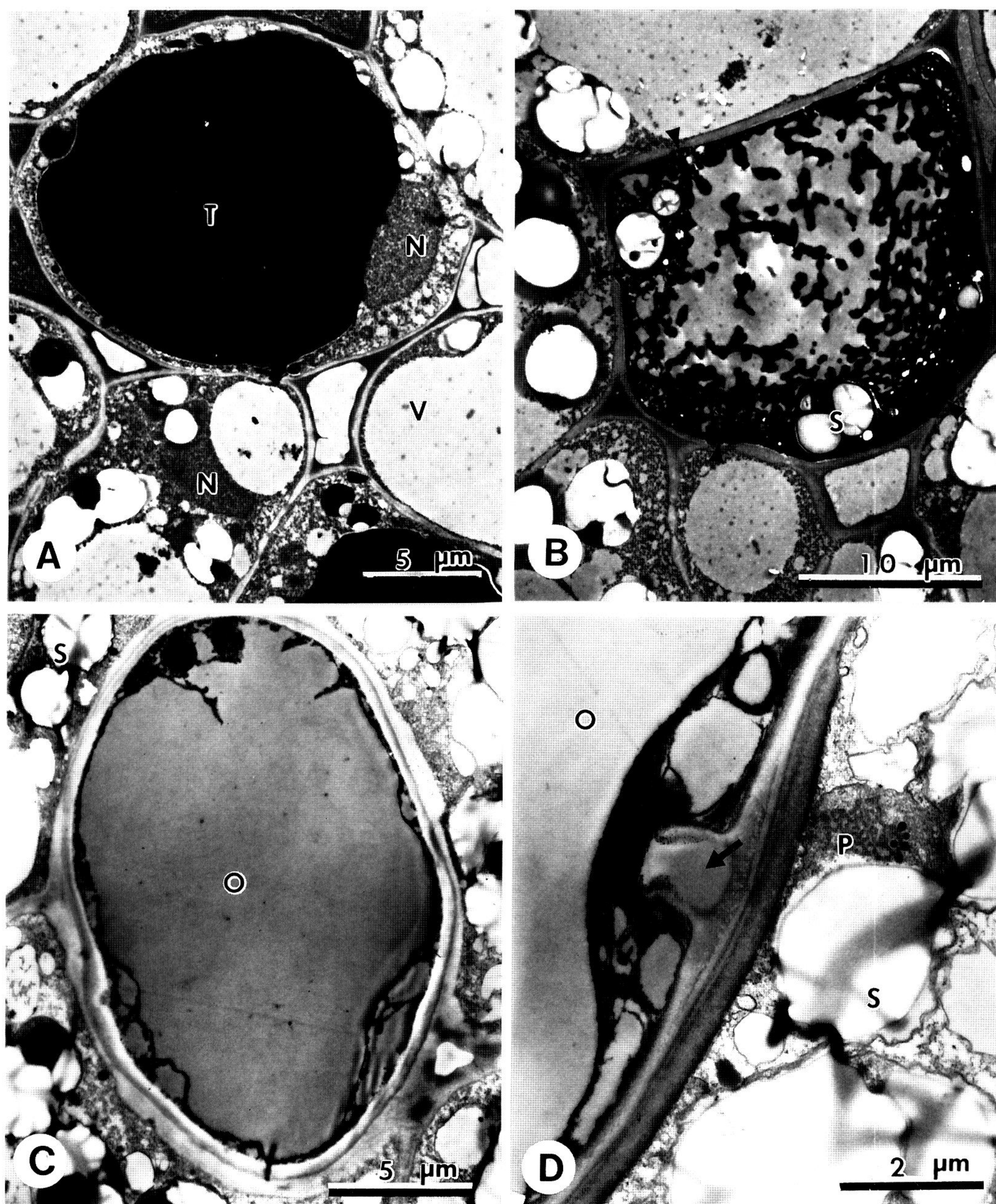


Fig. 3. TEM photographs of secretory idioblasts located in the cotyledons. A: Tannin cell with electron dense substance in the vacuole. B: Mucilage cell with mucilage substance. Groups of plasmodesmata (arrowheads) are located between the mucilage cell and the neighbor cells. C: Oil cell. The cell is with secretory substance in the large vacuole. The dense thin cytoplasm is around the cell periphery. D: Part of the oil cell. Oil substance is secreted between the cell membrane and wall from the cupule (arrow). Cell wall of the oil cell is deposited with suberin. N: Nucleus. O: Oil cell. P: Plastid. S: Starch grain. T: Tannin. V: Vacuole.

The extent of shoot apex (plumule) development in a mature seed is generally species- or family-specific (Bewley and Black, 1994). There are several primary leaves in some species, whereas in others the apex is quite rudimentary. In the mature seed of *M. thunbergii*, the embryo is well-developed and contains already 4-6 primary leaves. Also many young trichomes are located along the midrib of the abaxial surface of outer primary leaves. On the other hand, in the deep dormant seeds of *Taxus*, the plumule consists of only a dome-shaped

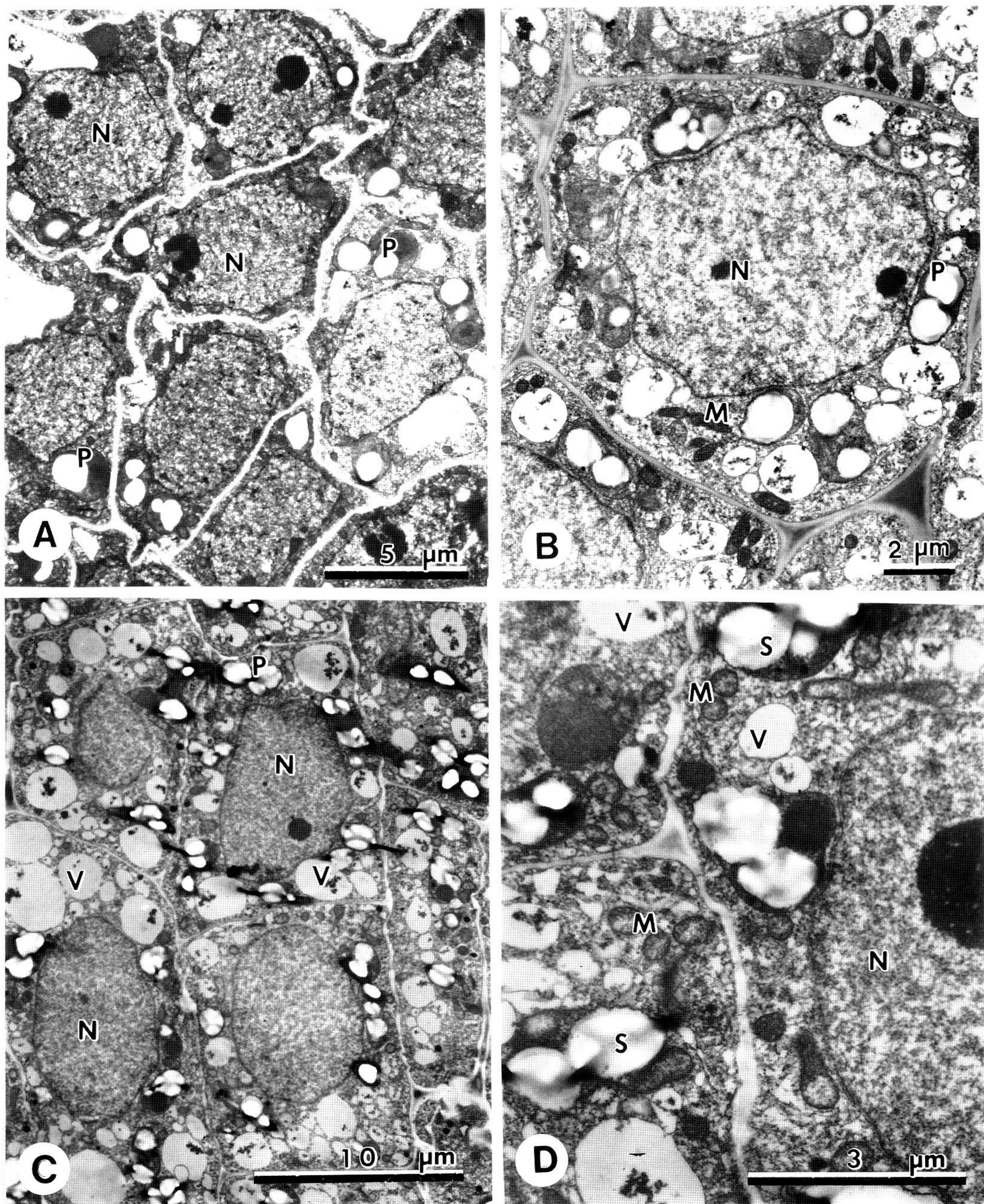


Fig. 4. TEM photographs of cells in the apical meristems of plumule and radicle of the fresh mature seeds. A and B: Cells in the apical meristem of plumule. They contain large nucleus and small vacuoles. The cytoplasm is dense with organelles. Mitochondria have well-defined cristae. There are some starch grains in the plastids. C and D: Cells in the radicle meristem showing the compact cytoplasm with large nucleus, small vacuoles, many mitochondria, and scattered plastids with starch grains. M; Mitochondrion; N: Nucleus; P: Plastid; S: Starch grain; V: Vacuole.

apical meristem (Chien *et al.*, 1998), and in the epicotyl-dormant mature seed of fringe tree, the embryo is lacking plumule (Lee and Kuo-Huang, 1999).

Many plants produce secretions that are believed to serve as animal attractants, defense, or storage rewards. Secretory cells constitute a type of internal secretory structure and are usually associated with parenchyma cells (Dickison, 2000). Different types of secretory

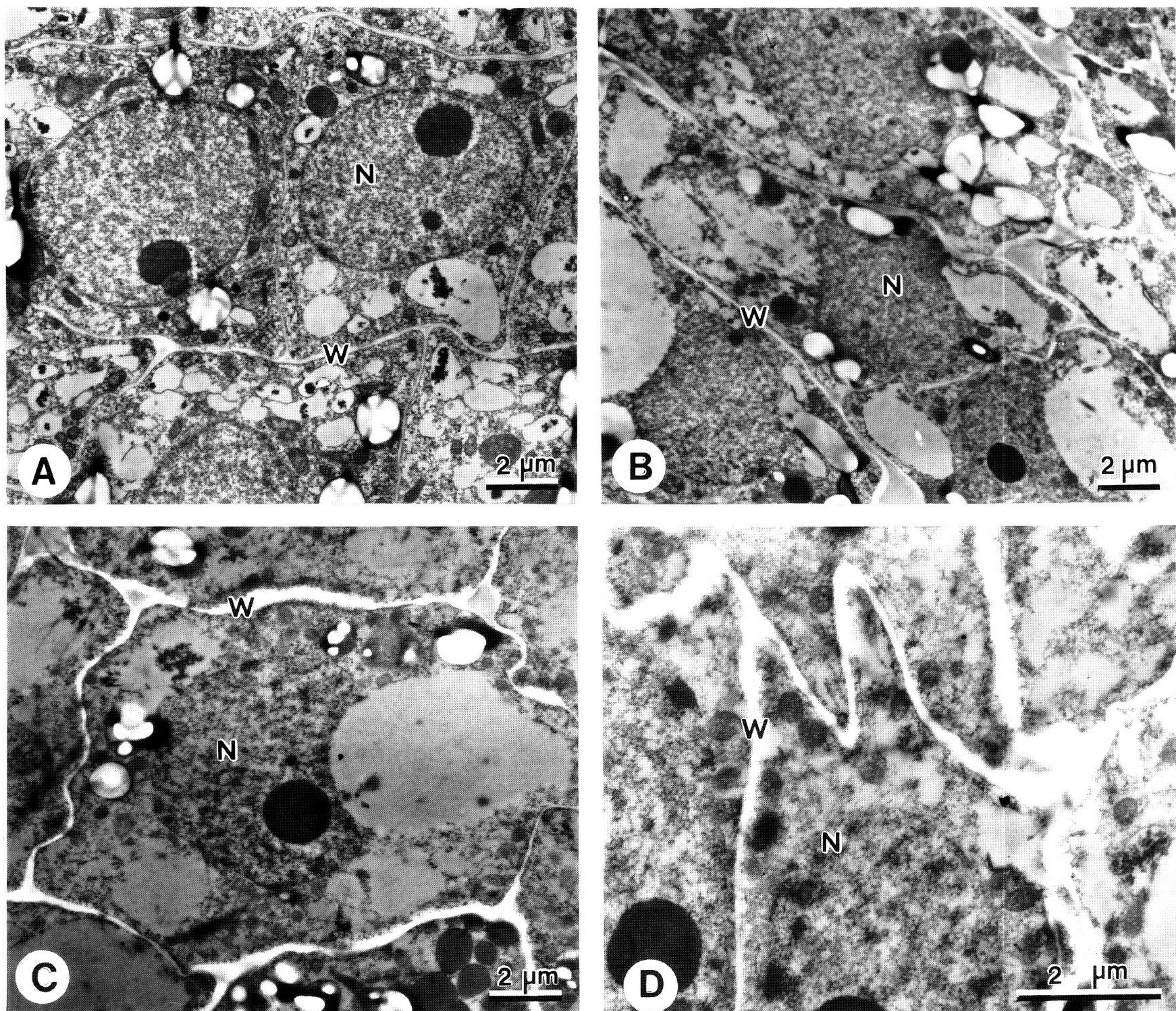


Fig. 5. TEM photographs of cells in the apical meristems of plumule (A and B) and radicle (C and D) of the mature seeds after desiccation processes. A: Seed in 100% relative humidity for one week. In compare to the cells in the apical meristems of plumule in fresh mature seed, there is no obviously ultrastructural change. B: Seed in 75% relative humidity for one week. The desiccation process causes the initiation of vacuolation and plasmolysis. C: In 75% relative humidity for one week. The Plasmalemma and nuclear membrane are broken. D: In 75% relative humidity for two weeks. The cells have distorted membrane structure. N: Nucleus; W: Wall.

structures often produce chemically identical products. In seeds, mucilage may serve as reserve food sources, for water retention, in regulation of germination of seeds, and probably for other as yet unknown purposes (Fahn, 1990). In the mature embryo of *M. thunbergii* (Lauraceae), two kinds of idioblastic secretory cells, i. e. mucilage and oil cells, were found. These two kinds of secretory cells are observed in the parenchymatous tissue of cotyledons, while only oil cells were found in the embryonic axes. In *Cinnamomum camphora* (Lauraceae), many oil cells can be found in the shoot apex, but the mucilage cells are only found in the expended leaves (Kuo, 1991). The occurrence of oil and mucilage cells in the developng leaves of *M. thunbergii* need to be checked.

Recalcitrant seeds continue to increase in dry weight up to the period of natural seed shedding. Right after shedding, the seeds are metabolically active and become increasingly desiccation-sensitive with the storage time (Berjak, *et al.*, 1992; Dodd *et al.*, 1989; Farrant *et al.*, 1988, 1992). If additional water is withheld, then the seeds are losing their viability. The

mature seeds of *M. thunbergii*, having a moisture content higher than 40%, tolerate water loss to only a minor degree during post-harvest drying (Lin and Chen, 1995). such moderate desiccation-tolerance at the mature stage resembles that of seeds of *Aesculus hippocastanum* L. (Tompsett and Pritchard, 1993).

The recalcitrant seeds maintained a constant germination percentage before the moisture content decreased obviously (Lin and Chien, 1995). From the investigation of the mature seeds of *M. thunbergii* stored under different relative humidity to achieve both rapid and slow drying conditions, Lin and Chen (1995) found a significant linear relationship between moisture content and germination percentage. For the seeds of *Avicennia marina* (recalcitrant seed) the germination process is initiated shortly after the seeds are shed and the associated subcellular events proceed in a short time. Dehydration results in irreversible metabolic disruption (Berjak *et al.*, 1984; Farrant *et al.*, 1985, 1986; Finch-Savage, 1992; Harrington, 1972). The desiccation sensitivity is mainly related to the degree of metabolic activity evidenced by ultrastructural characteristics. In this study, we have shown that the ultrastructure of the metabolically active cells of shoot and radicle meristem of the fresh mature embryo of *M. thunbergii* has severely damaged after two weeks of storage under 75% relative humidity.

ACKNOWLEDGEMENTS

We thank the National Science Council of Taiwan for a grant (NSC – 84 – 2311 - B002 - 021).

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紅楠異儲型種子微細構造的研究

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(收稿日期：2001 年 4 月 21 日；接受日期：2001 年 5 月 28 日)

摘 要

紅楠種子屬於不耐旱種子，其新鮮成熟種子內的胚芽已具四至六片初生葉。子葉含油細胞與黏液分泌細胞，然而胚主軸只有油細胞。胚芽與胚根頂端分生組織的細胞含大的細胞核與小液胞，細胞質緻密含各種胞器，色素體具澱粉粒與少量油滴。成熟種子經乾燥儲存後，頂端分生組織細胞之微細構造受到破壞（液胞化與細胞膜脫離等），且隨著貯存濕度之降低與貯存時間之延長而更為明顯，且其萌芽率也隨之降低。

關鍵詞：紅楠、異儲型種子、油細胞、黏液細胞、種子儲存、微細構造。

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