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Anatomical and Histochemical Changes in Berries of *Piper nigrum* L.

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ABSTRACT

Anatomical and histochemical changes taking place in *Piper nigrum* berries during their ripening are described. The important observations on the pericarp are the development of sclereids in the exocarp, a continuous band of oil cells in mesocarp and the wall thickening of the endocarpic cells. The mature seed with a single layer of seed coat, representing the innermost tegmen layer, encloses abundant perisperm. The endosperm and embryo are situated laterally at the terminal part of the seed. The perisperm is distinguished into an outer protein-rich zone and inner starch-filled zone. Starch and protein are also deposited in the mature pericarpic tissue. Lipid bodies are seen in the form of oil globules in oil cells.

INTRODUCTION

Johnson (1900, 1902) studied the floral morphology including seed and fruit structure of *Piper adunca* and *P. medium* and compared their floral structures with *Peperomia pellucida*. It was Cooper (1909) who first described the pericarp anatomy of *P. nigrum* berries. Murty (1959) made a brief account of fruit and seed structure of *P. nigrum*, *P. schmiditi* and *P. wighti*. A detailed embryological investigation on *P. nigrum* was carried out by Kanta (1962). She also gave some details about pericarp and seed histology. The pharmacognostic significance of *P. nigrum* berry was compiled by Trease (1966). Corner (1976) also described the seed structure of this *Piper* species.

In spite that *Piper nigrum* berries were described by many authors as above, several contradictory observations are found. The contradiction is possibly due to the lack of complete developmental study. The commercially important row products of *Piper* berries are 'black pepper' and 'white pepper'. The black pepper is prepared by drying the mature green coloured fruits and white pepper is prepared by drying the fully ripened berries after removing the outerpart of the fruit wall.

In the present communication we have comprised a detailed structural, developmental and histochemical observations on *Piper nigrum* berries.

MATERIALS AND METHODS

Flowering and fruiting spikes of *Piper nigrum* L. at sequential developmental stages (Table 1) were collected from different parts of Kerala and fixed in F.A.A. The fixed materials were dehydrated, embedded and microtomed according to the methods of Johansen (1940). Safranin, fast green and Haematoxylin were used for general staining. Starch grains and proteins were localized using periodic acid-Schiff's reagent (Jensen, 1962) and Coomassie brilliant blue (Eklavya, 1979), respectively. Lipid bodies are localized in unfixed materials using oil red 'O' (Lillie and Fullmer, 1976). The lignification was tested with phloroglucinol and HCl (Johansen, 1940). Photomicrographs are taken using Zeiss photomicroscope-I and phase contrast microscopes.

Table 1. Length and diameter of ovaries and fruits at different developmental stages

Stage	Length in mm	Diameter in mm	Remarks
1	0.4−0.8	0.4−0.8	Ovary
2	0.8−2.0	0.8−1.5	Young fruit
3	2.0−3.0	1.5−2.0	Developing fruit
4	3.0−4.5	2.0−3.0	Developing fruit
5	4.5−7.0	3.0−5.0	Mature fruit

RESULTS

P. nigrum is a glabrous climber with alternate coriaceous leaves. Flowers are borne in spike inflorescence (Fig. 1A). The sessile flowers in the spike are bisexual and/or unisexual (female) without perianth and covered by four lobed bracts. In transection of the spike only two lobes of the bract are seen arched over the ovary and stamen (Fig. 2). Mucilage canals are seen in the spike axis and bract (Fig. 2).

Ovary. The monocarpellary ovary is somewhat spherical in shape enclosing a single orthotropous ovule (Figs. 2 and 3). The ovary wall consists of 12–15 layers of cells. The outer epidermis and 1–2 hypodermal layers consist of square to rectangular cells with large globose centrally placed nuclei (Fig. 3). The mesoderm is formed of 9–11 layers of polygonal parenchyma (Fig. 3). Seven to nine developing vascular bundles are seen in the centre of the mesoderm tissue (Fig. 3). Oil cells are also seen in the mesoderm (Fig. 3). The inner epidermal cells are squarish and denser than that of the outer epidermis (Fig. 3). The ovule starts its differentiation before the complete development of the ovary wall tissue (i.e. before the completion of marginal carpel fusion) as a small mound of meristematic tissue at the basal part of the ovary (Fig. 4 at arrow). The fusing carpel margins are very distinct at the very early ovary stage (Fig. 5 at arrow). The meristematic mass of ovular tissue (nucellus) divides and enlarges in size (Fig. 3) and later differentiates into an orthotropous bitegmic and crassinucellate ovule (See also Kanta, 1962).

The ovary after fertilization develops into globose berries. These sessile berries are arranged spirally on the spike axis (Fig. 1B).

Pericarp. The fertilized ovule develops into the seed and the ovary wall transforms into the pericarp. The pericarp is distinguished into outer exocarp, middle mesocarp and inner endocarp. At the very early stage of the fruit maturation the pericarp consists of 18–22 layers of cells (Fig. 6). The cells of the outer epidermal layer divide only anticlinally and the cell walls become slightly thicker and covered by the cuticle. The underlying hypodermal cells divide and elongate radially to form a palisade layer (Fig. 6). These palisade cells divide both anticlinally and periclinally and differentiate into lignified and pitted rod shaped sclereids (Figs. 7 and 8). Both these sclereid layers and the outer thick walled epidermal layer together constitute the exocarp.

The parenchymatous mesocarp which consists of 15–19 layers of cells in the younger fruit is found to be homogenous in shape and size (Fig. 6). The conjoint and collateral vascular bundles, pass through the middle part of the mesocarp and terminate just below the stigma (Figs. 7–9). In the developing and mature fruit stages the mesocarp becomes 28–30 layered, and can be distinguished into two zones on the basis of cell size and their compact arrangement (Figs. 7 and 8). The mesocarpic cells upto the level of vascular bundles are larger vacuolated and loosely arranged than the cells lying beneath, which are smaller and compactly arranged (Figs. 7 and 8). When white pepper is prepared from ripe berries the berries part upto the inner compact zone of mesocarp is removed and thus the vascular strands are seen on its periphery (Figs. 10 at arrow). In young and developing berries scattered oil cells are seen in the mesocarp (Figs. 6 and 7). But in mature berries a continuous band of oil cells is also differentiated at the inner part of inner mesocarpic zone above the endocarp (Fig. 8 at arrows). The oil cells are larger and thicker-walled than the rest of the mesocarpic cells. Large number of small rhomboid crystals are seen in the mesocarpic cells (Fig. 11 at arrow).

The inner epidermis of the ovary wall transforms into the endocarp of berries. In young and developing fruit this endocarp consists of slightly radially elongated thin walled cells (Figs. 6 and 7). But when the berries are matured, the inner tangential wall and about 3/4 part of the radial walls of this endocarpic cells become lignified (Fig. 8). This thick walled endocarp forms a firm protective layer to the seed. Calcium oxalate druses are occasionally seen in the endocarpic cells (Fig. 12 at arrow head).

Even though the flower is monocarpellary in *P. nigrum,* the stigma is found to be trifid to pentafid. This stigma persists even in the mature fruit and it consists of radially elongated cells just above the pericarpic region (Fig. 9).

Histochemical tests show that starch or protein is not found deposited in the pericarp during developing stages of the berry. But lipid, in the form of oil globules, is seen in the oil cells. In ripe fruits starch grains are marked in the mesocarp, mostly near the middle region (Fig. 13). Few starch grains are also seen in the oil cells (Fig. 12 at arrow). Protein bodies are smaller, distributed throughout the pericarp but more in the peripheral layers (Fig. 14 circles). In mature berries oil globules are seen in the band of oil cells and in the isolated oil cells (Fig. 15 in circles). Cells with resinous contents are also seen here and there in the pericarp.

Seed. The mature seed is globose or oval in shape like the fruit. The seed and fruit wall tissues are closely packed in ripe berries. A mature seed consists of a single layered seed coat, large amount of perisperm and little endosperm tissue in which the embryo is embedded.

In developing seeds the seed coat has 6–8 layers of cells, which constitute outer and inner integuments (testa and tegmen in seed) (Fig. 16). Of these seed coat layers the cells of the innermost layer of tegmen are thick walled and large (Figs. 7 and 17 at arrow). During the course of development and maturation of the seed, all these seed coat layers except the innermost tegmen layer disintegrate and disorganize, and the seed coat becomes single-layered (Figs. 7 and 8). The cells of this seed coat layer are thick walled and have dense tanniniferous contents. In a fully mature seed this tegmen layer becomes highly compressed and streak-like. The cells of this seed coat layer lying opposite to the endosperm tissue produce finger-like protrusions inwardly, which unite with similar protrusions from the epidermal layer of perisperm tissue lying over the endosperm (Fig. 18).

Inside the seed coat the major part of the seed (90–95%) is filled with perisperm (Figs. 19 and 20). This perisperm is the most economically important part in *Piper* berries. The perisperm is formed by the division and enlargement of the persistent nucellar tissue. The remaining part of the seed consists of the endosperm and embryo which develop laterally at the terminal part (Fig. 19 at arrow). In developing and mature stages of the seed, the perisperm cells lying along the periphery are found to be small and dividing (Figs. 17 and 20). A thick cuticle layer is seen below the seed coat above the perisperm epidermis (Fig. 21). The perisperm can be distinguished into a peripheral 5–7 layers of protein-rich zone and inner starch-filled zone. The protein particles are found to be of different size (Fig. 21). Starch grains are also found in the peripheral protein-rich cells. Some of the starch-filled cells become larger by the breaking and disorganization of the adjacent cell walls. The starch grains are globular, abundantly seen in the cell lumen (Fig. 22). Large oil cells are found scattered in the perisperm tissue (Fig. 17). Certain cells containing resinous substances are also seen in the perisperm.

The endosperm is somewhat dome-shaped having the peripheral smaller and regular cells and the central larger and vacuolated cells (Figs. 18 and 23). Starch grains are not found in the endosperm tissue but protein bodies are seen. The embryo lies more or less in the centre of the endosperm tissue (Fig. 23).

DISCUSSION

In *P. nigrum* the berries develop from monocarpellary unilocular ovaries. In the very young stage of the ovary, the margins of the fusing carpel is distinct. Thus it can be said that the ovary is formed by ontogenetic (post-genital) fusion. The ovule starts its differentiation before the fusion of the carpel margins. The differentiation of the ovule before the complete development of the ovary wall was also reported in *Peperomia* (Murty, 1958). This type of ontogenetic fusion was reported in several angiosperm families by Baum (1948a, 1948b). Walker (1975, 1978) made a detailed investigation on post-genital carpel fusion in *Catharanthus roseus*. Even though a fusion zone is well distinguished in the young ovary stage of *P. nigrum*, this fused zone is not evident in the later stages of developmet. According to Baum (1943a), the earlier the fusion occurs in ontogeny, the less descernible is the line of fusion.

In ripe berries the pericarp is distinguished into outer exocarp, middle mesocarp and inner endocarp; and the seed consists of a single layer of tegmen, perisperm tissue, endosperm and embryo. The 2–3 layered exocarp is developed from the outer epidermis and hypodermal layer of the ovary wall. The division and contribution of the hypodermal layer in exocarp formation is well understood in fruit development. But Murty (1959) and Kanta (1962) described the exocarp of *P. nigrum* berries as the complete layers of cells lying above the zone of vascular bundles. This interpretation was made because of the lack of detailed observations in developing fruit stages. The reports on the pericarp structure of other species of *Piper* (Johnson, 1902; Murty, 1959) show that a sclereid zone is formed only in *P. nigrum*.

The mesocarp is formed from the mesoderm of the ovary wall. An increment in number of cell layers in the pericarp is seen during the developing stages of the fruit, and mostly in the mesocarp. In mature berries the mesocarp is distinguished into an outer zone of larger and highly vacuolated cells and an inner zone of smaller and compactly arranged cells. The vascular bundles lie in between these mesocarpic zones. Murty (1959) and Kanta (1962) explained that the mesocarp of *P. nigrum* berry is constituted of only the inner zone of compact parenchyma lying below the vascular bundles. But the developmental study of this berry and its comparison with other berries (Schroeder, 1958; Roth and Lindorf, 1972; Dave *et al.*, 1975, 1979, 1982; Roth, 1977; Dave, 1986) clearly showed that the mesocarp is wholly constituted of parenchymatous cells lying below the sclereid zone. The occurrence of oil cells in the mesoderm and mesocarp was a distinguishing feature of Piperaceae and is also reported by Johnson (1900, 1902) and Murty (1958, 1959). The occurrence of oil cells were also reported in the berries of *Persea americana* (Scott *et al.*, 1963; Roth, 1977; Plat-Aloia *et al.*, 1983). But the differentiation of a continuous band of oil cells in the mesocarp above the endocarp appears to be a unique feature of *P. nigrum* berries. The partial wall thickening of the endocarpic cells accounts for the protection of the seed. Generally the endocarpic cells of berries remain thin walled throughout (Roth, 1977). Lignification of endocarpic cells was also reported in *Capsicum* berries (Dave, 1986).

Even though the ovule is bitegmic, the seed coat remains single layered by the disorganization of all the integument layers except the innermost layer of inner integument. Murty (1959) and Corner (1976) reported that the seed coat in *P. nigrum* is composed of two or three layers. This must have happened because of their limited observations on the developing seed. In developing stage of the fruit the innermost tegmen layer is found to be composed of distinctly distinguished thick-walled dense cells, but later towards the maturity of the fruit, it is found as a very indistinct layer of cells. The special type of cell wall thickenings of the seed coat layer and perisperm epidermis are formed at the region of endosperm function as a shock-proof layer to the developing embryo. The major portion of the seed is filled with the starch and protein-rich perisperm tissue. Oil cells and resin-filled cells are also found scattered in the perisperm tissue. This portion of the berry forms about 80–90% by weight and is the highly medicinally important part. The dome-shaped endosperm is nuclear-type (Kanta, 1962) and consists of peripheral small regular cells and central large looser cells enclosing the central embryo.

The important products of *Piper* berries are black pepper and white pepper and pepper oil, piperonal, pepper oleoresin, soluble pepper, pepper shell, etc. are extracted from these dried fruits (Randhawa, 1963). The medicinal and economical properties of *P. nigrum* berries are evaluated by Trease (1966). According to him the *Piper* fruit connsists of a volatile oil (about 1.00–1.25%), crystalline alkaloid (about 5–9%), piperine, pipertine and an oleoresin. Trease (1966) pointed out that the aroma of the spice is due to the presence of the volatile oil that consists largely terpenes while the pungency is due to the pipertine and the resin. From the structural and histochemical studies it can be concluded that a major proportion of the above mentioned components are situated in the inner pericarp and seed tissues. A portion of these components is also present in the outer pericarpic tissues, which is removed during the preparation of white pepper. Hence we can say that black pepper is medicinally or economically more valuable than white pepper.

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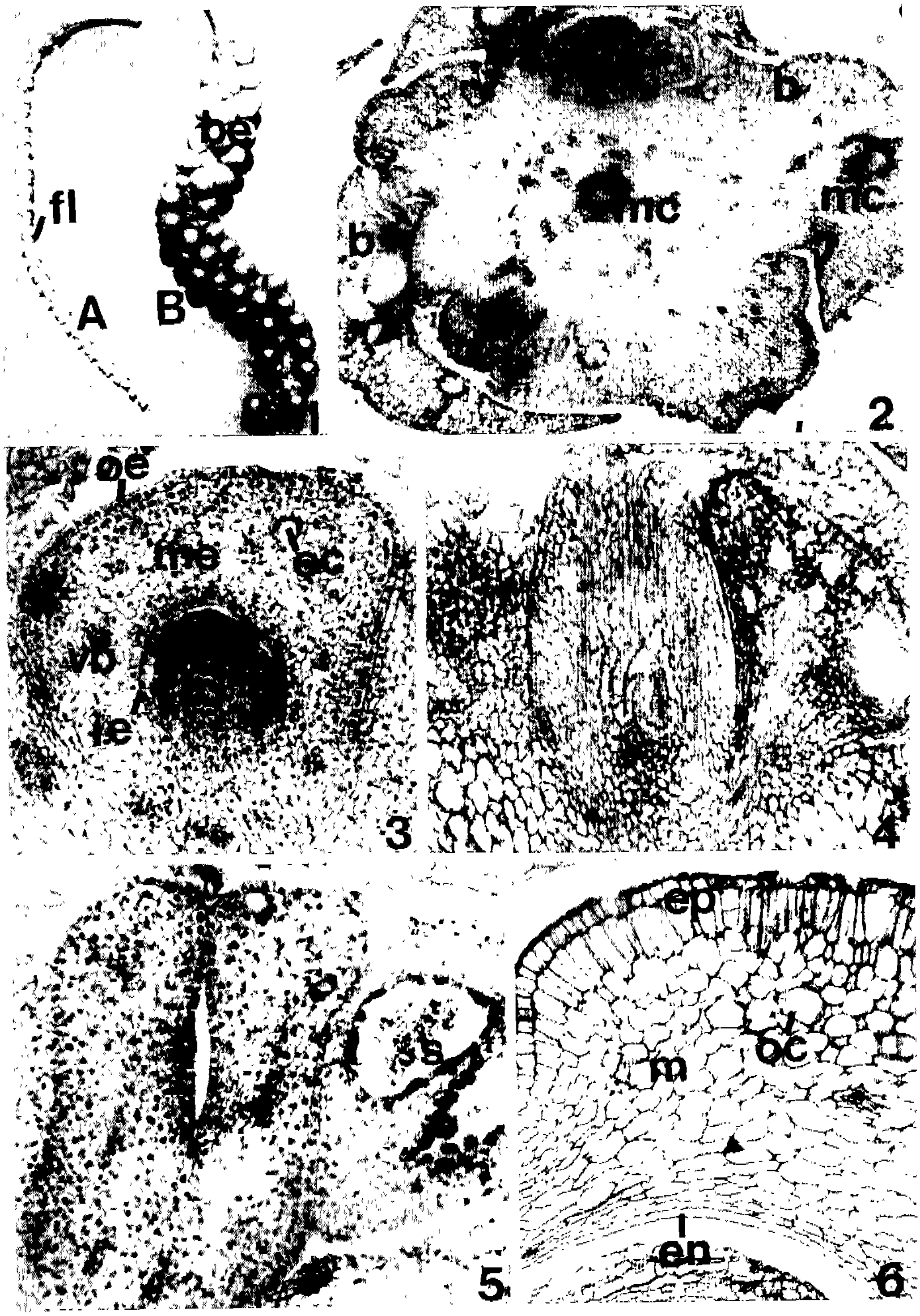
Explanation of Figures

Figs. 1- 6. Fig. 1A. Flowering spike, 1B. Fruiting spike ×0.9. Fig. 2. Transection of a flowering spike × 45. Fig. 3. Ovary in longisection ×190. Fig. 4. Developing stage of the ovary, see the meristematic mound of ovular tissue at arrow ×156. Fig. 5. Developing stage of the overy, see the unfused carpel margins at arrow ×175. Fig. 6. A portion of pericarp from young fruit × 195. (b-bract; be-berry; en-endocarp; ep-exocarp; fl-flower; ie-inner epidermis; m-mesocarp; mc-mucilage canal; me-mesoderm; o-ovule; oc-oil cell; oe-outer epidermis; ov-ovary; s-stamen; vb-vascular bundle).

Figs. 7-15. Fig. 7. Transection of pericarp from developing fruit ×115. Fig. 8. Transection of pericarp from mature fruit, see the continuous band of oil cells at arrows ×110. Fig. 9. Longisection of a developing fruit, see the vascular strands and persistent stigma ×35. Fig. 10. Morphology of white pepper, see the vascular strands at arrows ×8.5. Fig. 11. Phase contrast micrograph of mesocarpic cells with crystals ×410. Fig. 12. Phase contrast micrograph of inner pericarpic portion, see crystal in endocarpic cell and starch grains in the oil cell ×380. Fig. 13. Fluorescence micrograph of mesocarp with starch grains ×230. Fig. 14. Protein particles in the pericarp (in circles) ×60. Fig. 15. Oil globules in oil cells (in circles)×140. (en-endocarp; ep-exocarp; im-inner mesocarp mesocarp; p-perisperm; sc-seed coat; st-stigma; vb-vascular bundle; vs-vascular strand).

Figs. 16-23. Fig. 16. Transection of inner pericarp and seed coat layers from young fruit, see the disorganization of outer seed coat layers ×205. Fig. 17. A portion of inner pericarp, seed coat and perisperm from developing fruit, see the prominent inner seed coat layer at arrow ×140. Fig. 18. Transection of fruit through the endosperm region showing thickening of the seed coat layer and perisperm epidermis ×110. Fig. 19. Longisection of mature berrry, see the top shaped endosperm at the terminal part of the seed (at arrow) ×16. Fig. 20. Cellular detail of the perisperm tissue from mature fruit ×50. Fig. 21. Proteinaceous zone of perrisperm, see the protein particles in circles ×155. Fig. 22. Phase contrast micrograph showing starch grains in the perisperm cells ×320. Fig. 23. Endosperm and embryo from mature fruit ×95. (e-endosperm; em-embryo; en-endocarp; oc-oil cell; p-perisperm; pe-pericarp; sc-seed coat; t-testa; te-tegmen).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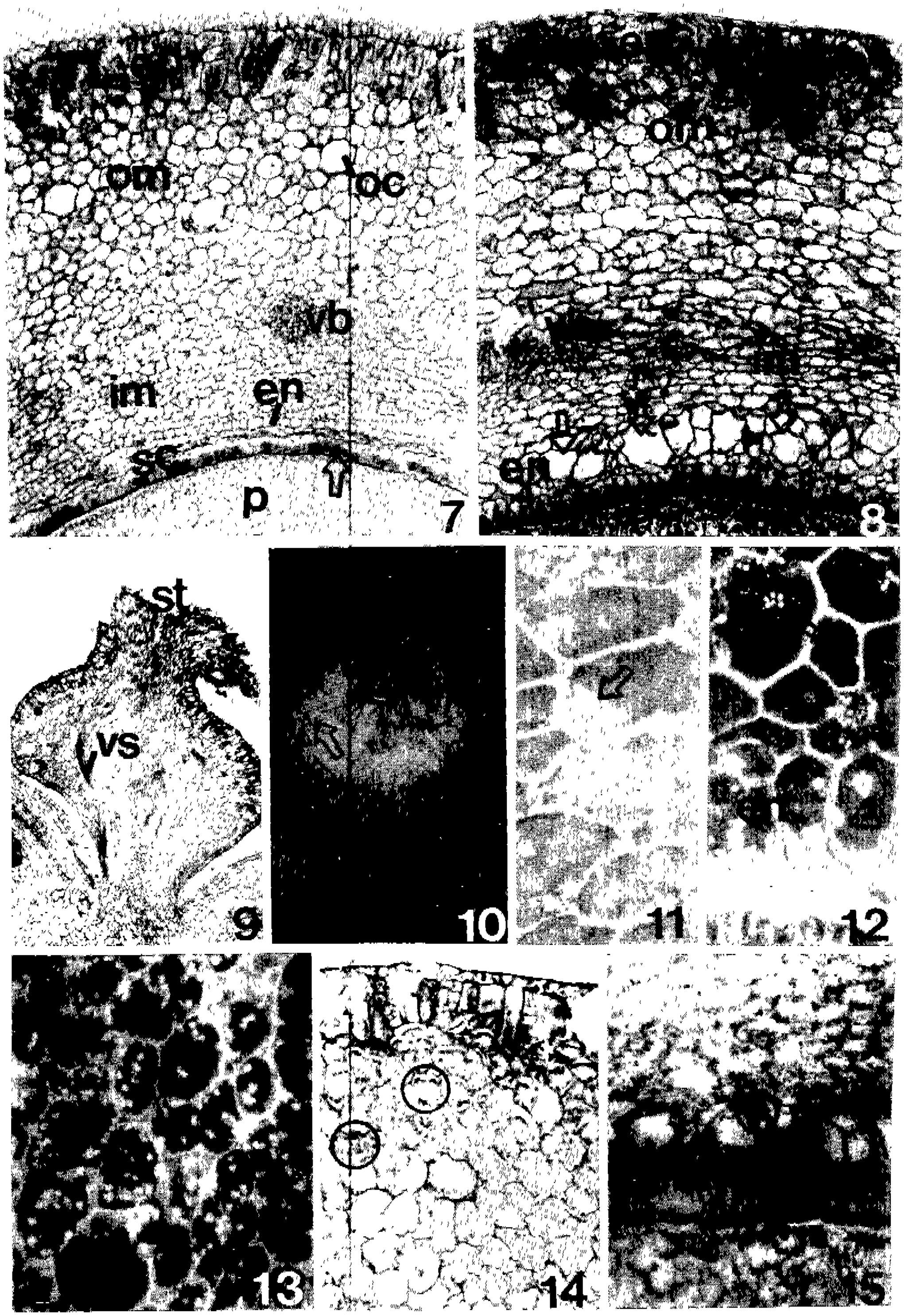
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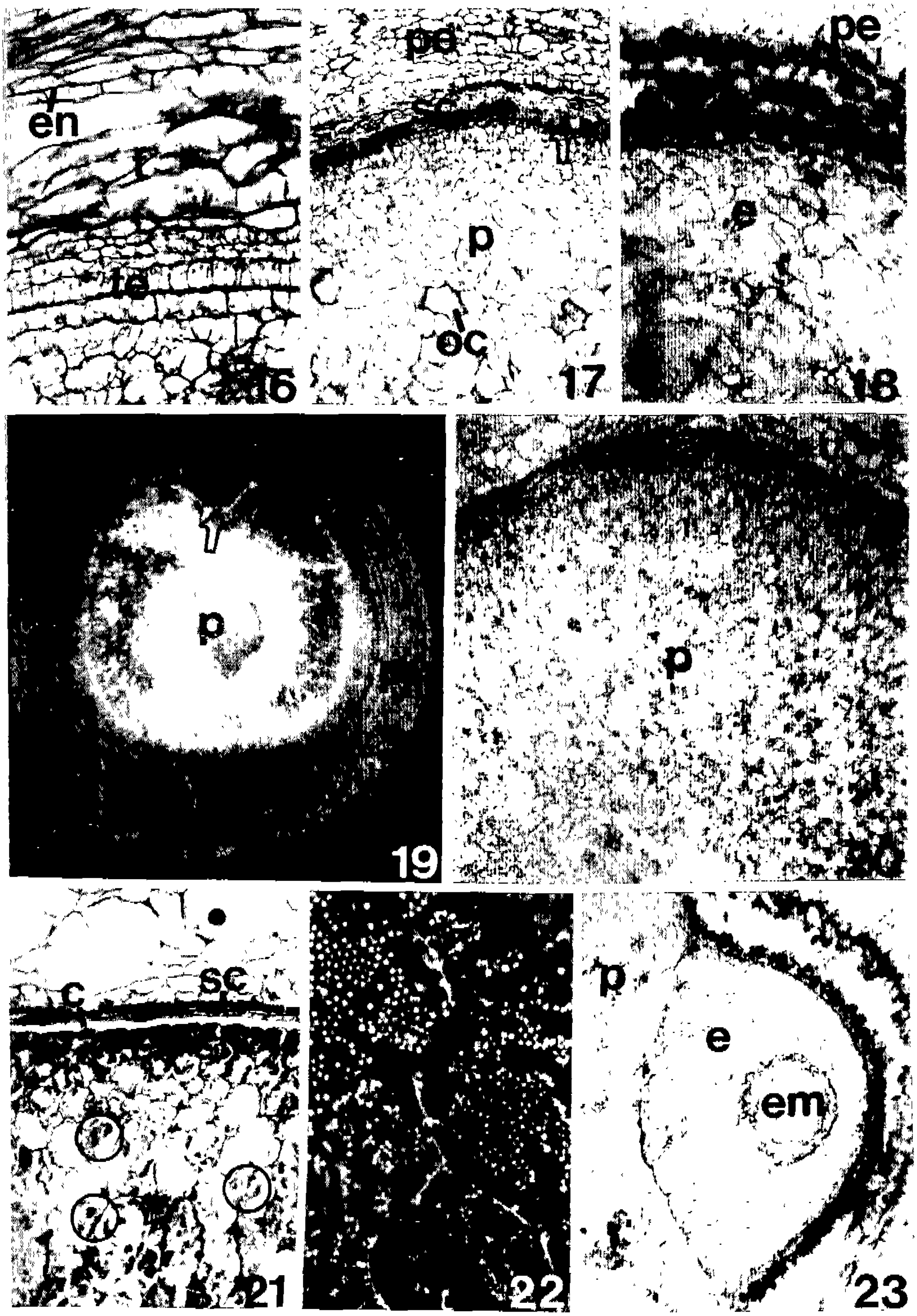
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