한국전자현미경학회지 제 33 권 제 1 호 2003
Korean J. Electron Microscopy 33(1), 79~92(2003)

Sequential Changes of Pericarp Ultrastructure in *Citrus reticulata* Hesperidium

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(Received March 11, 2003; Accepted March 28, 2003)

Citrus reticulata 감과 과피 내 미세구조 변화

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ABSTRACT

Ultrastructural changes of the pericarp in *Citrus reticulata* has been investigated during hesperidium abscission. The pericarp was composed of compactly arranged parenchyma cell layers during early stages of fruit development. The outermost exocarp was green and active in photosynthesis. However, cells in the exocarp soon changed into collenchyma cells by developing unevenly thickened walls within a short time frame. As the fruit approached maturation, the chlorophyll gradually disappeared and chloroplasts were transformed into carotenoid rich chromoplasts. In the mature fruit the exocarp consisted of large, lobed collenchyma cells with primary pit fields and numerous plasmodesmata. The immature mesocarp was a relatively hard and thick layer, located directly under the exocarp. With development, the deeper layers of the exocarp merged into the white, spongy mesocarp. Before separation of the hesperidium from the plant, some unusual features were detected in the plasma membrane of the exocarp cells. The number of small vacuoles and dark, irregular osmiophilic lipid bodies also increased enormously in the exocarp collenchyma after the abscission. They occurred between the plasma membrane and the wall, and invaginated pockets of the plasma membrane containing double membraned vesicles were also frequently noticed. The lipid bodies in the cytoplasm were often associated with other organelles, especially with plastids and mitochondria. The plastids, which were irregular or amoeboid in shape, contained numerous large lipid droplets, and occasional clusters of phytoferritin, as well as few loosely oriented peripheral lamellae. Myelin like configurations of membrane were frequently observed in the vacuoles, as was the association of lipid bodies with the vacuolar membrane. Most vacuoles had an irregular outline, and lipid bodies were often connected to the tonoplast of the vacuoles. The structural changes underlying developmental, particularly to senescence, processes in various hesperidium will be reported in the separate paper.

Key words : Abscission, *Citrus reticulata*, Pericarp, Ultrastructure, Lipid bodies

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INTRODUCTION

An ovary wall matures into a fruit wall as the number of cells comprising the ovary increases with the fruit development. The fruit wall may be a highly differentiated structure with two or three distinct layers that can be readily recognizable in some fruits. The *Citus* fruit, hesperidium, is composed of three very different morphological components. They are referred to, beginning with the outer most layer, as exocarp, mesocarp, and endocarp (Esau, 1979; Fahn, 1990). In the hesperidium, the combined exocarp and mesocarp are generally called the pericarp, commonly known as the rind or peel (Kale & Adsule, 1995). Within the pericarp, the external colored portion is the exocarp, while the internal, white layer is the mesocarp.

Studies of hesperidium development have generally focused on a specific cultivar such as *Citrus sinensis*, which is one of the most important economic fruits, orange. However, when the entire spectrum of citrus fruit research is considered, much attention has been given to the diversity of peel/pulp (pericarp/endocarp) ratios in mature fruit. Among diverse ratios, *C. medica* is at one extreme where a fleshy pulp is lacking in contrast with relatively thin-peeled *C. reticulata*, also known as *C. unshu*, where the predominating pulp is concentrated towards the other end. The mesocarp occupies the rest of the fruit in the former (Spiegel-Roy & Goldschmidt, 1996), while a degenerating mesocarp almost disappears in the latter, leaving only thin mesocarp with reticulate vascular elements between the two layers. According to Spiegel-Roy and Goldschmidt (1996), when peel growth predominates during the early phase of hesperidium development in *Citrus*, the mesocarp may occupy 60 to 90% of fruit volume. Later, when pulp growth generally takes over, the mesocarp becomes thinner and the portion of the mesocarp declines in most cases.

The present study attempts an investigation of the sequential changes of structure which occur during development and maturation in a rather extreme case of hesperidium pericarp in thin-peeled *Citrus reticulata*, at the time of abscission. *C. reticulata* is a very popular fruit with much commercial value in Korea. The study is mainly concerned with the ultrastructural changes underlying development of the pericarp, particularly, the changes that occur after abscission to attain the post-abscission effect seen in this case. The author has limited the study mostly to the region of the epidermis to the innermost peel to avoid difficulties which could arise from ambiguity in differentiating the mesocarp and endocarp. No attempt was made to correlate changes in hormonal conditions or pigmentation with fruit growth. The structural changes underlying developmental, particularly in reference to senescence and processes in various hesperidium will be reported in a separate paper.

MATERIALS AND METHODS

In *Citrus reticulata*, according to Kim (1996), the pericarp was peeled from the fruit, and most of the epidermal exocarp was trimmed away with a razor blade. The remaining exocarp and mesocarp, consisting mainly of the outer colored and inner white portions, were cut into small sections, approximately 2 mm^2. These sections were fixed for 3 hours in 4% formaldehyde and again in 2% osmium tetroxide buffered at pH 7.0 with a 0.01 M phosphate buffer (4~16 hrs). The samples were rinsed four times with the same buffer after each fixation. They were dehydrated in graded acetone series and embedded in Spurr's resin. All blocks were carefully trimmed under a dissecting microscope, particularly in the exocarp, so that the oil glands could be avoided. Ca. 60~90Å thin sections were cut on a Reichert Ultracut S ultramicrotome, picked up on formvar-coated grids (Ji & Kim, 2002), stained on the grids for 30~45 min either with 2% aqueous or 30% methanol uranyl acetate and lead citrate. They were studied with a Hitachi 7100 electron

microscope operated at Korea Basic Science Institute, Daegu Branch.

A staining procedure for lipase has been also carried out to reveal the nature of osmiophilic irregular bodies, present in almost all of the exocarp collenchyma cells of later development. The enzyme appears to be highly resistant to aldehyde fixation, since the original technique suggests fixation in 4% formaldehyde at 4°C for 24 hrs (Lewis, 1982). However, shorter fixation, 6 hrs, was performed, since it was considered to be adequate and preferable. Then washing in a suitable buffer solution, preferably overnight, was followed. As with all simultaneous coupling azo-dye techniques, sections prepared for incubation should be as thin as compatible with preservation of structure, and the sections should be pre-incubated in complete medium lacking substrate. Incubation medium consisting of the following ingredients was used; sucrose 1.5 g, 0.2 M Tris buffer (pH 7.4, 10 ml), sodium taurocholate (2.5%, 25 mg/ml) 1.0 ml, 2-thiononanoylbenzanilide in diemthyacetamide (40 mg /ml) 0.1 ml, and Fast Blue BBN 10 mg. After incubation of sections for 2~4 hrs at 37°C or for somewhat longer at room temperature, the sections were transferred to freshly prepared medium every 30 min. It is recommended the sections should be pre-incubated for 30 min in a medium made up as above with omission of the substrate (Lewis, 1982). With a normal period of fixation it was probable that the incubation time could be drastically shortened. Then, followed a washing in a buffered sucrose solution and a treatment with OsO_4. Adequate contrast was also obtained by treatment with a 2% unbuffered OsO_4 at room temperature. The sections were processed by the same method as mentioned above beyond the OsO_4 treatment.

RESULTS

The pericarp ultrastructure, particularly the exocarp, in *Citrus reticulata* changed noticeably during later development and abscission of the heperidium. In the early stages of fruit development, the outer part of the pericarp was somewhat green, where photosynthetically active layers composed of compactly arranged parenchyma cells (Fig. 1). They contained the normal constituent of organelles such as plastids, mitochondria, rER, and Golgi apparatus, among others. Chloroplasts with well-developed grana exhibiting the peculiar pattern of peripheral thylakoid (Fig. 2) were found in growing exocarp. The exocarp cells began to initiate the primary pit field with numerous plasmodesmata between neighboring walls. However, cells in the exocarp soon changed into collenchyma cells by developing unevenly thickened walls within a very short time frame (Fig. 3). In the latter part of the developing fruit, the exocarp already consisted of large, lobed cells with some intercellular spaces and scattered vascular elements.

As the fruit approached maturation and then underwent abscission, the chlorophyll gradually disappeared and most of the chloroplasts were transformed into carotenoid-rich chromoplasts. The chromoplasts contained numerous osmiophilic droplets and peripherally-located, single-stranded curved thylakoids (Fig. 4). Before the hesperidium was separated from the plant, unusual outgrowth of the plasma membrane took place at the periphery of the collenchyma cells. Vacuolar compartments varying in size and shape were also scattered throughout the cytoplasm. But mitochondria and microbodies were still abundant at this stage of the exocarp collenchyma (Fig. 5). Direct beneath the exocarp was the immature mesocarp which was colorless, slightly thick, and had relatively hard layers. With development, the deeper layers of the exocarp merged into the white and spongy mesocarp.

Further intriguing features noticed in almost all of the exocarp cells was the peculiarity of the plasma membrane. The plasma membrane clearly demonstrated condensation of electron-dense materials and/or vesicle formation that originated from the membrane itself. Direct involvement of the plasma membrane (Fig. 6) or

dilation of the membrane enclosing numerous small vesicles and fibrous materials (Fig. 7) was highly common. In addition to these, membrane-bounded vesicles were frequently derived from the vacuolar membrane, forming a cluster. In some cases, they showed close proximity to other organelles (Fig. 8). Accumulation of these vesicle clusters was made possible by the plasmodesmata between two cells (Figs. 9-10). Along with this phenomenon, cells were packed with numerous, small to large, vacuolar compartments and other scattered organelles (Fig. 11). Once a cluster of vesicles were formed, they had a tendency to move towards the organelles that were usually distributed near the cell wall (Fig. 12). Moreover, vesicles or electron-dense bodies (EDBs), presumably originating from the Golgi apparatus, approached chromoplasts or mitochondria from both the central vacuole and cytoplasm (Fig. 13). The EDB was probably formed by the accumulation and further condensation of the vesicles stated above. They occurred between the plasma membrane and the wall, and invaginated pockets of the plasma membrane containing double-membraned vesicles were easily revealed. The osmiophilic bodies in the cytoplasm were commonly associated with other organelles, particularly with plastids and mitochondria. Myelin-like configurations of membranes with the vacuoles were frequently noted, as was the association of osmiophilic bodies with the vacuolar membrane. Occasionally, EDBs of different origin, but mostly from the plasma membrane, enclosed coated vesicles (Fig. 14). With further aging, numerous EDBs surrounded most of the cellular components (Fig. 15).

Typical collenchyma cells at this stage contained a large central vacuole with vacuolar compartments, abundant EDBs, chromoplasts, and unevenly thickened walls (Fig. 16). Well-developed primary pit fields with numerous plasmodesmata, often forming median cavity, were encountered in a portion of the inner wall (Figs. 17-18), while most of irregularly-shaped EDBs were dispersed throughout the peripheral cytoplasm (Fig. 19). According to the results of the staining method for lipase, the EDBs were shown to be lipoidic in nature. The sections having chromoplasts with EDBs clearly showed the lack of electron-dense lipid droplets in the chromoplast and in neighboring, irregularly-shaped, osmiophilic body (Fig. 20). The plastids, irregular or amoeboid in shape, contained numerous large lipid droplets and occasional clusters of phytoferritin within the stroma (Fig. 21).

In contrast, the mesocarp did not show any drastic structural changes like those seen in the exocarp. Most sections of the mesocarp layer exhibited more or less dense cytoplasm (Fig. 22) when they were young and immature. But, the initial changes of irregular, unevenly thick walls noted earlier along the cell wall did occur between the cells. Also, cellular degradation persisted throughout the cytoplasm. Components of the cell wall became loose and microtubules became apparent (Fig. 23). During early developmental stages cell layers in the mesocarp underwent rapid plasmolysis and further deterioration continued (Fig. 24). The cells were almost empty, yet exhibited numerous, irregularly-outlined vacuolar compartments (Fig. 25). Cell walls were eventually crushed, leaving little space with cellular remnants (Fig. 26) during later development. The mesocarp remained such condition for some time during post-abscission.

DISCUSSION

The amount of growth and modification that can occur during fruit development is known to be quite variable (Mauseth, 1988). Development of hesperidium corresponds with changes in growth rate. The development of hesperidium fruit has been distinguished into three major stages in several studies; namely stage I, II and III (Spiegel-Roy & Goldschmidt, 1996, references therein). The peel, pericarp, begins to change color when the fruit approaches maturity. The stage III is the fruit

maturation and ripening phase, in which the exocarp changes to orange and the peel increases a little in thickness. Although the oil glands in the exocarp was not included in the present study, the shape of the oil glands is also known to change due to stretching of the pericarp (Holtzhausen, 1982). Further, a direct correlation is known to exist between the oil content and the surface area of the hesperidium (Kesterson & Braddock, 1995).

The pericarps of the hesperidium do differ among various *Citrus* taxa (Spiegel-Roy & Goldschmidt, 1996). Ripening of hesperidium is quite different from that of most other fruits by not having starch components approaching maturation stage. The pricarp is particularly rich in pectic substances which may make up 20~40% of dry matter (Kale & Adsule, 1995), but in *C. reticulata* these substances are probably in the exocarp, if produced, since *C. reticulata* has very thin mesocarp within the pericarp.

Unlike typical hesperidium having three morphologically distinct regions of pericarp, *Citrus reticulata* examined develops very thin mesocarp layer. The exocarp consists of small, dense collenchyma cells which contain chromoplasts including oil cavities. This layer consists of the colored portion of the peel with multicellular oil glands and contains the carotenoids, which give the characteristic color to the different hesperidium. The exocarp cells of the developing fruits contains the normal constituent of organelles such as plastics, mitochondria, rER, and ribosomes. This is an indication that they are reasonably active cells. Therefore, it is highly possible that the metabolic functions in the exocarp cells of hesperidium are operative throughout the development and maturation (Spigel-Roy & Goldschmidt, 1996). On the other hand, the mesocarp consists of loosely connected cells. This tissue has a spongy nature and is colorless to white because of the numerous air spaces in it. Regarding on features of the hesperidium endocarp, the following references are recommended, since the present study dealt with only the pericarp features (Fahn et al., 1974; Shomer & Ben-Gera, 1975; Koch et al., 1986; Nii & Coombe, 1988; Koch & Avigne, 1990; Tisserat et al., 1990; Burns et al., 1992, 1994; Spiegel-Roy & Goldschmidt, 1996).

The alteration of the chloroplast structure and the subsequent formation of chromoplasts are the well-known main ultrastrural changes in the exocarp associated with maturation in hesperidium. The mature chromoplasts are characterized by large osmiophilic globules and only few internal membranes. With some coordination, the pericarp layers in *Citrus reticulata* wall behave as separate tissues during maturation. The changes examined in the exocarp are comparable to the senescence of other chlorophyllous tissues (Goldschmidt, 1988), as shown in transformation of the chloroplasts into chromoplasts. Accumulation of carotenoid almost coincides with the disappearance of chlorophyll. Just prior to the carotenoid build-up, the transition from carotenoids of the photosynthetic chloroplasts to the intensely colored carotenoids of the chromoplasts are supposedly occurred (Thomson, 1966; Gross, 1987; Spiegel-Roy & Goldschmidt, 1996).

The variations in the vacuoles, and the changes in the ultrastructural features of the vacuoles during the development were of particular interest. The cells in the exocarp had several to numerous vacuoles in most of the examined materials. Considerable variation in the vacuoles, membranous configuration associated with the tonoplast, and changes in the vacuolar content has been revealed in *Citrus sinensis* (Thomson & Platt-Aloia, 1976) and other hesperidiums (Spiegel-Roy & Goldschmidt, 1996). These changes and variations probably indicate that the vacuoles have an important role as lysosomal compartments in accumulating and recycling cellular material during these stages. At later stage of the development, lipid bodies filled the vacuoles are frequently associated with vacuoles, chromoplasts, and other organelles. A reduction in the lytic and recycling capacity has been strongly suggested for the loss of cytoplasmic components (Thomson & Platt-Aloia, 1976).

Concomitant with the cytoplasmic changes, changes

associated with the plasma membrane, vacuolar membrane, and cytoplasmic vesicles are also noted. With the development, membrane outgrowth increases considerably in size and number. The cytoplasmic vesicles are often associated with the plasmodesmata within primary pit fields, and some are apparently free within either the vacuoles or inner cytoplasm. The small electron-dense globules, lipoidic nature, are attached to these membranes in *Citrus reticulata*. The variations in the plasma membrane, and the changes in the ultrastructural features of the vacuoles during the development are of particular interest. Occasionally small vesicles forming a large cluster are present within the vacuoles and/or in the cytoplasm, and small lipid bodies are associated with both inner and outer surface of the vacuolar membrane. Of particular interest was the presence of numerous membranous configurations within the vacuoles. Myelin-like membranous configurations extended from the vacuolar membrane, as well as from the cytoplasm and plasma membrane.

Formation of large osmiophilic globules are noticed in the exocarp cytoplasm. During later development and towards the maturation, there is an increase in the number and size of the large globules. Eventually, small vesicles or globules probably fuse together with the existing vesicles or larger globules. In many instances the membranes are in contact with the globule or closely adjacent to the globules. The lipid bodies in various sizes and shapes are often associated with the vacuolar membrane. Small to large lipid bodies are also common in the cytoplasm and they are often associated with organelles, particularly mitochondria and plastids.

The cytoplasmic vesicles and vacuolar pockets presumably represent a pathway for movement of material into the vacuole (Thomson & Platt-Aloia, 1976). The vacuoles in plants has probably a lysosomal or lytic function. In the late-senescent fruits, large osmiophilic bodies are also associated with, and accumulated within the vacuoles. The migration of lipid material into vacuoles or towards other cellular organelles as noted in the current study is probably a normal function in many plant tissues, and may be related to the lytic function as suggested by Thomson & Platt-Aloia (1976). These lipid bodies are supposedly playing an important role in cellular degradation particularly during senescence and post-abscission stage.

Invagination and fission of the prevacuolar compartment (PVC) membrane creates small internal vesicles, which upon fusion of the PVC with the vacuole are released into the interior of the vacuole. A number of different types of protein at the surface of the membrane are known to be involved in vacuolar vesicle targeting (Robinson et al., 2000). Plant cells have a PVC which may be a multivesiculate in nature. Multivesicular bodies have been frequently described in the plant literature (Robinson et al., 1998a, b). Three possibilities exist for the arrival of the contents and the membrane of the PVC into the vacuole; maturation and gradual transformation, fusion of the PVC with a pre-existing vacuole, and vesicle transport (Robinson et al., 2000). The fusion of a cluster of vesicles, so-called multivesicular bodies, with vacuoles has been frequently observed in *Citrus reticulata*. A possibility of transport out of the vacuoles to the cytoplasm, however, can not be ruled out.

Transformation of plastids and membrane outgrowth is considered to be the major physiological trait affected by growth substances in the hesperidium (Spiegel-Roy & Goldschmidt, 1996). The pericarp has been suggested as a center of hormonal regulation in the developing hesperidium (Monselise, 1978, 1986; Spiegel-Roy & Goldschmidt, 1996). The structural changes underlying developmental, particularly to senescence, processes in various hesperidium will be reported in the separate paper.

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<국문초록>

Citrus reticulata 감과 과피조직 내에서 탈리를 전후하여 신속하게 진행되는 특정 미세구조의 분화양상을 전자현미경적으로 연구하였다. 특히, 색소체 전환이 진행되어 뚜렷한 외과피층을 이루는 후각세포 내에서 일어나는 미세소기관 분해물질의 형성 및 특성에 초점을 두어

연구하였다. 색 변화가 수반된 발달 중의 외과피 유세포들은 세포벽이 비후되면서 1차공역이 잘 발달하는 후각세포로 분화하며, 대부분의 색소체는 유색체로 전환되어 티라코이드는 거의 소실되고 지질입자를 다량 함유하는 불규칙적인 형태로 변형되었다. 이때 세포막이나 액포막에서는 국부적으로 형성된 수많은 소낭들이 집적되어 세포질 내부로 이동하였고, 세포간 1차공역에 발달하는 원형질연락사를 통한 소낭의 집적현상 또한 빈번히 일어났다. 집적된 소낭들은 융합되어 전자밀도가 높은 구형 또는 일정한 형태가 없는 구조(EDB)로 되며, 이동하여 엽록체, 미토콘드리아 등의 주요 세포소기관을 점차 포위하였다. 소액포들은 융합하여 거대액포가 되며, 세포질 및 EDB에 포위된 소기관들은 점차 분해되기 시작하였다. EDB는 리파제 확인실험에 의해 지질성 물질로 이루어진 구조로 확인되었다. 과피발달 초기에 선행된 중과피 분화는 세포 내에서 급격히 진행된 융해현상에 의해 수많은 소액포들로 이루어진 거대한 세포 공강을 형성하며 밀착되어 불규칙적인 세포벽과 함께 해면성으로 되었다. 이와 같이 본 연구에서는 감과가 식물체에서 분리되기 직전에서부터 탈리 후까지 일어나는 과피의 발달 및 노화현상을 추적하였다.

FIGURE LEGENDS

Abbreviations: C = chloroplast, Cm = chromoplast, E = pericarp epidermis, EDB = electron dense body, F = phytoferritin, G = Golgi vesicles, L = lipid droplet, M = mitochondria, Mb = microbody, N = nucleus, P = parenchyma cell, Pl = plastid, PD = plasmodesmata, pm = plasma membrane, T = tonoplast, V = vacuole, W = cell wall.

Figs. 1 21: exocarp, Figs. 22 26: mesocarp. Figures 1 3 exhibit the pericarp before the hesperidium abscission, while the others represent the pericarp post abscission.

Fig. 1. Cells from the outermost exocarp parenchyma with no intercellular spaces. Bar = 5.5 μm.

Fig. 2. Chloroplast with grana found in growing exocarp. Note initiation of the primary pit field with numerous plasmodesmata (arrows) and the peculiar pattern of peripheral thylakoid in the stroma (arrowhead). Bar = 1.2 μm.

Fig. 3. Cells changing into collenchyma cells by developing unevenly thickened walls. Most of the plastids are chromoplasts. Bar = 5 μm.

Fig. 4. Chromoplasts with lipid droplets and peripherally located, single stranded curved thylakoids. Bar = 1.5 μm.

Fig. 5. Mitochondria and microbodies within the dense cytoplasm of the exocarp collenchyma. Bar = 0.75 μm.

Fig. 6. Part of the cell membrane, clearly demonstrating condensation of electron dense materials (left, 1), and vesicle formation (right, 2) originating from the plasma membrane. Arrows indicate direct involvement of the plasma membrane. Bar = 0.45 μm.

Fig. 7. Dilated plasma membrane (arrows) enclosing numerous small vesicles and fibrous materials (arrowhead). Bar = 0.25 μm.

Fig. 8. Membrane bounded vesicles (arrowheads) originating from the tonoplast. Bar = 0.7 μm. Inset: Low magnification of Fig. 8 showing close proximity of the vesicle cluster (double arrowheads) to the chromoplast. Bar = 0.25 μm.

Figs. 9 10. Clustering of small vesicles (arrowheads) via plasmodesmata. Bar equals 2.4 μm and 4 μm in Figures 9 and 10, respectively.

Fig. 11. Cells packed with numerous, small to large, vacuolar compartments (arrowheads) and other scattered organelles. Arrows indicate primary pit fields. Bar = 4.2 μm.

Fig. 12. Chromoplast with a cluster of vesicles (arrowheads) near the cell wall. Bar = 0.65 μm.

Fig. 13. Vesicles presumably originating from the Golgi apparatus approach mitochondria from both the central vacuole (arrows) and cytoplasm (arrowheads). Bars = 0.7 μm.

Fig. 14. Coated vesicles (arrowhead), most likely originating from the plasma membrane, enclosed by electron dense bodies of different origin (1, 2). Note tail like strand on second body (2). Bar = 0.4 μm.

Fig. 15. Plastids surrounded by numerous EDB (arrowheads) before further aging. Bar = 2.3 μm.

Fig. 16. Typical collenchyma cells with a large vacuole, chromoplasts (arrowheads), and unevenly thickened walls. Arrows indicate a primary pit field. Bar = 7.7 μm.

Fig. 17. Part of collenchyma cell wall with primary pit fields (arrows). Bar = 0.85 μm.

Fig. 18. Close up of plasmodesmata from Fig. 17, forming median cavity (arrowhead, longitudinal section). Inset: Cross section of a primary pit field exhibiting numerous plasmodesmata. All bars = 333 nm.

Fig. 19. Enlarged view of chromoplasts with irregularly shaped EDBs (arrowheads) shown in Fig. 16. Bar = 2.5 μm.

Fig. 20. Chromoplast processed by the lipase staining method. Note the lack of electron density in lipid droplets from the chromoplast and also note the neighboring irregularly shaped body (arrowhead). Compare to Fig. 19. Bar = 1 μm.

Fig. 21. Part of chromoplasts processed by the same method as in Fig. 15, showing phytoferritin within stroma. Bar = 1.5 μm.

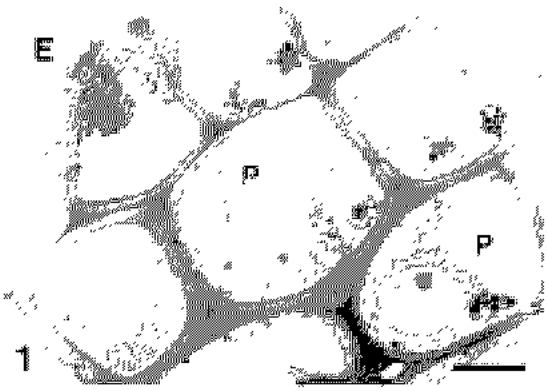
Fig. 22. Glancing section exhibiting relatively dense cytoplasm of young mesocarp cell. Note irregular, uneven, thick wall between cells. Bar = 2.4 μm. Inset: Plastids found within mesocarp cell. Bar = 1 μm.

Fig. 23. Periphery of the cell exhibiting cellular degradation. Components of the cell wall become loose and microtubules become apparent (arrows). Bar = 1 μm.

Fig. 24. Cell layers undergoing drastic plasmolysis (arrowheads) and further deterioration. Bar = 4.2 μm.

Fig. 25. Transverse section of the cell shown in Fig. 24, exhibiting numerous, irregularly shaped vacuolar compartments. Bar = 3 μm.

Fig. 26. Cell walls being crushed, leaving little space (arrowheads) with cellular remnants during later development. Bar = 5.8 μm.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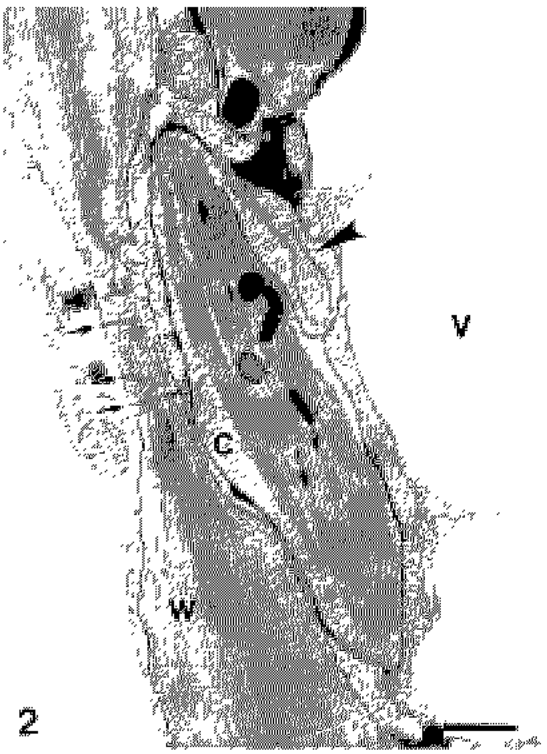
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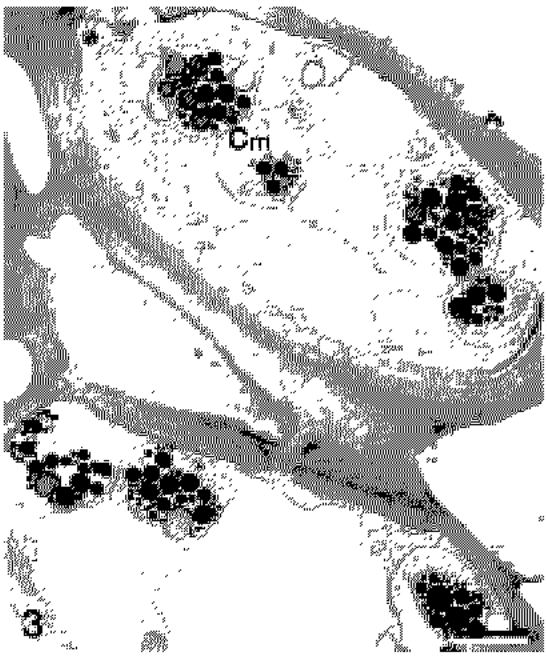
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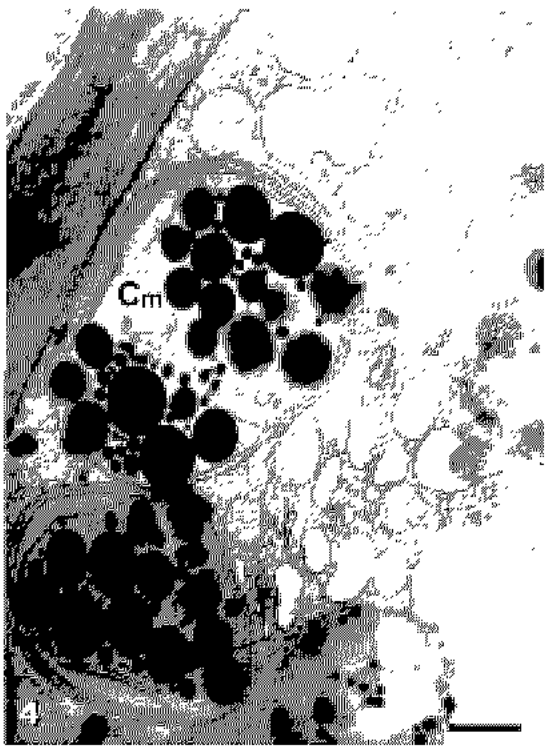
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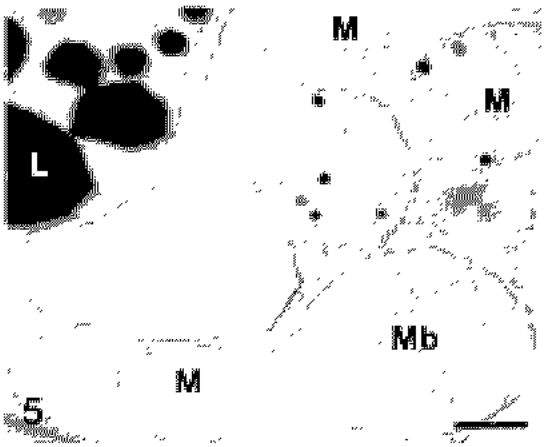
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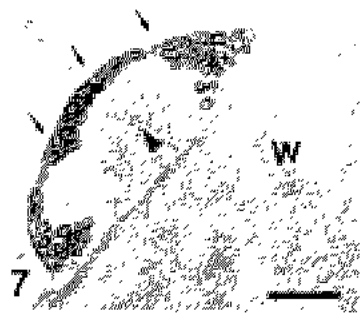
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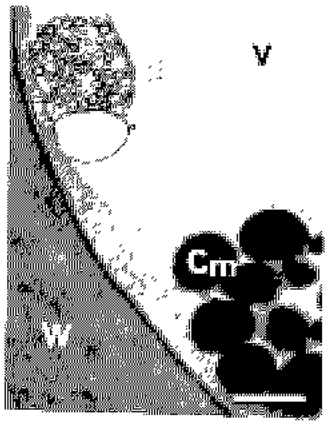
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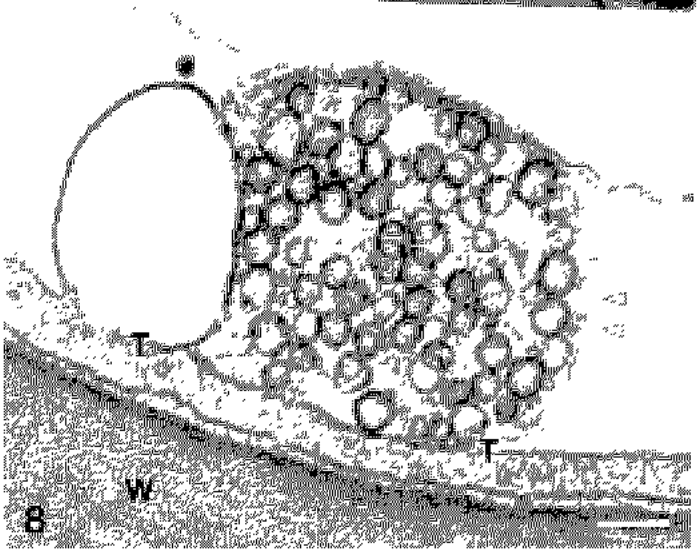
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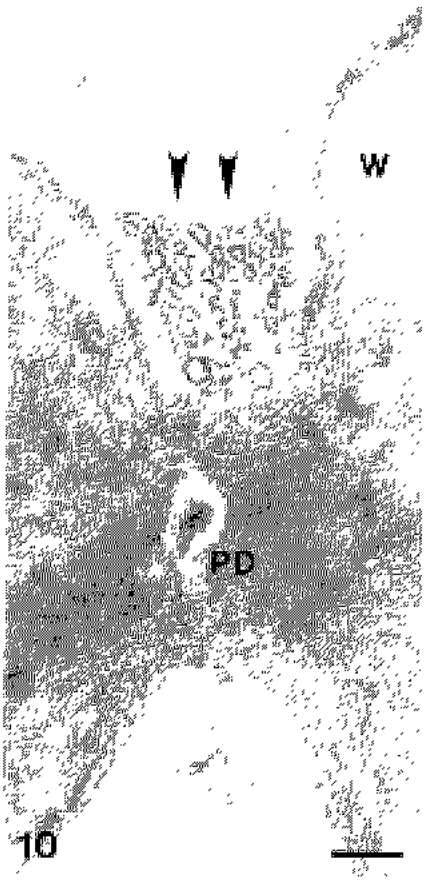
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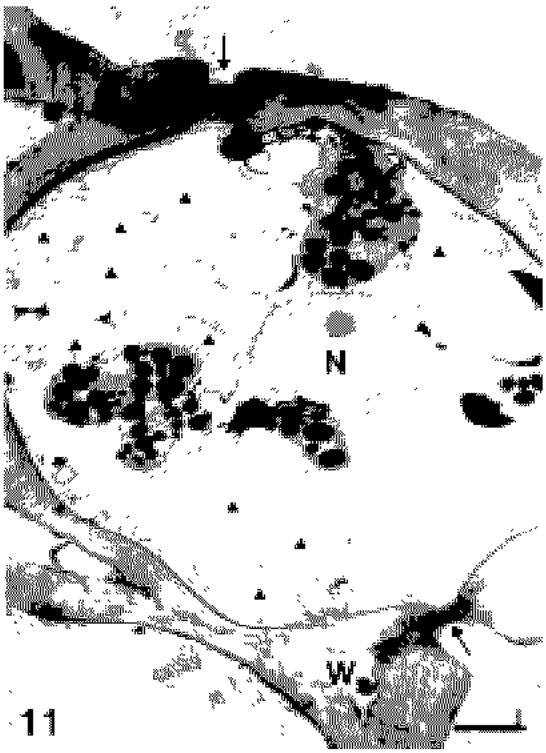
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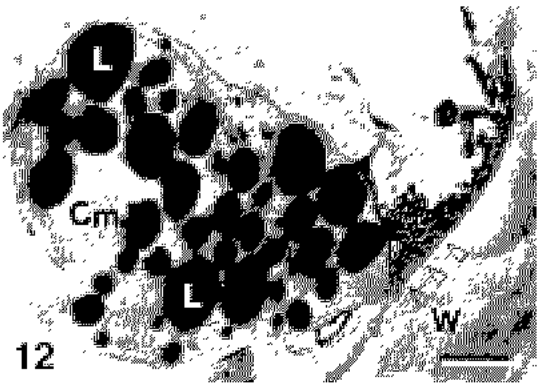
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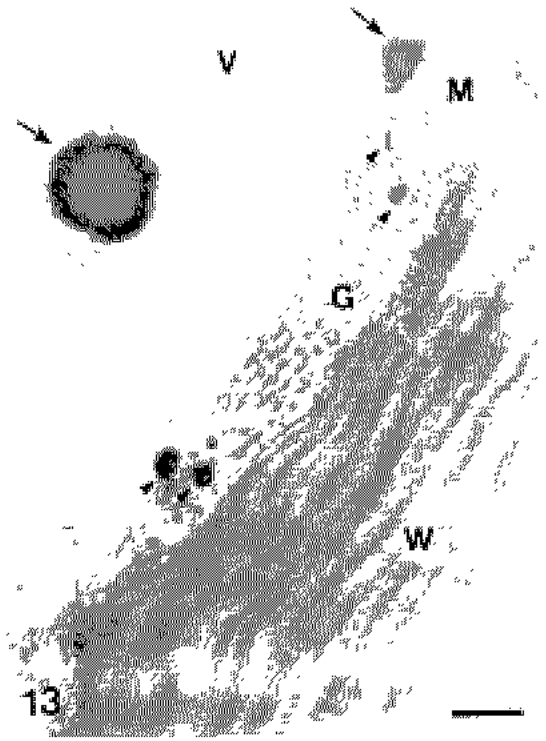
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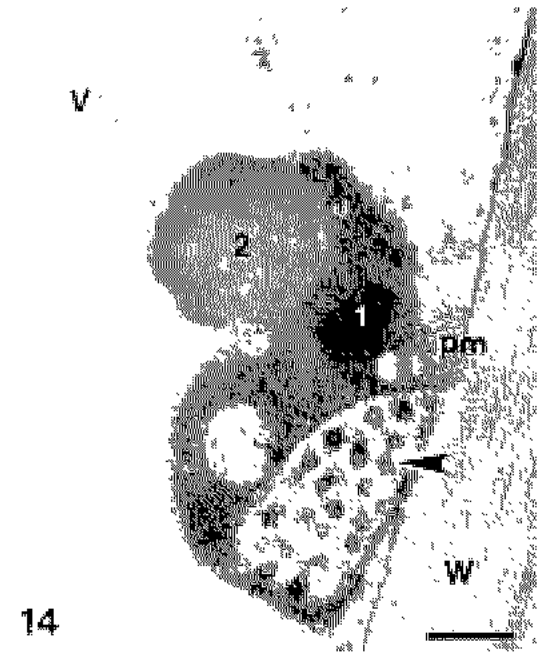
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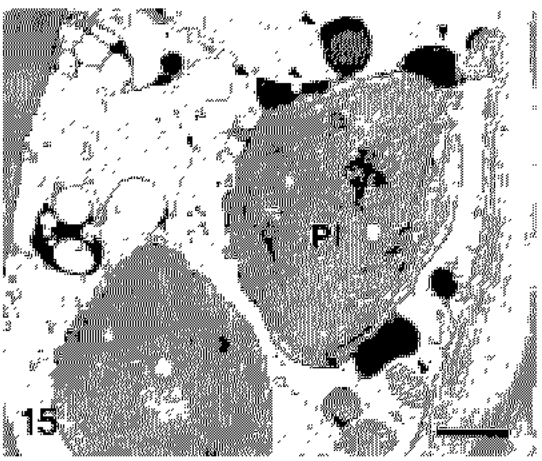
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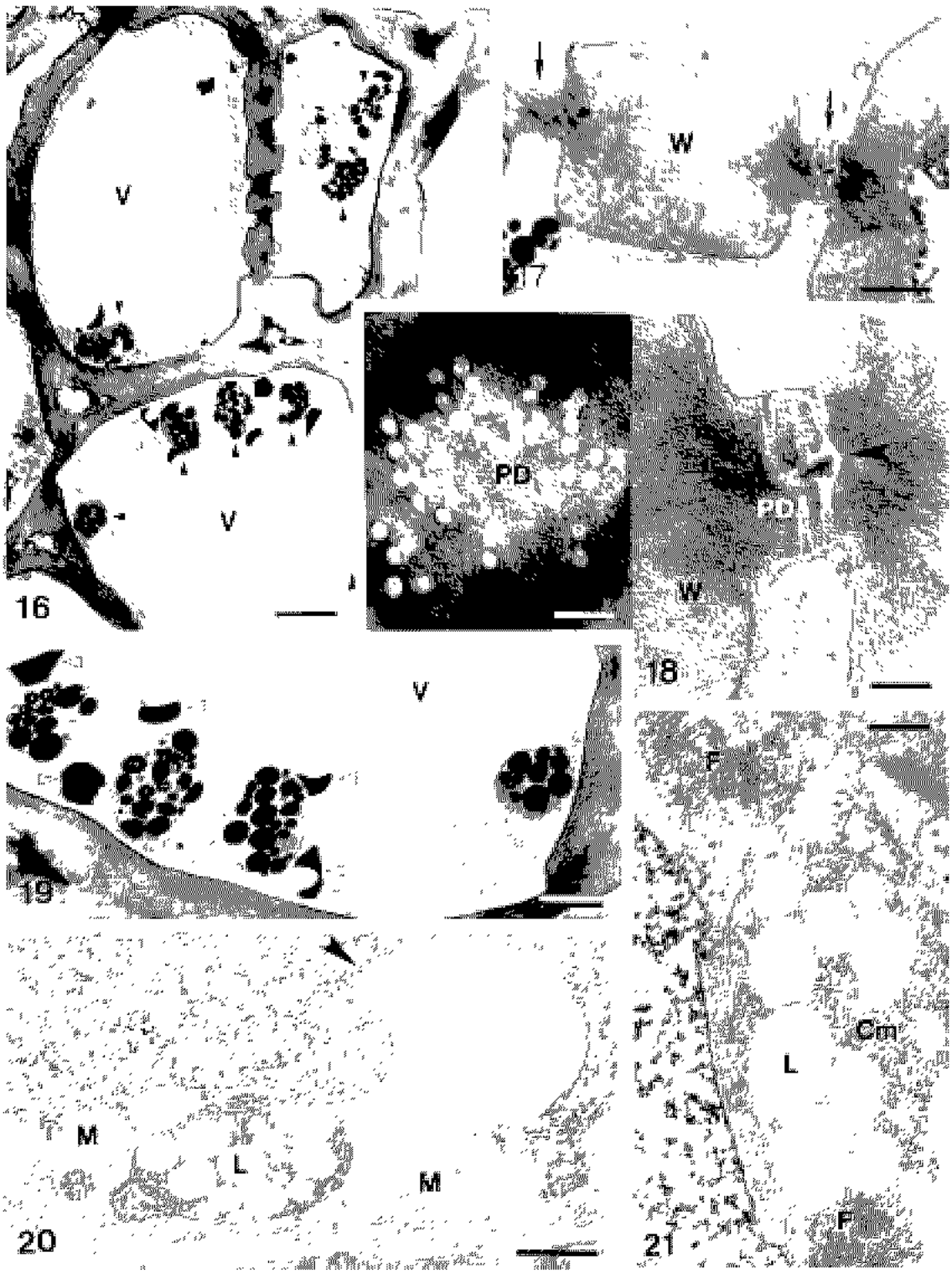
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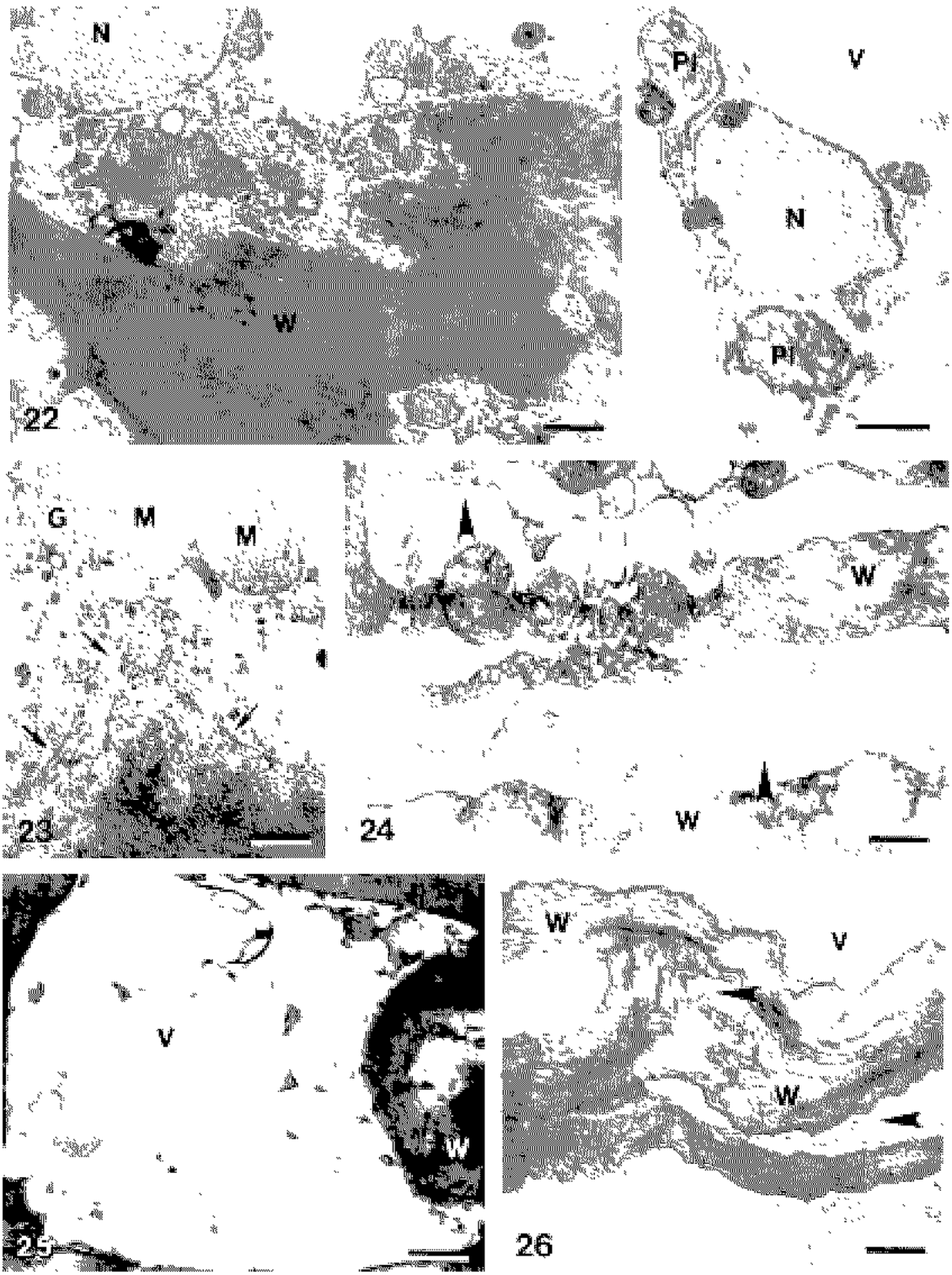
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