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Histochemical and Ultrastructural Changes in the Haustorium of Date (*Phoenix dactylifera* L.)

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Summary

During imbibition of *Phoenix dactylifera* embryos, all cotyledon cells show the same changes: protein and lipid bodies degrade, smooth endoplasmic reticulum (ER) increases in amount, and dictyosomes appear. At germination, the distal portion of the cotyledon expands to form the haustorium. At this time, epithelial cells have a dense cytoplasm with many extremely small vacuoles. Many ribosomes are present along with ER, dictyosomes, and mitochondria. The parenchyma cells have large vacuoles and a small amount of peripheral cytoplasm. Between 2 and 6 weeks after germination, epithelial cells still retain the dense cytoplasm and many organelles appear: glyoxysomes, large lipid bodies, amyloplasts, large osmiophilic bodies, and abundant rough and smooth ER which appear to merge into the plasmalemma. A thin electron-transparent inner wall layer with many small internal projections is added to the cell walls. Starch grains appear first in the subsurface and internal parenchyma and subsequently in the epithelium. Lipid bodies, glyoxysomes, protein, and osmiophilic bodies occur in the epithelial and subepithelial cell layers but not in the internal parenchyma. At 8 weeks after germination, the cytoplasm becomes electron transparent, vacuolation occurs, lipid bodies and osmiophilic bodies degrade, and the endomembranes disassemble. After 10 weeks, the cells are empty. These data support the hypothesis that the major functions of the haustorium are absorption and storage.

Keywords: Palm; Haustorium; Date; *Phoenix;* Germination.

1. Introduction

Palms exhibit many peculiar features during the processes of germination and seedling development. Seeds contain small embryos and copious amounts of endosperm. The distal portion of the cotyledon expands to form a haustorium which remains within the seed and expands tremendously as the endosperm disappears, until it nearly fills the seed. For example, coconut (*Cocos nucifera*) has a haustorium which grows for months and first absorbs the liquid endosperm (coconut milk) and finally occupies the entire cavity of the nut (TROLL 1935). This process takes only 10 weeks for date (*Phoenix dactylifera*) seedlings (DEMASON 1984). The proximal portion of the cotyledon elongates to "plant" the root and epicotyl axis.

The original structural and physiological observations of date germination were made by SACHS (1862). LLOYD (1910) also touched on structural and histochemical changes in the endosperm and haustorium of germinating date seedlings. Although no ultrastructural studies and no further anatomical studies have been done on the haustorium of palms, physiological and biochemical studies have been conducted on the endosperm cell wall composition in date and other genera (MEIER and REID 1982) and on lipid metabolism during germination of the oil palm (Oo and STUMPF 1983).

The scutellum of the grass embryo is also a cotyledon, and is therefore partially homologous to the palm haustorium. It may also perform functions similar to those of the haustorium (BROWN and MORRIS 1890, SMART and O'BRIEN 1979, NEGBI 1984). Much anatomical and some recent ultrastructural and histochemical work has been done on the scutellum. NEGBI (1984) has described 4 types of scutella in grasses based on structural and developmental differences. There are structural similarities in cell types, storage bodies, and vascular tissue arrangement in all grass scutella and the

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date cotyledon (DEMASON and THOMSON 1981); however, there are significant differences in growth parameters or in developmental timing the two organs (DEMASON 1984). These differences probably reflect the greater longevity of the haustorium over the scutellum.

This paper is one of a series which recounts the sequences of germination in the date palm. The structure of the tissues in the resting seed has been described (DEMASON and THOMSON 1981, DEMASON *et al.* 1983). The sequence of endosperm degradation has been described (DEMASON *et al.* 1985). And the mechanism of haustorium expansion and vascular tissue maturation have been examined (DEMASON 1984). The ultimate goal of this research is to determine the function of the haustorium.

In this paper, the histochemical and ultrastructural changes which occur during imbibition, development, and senescence of the date haustorium are described. I was especially interested in 1. characteristics which relate to its function, and 2. features comparable to the changes observed during the same sequence in the grass scutellum. The grass scutellum is thought to play a major role in controlling the mobilization of endosperm reserves.

2. Materials and Methods

Seeds of *Phoenix dactylifera* L. var. Medjool were obtained from the Coachella Valley, California. They were soaked in aerated water for 4–6 days before planting in vermiculite. Embryos and seedlings were dissected carefully from seeds at regular intervals after planting or after germination.

For light microscopy, tissue was fixed in 2% glutaraldehyde in 50 mM phosphate buffer, pH 7.2, dehydrated in an ethanol or acetone series, and embedded in JB-4 plastic (Polysciences) or Spurr's epoxy resin (SPURR 1969). Tissue embedded in Spurr's resin was post-fixed in 1% osmium tetroxide in the same buffer in the refrigerator overnight. Sections were stained with buffered toluidine blue at pH 7, aniline blue black (according to FISHER 1968), or PAS (according to O'BRIEN and MCCULLY 1981). PAS-stained material was first blocked with 2,4-dinitrophenyl-hydrazine. Sections were photographed on a standard Zeiss microscope and a Nikon microflex AFM on Kodak 2415 film.

For electron microscopy, tissue was fixed in 2% glutaraldehyde in 50 mM phosphate buffer or cacodylate buffer at pH 7.2 for 4–6 hours at room temperature, rinsed in buffer, and post-fixed in 1% osmium tetroxide in the same buffer in the refrigerator overnight. Some tissue was fixed in 2% glutaraldehyde and 2% tannic acid in 50 mM cacodylate buffer (pH 7.2), rinsed in buffer, and post-fixed in osmium as above. Tissue was dehydrated in an acetone series, embedded in Spurr's epoxy resin, sectioned with glass knives on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed on a Philips 400 electron microscope.

Catalase staining was performed with diaminobenzidine (DAB) as described by SEXTON and HALL (1978), except that the pH was adjusted to 10 instead of 9. The three controls which were run simultaneously included no substrate, KCN (inhibitor of catalase and peroxidase), and 3-amino-1,2,4-triazole (inhibitor of catalase) (SEXTON and HALL 1978). Fixation, dehydration, embedding, and sectioning were performed for the electron microscope as above. Grids were stained with lead citrate only before viewing on the Philips 400.

3. Results

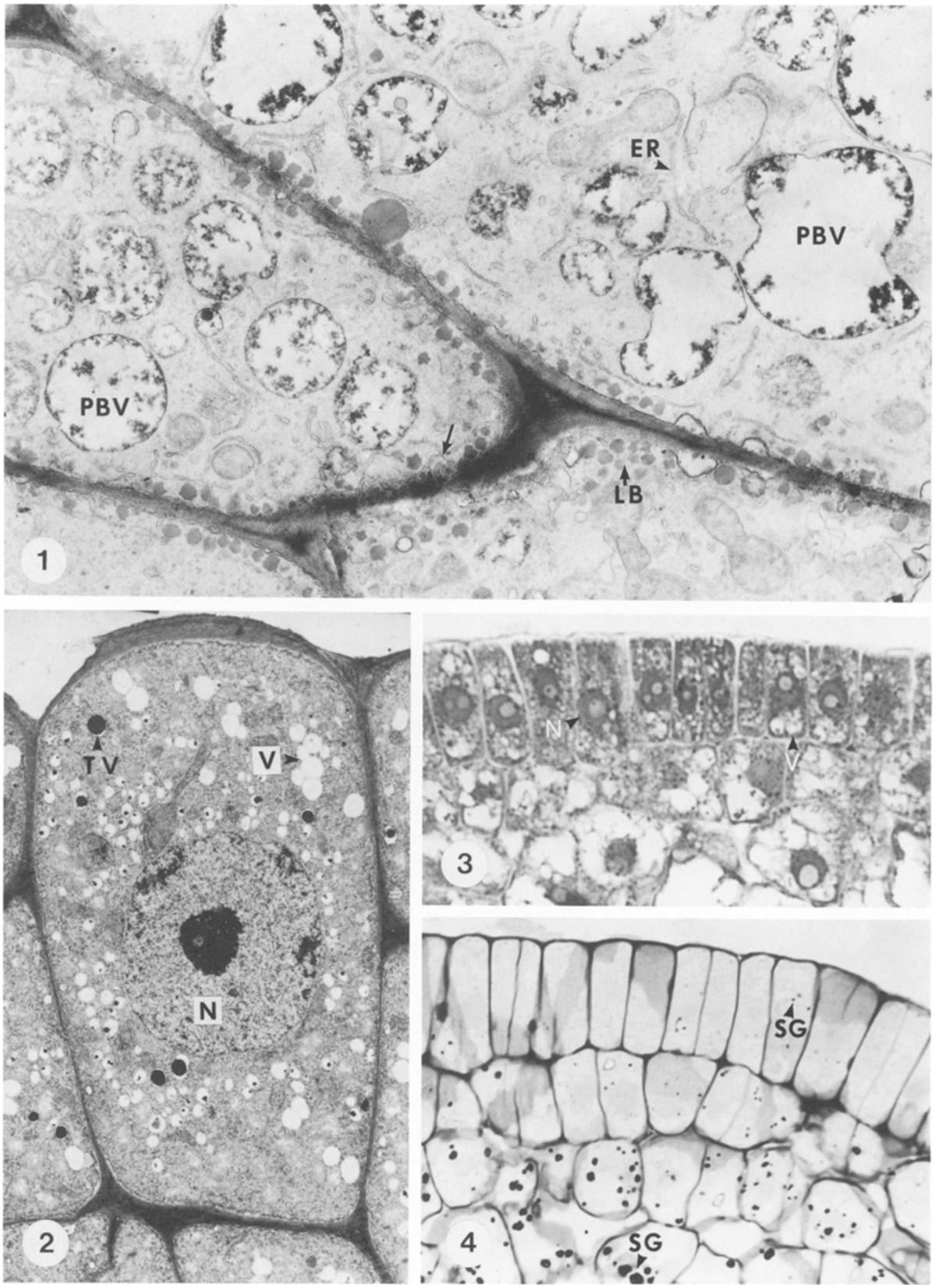
3.1. Imbibition

In the resting seed, epithelial cells and parenchyma cells of the cotyledon contain prominent and variably sized protein bodies and numerous small lipid bodies (0.5 μm diameter) that surround the plasma membrane and sometimes the protein bodies. No endoplasmic reticulum (ER) or dictyosomes are seen (DEMASON and THOMSON 1981). Within 3–6 days of planting, however, the protein bodies' contents consist of dark flocculent material arranged peripherally along the membrane (Fig. 1). The outlines are no longer spherical but are irregularly shaped. The small lipid bodies also take on an irregular outline. Long, tubular and branched smooth ER becomes evident, and it often appears to merge into the plasmalemma (Fig. 10 and arrow, Fig. 1). The plasmalemma itself is not smooth along the cell wall but has an undulating appearance. Dictyosomes also appear (not shown). The cytoplasm appears thin, and few ribosomes are seen.

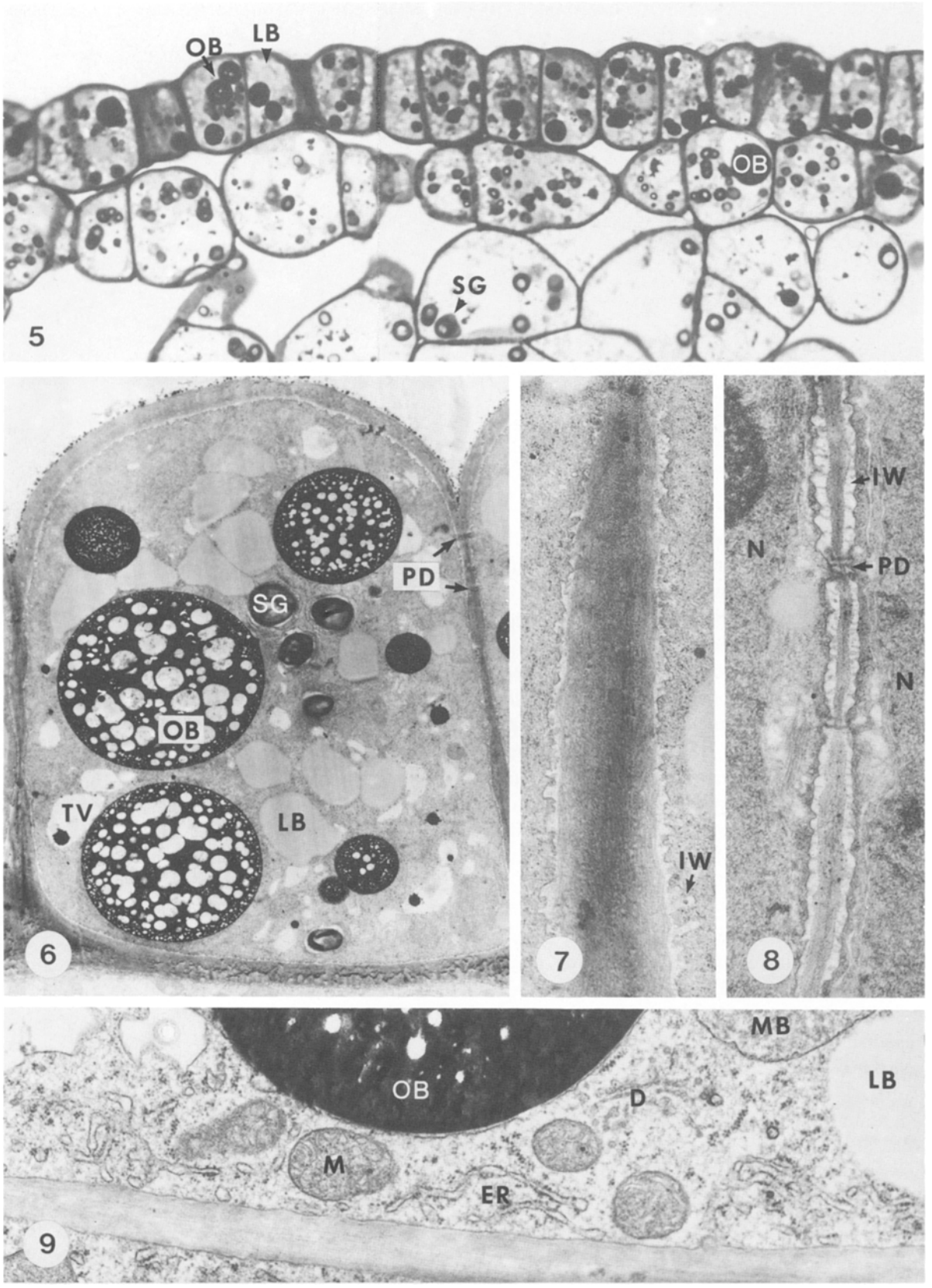
3.2. Germination

At germination, the protein and lipid bodies have disappeared. In the epithelial cells, there is a centrally located nucleus and numerous small vacuoles, some of which contain a uniformly dense, osmiophilic inclusion which may be tannin (Figs. 2 and 6). The general cytoplasm is very dense and, consequently, stains darkly for protein (Fig. 3). Numerous ribosomes are present in the cytoplasm, and some rough ER occurs. The plasmalemma remains undulate in outline, and an inner electron-transparent wall material accumulates between the plasmalemma and the thick primary wall. The cell wall does not stain with protein stains (Fig. 3) but does stain positively with PAS (Fig. 4).

The subepithelial cells contain large vacuoles and are not so densely staining for protein (Fig. 3). Starch accumulates at this stage. It appears first in internal parenchyma, with a few grains present in the subepithelial and epithelial cells (Fig. 4).



Figs. 1–4. Imbibition and germination. Fig. 1. Epithelial cells during imbibition (6 days after planting). Arrow indicates area where ER apparently grades into plasma membrane. × 11,000. Fig. 2. Epithelial cell at germination. × 5,000. Fig. 3. Protein-stained section of epithelium, subepithelium, and adjacent parenchyma from haustorium at germination. × 325. Fig. 4. PAS-stained section of epithelium, subepithelium, and adjacent parenchyma from haustorium at germination. × 325. *ER* endoplasmic reticulum, *LB* lipid body, *N* nucleus, *PBV* protein body vacuole, *SG* starch grain, *TV* tannin (?) vacuole, *V* vacuole



Figs. 5–9. Mature haustorium. Fig. 5. Epoxy section of epithelium, subepithelium, and adjacent parenchyma of haustorium 4 weeks after germination. × 325. Fig. 6. Epithelial cell from haustorium 4 weeks after germination. × 5,100. Fig. 7. Cell wall between epithelial cells. × 20,000. Fig. 8. Cell wall between parenchyma cells. × 18,000. Fig. 9. Tannic acid fixation of epithelial cell. × 22,000. *D* dictyosome, *ER* endoplasmic reticulum, *IW* inner wall, *LB* lipid body, *M* mitochondrion, *MB* microbody, *N* nucleus, *OB* osmiophilic body, *PD* plasmodesmata, *SG* starch grain, *TV* tannin (?) vacuole

Table 1. *Summary table*

Organelles	Imbibition	Germination	2–6 weeks	8 weeks	10 weeks
Protein bodies	degrading diameter 4–8 μm	absent	absent	absent	absent
Lipid bodies	degrading diameter 0.5 μm	absent	reappear diameter 3.5 μm	decrease in number	absent
Amyloplasts	absent	absent	present	disappearing	absent
Osmiophilic bodies	absent	absent	present diameter 7 μm	degrading	absent
Glyoxysomes	?	?	present diameter 2 μm	present	present
Dictyosomes	present	present	present	present	absent
Endoplasmic reticulum	increasing	abundant	abundant	decreasing and disorganized	absent
Cytoplasm	thin	dense	dense	thin	disorganized

3.3. Mature Haustorium (2–6 weeks)

The mature, functional haustorium has a distinct epithelial layer in which the cells are densely purple-staining (Fig. 5) and are tightly appressed to one another. A single subepithelial layer is apparent, although the cells are not so tightly appressed to one another or to the epithelium. The internal parenchyma is more irregularly shaped (Fig. 5). The subepithelium and internal parenchyma consist of vacuolate cells which are not densely staining with toluidine blue. Many storage bodies are present in the cells. Starch is present in the internal parenchyma, and starch, lipid, and osmiophilic bodies are present in the sub- and epithelial cells (Fig. 5). Starch is abundant in all cells (Fig. 13).

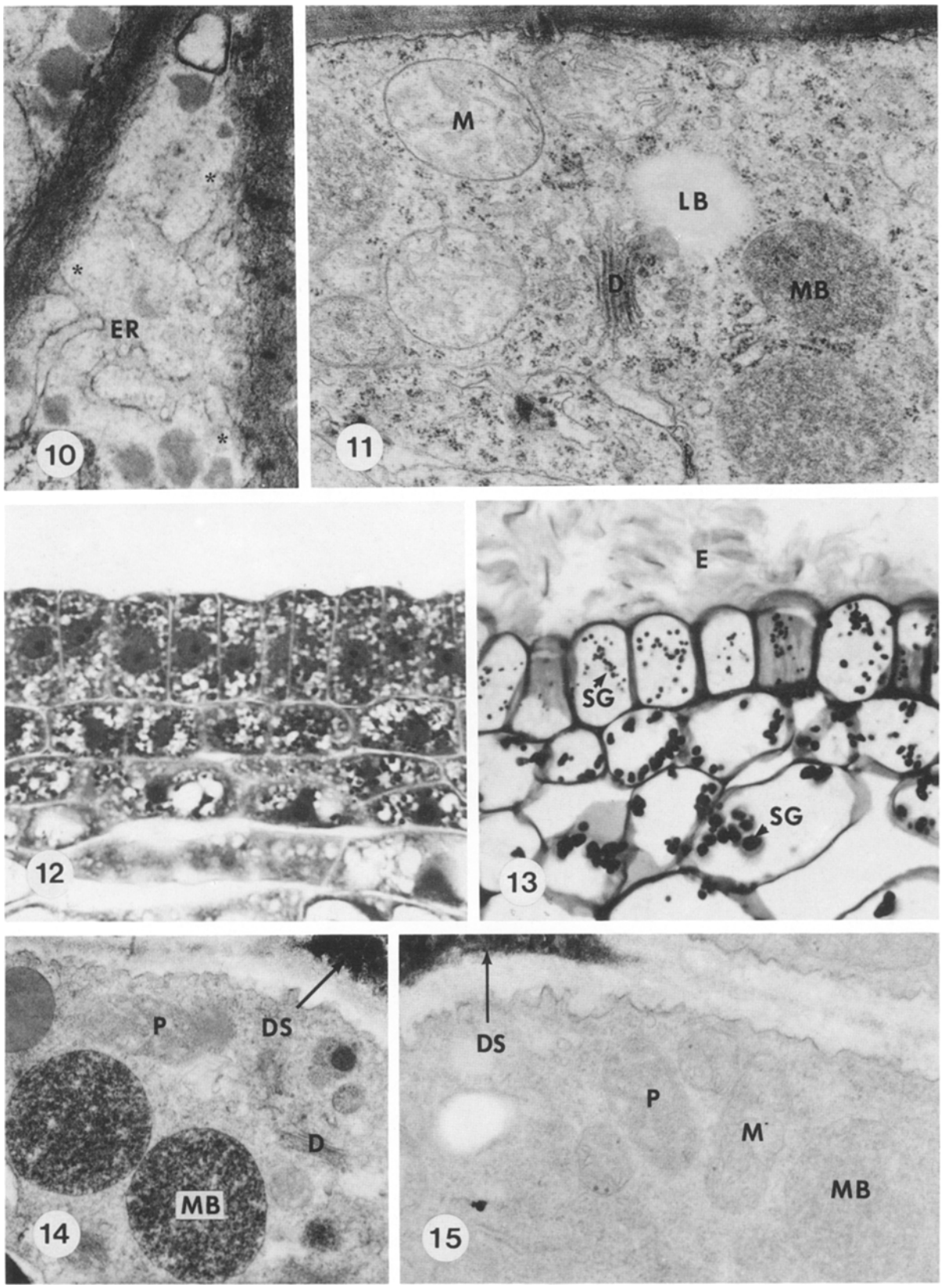
Ultrastructurally, the epithelial cells have dense cytoplasm, few small vacuoles, and are filled with inclusions. The inclusions consist of starch, large lipid bodies (approximately 3.5 μm in diameter) (Figs. 5 and 6), aniline blue-black positive bodies (approximately 2 μm in diameter) (Figs. 9, 11, 14, and 15), and extremely large osmiophilic bodies (approximately 7 μm in diameter) (Figs. 5, 6, and 9).

The aniline blue-black positive bodies have a single bounding membrane and a fairly dense granular content with no crystalline inclusions. Diaminobenzidine (DAB) staining occurs in these bodies (Fig. 14) and in the outer thickened wall of the epithelium. They do not stain when incubated in the aminotriazole control, but the cell walls do show staining (Fig. 15). No DAB staining occurs in the KCN control. I interpret these results along with the structural characteristics to mean that they are glyoxysomes and that peroxidase occurs in the walls.

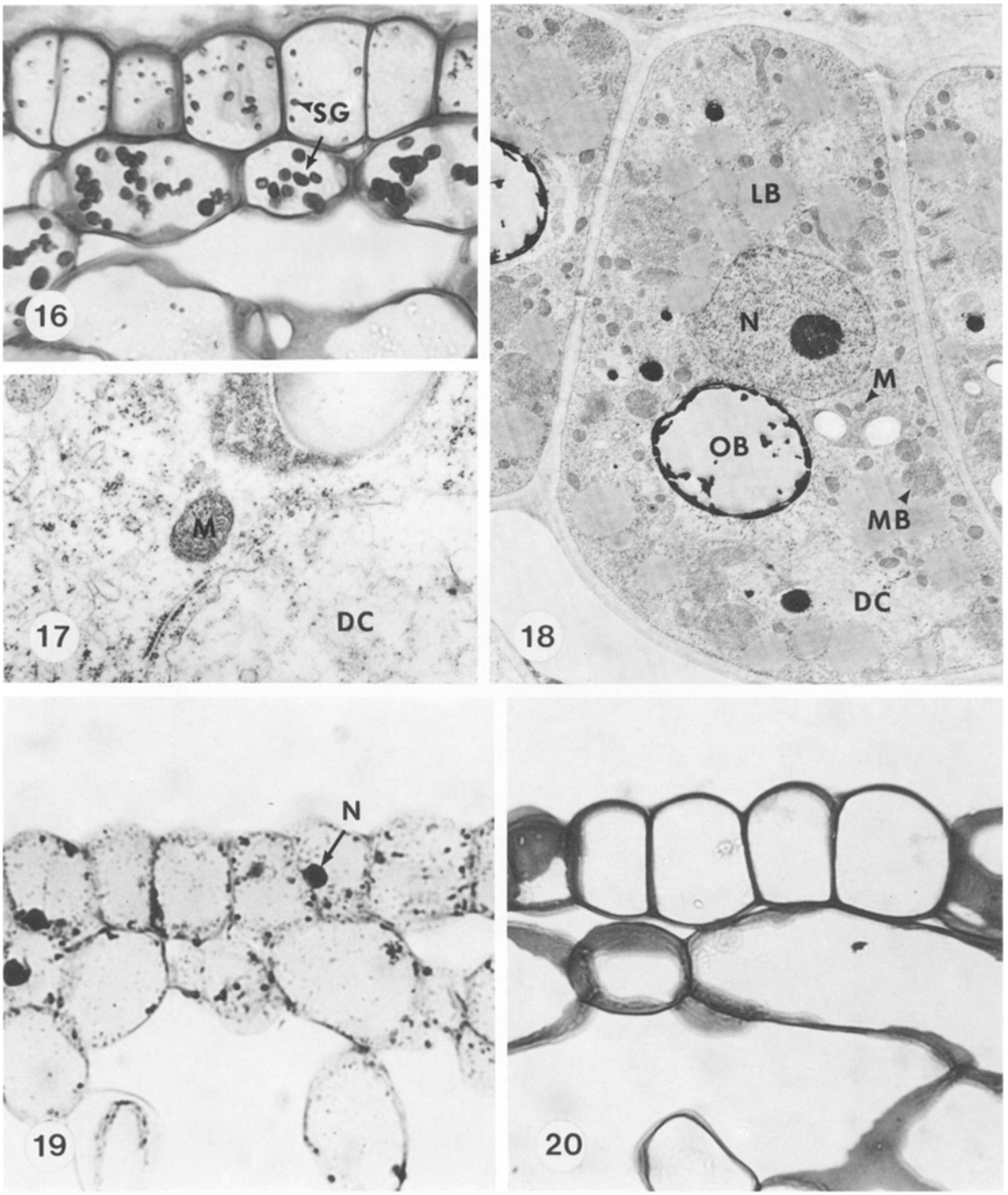
The osmiophilic bodies have a well-defined internal structure. Around the periphery, there are numerous small non-osmiophilic holes, whereas in the center of the body, there are large non-osmiophilic holes. Median and non-median sections of the bodies can be readily distinguished on the presence of the large holes. The tannic acid fixation shows that the bodies are membrane-bounded (Fig. 9). These bodies are dissolved during dehydration after fixation by glutaraldehyde alone and, therefore, do not occur in methacrylate sections (Fig. 12).

Cells in the epithelial layers also possess numerous polyribosomes and branching rough ER are present in the cytoplasm, especially abundant near the plasmalemma (Fig. 9). Grazing sections of cell corners continue to show ER merging into the plasmalemma as in Fig. 10. Small tannin-like inclusions are present in vacuoles (Fig. 6). Dictyosomes are present, although not particularly abundant, and are usually positioned near the cell periphery (Figs. 9 and 11). Mitochondria are abundant everywhere within the cell (Figs. 9 and 11).

The cell wall surrounding the epithelial cells is very thick (Fig. 7), although that around the internal parenchyma cells is thin (Fig. 8). Numerous plasmodesmata exist between epithelial cells and in contact areas between epithelial and parenchyma cells or between parenchyma cells alone (Figs. 6 and 8). The inner, electron-transparent wall layer also occurs around the entire epithelial cells and in contact areas between parenchyma cells (Figs. 6–8). This layer has many small inwardly directed projections reminiscent of transfer cell walls (Fig. 7). The cell walls stain with PAS but not with protein stains (Figs. 12 and 13).



Figs. 10–15. Epithelial structure. Fig. 10. Tubular endoplasmic reticulum in corner of epithelial cell during imbibition. * indicate areas where ER apparently grades into plasma membrane. × 27,000. Fig. 11. Ultrastructure of epithelial cell. × 31,000. Fig. 12. Protein-stained section of epithelium, subepithelium, and adjacent parenchyma from haustorium 4 weeks after germination. × 325. Fig. 13. PAS-stained section of epithelium, subepithelium, and adjacent parenchyma from haustorium 4 weeks after germination. × 325. Fig. 14. DAB-stained microbodies. × 20,000. Fig. 15. Aminotriazole control. × 25,000. *D* dictyosome, *DS* DAB-staining of outer wall, *E* endosperm, *ER* endoplasmic reticulum, *LB* lipid body, *M* mitochondrion, *P* plastid, *SG* starch grain



Figs. 16–20. Senescence. Fig. 16. PAS-stained section of epithelium, sub-epithelium, and adjacent parenchyma of haustorium 8 weeks after germination. × 325. Fig. 17. Fine structure of epithelial cytoplasm from haustorium 8 weeks after germination. × 5,000. Fig. 18. Epithelial cell from haustorium 8 weeks after germination. × 5,000. Fig. 19. Protein-stained section of epithelium, sub-epithelium, and adjacent parenchyma from haustorium 10 weeks after germination. Numerous small, darkly stained bodies are microbodies and mitochondria. × 325. Fig. 20. PAS-stained section of epithelium, subepithelium, and adjacent parenchyma from haustorium 10 weeks after germination. × 325. *DC* degenerating cytoplasm, *LB* lipid body, *M* mitochondrion, *MB* microbody, *N* nucleus, *OB* osmiophilic body, *SG* starch grain

These ultrastructural characteristics remain unchanged until approximately 8 weeks after germination.

3.4. Senescence (8–10 weeks)

At approximately 8 weeks after germination, changes in ultrastructure and storage body content occur. The cells on the lowermost and uppermost surfaces undergo these changes before those cells at the tips of the haustorium. Starch grains become smaller and less numerous in the epithelium first (Figs. 16 and 18). The osmiophilic bodies empty from the inside (Fig. 18). Lipid bodies remain longer than starch grains and the osmiophilic bodies. The ribosomes become disassociated from the ER, which also becomes disorganized (Fig. 17). The cytoplasm becomes less dense, and degenerating areas form which do not have continuous bounding membranes and contain thin flocculent material (Figs. 17 and 18). These vacuole-like areas continue to spread until they make up almost the entire cell (Fig. 19). Mitochondria and glyoxysomes remain abundant until the cytoplasm is lost (Figs. 18 and 19). By 10 weeks, starch is completely absent from the entire haustorium (Fig. 20). Negative cell wall staining by aniline blue-black and positive staining by PAS remains unchanged. After 10 weeks, all cells lack cytoplasmic contents, and the basal region of the cotyledon has dried and is severed from the seedling.

Tab. 1 summarizes the changes which occur in the epithelial cells over time from imbibition of the cotyledon to senescence of the haustorium at 10 weeks after germination.

4. Discussion

In a recent review on the structure and function of the scutellum in grasses, NEGBI (1984) divides scutella into four groups based on structure and development. The scutellum, which shows the most enlargement and therefore the most similarity in structure and probably also in function to the date haustorium, is the "*Melocanna* type". It enlarges significantly during seed maturation at the expense of the endosperm. The scutellum in these viviparous species is thought to function as a storage tissue during germination. The date haustorium, in contrast to the "*Melocanna* type", enlarges at the expense of the endosperm after germination, not before. No structural or ultrastructural studies have been done on this scutellum type for comparison with the palm haustorium.

The vascularization and ultrastructural studies which have been done on scutella have been done on species with the "scutellum *sensu stricto*" type of NEGBI (1984). The remainder of the discussion will be devoted to a comparison of the vascular structure and ultrastructural features of scutella and the haustorium of date. In this way we may be able to deduce an hypothesis of the major function of the haustorium.

Protein and lipid bodies are abundant in scutellar cells of resting cereal embryos, and as in date, they are degraded before germination (NIEUWDORP and BUYS 1964, SWIFT and O'BRIEN 1972, ZAMSKI 1973, SMART and O'BRIEN 1979 a). Degradation and metabolism of these storage materials probably provide energy and metabolites for the initial growth resulting in germination. By the time germination occurs, no protein or lipid bodies are present in any of the cotyledonary cells.

During the next stage of seedling development, many structural and ultrastructural changes occur in both the scutellum and the haustorium. The epithelium cells elongate normal to the scutellum surface and separate from one another along their anticlinal walls. Although in most species no increase in scutellum length or breadth occurs, often the epithelium is thrown into convolutions. The haustorium in date, in sharp contrast to the scutellum, enlarges dramatically in size and therefore in surface area. This enlargement is by a factor of 90 within the first 4 weeks after germination and several times more by 10 weeks (DEMASON 1984). The epithelial cells also increase in size in the later stages of haustorial growth; they approximately double in surface area. No cell separation occurs in the epithelium, but wrinkling or invagination does occur on the surface (DEMASON *et al.* 1985). The major advantage of enlargement, cell separation, and invagination might be to increase the surface area of contact between the haustorium/scutellum and the endosperm. This would facilitate secretion and/or absorption.

Starch and lipids build up during the first 2–6 weeks of seedling development. Concomitant with this is the increase in numbers of glyoxysomes and the appearance of large osmiophilic bodies. By 8 weeks after germination, lipid bodies, starch, and the osmiophilic bodies all disappear. The simultaneous appearance and disappearance of the starch and osmiophilic bodies suggest that the latter are composed of some sort of storage material. SACHS (1862) observed what he believed were large tannin bodies in the haustorium. LLOYD (1910) claimed that no tannin exists in the haustorium itself, but only in the proximal portion of

the cotyledon. Further characterization of these osmiophilic bodies will be made in a separate study.

Glyoxysomes have been observed in the scutella of corn (ZAMSKI 1973) and wheat (SWIFT and O'BRIEN 1972). They have also been isolated from corn scutella and characterized physiologically (LONGO and LONGO 1970, LONGO *et al.* 1972). Although no glyoxysomes have been observed in the haustorium of oil palm, it contains isocitrate lyase and malate synthetase (OO and STUMPF 1983), so glyoxysomes are undoubtedly present in it. The glyoxylate cycle is of course important in lipid metabolism.

Vascular bundle maturation and arrangement demonstrates similarity between scutella and the palm haustorium. The procambial strands in the cotyledon of both grasses and date palm are numerous and run a few cells below the epithelium proper (DEMASON and THOMSON 1981). Maturation of the vascular bundles to form a continuous vascular connection between seedling and haustorium does not occur until 4 weeks after germination (DEMASON 1984). This vascular discontinuity supports the hypothesis that the major function of the haustorium is storage and transport of materials to the seedling, rather than transport of secreted substances produced in the seedling. The large phloem to xylem ratio in the vascular bundles of both scutella and the date palm haustorium suggests a function in translocation of carbon compounds.

Further structural similarities between the scutellum/haustorium include the abundance of ribosomes, ER, dictyosomes, and the addition of an inner wall with small internally-directed projections. Tubular extensions of ER which appear to merge into the plasmalemma undulating over the wall projections is especially evident in grazing sections of cell corners. This configuration is termed "plasmatubules" by HARRIS *et al.* (1982) in the epithelium of barley. These also occur in phloem parenchyma (HARRIS 1981) and sieve elements (EVERT 1976). HARRIS (1982) used a dye experiment to demonstrate that this membrane amplification of the apoplast-symplast interface may aide solute transport. Along these same lines, epithelial, subepithelial, and parenchyma cells of date haustoria have many plasmodesmata. These characteristics would aide either absorption or secretory functions. Indeed, KEUSCH (1968) showed that 3-week-old haustoria absorbed both radioactively-labeled mannose and glucose.

Dictyosomes are present throughout haustoria development until the cytoplasm degenerates. They are always near the plasmamembrane. During enlargement of the haustorium, mitoses are frequent in the epithelium and these cells also enlarge in surface area. In addition, a thin inner wall is added to the cells. If plasmamembrane and wall material is constantly being added, one would expect abundant dictyosome activity. One then cannot either rule out or confirm from the presence of dictyosomes the possibility that the haustorium/scutellum is important in secretion of hydrolases.

Judging from the above-mentioned structural changes, absorption and metabolism of the products of hydrolysis from the endosperm, is probably the most important function of the date haustorium. The surface area between the haustorium is maximized in numerous ways: cell size and number increase in the epithelial layer and surface area of epithelial plasmalemma over the numerous inner wall projections. Structural features occur which would facilitate transport: location of vascular bundles, predominance of phloem in the vascular bundles, abundant plasmadesmata and elaborated ER-plasmalemma regions. The sequence of storage body build-up, maturation of vascular tissues and loss of storage bodies also support the hypothesis that the haustorium is mainly an absorptive and storage organ which supplies the seedling with products of hydrolysis until the seedling can support itself through photosynthesis.

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