THE ULTRASTRUCTURE OF COTYLEDONARY
TISSUE FROM GOSSYPIUM HIRSUTUM L. SEEDS

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ABSTRACT
Quiescent cottonseeds stored in a dry, anaerobic situation for over a year have been shown to contain cells whose contents are ultrastructurally similar to those of normal, fully hydrated plant cells. Plastids, mitochondria, and nuclei of the cells of cotyledon tissue in dry seeds possess normal-looking double membranes even under conditions of extreme desiccation. Previous reports have indicated on the basis of light microscopic work, that the cells of certain dry seeds do not possess nuclear membranes or mitochondria. The cells of the dry cottonseed do contain these structures, however. Dictyosomes have not been observed in the spongy parenchymal cells of the cotyledon tissue; it is suggested that they are concerned with translocation and/or utilization of material. The storage materials in the cells, protein and oil, are contained in vacuolar areas enclosed by a single membrane.

Seeds offer a convenient base line in ontogenetic studies of the plant. Many ultrastructural studies have been directed to the terminal stages of seed development (7, 8, 12, 17, 27) or to the initial stages of seed germination (4, 10, 18, 22, 28), but relatively few studies have been conducted on the dry seed per se. Yet an understanding of the final changes on maturation and the first events of germination requires information on the mature, quiescent seed as a point of reference.

Studies of biochemical changes in seeds which have initiated germination processes (1, 9, 31) show a dramatic rise in activity of many enzymes within a very short time. Wasserman (29), working with light microscopy, was unable to see mitochondria in cells of the dry seed. However, within 12 to 24 hours after the seeds had imbibed water, mitochondria in cells became visible, presumably arising de novo. Thus, it is obvious that changes occur quite rapidly in seeds after imbibition of water.

Unfortunately, most electron microscope studies have been conducted on seeds which were allowed to imbibe water for various periods prior to fixation (16, 18, 25, 28). Since rapid changes do occur in seeds upon imbibition of water, any technique involving imbibition of water, even for short periods, may not reflect their original condition.

The paucity of studies on dry seeds is probably due to the extreme difficulty of working with them. This study, therefore, was undertaken to determine the structure of cells in dry, quiescent seeds fixed directly without prior hydration.

MATERIALS AND METHODS
A master sample of cottonseed (Gossypium hirsutum L., var. Acala 4-42-77, Glandless) harvested in Fall, 1962 was stored in a vacuum desiccator with a desiccant at 3°C and subsamples were removed as needed. Sections of tissue approximately 0.3 mm in diameter were cut carefully from the seed and fixed immediately in two different fixatives, osmium tetroxide and permanganate salts.

OSMIUM: Since earlier work indicated that various solvents deranged the normal structure of the dry
were placed into a glass-stoppered flask containing osmium tetroxide fumes. Dry tissue sections on carbon-coated grids and poststained with lead citrate (23). The tissue was observed in Phillips EM-75, EM-100, and EM-200 microscopes.

RESULTS AND DISCUSSION

Electron micrographs at very low magnifications are very similar to pictures obtained with light microscopy. The upper and lower epidermis of the cotyledons has a cuticular covering which is extremely resistant to permanganate fixatives. The mesophyll is composed of a palisade layer and spongy parenchymal cells. The typical mesophyll cell is filled with numerous aleurone grains, which are interspersed in a fine, cytoplasmic network that forms the matrix of the cell. Within the interstices of the matrix are minute lipid droplets. Certain cells in the epidermis and in the provascular regions contain much more cytoplasmic material than the typical mesophyll cells. These cells are richer in organelles.

Observations under higher magnifications will be given in the following sections which detail the various cytoplasmic constituents.

HYALOPLASM: One of the most striking aspects of the dry seed parenchymal cell is the morphology of the hyaloplasm. In the electron microscope image it assumes a reticular appearance; within the groundwork of the reticulum can be found the organelles, the endoplasmic reticulum, and others (Figs. 1, 3 to 5). Occasionally, cytoplasmic connections, the plasmodesmata, can be seen between two cells (Fig. 1).

SPHEROSOMES: Numerous vacuolar areas within the cytoplasm are responsible for the reticular appearance of the hyaloplasm. They range in size from 0.3 to 3 μ in diameter and are one of the dominant features of the cotton seed parenchymal cells (Figs. 1, 5).

After osmium fixation the vacuolar areas are electron-opaque (Fig. 2). This is caused by the high content of unsaturated fatty acids in cottonseed oil (5) and indicates that the vacuolar areas are the sites of oil storage.

On the other hand, after permanganate fixation the vacuolar areas are electron-transparent. Since aqueous permanganate is not miscible with lipids, this tends to confirm that the material occupying the vacuoles is probably lipid (13).

The edges of the vacuolar spaces are characteristically bounded by an apparent membrane which has not yet been resolved into the unit membrane structure. Generally, this line of demarcation is more densely stained than an ordinary membrane. Part of the reason for the density of the line could be the deposition of manganese at the interface of a lipid droplet and the aqueous permanganate fixing solution.

Frey-Wyssling et al. (10) have shown a similar morphology for rapeseed and have identified the oil droplets with the classical spherosomes found in the epidermis of onion bulbs. They state that

1 Reference to commercial products does not imply their recommendation over others equally suitable.
the spherosomes are bounded by a unit membrane. Nieuwdorp (16) has also observed similar bodies in the scutellum of barley endosperm and has tentatively classified them as spherosomes. The oil droplets will, therefore, be called spherosomes.

**aleurone grains:** The most obvious subcellular constituents in the cottonseed parenchymal cell, even at the light microscope level, are the aleurone grains. These were among the few contents of cells known before 1840 (24), and by 1855 Hartig (11) had succeeded in isolating them from various oilseeds.

The aleurone grains in cottonseeds are spherical bodies with an apparent homogeneous matrix which stains evenly with both osmium and permanganate fixatives. They range in size from about 1 to 20 μ in diameter, averaging around 4 to 6 μ. The aleurone grain of the cottonseed is bordered by a single membrane which has been resolved into the unit membrane structure (Fig. 6).

Embedded within the homogeneous matrix of the aleurone grain are the globoids (Figs. 1, 5). They are not delineated by a membrane, although occasionally an interface line is visible. The globoids are electron-transparent after permanganate fixation and often electron-opaque after osmium fixation.

The aleurone grains are the site of protein storage. The storage proteins of seeds have recently been named “aleurins,” denoting a cytological localization of the major seed proteins (2).

**nucleus:** Although the aleurone grains of the cottonseed parenchymal cell tend to be spheroids, the nuclei assume various shapes. Generally, the nuclei are irregularly shaped and their contents are mottled after permanganate fixation. Each nucleus is surrounded by a double membrane which exhibits discontinuities, the pores. Light microscope studies have indicated a lack of membranes around the nucleus of a dry seed (14), but this is not true in the case of dry cottonseed.

**mitochondria:** The population density of these organelles varies among the cell types. The typical mesophyll parenchymal cell contains very few, whereas the cells in the provascular region contain numerous mitochondria (Fig. 3).

The mitochondria in the cells of the dry seed resemble those found in normal cells. They average about 0.5 to 0.7 μ in width by 1.0 and 1.3 μ in length. They possess the typical double membranes and the conventional cristae. The cristae range from sparse to moderate in number.

Generally, the mitochondria are ovoid in shape, although it is not uncommon to find irregularly shaped ones. Quite often small granules or globules can be seen within the matrix of the mitochondria (Fig. 3).

**proplastids:** Most of the cells which have been examined contain proplastids. The proplastids are bounded by a double membrane whose inner membrane invaginates to form variously shaped infoldings, some of which resemble cristae mitochondriales. The infoldings are usually less numerous than in the mitochondria and are apt to assume strange configurations such as rings or lamellar structures (Fig. 3).

The proplastids generally are ovoid in shape, although it is common to see misshapen ones. They range in diameter from slightly less than 1 μ to about 3 μ. They usually possess globules which vary in size, from approximately 300 to 2,000 A in diameter. The globules stain with both osmium and permanganate.

Although many seeds contain amyloplasts with starch grains, no starch grains have been seen in the dry, mature cottonseed.

**dictyosomes:** Dictyosomes in the cells of parenchymal tissue are conspicuous by their absence. It may be that their membranes are stacked so closely together within the framework of the cytoplasmic network that they are not readily apparent. Closely appressed lamellar structures have been observed within the matrix of the cytoplasmic network occasionally; however, they did not possess the typical morphology of the dictyosome.

On occasion, dictyosomes have been observed in the cells of provascular tissue (Fig. 4), but even there they are very sparse.

In a preliminary study, dictyosomes were seen in cells of the cotyledon tissue of dry castor beans. However, in the castor bean the storage tissue is the endosperm. In this case, the cotyledon would be a recipient tissue for stored food in contrast to the cottonseed cotyledon which is a donor tissue. Nieuwdorp (16) observed dictyosomes in the scutellum of barley which is also a recipient tissue.

**endoplasmic reticulum:** The endoplasmic reticulum is rather poorly defined in mesophyll parenchymal cells of the dry seed, but tubules and cross-sections of the endoplasmic reticulum can be seen within the groundwork of the hyaloplasmic network. Since the micrographs are of material fixed in permanganate fixation, no ribosomes are visible.
Although we do not have electron microscopic evidence for the presence of ribosomes in cells of the dry cottonseed, Phillips (21) has recently succeeded in isolating ribosomes from the cottonseed and has characterized them chemically and physically.

REFERENCES


Abbreviations Used in Figures

Al, aleurone grains
CW, cell wall
D, dictyosome
ER, endoplasmic reticulum
Gl, globoid
Mt, mitochondria
N, nucleus
Pd, plasmodesmata
Pp, proplastid
S, spherosome

Figure 1 A typical spongy parenchymal cell from the cotyledon of a dry cottonseed. Note the sparsity of hyaloplasmic material; organelles such as mitochondria and proplastids can be seen within the matrix of the hyaloplasm. The electron-transparent circular areas, the spherosomes, are the sites of oil storage, and the large electron-opaque circular areas, the aleurone grains, are the sites of protein storage. Lithium permanganate fixation. × 7,000.

Figure 2 A portion of a similar cell fixed in osmium tetroxide. Notice that the spherosomes (S) are electron-opaque, in contrast to the electron-transparent appearance in the previous picture. This is the only micrograph presented of material fixed in osmium tetroxide. × 7,000.

Figure 3 A cell observed within the provascular region of the cotyledon tissue. The cells in the provascular region tend to be richer in cytoplasm and have a higher density of organelles. This cell was unusually rich in mitochondria; note the proplastid with its characteristic globules. Lithium permanganate fixation. × 12,000.

Figure 4 A cell observed in the proximity of the previous cell (Fig. 3). This cell contains a profile of a dictyosome. The dictyosomes are very sparse or difficult to see in cells of the dry seed cotyledon tissue. Lithium permanganate fixation. × 16,000.

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**Figure 5** A higher magnification of a spongy parenchymal cell. Notice the double membranes of the mitochondria (double arrows) and the single membrane enveloping the aleurone grain (single arrows). The globoids which can be seen in this micrograph show no membranes. × 24,000.

**Figure 6** A much higher magnification of the single membrane enveloping the aleurone grain. This micrograph shows that the single membrane is a unit membrane. Lithium permanganate fixation. × 228,000.