Structural features of the leaf epidermis of *Halodule uninervis*

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Leaf blade epidermal cells of the marine angiosperm *Halodule uninervis* (Forssk.) Aschers resemble transfer cells since they possess a highly invaginated plasmalemma with which numerous mitochondria are often associated. However, wall ingrowths found in transfer cells of terrestrial plants are lacking. The plasmalemma is invaginated around fibrillar material which is not continuous with the cell walls and which appears to be structurally different from cell wall material. Epidermal cells possess an organelle-rich cytoplasm in which chloroplasts and electron-dense deposits feature prominently. The outer cell wall is about twice as thick as the other walls and seems to have a porous texture. A thin but distinct cuticle covers the leaf surface. Often minute cavities occur within the cuticle adjacent to the wall. Epiphytic algae and bacteria are frequently found in close association with the epidermis. Based on structural features, it would appear that leaf blade epidermal cells of *H. uninervis* play an important role in the leaf's activities such as synthesis of carbohydrates, absorption and secretion of solutes and osmoregulation.

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Blaarlamina-epidermisselle van die marine angiosperm *Halodule uninervis* (Forssk.) Aschers stem ooreen met oordragselle aangesien hulle soos laasgenoemde ook instulpings van die plasmalemma besit, wat met talryke mitochondrions geassosieer is. Die selwandingroeiings soos bekend by die oordragselle van landplante ontbreek egter. Die plasmalemma vou om instulpings van fibrillêre materiaal te vorm wat struktureel van die selwand verskil en waarskynlik nie aan die selwand geheg is nie. Die epidermisselle besit 'n organelryke sitoplasma met prominente chloroplaste en elektrondigte afsettings. Die buitenste tangensiale selwand is ongeveer twee keer so dik as die ander wande en het blykbaar 'n poreuse tekstuur. Die blaaroppervlak word deur 'n dun, maar opvallende kutikula bedek. Klein holtes kom dikwels in die kutikula, aangrensend aan die selwand, voor. Epifitiese alge en bakterieë kom dikwels in noue assosiasie met die epidermis voor. Op grond van strukturele kenmerke wil dit voorkom of die blaarlamina-epidermisselle van *H. uninervis* 'n belangrike rol in die aktiwiteite van die blaar vervul, soos die sintese van koolhidrate, absorbsie en sekresie van opgeloste stowwe en osmotiese regulering.

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Introduction

Marine angiosperms (also known as sea-grasses) are monocotyledonous plants that grow submerged in seawater. They occur in shallow coastal waters and are able to penetrate some short distance into estuaries and other brackish waters. Their adaptation to a marine environment makes them plants of interest. In a continuing study of the ultrastructural morphology of South African marine angiosperms the structure of leaf blade epidermal cells of *Halodule uninervis* (Forssk.) Aschers was investigated. Preliminary results of this study have appeared in abstract form (Barnabas & Kasavan 1980). In this paper further information on leaf epidermal structure is reported.

Materials and Methods

Plants of *H. uninervis*, growing submerged in rock pools, were collected at Mapelane along the Zululand coast. The salinity of the water was 35‰ at the time of collection. The material was transported to the laboratory in seawater in plastic bags.

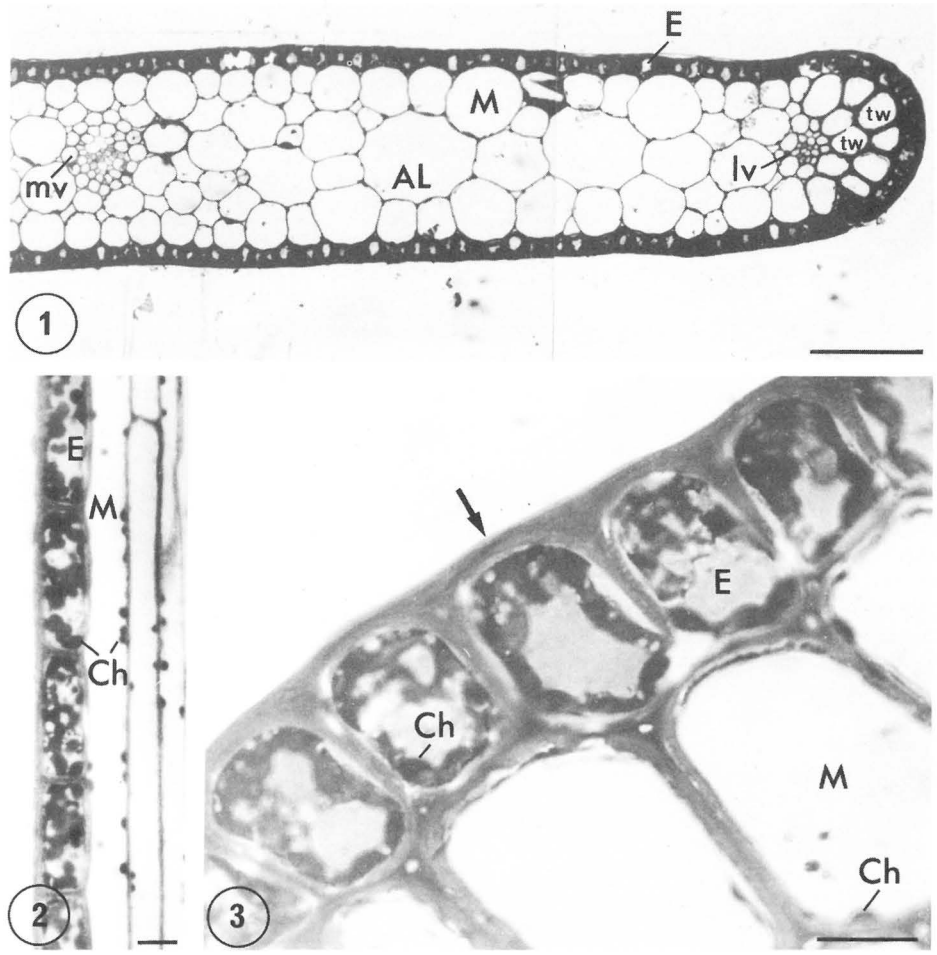
Samples for light and electron microscopy were taken from the middle of mature leaf blades. For electron microscopy material was fixed under vacuum in cold 6% glutaraldehyde for 6 h, post-fixed in 2% osmium tetroxide, stored overnight in a refrigerator at 4 °C, dehydrated in a graded ethanol series and embedded in Spurr's (1969) resin. Ultrathin sections were mounted on copper grids and stained with 2% aqueous uranyl acetate followed by lead citrate (Reynolds 1963). The sections were examined and photographed with a Philips 301 electron microscope.

For light microscopy, material embedded in Spurr's medium was sectioned 0,5 μm thick and stained with Azur II and methylene blue (Richardson *et al.* 1960).

Results

The general anatomy of a leaf blade is shown in Figure 1. Three major tissue systems — dermal, ground and vascular — constitute the blades. The epidermis is uniseriate. The cells are generally smaller than those of the mesophyll, have an abundance of chloroplasts and relatively thick outer walls (Figures 1 – 3). In longitudinal section the epidermal cells appear to be elongated (Figure 2).

Mesophyll tissue is undifferentiated and is composed of highly vacuolate cells lined by a thin parietal layer of cytoplasm in which few chloroplasts occur (Figures 1 & 2). Prominent longitudinal air canals are present in the mesophyll. Walls of mesophyll cells at the leaf margins are thickened (Figure 1) and form the main mechanical tissue of the blade. The vascular



Figures 1 – 3 Light micrographs of portions of leaf blades of *H. uninervis*. 1. Epidermal (E), mesophyll (M) and vascular tissues (mv median vein; lv lateral vein) comprising leaf blade. Note cells with thickened walls (tw) at leaf margin and air lacunae (AL). Scale line = 100 μm. 2. Longitudinal and 3. transverse sections of epidermal (E) and mesophyll (M) cells. Chloroplasts (Ch) more abundant in epidermis. Arrow points to thick outer tangential wall of epidermal cells. Scale lines = 100 μm for 2 & 3.

system consists of three longitudinal veins (a median and one lateral on either side — Figure 1) interconnected by small transverse veins.

The general ultrastructure of a whole epidermal cell and parts of the adjacent cells is shown in Figure 4. A striking feature of the cells is the highly invaginated plasmalemma, especially along the radial walls (Figures 4, 6 & 9). A labyrinthine extracytoplasmic space filled with fibrillar material (sometimes loosely distributed) occurs between the invaginated plasmalemma and the cell walls (Figures 4 – 6, 8 & 10 – 12). This space is particularly prominent along the radial walls (Figures 4, 6 & 9). The presence of isolated portions of plasmalemma-bound fibrillar material in the cytoplasm attests to the labyrinthine nature of the extracytoplasmic region (Figures 5 & 11 — arrowheads). Sometimes a large number of membranous structures, which may represent portions of the highly invaginated plasmalemma, are seen (Figures 5 – 7). Mitochondria with many cristae are abundant and are especially numerous in the vicinity of the plasmalemma invaginations (Figures 4, 5 & 9).

Chloroplasts with well developed grana feature prominently in the cytoplasm (Figure 4). Grana are often highly stacked (Figure 11). Occasionally, a poorly developed peripheral reticulum system is visible in the chloroplast stroma (Figure 10). Another prominent feature of the cytoplasm is the presence of relatively large globular deposits of electron-dense material (Figures 4, 8, 12 & 15).

Ribosomes have a scattered distribution throughout most of the cytoplasm and short peripheral profiles of endoplasmic reticulum are a common feature (Figures 8 & 9). Dictyosomes and microtubules are sparse (Figures 8 & 9). Microbodies occur more frequently and are usually found in close association with chloroplasts (Figures 8 & 12). Nuclei are often observed close to the inner tangential wall (Figure 4).

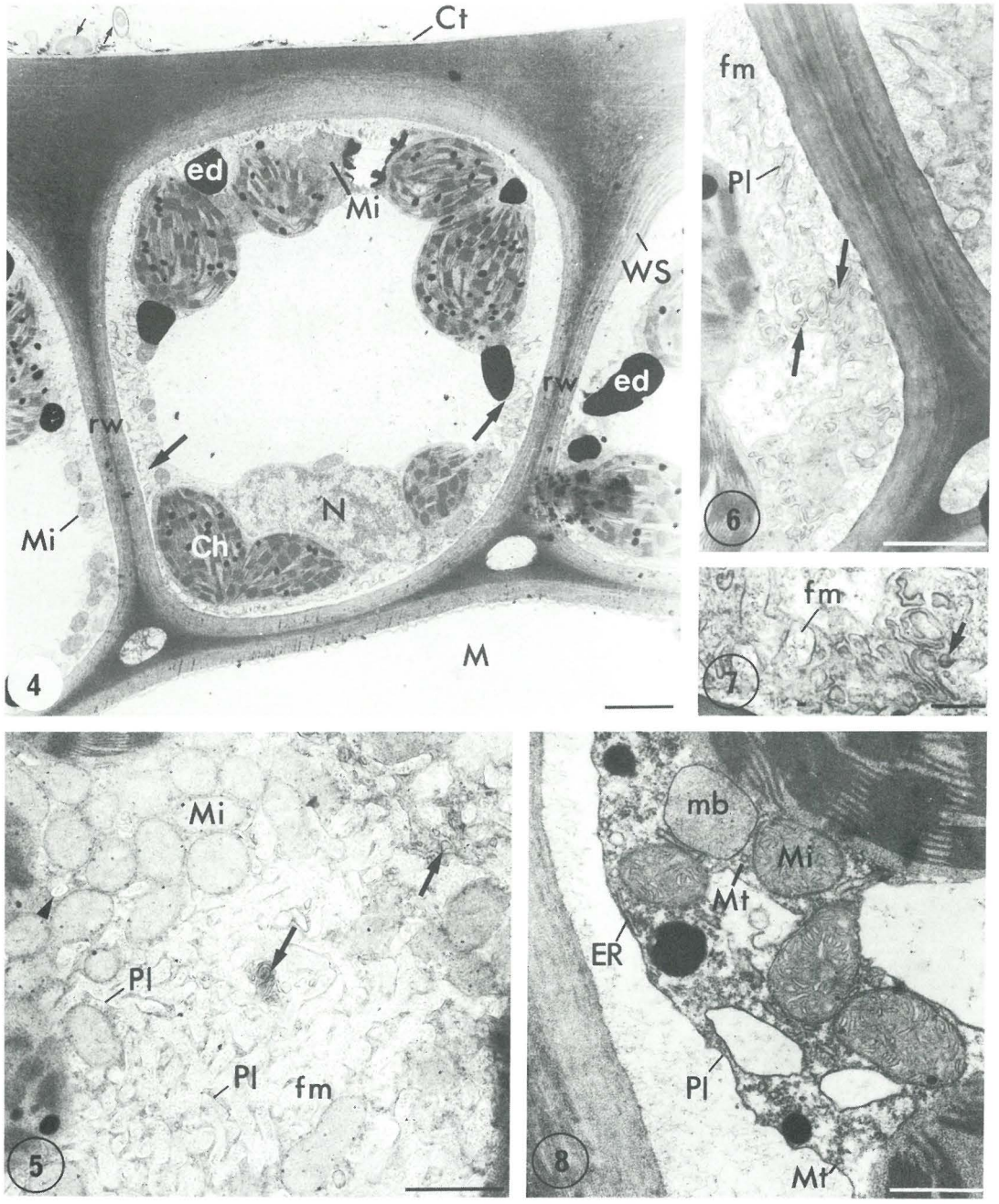
The epidermal walls have an interesting structure. The outer tangential walls are thicker than the radial and inner tangential walls (Figure 4). Radial walls often have a striated or lamellated appearance (Figures 4 & 9). Occasionally, parts of the outer wall appear electron-dense (Figure 15).

Under high magnification the outer tangential wall seems to have a porous texture (Figures 14 & 16). A distinct but thin cuticle covers the epidermis (Figures 4 & 14 – 16). The cuticle exhibits two interesting structural features: (i) minute spaces or cavities often occur within the cuticular layer adjacent to the wall (Figures 13 & 14) and (ii) strands of cell wall material may permeate the cuticle (Figure 16).

Epiphytic algae and bacteria (which are often embedded in an amorphous substance) are frequently found on the surface of the epidermis (Figures 4 & 13). Complete plasmodesmatal connections between epidermal cells and between epidermal and mesophyll cells are not present in mature cells. Remnants of plasmodesmata that probably interconnected the cells at an earlier stage of their development are shown in Figure 12.

Discussion

This study has shown that mature leaf blade epidermal cells of *H. uninervis* resemble transfer cells (Gunning & Pate 1969)

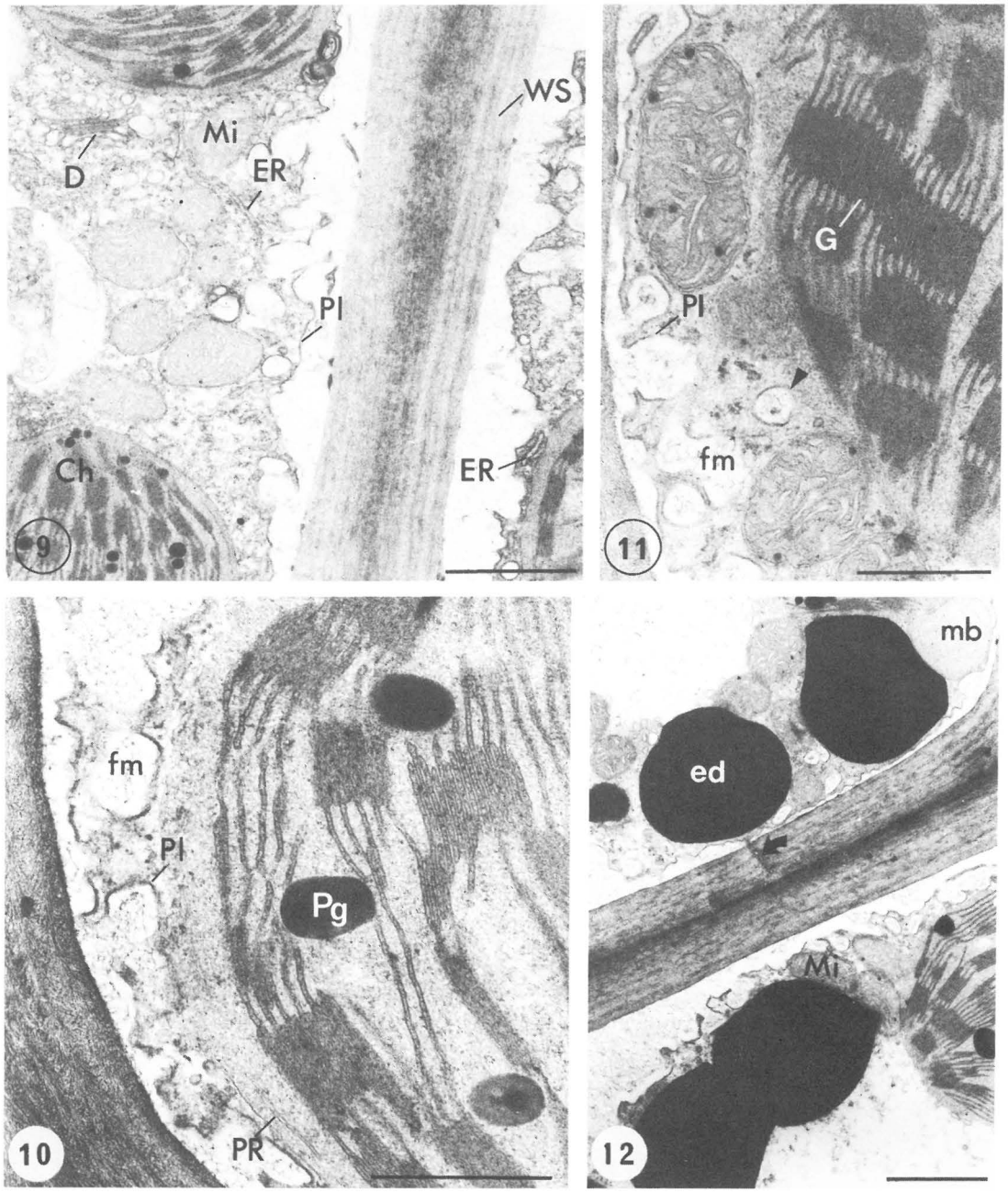


Figures 4 – 8 Electron micrographs of leaf blade epidermal cells of *H. uninervis*. 4. Cell with thick outer tangential wall covered by a thin cuticle (Ct), highly invaginated plasmalemma (large arrows) especially along radial walls (rw), chloroplasts (Ch), mitochondria (Mi), electron-dense deposits (ed) and part of a nucleus (N). M mesophyll; WS wall striations; note epiphytes (small arrows). Scale line = 2 μm. 5. Section through cytoplasm adjacent to transverse wall showing highly invaginated plasmalemma (Pl) around fibrillar material (fm), numerous mitochondria (Mi) and membranous structures (arrows). Scale line = 1 μm. 6. Plasmalemma (Pl) invaginated around fibrillar material (fm) along radial wall. Arrows point to membranous structures. Scale line = 1 μm. 7. Detail of part of Figure 6. fm fibrillar material. Arrow points at a membranous structure. Scale line = 0,5 μm. 8. Cytoplasm showing a microbody (mb), microtubules (Mt), endoplasmic reticulum (ER), mitochondria (Mi) and plasmalemma (Pl). Scale line = 0,5 μm.

since they possess a highly invaginated plasmalemma with which numerous mitochondria are often associated. The invaginations of the plasmalemma are believed to increase the surface area of this membrane. Epidermal cells with transfer cell characteristics have also been reported in the leaves of nearly all other marine angiosperms studied to date with the electron microscope. The only species found so far to possess leaf blade epidermal cells without transfer cell features are *Posidonia australis* (Kuo 1978) and *Posidonia sinuosa* (Cambridge & Kuo 1982).

Although epidermal cells of *Halodule uninervis* resemble transfer cells, they lack 'true' wall ingrowths. The material around which the plasmalemma is invaginated is not continuous with the cell walls and appears structurally different from cell wall material. In this respect epidermal cells of *H. uninervis* resemble those of *Thalassodendron ciliatum* (Barnabas 1982), *Thalassia testudinum* (Jagels 1973) and *Cymodocea rotundata* (Doohan & Newcomb 1976). In other marine angiosperms such as *Halophila ovalis* (Birch 1974; Barnabas & Naidoo 1979) and *Zostera capensis* (Barnabas *et al.* 1977) ingrowths of leaf blade epidermal cells are continuous with the wall and are structurally similar to wall ingrowths of transfer cells in terrestrial plants. The reason for this difference in the nature of the wall material around which the plasmalemma is invaginated in various sea-grasses, is presently not clear.

Transfer cells are believed to be involved in absorptive or

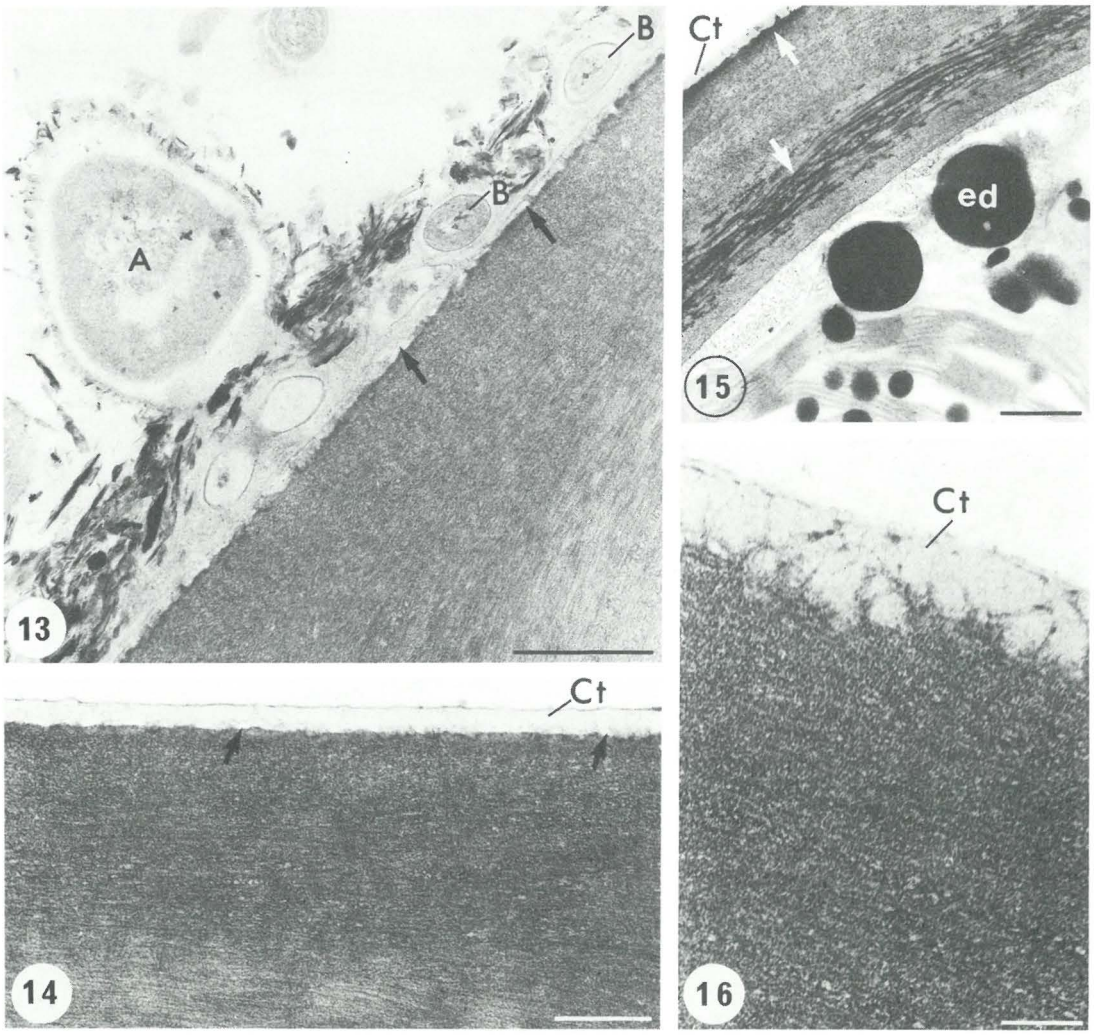


Figures 9 – 12 Electron micrographs of leaf blade epidermal cells of *H. uninervis*. 9. Cytoplasmic details along radial wall. Ch chloroplasts; D dictyosome; ER endoplasmic reticulum; Mi mitochondria; Pl plasmalemma; WS wall striations. Scale line = 1 μm. 10. Portion of a chloroplast with a poorly developed peripheral reticulum system (PR). fm fibrillar material; Pg plastoglobuli; Pl plasmalemma. Scale line = 0,5 μm. 11. A highly stacked granum (G) of a chloroplast. fm fibrillar material; Pl plasmalemma. Scale line = 0,5 μm. 12. Remains of a previous plasmodesmatal connection (curved arrow) between cells. mb microbody; Mi mitochondria; ed electron-dense deposits. Scale line = 1 μm.

secretory activities (Gunning & Pate 1969; Gunning 1977). It is possible therefore that leaf epidermal cells of sea-grasses with transfer cell characteristics may perform similar activities. For example, ^{32}P has been shown to be absorbed as well as excreted by the leaves of *Zostera marina* (McRoy & Barsdate 1970; McRoy *et al.* 1972) and *Phyllospadix* (Harlin 1973). Another structural feature that may enhance absorptive or secretory processes in these cells is the apparently porous outer wall observed in *Halodule uninervis* and *Thalassodendron ciliatum* (Barnabas 1982).

Since marine angiosperms grow submerged in a saline medium, it is probable that the epidermal cells also play a major role in osmoregulation. Jagels (1973 & 1982) proposed that the plasmalemma of these cells may be implicated in salt regulation. He suggested that salt may be either secreted actively across the plasmalemma of the epidermal cells or excluded at the membrane boundary. His hypothesis is supported by findings of the internal salt content of sea-grass tissues. Beer *et al.* (1980) investigated the internal distribution of ions in various leaf tissues of *Halodule uninervis* and *Halophila stipulacea*. They found that the epidermal concentrations of Na^+ and Cl^- were low when compared with ambient and tissue concentrations, indicating selective exclusion of Na^+ and Cl^- from the epidermis. The finding in the present study of a highly invaginated plasmalemma with which numerous mitochondria are often associated, provides further evidence for the possible role of the plasmalemma in osmoregulation. The mitochondria could provide ATP for an active transmembrane movement of solutes.

Another possible function of the enlarged plasmalemma may be to expedite the transfer of photosynthate into the cell walls for subsequent transport to the adjacent tissues. Apoplastic



Figures 13 – 16 Electron micrographs of leaf blade epidermal cells of *H. uninervis* showing portions of the outer wall. 13. An epiphytic alga (A) and bacteria (B) embedded in amorphous material, occur in close association with the wall. Note small cavities (arrows) between wall and overlying amorphous material. Scale line = 0,5 μm. (14). Porous texture of wall. Arrows point to small cavities between wall and cuticle (Ct). Scale line = 0,2 μm. 15. Electron-dense appearance of portions of outer wall (arrows). Ct cuticle; ed electron-dense cytoplasmic deposits. Scale line = 0,5 μm. 16. Strands of cell wall material permeating the cuticle (Ct). Note porous texture of wall. Scale line = 0,1 μm.

movement of photosynthate from the epidermis (the main photosynthetic tissue) to the interior tissues of the leaf probably occurs, since no plasmodesmata have been found to interconnect mature epidermal and mesophyll cells when epidermal cells possess transfer cell characteristics. Lüttge (1971) suggested that the enlarged plasmalemma of transfer cells could provide more space for diffusion or could accommodate more membrane-bound carrier systems. It is interesting to note that leaf blade epidermal cells of the two species of *Posidonia*, viz *P. australis* (Kuo 1978) and *P. sinuosa* (Cambridge & Kuo 1982), which do not possess transfer cell features, have numerous plasmodesmata which interconnect epidermal and mesophyll cells. In these sea-grasses therefore, an enlarged plasmalemma for the transfer of photosynthate might not be necessary — hence a plasma membrane without invaginations.

The close association of bacteria and other epiphytes with leaf blades, as observed in *Halodule uninervis*, has also been reported in other sea-grasses (Doohan & Newcomb 1976; Kuo 1978; Barnabas 1982). With the aid of radio-isotopes, the close relationship between sea-grass leaves and their epiphytes, has been demonstrated. Penhale & Thayer (1980) using ^{14}C and ^{32}P showed that both carbon and phosphorus were taken up by the roots of *Zostera marina* and transferred through the plants to the epiphytes on the grass blades. In addition, Goering & Parker (1972), employing the acetylene reduction technique, demonstrated N_2 fixation by epiphytes on sea-grass leaves. It is possible, therefore, that some kind of mutual relationship might exist between sea-grasses and their epiphytes. The discovery in the present study of minute spaces or cavities within the cuticle adjacent to the outer wall, as well as an apparently porous outer wall, could indicate that they represent structural features that facilitate the movement of substances into and out of the leaves.

In this investigation the amorphous substance within which bacteria are frequently embedded (Figure 13) and to which algae and other epiphytes often adhere might be a mucoid substance similar to mucigel (Smith *et al.* 1982). Mucigel is thought to be composed of both bacterial capsular material and plant organic products (Rovira 1965). This mucilaginous material could provide a suitable microhabitat for the epiphytes.

Other structural features of the epidermal cells of *Halodule uninervis* are generally similar to those reported in leaf blades of some other marine angiosperms, viz a thick outer wall, a striated or lamellated appearance of certain walls, a chloroplast-rich epidermis, highly stacked grana in many chloroplasts, a poorly developed peripheral reticulum system in the chloroplast stroma and electron-dense deposits (probably polyphenolics)

within the cells. It would appear that leaf blade epidermal cells of marine angiosperms with their organelle-rich cytoplasm are centres of high metabolic activity and must therefore play an important role in the leaf's activities e.g. synthesis of carbohydrates, osmoregulation and absorptive-secretory processes.

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