ULTRASTRUCTURAL FEATURES OF THE LEAF BLADE EPIDERMIS AND SQUAMULAE INTRAVAGINALES OF THE MARINE ANGIOSPERM *HALOPHILA OVALIS* (R.Br.)Hook.f.

BY

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LIST OF ABBREVIATIONS

(PLATES 1 - 4)

AL	-	Air Lacuna
AS	-	Air Space
BM	-	Basal Meristem
BS	-	Bundle Sheath
CC	-	Companion Cell
Ct	-	Cuticle
D	-	Diaphragm
E	-	Epidermis
FVB	-	Fibro Vascular Bundle
L	-	Lamina
LE	-	Lower Epidermis
LT	-	Leaf Tip
LVB	-	Lateral Vascular Bundle
Ms	-	Mesophyll
MV	-	Mid/Main Vein
P	-	Petiole
Pa	-	Parenchyma
Ph	-	Phloem
SG	-	Starch Grain
ST	-	Sieve Tube
UE	-	Upper Epidermis
XL	-	Xylem Lacuna

LIST OF ABBREVIATIONS

(PLATES 5 - 24)

AS	-	Air Space
Ch	-	Chloroplast
Ct	-	Cuticle
CE	-	Chloroplast Envelope
D	-	Diatom
Di	-	Dictyosome
DLe	-	Developing Leaf
E	-	Epiphyte
EP	-	Extracytoplasmic Pocket
ER	-	Endoplasmic Reticulum
ES	-	Extracytoplasmic Space
G	-	Granum
IG	-	Intergranum
IS	-	Intercellular Structure
ITW	-	Inner Tangential Wall
L	-	Lamina
LB	-	Lipid Body
LM	-	Leaf Margin
LT	-	Leaf Tip
M	-	Midrib
Mi	-	Mitochondria
Mt	-	Microtubule
Mu	-	Mucilage
MB	-	Microbody
MS	-	Membranous Structure

MVS	-	Multivesicular Structure
N	-	Nucleus
Nu	-	Nucleolus
NA	-	Nucleoid Area
NP	-	Nuclear Pore
NSR	-	Non-Striated Region
OTW	-	Outer Tangential Wall
P	-	Polysome
Pd	-	Plasmodesmata
Pg	-	Plastoglobuli
Pl	-	Plasmalemma
Pr	-	Proplastid
Pt	-	Plastid
PB	-	Paramural Body
PrB	-	Prolamellar Body
PR	-	Peripheral Reticulum
R	-	Ribosome
RER	-	Rough Endoplasmic Reticulum
Rh	-	Rhizome
Ro	-	Root
RW	-	Radial Wall
SL	-	Scale Leaf
SqI	-	Squamulae Intravaginales
SR	-	Striated Region
T	-	Tonoplast
V	-	Vacuole
Ve	-	Vesicle
WI	-	Wall Ingrowth
WS	-	Wall Striations
YCh	-	Young Chloroplast

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I. INTRODUCTION

Marine angiosperms, commonly known as seagrasses, have a world-wide distribution and are adapted to life in sea water. They are the only group of vascular plants which have successfully invaded the sea from the land. Seagrasses possess structures typical of vascular flowering plants viz.: rhizomes, leaves, flowers, seeds and a vascular system. They have a preference for shallow coastal waters and estuaries.

According to Den Hartog (1970) there are 12 genera and 49 known species of seagrasses. Seven of the genera have a preference for tropical waters while the remaining five are confined mainly to temperate waters. Although seagrasses are not true grasses, they are all monocotyledons and are placed in the single order Helobiae. They are classified into two families, Hydrocharitaceae and Potamogetonaceae (Refer to Table 1).

Table 1. Classification of seagrasses (Den Hartog, 1970).

Family	Subfamily	No. of Genera
Potamogetonaceae	Zosteroideae	1. *Zostera* 2. *Phyllospadix* 3. *Heterozostera*
	Posidonioideae	1. *Posidonia*
	Cymodoceoideae	1. *Halodule* 2. *Cymodocea* 3. *Syringodium* 4. *Thalassodendron* 5. *Amphibolis*
Hydrocharitaceae	Vallisnerioideae	1. *Enhalus*
	Thalassioideae	1. *Thalassia*
	Halophiloideae	1. *Halophila* Sect. Halophila Sect. Microhalophila Sect. Spinulosae Sect. Americanae

Arber (1920) and Den Hartog (1970) stressed four requirements that are essential for seagrasses to exist in a marine environment:

i) the ability to tolerate a saline medium;
ii) the ability to grow while fully submerged;
iii) the production of a strong anchoring system to withstand wave action and tidal currents; and
iv) the capacity for hydrophilous pollination.

These major criteria which are essential for the colonization of a marine habitat, are satisfied by all twelve known genera of seagrasses. According to Den Hartog (1970) the genus *Halophila*, of the subfamily, Halophiloideae, comprises nine herbaceous species. *H. ovalis* is classified under the section *Halophila*.

Halophila ovalis is distributed along the east coast of Africa, extending from the Red Sea as far south as Madagascar and temperate South Africa. In Asia and Australia these plants are common virtually everywhere along their coasts. *H. ovalis* has also been recorded in Japan, Tasmania and in the islands of Hawaii, Samoa and Tonga.
The habitat of *H. ovalis* ranges from mid-tidal level to a depth of about 10 - 12m. These plants tolerate a range of substrata such as coarse coral rubble to soft sands. The plants thrive in tropical and warm temperate waters.
H. ovalis is well suited for life on unstable substrata since rooting is facilitated by rhizomatous fragments and the plants can tolerate being covered by silt.

Plants of *H. ovalis* form dense stands in sheltered habitats and in so doing

retain accumulated sediments thus causing the sea bottom to rise (Den Hartog, 1970).

According to Den Hartog (1970), *H. ovalis* is somewhat euryhaline and penetrates into estuaries and sea-inlets where, often, the water salinity is much lower. *H. ovalis* is able to tolerate low water salinities (10^{o}/oo) as is the case in the Swan River near Perth in Western Australia (Den Hartog, 1970). *H. ovalis* has been reported to tolerate hyperhaline conditions on the island of Oahu in Hawaii (Doty & Stone, 1966).

The importance of seagrasses as primary producers has often been overlooked because of their submerged habit. Seagrass productivity has been estimated at about 300 - 600 g dry wt/m^2/year, which is even higher than the average world productivity of maize and rice (Thayer, Wolfe & Williams, 1975).

Although one would associate the high productivity of seagrasses with a multitude of grazers, there are only a few organisms which actually consume them. The leaves of certain seagrasses contain tannins which make them unpalatable to many herbivores (Feeny & Bostock, 1968). Seagrasses contribute to the food web primarily through detrital food chains (Fenchel, 1977). This conclusion was based on direct observations on herbivore feeding behaviour, analysis of gut content and faeces of grazers and by the use of labelled plant material. The vertebrates unlike the invertebrates are the chief grazers of seagrasses due to the fact that marine invertebrates lack enzymes necessary to break down high molecular carbohydrates (Kristensen, 1972). Seagrass beds serve as a significant food source for several groups of animals, herbivorous birds, adult green turtles, some amphipods, isopods and sea urchins.

In many estuarine and sheltered coastal localities the leaves and stems of

seagrasses provide one of the few stable substrates available for the attachment of epiphytic microalgae, invertebrates and microscopic organisms. Seagrass epiphytes serve a multitude of functions: they contribute to the productivity of the system (Jones, 1968); serve as a nutrient reservoir for seagrasses between growing seasons (Harlin, 1975); supply food for grazers (Zimmerman, Gibson & Harrington, 1979); and provide a habitat for small animals.

According to Kikuchi and Pèrés (1977) seagrass beds may function in a number of different ways as a structural habitat or shelter:

i) seagrass beds provide a range of substrate types for epiphytic algae and other associated organisms. The dense vegetation of the seagrass beds reduces water flow, thus offering protection;

ii) the photosynthetic activity of the seagrass beds brings about profound changes in O_2, CO_2 and pH values in the surrounding water; and

iii) the leaf canopy formed by the seagrasses offers protection from excessive illumination. In intertidal situations the leaf canopy protects other organisms from desiccation and extremes in salinity and temperature.

The above-mentioned factors together with the availability of food are believed to be responsible for the importance of seagrass beds as spawning areas or nursery grounds for a variety of fish and shrimp.

Research on seagrasses to date has covered aspects of their taxonomy, ecology, morphology, physiology, general anatomy and ultrastructure.

The distribution, general morphology, ecology and taxonomy of seagrasses have been described *inter alia* by Ostenfeld (1915, 1918), Miki (1932, 1933, 1934), Phillips (1960), Den Hartog (1970) and Kirkman (1985).

Some physiological aspects of seagrasses were reported by Bender (1971), McRoy *et al* (1972), Kirkman *et al* (1979), Benedict *et al* (1980), Iizumi *et al* (1982) and Wahbeh and Mahasneh (1985).

Several workers have studied the general anatomy of marine angiosperms and these include Balfour (1878), Cunnington (1912), Cohen (1938), Tomlinson (1969, 1972, 1982) and Wahbeh (1985).

Most of the ultrastructural research on seagrasses has been conducted on the leaf blade epidermal cells. The fine structure of leaf blade epidermal cells has been investigated in *Thalassia testudinum* (Jagels, 1973; Benedict & Scott, 1976), *Cymodocea* spp. (Doohan and Newcomb, 1976), *Zostera capensis* (Barnabas *et al*, 1977, 1982), *Posidonia* spp. (Kuo, 1978; Cambridge and Kuo, 1982; Colombo and Cinelli, 1983), *Thalassodendron ciliatum* (Barnabas, 1982) and *Halodule uninervis* (Barnabas & Kasavan, 1983b).

These studies have shown that leaf blade epidermal cells of seagrasses possess specialized features, many of which appear to be related to the submarine existence of these plants. Although some aspects of the fine structure of leaf blade epidermal cells of *H. ovalis* were reported (Birch, 1974; Barnabas and Naidoo, 1979), these studies were not comprehensive. For example, it was shown that mature leaf blade epidermal cells possessed transfer cell features (Gunning & Pate, 1969). However, it was not known at which stage in cell development structures typical of transfer cells arose. Such information would give clues about the functional significance of these structures in the epidermal cells. In view of the paucity of information concerning cytological features of this unusual epidermis in

the leaves of *H. ovalis*, it was felt that a more detailed investigation was necessary. Thus, the present study was initiated.

Leaf blade epidermal cells were examined closely from the point of view of not only their general structure but also developmental changes of the cells and their histochemistry. In addition, the fine structure of axillary scales (squamulae intravaginales) associated with the bases of leaves were also investigated. In the layout of this dissertation the first section deals with observations and discussion of the gross morphology, anatomy and histochemistry of the leaves. In the remaining sections, ultrastructural changes occurring during the development of leaf blade epidermal cells, the ultrastructural morphology of unusual cell wall structures called annuli, and the fine structure of the squamulae intravaginales, are presented and discussed.

II. MATERIALS AND METHODS

1. Collection and Culture of Plants

Plants of *H. ovalis* were collected from near the mouth of the Nahoon river in East London. The salinity of the water was 35°/oo at the time of collection. Rhizome segments showing vigorous growth were removed, together with substrate, and transported in plastic containers to the laboratory.

The rhizome segments were cultured in aquaria at a salinity of 35°/oo under glasshouse conditions. The aquarium water was circulated and filtered continuously by power filter pumps. Under these conditions, plants grew well.

2. Sampling

Vegetative buds and leaves of various ages (some of which were dissected from the buds) were sampled from vigorously growing plants. This type of selection ensured that epidermal cells of different ages were studied. In addition, examination of portions of leaf blades close to and away from the basal meristem yielded material containing cells in different stages of development. Petioles of mature leaves were also sampled.

3. Preparation for Transmission Electron Microscopy

Leaf material was trimmed and diced in 0,05M sodium cacodylate buffer (pH 7,2) in a petri dish. The material was then fixed under vacuum in cold 6% glutaraldehyde buffered with 0,05M sodium cacodylate for 6 hours,

washed several times in the cacodylate buffer, post-fixed in 2% osmium tetroxide made up in the same buffer and stored overnight in the refrigerator at 4°C. The samples were washed twice in cacodylate buffer, dehydrated through a graded series of ethyl alcohol and embedded in Spurr's (1969) low viscosity resin.

Resin blocks containing leaf material were mounted on stubs. Block faces were trimmed with glass knives. Ultrathin sections were cut with glass or diamond knives, mounted on uncoated 200 mesh copper grids and stained with 2% aqueous uranyl acetate followed by lead citrate (Reynold's, 1963). The sections were examined and photographed with a Philips 301 electron microscope.

4. Preparation for Scanning Electron Microscopy

Vegetative buds and leaves of different ages were fixed in 6% glutaraldehyde buffered with 0,05M sodium cacodylate and prepared as for TEM studies up to the 100% ethanol dehydration stage. The material was then critical point dried, fixed to stubs with double-sided adhesive tape and coated with gold for observation. The leaves and buds were examined and photographed with a Philips SEM 500.

5. Preparation for Light Microscopy

For light microscope studies, sections of leaf material embedded in either resin or wax were used.

a. Spurr-(1969) Embedded Material

Material that was embedded in Spurr's resin for TEM studies (as described earlier) was sectioned (0,5 µm thick) and stained with Azur II and Methylene Blue (Richardson, Jarett & Finke, 1960).

b. Glycol Methacrylate-Embedded Material

Leaf material was fixed in 6% glutaraldehyde, dehydrated in a graded ethanol series and embedded in LKB historesin. Sections ranging from 0,5 - 4 µm in thickness were stained with Toluidine Blue in benzoate buffer at pH 4,4 (Feder & O'Brien, 1968) or used unstained for histochemical tests.

c. Wax-Embedded Material

Leaves and vegetative buds were fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin wax. Sections ranging from 8 - 10 µm in thickness were mounted onto glass slides, dewaxed, rehydrated and used for histochemical tests.

d. Histochemistry

The following histochemical tests were applied to sections of leaves and buds to determine the chemical nature of cell walls and other cellular components.

Chemical Distinguished	Histochemical Test	Reference
Cellulose	IKI - H_2SO_4	Jensen (1962)
Cutin	Sudan III & IV	O'Brien & McCully (1981)
Total carbohydrates	Periodic-Acid-Schiff's reagent	Jensen (1962)
Lignin	Phloroglucinol	Jensen (1962)
Mucilage	Alcian Blue and Alcian Yellow	Parker & Diboll (1966)
Pectin	Ruthenium Red	Jensen (1962)

Appropriate controls were used for each of the above histochemical tests.

In addition fresh leaf material was immersed in 0,01N silver nitrate (Birch, 1974) for the detection of chlorides.

III. MORPHOLOGY, ANATOMY AND HISTOCHEMISTRY

Results

The external morphology of *H. ovalis* is shown in Figure 1. **Plants of** *H. ovalis* consist of smooth, procumbent, monopodially branched rhizomes which are fixed to the substrate by long, slender roots borne in the region of the nodes. Usually one root is present below each erect shoot. The roots are unbranched and covered with root hairs. The erect shoot is short and consists of a pair of foliage leaves borne on each node. The foliage leaves have long, slender petioles and thin oblong-elliptic to ovate blades with rounded apices. Leaves vary in length from 5 - 8 cm. The margins are entire. Each leaf has 12 - 22 pairs of cross veins which are often branched (Fig. 2). The midrib is connected to the intramarginal veins at the leaf apex. The slender petioles which support the foliage leaves are about 30 - 50 mm long. The scale leaves are sessile, transparent, suborbicular, about 3 - 8 mm long by 4 mm broad, and are borne in pairs along the rhizome at the bases of the petioles.

A basal meristem is found at the junction of the leaf blade and petiole (Fig. 2, arrow). Cells of the basal meristem have dense protoplasts (Fig. 13). The meristem gives rise to a major portion of the leaf blade, the cells maturing in a basipetal direction, with the oldest cells occurring at the leaf tip region (Fig. 11, LT arrow) and the youngest cells close to the meristem (Fig. 11).

Vascular supply to the petiole consists of an axial fibro-vascular bundle derived from the axial bundle of the rhizome and two lateral bundles derived from the peripheral bundles of the rhizome (Fig. 9). In the region of the leaf the axial bundle is continued as the main-vein.

The lateral bundles form the intramarginal veins which run parallel with the

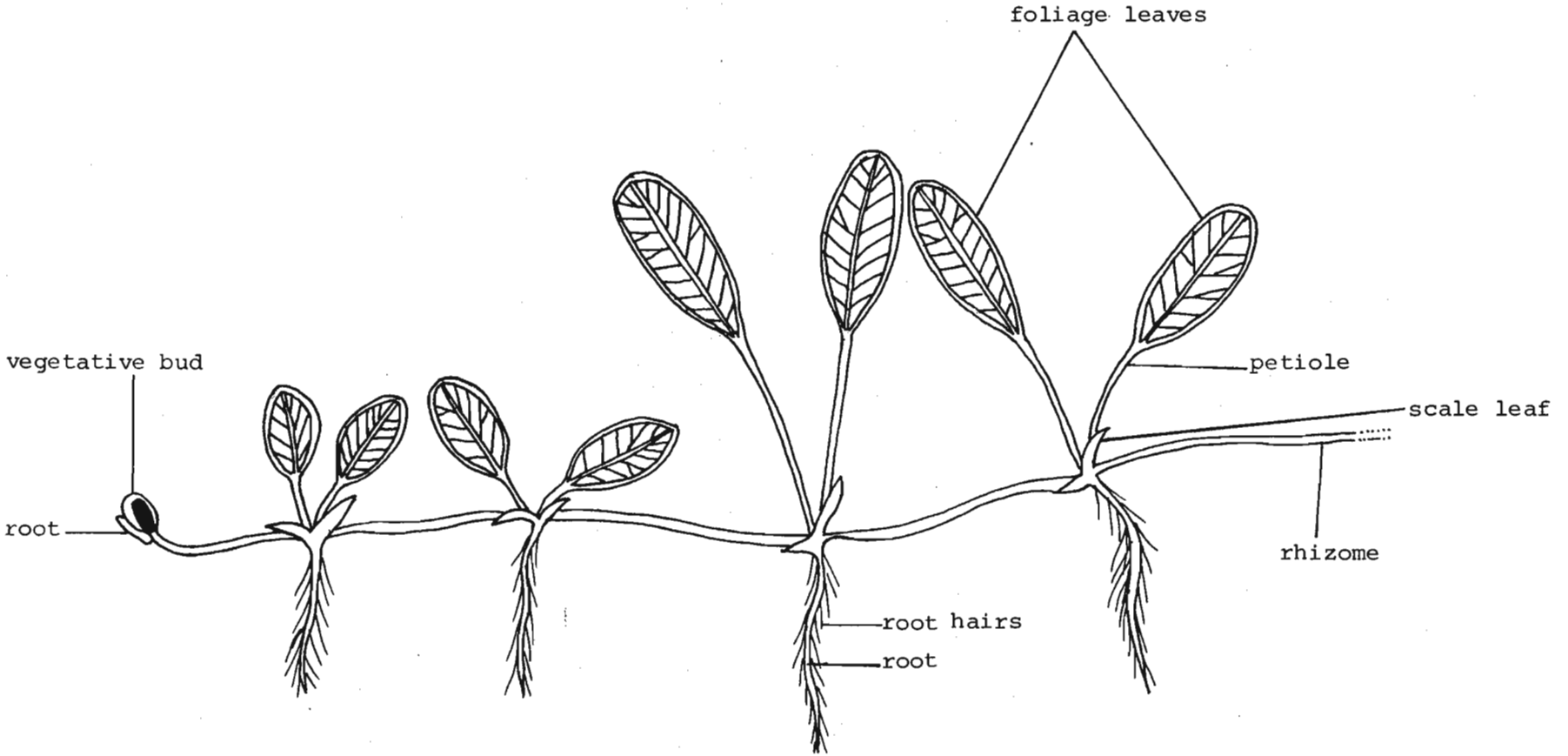


Fig. 1. Habit sketch of part of the plant of Halophila ovalis X2.

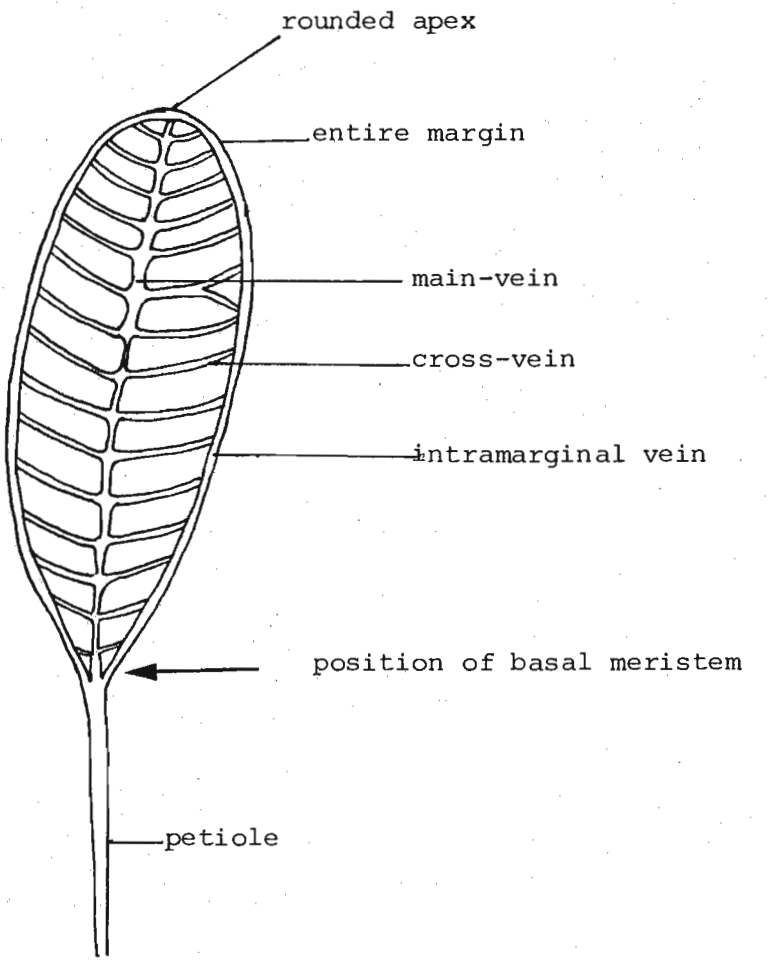


Fig. 2. Foliage leaf of Halophila ovalis X5.

leaf margin to the apex where they unite with the midrib. The outer epidermal cell walls of the petiole are thicker than the radial walls (Fig. 10).

The general anatomy of mature leaves is illustrated in Figures 3 - 8. A transverse section of a mature leaf indicates that three major tissue types (epidermal, mesophyll and vascular) make up the leaf blade. The main-vein is surrounded by an indistinct bundle sheath and is separated from the epidermis (upper and lower) by mesophyll cells. Mesophyll tissue is developed only in the midrib region and around the intramarginal veins. Large air lacunae are present in the mesophyll on either side of the main-vein. The lacunae are delimited by mesophyll cells. The lacunae of the blades are continuous with those of the petioles, rhizomes and roots (Roberts, McComb & Kuo, 1984). The lamina is made up of two rows of epidermal cells which are at times interrupted by lateral veins. Epidermal cells at the edge of the lamina have thicker walls than the cells of the lamina region (Fig. 5).

Epidermis

A uniseriate epidermis covers the leaf surface. Both upper and lower epidermi have similar structural features. Stomata are absent. Treatment of the cells with Sudan III and IV revealed the presence of a very thin cuticle (Fig. 15, arrows). In cross-sectional view the cells are more or less cuboidal whereas in longitudinal section they are longer than broad (Fig. 8). Epidermal cells are smaller than adjacent mesophyll cells (Fig. 3). Mature epidermal cells are highly vacuolate with a thin parietal layer of cytoplasm. Most of the organelles are restricted to the peripheral cytoplasm (Fig. 7). Epidermal cells have an abundance of chloroplasts and relatively thick outer walls (Fig. 7, arrow). Treatment of the cells with

PLATE 1

Figs. 3 - 10. Light micrographs of transverse and longitudinal sections of mature leaf blades and petiole.

Fig. 3. General anatomy of blade in midrib region, showing upper epidermis, lower epidermis, mesophyll, air lacuna and mid-vein.

Fig. 4. Details of midrib region, showing highly vacuolate cells of upper and lower epidermis and mesophyll, mid-vein composed of adaxial xylem and abaxial phloem.

Fig. 5. Details of intramarginal vein showing adaxial xylem, abaxial phloem, mesophyll. Note thick walled epidermal cells of leaf margin and lamina.

Fig. 6. Details of mid-vein and surrounding mesophyll tissue. Mid-vein surrounded by indistinct bundle sheath. Xylem lacuna surrounded by a row of inflated cells (arrow). Phloem consists of sieve tubes, companion cells and phloem parenchyma.

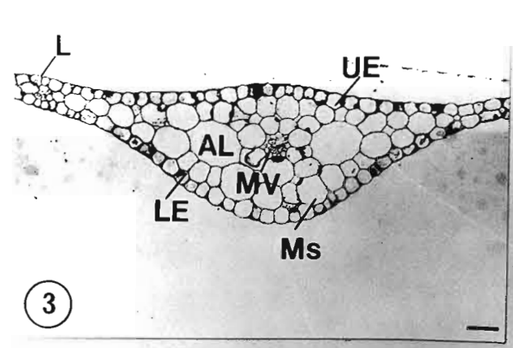
Fig. 7. High magnification view of epidermal and mesophyll cells. Notice outer tangential cell walls (arrow) thicker than radial walls (arrow head). Epidermal cells contain more chloroplasts than mesophyll cells.

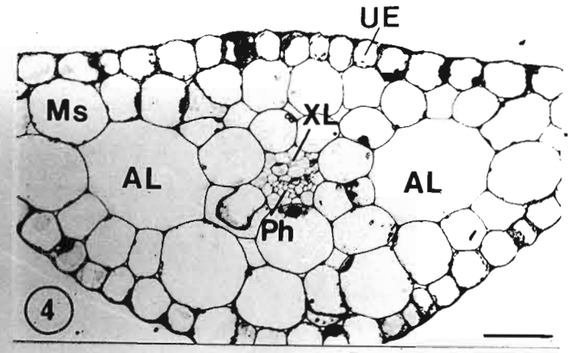
Fig. 8. Longitudinal section of leaf blade showing epidermal cells longer than broad and elongated mesophyll cells.

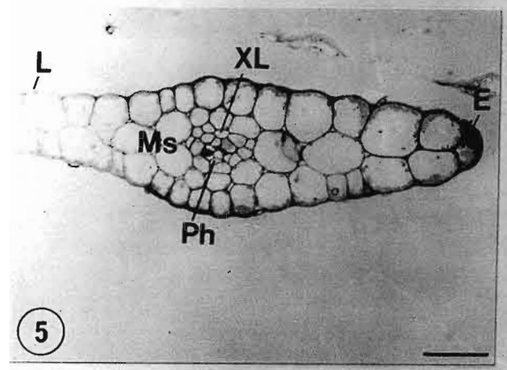
Fig. 9. General anatomy of petiole showing epidermis, fibro axial, lateral vascular bundles and air lacunae.

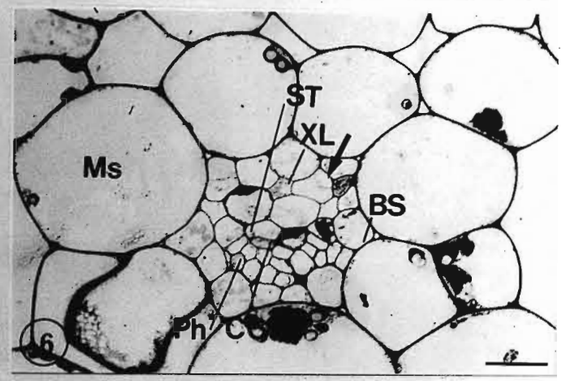
Fig. 10. High magnification view of petiole epidermis showing thick outer tangential walls (small arrow) in comparison to radial (thick arrow) and inner tangential walls (arrow head). Note large vacuolate mesophyll cells.

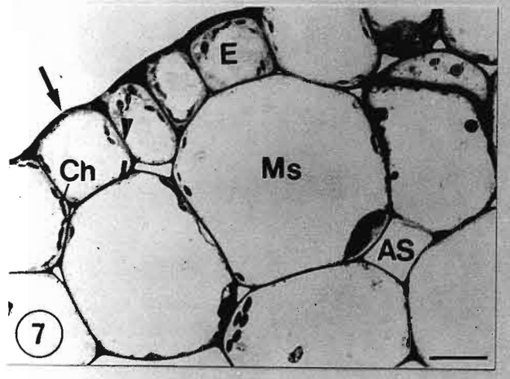
Bars represent: 30 µm (Fig. 3)
20 µm (Figs. 4, 5, 8, 9)
8 µm (Figs. 6, 7, 10)

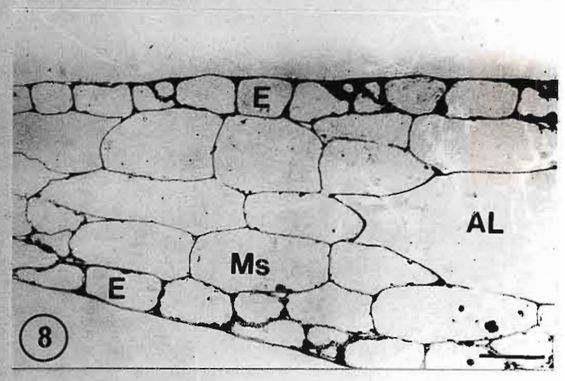


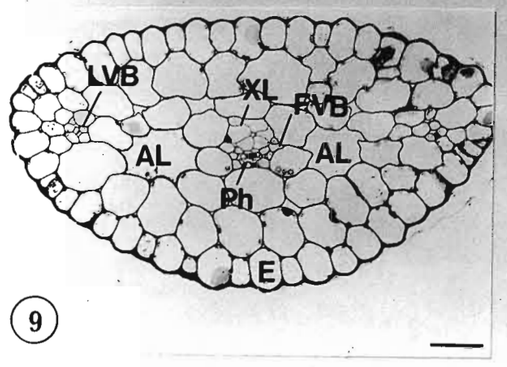


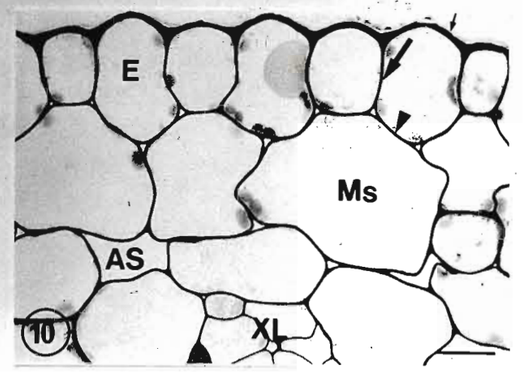












IKI-H_2SO_4 revealed the cellulosic nature of the walls. The cell walls stained purplish-blue (Fig. 16, arrows). In addition, a positive reaction of the walls with Ruthenium Red showed that the walls were rich in pectins (Fig. 17, arrows). Treatment of sections with Alcian dyes (Alcian Blue and Alcian Yellow) showed that epidermal cell walls were composed of both sulphated and acid non-sulphated polysaccharides, since they stained green (Fig. 18, arrows). Cell walls showed a negative reaction when treated with Phloroglucinol indicating that lignin was not present. The cell walls were PAS positive (Fig. 19).

Mesophyll

Mesophyll tissue is developed only in the midrib region (Fig. 3) and around the intramarginal veins (Fig. 5) as mentioned earlier. In transverse section the cells are spherical whereas in longitudinal section (Fig. 8) they are somewhat elongated. The cells are large, highly vacuolate with the organelles restricted to the thin parietal cytoplasm (Fig. 7). Air-spaces are evident between the mesophyll cells (Fig. 7). Unlike the epidermis, mesophyll cells contain many starch grains (Fig. 16).

Longitudinal veins

The longitudinal veins are conspicuous and include a median vein and two intramarginal veins (Figs. 4, 5). As mentioned earlier the three veins unite at the apical region of the leaf. Xylem occurs in the adaxial position and phloem is abaxial (Fig. 6). An ill-defined bundle sheath encloses the vascular tissues (Fig. 6). The xylem consists of a narrow lacuna surrounded by a row of inflated parenchyma cells (Fig. 6, arrow). Phloem consists of sieve tubes, companion cells and parenchyma (Fig. 6). The arrangement of the

PLATE 2

Figs. 11 - 14. Light micrographs of longitudinal sections of part of leaf blade and petiole of young leaves.

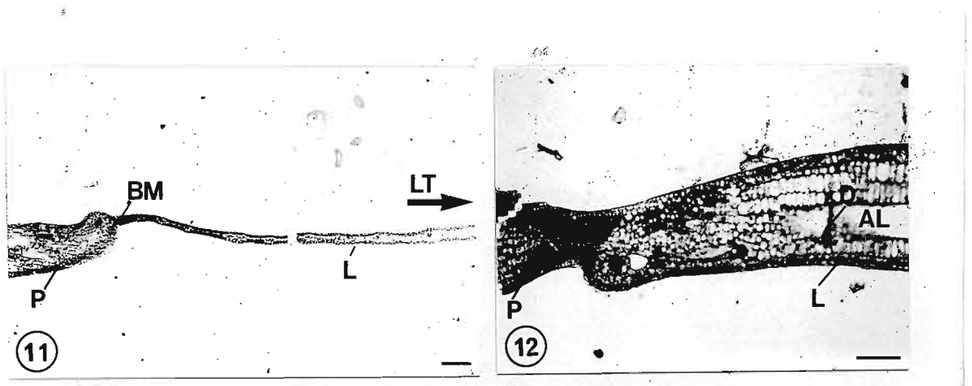
Fig. 11. Low magnification view of leaf blade and petiole indicating basipetal development of cells, the oldest cells occurring close to leaf tip region (LT, arrow) and youngest cells close to meristem.

Fig. 12. High magnification view showing probable position of basal meristem at junction of leaf blade and petiole. Note air lacuna interrupted by diaphragm.

Fig. 13. Details of basal meristem showing dense protoplasts of cells.

Fig. 14. General view of part of leaf blade and petiole, showing basal meristem and air lacuna interrupted by one cell thick diaphragm.

Bars represent: 20 µm (Figs. 11, 12, 14)
8 µm (Fig. 13)



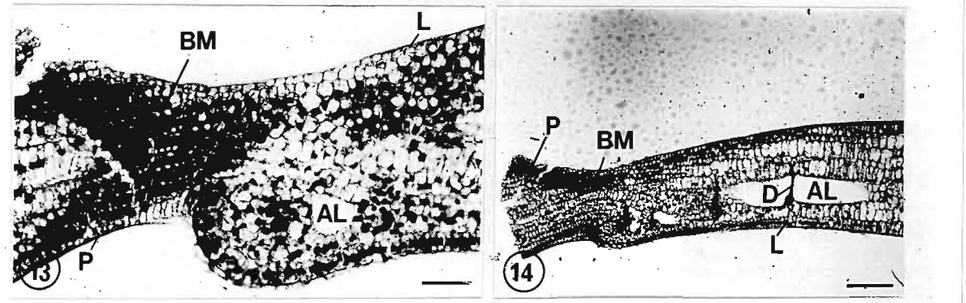


PLATE 3

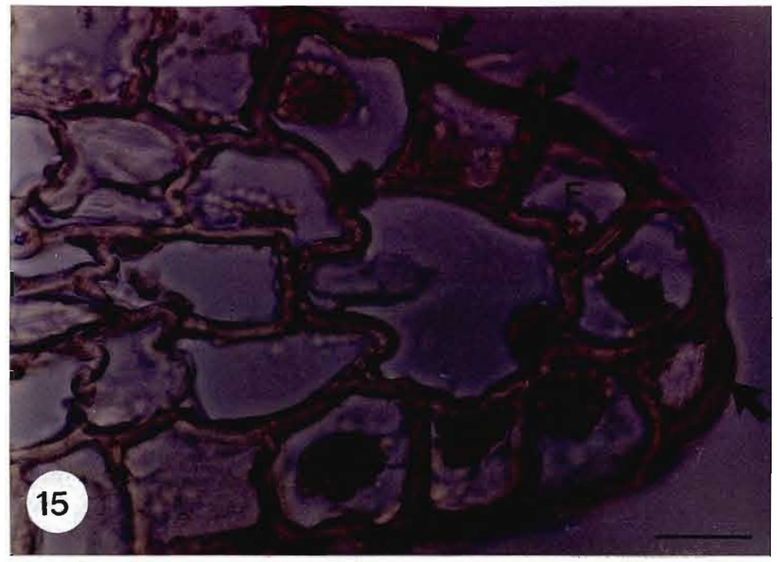
Figs. 15 - 17. Light micrographs of transverse sections of mature leaf blades showing the results of various histochemical tests.

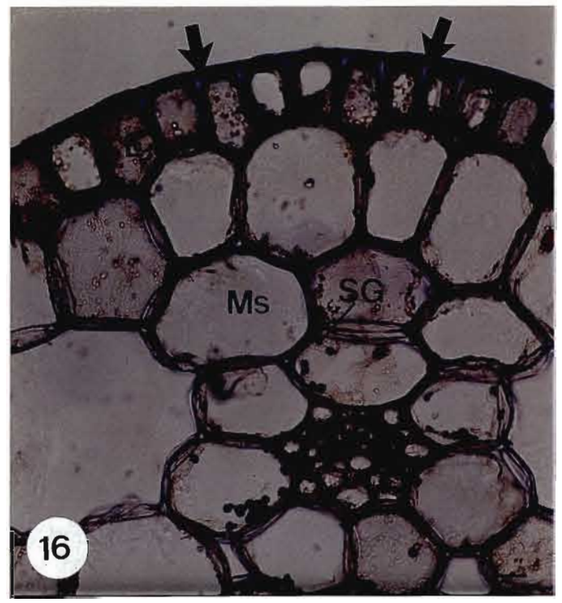
Fig. 15. Treatment with Sudan III and IV showing the presence of a very thin cuticle (arrows).

Fig. 16. Treatment with IKI-H_2SO_4 showing the cellulosic nature of the walls (arrows). Note starch grains of mesophyll cells also stained.

Fig. 17. The walls stain intensely with Ruthenium Red (arrows) indicating the presence of pectins.

Bars represent: 10 µm (Figs. 15, 16, 17)





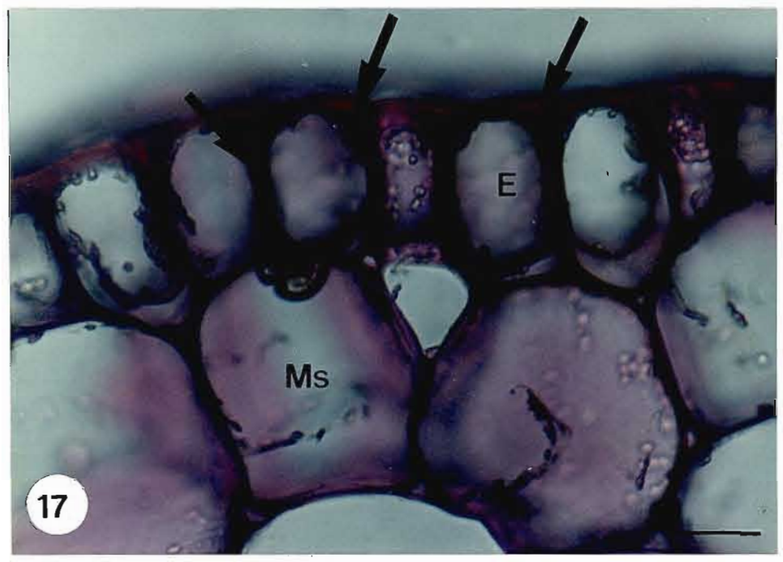


PLATE 4

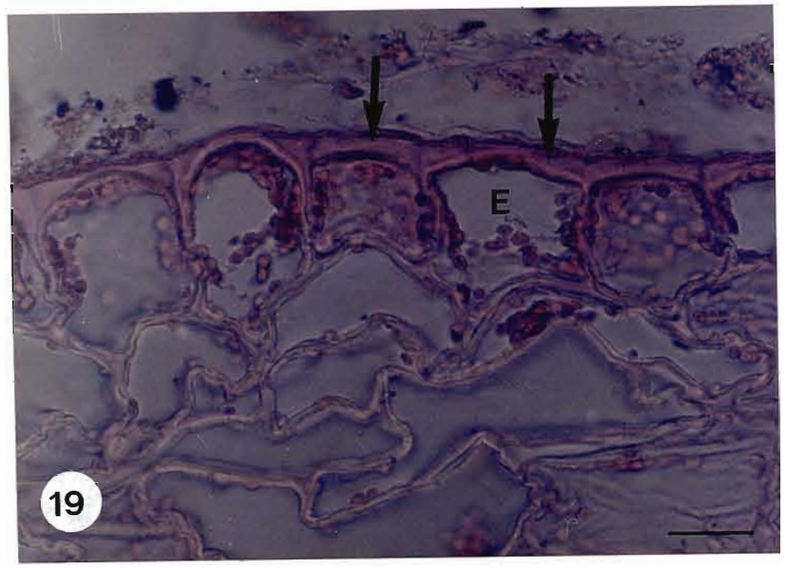
Figs. 18 - 20. Light micrographs of transverse sections of mature leaf blades showing the results of various histochemical tests.

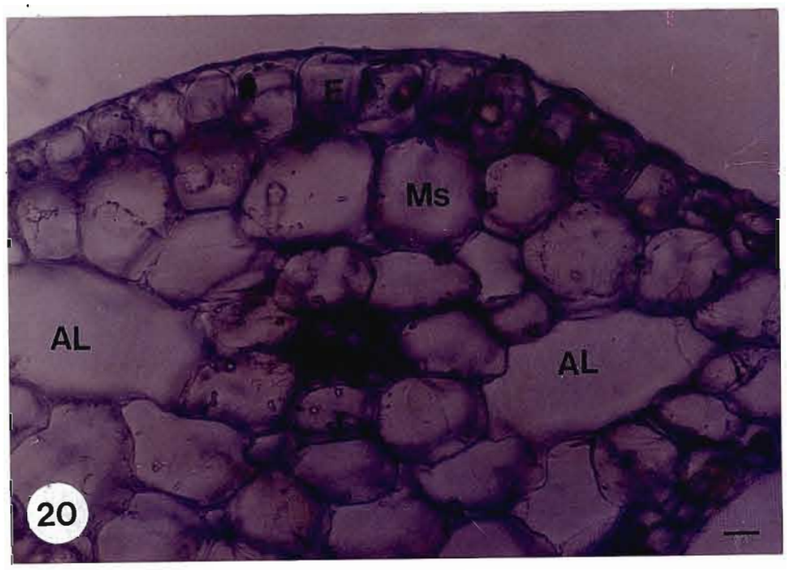
Fig. 18. The walls stain green with Alcian Blue and Alcian Yellow treatment.

Fig. 19. The walls stain pink with Periodic-Acid-Schiff's (PAS) reagent indicating a positive reaction for the presence of total carbohydrates.

Fig. 20. Micrograph of unstained control.

Bars represent: 10 µm (Figs. 18, 19, 20)





vascular tissues in the intramarginal vein (Fig. 5) is similar to that described for the main-vein.

Discussion

Like other seagrasses, *H. ovalis* possesses branched rhizomes located near the surface of the substrate. The creeping rhizomes bear roots and leaves at each node. However, unlike other seagrasses, the leaves of *H. ovalis* possess long, slender petioles which support the thin ovate blades. In this respect *H. ovalis* does not resemble seagrasses and monocotyledonous plants in general.

As seen in other seagrasses epidermal cells of *H. ovalis* possess more chloroplasts than any other tissues of the leaves and appear therefore to be the main photosynthetic tissue. A thick outer epidermal cell wall as seen in *H. ovalis* has also been reported in leaves of *Zostera capensis* (Barnabas *et al*, 1977), *Thalassodendron ciliatum* (Barnabas, 1982) and *Thalassia testudinum* (Jagels, 1973). These thickened walls together with the pronounced thickening of epidermal cell walls along the leaf margin (Fig. 5) probably provide the leaves with mechanical support since lignified tissue is absent. Like other seagrasses such as *Zostera capensis* (Barnabas, 1979, unpublished), *Posidonia australis* (Kuo, 1978) and *Posidonia oceanica* (Colombo *et al*, 1983) epidermal cell walls are pecto-cellulosic in nature. Additional support of the leaf blades is offered by the petiole since epidermal cells of the petiole have greatly thickened outer walls. It is interesting to note that structural features of petiole epidermal cells resemble those of leaf sheath epidermal cells of *Zostera capensis* (Barnabas, 1979, unpublished).

PLATE 5

Figs. 21 - 27. Electron micrographs of category A epidermal cell.

Fig. 21. A series of differentiating cells, showing the youngest cells at leaf margin, older cells towards midrib (M, arrow). Vacuoles increase in number in cells away from marginal area. Outer tangential wall thicker in wedge-shaped regions between adjacent epidermal cells (small arrows).

Fig. 22. Details of whole cell typical of category, showing large nucleus containing condensed nucleolus, proplastids, young mitochondria and dictyosomes. Numerous plasmodesmatal connections evident along radial and inner tangential cell walls.

Fig. 23. Details of part of epidermal cell, showing dense cytoplasm, proplastids, mitochondria, short profiles of ER around nucleus and wavy appearance of cuticle.

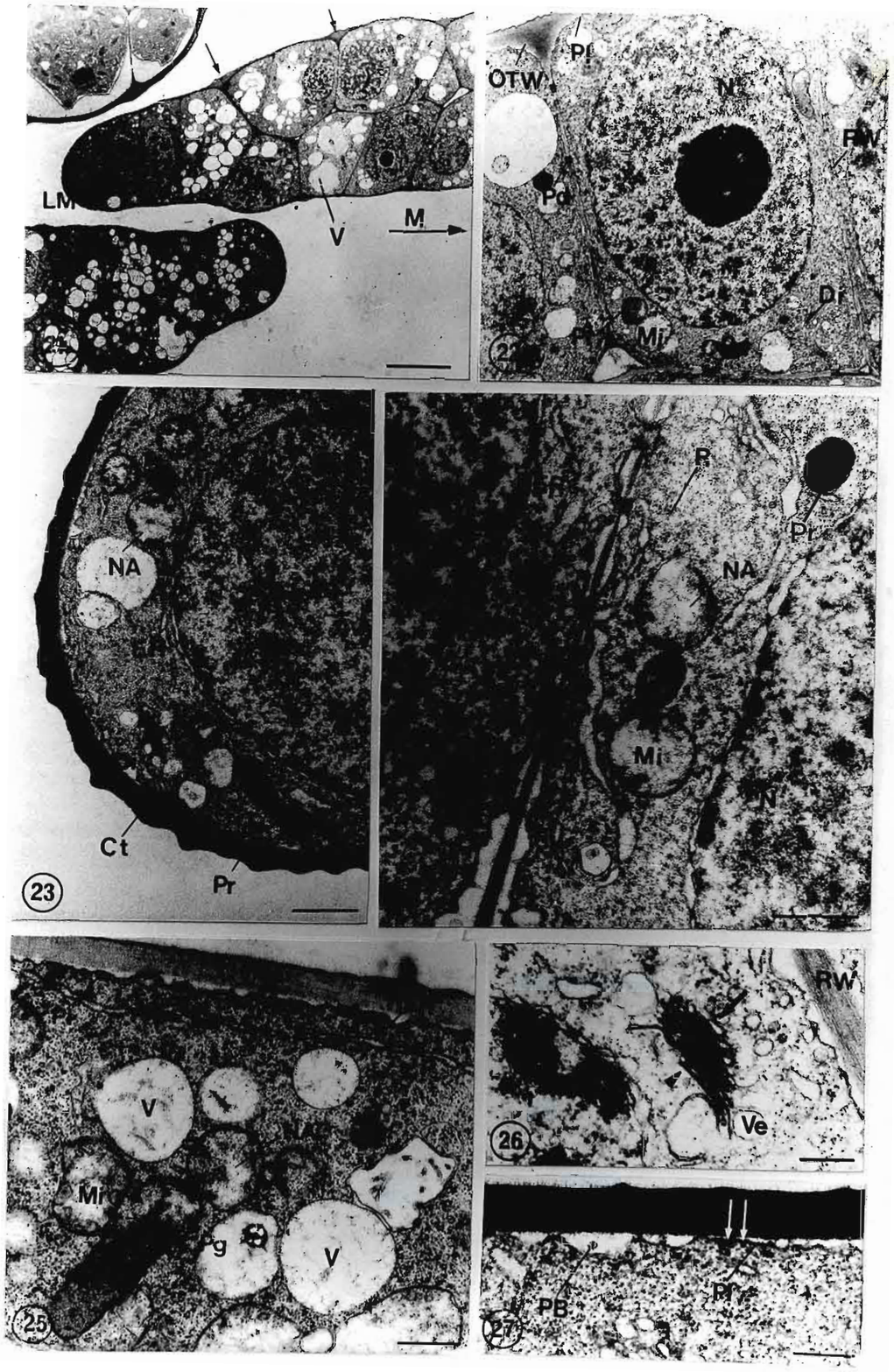
Fig. 24. Details of cytoplasm on either side of radial wall showing dictyosomes with associated vesicles close to wall, ribosomes, swollen profiles of RER, proplastids and circular mitochondria. Note numerous plasmodesmatal connections along radial cell wall.

Fig. 25. Long profiles of RER close to outer tangential wall, slight withdrawal of plasmalemma from wall, mitochondria, proplastids, clustered ribosomes (polysomes) and small vacuoles containing fine fibrillar material seen in cytoplasm.

Fig. 26. High magnification view of dictyosome with associated vesicles showing forming face (arrow) and maturing face (arrow head).

Fig. 27. Cortical microtubules (arrows) seen in transverse section immediately adjacent to plasmalemma. Paramural body in extracytoplasmic space.

Bars represent: 10 µm (Fig. 21)
1 µm (Figs. 22, 23, 24)
0,5 µm (Fig. 25, 27)
0,25 µm (Fig. 26)



The presence of large air-lacunae in the mesophyll tissue is a characteristic feature of the leaves of *H. ovalis*. These lacunae are continuous with the lacunae of the petioles, rhizomes and roots (Roberts, McComb & Kuo, 1984). Although continuous within the organs these lacunae were shown to be interrupted between organs by diaphragms one cell in thickness (Fig. 14). The diaphragms are perforated by interstitial pores which are believed to provide air continuity within the lacunar system. The presence of a well-developed extensive lacunar system in *H. ovalis* is probably an adaptive feature to colonize and survive in anoxic sediments.

The organization of the vascular tissues in *H. ovalis* closely resembles that of other seagrasses (Tomlinson, 1972; Barnabas *et al*, 1977, 1983a) in that xylem is reduced and phloem is well-developed. The poor development of xylem in seagrasses is a characteristic which is associated with submerged aquatics (Arber, 1920; Sculthorpe, 1967). Since all the organs of submerged aquatics are in contact with water, xylem is regarded as non-essential, hence its poor development. The indistinct bundle sheath around the phloem as seen in *H. ovalis* has also been reported in other seagrasses (Doohan & Newcomb, 1976; Benedict & Scott, 1976; Barnabas, 1979, unpublished).

Like most seagrasses, a well-defined basal meristem is present in *H. ovalis* at the junction of the leaf blade and petiole. According to Tomlinson (1980) seagrass leaves develop by means of basal meristems.

On the basis of the findings in the present study *H. ovalis* resembles other seagrasses both morphologically and anatomically.

IV. ULTRASTRUCTURE OF THE EPIDERMIS

1. ASPECTS OF DEVELOPMENT OF EPIDERMAL CELLS

RESULTS

Leaves of different ages were sampled so that cells in various stages of development, ranging from very young to mature cells were studied. Changes in plastid and mitochondrial structure, cell wall morphology (including initiation and subsequent development of wall ingrowths), and to a certain extent increase in the degree of vacuolation, were used as developmental indices to separate cells into different categories. Eight such categories were distinguished.

Category A

Category A cells represent the youngest epidermal cells studied. These cells are characterised by large nuclei, numerous ribosomes, proplastids, young mitochondria, dictyosomes and profiles of endoplasmic reticulum (ER). The most outstanding feature is the density of the cytoplasm in all the cells (Figs. 21-25) mostly due to the presence of ribosomes found singly or in clusters. Ribosomes remain abundant in the cells of categories B-H. Many long and short profiles of ER occur around the nucleus (Fig. 23) and close to the cell walls (Figs. 24, 25). The ER in Figure 25 is studded with ribosomes.

Few dictyosomes occur close to the radial cell wall (Figs. 24, 26). The maturing face of the dictyosome is associated with several vesicles (Fig. 26, arrow). The proximity of the dictyosomes and ER to the cell walls has been observed in categories B-H.

Young mitochondria, circular in thin section, with relatively few cristae, are well distributed in the perinuclear cytoplasm (Fig. 23). The immature mitochondria display electron transparent areas in the stroma (Figs. 23, 24, 25).

Proplastids with no differentiation of the internal lamellae are observed in Figures 23 and 25. These structures are mainly elongate to spherical in shape. Plastoglobuli are evident in the stroma of the proplastids which appears more electron dense than the surrounding cytoplasm and other organelles (Fig. 25).

Nuclei are large, smoothly circular in outline and occupy most of the space in the cells (Fig. 22). The nucleolus is condensed and highly electron dense (Fig. 22). Areas within the nucleolus are electron transparent (Fig. 22) probably suggestive of nucleolar vacuoles. The heterochromatin within the nucleoplasm appears to be very diffuse (Figs. 22, 23). The morphology of the nucleus in the other categories is similar to that of Category A cells.

The plasmalemma is regular in outline and appears closely appressed to the cell walls (Fig. 22). Occasionally there is slight withdrawal of the plasmalemma from the cell wall thus creating an extracytoplasmic space occupied by few paramural bodies (Figs. 24, 27).

The outer tangential cell wall is thicker than the walls in contact with adjacent epidermal cells (Fig. 22). The thickness of the outer tangential wall is more pronounced in the outer wedge-shaped regions between adjacent epidermal cells (Fig. 21, small arrows). The cuticle is observed to have a wavy appearance (Fig. 23) and is sudanophilic (Fig. 15).

Plasmodesmata are common along the walls between adjacent epidermal cells and between epidermal and mesophyll cells (Figs. 22, 24).

Microtubules are evident immediately adjacent to the plasmalemma along the outer tangential cell wall (Fig. 27 arrows). Few small vacuoles occur in the marginal epidermal cells (Fig. 21). They tend to increase in number and coalesce in epidermal cells away from the marginal area. Material of a fine fibrillar nature forms part of the contents of the vacuoles (Fig. 25).

Category B

The cells of Category B show signs of further differentiation compared to Category A cells in that there is a greater degree of vacuolation and a change in proplastid number and morphology.

The density of the protoplasts is similar to the cells of Category A and remains unchanged in the other categories. Numerous polysomes are discernible in Figure 33. Profiles of Rough Endoplasmic Reticulum (RER) are abundant in the vicinity of the cell walls (Figs. 33, 34).

The dictyosome population remains similar to the cells of the previous category (Figs. 33, 34).

Immature mitochondria, assuming circular, ovoid or elongate shapes are fairly abundant in the cytoplasm (Fig. 32). More development of cristae is seen in the mitochondria of the cells of this category (Fig. 32) than those of the previous category.

There is an increase in the proplastid population as compared to the cells of Category A. These structures assume various shapes and are electron dense (Figs. 30, 31). No development of internal lamellae is evident in the stroma of the proplastids (Figs. 28, 30). Some of these organelles possess prolamellar bodies (Gunning and Jagoe, 1967), (Fig. 28). Plastoglobuli and electron transparent areas termed nucleoid regions (Ris and Plaut, 1962) occur in the proplastid stroma (Fig. 28).

An interesting nucleolus is seen in Figure 28. A conspicuous electron transparent area, suggestive of a nucleolar vacuole occurs within the nucleolus.

PLATE 6

Figs. 28 - 35. Transverse sections of category B cells.

Fig. 28. Epidermal cell typical of category, with many small vacuoles, proplastids containing prolamellar bodies, large nucleus and numerous plasmodesmatal connections along radial cell walls and also between epidermal and mesophyll cell walls.

Fig. 29. Part of cytoplasm showing withdrawal of plasmalemma from outer tangential wall, paramural bodies in extracytoplasmic space and vacuole containing cytoplasmic matrix (arrow).

Fig. 30. Elongate proplastids, circular mitochondria and lipid body in cytoplasm.

Fig. 31. Details of cytoplasm along inner tangential wall indicating electron dense proplastids and their close association with mitochondria and ER.

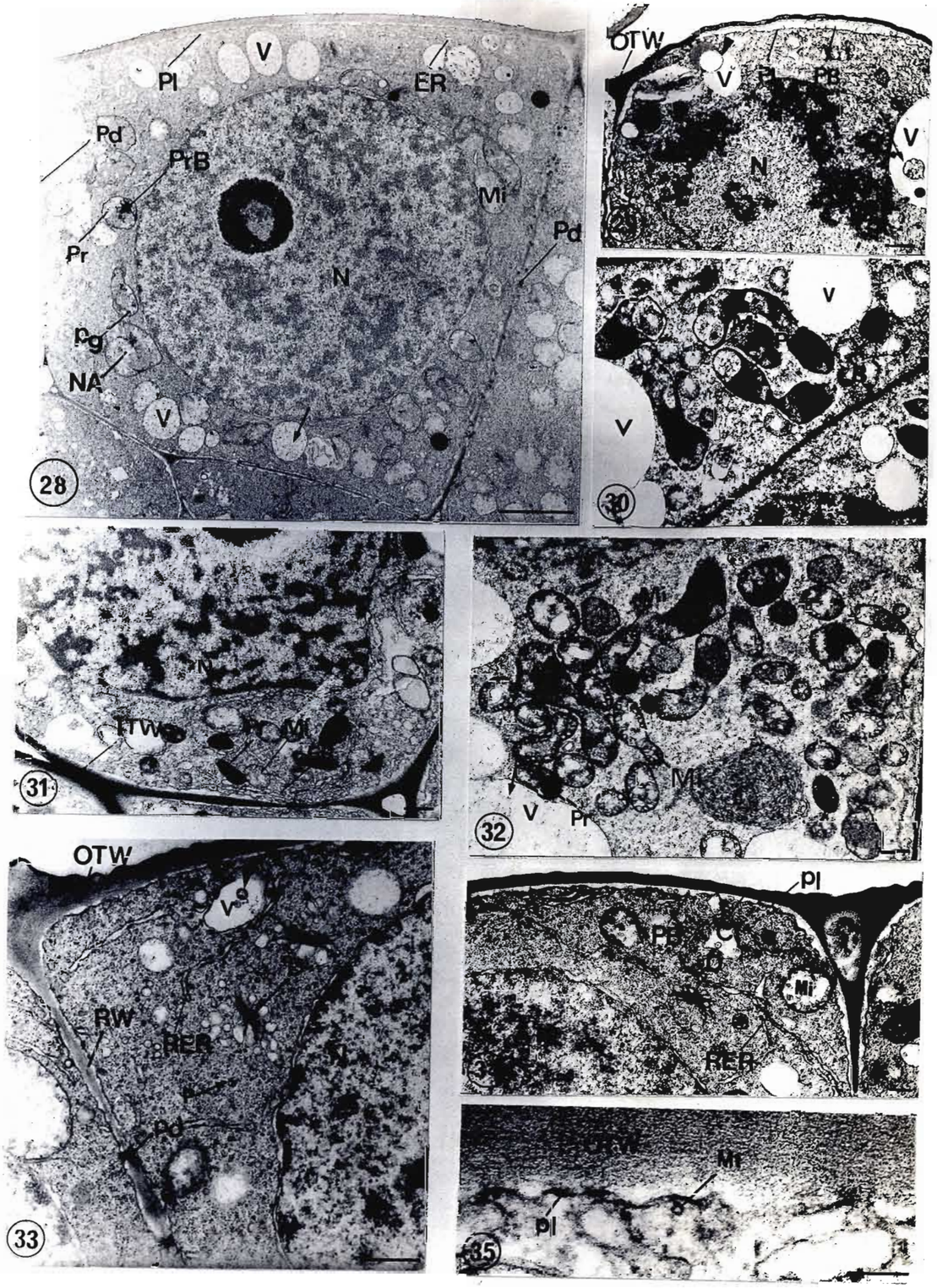
Fig. 32. Group of immature mitochondria, seen in transverse and longitudinal views. Vacuole contains material of fine fibrillar nature (arrow).

Fig. 33. High activity of dictyosomes and associated vesicles and RER in cytoplasm close to junction of outer tangential and radial cell walls. Note clustered ribosomes (polysomes) in cytoplasm (arrow) and vesicles in vacuole (arrow head).

Fig. 34. Details of cytoplasm along part of outer tangential wall showing profiles of RER, dictyosome, mitochondria, withdrawal of plasmalemma from cell wall and wavy appearance of cuticle. Paramural bodies in extracytoplasmic space.

Fig. 35. Part of thick outer tangential wall and microtubule adjacent to plasmalemma.

Bars represent: 1 µm (Figs. 28, 29, 30, 31, 32, 34)
0,5 µm (Fig. 33)
0,25 µm (Fig. 35)



The plasmalemma closely follows the contours of the cell wall (Fig. 28). In some cells the membrane withdraws slightly from the wall (Figs. 29, 34) thereby forming the extracytoplasmic space occupied by paramural bodies. The cell walls show an increase in thickness at this stage of their development (Figs. 33, 34). The uneven wavy appearance of the cuticle persists (Fig. 34).

Plasmodesmatal connections between adjacent epidermal cells are numerous (Figs. 28, 33).

Cortical microtubules are present immediately inside the plasmalemma (Fig. 35).

A few lipid bodies are evident in the cytoplasm (Figs. 28, 30, 32).

High vacuolar activity is observed in the cells of this category. Coalescence of vacuoles, to form larger structures is evident in Figure 29 (arrow head). The nature of the vacuolar contents is variable and includes material of a fine fibrillar nature (Figs. 28, 32, arrows) vesicles (Fig. 33, arrow head) and portions of cytoplasmic matrix (Fig. 29, arrow). The presence of larger vacuoles indicates that the cells of this category are more advanced in development than Category A cells.

Category C

Important changes regarding proplastid and mitochondrial morphology, cell wall thickness, distribution of ER and dictyosomes are noticeable in the cells of this category.

Profiles of RER (Fig. 40) are aligned close to and almost parallel to the outer tangential and radial cell walls.

Dictyosomes and their associated vesicles are evenly distributed in the cytoplasm and are active in the vicinity of the outer tangential and partly along the radial cell walls (Figs. 41, 42, 44).

The cytoplasm is well populated with young developing mitochondria that are predominantly circular in thin section and appear to be larger than those encounterd in the cells of the previous categories (Fig. 41). Cristae are relatively few and large electron transparent areas are seen in the mitochondrial stroma (Figs. 41, 45, thick arrows).

Microtubules are present adjacent to the plasmalemma in the region of the outer tangential wall (Fig. 47, arrow).

The plasmalemma is irregular. The extracytoplasmic space formed by the withdrawal of the membrane from the wall contains paramural bodies and other membranous structures (Fig. 37). Sometimes extracytoplasmic pockets lined by the plasmalemma and containing numerous spherical paramural bodies are observed in the vicinity of the outer tangential wall (Fig. 39) and the radial wall (Fig. 41). These bodies appear to arise from projections emanating from the plasmalemma (Fig. 41, small arrow).

PLATE 7

Figs. 36 - 41. Transverse sections of category C cells.

Fig. 36. Cell typical of category showing large central nucleus, withdrawal of plasmalemma from wall, paramural bodies in extracytoplasmic space, ER close to cell walls, electron dense proplastids, mitochondria and numerous plasmodesmatal connections along radial cell walls.

Fig. 37. Details along part of outer tangential wall, showing increase in thickness in wall, paramural bodies and membranous structures in extracytoplasmic space.

Fig. 38. Details of outer tangential wall, showing cuticle not closely appressed to cell wall.

Fig. 39. Extracytoplasmic pocket lined by plasmalemma contains many paramural bodies apparently contributing material to differentiating wall.

Fig. 40. Close association of mitochondria, proplastids and RER in vicinity of outer tangential wall; nucleus contains nucleolus with electron transparent regions indicative of nuclear vacuoles (arrow).

Fig. 41. Details of cytoplasm at junction of outer tangential and radial cell walls showing extracytoplasmic pocket with paramural bodies probably contributing material to radial wall. Note dictyosome, RER and mitochondria close to wall.

Bars represent: 0,5 µm (Figs. 36, 37, 40, 41)
1 µm (Fig. 38)
0,25 µm (Fig. 39)

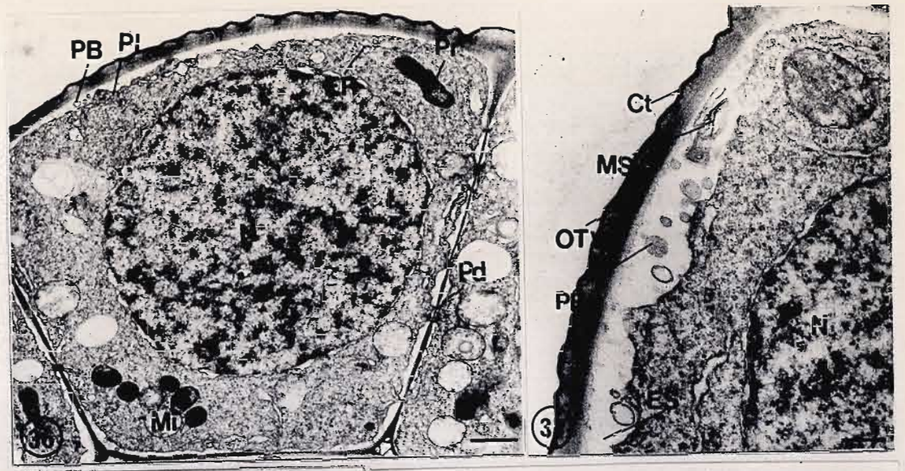


PLATE 8

Figs. 42 - 47. Transverse sections of category C cells.

Fig. 42. Details of cytoplasm along either side of radial wall showing dictyosomes and mitochondria close to cell wall. Note large prolamellar body in proplastid.

Fig. 43. Part of cytoplasm showing vacuole containing cytoplasmic material (small arrows) and many mitochondria.

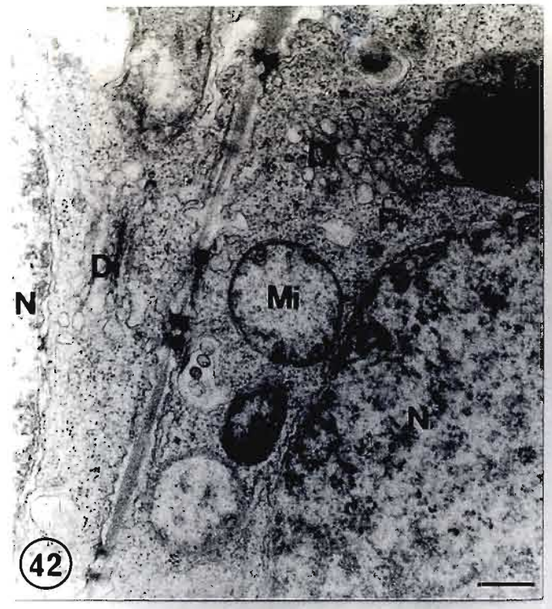
Fig. 44. Part of cytoplasm at junction of outer tangential and radial cell walls indicating dictyosome and profiles of RER close to cell walls, proplastid containing prolamellar body and plastoglobuli. Note withdrawal of plasmalemma from wall and uneven appearance of cuticle (arrow).

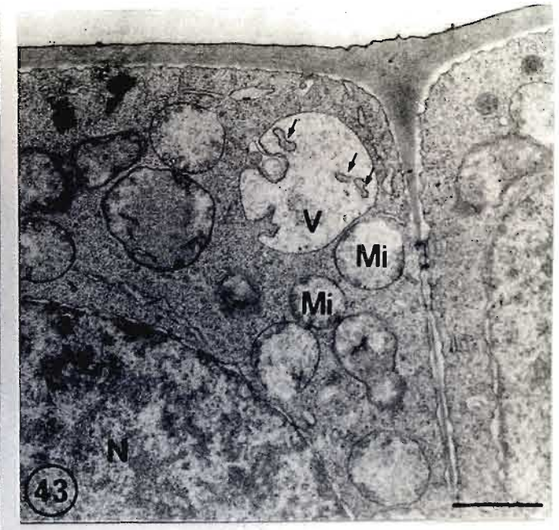
Fig. 45. Details of cytoplasm showing profiles of RER almost parallel to outer cell wall, mitochondria with prominent nucleoid areas (thick arrow), elongate proplastid containing prolamellar body and plastoglobuli, few primary thylakoids emanating from prolamellar body (arrow) and double nature of proplastid envelope (arrow head). Note thick outer tangential wall.

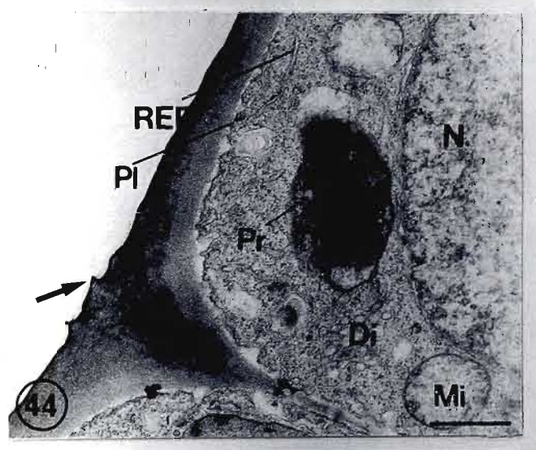
Fig. 46. High mangification view of proplastid showing few primary thylakoids (arrows), plastoglobuli, nucleoid area and double nature of proplastid envelope (arrow head).

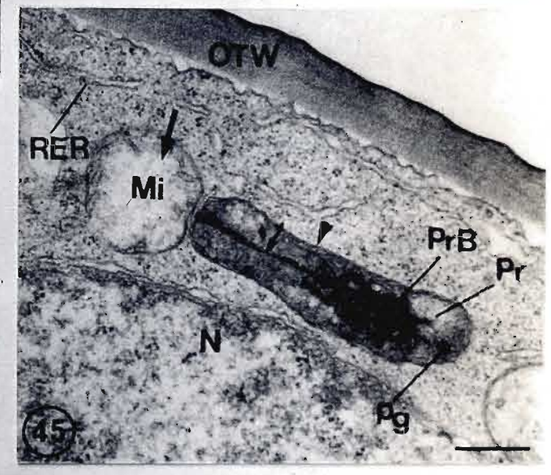
Fig. 47. Microtubules (arrows) adjacent to plasmalemma. Note density of cytoplasm due to ribosomes.

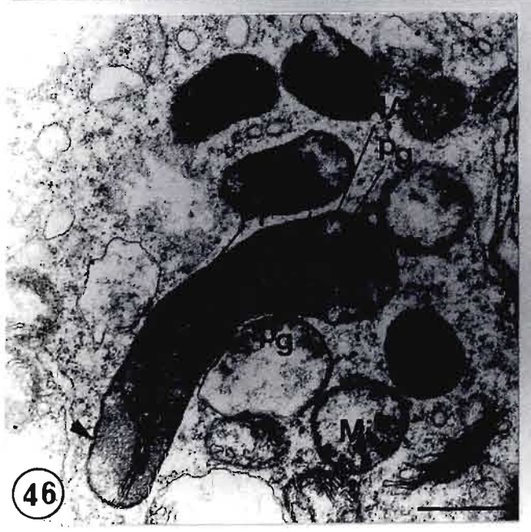
Bars represent: 0,25 µm (Fig. 42)
1 µm (Figs. 43, 44)
0,5 µm (Figs. 45, 46, 47)

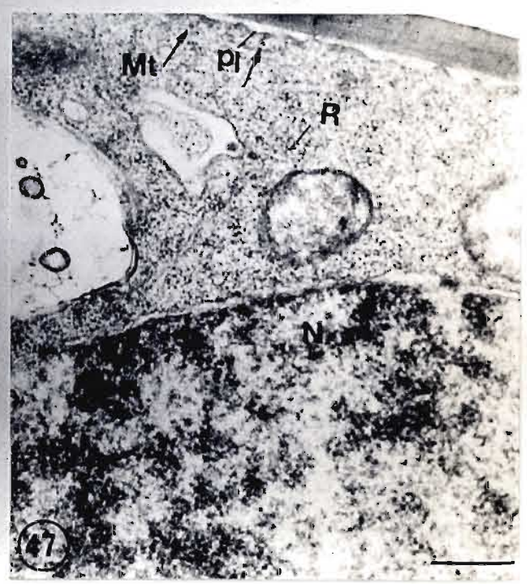












The cell walls continue to increase in thickness, this feature being most prominent in the outer tangential walls (Figs. 37, 45). The irregular outline of the cuticle is again evident (Fig. 37). The cuticle in Figure 38 is not closely appressed to the cell wall. Plasmodesmatal connections are evident between adjacent epidermal cells (Fig. 36).

Nucleolar vacuole occurs in the nucleolus of the cell seen in Figure 40.

Vacuolar activity remains high. The vacuole in Figure 43 (small arrows) is actively ingesting cytoplasmic matrix since the inclusions within bear resemblance to the matrix of the cytoplasm.

Plastids are elongate - ovoid structures. They display a definite double membrane (Fig. 45, arrow head) and a few primary thylakoids which emanate from the prolamellar body (Fig. 45, arrow). Plastoglobuli are prominent in the plastid stroma which is electron dense (Figs. 45, 46). More development of the internal plastid membranes is encountered in the cells of this category (Fig. 46, arrows).

Category D

A dramatic change is observed in plastid number and structure. The most striking feature in the cells of this category is the morphology of the plastids which bear resemblance to young chloroplasts (Figs. 52, 53). The cytoplasm of these cells is well populated with young chloroplasts. They have a tendency to occur in groups in different regions of the cells (Figs. 48, 50). The double nature of the chloroplast envelope is visible in Figure 53 (arrow). The young chloroplasts possess prominent membrane-organizing centres termed prolamellar bodies which are positioned at the poles or the centre of the chloroplast (Fig. 53). The prolamellar bodies display a typical crystal-lattice structure (Fig. 53). The young chloroplasts in Figures 52 and 53 display large prolamellar bodies from which emanate the primary thylakoids. The thylakoid lamellae run parallel to the long axis of the young chloroplast (Fig. 53, arrow head). Plastoglobuli occur in the prolamellar bodies as well as the stroma of young chloroplasts (Figs. 51, 53). Nucleoid areas (electron transparent regions) are another feature in the stroma of young chloroplasts (Fig. 53).

Mitochondria are large, spherical with little development of cristae (Fig. 51). They maintain a peripheral distribution in the cytoplasm (Fig. 51). Large nucleoid areas are evident in the mitochondrial stroma (Fig. 51). They have a tendency to occur in clusters (Fig. 51).

Occasionally twin nucleoli are observed in the nucleus (Fig. 54).

Dictyosomes (Fig. 56) and RER (Fig. 55) maintain a peripheral distribution.

The outer cell wall has thickened considerably (Figs. 50, 56).

PLATE 9

Figs. 48 - 53. Transverse sections of category D epidermal cells.

Fig. 48. Cell representative of category showing young chloroplasts, vacuole and nucleus containing nucleolus.

Fig. 49. Vacuoles coalescing to form larger structures present in one half of cell, nucleus and young chloroplasts aggregated in other half. Electron dense inclusions in large vacuole (arrow).

Fig. 50. Part of cytoplasm showing clustering of young chloroplasts, cytoplasmic material in vacuole and wall striations evident in thick outer tangential wall.

Fig. 51. Details of cytoplasm on either side of radial cell wall showing mitochondria, plastoglobuli and prolamellar body in stroma of young chloroplast.

Fig. 52. Young chloroplasts with prolamellar bodies. Note small vacuoles in cytoplasm and few plasmodesmatal connections along radial cell wall.

Fig. 53. Details of a young chloroplast depicting prominent prolamellar bodies giving rise to primary thylakoids (arrow heads). Note plastoglobuli and nucleoid areas in chloroplast stroma and double nature of chloroplast envelope (arrow).

Bars represent: 2 µm (Figs. 48, 52)
1 µm (Fig. 49)
0,5 µm (Figs. 50, 51, 53)

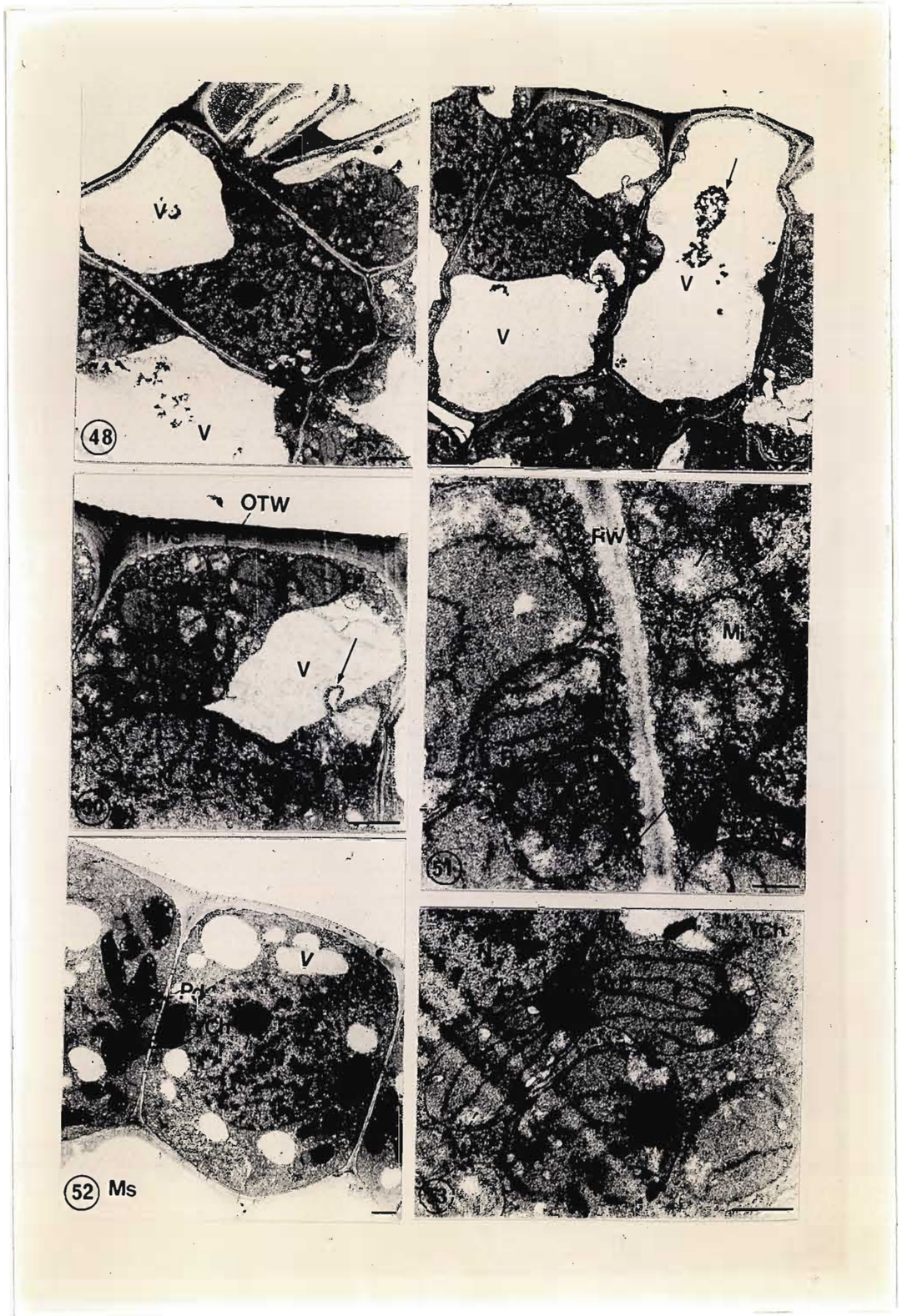


PLATE 10

Figs. 54 - 57. Transverse sections of category D epidermal cells.

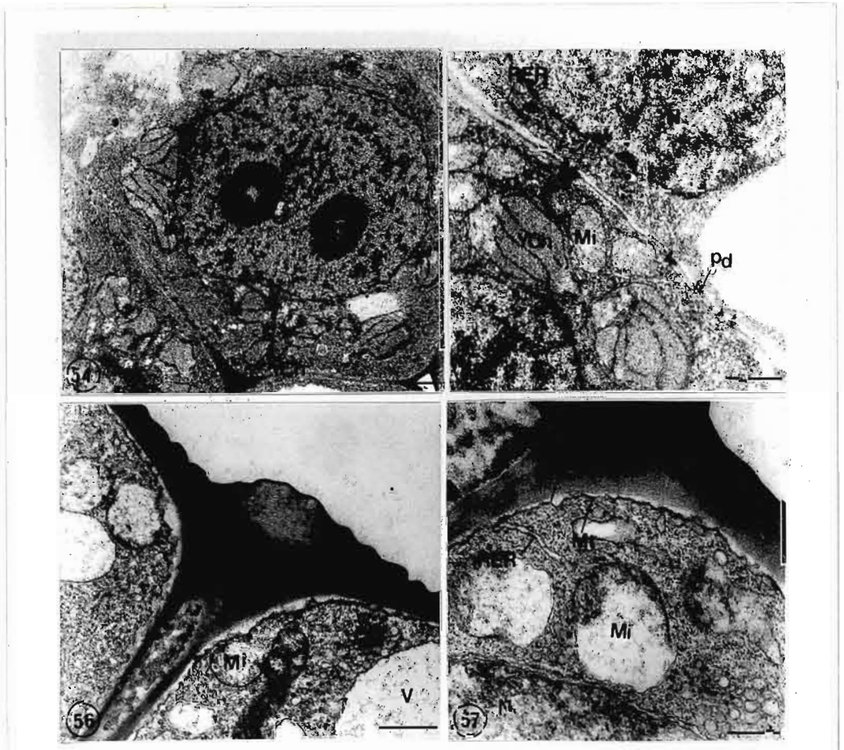
Fig. 54. Nucleus containing twin nucleoli, some young chloroplasts in cytoplasm.

Fig. 55. Details of cytoplasm along radial cell wall showing plasmodesmatal connections, RER close to wall, young chloroplasts and mitochondria.

Fig. 56. Thick outer tangential wall, striations evident in wall, close association of dictyosome with associated vesicles and mitochondria to wall.

Fig. 57. Details of cytoplasm at junction of outer tangential and radial cell walls showing microtubule adjacent to invaginated plasmalemma, mitochondria and RER close to cell wall.

Bars represent: 1 µm (Figs. 54, 55)
0,25 µm (Fig. 56)
0,5 µm (Fig. 57)



Striations in the outer cell wall are evident in this stage of development of the epidermal cells (Fig. 50).

Few plasmodesmatal connections between adjacent epidermal cells are noticed in the radial cell walls (Fig. 55).

Cortical microtubules are seen inside the plasmalemma at the corner of the outer tangential wall (Fig. 57, arrow).

Vacuoles have coalesced to form large structures (Fig. 49). Within some of the large vacuoles there is some cytoplasmic material (Fig. 50, arrow) and electron dense inclusions (Fig. 49, arrow) suggestive of lysosomal activities.

Category E

A striking feature of this category is the highly vacuolate cells. In addition, wall ingrowths are initiated at the junction of the outer tangential and radial cell walls.

Long and short profiles of RER occur singly and maintain a peripheral distribution close to the cell wall (Figs. 59, 64). The ER is observed to be studded with ribosomes (Fig. 63).

Many dictyosomes and associated vesicles are located close to the outer tangential and radial walls (Figs. 59, 60, 64, 65) and in the vicinity of the developing wall ingrowths (Fig. 59). The contents of some of the vesicles resemble those of the inner region of the outer cell wall (Fig. 59, arrows) suggesting that these vesicles could be contributing material to the wall.

The peripheral cytoplasm is well populated with mitochondria which are closely aligned along the radial and outer tangential walls (Figs. 61, 66). Further development of the cristae is noticed in these organelles (Fig. 64).

Microbodies are occasionally present (Fig. 61). Lipid bodies are present in the cells of this category (Fig. 69).

Cortical microtubules occur immediately inside the plasmalemma partly along the outer tangential and radial cell walls (Figs. 59, 60, 63).

The chloroplasts are large, ovoid-elliptic structures dominating the peripheral cytoplasm (Fig. 58). The double nature of the chloroplast

PLATE 11

Figs. 58 - 63. Transverse sections of category E cells.

Fig. 58. Cell typical of category showing large vacuole, initiation of wall ingrowths at junction of outer tangential and radial cell walls (arrows) and some young chloroplasts.

Fig. 59. Intense dictyosome activity close to developing wall ingrowths, contents of some vesicles resemble those of inner region of outer cell wall (arrows), microtubule adjacent to plasmalemma and striations evident in wall. Close association exists between RER, dictyosomes and mitochondria in vicinity of developing wall ingrowths.

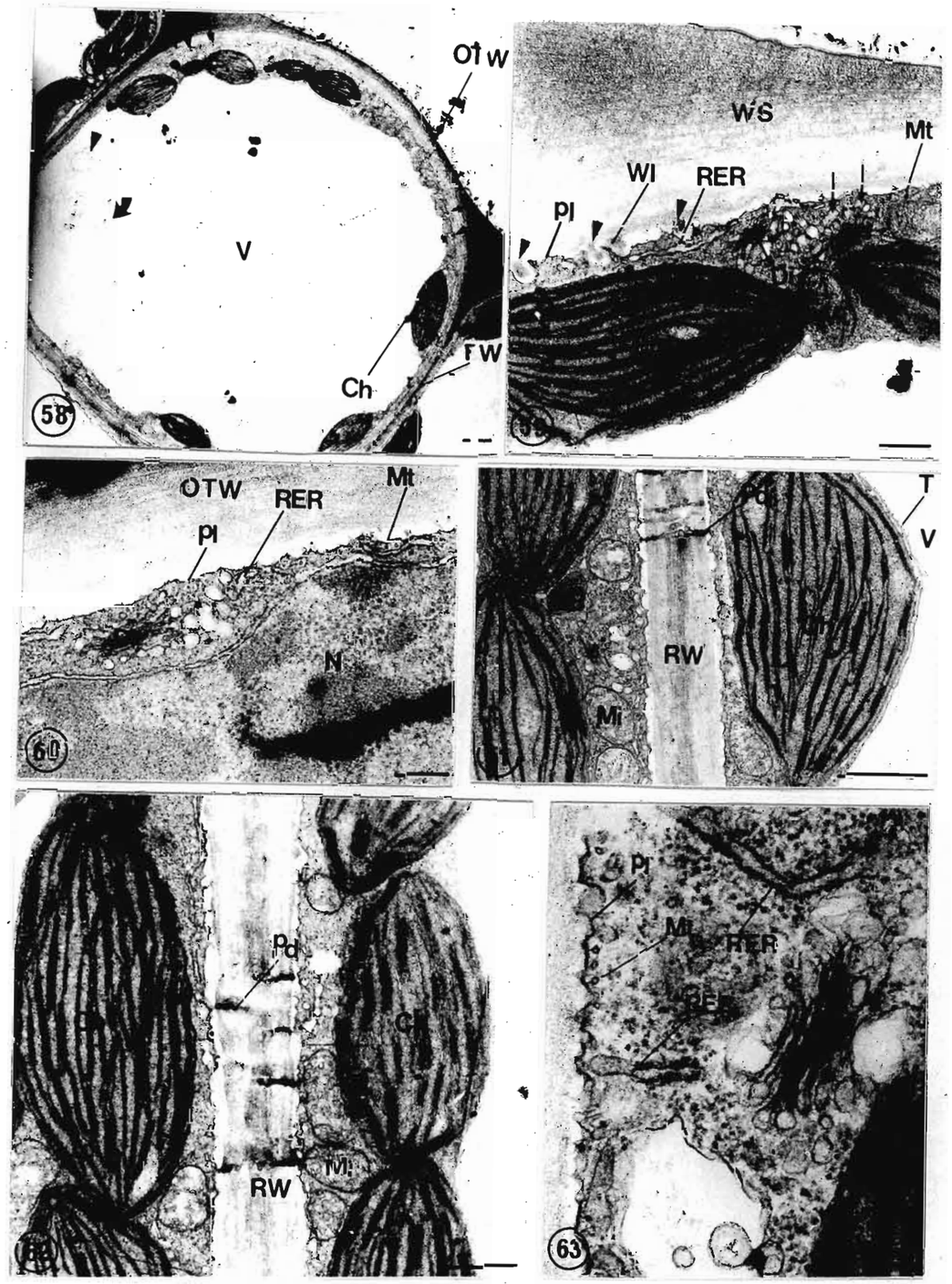
Fig. 60. Details of cytoplasm along part of outer tangential wall showing withdrawal of plasmalemma from wall, intense dictyosome and RER activity close to wall, cortical microtubules adjacent to plasmalemma, nucleus displaced to peripheral cytoplasm.

Fig. 61. Details of cytoplasm along radial wall showing mitochondria, dictyosome, microbody, young chloroplasts, plasmodesmatal connections and tonoplast of vacuole.

Fig. 62. Discontinuous plasmodesmatal connections along radial cell wall, mitochondria and chloroplasts close to wall.

Fig. 63. Microtubules adjacent to plasmalemma, intense activity of dictyosome and associated vesicles and RER close to wall.

Bars represent: 3 µm (Fig. 58)
0,5 µm (Fig. 59)
0,25 µm (Fig. 60)
1 µm (Figs. 61, 62, 63)



envelope is evident in Figure 64 (arrow). Several nucleoid areas and plastoglobuli are prominent within the granular stroma of the chloroplasts (Figs. 66, 67). A peripheral reticulum system is present inside the chloroplast envelope (Fig. 64). Chloroplasts of this category are young since the granal stacks connected by intergranal thylakoids are not high.

The plasmalemma is irregular in outline and tends to pull away from the cell wall (Figs. 60, 64). With progressive development of the cells the extracytoplasmic space becomes more pronounced (Fig. 64). Occasionally paramural bodies are evident in the extracytoplasmic space (Fig. 65). Plasmalemma invaginations precede wall ingrowth formation. Wall ingrowths in the form of irregular projections are initiated at the junction of the outer tangential and radial cell walls (Figs.59,arrow heads 58, 68, 69, arrows) and develop centripetally along the inner tangential wall (Fig. 58). Developing ingrowths are unbranched and appear as small finger-like projections emanating from the innermost region of the cell wall (Figs. 59, arrow heads; 68, arrow; 69, arrow). ER, dictyosomes, mitochondria and microtubules occur in close proximity to the forming ingrowths (Fig. 59). Developing wall ingrowths conform to the shape of the invaginated plasmalemma (Figs. 59, arrow heads; 68,arrow).

The outer tangential wall continues to increase in thickness with progressive growth of the epidermal cells (Figs. 59, 66). The outer region of the outer cell wall appears more electron dense than the inner region (Figs. 59, 66). Furthermore, the striated nature of the outer cell wall is apparent in Figure 59.

Some plasmodesmata occur along certain regions of the radial cell wall (Figs. 61, 62, 67). Some penetrate the radial wall fully (Fig. 61)

PLATE 12

Figs. 64 - 69. Transverse sections of category E epidermal cells.

Fig. 64. Details of cytoplasm at junction of outer tangential and radial cell walls showing intense activity of dictyosomes and associated vesicles, RER and mitochondria. Part of chloroplast showing grana and intergrana thylakoids, plastoglobuli, nucleoid area, peripheral reticulum and double nature of chloroplast envelope (arrow).

Fig. 65. Intense activity of dictyosomes and associated vesicles, ER and mitochondria close to wall. Microbody close to mitochondria, paramural bodies in extracytoplasmic space.

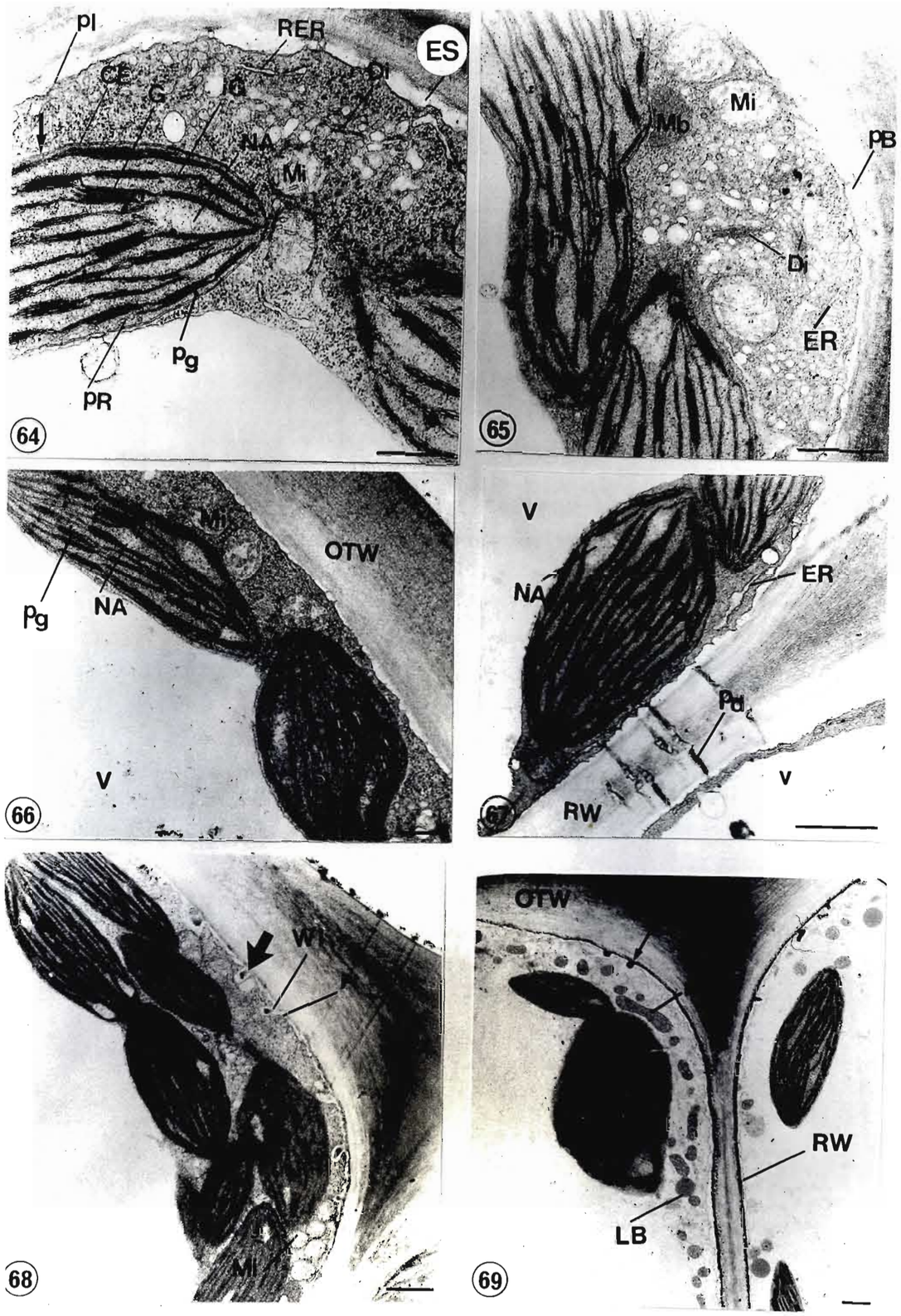
Fig. 66. Thick outer tangential wall with outer region more electron dense than inner region. Mitochondria and chloroplasts containing nucleoid areas and plastoglobuli in cytoplasm.

Fig. 67. Plasmodesmatal connections along radial cell wall, chloroplasts with prominent nucleoid areas, ER close to plasmalemma.

Fig. 68. Wall ingrowths arise from innermost region of cell wall (arrow). Chloroplasts and mitochondria aggregate in region of developing wall ingrowths.

Fig. 69. Lipid bodies closely associated with mitochondria in vicinity of radial cell wall. Ingrowths arising from innermost intensely staining layer of wall (arrow).

Bars represent: 0,5 µm (Figs. 64, 65, 66, 68)
1 µm (Figs. 67, 69)



whereas others are discontinuous (Figs. 62, 67).

Microbodies are occasionally present (Figs. 61, 65). A few lipid bodies occur in the cytoplasm (Fig. 69).

As mentioned earlier, the cells are highly vacuolate. The vacuole occupies most of the space in the cytoplasm, displacing all other organelles to the cell periphery (Fig. 58). The tonoplast of the vacuole is seen in Figure 61. Vesicles (arrow head) and material of a fine fibrillar nature (curved arrow) form part of the contents of the vacuole (Fig. 58).

Category F

The cells of this category display further development of wall ingrowths, chloroplasts, mitochondria and an increase in the thickness of the cell walls (Fig. 70).

As seen in cells of the previous category ER and dictyosomes occur in close proximity to developing wall ingrowths (Figs. 72, 74). Like the ER and dictyosomes mitochondria with well developed cristae also occur close to the developing wall ingrowths (Figs. 72, 74, 75).

There is an increase in the number and size of the chloroplasts (Figs. 70, 72). These organelles display a pronounced ovoid-elliptic shape and dominate the area of the peripheral cytoplasm especially in the region of the outer tangential wall and partly along the radial cell wall (Fig. 70). The internal membrane system is well organized. There is greater stacking of the granal thylakoid membranes (Fig. 72). The internal membranes run parallel to the long axis of the chloroplast (Fig. 72). Plastoglobuli and nucleoid areas are evident in the granular stroma of the chloroplasts (Fig. 72).

The plasmalemma is irregular and follows the outline of the developing wall ingrowths (Figs. 70, 72, 74). As mentioned previously, plasmalemma invaginations precede wall ingrowth formation (Fig. 76, curved arrow). Often paramural bodies (arrow) and membranous structures (arrow head) occur within the extracytoplasmic space (Fig. 72). Paramural bodies probably contribute to wall ingrowth formation (Fig. 76,arrow head). Further development of wall ingrowths is noticed in the cells of this category. Wall ingrowths in the form of small conical projections develop mainly along the inside of the outer tangential cell wall and partly along the radial

PLATE 13

Figs. 70 - 76. Transverse sections of category F cells.

Fig. 70. Cell representative of category showing further development of wall ingrowths, chloroplast accumulations in vicinity of wall ingrowths, thick outer cell wall and few plasmodesmatal connections in radial cell walls.

Fig. 71. Wide outer tangential wall, probably an oblique section through the transverse wall. Wall striations are evident.

Fig. 72. Details of cytoplasm along part of outer tangential wall showing wall ingrowths arising from innermost electron dense layer of wall, (curved arrow), plasmalemma follows contours of ingrowths, paramural bodies (arrow) in extracytoplasmic space. RER, mitochondria and dictyosome close to developing wall ingrowths, spindle-shaped chloroplasts with granal and intergranal thylakoids, plastoglobuli and nucleoid areas.

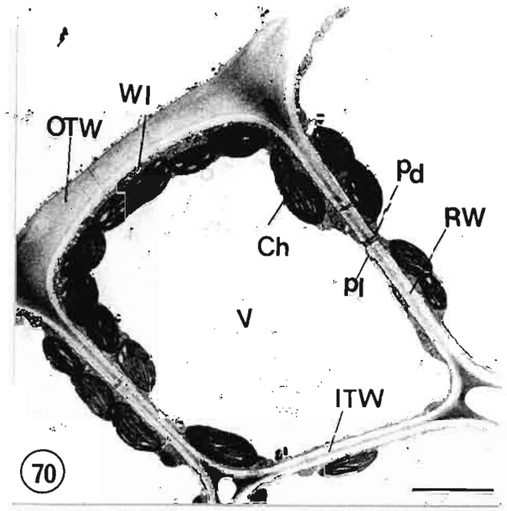
Fig. 73. Details of cytoplasm at junction of outer tangential and radial cell walls showing small finger-like wall projections and discontinuous plasmodesmatal connections in radial cell walls.

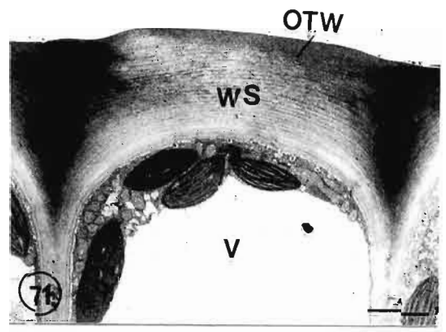
Fig. 74. Close association of chloroplasts, mitochondria, ER and dictyosome to developing wall ingrowths. Note tonoplast of vacuole. Note apparent continuity of ER with plasmalemma (arrow).

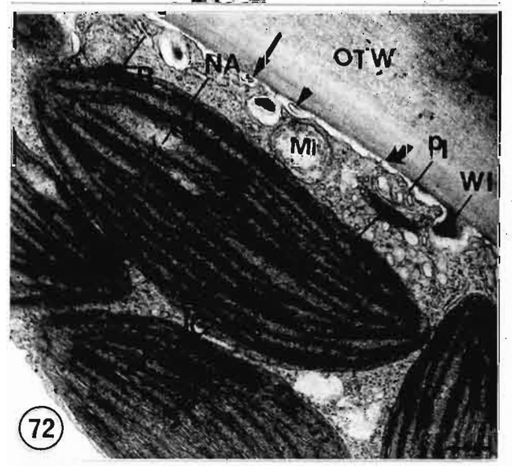
Fig. 75. Discontinuous plasmodesmatal connections in radial cell wall. Note close association of dictyosome, microbody, mitochondria and chloroplast in cytoplasm alongside radial wall.

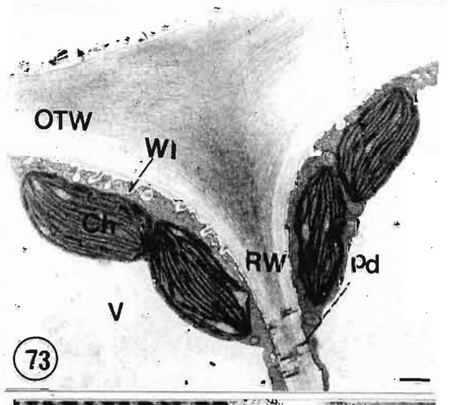
Fig. 76. Microtubules (small arrows) adjacent to plasmalemma, paramural bodies in extracytoplasmic space. Developing wall ingrowth (arrow head) and paramural bodies fusing with ingrowth.

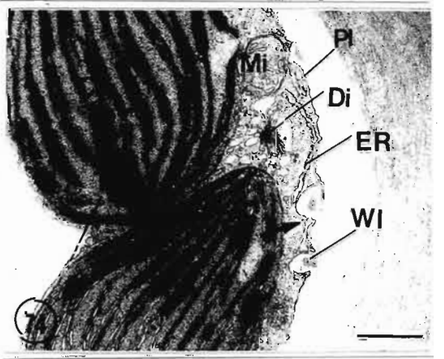
Bars represent: 1 µm (Figs. 70, 74)
0,5 µm (Fig. 71)
2 µm (Fig. 72)
0,25 µm (Figs. 73, 75, 76)

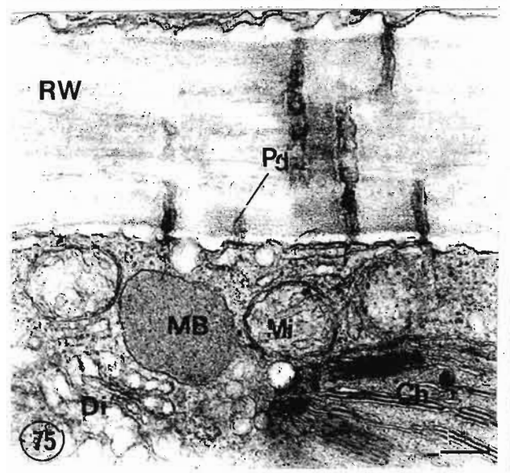


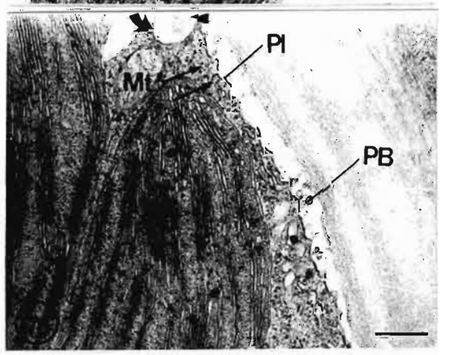












cell wall (Figs. 70, 73, 74). ER, dictyosomes and mitochondria continue to occur in close proximity to the developing wall ingrowths (Figs. 72, 74).

The outer cell wall is wider in comparison to the radial and inner tangential cell walls (Fig. 70). Wall ingrowths appear to arise from an inner narrow electron dense layer (Fig. 72, curved arrow). An extremely wide outer tangential cell wall is observed in Figure 71. This is probably an oblique section through the transverse wall. The striated nature of the outer cell wall is apparent in Figure 71.

Fewer plasmodesmatal connections between adjacent epidermal cells have been noticed with progressive growth of the cells (Fig. 70). Discontinuous plasmodesmatal connections between adjacent epidermal cells are observed in Figure 75.

Microbodies are sparse (Fig. 75).

Some microtubules are present just inside the plasmalemma, close to the corner of the outer tangential wall (Fig. 76, arrows).

Category G

Category G cells can be considered as being nearly mature owing to changes in wall ingrowth structure, cell wall thickness, chloroplast morphology, an increase in number of mitochondria and lipid bodies.

The dictyosomes continue to be active producing numerous vesicles in the vicinity of the wall ingrowths (Figs. 84, 86). The apparent fusing of a dictyosome vesicle with the plasmalemma is occasionally observed (Fig. 84, arrow). The contents of some dictyosome vesicles appear to resemble wall ingrowth material (Fig. 86, arrow), thus indicating that the vesicles could be contributing wall material to the ingrowths.

Numerous mitochondria with well developed cristae appear to be closely juxtaposed to the wall ingrowths (Figs. 83, 85, 86). Mitochondrial shape varies from circular to elongate (Figs. 83) in thin section. In these nearly mature epidermal cells a close relationship exists between the ER, mitochondria, dictyosomes and wall ingrowths (Fig. 85).

The chloroplasts are not fully mature, they display well organized membrane systems which run parallel to the long axis of the organelles (Figs. 80, 82, 84). Granal stacks are not very high. Vesicles of the peripheral reticulum system are evident immediately inside the chloroplast envelope (Fig. 82). Plastoglobuli and nucleoid areas are observed in the granular stroma of the chloroplasts (Figs. 80, 82).

Interesting spherical to elongate membranous structures (paramural bodies) are visible in the extracytoplasmic space of Figures 85 and 89. The outer tangential wall shows a pronounced increase in thickness (Fig. 83). The outer region of the wall (Fig. 83) appears to be more

PLATE 15

Figs. 84 - 90. Transverse sections of category G cells.

Fig. 84. Part of cytoplasm along outer tangential wall, showing well organized membrane system of chloroplast and close association of dictyosome, ER and mitochondria to wall ingrowth. Note dictyosome vesicle apparently fusing with plasmalemma (arrow).

Fig. 85. Mitochondria and dictyosome closely associated with wall ingrowth, paramural bodies and membranous structures in extracytoplasmic space.

Fig. 86. Details of cytoplasm showing nucleus with large nuclear pores, ER partly ensheathing wall ingrowth, contents of dictyosome vesicle resembling wall ingrowth material (arrow).

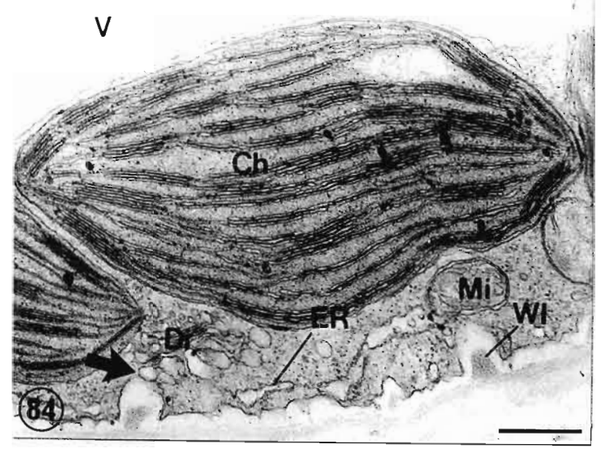
Fig. 87. Part of cytoplasm showing wall ingrowths, paramural bodies in extracytoplasmic space, mitochondria and microbody.

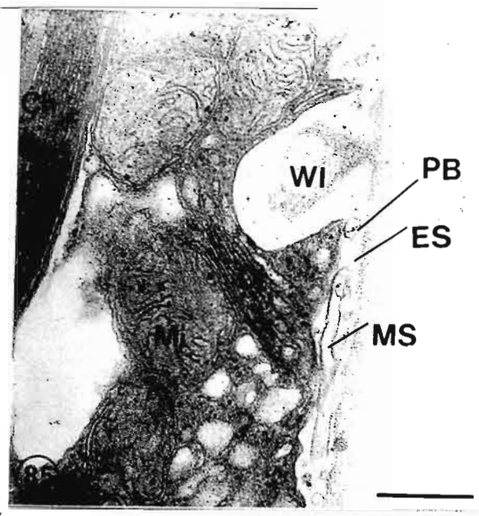
Fig. 88. Dividing microbody close to wall ingrowth. Note RER very close to wall ingrowth.

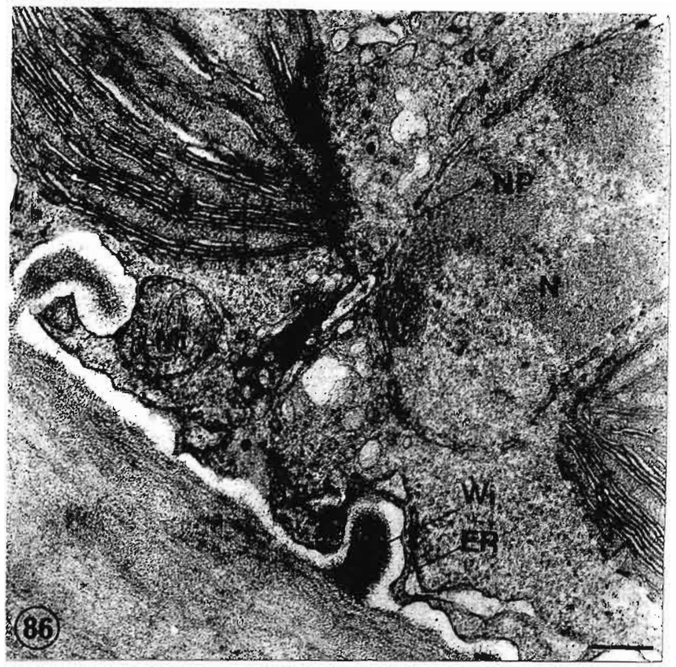
Fig. 89. Elongate membranous structures in extracytoplasmic space, mitochondria close to wall ingrowth, microbodies in cytoplasm.

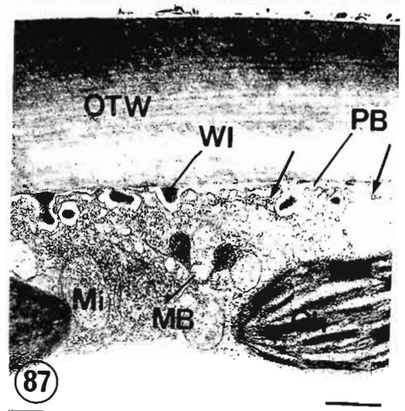
Fig. 90. Details at junction of outer tangential and radial cell walls showing chloroplasts, circular mitochondria and wall ingrowths arising from inner intensely staining layer of wall.

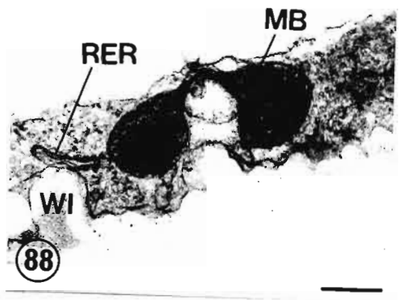
Bars represent: 0,5 µm (Figs. 84, 87)
0,25 µm (Figs. 85, 88, 89)
0,1 µm (Fig. 86)
1 µm (Fig. 90)

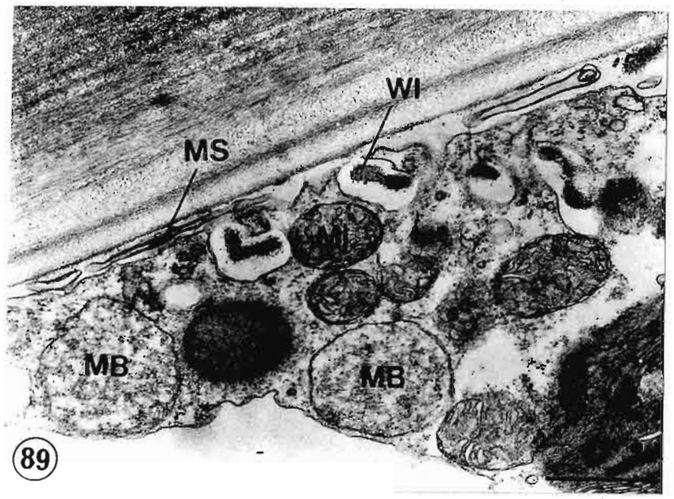


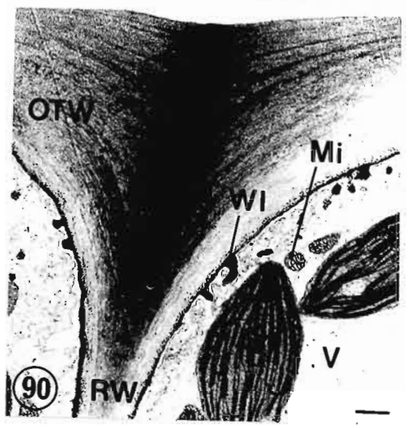












electron dense than the inner region. The innermost layer of the electron transparent region stains intensely (Fig. 83, arrow). However, not all cell walls display this feature.

With progressive maturation of the cells the wall ingrowths have a tendency to become more specialized in their morphology. The finger-like wall ingrowths of these cells have increased in length (Fig. 79) and some appear to be branched (Fig. 83, arrow head). An interesting feature observed in some of the cells is the presence of multivesicular structures (Figs. 79, 80). These structures embody numerous vesicles which appear to contribute wall material to the ingrowths. Also, vesicles of apparently ER origin seem to contribute to the growth of the developing ingrowths (Fig. 78). A close association exists between the RER and the wall ingrowth (Fig. 88).

Nuclei are no longer as massive as they used to be in the young epidermal cells. They are displaced to the cell periphery and assume various lobed shapes (Fig. 83). The nuclear invaginations commonly house mitochondria (Fig. 83). Large nuclear pores are evident in the nucleus of Figure 86.

The contents of the vacuole vary from electron dense droplets (Fig. 83, curved arrow) to a fine fibrillar material (Fig. 77, arrow) and membranous vesicles (Fig. 82).

Large lipid bodies are apparent in the cells of this category (Figs. 81, 83) being closely associated with mitochondria and chloroplasts. Microbodies are present (Figs. 87, 88, 89). Some of the microbodies appear to be dividing (Figs. 87, 88). Microtubules occur inside the plasmalemma close to the wall ingrowths (Fig. 87, arrows).

Category H

The cells of this category represent mature epidermal cells and are characterized by the presence of complex wall ingrowths, highly stacked chloroplast grana and thick cell walls.

In mature cells the presence of ER and dictyosomes is not so obvious.

The mitochondria tend to be closely associated with wall ingrowths (Figs. 95, 105). The cristae are well developed and electron dense granules are visible within the mitochondrial stroma (Fig. 95, arrow). An intersting feature is the close association of the mitochondria and the lipid bodies (Figs. 95, 97).

Wall ingrowths in the cells of this category appear to be more complex in structure than those of the previous category (Figs. 91, 92). Often the ingrowths are highly branched (Figs. 92, 93). Adjacent formerly slender curved ingrowths merge to form complex structures inbetween which cytoplasmic material is present (Figs. 92, 105, arrows). The presence of cross-sectional profiles of wall ingrowths isolated in the cytoplasm attests to the branching of the ingrowths (Fig. 93, arrow) Occasionally paramural bodies are associated with the wall ingrowths in the extracytoplasmic space (Fig. 98).

During maturation of the epidermal cells, the outer tangential wall achieves maximum thickness (Fig. 98). The radial and inner cell walls are not as thick as the outer tangential wall (Fig. 91). A high magnification view of the outer tangential wall is seen in Figure 103. The cell wall appears to consist of a cuticular layer on the outside followed by a narrow intensely staining layer (arrow) immediately inside

PLATE 16

Figs. 91 - 98. Transverse sections of category H cells.

Fig. 91. Cell representative of category showing well-developed wall ingrowths, lens-shaped chloroplasts, mitochondria and thick outer tangential wall. Plasmodesmatal connections absent along radial walls, single connection seen in wall between epidermal and adjacent mesophyll cell (arrow).

Fig. 92. Part of cytoplasm along outer tangential wall showing branched wall ingrowths (arrow).

Fig. 93. Wall ingrowths branched, arise from inner intensely staining layer of the wall, cross-sectional profiles of ingrowths seen in cytoplasm (arrow).

Fig. 94. Nuclear invagination housing mitochondrion.

Fig. 95. Details at junction of outer tangential and radial cell walls showing numerous mitochondria in close proximity to wall ingrowths, microbodies and lipid body occur close to mitochondria.

Fig. 96. Intercellular structure in air-space. Note membranes (arrow), vesicles (arrow head) and ribosomes.

Fig. 97. Close association of mitochondria and lipid bodies in cytoplasm along radial cell wall.

Fig. 98. Details along part of outer tangential wall showing paramural bodies associated with wall ingrowths in extracytoplasmic space. Note thick outer tangential wall.

Bars represent: 10 µm (Fig. 91)
1 µm (Figs. 92, 93, 95, 97, 98)
0,25 µm (Figs. 94, 96)

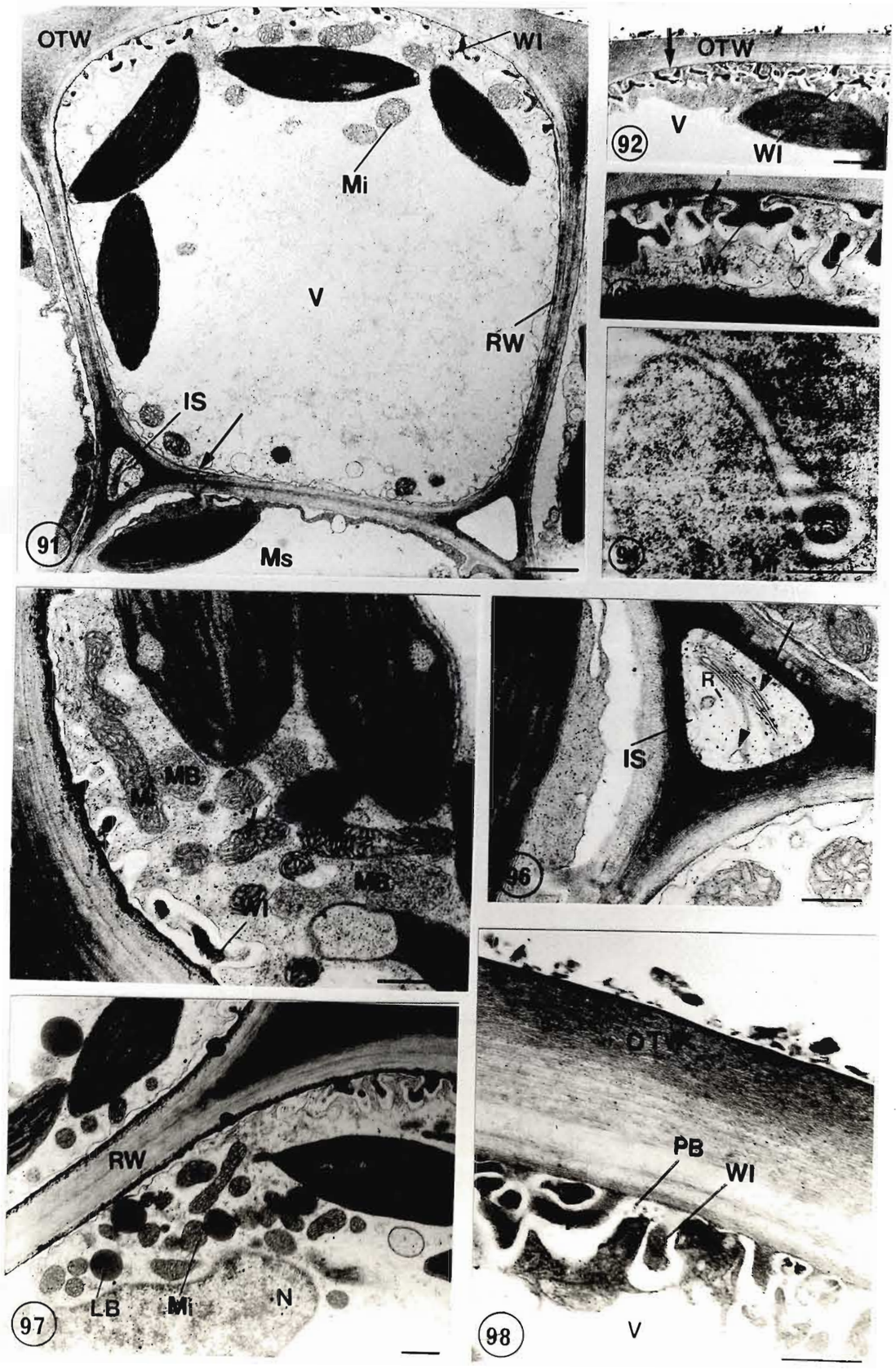


PLATE 17

Figs. 99 - 106. Transverse sections of category H cells.

Fig. 99. Details of internal membrane system of chloroplast showing highly stacked granal thylakoids connected by intergranal thylakoids.

Fig. 100. Well-organized membrane system of chloroplast showing granal and intergranal thylakoids, plastoglobuli, nucleoid area.

Fig. 101. Epiphyte on surface of epidermal cell.

Fig. 102. Cytoplasmic details showing wall ingrowths, microtubule adjacent to plasmalemma, mitochondria and chloroplasts.

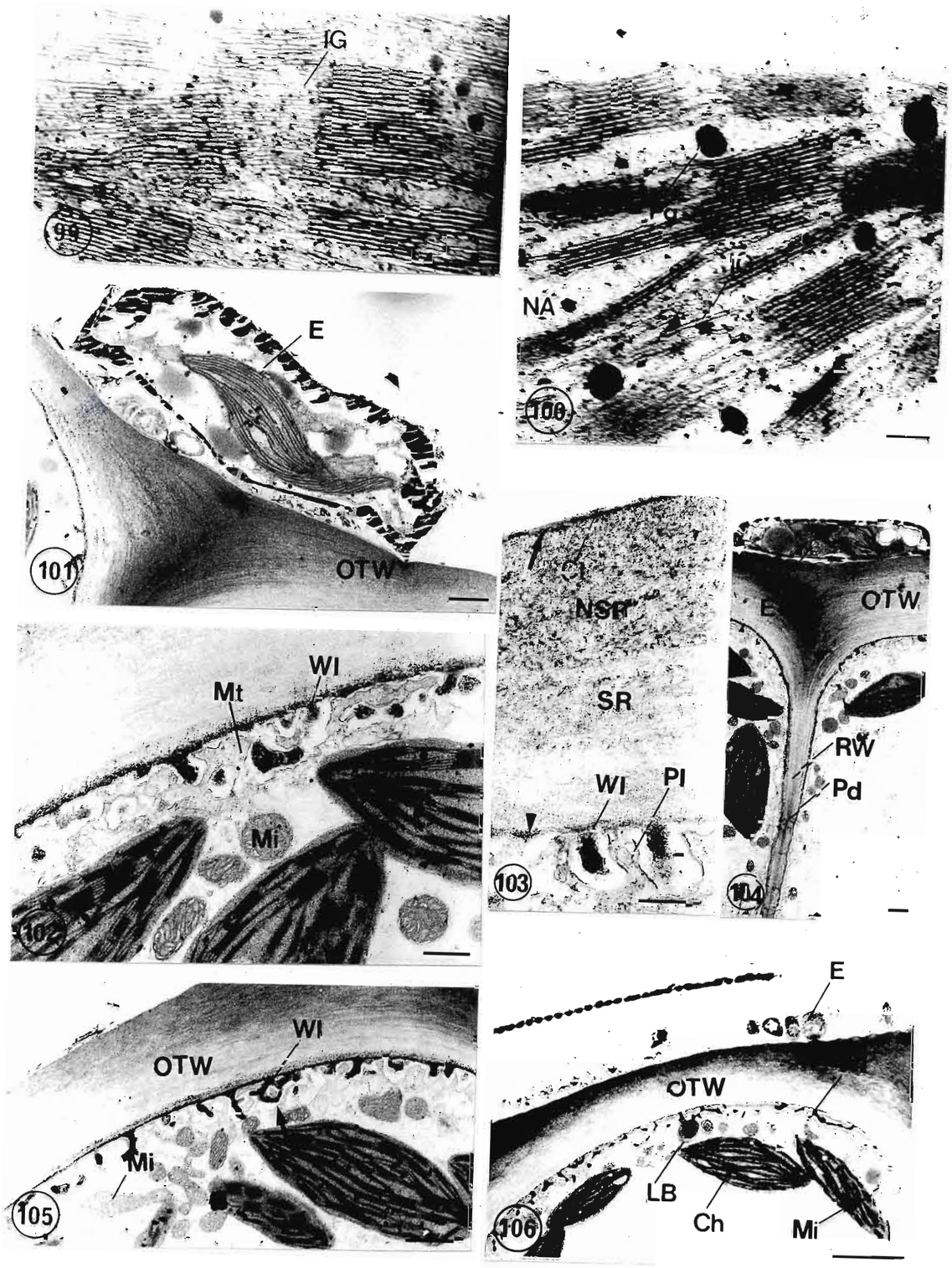
Fig. 103. Details of outer tangential wall, showing various layers and wall ingrowths arising from innermost intensely staining layer.

Fig. 104. Epiphyte on surface of cell. Note thick outer tangential wall and discontinuous plasmodesmatal connections in radial cell wall.

Fig. 105. Cytoplasmic details along outer tangential wall showing branched wall ingrowths, chloroplasts and numerous mitochondria.

Fig. 106. Cytoplasm along outer tangential wall showing branched wall ingrowths, chloroplasts, mitochondria and lipid bodies. Note epiphytes on surface of cell.

Bars represent: 0,25 µm (Figs. 99, 103)
1 µm (Figs. 100, 101, 104, 105)
0,5 µm (Figs. 102, 106)



the cuticle. This is followed by a non-striated region and a wide striated region. An inner, narrow, darkly staining border occurs immediately inside the striated layer (arrow head). Wall ingrowths arise from this intensely staining layer (Figs. 93, 98, 103).

The chloroplasts are lens-shaped with well defined internal membrane systems (Figs. 91, 99). The granal stacks are higher than those encountered in the chloroplasts of the previous category (Figs. 99, 100). Plastoglobuli and few nucleoid regions are observed in the granular stroma of the chloroplasts (Fig. 100).

In some instances, deep channels penetrate into the nucleoplasm of the nucleus (Fig. 94). These channels are lined with the nuclear envelope and contain cytoplasmic components such as mitochondria.

Although microbodies (Fig. 95) occur in these cells, microtubules are less obvious (Fig. 102). Remnants of plasmodesmata are evident in Figure 104. An interesting feature seen in Figures 91 and 96 is the presence of an unusual structure within the intercellular air-space. Part of a membrane system (arrow), some vesicles (arrow head) and ribosomes of the organism is evident (Fig. 96).

Epiphytes (probably algae) are common on the surface of mature leaves (Figs. 101, 104, 106). The leaves are used as substrate for attachment of epiphytes.

2. ANNULI IN OUTER EPIDERMAL CELL WALL

Leaf epidermal cells possess an unusual structure in their outer cell walls which Birch (1974) called an annulus. The distribution and structure of the annuli in young and mature leaves were investigated.

RESULTS

Annuli are ring-like structures present in the mid-region of the outer cell walls. A typical annulus consists of a rim (Fig.111, arrow) which encloses a large central unthickened area (Fig. 111, arrowhead). This central unthickened area is generally raised (Fig. 111) but might also be depressed (Fig. 108, curved arrow). Scanning electron micrographs show that annuli occur in epidermal cells on both surfaces of young and mature leaf blades.

a. Young leaves

Annuli of epidermal cells over the main vein, intramarginal vein (arrow) and other regions of the blade are seen in figure 107. The shape of the annuli varies in different parts of young leaf blades. For example, annuli tend to be oval in cells over the main vein (Fig. 108, arrowhead) while they are circular elsewhere (Fig. 109, arrows). The cuticular layer around the annuli, often has an uneven togography (Figs. 109, 111, curved arrows). In transverse sections of epidermal cells, depressions in the wall (Fig. 112, arrows)probably mark the position of the annulus rim. However annuli are absent from epidermal cells of the petiole (Fig. 117).

PLATE 18

Figs. 107 - 112. Scanning Electron Micrographs of young annuli.

Fig. 107. The distribution of annuli in midrib, lamina, intramarginal vein (arrow) and leaf tip regions.

Fig. 108. Oval shaped annuli in mid-vein (arrow head) and lamina (arrow). Note depressed annulus (curved arrow).

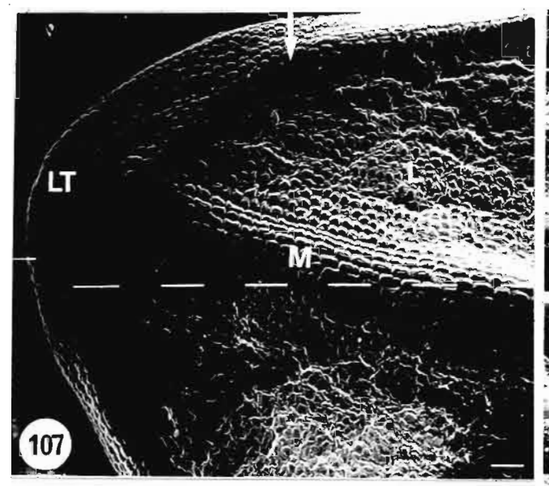
Fig. 109. Circular elevated annuli (arrows). Note uneven topography of cuticle (curved arrow).

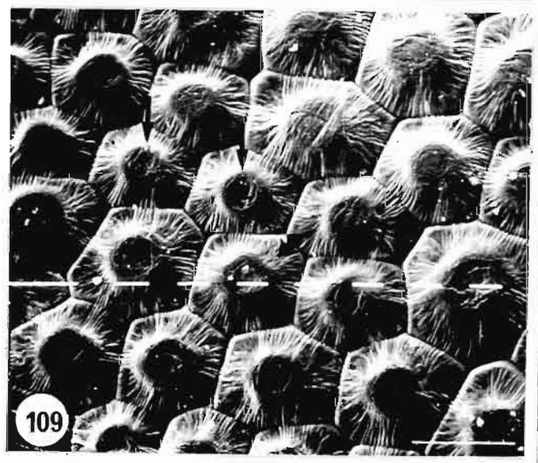
Fig. 110. Elevated annuli with distinctive rim (arrow).

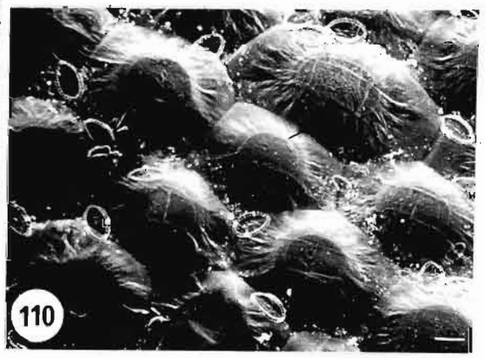
Fig. 111. Annuli consisting of outer rim (arrow) and wide central region (arrow head).

Fig. 112. Transverse section of outer tangential cell wall showing probable position of rim of annulus (arrows).

Bars represent: 20 µm (Fig. 107)
3 µm (Fig. 108, 110)
1 µm (Fig. 109)
2 µm (Fig. 111)
0,5 µm (Fig. 112)







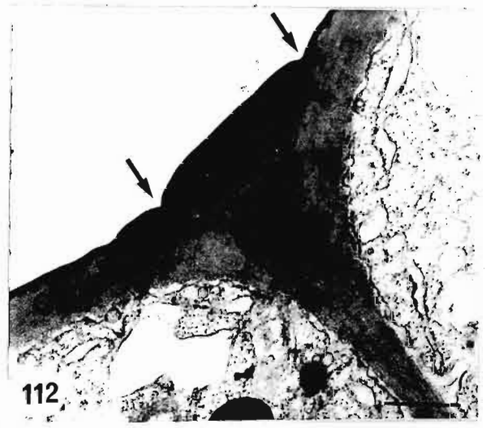


PLATE 19

Figs. 113 - 116, 118. Scanning electron micrographs of annuli of mature leaves.

Fig. 113. Oval shaped annuli (arrows) in lamina region. Note pennate diatoms on leaf surface.

Fig. 114. Oval shaped annuli in midrib region (arrows).

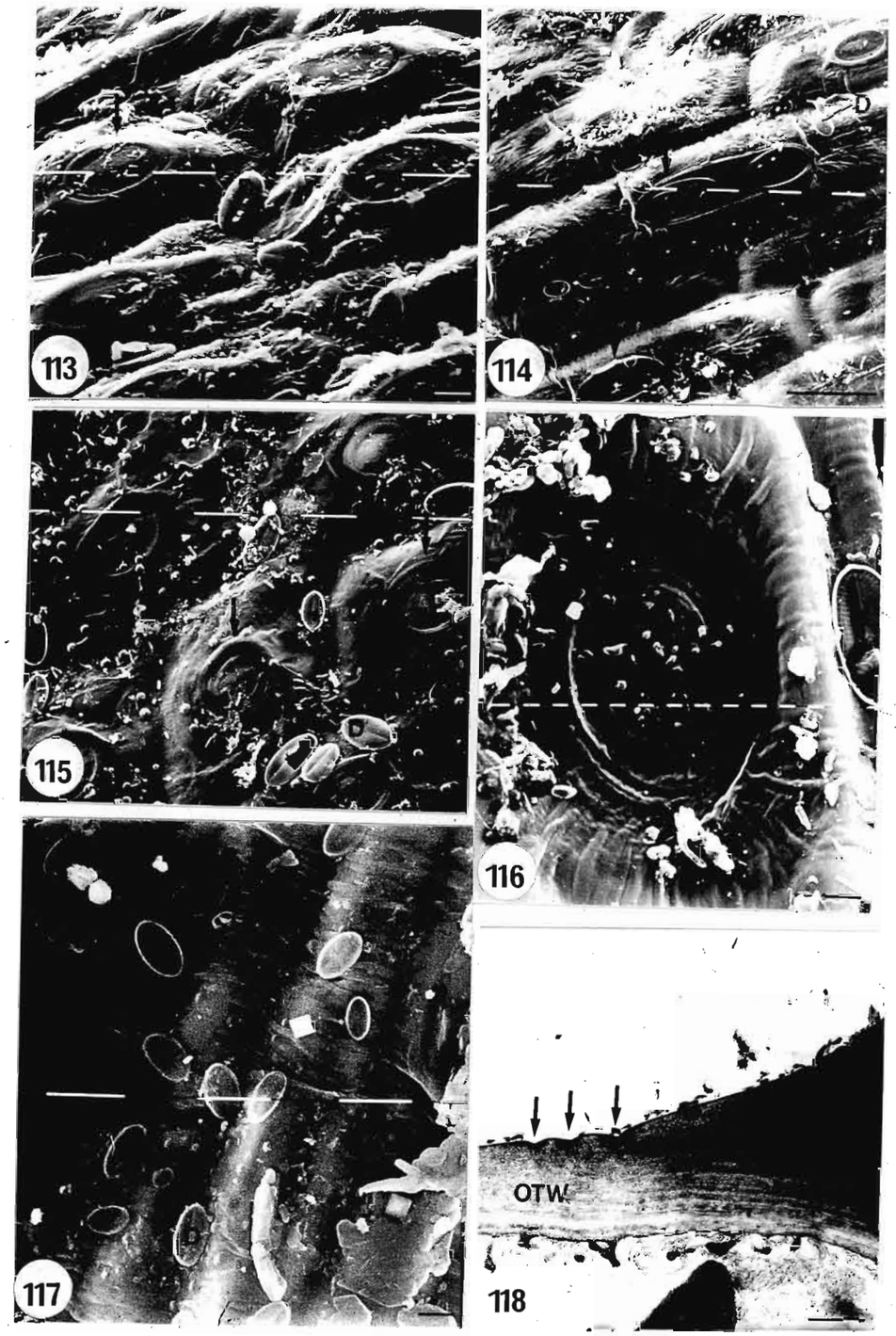
Fig. 115. Elevated annuli with multiple rims (arrows). Note pennate diatoms on leaf surface.

Fig. 116. Multiple rim annulus sunk in wall of epidermal cell (arrow).

Fig. 117. Surface of epidermal cells of petiole showing absence of annuli (arrows).

Fig. 118. Transverse section of outer tangential cell wall showing probable position of multiple rim of annulus (arrows).

Bars represent: 1 µm (Figs. 113, 116, 118)
10 µm (Fig. 114)
5 µm (Fig. 115)
0,5 µm (Fig. 117)



b. Mature leaves

Annuli in walls of epidermal cells of mature leaves tend to be more oval in shape (Figs. 113, 114, arrows). Rim structure is generally more complex in mature leaves (Figs. 115, 116, arrows). The annulus in figure 116 is made up of multiple rims. In transverse section depressions in the outer wall of epidermal cells (Fig. 118, arrows) mark the positions of the multiple rims. Complex annuli are seen in figure 114. Some of the annuli of mature leaves appear to be raised (Fig. 113, curved arrow) whereas others appear to be sunk into the cell wall (Fig. 116).

The annuli of both young and mature leaves show a positive reaction when fresh leaves are treated with silver nitrate. The central regions of the annuli react strongly with silver nitrate and stain intensely (Figs. 119, 120, arrows). Other portions of the epidermal cells show no reaction with silver nitrate.

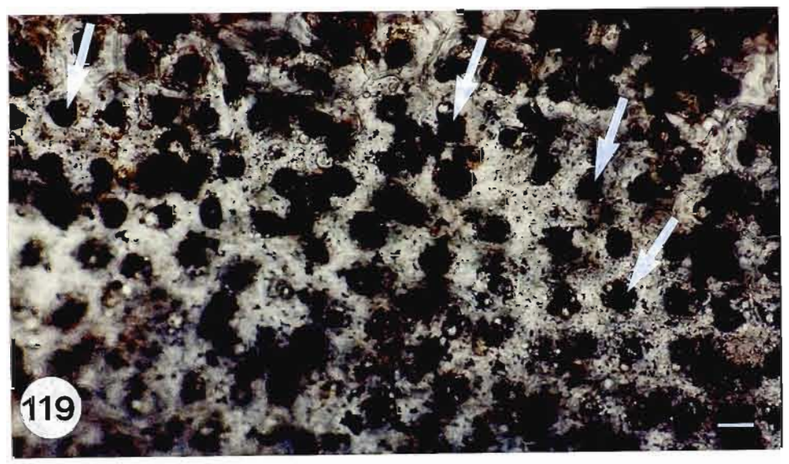
PLATE 20

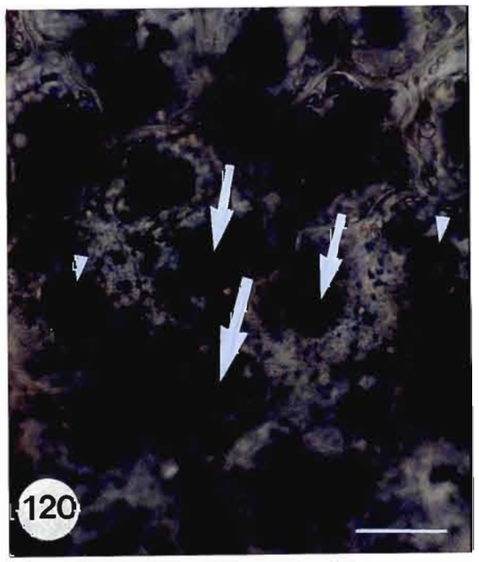
Figs. 119 - 120. Light micrographs of surface of leaves showing localization of chloride ions.

Fig. 119. Annuli on surface of epidermal cells stain intensely with Silver Nitrate (arrows).

Fig. 120. High magnification view of annuli showing intense staining of central region (arrow). Note less intense staining on rim of annulus (arrow head).

Bars represent: 10 µm (Figs. 119, 120)





DISCUSSION

This investigation of the structure and histochemistry of leaf blade epidermal cells of *H. ovalis* has yielded interesting information about the cytology and probable functions of these cells. One of the most striking changes observed during the study of epidermal cell development was the transformation of these cells into cells with transfer cell characteristics. Transfer cells (Gunning & Pate, 1969) are cells which possess ingrowths of wall material. These wall ingrowths are a specialized type of secondary wall and are deposited on the inner face of the primary wall. The plasmalemma always conforms to the outline of the wall ingrowths and follows their contours no matter how irregular and labyrinthine they are. Mitochondria usually occur in close association with the ensheathing plasmalemma of the ingrowths. According to Gunning and Pate (1969) and Gunning (1977) transfer cells are probably involved in absorptive or secretory activities. They are known to occur in a vast array of tissues, some of which include minor veins of leaves, reproductive tissues, nectaries, salt glands and root systems (Gunning, 1977).

Epidermal cells with transfer cell characteristics have been reported in the leaves of other seagrasses such as *Thalassia hemprichii*, *Cymodocea serrulata*, *Cymodocea rotundata* (Doohan & Newcomb, 1976), *Zostera capensis* (Barnabas *et al*, 1977), *Amphibolis antartica*, *Amphibolis griffithii* (Ducker *et al*, 1977), *Thalassodendron ciliatum* (Barnabas, 1982) and *Halodule uninervis* (Barnabas & Kasavan, 1983**b**). The only seagrass species found so far to possess leaf blade epidermal cells without transfer cell characteristics are *Posidonia australis* (Kuo, 1978) and *Posidonia sinuosa* (Cambridge and Kuo, 1982).

In fresh water aquatics, epidermal cells with transfer cell features have been found in submerged leaves of *Ranunculus fluitans* and *Hydrilla verticillata* (Gunning & Pate, 1969) and in *Elodea* sp. (Sitte, 1963).

1. Development of Wall Ingrowths and Cytoplasmic Changes Accompanying Ingrowth Formation

Wall ingrowths start forming in cells when chloroplasts possess a relatively well-developed membrane system in their stroma (category E cells).

Prior to wall ingrowth formation, some cytoplasmic changes occur in regions where ingrowths start forming. These cytoplasmic changes include:

i) the withdrawal of the plasmalemma from the cell wall, thereby creating an extracytoplasmic space;

ii) the association of microtubules with the withdrawing plasmalemma;

iii) the presence of paramural bodies (Marchant & Robards, 1968) in the extracytoplasmic space and

iv) the appearance of many dictyosomes and ER profiles close to the sites of wall ingrowth formation.

Wall ingrowths start forming at the junction of the radial and outer tangential walls and continue development in a centripetal direction along the outer tangential wall. Throughout wall ingrowth development, dictyosomes with associated vesicles, profiles of ER, paramural bodies and microtubules usually occur at the sites of ingrowth formation.

A brief account of cytoplasmic components apparently involved in wall ingrowth formation follows:

a. Dictyosomes

The role of dictyosomes and dictyosome-derived vesicles in cell wall formation has been well documented *inter alia* by Morré, Mollenhauer and Bracker (1971). It is known that wall matrix and, in some plants, cellulose microfibrils are generated within dictyosome cisternae or vesicles and delivered to growing regions of cell walls (Engels & Kreger, 1974; Gunning & Steer, 1975).

The occurrence of dictyosomes close to sites of forming ingrowths and the observation of vesicles (apparently of dictyosome origin) appressed to the plasmalemma, in the present study, suggest that this organelle probably plays a role in wall ingrowth synthesis. Intense dictyosome activity near developing wall projections, has also been reported in cells of other plants (Gunning & Pate, 1969; Peterson & Yeung, 1975; Schnepf & Pross, 1976; Briarty, 1978; Yeung & Clutter, 1978; Fineran, 1980). In leaf blade epidermal cells of other seagrasses such as *Cymodocea serrulata* and *Thalassia hemprichii* (Doohan & Newcomb, 1976) and *Zostera capensis* (Barnabas *et al*, 1977, 1982) high dictyosome activity associated with wall ingrowth formation was noticed.

b. Endoplasmic Reticulum

The role of ER in the synthesis or transport of cell wall material (polysaccharides) is not yet understood, Bowles & Northcote (1972, 1974) have proposed a direct role for the ER in hemicellulose

biosynthesis. Westafer and Brown (1976) have implicated the ER in the synthesis and secretion of secondary wall materials. However, Ray *et al* (1969, 1976) did not find any evidence for a direct role of the ER in hemicellulose synthesis.

The occurrence of ER in regions where wall ingrowths form and the close association of ER with forming ingrowths in the present study suggest that the ER might be involved in the synthesis of wall material in epidermal cells of *H. ovalis*.

In developing wall ingrowths of transfer cells in the leaves of *Linaria* (Cresti, Ciampolini & Kapil, 1983), the suspensor of *Phaseolus coccineus* (Yeung & Clutter, 1978) and in leaf blade epidermal cells of *Zostera capensis* (Barnabas *et al*, 1977, 1982) a similar relationship between ER and developing wall ingrowths has been reported.

c. Plasmalemma

There is evidence which suggests that cellulose biosynthesis and microfibril assembly occur at the cell surface, probably in association with multi-enzyme complexes on the plasma membrane (Willison & Brown, 1978; Mueller *et al*, 1976). According to Gunning and Steer (1975) the cellulose synthesizing systems probably originate in the Golgi apparatus and are transported to the plasmalemma by the golgi vesicles.

In addition to the probable role of the plasmalemma in the synthesis of wall materials, the plasmalemma in the present study appeared to have an influence on the morphology of the wall ingrowths. Often plasmalemma invaginations preceded wall ingrowth formation and developing wall ingrowths closely resembled the shape of the

invaginated plasmalemma.

A similar phenomenon was reported in developing wall ingrowths of leaf blade epidermal cells of *Zostera capensis* (Barnabas *et al*, 1982). According to Gunning and Steer (1975) the morphology of the plasmalemma predicts the morphology of the wall.

d. Microtubules

Prior to and during wall ingrowth formation microtubules were often observed adjacent to the plasmalemma. This feature has also been reported in developing wall ingrowths in leaf blade epidermal cells of *Zostera capensis* (Barnabas *et al*, 1982). Functions attributed to the microtubules in this situation include the possible formation of extracytoplasmic spaces prior to wall ingrowth development (Schnepf, 1974) and the formation of wall ingrowths in the transfer cells of some plants (Tu & Hiruki, 1971; Jones & Northcote, 1972). Microtubules have been implicated in lifting off the plasmalemma in the sieve cells of the needle trace of *Pinus radiata* (Singh, 1984) during growth of the cell wall. Microtubules were also evident alongside the plasmalemma during differentiation of the walls of the pedestal cell of *Utricularia monanthos* (Fineran, 1980) prior to ingrowth formation.

It is probable, in the present study, that microtubules function in lifting off the plasmalemma from the cell wall thereby creating extractyoplasmic spaces prior to wall ingrowth formation.

e. Paramural Bodies

Paramural bodies (Marchant & Robards, 1968) are a common feature in the

extracytoplasmic spaces of young epidermal cells of *H. ovalis* whose outer tangential walls are undergoing changes in thickness prior to wall ingrowth formation. These bodies probably contribute material to the differentiating cell walls as suggested by Chafe (1974). The close association of paramural bodies and other membranous structures with developing wall ingrowths in the present study suggests that they also contribute material to the forming ingrowths. Occasionally conspicuous multivesicular structures containing numerous paramural bodies were observed in close association with developing wall ingrowths. According to Jones and Northcote (1972) paramural bodies are a form of increased plasmalemma essential for the synthesis of wall materials for ingrowth formation.

Paramural bodies have also been implicated in wall ingrowth formation in *Zostera capensis* (Barnabas *et al*, 1982) and in transfer cells of other plants (Tu & Hiruki, 1971; Jones & Northcote, 1972; Peterson & Yeung, 1975; Newcomb & Peterson, 1979).

2. Mitochondria

Mitochondria, although not actively involved in formation and subsequent development of wall ingrowths, play an important role with regard to the function of the wall-membrane apparatus (see later). Mitochondria showing little development of cristae are fairly well distributed in the cytoplasm of young epidermal cells of *H. ovalis*. With progressive growth of the epidermal cells and formation of wall ingrowths, the mitochondrial population increases and becomes peripherally distributed in the cytoplasm. In mature epidermal cells with well formed ingrowths, mitochondria with well-defined cristae lie closely juxtaposed to the

ingrowths.

Studies concerning the development of the suspensor of *Phaseolus coccineus* (Yeung & Clutter, 1978) indicated a concomitant increase in mitochondrial numbers with the formation of wall ingrowths. According to these workers the close association of the mitochondria and wall ingrowths implies that energy is necessary for the transport of solutes across the plasmalemma. Mitochondria are prominent after wall ingrowth formation in the pedestal cells of the external glands of *Utricularia monanthos* (Fineran, 1980). Mitochondrial-wall ingrowth associations have also been noted in the A-type transfer cells of the minor veins of *Linaria* (Cresti, Ciampolini & Kapil, 1983), in transfer cells of the digestive glands of *Pinguicula* (Heslop-Harrison & Heslop-Harrison, 1981) and in leaf epidermal transfer cells of *Zostera capensis* (Barnabas *et al*, 1977, 1982).

3. Chloroplasts

In the mature leaf epidermal cells of *H. ovalis*, chloroplasts, like mitochondria, remain the dominant organelles in the cytoplasm when wall ingrowths form. As seen in the study of the development of the epidermal cells, chloroplasts are derived from proplastids which are characteristic of young cells. Various stages in the transformation of proplastids into chloroplasts were observed and these transformations resembled those of terrestrial plants (Thomson & Whatley, 1980). The development of the chloroplasts in *H. ovalis* occurs via the following stages, proplastid - young chloroplast - mature chloroplast. Proplastids containing prolamellar bodies are characteristic of etioplasts (Rascio *et al* 1984) and are common in very young leaves totally enclosed in the

vegetative buds of *H. ovalis*.

Prolamellar bodies were also noticed in developing chloroplasts of leaf blade epidermal cells of *Cymodocea rotundata*, *Cymodocea serrulata* and *Thalassia hemprichii* (Doohan & Newcomb, 1976), *Zostera capensis* (Barnabas *et al*, 1977) and *Posidonia oceanica* (Colombo *et al*, 1983). Fine structure of mature chloroplasts of *H. ovalis* closely resembles those of other seagrasses studied.

The poorly developed peripheral reticulum system seen in *H. ovalis* is similar to that of other seagrasses studied (Barnabas, 1982, 1983b; Doohan & Newcomb, 1976). A peripheral reticulum system is associated with C_4 plants, particularly grasses (Rivera & Arnott, 1982) and has been implicated with high photosynthetic activity.

Regions of low electron density, i.e., nucleoid areas (Ris & Plaut, 1962), were observed in most stages of chloroplast development, represent the genetic system of the chloroplasts.

4. Probable Functions of the Wall-Membrane Apparatus

The cytoplasmic specializations of transfer cells which include wall ingrowths, ensheathing plasmalemma and associated mitochondria, have been referred to as the wall-membrane apparatus by Gunning and Pate (1969). As mentioned previously, transfer cells are thought to have absorptive and/or secretory functions. They are believed to be involved in the short distance transport of solutes with minimal movement of water (Pate & Gunning, 1972). Wall ingrowth formation seems to coincide with intensive solute transport, but as compared with the development of the cell as a whole, the ingrowths develop fairly late. Gunning and Pate (1969) reported that the secretory cells of the extra-floral nectaries of

Vicia faba L. do not form ingrowths until sugar secretion starts. In addition, the epithelial cells of the septal nectaries of *Gasteria Aloe* also develop wall ingrowths before the secretion phase (Schnepf & Pross, 1976).

In leaf blade epidermal cells of *H. ovalis* wall ingrowth formation occurs late in the development of the epidermal cells, probably just before the onset of intensive solute transport. Initiation of ingrowths appears to be correlated with chloroplast development. Wall protuberances start forming when the chloroplasts possess well-developed membrane systems in their stroma. Presumably at this stage of chloroplast development photosynthate becomes available for export and this acts as a stimulus for the formation of ingrowths.

The enlarged plasmalemma would expedite the transfer of photosynthate from the epidermis (the main photosynthetic tissue) to the interior tissues of the leaf. Apoplastic movement of photosynthate probably occurs since discontinuous plasmodesmata interconnect mature epidermal and mesophyll cells. 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 *Posidonia sinuosa* (Cambridge & Kuo, 1982), which do not possess transfer cell features, have numerous plasmodesmata which interconnect epidermal and mesophyll cells. In these seagrasses therefore, an enlarged plasmalemma for the transfer of photosynthate might not be necessary - hence a plasmalemma without invaginations.

5. Absorption and Secretion

Physiological studies have shown that seagrass leaves are capable of absorptive and/or secretory 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). In addition carbon and phosphorus have been shown to be taken up by roots of *Zostera marina* and subsequently transferred through the plants to epiphytes on the seagrass leaves (Penhale & Thayer, 1980). Although physiological studies have not been carried out, it is possible that transfer cell features in leaf blade epidermal cells of *H. ovalis* enhance absorptive and/or secretory processes of the leaves.

6. Osmoregulation

Salt glands which are exposed to the atmosphere are considered to be desalination devices that apparently maintain the salt balance in the leaves by secreting excess salts (Esau, 1977). The secretory cells of most salt glands develop wall ingrowths characteristic of transfer cells (Thomson *et al*, 1969; Levering & Thomson, 1971). Many halophytes of salt marshes and mangrove swamps possess specialized structures (salt glands) in their leaves for salt excretion. These include the salt marsh monocot, *Spartina foliosa* (Levering & Thompson, 1971), the halophyte, *Tamarix aphylla* (Thomson *et al*, 1969) and the mangrove *Avicennia marina* Shimony *et al*, 1973).

Although marine angiosperms are constantly bathed in a salt solution, the leaves of the species that have been investigated so far at the ultrastructural level, do not appear to have structures as specialized

as salt glands for salt secretion.

On the basis of an ultrastructural comparison with the salt gland cells of the salt marsh monocot, *Spartina*, Jagels (1973) postulated that the leaf epidermal cells of the marine angiosperm, *Thalassia testudinum*, function as salt secreting cells. 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 seagrass 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 compared with ambient and tissue concentrations, indicating selective exclusion of Na^+ and Cl^- from the epidermis.

The leaf blade epidermal cells of *H. ovalis* are probably also capable of osmoregulation, with the plasmalemma possibly playing a role in salt regulation in a manner similar to that proposed for *Thalassia*. The fact that wall ingrowths are absent from young leaf blade cells suggests that osmoregulation can be accomplished without the development of transfer wall configurations and the cytoplasmic specializations that accompany them. It is probable that exclusion of salt at the plasmalemma boundary is the main mechanism operative in osmoregulation.

The presence of plasmodesmatal connections between mesophyll and adjacent young epidermal cells and their absence in mature cells of *H. ovalis* indicate a shift from symplastic to apoplastic transport. According to Jagels (1983) the loss of plasmodesmata in mature epidermal cells directly precedes the development of the osmoregulatory system. The loss of plasmodesmatal connections probably prevents the symplastic back

flow of salts as is the case in *Thalassia testudinum* (Jagels, 1973) and *Zostera capensis* (Barnabas *et al*, 1977).

7. Vacuoles

In contrast to young epidermal cells, the vacuole was a prominent feature of mature epidermal cells of *H. ovalis* since it occupied most of the cytoplasmic area. Vacuolar contents included material of a fine fibrillar nature, portions of cytoplasmic matrix, myelin-like structures and vesicles. The presence of these structures within the vacuoles is suggestive of autophagic activity (Esau, 1975). Evidence for the lysosomal function of the vacuole has been provided by many workers (Fineran, 1970; Matile, 1978).

Highly vacuolate cells, as seen in the present study, are not encountered in the leaf blade epidermal cells of other seagrasses. It is possible that the large vacuoles in *H. ovalis* probably play a role in maintaining the turgidity of the leaves since the leaves lack mechanical supporting tissue.

8. Nucleus

In general, changes in nuclear size, structure and position observed during development of leaf blade epidermal cells of *H. ovalis* resemble similar changes reported in cells of other plants. Nucleoli number varied from one to two per cell and occasionally nucleolar vacuoles were prominent.

An interesting feature of some nuclei (observed both in epidermal cells

as well as in cells of the leaf associated glands) was the presence of long channels which penetrated deep into the nucleoplasm. These invaginations were often associated with mitochondria. Nuclear invaginations have also been reported in reproductive plant tissues (Sheffield *et al*, 1979) and similar invaginations, commonly housing mitochondria, were observed in the sieve cells of the needle trace in *Pinus radiata* (Singh, 1984). According to Singh (1984) mitochondria are thought to provide energy for the formation of the nuclear invaginations. These are believed to increase the surface area of the nucleus presumably leading to an increase in activities associated with the nucleus.

9. Lipid Bodies

In the present study lipid bodies were common in nearly mature epidermal cells with developing wall ingrowths. In most cases the lipid bodies were closely associated with mitochondria and chloroplasts. Lipid bodies have also been reported in leaf blade epidermal cells of other seagrasses such as *Zostera capensis* (Jagels, 1983). According to Cecich (1979) lipid bodies serve as energy reserves and their close association with other membranous structures suggests that these bodies are a source of membrane lipid. Studies in jack pine apices indicated that lipid bodies may contain phospholipid precursors for membrane synthesis (Cecich, 1979).

10. Cell Wall

As reported in other seagrasses, leaf blade epidermal cells are covered by a thin cuticle. The cuticle of *H. ovalis* does not appear to be

perforated as in *Zostera marina* (Gessner, 1968, 1971), *Posidonia australis* (Kuo, 1978) and *Thalassodendron ciliatum* (Barnabas, 1982). The cuticle probably serves a protective function, possibly protecting the leaf blades against damage from abrasive sand particles and epiphytes associated with leaves.

As seen in mature epidermal cells the outer tangential cell wall has an interesting structure. The narrow darkly staining layer bordering the extracytoplasmic space, as seen in the present study, is a common feature of the walls of *Thalassodendron ciliatum* (Barnabas, 1982), *Halodule uninervis* (Barnabas & Kasavan, 1983b), *Thalassia testudinum* (Jagels, 1973) and *Zostera capensis* (Barnabas *et al*, 1977). In *H. ovalis* and *Zostera capensis* (Barnabas *et al*, 1977) wall ingrowths have been reported to arise from this layer. This intensely-staining layer forms a continuous band and is probably a form of secondary wall deposited on the inner face of an ordinary wall. Wall ingrowths are regarded as a specialized form of secondary wall (Pate & Gunning, 1972) and their continuity with the intensely staining border supports the conclusion for the secondary nature of this layer.

In most marine angiosperms, the inner half of the outer tangential wall has a characteristic striated or lamellated appearance. According to Birch (1974) the striated nature of the epidermal cell walls of *H. ovalis* and a *Cymodocea* sp. resemble the collenchymatous cell walls in land plants.

The outer region of the cell wall is comprised of a narrow intensely staining layer inside the cuticle and a non-striated layer (Fig. 103). A similar narrow intensely-staining layer in the outer epidermal cell

walls of *Zostera capensis* (Barnabas *et al*, 1977) was found to be composed of pectins. Histochemical observations in *H. ovalis* indicated that the walls are rich in pectins (Fig. 17). Investigations by Maeda *et al* (1966) on cell wall constituents, especially pectic substances in the leaves of *Zostera marina* revealed that the methoxyl content of the pectic substance was very low compared to that of terrestrial plants and that an ester-like sulphate was also present. Because of this unusual chemical composition, these investigators suggested that the pectic substance might be concerned with ionic absorption. The cell walls of *H. ovalis* could therefore play an important role in ionic absorption.

11. Annuli in outer epidermal cell wall

Solereder(1913) was the first to notice and comment on the unusual surface structural features in leaves of *H. ovalis*. He described them as "weak places" of the epidermal cells. Subsequently Birch (1974) described briefly the structure of the annuli. The present investigation has provided more information about the distribution and structure of the annuli.

Annuli are known to occur in leaf blade epidermal cells of other species of *Halophila* but are not present in the leaves of other seagrasses such *Cymodocea, Enhalus, Halodule, Syringodium, Thalassia* and *Zostera* (Birch, 1974). To date annuli have not been found in epidermal cells of other seagrasses.

Franke (1969) and Yamada et al (1966) have suggested that annuli may represent vestiges of stomata since functional stomata are not needed in marine angiosperms. However, this seems to be unlikely owing to the regularity of their occurrence in almost all the epidermal cells. Another explanation of Franke (1969) is that the annuli could represent vestigial scars of trichomes such as those found in *Halophila decipiens* on the leaf scales and spathe. This is also unlikely since the leaves of *H. ovalis* are glabrous.

The exact function of the annuli is not known. On the basis of the reaction with silver nitrate, it would appear that the annuli represent sites of chloride accumulation. A similar conclusion was also reached by Birch (1974). Further evidence that the annuli could represent sites of solute accumulation or secretion is the fact that the annuli occur in close proximity to the well-developed wall membrane apparatus in the outer wall of leaf blade epidermal cells. As mentioned earlier this region probably represents a site of intense solute fluxes.

Further evidence for a physiological role of the annuli is seen in the comparison of the annuli with the hydropoten of aquatics. Hydropoten are localized regions of irregular flat cells on the undersurface of floating leaves or on submerged leaves in the Alismataceae, Apogetonaceae, Hydrocharitaceae and Potamogetonaceae but are absent in seagrasses (Mayr, 1915). Functions ascribed to these structures are:

a. they are regions of localized salt uptake or sites of ion exchange which may involve active transport;

b. they are regions which probably facilitate either water loss or water uptake.

The annuli and hydropoten although variable in structure probably function as regions of localized salt uptake.

It is probable that the annuli play a role in maintaining the salt balance in the leaves by preventing the entry of excess salt into the epidermal symplasts. The presence of annuli in leaf blade epidermal cells of species of *Halophila* represents an unusual structural feature in the leaves of seagrasses.

12. Intercellular Structure

The unusual structure seen in an intercellular air-space of a mature leaf of *H. ovalis* is probably an alga orientated in the direction of the longitudinal axis of the leaf. Membranes with parallel arrangement, some ribosomes and small vesicles are visible in this apoplastic structure. The membrane system which resembles thylakoid membranes of an algal chloroplast is not enclosed by a double membrane envelope. This suggests that the intercellular structure may be a prokaryote, possibly a blue-green alga. The presence of the organism in the air-space is not of common occurrence and has only been observed once in the present study.

A number of workers (Wilson & Mahlberg, 1980; Dell *et al*, 1982) have reported the presence of intercellular structures in plants. There are reports of fungi and bacteria in air-spaces of certain aquatic plants (Kohlmeyer & Kohlmeyer, 1979, Kuo *et al*, 1981). However, very little information is available on the occurrence of intercellular structures in marine angiosperms.

Kuo (1984) reported the presence of fungal hyphae in the leaf apoplast of the subtidal form of the seagrass *Zostera muelleri*. He suggested that the apoplastic fungus and seagrass derive mutual benefit from each other. The fungus obtains nourishment for development via apoplastic solute transport and oxygen from the air lacuna system. The extensive network of hyphae of the apoplastic fungus is thought to enhance solute transport between the leaf epidermal cells and sieve tubes, the epidermal cells being the main sites of photosynthesis. The intercellular structure in the air-

space system of the leaf of *H. ovalis* apparently does not form an extensive network as the fungus in *Zostera muelleri*.

Although the intercellular structure in *H. ovalis* apparently does not form an extensive network as the fungus in *Zostera muelleri*, its presence might confer the same mutual relationship as suggested by Kuo (1984).

V. ULTRASTRUCTURE OF THE SQUAMULAE INTRAVAGINALES

Introduction

Irmisch (1858) first reported the presence of axillary scales, termed 'squamulae intravaginales', which are characteristic of many families of aquatic monocotyledons belonging to the order Helobiae. The squamulae occur in the axils of foliage and scale leaves (including prophylls and cotyledons) and may also be associated with bracts. They are seldomly associated with the floral parts of the plant.

In the vegetative buds of *H. ovalis* the squamulae develop early and are commonly found associated with very young leaves. However, in *Elodea* the squamulae appear late during bud development and according to Dale (1957) the delay in their appearance may be due to the unusual shape of the shoot apex.

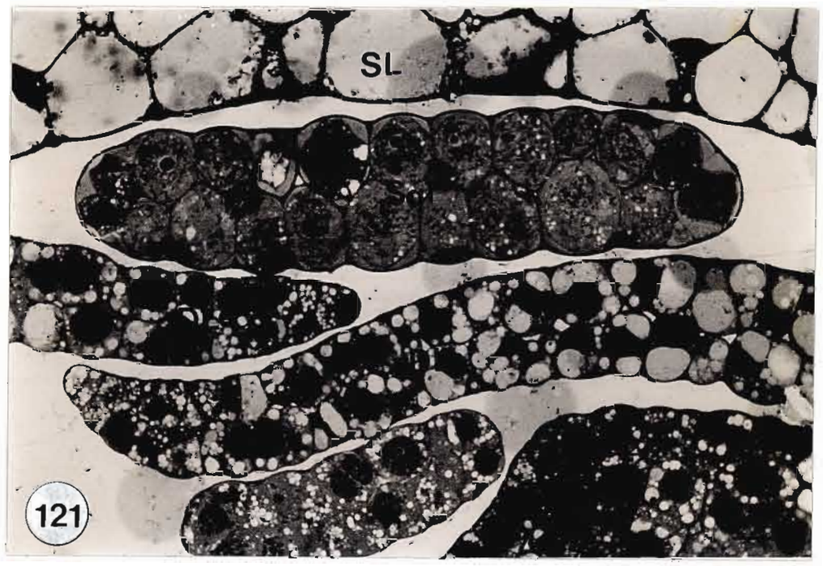
Studies concerning the frequency and distribution of the squamulae in the Helobiae include those by Wilder (1975), Gibson (1905) and Arber (1923, 1925). The fine structure of the squamulae in *Elodea canadensis* and *Potamogeton perfoliatus* was described by Rougier (1965). Other aspects of study of the squamulae are those by Dale (1957), Wilder (1974) and Ancibor (1979).

The present ultrastructural study of the squamulae of *H. ovalis* was initiated because no information is available on the fine structure of squamulae in marine angiosperms. In addition, the proximity of the squamulae to the foliage leaves in vegetative buds, facilitated the investigation of their ultrastructural morphology and probable functions.

PLATE 21

Fig. 121. Light micrograph of transverse section of part of vegetative bud showing squamulae intravaginales , scale leaves and developing foliage leaves.

Bar represents: 5 µm



Results

In the present investigation only squamulae associated with young developing leaves in the vegetative buds were examined, because the squamulae in the axils of older leaves fall off as the leaf matures. Figures 122 and 123 (arrows) show the position and size of the squamulae relative to developing young leaves in partially dissected vegetative buds. The squamulae of *H. ovalis* are sessile or subsessile membranous structures situated at the base on either side of each leaf. These membranous scales arise from the axis in pairs on either side of each pair of leaves. The scales are ovoid-elliptical in shape, approximately 0,5 mm long, 0,2 mm wide and are comprised of two layers of cells orientated in the direction of the leaf axis (Fig. 121). They are non-vasculated. Squamulae cells are similar in shape and composition. In cross-sectional view the cells are cuboidal in shape (Fig. 121) whereas in longitudinal section they are longer than broad (Fig. 125).

Fine structure of the squamulae cells revealed interesting features characteristic of glandular or secretory tissues. The most striking feature of the cells is their organelle-rich cytoplasm and pronounced accumulation of material (probably mucilage - see later) between the plasmalemma and cell walls (Fig. 124). The density of the cytoplasm is due to the presence of numerous ribosomes (Fig. 126, small arrow) and organelles (Fig. 125, arrow). Many dictyosomes, mitochondria, RER cisternae and some plastids are observed in the cytoplasm (Fig. 124). The dictyosomes and RER cisternae appear to be the most active and abundant structures in the cytoplasm (Fig. 124).

The nucleus, although fairly large, does not dominate the area of the cytoplasm (Fig. 124). Areas of heterochromatic material are evident in the nucleoplasm (Figs. 124, 125). Many mitochondria, varying in cross-sectional

PLATE 22

Figs. 122 - 123. Scanning electron micrographs of dissected vegetative buds showing position of squamulae intravaginales (arrows) relative to young developing leaves.

Figs. 124 - 128. Transverse and longitudinal sections of cells of squamulae intravaginales.

Fig. 124. Transverse section of squamulae cell showing organelle rich cytoplasm with central nucleus, RER surrounding nucleus, abundant dictyosomes and mitochondria, plastids, plasmalemma, plasmodesmatal connections in radial cell wall and the accumulation of mucilage between cell wall and plasmalemma, Note outer tangential wall thicker than radial and inner tangential walls.

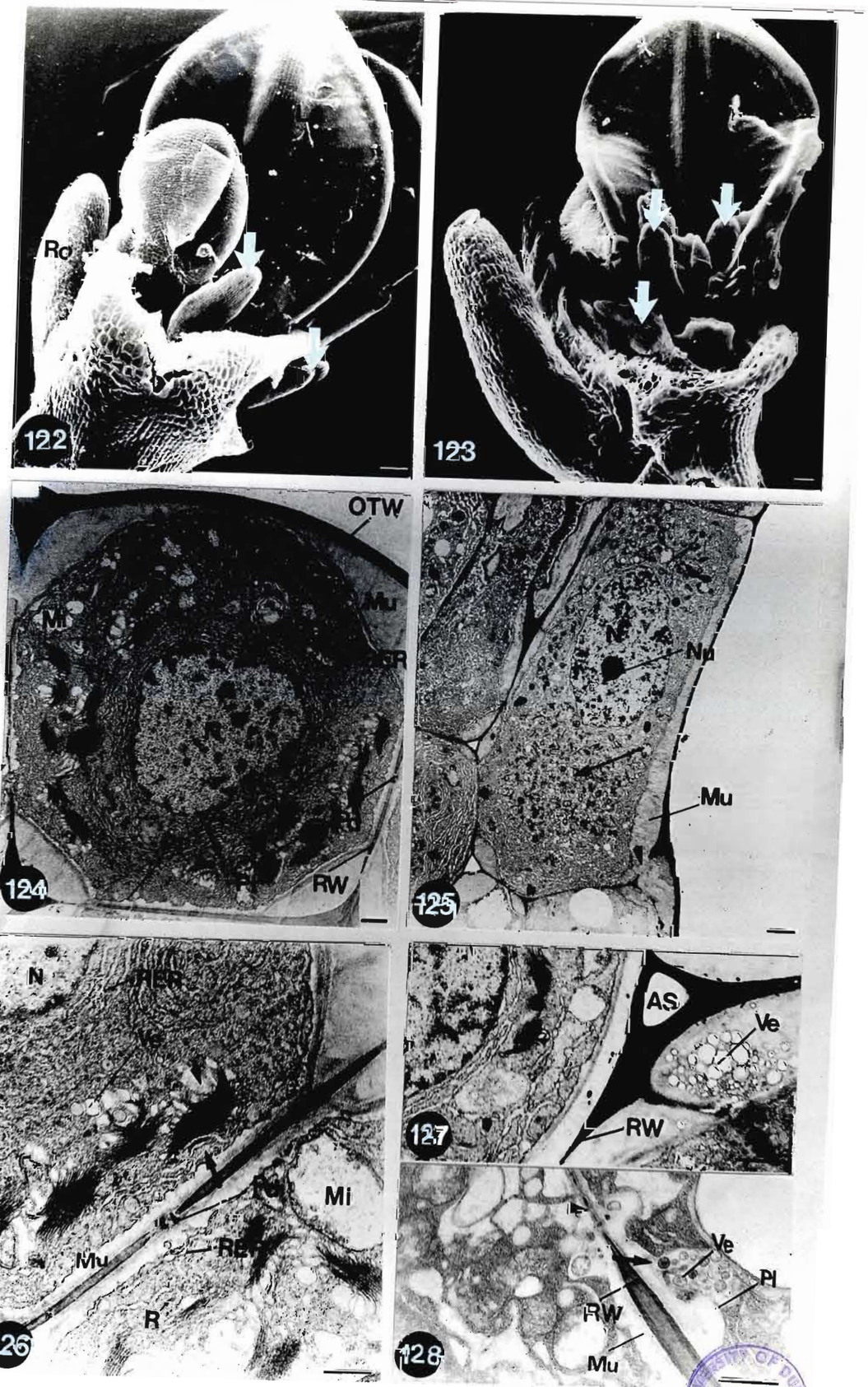
Fig. 125. Longitudinal section of squamulae cell showing nucleus with condensed nucleolus, organelle rich cytoplasm (arrow), accumulation of mucilage between cell wall and plasmalemma, dissolution of radial cell wall (arrow head).

Fig. 126. Details of cytoplasm along radial cell wall showing ribosomes (small arrow), RER parallel to plasmalemma, abundant dictyosomes with forming (arrow) and maturing (arrow head) faces. Mucilage seen between cell wall and plasmalemma, Note plasmodesmatal connection in radial cell wall.

Fig. 127. Vesicles in extracytoplasmic space at corner of cell wall.

Fig. 128. Vesicles in extracytoplasmic space containing mucilage (arrow). Note plasmalemma and breakdown of radial wall (arrow head).

Bars represent: 30 µm (Figs. 122, 123)
1 µm (Figs. 124, 127)
3 µm (Figs. 125)
0,5 µm (Figs. 126, 128)



profile from circular to oval (Figs. 124, 133) occur in the cytoplasm. Plastids are not numerous, the shapes vary from round to elongate depending on the plane of section (Fig. 133). These plastids are more electron dense than the surrounding cytoplasm (Fig. 133). Little development of the internal lamellar system is seen in the plastids of Figures 132, 133 (arrows). Plastoglobuli are a common feature of the plastid stroma (Figs. 132, 133).

Dictyosomes are numerous and have a tendency to occur close to those parts of the cells where extruded material has accumulated between the plasmalemma and cell walls (Fig. 124). Each dictyosome is highly stacked with an average of 12-14 cisternae per stack (Fig. 126). There is distinct polarisation of their structure comprising a forming face (arrow) and a maturing face (arrow head) as seen in Figure 126. The maturing face has a curved appearance (Fig. 126, arrow head) and bears a number of cisternae of varying sizes. The large production of vesicles at the dilated edges of the cisternae is indicative of a high degree of activity of the dictyosomes (Figs. 124, 126).

Another conspicuous structural component of the squamulae cells is the well-developed endoplasmic reticulum especially of the rough type (Figs. 124, 126). Rough Endoplasmic Reticulum is abundant in squamulae cells. Often ER cisternae appear swollen (Figs. 129, 131, 132).
By a concentrical arrangement of numerous RER cisternae, the RER frequently forms myeline-like structures (Fig. 133). This concentric arrangement of the RER cisternae also occurs around the nucleus (Figs. 124, 130). Deep invaginations of the nucleus are lined with RER cisternae (Fig. 130). Short cisternae of RER appear to connect concentric ER around the nucleus with RER located along the periphery of the cell (Fig. 124, arrow). A close association exists between the dictyosomes and ER, the two most active

PLATE 23

Figs. 129 - 134. Transverse sections of cells of 'squamulae intravaginales'.

Fig. 129. Group of glandular cells showing breakdown of radial cell walls with increasing mucilage production (arrows). Note mucilaginous sheath surrounding gland cells on outside (arrow heads). Note spherical outline of protoplasts (curved arrow) and some vacuoles in cytoplasm.

Fig. 130. Nuclear invagination lined by RER cisternae.

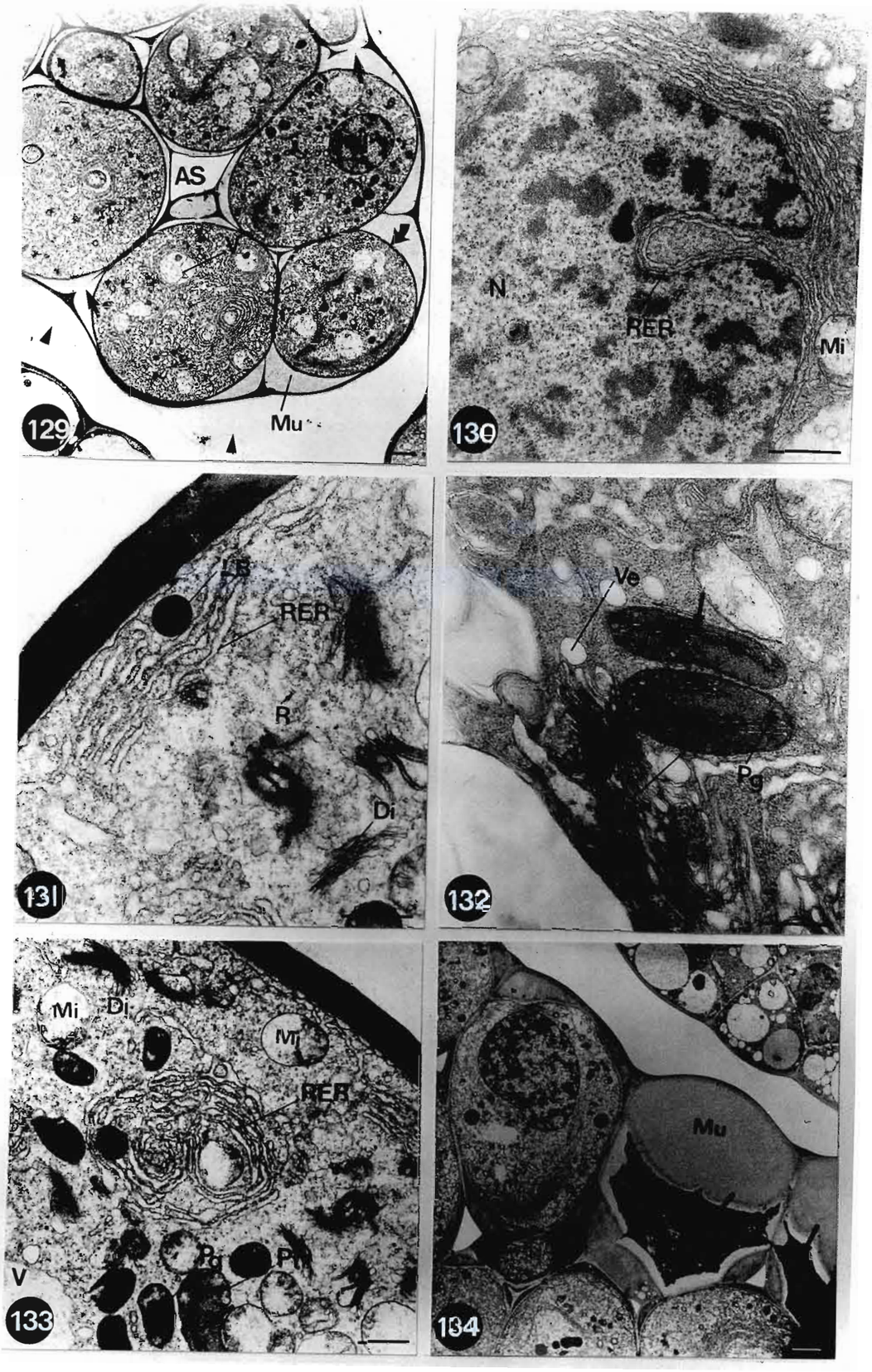
Fig. 131. Details of cytoplasm along outer tangential wall showing lipid body, parallel profiles of swollen RER cisternae, dictyosomes and ribosomes (arrow).

Fig. 132. Details of cytoplasm showing elongate plastids with plastoglobuli and internal lamellae (arrow), dictyosomes and vesicles. Note swollen ER cisternae.

Fig. 133. Cytoplasmic details showing circular to elongate plastids, concentric rings of RER, numerous dictyosomes and mitochondria.

Fig. 134. Degeneration of protoplast (arrows) coupled with increasing mucilage production.

Bars represent: 3 µm (Figs. 129, 134)
0,5 µm (Figs. 131, 132, 133)
1 µm (Fig. 130)



components of the squamulae cells (Figs. 126, 132).

Often vesicles, probably of ER or dictyosome origin occur within the extruded material between the cell wall and plasmalemma (Figs. 127, 128).

Lipid bodies are sparse in squamulae cells (Fig. 131).

As mentioned earlier, a characteristic feature of squamulae cells is the accumulation of material within the prominent extracytoplasmic spaces between the cell walls and plasmalemma, especially at the corners of the cells. Transverse sections of vegetative buds treated with Alcian Blue and Alcian Yellow showed that this accumulated material within the cells (e.g. Figs. 124, 125; 135, 136, arrows) and around the squamulae (Figs. 129, arrow heads; 135, 136, arrow heads) was positive to Alcian Yellow. This suggests that the material is carbohydrate-like in nature and is probably mucilage. Since Alcian Yellow is specific for acid non-sulphated polysaccharides it is possible that the mucilage is composed mainly of acid non-sulphated polysaccharides. The homogeneity of the mucilaginous substance is more or less consistent in the squamulae cells.

The outer cell wall of squamulae cells is thicker than radial and inner tangential cell walls (Figs. 124, 129). Plasmodesmatal connections between adjacent squamulae cells are visible in the radial cell walls only (Figs. 124, 126). With further development of the cells, parts of the radial cell walls begin to disintegrate (Fig. 129, arrow). The plasmalemma does not follow the outline of the cell walls (Fig. 124).

PLATE 24

Figs. 135 - 136. Light micrographs of transverse sections of part of vegetative bud showing reaction of squamulae intravaginales to Alcian dyes.

Fig. 135. Mucilage accumulation between developing leaves and the squamulae intravaginales (arrow heads). Note presence of mucilage within squamulae cells (arrows).

Fig. 136. Mucilage extruded from squamulae intravaginales (arrow heads) stained yellow with Alcian dyes. Note presence of mucilage within squamulae cells (arrows).

Bars represent: 10 µm (Figs. 135, 136).

Discussion

This study has shown that the cells of the squamulae intravaginales have structural features similar to those of secretory cells. The presence of extruded material probably mucilage, within the extracytoplasmic spaces especially at the cell corners as well as around the squamulae, is indicative of the secretory function of these cells.

A striking feature of the squamulae cells is the large population of dictyosomes and profiles of RER in the cytoplasm. The highly-stacked dictyosomes together with the many dictyosome-derived vesicles suggests a high level of activity. The occurrence of dictyosomes especially in the peripheral regions of the cells suggests that they may play a role in secretion. Dictyosomes have been implicated in mucilage secretion in many plants (Mollenhauer & Morré 1966, Fahn 1979, Fineran & Lee 1974).

A close association exists between the dictyosomes and ER in squamulae cells. The presence of ER cisternae close to the forming face of some dictyosomes indicates a membrane flow from the ER to dictyosome (Fig. 126, arrow). This step in the endomembrane system (Morré *et al*, 1971) is well documented in animals and lower plants and has only recently been reported to occur in higher plants (Robinson & Hammerl, 1980). ER and dictyosome relationships in this study appear to be in line with the endomembrane concept. It is possible that the extensive profiles of ER in squamulae cells also play a role in secretion. Involvement of ER in polysaccharide production has been suggested for some secretory structures (Werker & Kislev, 1978).

Although histochemical tests for the presence of proteins in the extruded mucilage from the cells were not carried out it is possible that RER elements are associated with the bulk production of protein, an often-

reported component of mucilage. The relationship between RER and bulk protein production in secretory cells has been reported by Gunning and Steer, 1975; Werker and Vaughan, 1976. The occurrence of numerous ribosomes in the cytoplasm also indicates enhanced protein synthesis in these cells.

Concentric rings of ER, as seen in the present study, has also been recognised in young club-shaped trichomes of potato (Lyshede, 1980) and in apical meristematic cells of dormant potato tuber buds (Marinos, 1967; Shih & Rapport, 1971; Lyshede, 1980). According to Lyshede (1980), concentric rings of ER are probably temporary structures since they disappear or are transformed into normal ER during maturation of the cells.

The deep nuclear invaginations observed sometimes in cells of the squamulae have also been encountered in secretory cells associated with latex ducts in *Mammillaria guerreronsis*, a member of the Cactaceae (Wittler & Mauseth, 1984). These invaginations are believed to increase the surface area of the nucleus, presumably leading to an increase in activities associated with the nucleus.

Mitochondria are well distributed in the cytoplasm of the squamulae cells. According to Lüttge (1971) high mitochondrial numbers appear to be a characteristic feature of secretory cells. Involvement of mitochondria has been suggested for the production of slime drops in root hairs of *Sorghum* (Werker and Kislev, 1978). Mitochondria probably play a role in the metabolism of secretory products.

As seen in the present study, the plasmalemma becomes detached from the cell walls, especially at the corners thus giving rise to extracytoplasmic spaces in which mucilage accumulates. Such spaces, filled with secretory

products between the cell wall and plasmalemma, are common in secretory cells (Fahn, 1979).

Dissolution of the radial walls of the squamulae cells of *H. ovalis* parallels the accumulation of secreted mucilage in the extracytoplasmic spaces. Similar observations have been reported in *Hibiscus* during pedicel abscission (Gilliland et al, 1970) and in the extrafloral nectaries of *Plumeria rubra* (Mohan & Inamdar, 1986). Mucilage accumulation between the cell wall and protoplast also occurs in mucilage cells of *Opuntia ficus-indica* (Trachtenberg & Fahn, 1981), in the glands of *Mimulus tilingii* (Schnepf, 1976) and in the extrafloral nectaries of *Plumeria rubra* (Mohan and Inamdar, 1986). According to Morré *et al* (1967) this feature occurs in cells with reduced turgor. Progressive growth of the squamulae cells of *H. ovalis* is coupled with increasing mucilage production. Eventually the whole protoplast degenerates thus providing space for the copious mucilaginous secretion.

The results of this ultrastructural study as well as histochemical tests, strongly support the conclusion that the squamulae intravaginales of *H. ovalis* serve as secretory organs. Histochemical tests have shown that mucilage is probably the principal secretory product. The function of mucilaginous secretions in aquatics is not clear. It has been postulated that the secretion inhibits the growth of micro-organisms favoured by the wet conditions (Schilling, 1894). It is probable that the mucilage in the squamulae of *H. ovalis* act as a deterrent against invasion by micro-organisms. The squamulae may also act as close packing organs which fill the spaces between developing leaves (Tomlinson, 1982).

VI. CONCLUSION

This investigation has shown that *H. ovalis* possesses structural features that are in general, similar to those of other marine angiosperms. The most striking morphological differences between *H. ovalis* and other seagrass genera are: i. the petiolate leaves; and ii. the ovate to elliptical-shaped leaf blades (unlike the grass-like leaves typical of the other seagrasses).

Like other seagrasses and submerged fresh water aquatics in general, leaves have a simple anatomy. The xylem is greatly reduced, phloem is well-developed and a well-developed air-space system is present. The epidermis appears to be the main photosynthetic tissue since epidermal cells possess more chloroplasts than any other tissue of the leaf.

Young epidermal cells have an organelle-rich cytoplasm with many plasmodesmata interconnecting adjacent epidermal and mesophyll cells. As the cells mature they acquire characteristics of transfer cells. Wall ingrowths form in cells when chloroplasts have well-developed membrane systems in their stroma. The plasmalemma follows the contours of the ingrowths and this leads to an increase in its surface area . Since plasmodesmatal connections become discontinuous as epidermal cells mature, the enlarged plasmalemma probably facilitates the transfer of photosynthates from the epidermal cells to the interior tissues of the leaf.

As structures such as salt glands do not exist in the leaves, osmoregulation seems to be accomplished by the plasmalemma of the epidermal cells. The enlarged plasmalemma in mature cells probably leads to an increase in the osmoregulatory capacity of the cells. An enlarged plasmalemma

might also enhance absorptive and/or secretory activities between the epidermal cells and surrounding sea water.

Unlike other seagrasses, unusual ring-like structures called annuli occur in the outer wall of leaf blade epidermal cells. On the basis of their histochemistry, it would appear that the annuli represent sites of chloride localization. The annuli are probably the equivalent of salt glands of terrestrial halophytes.

Although the leaves of *H. ovalis* lack lignified strengthening tissues, support of the leaf blades appears to be accomplished by the thickened outer wall of the epidermal cells. In addition, the greatly thickened outer wall of epidermal cells of the petiole probably offers further support to the leaf blades.

Studies of the squamulae intravaginales showed that their cells have features in common with secretory cells. Material extruded from the cells accumulates within large extracytoplasmic spaces at the cell corners, as well as outside the cells. On the basis of histochemical tests the extruded material appears to be mucilage.

This investigation of the structure of leaf blade epidermal cells and squamulae intravaginales of the marine angiosperm, *H. ovalis* has contributed to our understanding of structural features in this unusual group of vascular plants which have successfully invaded the sea from the land.

SUMMARY

The structure and histochemistry of leaf blade epidermal cells and leaf-associated axillary scales (squamulae intravaginales) of the marine angiosperm, *H. ovalis*, were investigated by light, scanning and electron microscopy. The epidermis appears to be the main photosynthetic tissue because the cells possess more chloroplasts than any other tissue of the leaf. Mature epidermal cells possess transfer cell features since wall ingrowths and an invaginated plasmalemma with closely-associated mitochondria are present. Developmental studies have shown that wall ingrowths form in cells when chloroplasts have well-developed membrane systems in their stroma.

Since structures such as salt glands do not exist in the leaves, osmoregulation seems to be accomplished by the plasmalemma of the epidermal cells. The enlarged plasmalemma probably also results in an increase in the osmoregulatory capacity of the cells. Unusual ring-like structures (called annuli) in the outer wall of leaf blade epidermal cells may also have a role in salt regulation. On the basis of their histochemistry, the annuli appear to represent sites of chloride localization.

Squamulae intravaginales, which are closely associated with the bases of young leaves, are non-vasculated scale-like structures only two cells in thickness. The cytology of their cells suggests that they have features in common with secretory cells. Material extruded from the cells accumulates within large extracytoplasmic spaces at the cell corners as well as outside the cells. On the basis of histochemical tests, the extruded material appears to be mucilage.

This investigation of the structure of leaf blade epidermal cells and squamulae intravaginales of the marine angiosperm *H. ovalis*, has contributed to our understanding of structural features in this unusual group of vascular plants which have successfully invaded the sea from the land.

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PLATE 14

Figs. 77 - 83. Transverse sections of category G cells.

Fig. 77. Cell typical of category showing further development of wall ingrowths, well developed chloroplasts and an increase in the thickness of the cell walls. Few plasmodesmatal connections are evident in radial cell walls.

Fig. 78. Details of cytoplasm along part of outer tangential walls showing multivesicular structure containing many paramural bodies in close association with wall ingrowth. ER apparently contributing vesicle to wall ingrowth.

Fig. 79. Multivesicular structure containing numerous paramural bodies protruding into vacuole. Paramural bodies apparently contributing material to wall ingrowth (arrow).

Fig. 80. Details of cytoplasm along radial cell wall showing multivesicular structure, plasmodesmatal connections penetrating radial wall fully, mitochondria, ER and chloroplasts.

Fig. 81. Lipid bodies closely associated with mitochondria and chloroplast.

Fig. 82. Well developed membrane system of chloroplast showing granal and intergranal thylakoids, plastoglobuli, nucleoid area and peripheral reticulum.

Fig. 83. Details along outer tangential wall showing thick outer cell wall, innermost intensely staining layer from which wall ingrowths arise (arrow), branched wall ingrowth (arrow head), lipid bodies and lobed nucleus housing mitochondrion. Vacuole contains electron dense material (curved arrow).

Bars represent: 2 µm (Fig. 77)
0,5 µm (Figs. 78, 82)
0,1 µm (Fig. 79)
0,25 µm (Fig. 80)
1 µm (Figs. 81, 83)