Ultrastructural Study on *Lemna perpusilla*¹

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Lemna perpusilla is a small aquatic plant that has been frequently used for physiological studies on flowering (Hillman and Posner 1971, Hudson and Hamner 1971). Early work by Hegelmaier (1868) and Brooks (1940) provided information on the structure of plant body and later the reproductive structure was also described (Maheshwari and Kapli 1963) at the light microscopic level. There is no electron microscopic examination on the plant has been reported other than that of Arnott and Pautard (1970) which dealt largely with calcium crystal structure and metabolism. As abscission can occur rapidly in this plant, the fine structures of frond cells as well as the cells in abscission zone are studied in a detail investigation.

Materials and methods

Lemna perpusilla, Torr. strain 6746, was grown aseptically in half strength Hutner's medium made 1% (W/V) with sucrose (Hillman 1971a). Continuous illumination was provided by cool white fluorescent lamps and incandescent lamps at an intensity of 320 ft-c. The plants were maintained at a temperature of 22°-24°C.

Frond tissue was fixed for 2-3 hrs for electron microscopy in formaldehyde-glutaraldehyde buffered with 0.1 M cacodylate (pH 6.8) (Karnovsky 1965). This was followed by a post-fixation in unbuffered 1% (W/V) osmium tetroxide for two hours at room temperature. All tissues were rinsed thoroughly in buffer and dehydrated in an acetone series. Spurr's epoxy resin (Spurr 1968) was used for embedding.

Sections were stained with 1% (W/V) uranyl acetate and lead citrate and examined with the Zeiss EM 9 electron microscope. A few whole plants were fixed in osmium tetroxide vapor and prepared for scanning electron microscopy (Falk *et al.* 1971). Scanning electron microscopic examination were made with a Cambridge S-4 Steroscan using an accelerating potential of 20 KV.

Observations

Daughter fronds arise from two pouches situated at the nodal region of the mother frond (Figs. 1, 2). Young daughter frond consists of essentially meristematic cells with a large, centrally located nucleus. Many mitochondria are observed and they sometimes assume a dumbbell morphology. The chloroplasts of

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Key to labelling and explanation of figure in page 000.

Fig. 11. Crystal idioblast showing ribbon-like arrays of crystal elements. (Crystals no longer present). $\times 4,420$.

these cells, contain substantial amounts of starch and possess a well developed granal system. Rough endoplasmic reticulum and dictyosomes are abundantly

Figs. 1-11. Micrographs of *Lemna perpusilla*. Key to labeling: AZ, abscission zone; CE, crystal element; Co, Core; Cp, chloroplast; CW, cell wall; D, dictyosome; DC, dead cell; DF, daughter frond; ER, endoplasmic reticulum; F, fibril; IS, intercellular space; M, mitochondrion; Me, membrane; MF, mother frond; ML, middle lamella; Mt, microtubule; My, myelin figure; N, nucleus; Nu, nucleolus; Pd, plasmodesmata; Po, pouch; RL, ribosome-like particles; S, starch; Va, vacuole; Ve, vesicles.

Figs. 1-2. 1, scanning electron micrograph of *Lemna* showing vegetative morphology. $\times 25$. 2, light micrograph of abscission zone situates between mother and daughter fronds in paradermal section. $\times 150$.

Figs. 3-4. 3, a meristematic cell of young frond. $\times 19,000$. 4, microtubules associated with cell walls of young frond. $3,400\times$.

Figs. 5-6. 5, cells in abscission zone are rectangular in shape and with large amount of long, rough endoplasmic reticulum. $\times 5,100$. 6, random orientation of cell wall associated microtubules in abscission zone cells. $\times 54,600$.

Figs. 7-8. 7, abscission zone cells in a late stage of abscission showing the appearance of myelin figures and dissolution of cell wall materials. $\times 18,200$. 8, a mature frond cell is elliptical in shape with an irregular shaped nucleus. $\times 5,100$.

Figs. 9-10. 9, intercellular space development-showing dead cell. $\times 24,500$. 10, young crystal elements have distinct cores from which many fibrils radiating out. Many ribosome-like particles distributes over the crystal elements. $\times 23,400$.

distributed in the cytoplasm (Fig. 3). Microtubules are commonly seen associated with the cell walls of young frond (Fig. 4).

Daughter fronds eventually separate from the mother frond by the process of abscission. An abscission zone differentiates between mother and daughter fronds to effect this process (Fig. 2). The zone consists of 4–5 rows of more or less rectangular cells which exhibit an irregular outline (Figs. 2, 5). Long segments of rough endoplasmic reticulum are often observed to be associated with the plasmodesmata of these cells (Fig. 5). Microtubules in cells of the abscission zone just prior to abscission often assume a random orientation (Fig. 6). Prior to abscission, myelin figures begin to appear with considerable frequency along the cell wall (Fig. 7). Finally, cell separation is due to cell wall breakdown and middle lamella degradation. Abscission can occur in as short as a period as 1 day in *Lemna*.

As maturation occurs, the cells of the daughter frond are changed to elliptical in paradermal section. Their protoplasts contain a centrally located, irregularly shaped nucleus. Endoplasmic reticulum is often found associated with the plasmalemma. There are numerous small vesicles in these cells rather than a large central vacuole. Mitochondria are common and possess well developed cristae. The chloroplasts of cells found in this tissue are rather elongate but are not common. Dictyosomes remain unchanged.

Intercellular spaces develop lysigenously in a random manner (Fig. 9).

In some of the cells of this tissue, crystals form usually within a vacuole. Young crystal elements appear to consist of a central core with fibrils that radiated from this core. The entire crystal element is double membrane bound and ribosome-like particles are associated with the membrane, although not in great number. New crystal elements seem to bud from older ones (Fig. 10). As crystal formation progresses, the crystal elements line up in ribbon-like arrays connected by parallel membranes (Fig. 11). The crystals themselves are frequently dissolved during fixation. Crystal containing cells often occupy as much as five times the volume of other cells.

Discussion

Abscission is a regularly occurring process in *Lemna perpusilla*. The presence of numerous myelin figures and long segments of rough endoplasmic reticulum adjacent to the cell wall in the abscission zone is one of the most interesting observations reported in this study. Jensen and Valdovinos' (1968) work on the abscission zone of flower pedicels suggested that the presence of rough endoplasmic reticulum may indicate the synthesis of enzymes associated with cellwall degradation in the abscission zone. Myelin figures, structures found in algae, fungi and higher plants, have had several functions suggested for them: cytoplasmic alteration (Carbonell and Pollak 1962); wall formation (Wilsenach and Kessel 1965, Esau *et al.* 1966). In as much as abscission occurs quite rapidly in *Lemna perpusilla* and that the structural basis for the containment of lytic enzymes remains unknown, the observed myelin figures might well be implicated as containers.

The cells in the region of the presumptive abscission zone undergo mitosis

frequently. Microtubules are found regularly in cells of this tissue. If microtubules are considered as necessary for the deposition and orientation of a cell wall component during wall formation, then the loss of orientation of microtubules observed in abscission cells could be related to the onset of abscission.

In their study of abscission of flower pedicles, Jensen and Valdovinos (1968) observed plasmodesmata with branches in the region of the middle lamella. They speculated that dissolution of the middle lamella between cells in the abscission zone might be initiated where the branches plasmodesmata occurred. In *Lemna perpusilla*, plasmodesmata vanish into the cell walls of cells of the abscission zone. Swelling of the cell wall in these regions is also observed. Therefore, it is suggested that enzymes synthesized by rough endoplasmic reticulum and/or enzymes stored in myelin figures in the region could be discharged into the plasmodesmata and lead to middle lamella and cell wall breakdown with eventual abscission.

Arnott and Pautard (1970) observed several stages of development of crystals in *Lemna minor*. They reported that paired membranes within the central vacuole arranged themselves in a parallel manner and at discrete points, generated crystal chambers containing electron dense material which crystallized to become calcium oxalate. Crystal development in *Lemna perpusilla* appears to follow a similar course. However, we were able to make a few additional observations. We found that a central core is present in each young crystal element which may be the center of the crystallization process. We also noted fibrils associated with the crystal chamber membranes. Frank and Jensen (1970) in their study of crystal formation in *Canavalia ensiformis* reported tubules associated with crystals and suggested that they might function in transporting materials for crystal formation. The fibrils observed in *Lemna perpusilla* could function similarly. Moreover, if the particles associated with the crystal chamber membranes are ribosomes, it would suggest that protein synthesis is necessary for crystal synthesis and that perhaps the fibrils represent some form of endoplasmic reticulum.

Summary

The structure of young frond cells of *Lemna perpusilla* is similar to meristematic cells. The abscission zone which forms in *Lemna* is found to consist of 4-5 rows of rectangular cells. Early in development, cells in this zone often have long segments of endoplasmic reticulum associated with plasmodesmata. Later, microtubules associated with the walls of cells in this zone assume a random orientation. Myelin figures appear and as abscission progresses, cell separation occurs. The development of crystal and the association of ribosome-like particles are also observed.

Literature cited

- Arnott, H. J. and Pautard, P. G. E. 1970. Calcification in Plants. In: Biological Calcification, H. Schraer, ed. New York. Appleton-century-Crofts.
- Brooks, J. S. 1940. The cytology and morphology of the Lemnaceae. Ph. D. Thesis, Cornell
- Carbonell, L. M. and Pollak, L. 1962. "Myelin figures" in yeast cultures of *Paracoccidioides*

- brasiliensis*. J. Bacteriol. **83**: 1356–1357.
- Esau, K., Cheadle, V. I. and Gill, R. H. 1966. Cytology of differentiating treacheary elements II. Structures associated with cell surfaces. Amer. J. Bot. **53**: 765–771.
- Falk, R. H., Gifford, E. M. Jr. and Cutter, E. G. 1971. The effects of various fixation schedules on the scanning electron microscopic image of *Tropaeolum majus*. Amer. J. Bot. **58**: 678–680.
- Frank, E. and Jensen, W. A. 1970. On the formation of the pattern of crystal idioplasts—*Canavalia ensiformis* D. Planta **95**: 202–217.
- Hegelmaier, F. 1868. Die Lemnaceen. Leipzig. 1969 pp.
- Hillman, W. S. 1971a. Nitrate and the course of *Lemna perpusilla* carbon dioxide output under daily photoperiodic cycles. Plant Physiol. **47**: 431–434.
- and Posner, H. B. 1971. Ammonium ion and the flowerine of *Lemna perpusilla*. Plant Physiol. **47**: 586–587.
- Hudson, H. K. and Hamner, K. C. 1971. A comparison of the effects of autoclaved and non-autoclaved gibberellic acid on *Lemna perpusilla* 6746. Plant Physiol. **47**: 726–728.
- Jensen, T. E. and Valdovinos, J. G. 1968. Fine structure of abscission zones III. Cytoplasmic changes in abscising pedicles of tobacco and tomato flowers. Planta **83**: 303–313.
- Karnosky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. **27**: 137–138. (Abstr.)
- Maheshwari, S. C. and Kapil, R. N. 1963. Morphological and embryological studies on the Lemnaceae I. The floral structure and gametophytes of *Lemna paucicostata*. Amer. J. Bot. **50**: 677–686.
- Spurr, A. R. 1968. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultra. Res. **26**: 31–43.
- Wilsenach, R. and Kessel, M. 1965. The role of lomasomes in wall formation in *Penicillium vermiculatum*. J. Gen. Microbiol. **40**: 401–404.
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