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Histochemistry and ultrastructure of paraphyses in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner

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Sargassum vulgare and *S. johnstonii* bear androgynous receptacles with unisexual conceptacles. The fully developed conceptacles are filled with secretions and communicate with the external environment through the ostiole plugged with polysaccharidic material. The conceptacles are lined by flat cells–the conceptacle-wall cells, which are the progenitors of either antheridia or oogonia or paraphyses. In a young conceptacle, the paraphysial and the conceptacle-cell walls contain alginic acid and sulphated polysaccharides. During the early stages of conceptacle development, the cytoplasm is organelle-rich but in the old conceptacles, the organelles lyse and the paraphysial and conceptacle-wall cell cytoplasm becomes vacuolate.

*Key Index Words: alginic acid—histochemistry—paraphysis—Phaeophyceae—*Sargassum*—secretions—sulphated polysaccharides.*

In Sargassaceae (Fucales), the histochemical and ultrastructural information on the paraphyses and the conceptacle-wall cells is meagre. The mucilaginous secretions have been reported from the conceptacles of *Fucus edentatus* (McCully 1968) whereas elaborate wall projections have been observed in the conceptacle wall cells of *Durvillaea potatorum* (Clayton et al. 1987). The present study on *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner is undertaken to correlate the histochemical, developmental and ultrastructural aspects of the paraphyses and the conceptacle-wall cells.

Materials and Methods

The plants of *Sargassum vulgare* and *S. johnstonii* were collected during the low tide period from Port Okha, Gujarat, through the months of January, February and November of 1987–89. The selected parts of the plants thus collected were processed for light, transmission and scanning electron microscopy.

For light microscopy, the plant parts were fixed in 10% acrolein, washed in distilled water and postfixed in 1% $HgGl_2$. This was followed by dehydration, and infiltration. The infiltrated materials were embedded in glycol methacrylate (Vijayaraghavan & Shukla 1990), and sectioned on a Spencer (AO) rotary microtome fitted with a locally made adaptor to hold glass knives. Two micron thick sections were serially transferred to small drops of distilled water and later stained with PAS reagent for insoluble polysaccharides (Vijayaraghavan and Shukla 1990); with Alcian Blue for sulphated polysaccharides (Parker and Diboll 1966); with TBO for sulphated and carboxylated polysaccharides (McCully 1966); with Coomassie Brilliant Blue for proteins (Weber and Osborn 1975) and with Feulgen reagent for DNA (Vijayaraghavan and Shukla 1990). For SEM, the plant parts were fixed in 4% formalin, dehydrated in graded acetone series, critical point dried and scanned for topographical details. For transmission electron microscopy, the desired stages were cut into small pieces, fixed in 6% glutaraldehyde prepared in phosphate buffer, dehydrated, infiltrated and embedded in Epon-Araldite mixture (Mollenhauer 1964). Ultrathin sections were stained and then observed through

Philips EM 300.

Results

Light microscopic Studies

The reproductive phases are seasonal in *Sargassum vulgare* and *S. johnstonii*. The axillary receptacles occur in clusters (Figs. 1, 2). Unisexual conceptacles are embedded in the receptacular tissues. The antheridial and oogonial conceptacles are borne in the same receptacle but in the conceptacles, either the antheridia or oogonia cooccur with paraphyses. Thus, the plants are neither monoecious nor dioecious in the generally accepted sense as both types of reproductive organs are present in the same receptacle but always in separate conceptacles. Androgynous condition thus prevails in these two species of *Sargassum*.

Early in the ontogeny, the conceptacle-wall cells enlarge and develop into papillae which through repeated transverse divisions develop into multicellular paraphyses whose terminal cells are globose (Fig. 3). Along with these changes either the oogonia or the antheridia also codevelop inside the conceptacle.

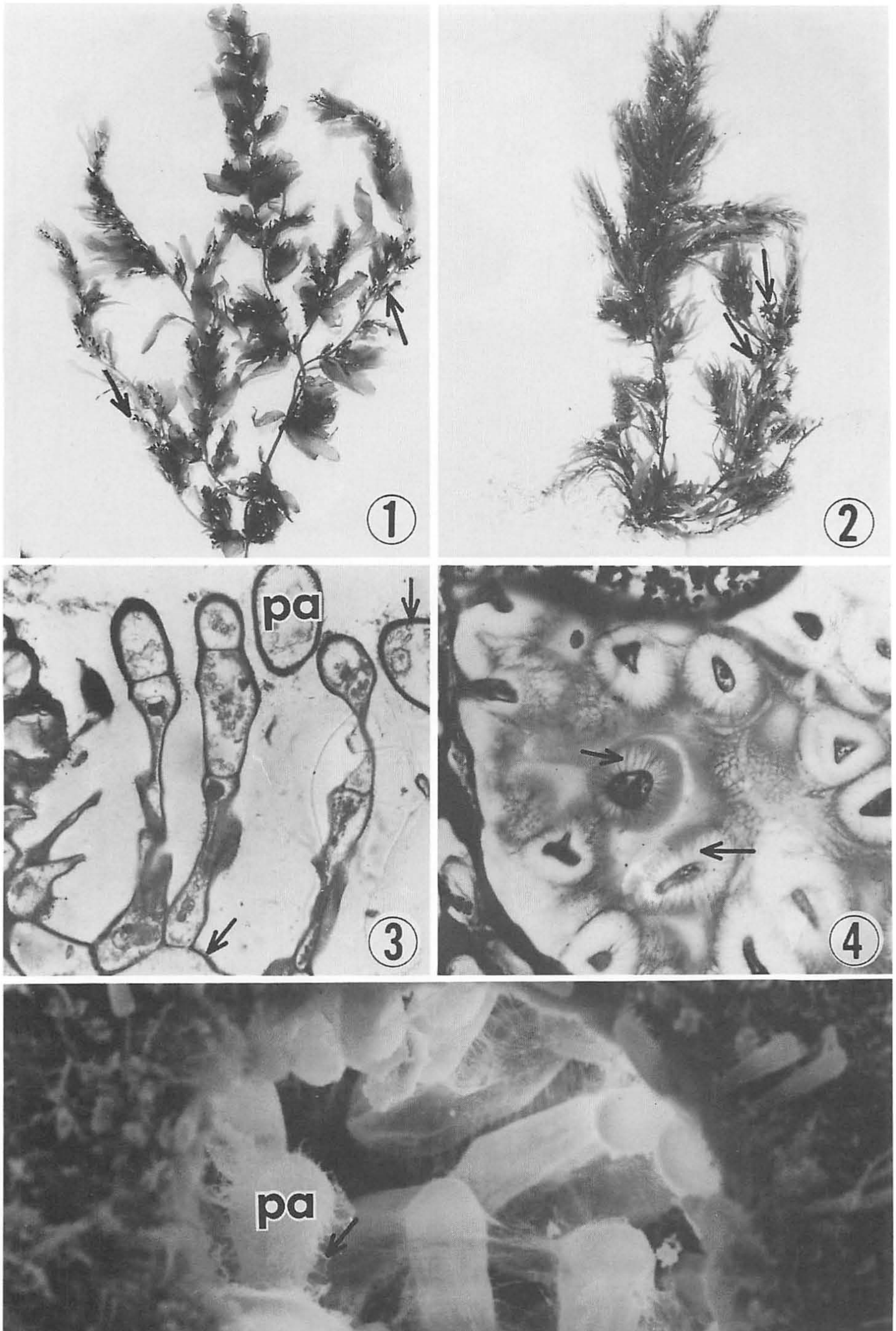
The young paraphysial cells show walls which stain reddish-violet with TBO and moderate magenta with PAS reagent and are hence rich in alginic acid. The cytoplasm stains well for proteins and contains moderate amount of polysaccharides. Also enclosed in the cytoplasm are a few small vacuoles and phenolic compounds which occur in large amounts. In the developing paraphyses, the cell walls reveal identical staining reactions. The cell walls, in addition, reveal small thread-like structures that stain intense violet with TBO (Figs. 3, 4). The paraphysial cells, in transverse sections, reveal core filled with phenolic materials (Fig. 4). The cytoplasm gradually becomes vacuolate and reveals a low amount of proteins and moderate polysaccharides. The cells are uninucleate and the nucleus stains well for DNA. Hence, the paraphyses which codevelop with sex organs, remain rich in cytoplasmic contents during the early stages of oogonial/antheridial development. Later as the sex organs are ready for gamete release, the paraphysial cytoplasm shows vacuolation.

The conceptacle-wall cells when young contain abundant proteins, moderate polysaccharides and phenolic materials. At maturity, the wall cells become more vacuolate and the proteins are meagre. The conceptacular cavity reveals polysaccharidic secretions which stain turquoise with Alcian Blue, magenta with PAS reagent and violet with TBO–thus indicating a mixture of sulphated and carboxylated polysaccharides (Fig. 4). This polysaccharidic material also acts as a plug in the female conceptacle where it keeps the ostiole closed while the oogonia are developing.

Electron Microscopic Studies

The paraphysial cell when viewed under scanning electron microscope reveals many strands that radiate from the wall into the conceptacle cavity (Fig. 5). At ultrastructural level, this wall is well differentiated, thick, lacks an orderly arrangement of microfibrils and is overlaid by material that is differentiated into an inner light and an outer dark zones. The latter radiates a few electron dense strands into the conceptacle cavity (Fig. 7). Adjacent to the wall lie many vacuoles that contain electron dense materials. The material is released from the vacuoles and

Figs. 1–5. Fig. 1. *Sargassum vulgare*, a mature plant showing forked branches, leaves, bladders and receptacles (arrows). ×0.47. Fig. 2. *Sargassum johnstonii*, whole mount of a plant bearing dense leaves and receptacles (arrows). ×0.47. Fig. 3. *S. vulgare*, Mature female conceptacle showing paraphyses (pa) near the ostiolar region. The terminal paraphysial cells are globular and veneered by polysaccharidic materials. The paraphysis cytoplasm reveals phenolic materials and carboxylated polysaccharides. The paraphysial and the conceptacle-cell walls reveal small polysaccharidic projections (arrows). ×1474 (TBO stained). Fig. 4. *S. vulgare*, Transverse section of a conceptacle, showing paraphyses surrounded by metachromatic material. The core of the paraphysis encloses phenolic materials and polysaccharides. From the core radiate thin strands into the conceptacle cavity. ×1474 (TBO stained). Fig. 5. *S. johnstonii*. Scanning electron micrograph. The paraphyses that occlude the ostiole show blunt tips from which emerge many, small, thread-like structures (arrows). ×1180.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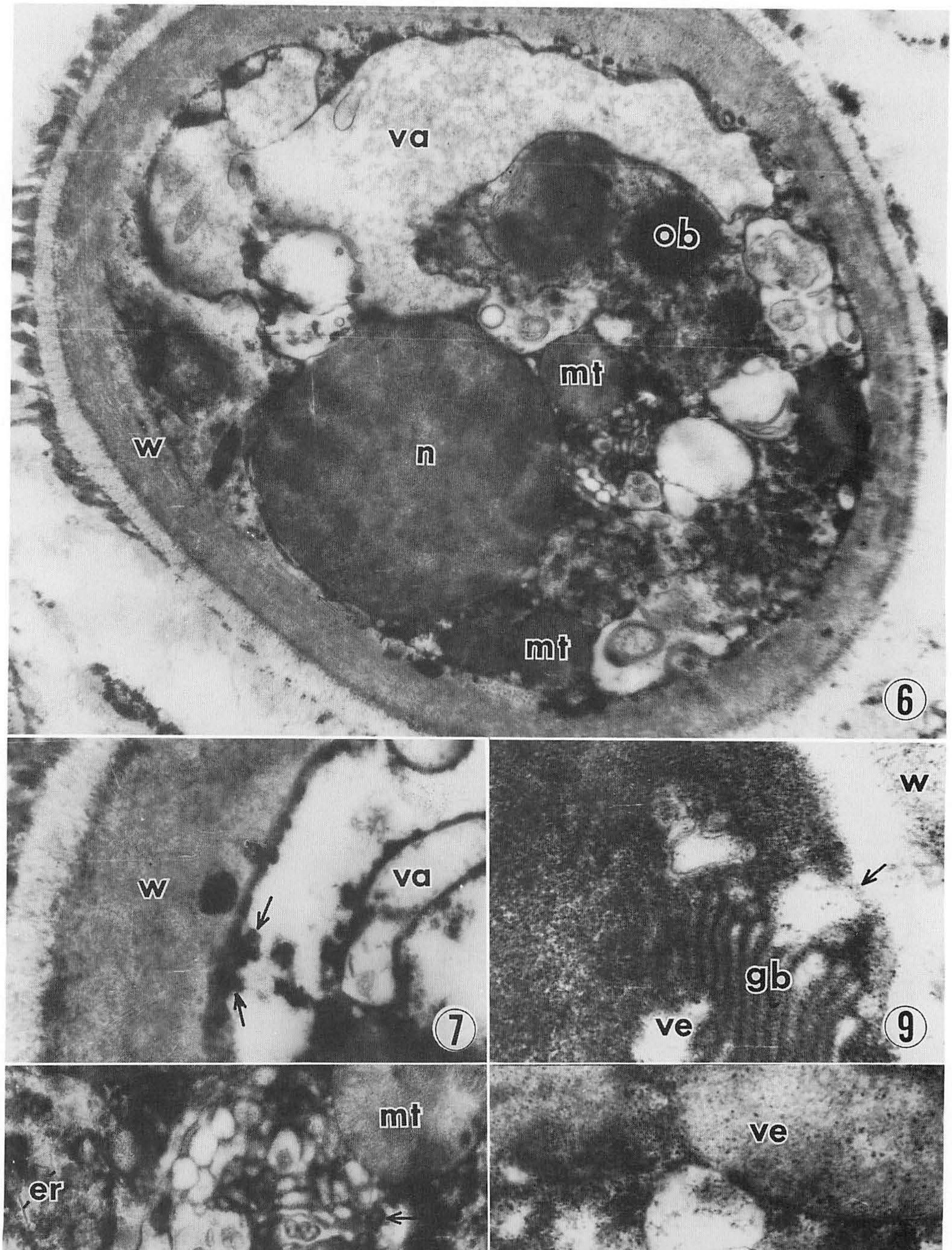
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deposited on the inner side of the wall (Fig. 7). The vacuoles containing electron dense materials are also abundant elsewhere in the cytoplasm.

The paraphyses possess vesicles that contain particulate electron-dense materials. These vesicles are identical to those found in the conceptacle-wall cell. The paraphyses have organelle-rich cytoplasm (Fig. 6). The nucleus is large, peripherally placed and has a well-defined nuclear membrane. Many vacuoles of different shapes and sizes; pleomorphic mitochondria, golgi bodies (Fig. 8), abundant osmiophilic droplets and endoplasmic reticula (Fig. 8) are present. In the mature paraphyses, organelles undergo lyses and the lysate is added to the wall material. Thus, the cell has an elaborate wall but vacuolate cytoplasm.

The conceptacle-wall cell cytoplasm reveals plastids, mitochondria, and golgi bodies. The golgi bodies are well developed and occur in both the formative and maturation faces. At the formative face many vesicles with fibrous material are produced (Fig. 9). The vesicles are pinched-off and later fuse between themselves. The golgi derived vesicles that are produced in the vicinity of the conceptacle-cell wall fuse with the wall and thus add to the wall material (Fig. 10). Also present inside the cell cytoplasm are vesicles which are filled with particulate, and electron-dense materials (Fig. 10).

Discussion

The walls of the paraphyses in *Sargassum johnstonii* and *S. vulgare* show short outgrowths that extend into the conceptacle cavities as seen in *Durvillaea potatorum* (Clayton *et al.* 1987). In addition, in *Sargassum* spp. the paraphysis cytoplasm has three types of vacuoles; 1) vacuoles with electron-dense particulate material, 2) vacuoles with electron-dense material and 3) with fibrous material. Such vacuoles are also present in the conceptacle-wall cells of *Sargassum* spp. In *Fucus edentatus*, the paraphyses cytoplasm contain vacuoles with different inclusions (McCully 1968).

In *Sargassum johnstonii* and *S. vulgare* the presence of numerous mitochondria and a few chloroplasts is another noteworthy feature of paraphysial cytoplasm which indicates a high rate of metabolic activity. Living *Fucus edentatus* paraphyses when viewed with fluoresence microscope suggest that the paraphyses cells possess abundant mitochondria (McCully 1968). Further, the conceptacle cavity in *Sargassum vulgare* and *S. johnstonii* is filled with sulphated and carboxylated polysaccharides that are perhaps secreted both by the paraphyses and the conceptacle-wall cells. Mucilage in the reproductive conceptacles in *Pelvetia canaliculata* is produced in cells lining the walls (Evans *et al.* 1973). A similar situation is found in *Fucus edentatus* (McCully 1968).

The role of alginic acid and sulphated polysaccharides is well known. According to Percival and McDowell (1967) they are mainly involved in prevention of desiccation. The polysaccharides in *Sargassum vulgare* and *S. johnstonii*, are presumed to form a soft, slippery cushion in which the sex organs develop. Once these organs are ready for release, the polysaccharides are at a low level in the conceptacle cavity. In *Cystophyllum sisymbrioides* paraphyses push their way through before the oogonia appear. The paraphyses are at first stiff but become soft after extrusion and form a slimy matrix in which the oogonia are en-

Figs. 6–10. *Sargassum johnstonii*. Transmission electron micrograph. Fig. 6. A paraphysis in transverse section to show a thick wall (w), peripherally placed nucleus (n), mitochondria (mt), vacuoles (va) of various sizes and osmiophilic bodies (ob). ×7,830. Fig. 7. Paraphysis wall (w) is the site of active discharge of phenolic materials (arrows). Many vacuoles (va) containing electron dense particulate material lie near the wall. ×21,140. Fig. 8. Portion of paraphyses to show abundant, electron dense osmiophilic bodies (arrows), and endoplasmic reticula (er) in the cytoplasm and different types of vacuoles (va) and pleomorphic mitochondria (mt). ×21,140. Fig. 9. Well formed golgi apparatus (gb) cuts off vesicles (ve) that aggregate below the conceptacle-cell wall (w). A vesicle is seen in the process of releasing its contents toward the wall (arrow). ×32,790. Fig. 10. Portion of conceptacle-wall cell cytoplasm to show coexistence of large and small vesicles filled with electron dense particulate materials. ×32,790.

closed (Tahara 1913). In *Bifurcaria brassiciformis*, the oogonia could be seen carried up on stalks which were themselves embedded in a tenacious common jelly, presumably derived from the walls of the paraphyses (Delf 1935).

The presence of phenolic materials in the conceptacular tissues is of great ecological significance. The ostioles in *Sargassum* spp. of the old receptacle are devoid of polysaccharidic plug-materials. The disappearance of plug-material upon release of oogonia/spermatozoids makes conceptacles susceptible to the attack by epiphytic and endophytic microbes. The abundance of the phenolic deposits in the persistent tissues of old conceptacles help to deter the attack of the microbes through invasion route. Phenolic materials also help to prevent attack by the herbivores (Clayton and Shankly, 1987). The paraphysial cytoplasm is packed with phenolic materials in *Sargassum* spp. throughout the conceptacle development. In *Scytosiphon* sp. the paraphyses have a protective role during gamete discharge, perhaps they help in preventing the excessive spread of lytic enzymes to the walls of immature gametangia (Clayton, 1984).

The conceptacles, during the last phase of reproductive season (January end to February) are totally devoid of contents. In *Sargassum johnstonii* and *S. vulgare* the conceptacle-cell wall, during this stage, is elaborate and possesses prominent, thread-like, projections as seen in *Durvillaea potatorum* (Clayton *et al.* 1987), where the conceptacle cells immediately adjacent to the conceptacle contents produce short filamentous outgrowths which extend into the central cavity.

The conceptacle-wall cells also reveal perinuclear golgi bodies which produce many vacuoles. The fibrous materials are frequently incorporated into the vacuoles at the formative region, undergo condensation and are released at the cell surface (present work). Such vacuoles have been termed as secretory vacuoles and have been shown to be released at the cell surface (Bouck 1962; Mollenhauer and Whaley 1963). The production of sulphated material by the meristoderm cells of *Pelvetia canaliculata* and secretory cells of *Laminaria saccharina* occurs in the golgi-rich, perinuclear areas (Evans et al., 1973). In pancreatic acinar cells, sulphation of material occurs in golgi bodies (Berg and Young 1971). Discharge of fibrous materials into the wall and cavity in *Sargassum johnstonii* and *S. vulgare* presumably occurs by the activity of golgi bodies present in the conceptacle-wall cells and the paraphyses and the energy required for the secretory process is provided by numerous mitochondria in the cell cytoplasm. These two cell populations, therefore, may be designated at least functionally as secretory cells.

References

Berg, N. B. and Young, R. W. 1971. Sulphate metabolism in pancreatic acinar cells. J. Cell Biol. **50:** 469–483.

Bouck, G. B. 1962. Chromatophore development, pits and other fine structure in the red alga, *Lomentaria baileyana* (Harv.) Farlow. J. Cell Biol. **12:** 553–569.

Clayton, M. N. 1984. An electron microscope study of gamete release and settling in the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta). J. Phycol. **20:** 276–285.

Clayton, M. N., Hallam, N. D. and Shankly, C. M. 1987. The seasonal pattern of conceptacle development and gamete maturation in *Durvillaea potatorum* (Durvillaeales, Phaeophyta). Phycologia **26:** 35–45.

Clayton, M. N. and Shankly, C. M. 1987. The apical meristem of *Splachnidium rugosum* (Phaeophyta). J. Phycol. **23:** 296–307.

Delf, E. M. 1935. Liberation of oogonia in *Bifurcaria* and other members of the Fucaceae. New Phytol. **34:** 245–259.

Evans, L. V., Simpson, M. and Callow, M. E. 1973. Sulphated polysaccharide synthesis in brown algae. Planta **110:** 237–252.

McCully, M. E. 1966. Histological studies on the genus *Fucus*. I. Light microscopy of the mature vegetative plant. Protoplasma **62:** 287–305.

McCully, M. E. 1968. Histological studies on the genus *Fucus*. II. Histology of the reproductive tissues. Protoplasma **66:** 205–230.

Mollenhauer, H. H. 1964. Plastic embedding mixture for use in electron microscopy. Stain Technol. **39:** 111–114.

Mollenhauer, H. H. and Whaley, W. G. 1963. An observation of the functioning of the golgi apparatus. J. Cell Biol. **17:** 222–226.

Parker B. C. and Diboll, A. G. 1966. Alcian stains for histochemical localisation of acid and sulphated poly-

saccharides in algae. Phycologia **6:** 37–46.
Percival, E. and McDowell, H. 1967. Chemistry and Enzymology of Marine Algal Polysaccharides. Academic Press, London.
Tahara, M. 1913. Oogonium liberation and the embryogeny of some Fucaceous algae. J. Cell Sci. Imp. Univ. Tokyo. **32:** 1–13.
Vijayaraghavan, M. R. and Shukla, A. K. 1990. Histochemistry: Theory and Practice. Bishen Singh Mahendra Pal Singh. Dehradun.
Weber, K. and Osborn, M. 1975. Proteins and sodium dodecyl sulphate: Molecular weight determination on polyacrylamide gel and related procedure: p. 179–223. In NEURATH, H. and HILL, R. L. [eds.] The Proteins Vol. I. 3rd ed. U.S.A.

M. R. Vijayaraghavan · Inderdeep Kaur: *Sargassum vulgare* C. Agardh と *Sargassum johnstonii* Setchell & Gardner の側糸の組織化学と微細構造

Sargassum vulgare C. Agordh と *S. johnstonii Setchell* & *Gardner* は雌雄異巣の生殖器托をもつ。十分発達した生殖器巣は分泌液で満たされており，巣口にある多糖物質を介して外界と連絡している。生殖器巣には，平板状の生殖器巣壁細胞が並んでおり，それらは造精器か造卵器のいづれか，または側糸となる。若い生殖器巣では，側糸および生殖器巣壁細胞は，アルギン酸と硫酸多糖を含んでいる。生殖器巣の発達初期過程では，細胞内小器官が多くみられるが，老成した細胞では，それらは溶解し，側糸や生殖器巣壁細胞は液胞化する。(Department of Botany, University of Delhi, Delhi 110007, India)