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Developmental and histochemical studies on carposporophyte of *Solieria robusta* (Greville) Kylin Solieriaceae, Gigartinales) from Port Okha, India

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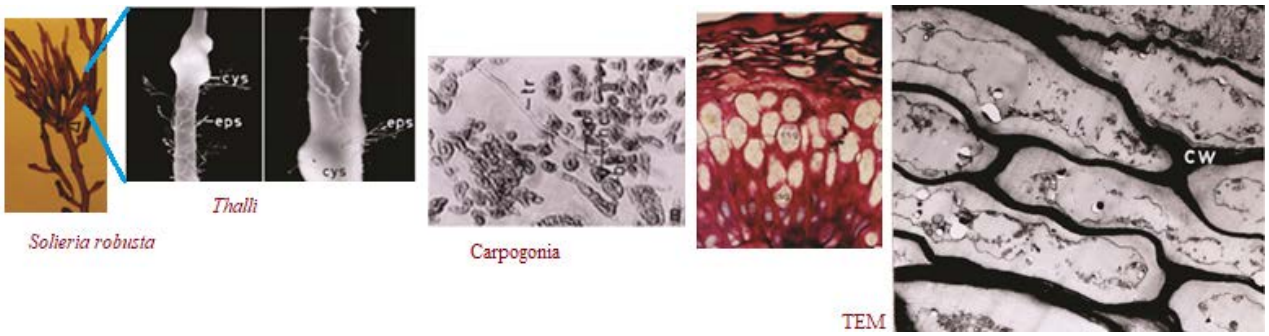
ABSTRACT



Solieria robusta shows isomorphic alternation of generation. The female thallus is terete with erect lateral branches that show attenuation at their bases. The thallus is differentiated into cortex and medulla. The female thallus surface shows many scattered cystocarps that appeared as black coloured dots. The development of the cystocarp is acropetal. The thallus surface is raised due to presence of cystocarps with a small circular ostiole in the centre. The complete gelatinization of extracellular mucilage and degeneration of surrounding vegetative cells makes an orifice above the thallus surface for the carpospores release. Mature cystocarp is embedded in the medullary region. A distinct gradient of cytoplasmic polysaccharides is seen from fusion cell to carpospores. The carposporangia and carpospores are replete with floridean starch grains and sulphated polsaccharides. The mature carposporangial cytoplasm is fully packed with floridean starch grains, fibrous vesicles and chloroplasts. Carposporangial wall is three to five layered and is composed of microfibrils.

Keywords: Carposporophyte, Carposporangia, Gonimoblast filaments, carpospores, Red Algae, Sea Algae,

INTRODUCTION

The taxa belonging to the Solieriaceae have been persumed to follow Polysiphonia-type of life-history. *Agardhiella subulata* normally exhibits isomorphic phases but dwaft male plants commonly develop in *situ* from tetrasporangia.[1] The Australian genera of Solieriaceae and Rhabdoniaceae possess isomorphic gametophytes and tetrasporophytes. According to West and Hommersand (1981) *Turnerella* and *Opuntiella* possess heteromorphic life cycles and probably do not belong to the Solieriaceae.[2]

The genus *solieria* has been included in the family Rhodophyllidaceae.[3] The multiaxial and non-procarpic taxa have been placed in the Solieriaceae whereas uniaxial and non-procarpic genera have been assigned to the Rhabdoniaceae. The *solieria* come under the family Solieriaceae(Gigartinales) and Solieriaceae is considered as a crucial one in the possible evolution of many specialized group of Gigartinales as it links many other families.[4]

Solieria robusta is a dioecious taxon and reveals isomorphic alternation of generation.[5] *Solieria robusta* from Indian coast is attached to the calcareous rocks by a discoid fibrous holdfast and occur in the rockpools of intertidal region. The female thallus is terete with erect lateral branches that show attenuation at their bases.[6] The female thallus surface shows many scattered

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cystocarps that appeared as block dots. The development of cystocarp is acropetal. The development of carposporophyte brings many changes in both the cytoplasm and the cell wall composition.[7,8] The earlier work on development of carposporophyte, carporporogenesis, and ostiole formation in the genera,[6] specially a correlative study of developmental, histochemical and ultrastructural aspect is meager. The present work aims to study development of carposporophyte at both light and electron microscopic levels. Ultrastructural studies have added a completely new dimension to our knowledge on the structure of carposporangia, carpospore and pericarp.

MATERIAL AND METHODS

Solieria robusta was collected at Port Okha(Gujarat)[9] during the low tide periods. The portions of the thalli were observed under steriomicroscope and also processed for light microscopic studies. Two-micron thick sections were cut with glass knives using a locally made adaptor that fits into the rotary microtome. The sections are stained with 0.05% Toluidine Blue O (TBO) at PH 4.4; Periodic - acid Schiff's reagent (PAS) and Coomassie Brilliant Blue (CBB).[10] Photomicrographs were taken on ORWO B/W film using Reichert Polyvar photomicroscope.

For transmission electron microscopy (TEM), Portions of thalli were fixed in 6% glutaraldehyde prepared in 0.02M phosphate buffer at pH 6.8 and post fixed in 1% osmium tetroxide in the same buffer. The tissues were dehydrated in ascending aqueous ethanol and propylene oxide series. Infiltration was done in Epon-Araldite mixture.[9]

Ultrathin sections were cut on Reichert Ultramicrotome using glass knives. Staining was done with uranyl acetate and lead citrate. Sections were observed under Philips EM 300 electron microscope.

For scanning electron microscopy selected portions of thalli were passed through a graded cold (4ºC) ascending acetone series, dehydrated in anhydrous acetone, treated for critical point drying (CPD), coated with gold and observed under Philips SEM 501-B.

RESULTS

Solieria robusta is a dioecious taxon and it attached to the substratum by a discoid or fibrous holdfast (Figure 1 C). The colour of the thalli is purple to brown red (Figure 1 A,B). The thallus consist of terete, erect lateral branches which usually attenuate at their base and subacute at their tips (Figure 1 A,B). Branching is irregular. The female thallus surface shows many scattered cystocarps that appeared as dark coloured dots (Figure 1 A,B). Mature cystocarps are present near the base and the younger ones towardsthe tip. The development of the cystocarp is acropetal (Figure 1 A,B). Scanning electron micrograph of plant surface shows a very thick cuticle which covers the epidermal cell walls. The thallus surface is raised due to the presence of cystocarp with a small circular ostiole in their centre. (Figure 1 D,E,F,G). The plant axis is differentiated into cortex and medulla. The cortical region comprises the epidermis, outer and inner cortices. The epidermis is unilayered. The cells of the outer cortex are small in size as compared to inner cortical cells. The cells of the outer cortex are gorged with floridean starch grains. In female thallus,

Figure 1: A to G: *Solieria robusta*. Morphology. (cys, cystocarp ; eps. epiphytes; holdfast; os, ostiole). (A).A female plant with swollen cystocarps (arrows). The branching is irregular and the branches show attenuation at their base (double arrow). Occasionally, 4 or 5 branches arise from the damaged branch and form an umbel (arrowhead), x 3. (B) The thallus surface shows many scattered cystocarps that appear as dots (arrowheads). Mature cystocarps occur near the base of the branches and young ones towards the tips. The development of cystocarp is acropetal. x 4. (C) Male plants with well developed discoid holdfasts, x 2. (D&E) Cystocarpic plants laden with Polysiphonia sp. that occurs as epiphyte, x 5. (F) *Solieria robusta*. Cystocarp. (Scanning electron micrographs). A portion of thallus surface to show the spherical cystocarps with small, circular, ostioles in the centre. These cystocarps cause the swelling of thallus surface. x 112. Fractured thallus to show mature cystocarp embedded in the medulla. (G) Magnified view of the ostiole (arrow), x 3500

the carpogonial branch is three celled and arise from cortical cells (Figure 2 A, B,). Occasionally, two carpogonial branches arise from a common supporting cell (Figure 2A,C). The carpogonium is elongate, conical shaped and has a long trichogyne (Figure 2C). Initially the basal cell of the carpogonial branch is larger than boththe hypogynous cell and the carpogonium (Figure 2B). After fertilization the carpogonium becomes large and spherical in shape and issues a single connecting filament from its terminal end (Figure 2 D).

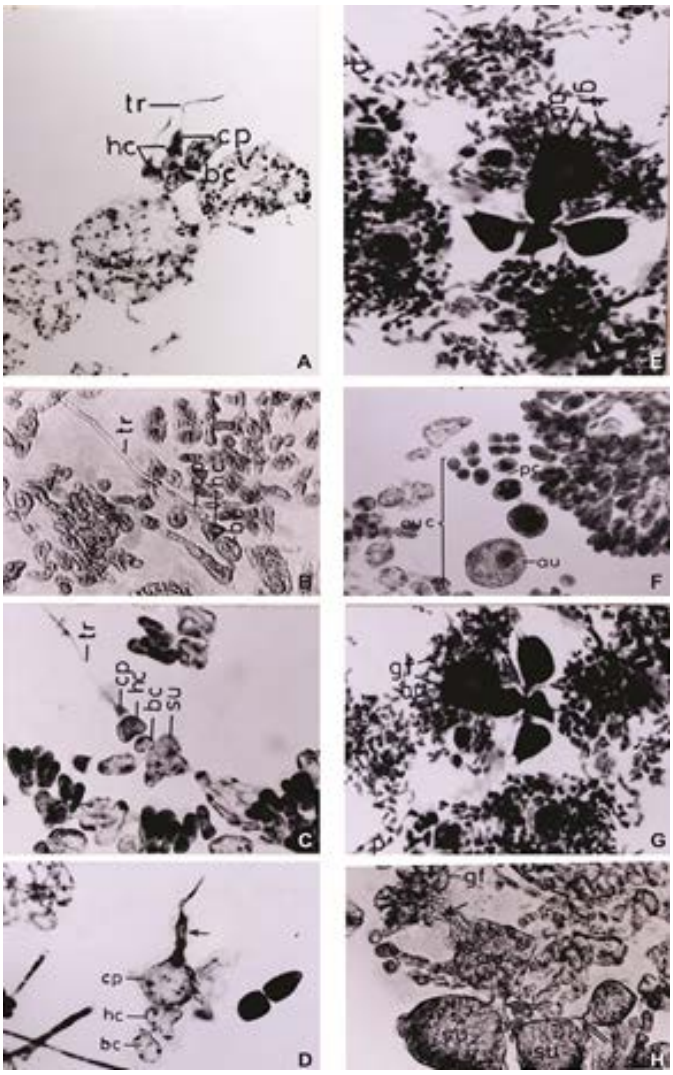

Figure 2: A to H: *Solieria robusta*. Carpogonial Branch, Auxiliary Cell & Auxiliary cell-complex. (au, auxiliary cell; auc, auxiliary cell complex; bc, basal cell; cf, connecting filament; cp; cp, carpogonium; gf, gonimoblast filament; hp, hypogynous cell; mf, medullary cell filament; pc, pericarp; pit-connection; su, supporting cell; tr, trichogyne). (A&B) Temporary mounts of thalli to show mature 3-celled carpogonial branches. The hypogynous cell is darkly stained as compared to other two cells of the branch. The carpogonia are conical in shape and have long trichogynes. In A, two carpogonial branches arise from a common supporting cell, x 1800. (C) Temporary mount of the thallus show that two carpogonial branches originate from a common supporting cell, x 1800. (D) Three-celled carpogonial branch after fertilization (arrow), x 4500. (E&G) Temporary mounts of the thalli showing the fusion of a few gonimoblast cells to the auxiliary cell'. Fusion among the auxiliary cell and other darkly stained cells of the auxiliary cell-complex leads to the formation of large and round fusion cell, x 1800. (F) Same, to show the presence of auxiliary cell-complex. The auxiliary cell is part of the inner cortex and contains darkly staining nucleus and remains uninucleate. As this cell matures the juxtaposed, multinucleate inner cortical cells stain intensely. The auxiliary cell together with these darkly staining inner cortical cells comprise the auxiliary cell-complex and is recognizable prior to diploidization. x 2250. (H) Gonimoblast initial (arrow) is cut off from the auxiliary cell. Later this initial divides to form a cluster of gonimoblast cells. The auxiliary cell fuses with supporting cells through pit-connections (arrow) and form the fusion cell, x 4500.

Auxiliary cell, auxiliary cell complex and cystocarp:-

Auxiliary cells are dedifferentiated inner cortical cells that contain the core darkly stained nuclei and remain uninucleate (Figure 2F). As this cell matures, juxtaposed, multinucleate, inner, cortical cells stain intensely (Figure 2F). The auxiliary cell together with these darkly staining cortical cells comprise the auxiliary cell complex. (Figure 2F). After diploidization of the auxiliary cell, the adjacent cortical cells, surrounding cells of auxiliary cell complex form the pericarp (Figure 2F). A single gonimoblast initial is cut off from the auxiliary cell and later divide transversely to form a compact cluster of gonimoblast cells (Figure 2B). A few gonimoblast cells fuse with the auxiliary cell (Figure 2 E,G,H) which in turn fuses with adjacent darkly stained supporting cortical cell (Figure 2 E,G,H)

The fusion product of all these cells lead to the formation of large fusion cell. Light and electron microscopic studies show lysis of cell walls inside the fusion cell cytoplasm. In later stages, gonimoblast filaments are seen distributed around the entire periphery of fusion cell entire periphery of the fusion cell (Figure 3 A : 3G). Those Gonimoblast cells that face towards the pericarp, do not participate in the formation of fusion cell, but divide and produce spreading carposporangia (Figure 2G,H;3G). The lower gonimoblast filaments that are contiguous with the fusion cell coalesce with the fusion cell. A few gonimoblast cells remain sterile ,unbranched,unseptate and connect the fusion cell with the pericarp (Figure 2G). The mature cystocarp shows a large fusion cell in the centre from which branched and unbranched gonimoblast filaments emanate. The terminal or two or three upper cells of these branched gonimoblast filaments mature into carposporangia (Figure 3 B,E,G,H). Light and Electron microscopic studies show that mature cystocarp is enveloped by three to five layered thick pericarp. (Figure 3 A,B,E). The Carpospores are initially elliptical in shape but at release, through the ostiole, (Figure 3C) become spherical. The cortical cells, adjacent to the cystocarp, proliferate and produce small cells (Figure 3 C) which raise above the thallus surface (Figure 1 D,E,F). The ostiole is formed by the gelatinization of the pericarp cells followed by lyses of adjacent cortical cells. The complete gelatinization of the extracellular mucilage and degeneration of vegetative cells makes an orifice (Figure 1G) above the thallus surface for the carpospore release (Figure 3D).

Histochemical Studies

Localizaton of sulphated and carboxylated polysaccharides Cystocarp: The intercellular spaces and cell walls of fusion cell, gonimoblast filaments and pericarp are rich in both carboxylated and sulphated polysaccharides (Figure 3D) the gonimoblast cell's cytoplasm shows abundant sulphated polysaccharides as compared to the carposporangial cytoplasm which stain moderately for this metabolite (Figure 3D). The fusion cell and pericarp cells cytoplasm are bereft of this metabolite (Figure 3 A), At the ostiolar region, both the pericarp and surrounding cortical cells of the thallus lyse and the lysate of all these cells is rich in sulphated and carboxylated polysaccharides (Figure 3C) This process helps in protection and the dispersal of carpospores.

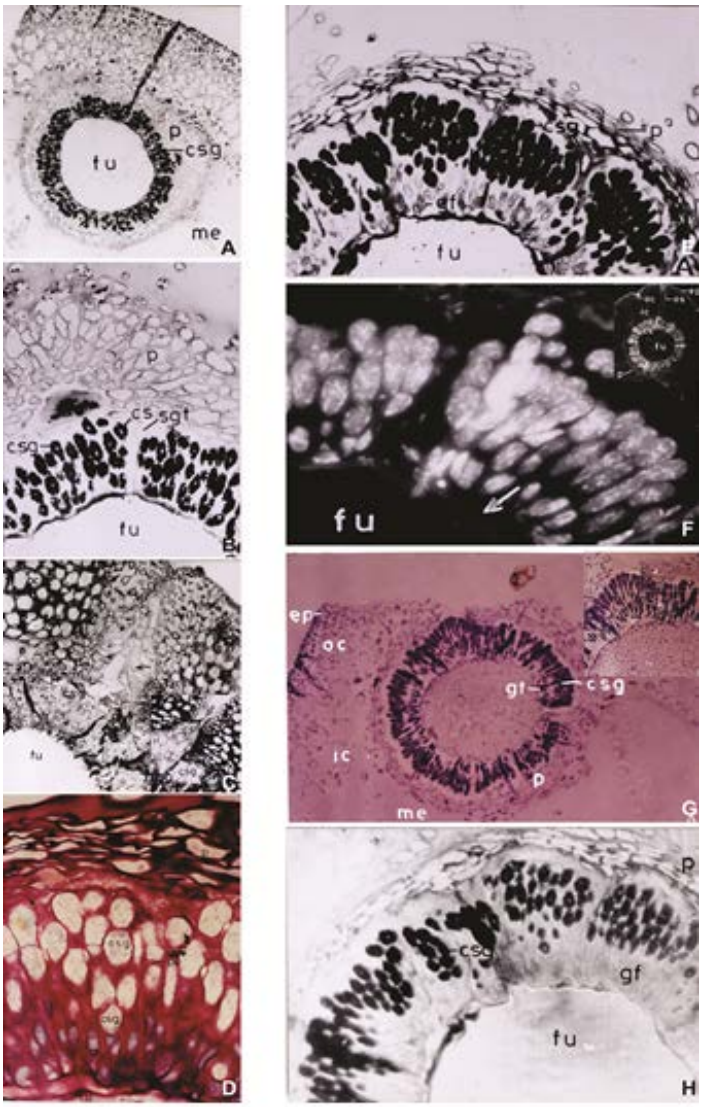

Figure 3 : A to H : *Solieria robusta*. Cystrocarp. Localization of insoluble polysaccharides, Protein, polysaccharides and Autofluorescent Compounds. (cs, carpospore; csg, carposporangium; ep, epidermis; fu, fusion cell; gf, gonimoblast filament; ic, im, intercellular mucilage; inner cortex; me, medulla; mf, medullary filament; n, nucleus; oc, outer cortex; os, ostiole; p, pericarp; pc, pit-connection; sgf, sterile gonimoblast filament. (A&B) Longitudinal sections of thalli through mature cystocarps to show that the upper 2 or 3 cells of the gonimoblast filaments mature into carposporangia. B x 450; C x 1800. (C) Longitudinal section of a portion of the mature cystocarp to show the formation of ostiole. The cortical cells, adjacent to the cystocarp, proliferate and produce small cells that raise the thallus surface. The cortical cells bordering the ostiole proliferate and produce small cells that raise the thallus surface, x 1800. The lysate (arrow) is rich in both sulphated and carboxylated polysaccharides (Photomontage), x 1800. (D) Longitudinal section of a portion of cystocarp to show mature carpospores at the peripheral region near the cystocarpic cavity (arrow). The cavity stains well for carboxylated and sulphated polysaccharides. The carposporangial cytoplasm stains moderately for sulphated polysaccharides whereas the gonimoblast filaments are rich in this metabolite. Intercellular mucilage stains well for both sulphated and carboxylated polysaccharides, x 5625. (E) Transverse section of a portion of cystocarp to show branched gonimoblast filaments bearing rows of carposporangia. After the formation of fusion cell, the gonimoblast filaments in succession segment and form carposporangia.x 1800. (F) Longitudinal sections of portions of mature cystocarps to show autofluorescent compounds in gonimoblast filaments and carposporangia. Gonimoblast filaments are rich in this compound as compared to carposporangia. The pericarp cells, however, show a low ebb. x 1800. Transverse section of mature cystocarp with well-developed ostiole. x 360. (G) Transverse section of mature cystocarp. Differential distribution of proteins is seen in the fusion cell, pericarp, gonimoblast filaments and carposporangium. Both gonimoblast filaments and carpospores are rich in cellular proteins, x 525. Same, magnified to show that gonimoblast filaments and carposporangia are replete with cellular proteins whereas the fusion cell and cells of the pericarp show depletion of this metabolite. x 2250. (H) The gonimoblast filaments show moderate amount of floridean starch grains whereas the carposporangia and carpospores are replete with these grains. All the carposporangia show centrally located nuclei (Phase contrast optics). x 1800

Localizaton of insoluble polysaccharides: The cell walls of fusion cell, gonimoblast filaments and pericarp are rich in insoluble polysaccharides (Figure 3A,B; 3E) but that of carposporangia stain with less intensity. A distinct polysaccharides gradient is visible commencing from the fusion cell to the carpospores (Figure 3A,B). The fusion cell cytoplasm is bereft of this metabolite whereas the gonimoblast is bereft of the metabolite whereas the gonimoblast filaments stain moderately (Figure 31 AB). The carposporangia and carpospores are gorged with floridean starch grains (Figure 3E, H).

Localization of Total Protein : The mature cystocarp shows differential distribution of cytoplasmic proteins (Figure 3G). The cytoplasm of gonimoblast filaments is rich in this metabolite as compared to that of the carposporangia (Figure 3 G). The carposporangia and the carpospores are uninucleate (Figure 3G) and contain abundant cytoplasmic proteins (Figure 34 A, B) whereas the fusion cell and the pericarp cells show a low ebb of this metabolic Figure (3G) The sterile gonimoblast filaments are unbranched and show presence of cytoplasmic proteins (Figures. 3G).

Localizaton of Nucleic Acid: In the cytoplasm of carporsporangia and carpospores, nuclei and extracellular materials stain well with aceto-iron-haematoxylin and chloral hydrate. The female thallus sectionswhen observed under UV light reveal many autofluorescent compounds that are localized in mature carposporophyte. In mature cystocarp, autofluorescent compounds are localized in carpospores, carposporangia and in gonimoblast filaments (Figure 3F). The gonimoblast filaments are rich in this compound as compared to the carpospores. The cell walls of pericarp as compared to the carpospores. The cell walls of the pericarp and fusion cells show very weak fluorescence (Figure 3F).

Ultrastructural Studies Carposporangia: The young carposporangial cytoplasm has dictyosomes, chloroplasts and a few floridean starch grains (Figure 4B). The dictyosomes produce vesicles (Figure4B). The mature carposporangia are packed with pleomorphic floridean starch grains and vesicles with dense cytoplasm (Figure 4B,C), the fibrous vesicles are present in the cell cytoplasm and near the cell wall.

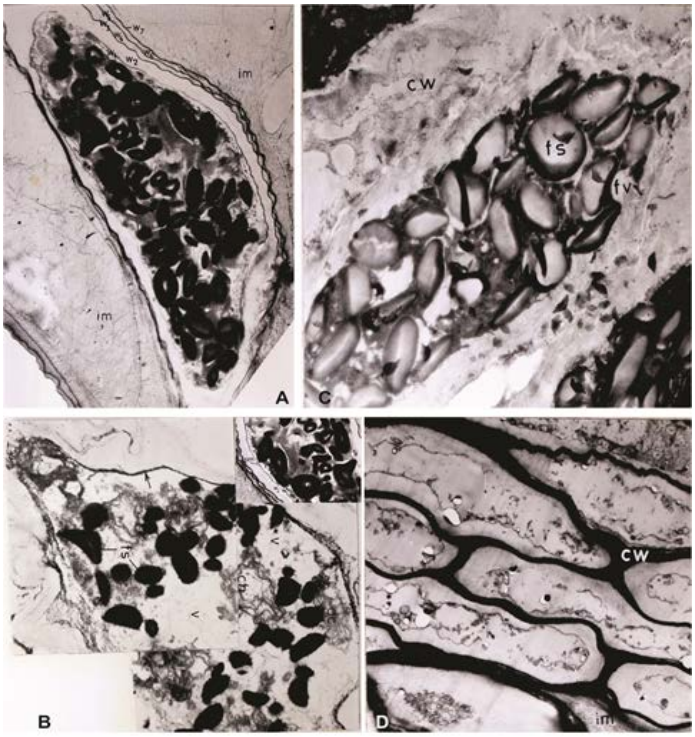

Figure 4: A to D: TEM of *Solieria robusta*. Carposporangium & Pericarp. (ch, chloroplast; cw, cell wall; dc, dictyosome; fs, floridean starch grain; fv, fibrous vesicle; im, intercellular mucilage; v, vacuole)

(A). A mature carposporangium showing 7 layered thick cell wall. The layer W1 is electron-dense and thin; W2 is electron-translucent and thick; W3 is electron-dense and thin; W4 is electron- translucent and thick; W5 is electron-dense and thin; W6 is electron-translucent and thick and W7 is electron-dense and thin. This is followed by intercellular mucilage with reticulate texture, x 8160

(B) A portion of young carposporangium cytoplasm with peripheral distribution of chloroplasts, dictyosomes and pleomorphic floridean starch grains. Many fibrous vacuoles are present. There is a single, electron-dense, wall layer (arrow) (Photomontage), x 15,000

(C) The carposporangial cytoplasm are enclosed in mucilaginous sheaths and are packed with floridean starch grains and fibrous vesicles. These vesicles fuse with plasmalemma and release their contents into the mucilaginous sheath to form additional wall materials. A x 9840.

The young carposporangium is enclosed in mucilaginous sheath. The mature carposporangium has multilayered cell wall and its base possesses a large vacuole that is filled with fibrous materials (Figure 4A). These layers show parallel arrangement of microfibrills (Figure4A). The intercellular mucilage between the developing carposporangia show reticulate arrangements of fibrils (Figure 4D).

Pericarp : Transmission electron micrographs of pericarp show thick walled and vacuoulate cells. Degenerating chloroplast lamellae are also seen in the cell cytoplasm. (Figure 4D). The intercellular mucilage reveals reticulate arrangement of fibrils (Figure 4D).

DISCUSSION

Solieria robusta, from Port Okha, grow luxuriantly in rock-pools of the intertidal regions either through discoid or hapteroid holdfasts. *Solieria robusta* is a red algae with many prominent applications potential in different fields including biomedical sciences,[7,11] nutraceuticals,[12–15] pigments,[14] cosmetics,[16] biomass fuels,[17] and biochemical synthesis.[18] Perrone *et.al.*[19] reported that all the Solieracean plants develop discoid holdfasts when they are attached to mussel-shells or small stones. *Solieria robusta* is a intertidal algae so are attached through discoid holdfast. In *Solieria robusta* the axis of the plant is differentiated into epidermis, outer and inner cortices and medulla. The epiphytism is known to be a common phenomenon in marine plants particularly in a biological communities where substratum is the limiting factor.[20] The reported morphological phylogeny of *S. robusta;*[3] *S. tenera* and *S. chordalis,*[1,21] has indicated that in these the axes are differentiated only into the cortex (outer and inner) and medulla. In *S. Robusta* (Present work) both ultrastructural and histochemical studies reveal that the cortical region can be divided into epidermis, outer and inner cortices. The epidermal cells are elongate and their cell walls show reticulate arrangement of micro fibrils. The cells of the outer cortex show parallel arrangement of micro fibrils and are gorged with floridean starch grains and cytoplasmic proteins whereas the inner cortical cells are large and show polarized distribution of floridean starch grains and proteins. This supports that the differentiation of tissue types can be correlated to the function and to stress conditions operating on them during development. It is desirable that both *S.Tenera* and *S. Chordalis* be reinvestigated using modern techniques of tissue preparation to ascertain the precise nature of tissue differentiation.[22,23] The tissue differentiation represents a highly specialized adaptation and division of labour strategy. In *S. robusta* the anatomical differentiation of the thallus into epidermis, outer and inner cortices and medulla with their associated polarized distribution of Floridean starch grains and cytoplasmic proteins is a remarkable adaptation which confers on the plant both ecological and physiological survival strategies to withstand tidal functions and grow luxuriantly in the intertidal regions. In *Solieria robusta*, the ultra structural studies show variable shape and size of floridean starch grains in the cell cytoplasm. The scanning electron microscopic observations on floridean starch grains of *Seirospora griffithsiana* show morphological variations of starch grains.[24] There literature reports indicatre fairly large range in starch grain size in carpospores of *Bonnemaisonia nootkana* as well as in carpospores and holdfast of *Rhodymenia pertusa*.[25–27] Meeuse et al. (1960) have observed wide range of diameter in starch granules in *Odonthalia flocosa.*[28] Floridean starch grains are variable in shape and size due to their scattered disposition in the cytoplasm where they are constantly subjected to stress conditions in the cytoplasm where they are constantly subjected to stress condition.[28]

The carpogonial branch of *Solieria robusta* is 3-celled and consists of carpogonium, hypogynous and basal cells. The supporting cell is an undifferentiated cell of the inner cortex. The hypogynous cell stains darkly than the other cells of the

carpogonial branch. Min-Thein and Womersley[3] in *S. robusta* and Gabrielson and Hommersand[1,21,29] in *S. tenera, S. chordalis* and *Agardhiella subulata* observed identical carpogonial branches and darkly staining hypogynous cells. In *Sarconema* sp. the carpogonial branch is either 3 or4 celled.[30]

Controversy exists whether *Solieria* is procarpic or nonprocarpic. Kylin(1932)[31] in *S. chordalis* and Min-Thein and Womersley (1976)[3] in *S. robusta* from southern Australia report them to be non-procarpic whereas Wynne and Taylor (1973)[32] in *S. chordalis* observed procarpic nature. The present work results on *S. robusta* obtained from Port Okha confirms it to be nonprocarpic.

In *Agardhiella subulata* and in *Sarcodiotheca gaudichaudii* two connecting filaments arise from the fertilized carpogonium.[29] In *Solieria robusta*, after fertilization, a single connecting filament emanates from each carpogonium and fuses with the remote auxiliary cell from which gonimoblast filaments originate. Further, cells of the auxiliary cell-complex and auxiliary cell stain intensely before diploidization and are recognized from other cortical cell. The genera *Agardhiella* and *Sarcodiotheca* also possess an auxiliary cell-complex that differentiates prior to diploidization of the auxiliary cell.[29] *Sarconema filiformis* and *S. scinaioides*[30] and Placentophora sp.,[33] however, have undifferentiated auxiliary cells prior to diploidization. Kylin (1932)[31] observed that the auxiliary cells in *Solieria* are not recognizable before fertilization. Contrary to this statement, in *Solieria robusta*, the auxiliary cell is prominent and can be distinguished from the cortical cells before diploidization (Present work). It appears that the dedifferentiation of the inner cortical cell to form the auxiliary cell confers on it special attributes where this cell differs both morphologically and functionally and produces only gonimoblast initials.

In *Solieria robusta* (Present work) after diploidization of the auxiliary cell, the adjacent cortical cells form branched chains of cells which envelope the auxiliary cell-complex and auxiliary cell. A few inner gonimoblast filaments and adjacent darkly stained cells fuse with auxiliary cell and their fusion product leads to the formation of large fusion cell with a common boundary. In early stages, fusion cell shows lysis of cell walls in the centre of matrix (Present work). Kylin (1932)[31] in *Solieria chordalis* and Min-Thein and Womersley (1976)[3] in *S. robusta* suggest that fusion cell is formed by the coalescence of the first formed gonimoblast cells with the auxiliary cell. Garbielson and hommersand (1982)[29] in *S. chordalis* and *S. tenera* report that fusion cell are formed by the fusion of auxiliary cell, gonimoblast cells and cells of the lateral vegetative filaments promixmal to the auxiliary cell. In *Callophycus* sp. A few first formed gonimoblast cells coalesce with the auxiliary cell to form the round fusion cell. The cells in the lateral filament containing the auxiliary cell later fuse progressively through their pit-connections to an axial cell to form a stalk-like extension of the central fusion cell (Min-Thein and Womersley, 1976).[3] The genera *Areschougia*, *Melanema* and *Erythroclonium* (Rhabdoniaceae) also form the fusion cell composed of gonimoblast cell, the auxiliary cell and cells of the lateral filaments bearing the auxiliary cell (Min-Thein and Womersley, 1976).[3] In *Sarconema filiformis* and *S. scinioides* (Solieriacea). The fusion cell is formed by the fusion of the first formed gonimoblast cells, the auxiliary cell and some neighbouring vegetative cells.[30] The present work on *Solieria robusta*, from the Port Okha, reveals that the fusion cell is formed by auxiliary cell, cells of the auxiliary cell-complex and a few inner gonimoblast cells and supports the contention of Gabrielson and Hommersand (1982).[1]

Tripodi (1974)[34] in *Polysiphonia;* and Tripodi and de Masi (1977)[35] in *Erythrocystis* studied carpsporophyte development and described the fusion cell as meristematic, and continuously building off cells that eventually develop into carpospores. Ducker et.al. (1978)[20] consider in Nemalion the fusion cell to be meristematic. Delivopoulos and Tsekos (1985)[36] show that fusion cell of *Gracilaria verrucosa* contains contains copious amount of floridean starch grains and protoplasts. The fusion cell of *Nizymenia* australis (Gigartinales), in addition to polysaccharides, is replete with proteins, shows transfer cell morphology and acts as a sink for reserve metabolites.[37] In *Solieria robusta*,from Port Okha, the fusion cellis bereft of floridean starch grains and lacks transfer cell morphology (Present work) as also reported in *Scinaia pseudocrispa*.[37] The gonimoblast filaments that originate from fusion cell eventually give rise to carpospores albeit from fusion cell eventually give rise to carpospores albeit a few sterile gonimoblast filaments that coexist span the fusion cell with the pericarp. More ultrastructural and histochemical studies are needed to understand the structure, nature and function of this enigmatic cell-complex in different orders of Rhodophyceae.

The reported studies in *Levringiella gardneri* (parasitic taxon), indicated that the cells of the pericarp are secretory in nature and produce mucilage that acts as bacteriostatic agent and protects the carpospores from desiccation.[38] In present study of *Solieria robusta*, from Port Okha, ultrastructural and histochemical studies reveal that the cell wall of pericarp is made up of electron-dense, fibrillary materials and is rich in complex polysaccharides. During ostiole formation the pericarp cells lyse and the lysate, rich in carboxylated and sulphated polysaccharides, plugs the ostiole and protects the immature carpospores from environmental hazards. It appears that pericarp, in both parasitic and nonparasitic taxa, although has a common function of carpospores protection may exhibit other roles as well. It is desirable that structure, ultrastructure and histochemistry of pericarp belonging to divergent Rhodophycean taxa be undertaken to ascertain the exact nature and function of this important postfertilization. Proliferative, tissue.

According to previous report in *Solieria robusta*, the terminal carposporangia arise from the gonimoblast filaments.[3] In *S. tenera* and *S. chordalis* only the terminal cells of the branched gonimoblast becomes carposporangia. In contrast, *Solieria robusta*, from Port Okha, reveals that both terminal and two or three upper cells of the gonimoblast filament differentiate into carposporangia. The work of Gabrielson and Hommersand[29] in *Agardhiella subulata* reported both terminal as well as carposporangia in succession. Papenfuss and Edelstein[30] observed chains of carposporangia from the tips of gonimoblasts of *Sarconema scinaioides*. The carposporangia development is

variable in different taxa and need not be used as taxonomic character.

In *Solieria robusta* four stages are recognized during carpospores differentiation.

Stage I : The carposporangial cytoplasm shows dictyosomes, chloroplasts and a few floridean starch grains. A mucilaginous electron-transparent sheath surrounds the carpospores cytoplasm.

Stage II: Carposporangium shows abundant floridean starch grains and fibrous vesicles. The fibrous vesicles fuse with mucilaginous sheath and contribute to wall deposition.

State III: The mature carposporangium is multi-layered and gorged with floridean starch grains and fibrous vesicles.

Stage IV: Carposporangium possesses a large fibrous vacuole at its base where as the upper half is rich in floridean starch grains.

Almost similar differentiated stages of carpospores development are observed by Kugrens and Delivopoulos[39] in *Plocamiocolax pulvinata*. Ultrastructural studies on carposporophyte and carposporogenesis have been conducted only in a few taxa. It is possible that ultrastructural studies coupled with histochmical evidence on divergent Rhodophycean taxa will help in distinguishing precisely the orders and families or red algae particularly those belonging to Cryptonemiales. Giagartinales and Rhodymeniales.

In early stages of carposporogenesis golgi apparatus produce vesicles that contribute to mucilage secretion.[38,40]

Chamberlain and Evans[25] observed that different types of vesicles release substances involved in fixing the spores to the substratum. According to Scott and Dixon[41] in *Ptilotahypnoides* fibrous vacuoles in the carposporangia play an important role in secretion of outer wall that is essential for spore liberation or attachment. The fibrous vacuoles are reservoirs of complex polysaccharides. The present work on *Solieria robusta* supports the views of Scott and Dixon[41] and reveals that fibrous vacuole at the base of the carpospores helps in the dispersal of carpospores.

SUMMARY

The thalli of *Solieria robusta* are collected from Port Okha (Gujarat), grows luxuriantly and is attached to the limestone rocks, in the rock pools, of intertidal region. Both gametophyte and tetrasporophyte plants are attached to the rocks through discoid holdfasts or hapteron-like structures. The thalli are purplish-red to brownish-red in colour and consist of terete of slightly compressed, erect lateral branches that are attenuate at the base and subacute at the tip. Branching is irregular and occasionally from the damaged portion 4 or 5 branches regenerate to form an umbel. The cystocarps show acropetal arrangement, are dispersed all over the thallus surface and possess well developed ostioles. The cystocarps elevate the thallus surface.

The 3-celled carporgonial branch, arises from the inner cortical cell and consists of carpogonium with long trichogyne, hypogynous cell and basal cell. The hypogynous cell is darkly stained as compared to the carpogonium and the basal cell. The basal cell is larger than both the hypogynous cell and the carpogonium. After fertilization the carpogonium enlarges, becomes spherical and produces a single connecting filament.

Auxiliary cell and the cells of the auxiliary cell-complex, stain darkly prior to diploidizatoin and can be easily distinguished from other cortical cells. It is a non-procarpic plant where connecting filament arises from the fertilized carpogonium and fuses with remote auxiliary cell, into which the zygote nucleus migrates. After diploidization, the inner cortical cells around the auxiliary cell-complex produce numerous branched filaments which envelope the developing cystocarp. After the formation of pericarp, the auxiliary cell cuts off gonimoblast initial which in turn gives rise to a compact cluster of gonimoblast cells. As the auxiliary cell matures additional gonimoblast filaments arise from its periphery. The auxiliary cell, along with a few basal gonimoblast cells and darkely stained adjacent cortical cells fuse and the fusion productof all these cells lead to the formation of large, round, fusion cell in the centre of the mature cystocarp. The terminal one or two cells of each branched gonimoblast filaments becomes the carposporangia. A few gonimoblast filaments remain sterile, become non-septate and unbranched and connect the pericarp with the fusion cell. The carpospores are released through a prominent ostiole that is formed by the gelatinization of pericarp and surrounding vegetative cells. A distinct gradient of cytoplasmic polysaccharides is seen from fusion cell to carpospores. The fusion cell and the pericarp are bereft of floridean starch grains and complex polysaccharides. The carposporangia and carpospores are replete with floridean starch grains and sulphated polysaccharides. The cytoplasm of gonimoblast filaments and carposporangia are rich in total proteins.

Carposporangial cytoplasm is fully packed with floridean starch grains, fibrous vesicles, and chloroplasts. Fibrous vesicles fuse with plasmalemma and participate directly in the cell wall formation. Carposporangial wall is 3 to 5 layered and is composed of parallel microfibrils. Abundant mucilage is present between the developing carposporangia. Mature carposporangium, at the base, forms a fibrous vacuole which help in the dispersal of carpospores.

CONCLUSIONS

Plant is dioecious with separate male, female and tetrasporic plants and occur in the intertidal region.

The thallus consists of terete lateral branches which at bases are attenuate and at tips appear subacute. The thallus is multiaxial.

The thallus consists of cortex and medulla. The cortex is further differentiated into epidermis, outer and inner cortices.

The epidermal cells and outer cortical cells are rich in starch grains and cytoplasmic proteins as compared to inner cortical cells. In the cortical region, intercellular mucilage reveals both sulphated and carboxylated polysaccharides.

The carpogonial branch is 3-celled and arises from the inner cortical cells. Hypogynous cell is darkly stained as compared to the hypogynous cell is darkly stained as compared to the carpogonium and the basal cell. After fertilization, the

carpogonium becomes large and spherical and puts forth a single, connecting filament.

Any enlarged inner corticalcell, with darkly staining nucleus, acts as an auxiliary cell. This cell in association with contiguous darkly stained cortical cells form the auxiliary cell-complex and is distinguishable before diploidization .The diploidization of auxiliary cell take place through the connecting filament and is followed by the formation of distinct pericarp.

Gonimoblast initial is cut off from the auxiliary cell and later divides to form a cluster of gonimoblast cells. The fusion product of auxiliary cell, inner gonimoblast cells and adjacent darkly stained cortical cells lead to the formation of large, round, fusion cell with a common boundary. Wall remnants are also seen in the fusion cell of young cystocarp.

The carpospores are released through the ostiole which is formed by the lyses of pericarp and vegetative cells. The lysate is rich in sulphated and carboxylated polysaccharides.

The mature cystocarp is embedded in the medullary region and is entangled by medullary filaments. The carpospores possess prominent nuclei and are gorged with floridean starch grains and proteins.

The outer layers of cell wall show parallel arrangement of microfibrils whereas the inner layer show lax arrangement of microfibrils.

The carpospores are replete with floridean starch grains, and fibrous vesicles that release their contents into the plasmalemma to form wall materials. The carposporangium wall is three to five layered thick.

Autofluorescent proteinaceous materials are present in epidermal and cortical cells. Spermatangia carposporangia, gonimoblast filaments and tetrasporangia also possess such autofluorescent materials.

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REFERENCES AND NOTES

1. Gabrielson P. W., Hommersand M. H. The Atlantic Species of Solieria (Gigartinales Rhodophyta): Their Morphology Distribution and Affinities. *J. Phycol.* **1982**.
2. J. West, M. Hommersand. Rhodophyta: Life Histories. In *The Biology of Seaweeds*; **1981**; pp 133–193.
3. U. Min-Thein, H.B.S. Womersley. Studies on southern Australian taxa of Solieriaceae, Rhobdoniaceae and Rhodophyllidaceae (Rhodophyta). *Aust. J. Bot.* **1976**, 24 (1), 1–166.
4. M.R. Vijayaraghavan, I. Nashier, B. Mam. In vitro studies of Callithamnion sp. (Ceramiaceae, Ceramiales): Growth and differentiation. *Seaweed Res. Utiln.* **1995**, 17, 13–22.
5. I. Nashier, A. Jha. Developmental and histochemical studies on Spermatangia in Solieria robusta (Greville) Kylin (Solieriaceae), Rhodophyta. In *Plant Form and Function*; Bhatia, B., Shukla, A. K., Sharma, H. L., Eds.; Angkor Publishers, **1998**.
6. I. Nashier, A. Jha, M.R. Vijayaraghavan. Tetraspore development in Solieria robusta (Greville) Kylin (Solieriaceae, Gigartinales) from Port Okha, India. *Phytomorphology* **1999**, 49 (3), 233–240.
7. K. Hori, S. Ikegami, K. Miyazawa, K. Ito. Mitogenic and antineoplastic isoagglutinins from the red alga Solieria robusta. *Phytochemistry* **1988**, 27 (7), 2063–2067.
8. I. Nashier, A. Jha, M.R. Vijayaraghavan. Wound regeneration in Solieria robusta (Greville) Kylin (Solieriaceae, Gigartinales) from Port Okha, India. *Seaweed Res. Utiln.* **1998**, 20 (1&2), 119–124.
9. H.H. Mollenhauer. Plastic Embedding Mixtures for Use in Electron Microscopy. *Stain Technol.* **1964**, 39, 111–114.
10. M. Kumar, A. Jha, M.R. Vijayaraghavan. Developmental and reproductive strategies of two marine algae Gracilaria corticata (Gigartinales, Rhodophyta) and Asparagopsis taxiformis (Bonnemaisoniales, Rhodophyta) from Port Okha (Gujarat), west coast of India. *Indian J. Mar. Sci.* **2000**, 29 (3), 248–252.
11. S. Sekar, K.V. Pugalendi, B. Kishnan, K.K. Ganesan. Evaluation of anti-hyperglycemic effect of Gracilaria corticata extract in Normal and Streptozotocin-induced Diabetic rats. *Chem. Biol. Lett.* **2015**, 2 (1), 6–11.
12. I. Nashier Gahlawat, P. Lakra. Contextual implicit role of PROBIOTICS in improving the Human Health. *J. Integr. Sci. Technol.* **2017**, 5 (2), 50-53.
13. P. Lakra, S. Sehgal, I. Nashier Gahlawat, M. Wadhwa nee Dasbas. Use of products developed from potato flour, defatted soy flour and corn flour in combating malnutrition. *Integr. J. Soc. Sci.* **2018**, 5 (1), 47–50.
14. I. Nashier Gahlawat. Emerging new insights into significance and applications of Plant Pigments. *J. Integr. Sci. Technol.* **2019**, 7 (2), 29–34.
15. P. Lakra, I. Nashier Gahlawat. Prospective Phytochemicals for alleviation of different chronic ailments. *Integr. J. Soc. Sci.* **2015**, 2 (1), 36–39.
16. M. Nurilmala, T. Hidayat, F. Sudirdjo. Characteristics of seaweed as raw materials for cosmetics. *Aquat. Procedia* **2016**, 7, 177–180.
17. K. Khatri, M.S. Rathore, S. Agrawal, B. Jha. Sugar contents and oligosaccharide mass profiling of selected red seaweeds to assess the possible utilization of biomasses for third-generation biofuel production. *Biomass and Bioenergy* **2019**, 130, 105392.
18. F. Weinberger. Preparative extraction of exometabolites from seaweed surfaces. *Protoc. macroalgae Res.* **2018**, 300–308.
19. C. Perrone, E. Cecere. Two Solieriacean Algae New To the Mediterranean: Agardhiella Subulata and Solieria Filiformis (Rhodophyta, Gigartinales). *J. Phycol.* **1994**, 30 (1), 98–108.
20. S.C. Ducker, R.B. Knox. Epiphytism at the Cellular Level with Special Reference to Algal Epiphytes. *Cell. Interact.* **1984**, 113–133.
21. L.J. Goff, M.H. Hommersand. Reproductive Development of the Parasitic Red Alga Gardneriella Tuberifera (Solieriaceae, Gigartinales). *J. Phycol.* **1982**, 18 (2), 295–304.
22. S.K. Singh, S.K. Shukla, N.K. Dubey, P.K. Shukla, R. V Lansdown. Morphological studies of the ligules of selected Indian species of Selaginella (Selaginellaceae). *Flora* **2019**, 252, 69–75.
23. S.K. Singh, N.K. Dubey, G.K. Srivastava. The microspore morphology of some species of Selaginella (Selaginellaceae) from India. *Palynology* **2016**, 40 (2), 216–229.
24. R.G. Sheath, J.A. Hellebust, T. Sawa. Ultrastructure of the floridean starch granule. *Phycologia* **1981**, 20 (3), 292–297.
25. A.D. Boney. Mucilage sheaths of spores of red algae. *J. Mar. Biol. Assoc. United Kingdom* **1975**, 55 (3), 511–518.
26. A.D. Boney. The liberation and dispersal of carpospores of the red alga

Rhodymenia pertusa (Postels et Rupr.) J. Ag. *J. Exp. Mar. Bio. Ecol.* **1978**, 32 (1), 1–6.

27. A.D. Boney. Survival and growth of alpha-spores of Porphyra schizophylla Hollenberg (Rhodophyta: Bangiophyceae). *J. Exp. Mar. Bio. Ecol.* **1978**, 35 (1), 7–29.
28. B.J.D. Meeuse, M. Andries, J.A. Wood. Floridean starch. *J. Exp. Bot.* **1960**, 11 (2), 129–140.
29. P.W. Gabrielson, M.H. Hommersand. the Morphology of Agardhiella Subulata Representing the Agardhielleae, a New Tribe in the Solieriaceae (Gigartinales, Rhodophyta). *J. Phycol.* **1982**, 18 (1), 46–58.
30. G.F. Papenfuss, T. Edelstein. The morphology and taxonomy of the red alga Sarconema (Gigartinales: Solieriaceae). *Phycologia* **1974**, 13 (1), 31–43.
31. H. Kylin. Die Florideenordnung Gigartinales. *Lunds Univ. Arsskr. NF Avd. 2* **1932**, 28, 1–88.
32. M.J. Wynne, W.R. Taylor. The status of Agardhiella tenera and Agardhiella baileyi (Rhodophyta, Gigartinales). *Hydrobiologia* **1973**, 43 (1–2), 93–107.
33. G.T. Kraft. Morphology of Placentophora (Solieriaceae, Gigartinales: Rhodophyta), a New Genus Based on Sarcodiotheca Colensoi From New Zealand. *J. Phycol.* **1975**, 11 (4), 399–410.
34. G. Tripodi. Ultrastructural changes during carpospore formation in the red alga Polysiphonia. *J. Submicrosc. Cytol.* **1974**, 6, 275–286.
35. G. Tripodi, F. De Masi. The post-fertilization stages of red algae. The fine structure of the fusion cell of Erythrocystis. *J. Submicrosc. Cytol.* **1977**, 9 (4), 389–401.
36. S.G. Delivopoulos, I. Tsekos. Ultrastructure of the Fusion Cell in Gracilaria Verrucosa (Huds.) Papenfuss (Rhodophyta, Gigartinales). *New Phytol.* **1985**, 101 (4), 605–612.
37. M.R. Vijayaraghavan, B. Bhatia. Developmental and histochemical studies on the spermatangium and carposporophyte of _Scinaia forcellata _(Nemaliales, Rhodophyta) from Port Okha, India. *Nov. Hedwigia* **1996**, 112, 135–146.
38. P. Kugrens, J.A. West. the Ultrastructure of Carposporogenesis in the Marine Hemiparasitic Red Alga Erythrocystis Saccata. *J. Phycol.* **1974**, 10 (2), 139–147.
39. P. Kugrens, S.G. Delivopoulos. Ultrastructure of the Carposporophyte and Carposporogenesis in the Parasitic Red Alga Plocamiocolax Pulvinata Setch, (Gigartinales, Plocamiaceae). *J. Phycol.* **1986**, 22 (1), 8–21.
40. A.H.L. Chamberlain, L. V. Evans. Aspects of spore production in the red alga Ceramium. *Protoplasma* **1973**, 76 (2), 139–159.
41. J.L. Scott, P.S. Dixon. Ultrastructure of Tetrasporogenesis in the Marine Red Alga Ptilota Hypnoides. *J. Phycol.* **1973**, 9 (1), 29–46.