Cytoplasmic metabolites study of Vegetative and Reproductive structures of *Solieria robusta*

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ABSTRACT

*Solieria robusta* exhibits triphasic isomorphic alternation of generation. The plants of the male, female and tetrasporophytes are terete with erect lateral branches that show attenuation at their bases. The thallus is differentiated into cortex and medulla. Spermatangial mother cell and spermatangial cytoplasm stain moderately for sulphated and carboxylated polysaccharides whereas mature tetrasporangia, tetraspores, and carpospores are rich in sulphated polysaccharides. The tetrasporangial cytoplasm like carposporangial cytoplasm show fibrous vesicles, chloroplast, mitochondria and floridean starch grains. A thick electron transparent mucilaginous sheath covers the tetrasporangial wall whereas carposporangial wall is 3 to 5 layered. The carpospores and tetraspores are replete with floridean starch grains which are of various shapes and size. *Solieria robusta* (Greville) Kylin shows regeneration.

Keywords: Red Algae, Histochemistry, Metabolites, Seaweed, Regeneration

INTRODUCTION

Indian resources in seaweeds, are sufficiently valuable but they are not fully explored and utilized. Sea weeds are important sources of algicin acid (alginites), agar and carageenans which are useful in food, textile and pharmaceutical industries. With depleting land and increasing population, one has to look towards the marine ecosystem for food and survival. Almost half the Florideophycean families belong to the Gigartinales one of the most diverse orders of the Florideophycideae. In Gigarinates the auxiliary cell is produced in the vegetative filament, Red algae cell wall contain copious amount of carboxylated and sulphated polysaccharides. These polysaccharides can be extracted from many seaweeds which grow luxuriantly in subtidal regions. To make this approach feasible, it is necessary to investigate the reproductive biology of these taxa using modern methods. Histochemistry helps in situ localization of important metabolites during progressive developmental stage in the life cycle. Electron microscopic studies help in revealing better resolution of cell organelles, relative distribution and their probable function. The red algae *Solieria robusta* grows in rock pools in Okha coast. The earlier reports on this taxon casts many doubts and lacunae in our knowledge with respect to its origin, development and ultrastructure of vegetative and reproductive thalli. The information on reproductive thalli, reproduction biology, particularly a correlative account of developmental, histochemical and ultrastructural aspects is very less. The histochemical studies detect all changes that occur in the wall polysaccharides and cytoplasmic metabolites during progressive stages of development of both vegetative and reproductive structures. The present work is aim to study histochemical details that occurs in the wall polysaccharides and cytoplasmic metabolites during progressive stages of development of both vegetative and reproductive structures. Detailed study of Carposporogenesis and tetrasporogenesis at both light and electron microscopic level is undertaken. This work on *solieria robusta* confirms that it is non-procarpic and show process of wound generation and add on a complete new aspects to our knowledge about *solieria robusta*.

MATERIAL AND METHODS

Red Alga *Solieria robusta* (Greville) Kylin was collected at Port Okha(Gujarat) during the low tide periods for light microscopic studies, the parts of the thalli were observed under stereomicroscope...
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). Photomicrographs were taken on ORWO B/W film using Reichert Polyvar photomicroscope.

For transmission electron microscopy (TEM), parts 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.

ELECTRON MICROSCOPY

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

B For scanning electron microscopy selected sections of the thalli were passed through a graded cold (4°C) increasing acetone medium, dehydrated in anhydrous acetone, treated for critical point drying (CPD), coated with gold and observed under Philips SEM 501-B.

RESULTS

Vegetative Morphology & Anatomy

In Okha Coast (Gujarat), there is narrow supralittoral zone which is inhabited by a few green algae and molluscs (Figure 1A). Red seaweeds occur mainly in the subtidal region whereas brown and green algae are abundant in the intertidal zone. The Okha-reef is characterized by hard, corrugated, limestone rocks that are covered with calcareous deposits (Figure 1B). Solieria robusta is attached to the calcareous rocks and occur in the rock-pools of the intertidal region (Figure 1B). Occasionally, during high tide periods plants are washed ashore by the waves and can also be collected as drift plants. The thalli are attached to the substratum by a fibrous or discoid holdfast (Figure 1C) or the hapteron-like structure. The height of the plant is 10 - 40 cm in length. The colour of the thalli is purple to brown red. Solieria robusta consists of terete or slightly compressed, erect lateral branches which are generally attenuate at their base (Figure 1C-D) and tapered at their tips. Branching pattern is irregular. In male plants, the spermatangia are scattered on the surface of young branches. The female thallus surface shows many scattered cystocarps that appear as dark coloured dots (Figure 1D). Mature cystocarps are present near the base of the branch and the younger ones toward the tip (Figure 1D). The development of the cystocarp is thus acropetal. Scanning electron micrograph of female plant surface shows a very thick cuticle which covers the epidermal cell walls (Figure 2C). The thallus surface is raised due to presence of cystocarp with a small circular ostioles in their centre. In male plants, the mucilage covering of epidermal cells lyse after the cutting of spermatangia (Figure 2A). The thalli surface show growth of many epiphytes notably Polysiphonla, Ceramium sp. and a number of diatoms. APEX : The apices in gametophytes and tetrasporophytes possess a group of apical cells (Figure 3A). The apical cell initially multiplies obliquely producing a subterminal cell (Figure 3A) which cuts off a periaxial cell (Figure 3A). A single periaxial cell is cut off from each axial cell and succeeding periaxial cells rotate in a snaky pattern by the side of the axial filaments (Figure 3A). Periaxial cells produce lateral filaments that form the cortex. Derivatives of the apical cells become the axial filaments which later form the medulla. AXIS : The axis is differentiated into cortex and medulla. The cortical region comprises the epidermis, outer and inner cortices. The epidermis is unlayered (Figure 2B,C,D). The cortex is 4 to 6 cells thick which is divided into outer and inner cortex (Figure 2B,CD). The former is made up of small and the latter of big cells (Figure 2D).
In mature thallus the cell walls of the cortical cell are thick (Figure 2D) and possess pit-connections (Figure 2B). Epidermal cells also show pit-connections with prominent septal-plugs (Figure 2B). The cells of the outer cortex are small in size as compared to inner cortex cells and are gorged with floridean starch grains (Figure 2D). The size and shape of the starch grain vary but most of them appear spherical. The tetrasporephyte thallus reveals identical anatomy. In main axis, the cortical cells show wall thickening that aids in mechanical function (Figure 2B,C,D).

**REPRODUCTIVE BIOLOGY**

**SPERMATANGIA:** The spermatangia are dispersed superficially on the top of the young branches (Figure 3D). The outer cortical cells cut off 2 to 4 spermatangial mother cells (Figure 3E,F,G). Each spermatangial mother cell cuts 1 or 2 spermatangia...
All the spermatangia are ovoid to ellipsoid in shape and have dark staining tips (Figure 3E,F,G). A few outer cortical cells do not cut spermatangia but produce epidermal cells (Figure 3E,F,G). The extracellular mucilage that veneers the spermatangial mother cells gelatinizes after the formation of spermatangia (Figure 2A) and thus enables the release of spermia.

**CARPOGONIAL BRANCH**: The carpogonial branch is 3-celled and arises from the inner cortical cells. Occasionally, 2 carpogonial branches arise from a common supporting cell (Figure 4C). The carpogonium is elongate, conical shaped (Figure 4C) and has a long trichogyne (Figure 4C). After fertilization, the carpogonium becomes large and spherical in shape and issues a single connecting filament from its terminal end.

**AUXILIARY CELL**: Auxiliary cells are the dedifferentiated inner, spherical Cortical cells that contain in the core darkly stained nuclei and uninucleate. As Auxiliary cell matures, the juxtaposed, polynucleate, inner cortical cells stain darkly. The auxiliary cell along with these darkly staining cortical cells comprise the auxiliary cell-complex (Figure 4D,E). After diploidization of the auxiliary cell, the neighbouring cortical cells adjoining the auxiliary cell-complex generate elongate, septate, filaments which form the pericarp (Figure 4D,E).

A single gonimoblast initial is cut off from the auxiliary cell and later divides transversely to form a closely packed group of gonimoblast cells (Figure 4D,E). As the auxiliary cell enlarges, it produces from its periphery many branched gonimoblast filaments. Interestingly, a few gonimoblast cells combine with the auxiliary cell (Figure 4D,E) which in turn merge, through pit-connection, with adjacent darkly stained supporting (cortical) cells (Figure 4F).

The fusion product of all these cells lead to formation of a big fusion cell. Gonimoblast filaments are seen around the fusion cell (Figure 4F,G). Those branched gonimoblast cells that face towards the pericarp, do not participate in the formation of initial fusion cell but divide and produce the spreading carposporangia (Figure 4F,G). A few of the gonimoblast filaments remain sterile, unbranched, unseptate and connect the fusion cell with the pericarp (Figure 4G). 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 4F,G). Light and Electron microscopic studies show that mature cystocarp is enveloped by three to five layered thick pericarp (Figure 4F) and is embedded in the medullary region. The carpospores are initially elliptical in shape but at release, through the ostiole, become spherical (Figure 4F).

**OSTIOLE FORMATION**: The cortical cells, adjacent to the cystocarp, proliferate and produce small cells (Figure 4A,B) which raise above the thallus surface (Figure 4A,B). The ostiole is formed by the gelatinization of the pericarp cells followed by dissolution of adjoining cortical cells. The progressive gelatinization of pericarp cells is followed by the dissolution of vegetative cells (Figure 4B). The resultant lysate assembles at the ostiolar region (Figure 4B). The complete gelatinization of extracelllar mucilage and degeneration of vegetative cells makes an orifice (Figure 4A,B) above the thallus surface for the carpospores release (Figure 4A,B).

**TETRASPORANGIA**: Tetrasporangia are scattered all over the thallus surface except at the branch apices and the main axis. Tetrasporangia are cut off laterally by longitudinal divisions of the medullary cells and mature tetrasporangia are gelatinized and released through the orifice of the cystocarp. Tetrasporangial initials enlarge in size (Figure 5A,B,C) and later divide transversely to produce one or two additional cells (Figure 5A,B,C). Tetrasporangial initials enlarge in size (Figure 5A,B,C) and

![Figure 4. Solieria robusta. Carpogonial branch, Auxiliary cell complex, Cystocarp, Fusion Cell, Gonimoblast Filament & Carpospore. (cs. carpospore; csg, carposporangium; ep, epidermis; fu, fusion cell; gf, gonimoblast filament; ic, inner cortex; oc, outer cortex; os, ostiole; me, medulla ; cp, carpogonium; he, hypogynous cell; su, supporting cell; tr, trichogyne; au, auxiliary cell; p, pericarp; sgf, sterile gonimoblast filament) (A) Transverse section of mature cystocarp with well developed ostiole. The carposporangia, carposporangia, gonimoblast filaments and pericarp cells contain autofluorescent compounds. The epidermal and outer cells are rich in this compound whereas the inner cortical cells show peripheral arrangement. Ax 360; B x 450 (B) Magnified to show that the pericarp and surrounding vegetative cells lyse. The lysate (arrow) is rich in both sulphated and carboxylated polysaccharides (Photomontage), x 1800 (C) 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 trichogyne. In A, two carpogonial branches arise from a common supporting cell, x 1800 (D&E) 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 form the fusion cell, x 4500 (F) A portion of the thallus to show the growth of the cortical cells around the cystocarp. Epidermal and outer cortical cells are rich in floridean starch grains as compared to inner cortical and medullary cells, x 1801 (G) 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.

![Diagram of Solieria robusta](image-url)
consists thick cytoplasm when equate to the adjacent cortical cells (Figure 5A,B,C). In transactions, mature tetrasporangia show thick and well stained wall (Figure 5A,B,C). A prominent, darkly stained nucleus is apparent in tetrasporangial mother cell (Figure 5E). Immature tetrasporangia are initially oblong in shape but during Progressive maturation elongate basally and expand laterally (Figure 5A,B,C) and appear juxtaposed betwixt the epidermal cells. The cleavage of the proplast is accompanied by the ingrowth of the septa from the wall of the sporangium (Figure 5A,B,C E). The mature tetrasporangium undergoes zonate, Presumably meiotic, division, to produce four spores of equal size (Figure 5A,B,CE).

**Histochemical Studies**

**Axis:** The extracellular mucilage that veneers the outer tangential walls of epidermal cells; the walls of epidermis, outer and inner cortical cells and rhizoidal cells stain deep violet with TBO (Figure 3B,C) indicating the presence of carboxylated and sulphated polysaccharides. In Solieria robusta the cell walls of the medullary filaments also show identical staining properties. The intercellular mucilage of the cortical cells contain both the above types of polysaccharides whereas that of medullary regions stain reddish violet confirming the abundance of sulphated polysaccharides (Figure 3 C). Spermatangia: The extracellular and intercellular mucilages between the epidermal cells and outer cortical cells stain deep-violet indicating the presence of both sulphated and carboxylated polysaccharides. As the cortical cells cut off spermatangial mother cells, which produce spermatangia, the extracellular mucilage gelatinizes and paves way for spermatia release. The spermatangial and spermaticial cytoplasm stain moderately and contain sulphated and carboxylated polysaccharides (Figure 3C). The cell walls of spermatangial mother cells and cortical cells stain deep violet indicating the presence of both carboxylated and sulphated polysaccharides. The cell walls of spermatangia bound by polysaccharide covering (Figure 3C). The intercellular mucilage between the spermatangia and spermatangial mother cells is rich in sulphated polysaccharides (Figure 3C). **Cystocarp:** The intercellular spaces and cell walls of fusion cell, gonimoblast filaments and pericarp are rich in both carboxylated and sulphated polysaccharides (Figure 4F). The gonimoblast cell's cytoplasm shows plentiful sulphated polysaccharides as compared to the carposporangial cytoplasm which stains moderately for this metabolite (Figure 4F). The fusion cell and pericarp cells cytoplasm are bereft of this metabolite (Figure 4F). At the ostiolar region, both the pericarp and surrounding cortical cells of the thallus lyse and the lysate of all these cells have copious amount of sulphated and carboxylated Polysaccharides. This process helps in Protection and the dispersal of carpospores. **Tetrasporangium:** The extracellular mucilage, and The cell wall the tetrasporangium stains intensely for wall polysaccharides and reveal carboxylated and sulphated polysaccharides (Figure 5B). The tetrasporangial cytoplasm, however, stains moderately for sulphated polysaccharides (Figure 5B).

**Localization of Insoluble Polysaccharides**

**Axis:** The epidermal and outer cortical cells are replete with floridean starch grains as compared to inner cortical cells(Figure3F).

![Figure 5. Solieria, robusta. Tetrasporangium, Mature carposporangium (ep, epidermis; ex, extracellular mucilage; fs, floridean starch grains; oc, outer cortex; tsg, tetrasporangium; im, intercellular mucilage; ep, epidermis; fs, floridean starch grain; fv, fibrous vesicle; ch, chloroplast; ic, inner cortex; me, medulla; mf, medullary filament; we, wound epidermis) (A). Longitudinal section of the thallus to show mature tetrasporangia. Protoplasm cleavage is accompanied by the ingrowth of the septum from the sporangium wall. Proteins are restricted only to cell cytoplasm. Cell walls and intercellular mucilage are bereft of this metabolite (CBB). x 5625. (B). Same, to show in a mature tetrasporangium the extracellular mucilage stains deep violet indicating the presence of sulphated and carboxylated polysaccharides. The tetrasporangium cytoplasm shows metachromasy and confirms the abundance of sulphated polysaccharides (TBO). x 5625 (C). Longitudinal section of thallus to show epidermis veneered by a thick mucilage that stains positively for insoluble polysaccharides. Epidermal cells and outer cortical cells show moderate amount of this metabolite. Tetrasporangium is gorged with floridean starch grains. The tetraspores are zonately arranged (PAS), x 5625 (D). 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 (E). Transmission electron micrograph to show tetrasporangium. The cleavage furrows are seen as invaginations of the plasmalemma. These furrows show centripetal extension (arrows) and partition the tetrasporangium into four zonately arranged tetraspores. x 10,600. L. S. of thalli showing autofluorescent compounds in tetrasporangium. (F). Scanning electron micrograph to show regenerate growth (arrow) from the tip of a wound tissue, x 224. Blade-like outgrowths (arrows) regenerate from wound portions of the plants, x 10 (G). Longitudinal section of a regenerating young branch from the wound surface. The epidermal layer covering the new tissue is easily distinguished from that of the wound surface. This indicates...
regeneration of cells from the wound surface of the medullary region. Both the original and the regenerated tissues are covered by thick mucilage that stains for both sulphated and carboxylated polysaccharides. The intercellular mucilage both in apical and cortical region reveals the presence of carboxylated and sulphated polysaccharides, x 1800.

Spermatangia : The cell wall of spermatangia and spermatangial mother cells stain feebly for wall polysaccharides. Cystocarp : The cell walls of fusion cell, gonimoblast filaments and pericarp are rich in insoluble polysaccharides but that of carposporangia stain with less intensity (Figure 4F). Tetrasporangium : The extracellular mucilage that covers both the outer tangential walls of the epidermis and tetrasporangium is replete with polysaccharide secretions. The walls of epidermal cells and tetrasporangium stain well for insoluble polysaccharides (Figure 5C). The tetrasporangium and tetraspores are gorged with floridean starch grains (Figure 5C).

LOCALIZATION OF TOTAL PROTEINS

Axis : The cytoplasmic proteins in the epidermal cells and the outer cortical cells show uniform distribution, whereas in the inner cortical cells they are polarized and restricted to cell periphery (Figure 3D E,F,G). In apical region the cytoplasm of apical cell and their derivative cells (axial filament) is rich cytoplasmic proteins. Spermatangia : Spermatangial mother cells and outer cortical cells are rich in cytoplasmic proteins (Figure 3F,G). The Spermatangia are polarized with dark staining, protein-rich, tips (Figure 3F, G). The lower-half of the spermatangium is, however, protein negative (Figure 3F,G). Cystocarp : The mature cystocarp shows differential distribution of cytoplasmic proteins (Figure 4G). The cytoplasm of gonimoblast filaments is rich in this metabolite as compared to that of the carposporangia (Figure 4G). The carposporangia and the carpospores are uninucleate and contain abundant cytoplasmic proteins (Figure 4G) whereas the fusion cell and the pericarp cell show a low ebb of this metabolite (Figure 4G). Tetrasporangia : The mature tetrasporangia and the tetraspores are replete with cytoplasmic proteins (Figure 5A).

LOCALIZATION OF NUCLEIC ACID

In the cytoplasm of carposporangia and carpospores, nuclei and extracellular materials stain well with aceto-iron-haematoxylin and chloral hydrate.

LOCALIZATION OF AUTOFLUORESCENT COMPOUNDS

The thallus sections when observed under UV light (blue-violet) reveal many auto fluorescent compounds that are localized in both epidermal and cortical cells. The epidermal cells are fully gorged with auto fluorescent compounds that are uniformly distributed in cell cytoplasm (Figure 3E). In the inner cortical cells, auto fluorescent compounds occur in peripheral cytoplasm or they lie close to the cell wall (Figure 3E). The tips of spermatangia and spermatangial mother cells (Figure 3E) are rich in auto fluorescent compounds. In mature cystocarp, auto fluorescent compounds are localized in carpospores, carposporangia and in gonimoblast filaments (Figure 4A). The gonimoblast filaments are rich in this compound as compared to the carpospores. The cell walls of pericarp and fusion cells show very weak fluorescence (Figure 4A). The tetrasporangium and tetraspores are replete with auto fluorescent compounds (Figure 5E).

ULTRASTRUCTURAL STUDIES OF GAMETOPHYTE AND TETRASPOROPHYTE THALII

Epidermis : The epidermal cells are embedded in an amorphous matrix and cell walls show reticulate arrangement of microfibrils. Outer Cortex : The outer cortical cells show multilayered fibrillar cell wall. The cytoplasm is replete with floridean starch grains. Inner Cortex : The inner cortical cells show less floridean starch grains (Figure 2D) as compared to epidermal and outer cortical cells. In tetrasporic plants both disc and elliptical shaped chloroplasts coexist. The mitochondria show tubular cristae which in cross section appear circular.

REPRODUCTIVE STRUCTURES: Carposporangia: The young carposporangial cytoplasm has dictyosomes, chloroplasts and a few floridean starch grains (Figure 5D). The dictyosomes produce vesicles. The young carposporangium is enclosed in mucilaginous sheath. The mature carposporangium has multilayered cell wall and at it's base possesses a large vacuole that is filled with fibrous materials. These layers show parallel arrangement of microfibrils (Figure 5D).

Tetrasporangia: A thick and electron-transparent, mucilaginous sheath produced through the release of fibrous contents of vesicles, encloses the tetrasporangial cytoplasm. The tetrasporangial wall is composed of electron-dense, microfibrils and is distinguished from epidermal cell wall which possesses Parallel arrangement of microfibrils (Figure5E). The mature tetrasporangium is gorged with floridean starch grains (Figure 5E) as compared the adjacent epidermal cells which show little storage materials (Figure 5E).

Mature tetrasporangial cytoplasm show fibrous Vesicles which fuse directly with plasmalemma and release their contents or they fuse with one another to form large, fibrous, vacuoles (Figure 5E). The chloroplasts are also seen in the cell cytoplasm. The protoplast cleavage is accompanied by the cleavage furrows from the plasmalemma (Figure 5E). These cleavage furrows show centripetal extension and partition the tetrasporangium into four zonately arranged tetraspores. The tetrasporangium undergoes simultaneous zonate division producing four spores. The initial median cleavage is arrested just short of completion, and other two cleavages are initiated at the same depth (Figure 5E).

WOUND REGENERATION

Tissue damaged is caused either by wave action or by heavy epiphytic growth. The cells of the wound surface become mitotically active and form the epidermal layer. After the formation of epidermis the medullary regions cells retain their mitotic activity and develop into the regenerated branch( Figure 5F,G). The anatomy of the regenerated branch is identical to that of the normal branch. Both the normal and the regenerated tissues have thick extracellular and intercellular mucilages that stain for both sulphated and carboxylated polysaccharides (Figure 5G).

DISCUSSION

The plants of Solieria robusta, from Port Okha, grow luxuriantly attached to the calcareous rocks in rock-pools of the intertidal regions either through discoid or hapteroid holdfasts. The red algae serve as important source for various phytochemicals, health
beneficial extracts\textsuperscript{5–5} and number of other uses. The learning of fundamental information about algae\textsuperscript{6–11} should be considered one important aspect to generate the value to rich information of different species. The constituent of algae depends upon the type of algae and life cycle to respective species. 

Generally, the Solieriacean plants develop discoid holdfasts when they are attached to mussel-shells or small stones. According to Chapman (1973),\textsuperscript{12} the macroscopic algae (seaweeds) that grow in the intertidal and subtidal areas are always attached to the substratum by discoid holdfasts which are either cellular being extension of basal cell; prostrate and filamentous; or discoid. Perrone and Cecere (1994)\textsuperscript{13} separate Agardhiella subulata and Solieria filiformis on the basis of holdfast morphology, the former being attached by means of simple discoid holdfast, whereas the latter grows erect from the fibrous basal system. My work on Solieria robusta agrees with that of Chapman (1973)\textsuperscript{12} that intertidal algae are attached through discoid holdfasts. Solieria robusta supports abundant epiphytic algal growth (Present work) as seen in many marine seaweeds (Sieburth and Tootle, 1981).\textsuperscript{14}

Gastroclonium iyengarii supports epiphytic bacterial growth on its thallus which is responsible for iridescence. According to Duckett and Knox (1984)\textsuperscript{15} epiphytism is a common process in marine plants particularly in a biological communities where substratum is the limiting factor. In Solieria robusta growth is started by a group of apical cells where the apical cell divides obliquely producing subterminal cells which cut off periaxial cells. Successive periaxial cells rotate around the axial filament and later divide to produce branched cortical filaments (Present work). Similar type of apical development is observed in S. Chordalis and S. Tenera and also in many other uniaxial taxa like Rhabdonia, Areschougla and Melanema.\textsuperscript{16} 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 fluctuations and grow luxuriantly in the intertidal regions (Present work). The scanning electron microscopic observations on floridean starch grains of Seirorspa griffithsiana show Morphological variations of starch grains.\textsuperscript{17} Boney (1975, 1978)\textsuperscript{18–20} observes large range of starch grains size in carpospores of Bonnemaisonia noodkana as well as in carpospores and holdfast of Rhodymenia pertusa. Meeuse et al. (1960)\textsuperscript{21} have observed wide range of diameter in starch granules in Odonthalia floccosa. Floridean starch grains are variable in shape and size due to their scattered disposition in the cytoplasm where they are constantly being extension of basal cell; prostrate and filamentous; or discoid. Perrone and Cecere (1994)\textsuperscript{13} separate Agardhiella subulata and Solieria filiformis on the basis of holdfast morphology, the former being attached by means of simple discoid holdfast, whereas the latter grows erect from the fibrous basal system. My work on Solieria robusta agrees with that of Chapman (1973)\textsuperscript{12} that intertidal algae are attached through discoid holdfasts. Solieria robusta supports abundant epiphytic algal growth (Present work) as seen in many marine seaweeds (Sieburth and Tootle, 1981).\textsuperscript{14}

that intercellular mucilage provides strength as well as flexibility to the thallus and protects the seaweeds against physical damage and constant environmental stress conditions. In Solieria robusta both epidermal, cortical and medullary cell walls stain deeply for carboxylated and sulphated polysaccharides (Present work). The walls of Nizymenia australis and Scinaia forcellata and Asparagopsis taxiformnis are composed mainly of carboxylated and sulphated polysaccharides.\textsuperscript{2} The cell walls of Sargassum vulgare and S. johnstonii are also made up of carboxylated and sulphated polysaccharides.\textsuperscript{25} The presence of polysaccharides in cell walls of a number of brown algae has been demonstrated by X-ray diffraction\textsuperscript{26} and differentialing techniques.\textsuperscript{27,28} Frei and Preston (1964)\textsuperscript{29} suggest that these polysaccharides, in addition to ion exchange, play a strong structural role. The walls of red algal cell typically have a layered fibrillar appearance enmeshed in a mucopolysaccharide matrix. In S. robusta the cell wall is a multilayered, fibrillar structure embedded in amorphous matrix. In Solieria robusta spermangia occur in large patches on the surface of young branches (Present work). Yamamoto (1975)\textsuperscript{30} recognized 5 types of spermangial developmental patterns. (a) Verrucosa type, (b) Textorii type, (c) Chorda type, (d) Symmetrica type, (e) Henriguesiana type. In Solieria robusta (Present work) the spermangial mother cells develop from the cells of outer cortex. A few cortical cells remain sterile and do not cut off spermangia and thus the spermangia do not form a continuous layer over the thallus surface. The development of the spermangia, thus, conforms to the Symmetrica type. According to Min-Thein and Womersley (1976)\textsuperscript{31} in Solieria robusta the cortical cells cut off 3 or 4 spermangial mother cells, each of which produces 2 or 3 ovoid spermangia. Gabrielson and Hommersand (1982),\textsuperscript{16} in Solieria tenera, observed surface cortical cells produce 2 to 5 spermangial mother cells, which in turn produce successively spermangia. My work supports the view of Min-Thein and Womersley (1976)\textsuperscript{16} regarding the development of spermangia. In Scinaia forcellata (Vijayaraghavan and Bhatia (1996)\textsuperscript{32} and Solieria robusta (Present work) histochemical studies reveal that spermangial cytoplasm contain mucopolysaccharides and probably contribute to the mucilage layer which covers the spermangia) It is possible that these mucilages exert constant pressure on spermangial wall and thus play an important role in spermangia release. The carpogonial branch of Solieria robusta is 3-cellled and consists of carpogonium, hypogynous and basal cells. Min-Thein and Womersley (1976)\textsuperscript{16} in S. robusta and Gabrielson and Hommersand (1982)\textsuperscript{32} in S. tenera and S. chordalis and Agardhiella subulata observed identical carpogonial branches. In Sarconema sp the carpogonial branch is either 3 or 4 cellled.\textsuperscript{33} Controversy exists whether Solieria is procarpic or nonprocarpic. Kylin (1932)\textsuperscript{34} in S. chordalis and Min-Thein and Womersley (1976)\textsuperscript{16} in S. robusta from southern Australia report them to be nonprocarpic whereas Wynne and Taylor (1973)\textsuperscript{35} in S. chordalis observed procarpic nature. My work on S. robusta from Port Okha confirms it to be nonprocarpic. The genera Agardhiella and Sarcotheca also possess an auxiliary cell-complex that differentiates prior to diploidization of the auxiliary cell, Sarconema filiformis and S. scinaoides and Placentophora sp.,\textsuperscript{36} however, have undifferentiated auxiliary cells prior to diploidization. Kylin (1932)\textsuperscript{34} 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)34 in Solieria chordalis and Min-Thien Womersley (1976)35 in S. robusta suggest that fusion cell is formed by the coalescence of the first formed gonimoblast cells with the auxiliary cell. Gabrielson and Hommersand (1982)15 in S. chordalis and S. tenera report that fusion cells are formed by the fusion of auxiliary cell, gonimoblast cells and cells of the lateral vegetative filaments proximal to the auxiliary cell. My 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).16 More ultrastructural and histochemical studies are needed to understand the structure, nature and function of this enigmatic cell-complex in different orders of Rhodophyceae. According to Kugren and West (1973)37 in Levriniella gardneri (parasitic taxon), the cells of the pericarp are secretory in nature and produce mucilage that acts as bacteriostatic agent and protects the carpospores from desiccation. In Solieria robusta, from Port Okha, ultrastructural and histochemical studies reveal that the cell wall of pericarp is made up of electron-dense, fibrillar materials and is rich in complex Polysaccharides. According to Min-Thien and Womersley (1976),35 in Solieria robusta, the terminal carposporangia arise from the gonimoblast filaments. 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. Gabrielson and Hommersand22 in Agardhiella subculata reported both terminal as well as carposporangia in succession. Papenfuss and Edelstein (1974)33 observed chains of carposporangia from the tips of gonimoblasts of Sarconema scinaoides. The carposporangia development is variable in different taxa and need not be used as taxonomic character. My work on Solieria robusta supports the views of Scott and Dixon (1973)38 and reveals that fibrous vacuole at the base of the carpospores helps in the dispersal of carpospores). In Florideophycideae two distinct types of zonately divided tetrasporangia occur. The first and most common is the successive type where median division occurs initially and is followed by two other divisions. In the second, simultaneous type, all the cleavages are simultaneously initiated. In Solieria robusta the ultrastructural studies reveal that the median cleavage is arrested just short of completion and other cleavages are later initiated (Present work). Guiry (1990)39 found that in Agardhiella subulata (Solieriacae, Gigartinales), after the first meiotic division, the median cleavage furrow is almost complete but stops just short of merging. Following the second meiotic division, the other two furrows invaginate and once they come at the same depth as the first cleavage furrow, all three simultaneously merge. Guiry (1990)39 called this as intermediate between successive and simultaneous zonate division. In Solieria robusta,2,3,40 mature tetrasporangial cytoplasm shows the presence of chloroplasts, fibrous vesicles and abundant floridean starch grains. The starch grains are variable in shape and size. Kugrens and West (1972)37 observed that thickening of tetraspore wall prevails as the tetraspore mother cell divides and a dark staining fibrillar structural shape outer wall layer in the middle of tetraspores and vegetative cell walls, causing the points of weakness. The inner layer is electron-transparent and is identical with that of fibrous vesicles. It clearly shows that these vesicles release mucilages that form the mucilaginous layer or sheath which encloses the tetrasporangial cytoplasm and further help in attachment of tetraspore to the substratum.

**Wound Regeneration:** The process of regeneration at the subcellular level has been studied in Sargassum muticum. The mitosis is preceded by increase in cytoplasmic density and cell organelle numbers suggesting increased metabolic activity. Similar results have been observed in other algae species.41 In solieria filiformis regeneration showed marked polarity by progressing differently at the proximal and distal ends of an explanatum.13 Solieria robusta also show the process of regeneration.

**SUMMARY**

The plants of Solieria robusta are collected from Port Okha (Gujarat). The Okha-reef comprises of limestone rocks that are occasionally covered with calcareous deposits. (S. robusta grows luxuriantly and is attached to the limestone rocks, in the rock pools, of intertidal region. A few plants are occasionally washed ashore as drifts. Both gametophyte and tetrasperophyte 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 or 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 thallus is multiaxial and at the apex has a group of apical cells which initially divide obliquely to produce subterminal cells which are the progenitor of periaxial cells that segment laterally cutting filaments that divide to form the cortex. The central derivatives of the apical cell becomes the axial filament. The medulla is composed of axial filament, interconnecting rhizoidal filaments. The thallus is, thus, differentiated Into cortex and medulla. The cortical region comprises the epidermis, outer and inner cortices. In male plants, the outer cortical cells produce 2 to 4 spermatangial mother cells. Spermatangia are ovoid to ellipsoid in shape and possess dark staining tips. The 3-celled carposporangial branch, arises from the inner cortical cell and consists of carpogonium with long trichogyne, hypogynous cell and basal cell. Auxiliary cell and the cells of the auxiliary cell-complex, stain darkly prior to diploidization and can be easily recognized from other cortical cells. It is a nonprocaryotic plant where connecting filament arises from the fertilized. As the auxiliary cell matures additional gonimoblast filaments arise from its periphery. The auxiliary cell, along with a few basal gonimoblast

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cells and darkly stained adjacent cortical cells fuse and the fusion product of 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 nonseptate and unbranched and connect the pericarp with the fusion cell. The carpospores are released through a ostiole that is formed by the gelatinization of pericarp and surrounding vegetative cells. Tetrasporangia are scattered all over the thallus surface except the branch apices and the main axis. The tetrasporangial initials enlarge, undergo meiotic divisions, and produce four, equal, zonate tetraspores. The mucilage that veneers the epidermis, cortical, rhizoidal and medullary cell walls stains deep violet indicating the presence of both carboxylated and sulphated polysaccharides. In cortical region, the intercellular mucilage shows both sulphated and carboxylated polysaccharides. The extracellular mucilage covers the thallus surface and gelatinizes only after the formation of spermatangia. This process enables the release of spermatia. The spermatangium is 3-celled and arises from the inner cortical cells. Any enlarged inner cortical cell, with darkly staining nucleus, acts as an auxiliary cell. 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. The carpospores are escaped through the ostiole which is formed by the lyses of pericarp and vegetative cells. The lysate is rich in sulphated and carboxylated polysaccharides. Autofluorescent proteinaceous materials are present in epidermal and cortical cells. Spermangia carposporangia, gonimoblast filaments and tetrasporangia also possess such autofluorescent materials. Tetrasporangia are scattered all over the thallus surface. The tetrasporangium cytoplasm is gorged with floridean starch grains and proteins and show fibrous vesicles and chloroplasts. The tetraspores are zonately arranged. Solieria robusta (Greville) Kylin shows regeneration without callus formation.

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