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A Comprehensive Light Microscopic Study on Sargassum Tenerrimum, an Alginate Producing Plant (Fucales, Phaeophyta)

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ABSTRACT

During early stages of regeneration, cells at the cut ends become mitotically active and undergone cell differentiation to produce some filamentous outgrowths. The fucoids show a high level of anatomical differentiation of the thallus into three zones; meristoderm, cortex and medulla. Interestingly, the tissue differentiation as a highly specialized adaptation and division of labor strategy. Anatomically, the thallus differentiated into meristoderm, cortex and medulla. However, the meristoderm is overarched by some extra cellular layers. This extra cellular layer periodically sloughed – off and the meristoderm cells plays in important role in the regeneration of tissue.

1. Introduction

The studies have revealed the role of polyamines in the modulation of a variety of physiological processes, such as stabilization of cell membranes (Schubert et al., 1983; Roberts et al., 1986; Kaur-Sawhney and Applewhite, 1993) , stress response (Flores 1990 ; Aurisiano et al., 1993; Das et al., 1995; Galston et al., 1997; Kakkar et al., 2000) and senescence (Slocum et al., 1984; Evans and Malmberg, 1989; Rey et al., 1994; Del Duca et al., 2000). In recent years, the interest in light microscopic research has increased tremendously and is now being applied to study important agronomic and horticulture crops (Rajam, 1997). Different species of *Sargassum* are presently utilized in commercial production in different parts of the world because of high alginate content.

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Thus, the objective of the present work was to study the light microscopic studies of *Sargassum tenerrimum* to increase the productivity of the study for field cultivation in India and to select the fast growing strains that yield high quality alginate content.

2. Material and Methods

2.1. Light Microscopic Studies

The Sample of *Sargassum tenerrimum* was collected from the Rann of Kutch in 2011 in the month of July (Figure 1).

Figure 1. *Showing the point of collection of Samples*

The Rann of Kutch is a seasonal salt marsh. It is a salt marsh in the Thar Desert in the Kutch District of Gujarat which covers about 7500 km^2 in the area. Selected parts from the vegetative thalli and the portion of regenerating tissue were fixed immediately in 4% Formalin: Seawater. Now the fixed materials were successively transferred to 2 methoxy ethanol (2 times for 24 hours each), 100% Ethanol (24 hours), n-propanol (24 hours), n-butanol (2 times for 24 hours) at 4^0C. In several changes of glycol methacrylate monomer mixture (2-2' Azobis i.e. 2 methyl propionitrile – 0.3 gm, 2 hydroxyl methacrylate – 92.2 ml and 7.5 ml of poly ethylene glycol 400). The glycol methacrylate containing infiltrated segments was polymerized at 60^0C for 48 hours in temperature controlled oven and sections were cut into 1-2 micrometer thick with glass knives on an A.O. Spencer 820 rotary microtone. Sections were placed on chops of filter sterilized distilled water on glass slides and dried overnight at room temperature. Now the sections were stained by following methods:

(a) Stained for 1-2 minutes in 0.5% of TBO (Toluidine Blue O) in benzoate buffer, then they were washed for 1-2 minute in running tap water.

(b) Stained for 6 minutes in 0.25 gm of CBB (Coomassie Brilliant Blue) in 5 ml of methanol, 7.5 ml of acetic acid and 100 ml of distilled water then they were washed for 5 minutes in running tap water.

Photographs were taken using Black and White (ILFORD) film under Nikon photomicroscopes. Photomicrographs were printed on Agfa-Brovira paper. Durst M-600 enlarger was used to print the photographs. The colour prints were printed at a QSS.

3. Results and Discussion

3.1. Light Microscopic Studies

In *sargassum tenerrimum,* the plant growth occurs by mean of a three sided cell which cuts off segments from all the three sides forming a promeristematic zone. From stipe and holdfast region, three distance tissue system was observed where the first in the columnar, assimilatory meristodern, the second is the storage system consist of several cortex and the third is the conducting system consist of medulla. In contrast to mistoderm layer, the cell cytoplasm contains more than one type of polysaccharide which stain violet with TBO and also reveal a dark core and a light rim (Uriostegui-Guzman et al 2002). Some proteinous bodies also observed which occasionally fuse to form band like structures which lie at the base of the cell. Cytological studies show that comparison between the cut surface epidermal layer and the epidermis of the intact stipe, leaves and regeneration leaves showed similarity that the of the regeneration leaves (Figure 2a). The regular cell deposition was shown in Figure 2b. Fagerberg of and Dawes (1976, 1977) reported the morphology and anatomy of the regeneration tissue resembled that of the leaves of the intact plant rather than that of the stipe form which it originated(Yoshida 1997, Yokoya1996, 1997, 2000). Similar observation was seen in the present study of *S. tenerrimum*. On the other hand, when the thallus segments were cultured in seawater enriched with Von stosch's solution, the thallus segments developed several branches (Yokoya 2004). However, when the explants of *S. tenerrimum* culture in polyamines enriched cultured media, medullary cells (Figure 2c) plays an important role in the regeneration of several vegetative branches alongwith several leaves and receptacles (Chen et al.1978, Moss 1971, Uchida1993, Yan 1984),. However the phenolic compound may associated with "clotting mechanism", leads to protein precipitation and may also involved in the stabilization of water soluble polysaccharides.

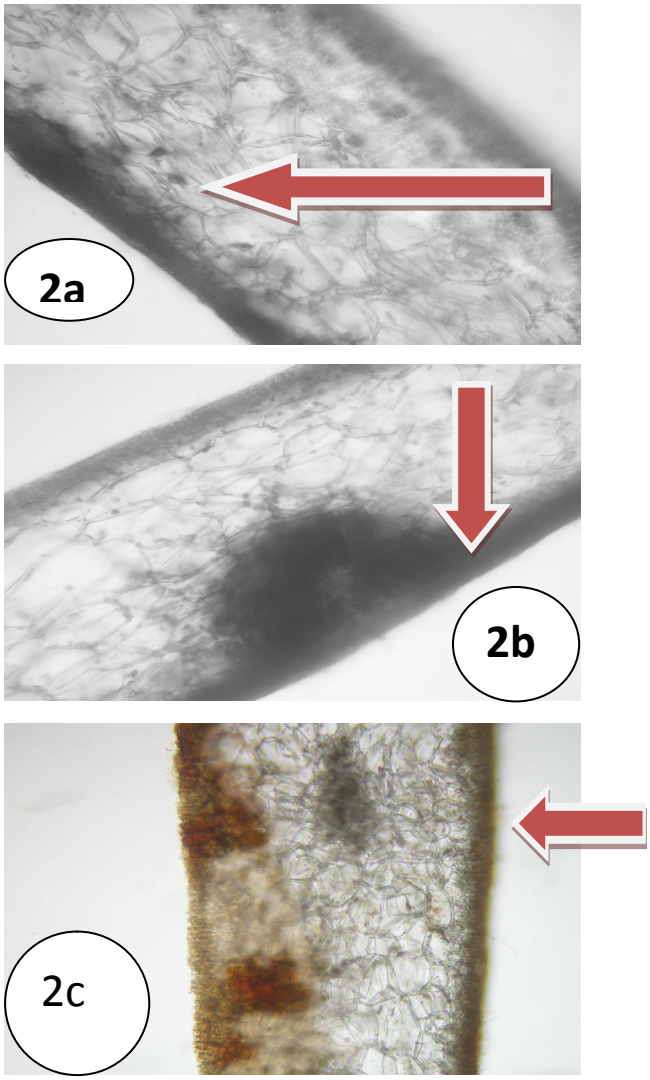

Figure 2. Light Microscopic Studies shown following impact : a) A slight deposition of cells at the epidermal layer. b) Heavy deposition of cellular deposition. c) A clump of cellular deposition at the middle layer

4. Conclusion

From the present study,. This study may also leads to micropropagation and seed stock in brown algae such as *Sargassum tenerrimum.* On the contrary, the regeneration tissue support similarities of the regeneration leaves to leaves of the intact plant/. Thus, the thallus segments of *Sargassum tenerrimum* showed growth of filaments formed by division of cortical, sub cortical and medullary cells.

Conflict of Interest: The authors declare no conflict of Interest.

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