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Changes in the pigment constituents of *Gracilaria edulis* (Gmelin) Silva cultured in open sea off Narakkal by reproductive method

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ABSTRACT

Red alga *Gracilaria edulis* was cultured by reproductive method in floating rafts in the sea off Narakkal, Kochi. Pigment constituents such as Chlorophyll-a, Phycocyanin, Phycoerythrin and Allophycocyanin of the seaweed were estimated and correlated with growth and environmental parameters such as atmospheric temperature, salinity and rainfall during the culture period. On 60th day after transplantation all pigment contents were found to be very high coinciding with heavy rainfall. Growth and pigment contents also showed changes at different depths of water column.

Introduction

Light and salinity are the major environmental factors associated with algal growth in marine environment. Light quality and quantity change with depth and also throughout the day. Light intensity determines the distribution, growth, morphology and physiological characteristics and productivity of red algae (Luning 1981; Lapointe and Duke 1984; Levy and Gnatt 1986; Friedlander *et. al.*, 1991) Salinity influences red algal growth (Rueness and Tanger, 1984; Daugherty and Bird, 1988) and also the level of photosynthetic pigments (Koach and Lawrence, 1987; Machler, 1988). Submerged aquatic plants are subjected to sharply decreasing light intensities along a depth gradient and respond to low light conditions by increasing pigments as well as accessory pigment chlorophyll ratios (Waaland *et. al.*, 1974; Rhee and Briggs, 1977; Lapointe 1981). According to Beer and Levy (1983), *Gracilaria* sp adapts to low light by increasing chlorophyll and phycoerythrin contents.

This paper gives an account of the variation in the pigment constituents at different depths in the water column during various stages of growth and also emphasizes the effect of environmental factors like atmospheric temperature, salinity, and intermittent rainfall during the culture period.

Materials and Methods

Gracilaria edulis was cultured by reproductive method in the sea off Narakkal, Kochi by installing two floating rafts at a distance of approximately 100 meters from the shore. Healthy cystocarpic plants of

Gracilaria edulis were collected from Mandapam (9°17'N and 70°11'E), southeast coast of India, cleaned off epiphytes, washed thoroughly in running seawater followed with sterilized seawater and transported to CMFRI, Kochi in enriched seawater. Enrichment was done by the standard procedure of Conway and Walne (Walne, 1974). The plants were weighed, transferred into perforated plastic packets and hung for spore liberation on the floating rafts in a randomized block design. This helped in natural collection of spores and attachment to the nylon fishing net.

The culture period was from November, 2000 to March, 2001. Regular collection of water samples and sample ropes were taken from the culture site. The culture rope was divided into 4 regions according to the depths in the water column i.e. 0-30cm, 30-60 cm, 60-90 cm and 90-120 cm. Growth, number of germlings per unit area and pigments such as chlorophyll-a, phycoerythrin, phycocyanin and allophycocyanin of plants at these 4 regions were estimated. Apical portion of the plants were cleaned thoroughly in sterilized seawater, blotted, weighed and ground in 90% acetone for extraction of chlorophyll-a. Water-soluble pigments such as phycoerythrin, phycocyanin and allophycocyanin were extracted in 0.5 M phosphate buffer (pH 6.8). Estimation was carried out by the standard procedure of Jeffrey & Humphrey (1975). Salinity of the water samples was estimated by a refractometer.

The number of germlings/unit area during different stages of culture period at various depth in the water column was found out by observing mesh cuttings (1cm² size) from the net pieces under the microscope till visible size plants appeared in the net. The size of the plants at different depths was also noted. The biofouling organisms on the sample ropes were also identified. Statistical analyses of pigments by ANOVA at different depths during different days of the culture period were undertaken.

Results

All the pigment constituents of *G.edulis* showed the highest concentration on the 60th day after transplantation. The chlorophyll content showed a marginal decline during the 45th day after which it increased reaching the peak value on the 60th day. It then declined till the 90th day and remained almost unchanged till harvest. It was observed that the chlorophyll content shows significant positive correlation with depth with maximum value at 90-120cm. (Fig.1).

As observed in chlorophyll, the phycoerythrin content also showed peak value on the 60th day, then declined sharply at all depths till the 75th day. The phycoerythrin content then increased marginally till harvest. The contents ranged between 86 and 1785 mg/g.fr.wt with maximum value at 60-90cm depths. (Fig.2) The phycocyanin showed a similar trend like chlorophyll with peak value on the 60th day at a depth of 90-120 cm. Further, it showed an irregular pattern till harvest. The phycocyanin content ranged between 70.87 and 821.44 mg/g.fr.wt. (Fig.3). The allophycocyanin showed different peaks at the 4 regions. Maximum concentration was found at 60-90 cm depths on the 60th day. Peak value at 0-30 cm depths was observed on the 75th day while at 30-60 cm depths it was observed on the 90th day. Not much variation was observed from 90-105 days, the pigment content then increased till harvest.

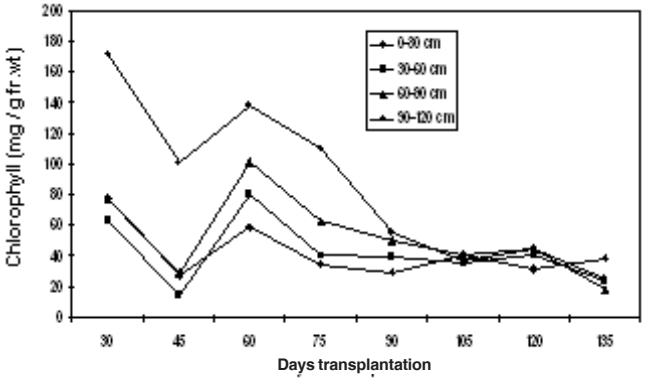

Fig. 1. *Chlorophyll-a content of* Gracilaria edulis

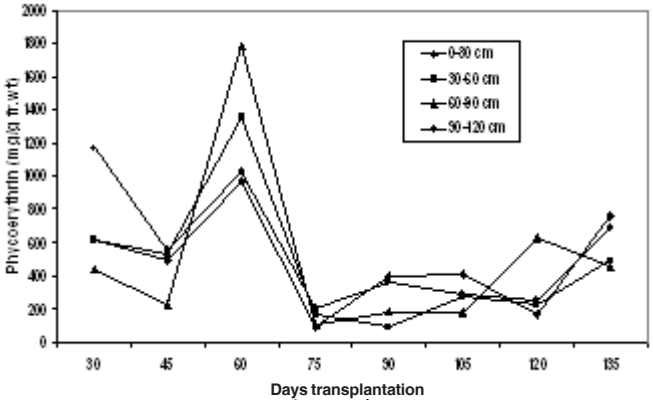

Fig. 2. *Phycoerythrin content of* Gracilaria edulis

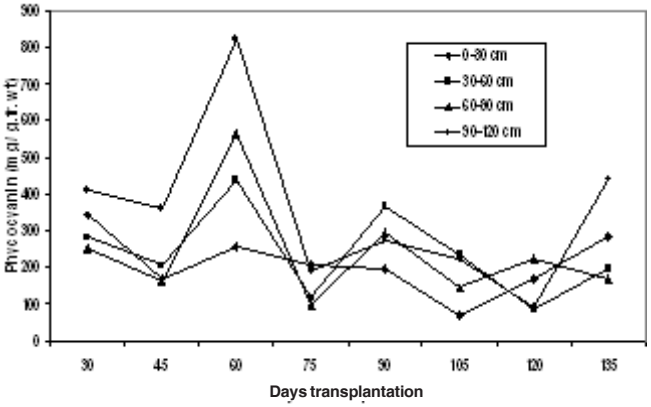

Fig. 3. *Phycocyanin content of* Gracilaria edulis

The values ranged from 152.18 to 1722.5mg/g.fr.wt (Fig.4)

Of the pigments analyzed, phycoerythrin was found to be maximum at all depths followed by allophycocyanin, phycocyanin and then chlorophyll-a. The pigment contents showed a positive correlation with depth. Peak value of all pigments was observed on 60th day, which coincides with rainfall and optimal salinity. Atmospheric temperature during the culture period varied between 28-32°C while salinity ranged from 30-34 ppt. Intermittent rainfall was observed during the 45th, 60th and 105th days of the culture period with a peak of 83 mm recorded on the 105th day (Fig.5).

The number of germlings/unit area increased along the culture period with maximum germlings observed on the 60th day after transplantation and then gradually declined as the size of the plant increased (Fig.6).

Statistical interpretation of the estimated pigments showed that chlorophyll-a was highly significant with growth and also with depth, while phycoerythrin and phycocyanin showed positive significance with growth of the plant during the culture period but was not significant along the depth gradient. Allophycocyanin did not show any significance along depth or with culture period (Table.1).

Biofouling was very much prominent on the culture ropes with heavy fouling of Amphipods, Isopods, Hydrozoan colonies, Polychaetes, Modulus, Barnacles, Bryozoans, Clams, Mussels and larvae of crustaceans and epiphytic growth of *Cladophora, Chaetomorpha* and *Centroceras.* The growth of plants was retarded due to heavy infestation of biofouling organisms.

Discussion

Peak value of all pigments was observed on 60th day. This coincides with high rainfall. The number of germlings per unit area showed maximum number of germlings on 60th day and then declined. This emphasized that the plants were in early stage of growth during this period and all the pigments levels were high during this period. Also high rainfall during this period reduced the salinity and temperature. This might have helped to increase their pigment contents to optimize their photosynthetic activity to maintain a balanced growth. According to Lapointe and Duke (1984) acclimatization to limiting light results in increased pigment levels suggesting that seaweeds maximize their photosynthetic capacity by optimizing pigment levels. Heavy infestation of fouling organisms also interfere the growth of the plants to some extent. These results are in agreement with the earlier reports. Molina *et. al.* (1991) opined that epiphytic growth interfere with light falling on the thallus thus decreasing the photosynthetic rate, the plants in turn compensate for the reduced light intensity by increasing the concentration of pigments. Epifauna reduce the algal growth because they interfere directly with light by shading and causing sinking of thalli. Mussels, amphipods and egg masses cause clumping of thalli (Cancino *et. al*., 1987)

During the heavy rainfall (83 mm) encountered on the 105th day, not much variation was observed in the pigment constituents and it remained almost constant till harvest. The plants were almost in mature stage which reduce further change in pigments level. Epiphytic growth was very scanty and also maximum growth rate of plants was observed during this period, which continued till harvest. Heavy rainfall during this period optimized the salinity (30 ppt) for proper growth of the plants. It may also be explained here that the reduction in salinity might have helped to reduce biofouling unlike observed on the 60th day.

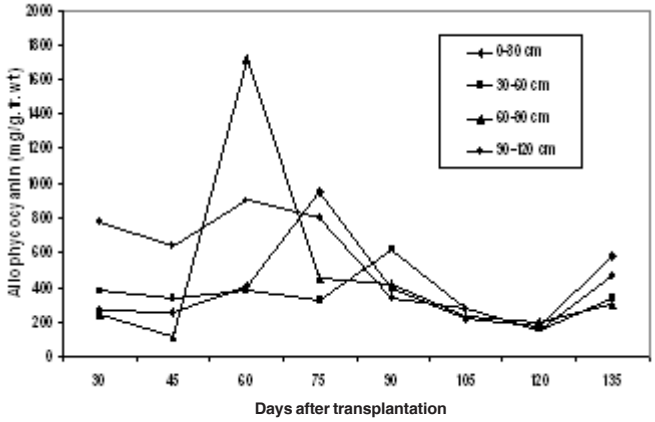

Fig. 4. *Allophycocyanin content of* Gracilaria edulis

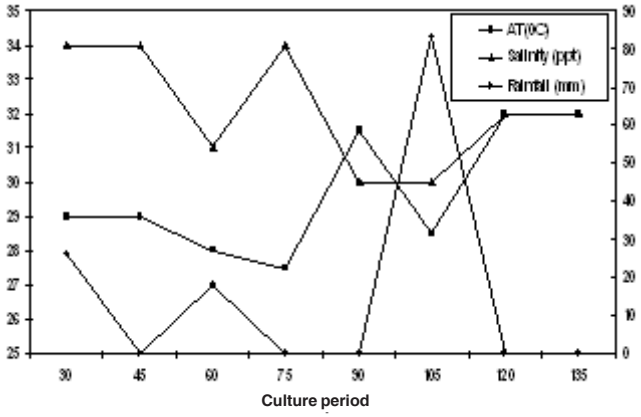

Fig. 5. *Environmental parameters during culture of* Gracilaria edulis

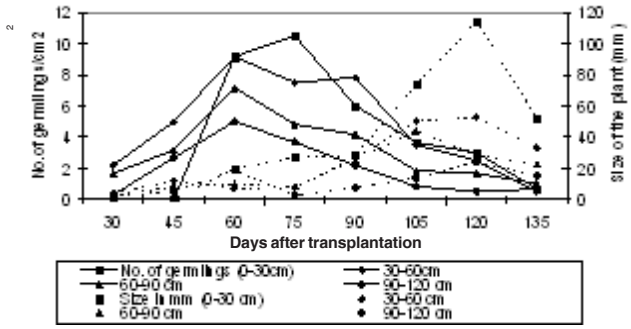

Fig. 6. *No. of germlings / unit area and size of the plant*

Table 1. *Annova : Two-factor without replication*

ANOVA for chlorophyll

rce of Varia	SS	df	MS	F	P-value	F crit
Rows	13494.32	6	2249.053	6.01059	0.004196	2.996117
Columns	4151.379	2	2075.689	5.547275	0.01968	3.88529
Error	4490.182	12	374.1818			
Total	22135.88	20				

ANOVA for phycocerythrin

rce of Varia	SS	df	MS	F	P-value	F crit
Rows	3227211	6	537868.5	12.20135	0.000169	2.996117
Columns	11803.58	2	5901.788	0.13388	0.875983	3.88529
Error	528992.4	12	44082.7			
Total	3768007	20				

ANOVA for phycocyanin

rce of Varia	SS	df	MS	F	P-value	F crit
Rows	472878.4	6	78813.07	8.081554	0.001177	2.996117
Columns	53193.3	2	26596.65	2.727241	0.105596	3.88529
Error	117026.6	12	9752.218			
Total	643098.3	20				

ANOVA for allophycocyanin

rce of Varia	SS	df	MS	F	P-value	F crit
Rows	1174912	6	195818.7	2.020404	0.141226	2.996117
Columns	147392.4	2	73696.2	0.760377	0.488745	3.88529
Error	1163047	12	96920.59			
Total	2485352	20				

In the present experiment it was observed that pigment constituents increase with depth, which is in conformity with the work carried out by Reeta and Sally (2000). In the present study pigment constituents at different depths showed variation, which in turn is reflected on the appearance on the plants. Plants grown in the lower depth appeared darker than that grown near the surface. According to Brody and Brody (1962) and Rhee and Briggs (1977) red alga changes its colour in relation to Chl/PE ratio.

Acknowledgement

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Literature cited

Beer, S and I. Levy 1983. Effects of photon fluence rate and light spectrum composition on growth, photosynthesis and pigment relation in *Gracilaria* sp. *J. Phycol.,* 19 : 516 - 522.

Brody, M. and S. S. Brody 1962. Induced changes in the photosynthetic efficiency of *Porphyridium cruentum*. *Arch. Biochem. Biophys.,* 96:354-359.

Cancino, J. M; M. Munoz and M. C. Orellana 1987. Effects of epifauna on algal growth and quality of the agar produced by *Gracilaria verrucosa* (Hudson) Papenfuss. *Hydrobiologia,* 151/152:233-237.

Daugherty, B. K. and K.T. Bird 1988. Salinity and temperature effects on agar production from *Gracilaria verrucosa* strain G-16. *Aquaculture,* 75:105-113.

Friedlander, M., M. D. Krom and A. B. Amotz 1991. The effect of light and ammonium on growth, epiphytes and chemical constituents of *Gracilaria conferta* in outdoor cultures. *Bot. Mar.,* 34: 161-166.

Jeffery, S. W. and G. F. Humphrey 1975. New spectrophotometric equations for determining chlorophyll-a, b, c and C^2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz,* 167 : 191 - 194.

Koach, E. W. and J. Lawrence 1987. Photosynthetic and respiratory responses to salinity changes in the red alga *Gracilaria verrucosa*. *Bot. Mar.,* 30 : 327 - 330.

Lapointe, B. E. 1981. The effect of light and nitrogen on growth, pigment content and biochemical composition of *Gracilaria foliifera v.angustissima (*Gigartinales, Rhodophyta) *J.Phycol*., 17 : 90 - 95.

Lapointe,B.E. and C.S.Duke 1984. Biochemical strategies for growth of *Gracilaria tikvahiae (*Rhodophyta) in relation to light intensity and nitrogen availability. *J.Phycol.,* 20 : 488 - 495.

Levy, I and E.Gnatt.1986. Light acclimation in *Porphyridium purpureum* (Rhodophyta) growth, photosynthesis and phycobilisome. *J.Phycol.,* 24 : 452 - 458.

Luning, K. 1981. Light. In : *The biology of seaweeds*. C.S.Lobban and J.Wynne (Eds.), Blackwell Scientific Publ., Oxford . pp. 326 -355.

Machler, B. A., 1988. Salinity effects on photosynthesis, carbon allocation and N2 assimilation in the red alga *Gelidium coulteri. Plant Physiol*., 88 : 690 - 694.

Molina, Cancino,J. M. and Montecino, V. 1991. Photosynthetic pigments shifts in *Gelidium rex* (Rhodophyta) induced by the epibiont Membranipora tuberculata (Bryozoa). *Rev. Chil. Hist. Nat.,* 64 (2) : 289-297.

Reeta Jayasankar and Sally Varghese, 2000. Growth and pigment constituents of the red alga *Gracilaria edulis* (Gmelin) Silva, grown at different depths. *Indian J. Fish.*, 47 (4) : 365 - 369.

Rhee,C. and W.R.Briggs 1977. Some responses of *Chondrus crispus* to light. I. Pigmentation changes in the natural habitat. *Physiol. Plant*, 43 : 35 - 42.

Rueness, J. and T. Tanger 1984. Growth in culture of four red algae from Norway with potential for mariculture. *Hydrobiologia*, 116 / 117 : 303 - 307.

Waaland, J.R., S.D.Waaland and G.Bates 1974. Chloroplast structure and pigment composition in the red alga, *Griffithsia pacifica:* regulation by light intensity. *J.Phycol.*, 10 : 193 - 199.

Walne, P.R. 1974. *Culture of bivalve molluscs: 50 years experience in Conway.* Fishing News (Books) Ltd.Surrey. 173 pp.