INFLUENCE OF DIFFERENT WAVELENGTHS OF LIGHT ON PHOTOSYNTHESIS AND PIGMENT CONSTITUENTS AND ABSORPTION SPECTRA OF *GRACILARIA* SPP.

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ABSTRACT

Three species of *Gracilaria* such as *G.edulis, G. crassa* and *G. corticata* were exposed to different broad band of lights. Red (> 600 nm), green (450-610 nm) and blue (390-570 nm) lights were provided to the plants kept in different growth chambers maintained with constant temperature of 28°C and photoperiod of 16:8 h light and dark period. The plants maintained under WL were considered as control. Photosynthetic activity and chlorophyll content were found to increase in all the species during 6th day of observation. Plants maintained GL exhibit higher concentration of chlorophyll in *G.edulis* and *G. corticata*. Prolonged period of treatment (12 days) showed less chlorophyll in above treatment but increased under RL. The photosynthetic activity declined in all the treatments on 6th day but retard significantly in all the treatments on 12th day. Plants maintained under red light possess higher P_0 activity. *G.corticata,* which possesses higher P_0 activity initially showed maximum reduction in activity in all the treatment but under red light, the plant still maintains to photosynthesize in a reduced rate. The decline in P_0 activity may not be due to the spectral effect but may relate to the decline of dissolved carbon in the seawater or other environmental parameters like decline in nutrient contents or change of pH. The observed better P_0 activity in red light may be due to high quantum yield and increase in chlorophyll content might induce the PSII activity and carbon fixation.

Key Words: *Gracilaria*, wavelength, photosynthesis, pigments and absorption spectra.

Light plays a very important role as a factor controlling plant morphology (Dring and Luning, 1983; Dring, 1988). Most reports analyze the effects of light quality on growth, morphology and pigmentation (Mathieson and Burns, 1975). The relationship between pigmentation and growth in different light qualities is not well understood and very little information is available for macroalgae (Figueroa *et al.*, 1995). Long term cultivation in either blue or red light produce adverse effects in few green and brown algae (Clause, 1970; Schmid, 1984; Wennicke and Schmid, 1987). For red seaweed very little work

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is being carried out on the spectral effect of growth performance or metabolism. Leukart and Luning (1994) demonstrated that green light at very low intensity (0.5 µmol m^{-2} s^{-1}) was more effective than red and blue light for germling growth in several red algae. The better growth rate in red than blue light of *Porphyra umbilicalis* was probably due to high photosynthetic efficiency and quantum yield (Figueroa *et al.*, 1995). Figueroa *et al.* (1994) showed that oxygen production was three times higher in algae grown for three weeks in red light than blue light at 70 µmol m^{-2} s^{-1}. The low photosynthetic efficiency of red algae in blue light has been well known from the photosynthetic action spectrum (Haxo and Blinks, 1950; Luning and Dring, 1985). The present work was aimed to find out the spectral effect on photosynthetic activity and pigment constituents of some *Gracilaria* species.

MATERIALS AND METHODS

Different species of *Gracilaria* such as *G. edulis, G. crassa* and *G. corticata* were collected from Gulf of Mannar near Mandapam (9°17´N & 70°11´E to 79°E). They were brought to the laboratory, washed thoroughly in seawater followed by filtered sterilized seawater. To avoid the transportation stress, the plants were kept in a growth chamber maintained at a temperature of 28°C, light intensity of 1000 lux and photoperiod of 16:8 light and dark cycle. After 24 hours of incubation, the healthy plants were sorted out and kept separately in 51 glass aquarium tanks with enriched seawater. The plants were exposed to different broad wavelength of light such as red (>600 nm), green (450-610 nm) and blue light (390-570 nm) in a growth chamber in different racks. Plants kept under white light were used as the control. Observations were made on before treatment (BT), 6 days after treatment (6DAT) and 12 days after treatment (12DAT). Enriched medium was changed after 6 days.

Absorption spectra of the thallus were recorded at room temperature (25°C) using Hitachi 557 spectrophotometer. The ground glass sides of matched cuvettes were kept in the light path so that reference and the sample beams are scattered to the same extent. The slit width of the measuring beam was narrowed down to 2.0 nm.

The oxygen electrode carried out determination of the photosynthetic rate. The apical portion of the plants were hung from the top inside the cylindrical oxygen electrode chamber (Hansatech, UK) containing 2 ml of filtered seawater. Saturated white light of 100 W/m^2 was passed through a round bottom flask (10 cm diameter water bath) from the slide projector (Photophone Ltd., India) before illuminating the chamber. The water inside the cylindrical tube was stirred continuously by a magnetic stirrer. The amount of oxygen evolved was monitored continuously at 25°C. Mean of three consecutive readings were taken for calculation. Rate of photosynthesis was expressed as µmol O_2 evolved/g fresh weight/h.

Estimation of pigments such as Chlorophyll a (chl), Phycoerythrin (PE), Phycocyanin (PC) and Allophycocyanin(APC) were carried out by the standard procedure of Jefferey and Humphrey (1975).

RESULTS

G. edulis, G. crassa and *G. corticata* were exposed to different broad band of lights Blue (BL), Red (RL), Green (GL) and White (WL) in a growth chamber. The changes in their photosynthetic activities and pigment compositions were as follows.

The Chl content of *G. edulis* increased in all treatments after 6 days of exposure. Maximal increase was noticed under green light compared to white light and red light. After 12 days of treatment, there as a marginal decline in Chl content in WL but significantly under GL. Whereas under BL and RL there was a gradual increase in Chl content. In *G. corticata* the Chl content increased to a greater extent compared to *G. edulis.* Maximum increase was noticed under GL followed by WL, RL and BL. On 12 days of treatment further increase of Chl pigment was noticed under BL and RL. Both WL and GL had caused a decrease. In *G.crassa,* the Chl content declined in all treatments except a marginal increase of 7.4% under RL. Maximum decline of 58.5% was noticed under WL (Fig. 1).

PE content of *G. edulis* registered an increase on 6th day of treatment in all the samples. It varied from 21% in WL to 61.5% in GL. On the 12th day of treatment, the PE content declined in GL but increased in all other light treatment. RL and BL had increased the PE content by 14.3% and 34.2% respectively. However, GL exhibited a 20.5% decline over the control on 12 days of treatment. In *G. corticata,* the PE content showed an increase in GL, followed by RL and BL but it declined in WL on the 6th day. Further, on 12 days of treatment, PE content declined under WL and BL. Under RL, there was only marginal decline (2.6%) of PE pigments. Whereas in GL it continued to increase further with reference to control. All the treated samples showed more PE content on 6th as well as 12th days of treatment and maximum was noticed under GL. In *G. crassa,* initially the PE content increased marginally in all the treatment conditions except under RL where it showed a decline by 17%. WL had produced an increase of 32.5% on 6th day of treatment. Further on 12th day, the PE content increased in all the treated samples except under WL where a decline by 32.1% was observed. With reference to control, the PE content was found to be less by 23% in GL, 25.2% in BL and 37.4% in RL on 6th day but on 12th day all the treated samples had more PE than the control. Maximum increase was noticed under GL (34.0%) followed by RL (20.3%) and BL (15.0%) (Fig. 1).

Phycocyanin content was also found to be more in all treatment on the 6th day of observation in *G. edulis* and *G. corticata.* With reference to control, the

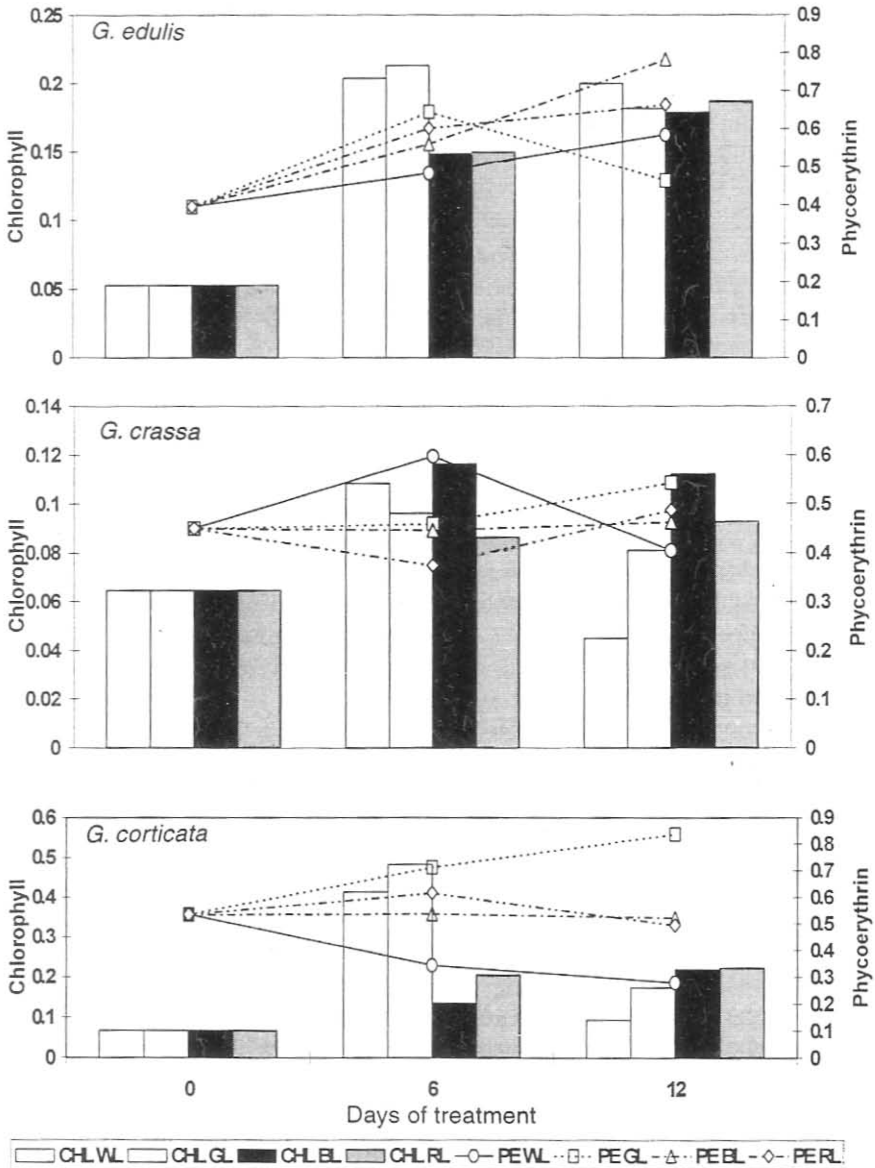

Fig. 1. Chlorophyll and phycoerythrin contents of *Gracilaria* species (expressed in mg/g FW).

PC content was found to be more in treated samples. Maximum increase was observed under GL followed by BL and RL. On the 12th day, the PC content declined in all the treated samples except under RL, which exhibited a further increase. WL caused marginal decline of 2.8% compared to 25% and 12.7% in GL and BL respectively. On the 12th day, *G. corticata* also registered a decline of PC content in all treatment conditions except for a marginal increase under BL. Maximum reduction was noticed under RL compared to WL and GL. Both *G. edulis* and *G. corticata* showed decline of PC content under all treatment conditions. Maximum decline was noticed under BL.

In *G. crassa,* the PC content was found to be more under WL than under GL but BL and RL decreased in levels by 8.2% and 9.6% respectively. On 12th day, this trend was reversed reducing PC content in WL and GL but increasing under BL and RL. With reference to control, the treated sample exhibited more PC content. BL registered the maximum increase compared to other treated samples (Fig. 2).

APC content of *G. edulis* gradually increased in all treated samples till 12th day. The plant exhibited a decline of 20.6% on 12th day under GL with reference to the level on 6th day. However, in general, the APC content increased in all treated samples with reference to 0 day. In *G. corticata* the APC content was found to be maximum in GL on 6th and 12th day of treatment. The APC content continued to increase in GL till 12th day of treatment whereas it declined in BL and RL. Recovery in APC content was noticed in control.

In *G. crassa,* the APC content was found to be maximum in WL on 6th day but on 12th day, GL treated samples showed maximum APC content compared to others. Initially, there was an increase of APC content in all the treated samples compared to 0 day of treatment. Such increase was maximum under WL (49.9%) compared to the marginal increase in other treatment. Further treatment declined the APC content only under WL by 30.6% but continued to increase in other treatments (Fig. 2).

The photosynthetic activity was found to be maximum in *G. corticata* among the three species of *Gracilaria.* Upon exposure to different light conditions, *G. edulis* exhibited an increase in activity in WL, GL and BL but declined by 19.9% under RL on 6th day. Maximum increase was observed under WL. On 12th day, the activity was found to be reduced in all samples except those under BL. In *G. corticata,* maximum decline of photosynthetic activity was noticed in RL. In *G. crassa,* the photosynthetic rate increased in WL and GL on 6th day but declined thereafter. Under BL, the activity continued to decline gradually till 12th day (Fig. 3).

The absorption spectrum of fresh incubated thallai of *G. edulis, G. crassa* and *G. corticata* showed many peaks at 676, 621, 565, 495 and 433 nm representing Chl, PE, PC, APC and carotenoids respectively. The peak at 541 nm was more prominent for *G. edulis* compared to the other two species. In *G. edulis* under WL, the peak at 433 nm was highly elevated forming more number of peaks from 433 to 495 nm. The peaks at 495 nm was shifted to 500 nm in WL on 12th day. The peak at 541 nm was most prominent under RL than in other treated samples. The peak at 565 nm was shifted to 561 nm in WL and 676 to 678 nm in RL (Fig. 4).

G. crassa showed differences in spectra after treatment. On 6th day, shoulders were noticed at 450 nm in RL which were more prominent under

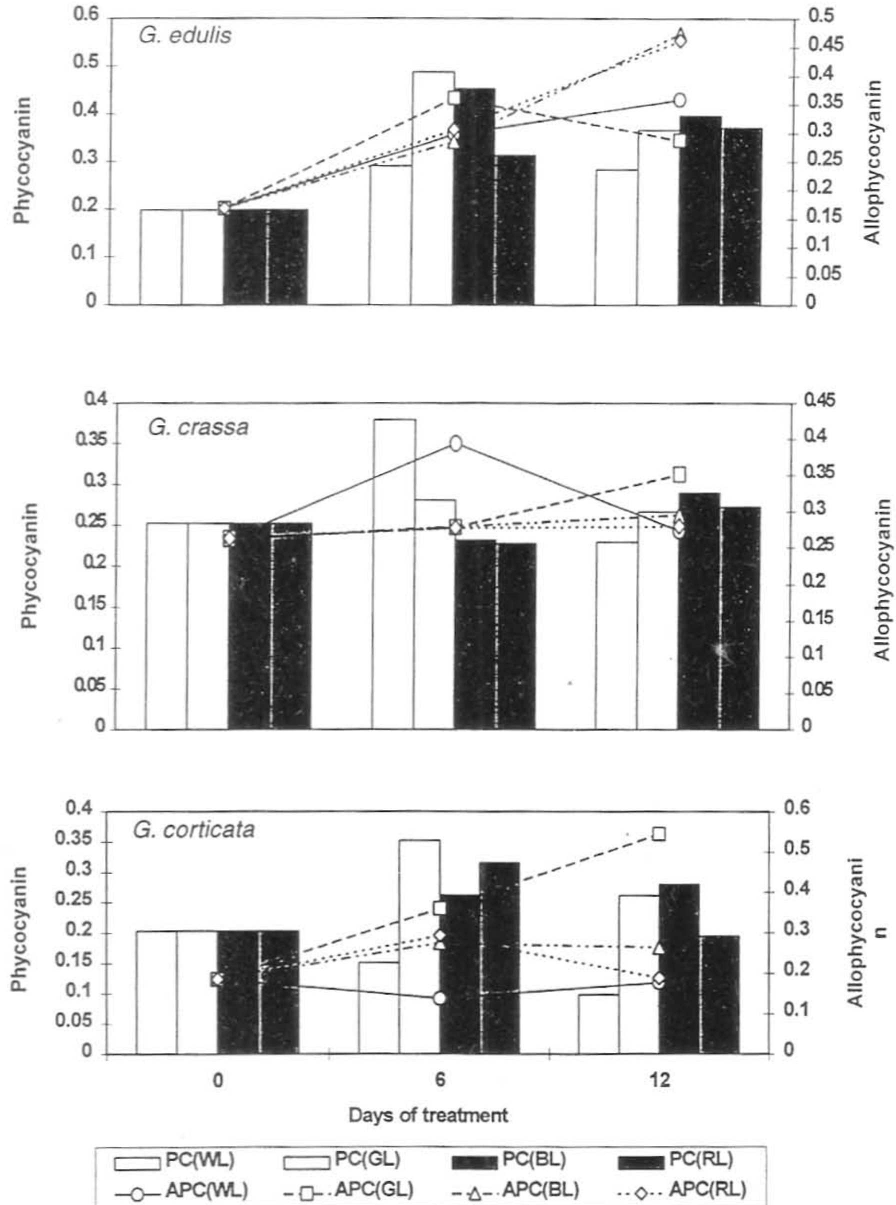

Fig. 2. Phycocyanin and allophycocyanin content of *Gracilaria* species (express in mg/g FW).

WL. The trough between 433 and 495 nm were found to be flat under GL and BL. On 12th day, the spectrum under RL declined sharply below 420 nm with a prominent shoulder at 450 nm. The peak at 495 nm was negligible with a flat peak at 621 nm under WL. The trough between 433 and 495 nm was

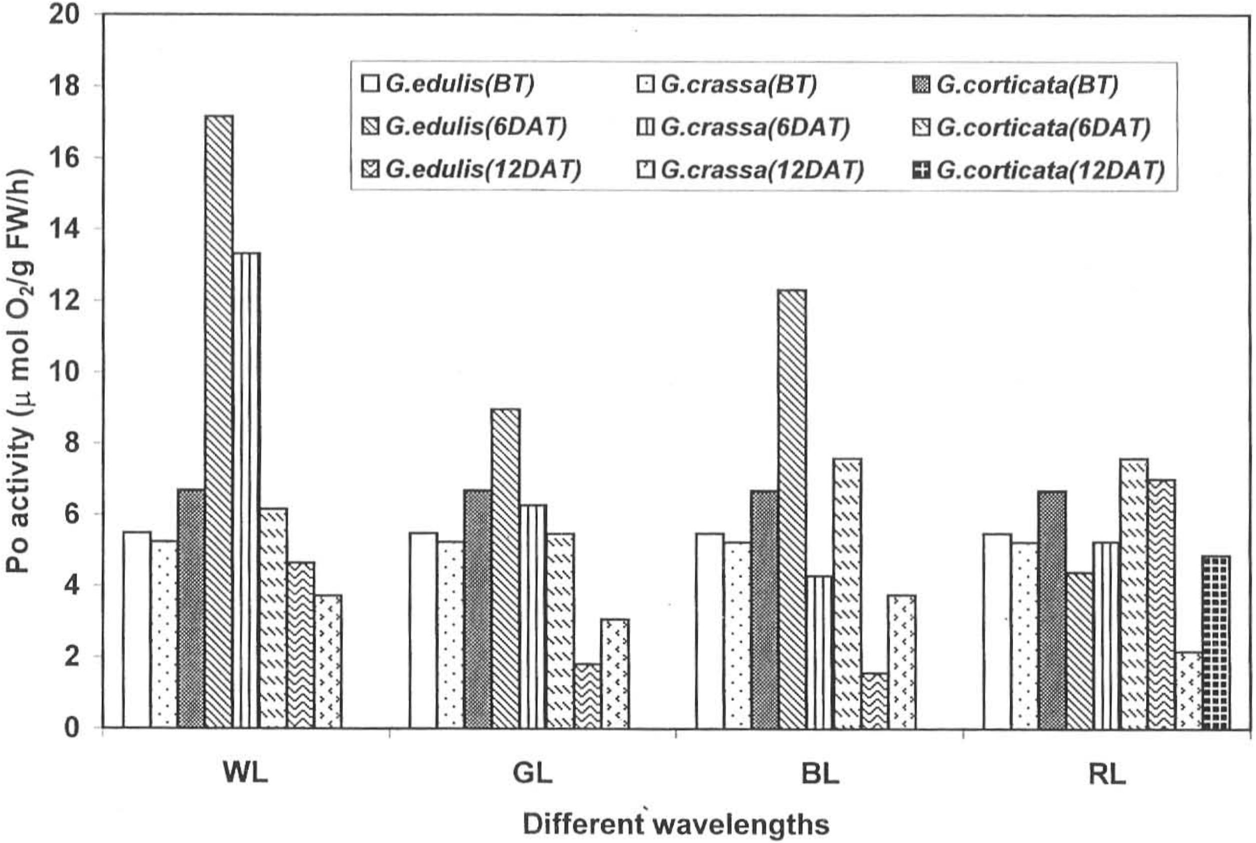

Fig. 3. Photosynthetic activity of *Gracilaria* species (average value of three replicates are expressed in μmol O_2/g FW/h)

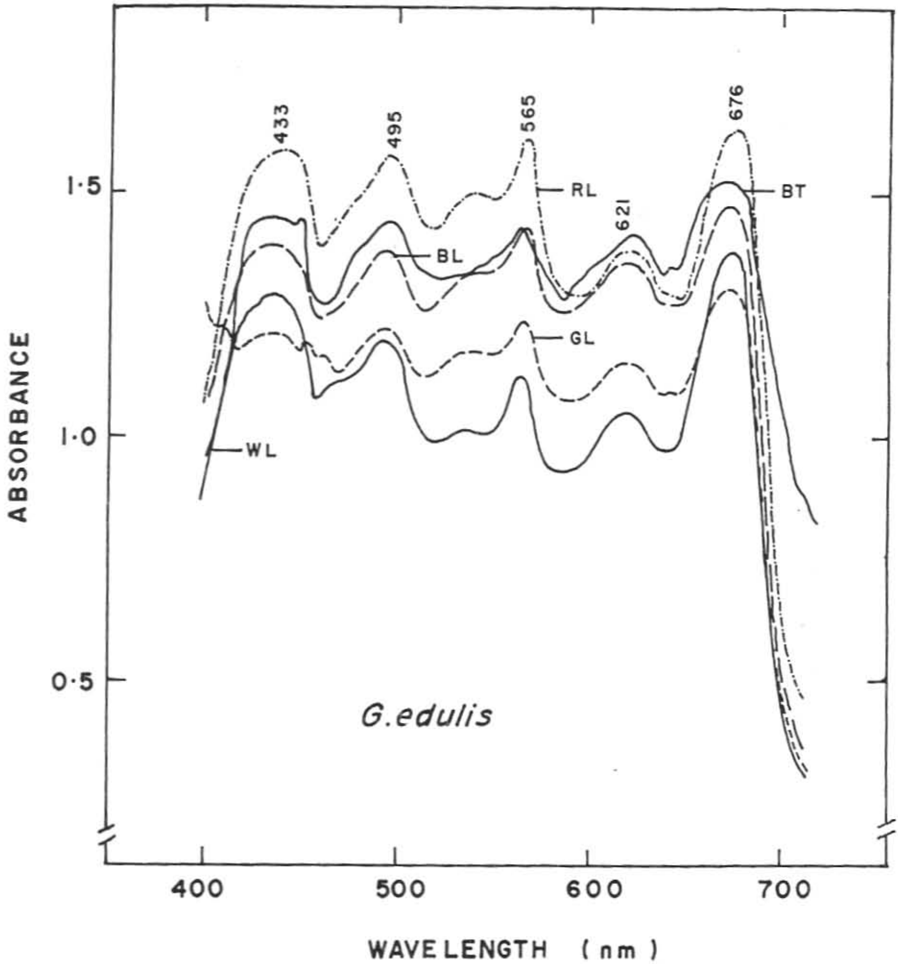

Fig. 4. Room temperature absorption spectra of fresh thalli of *Gracilaria edulis* under different wavelength of light.

conspicuously flat. The elevation at 541 nm was prominent under WL and RL but absent in GL and BL (Fig. 5).

In *G. corticata,* the spectrum was smooth with prominent elevation at 541 nm in untreated samples. Upon treatment for 6 days, RL dominated the peaks at 676, 495 and 433 nm. The humps at 541 nm was not as prominent as those in 0-day sample and absent under WL. BL treatment produced number of peaks and crest between 433 and 495 nm. A prominent shoulder was noticed at 450 nm in WL. RL treatment shifted the 676 peak to 672 nm. BL treatment caused sharp decline of absorbance at 400 nm. Maximum decline

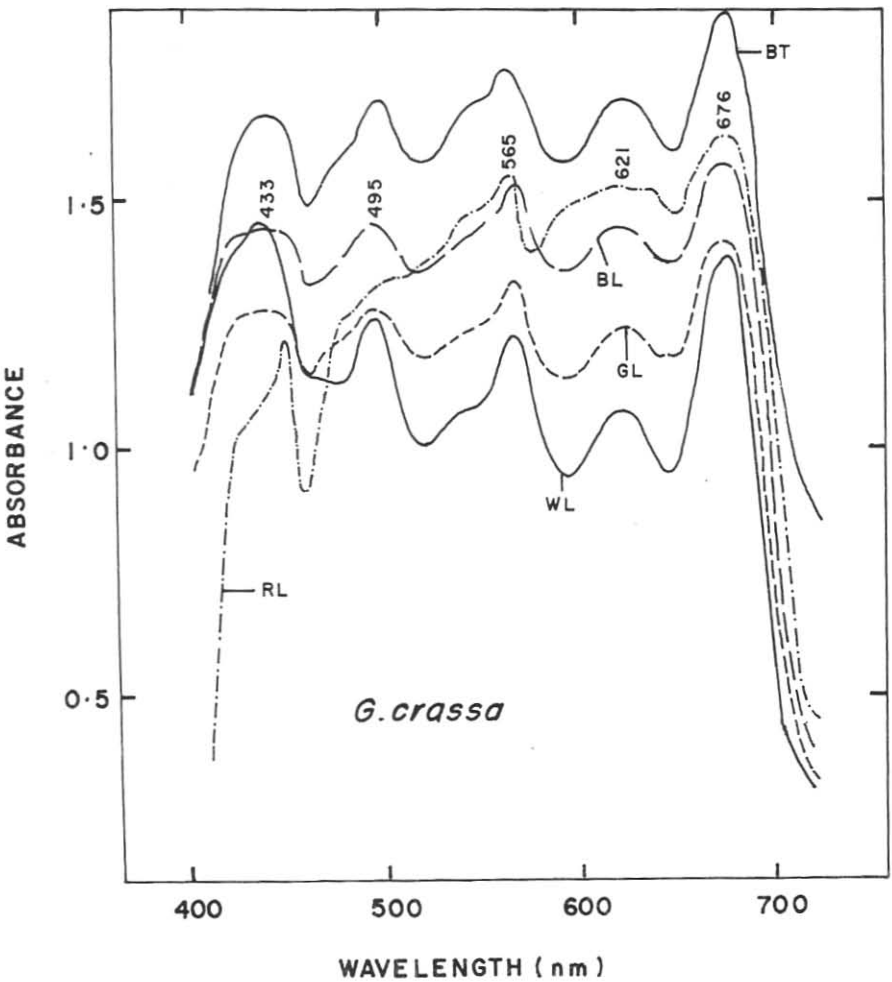

Fig. 5. Room temperature absorption spectra of fresh thalli of *Gracilaria crassa* under different wavelength of light.

was noticed under WL. On 12th day, the spectrum remained almost similar except for the presence of flat trough between 433 and 495 nm in RL and WL. The peak at 676 nm was shifted to 670 nm in WL and GL. RL exhibited the shift of peak from 621 to 615 nm (Fig. 6).

DISCUSSION

The theory of complementary chromatic adaptation suggests that red seaweeds are best adapted for life in the deep sea because of inclusion of PE

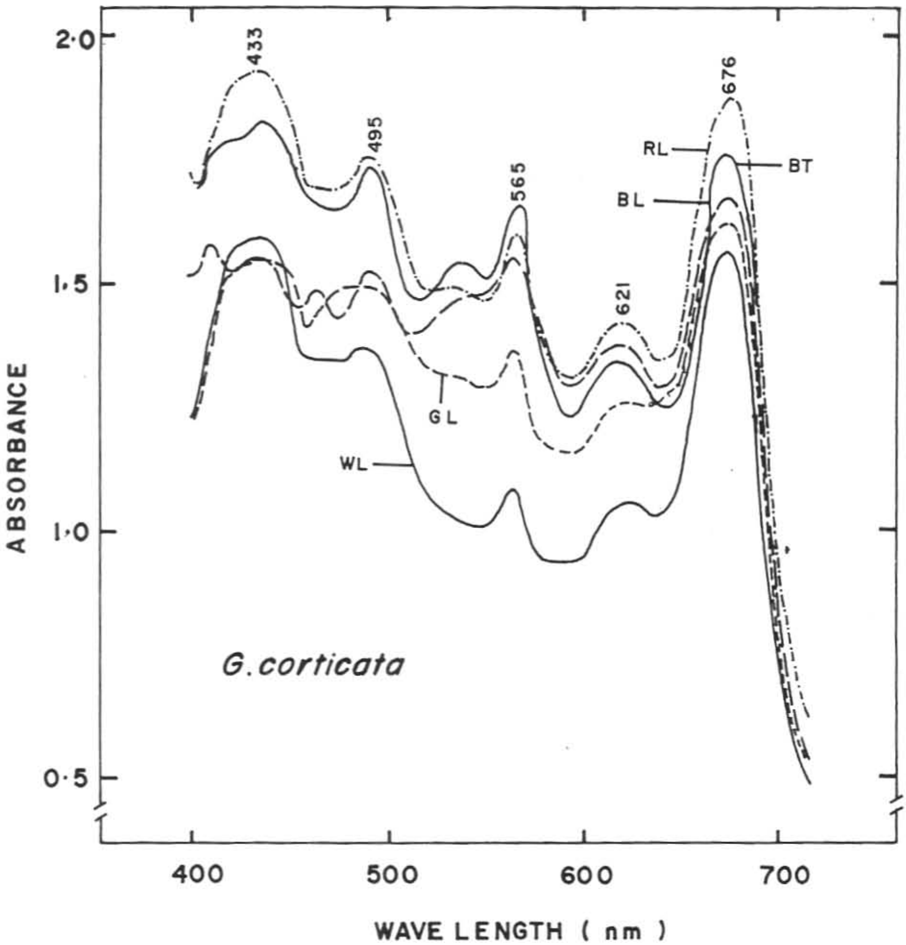

Fig. 6. Room temperature absorption spectra of fresh thalli of *Gracilaria corticata* under different wavelength of light.

in the photosynthetic unit. The mechanism involved in this process is well studied in cyanobacteria. However, there is still controversy about the occurrence of complementay chromatic adaptation in red algae.

In the present study, all the three species of *Gracilaria* exhibited different activities under spectral influence. The photosynthetic activities of *G. edulis* and *G. corticata* were influenced by the blue light in the initial stage but prolonged treatment retarded the activity. *G. edulis* and *G. corticata* retained the ability to maintain photosynthetic activity under red light. The growth of the thallus was also more prominent under this treatment. Plants were highly elongated and branched. Similar results were reported in other red algae such as *Porphyra, Palmaria palmata* (Luning, 1992) and *Gracilaria* (Beer and

Levy, 1983). Earlier workers suggested that red light favoured thallus expansion, cell division, carbon accumulation and its deposition in cell walls and intercellular matrix. Figueroa *et al.* (1995) opined that the thalli grown under red light showed smaller protoplasmic area and ample intercellular matrix, which are morphological indications that polysaccharide production is favoured. Figueroa *et al.* (1994) explained that oxygen production was three times higher in algae growing under red light than blue light. Similar observations were made in the present study. *G. corticata,* which presumed to exhibit photorespiratory activity in all the spectral range, showed photosynthetic activity under red light even after 12 days of treatment. No adverse effects of long time cultivation in red light were observed for *Porphyra umbilicalis,* which indicates that this spectral range serves both photosynthetic and morphogenetic demand of growth. In *G. crassa,* the photosynthetic activity was less in all treated samples including under red light. It may be due to the fleshy and rigid thallus structure of *G. crassa.*

Chlorophyll content of *G. edulis, G. crassa* and *G. corticata* were found to be higher in all light treatment for 6 days. Prolonged treatment retarded the pigment content in BL, WL and GL but RL enhanced the pigment content. Figueroa and Niell (1989) demonstrated the involvement of phytochrome in the control of chlorophyll synthesis in red algae, *Porphyra umbilicalis.* Like other algae, BL also helps in enhancement of chlorophyll content in *G. edulis, G. corticata* and *G. crassa.* In green algae chlorophyll synthesis is regulated by specific BL photoreceptors (Senger and Bauer, 1987).

The accessory pigments were found to be higher under GL in all the species of *Gracilaria* after 6 days of treatment but prolonged treatment exhibited reduction of all accessory pigments in *G. corticata* but increased under red, blue and green light in *G. edulis* and *G. crassa.*

The observed better growth and photosynthesis under RL than BL may be due to high quantum yield in the former. The spectral range for BL used may not be optimal for photosynthesis because only the PSI was well supplied with photons while PSII was inadequately served. Increase in chlorophyll content under red light might induce PSII activity and carbon fixation although the accessory pigments are comparatively less. Continued photosynthetic activity in *G. corticata* under RL may support the hypothesis that chloroplast activity and long wavelength light are critical for pigment biosynthesis in *G. corticata.*

The absorption peaks of the spectra of plants maintained under different light quality showed shift of peaks at 565, 621 and 676 nm, which were attributed to the change in the accessory pigments. In all the species of *Gracilaria,* it was observed that the peaks under red light were more in height. *G. crassa* and *G. corticata* showed prominent shoulders at 450 nm and several peaks and troughs between 433 and 450 nm that have been attributed to changes in the compositions of chlorophyll as observed by Smit and Alberte (1994). The absorbance change at 515 nm has been attributed to a number of components (Chlorophyll and carotenoids) functioning in the electron transport chain near PSII. This may partly indicate a change in membrane potential. Thus the change at 515 nm presumably shows participation of carotenoids in photosynthesis of algae.

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