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Effect of Light Intensities on the Fluorescent Yield of *Gracilaria* spp.

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Abstract

The fluorescent kinetics of *Gracilaria* spp. showed variation among different species when subjected to varying treatment of light intensities. In control (untreated fresh sample), the variable fluorescence and the quantum yield were maximum in *G. corticata* than *G. edulis* and *G. crassa*. Upon exposure to different treatments for 12 days, it was observed that the values of fluorescent yield increased in all treated samples of *G. edulis* and such increase was maximum only in the sample kept under intermediate light (2.0 w/m^2). In *G. crassa*, the variable fluorescence and the quantum yield increased under intermediate light. Further treatment resulted in decline of the above value under high light (3.0 w/m^2) and intermediate light intensities. In *G. corticata*, the Fv and the Fv/Fm values declined within 6 days of treatment but marginal recovery occurred on 12th day of treatment.

Key words: Light intensity, fluorescent yield, *Gracilaria*

Introduction

The productivity of macrophytes is influenced by multiple environmental factors as well as morphological structure of the plants (Chapin *et al.*, 1987). Several methods such as ^{14}C uptake, oxygen evolution and ATP changes are being adopted to study the productivity. It is reported that variable chlorophyll fluorescence under a strong excitation light saturate the photosynthesis and produce a typical oscillatory pattern of fluorescence (Kulandaivelu and Daniell, 1980; Samuelsson and Qquist, 1977). Of many possible applications of chlorophyll fluorescence to investigation of photosynthesis, the ratio of room temperature, dark-adapted variables to maximum fluorescence (Fv/Fm) is widely used to evaluate the changes in the quantum yield from PSII. The time course of fluorescence induction kinetics of the dark-adopted sample shows very fast and slow components. It changes in a time duration ranging from some nanoseconds to a few minutes depending on the process of energy migration within the antennae and the photosystems and finally the dark reaction or the carbon fixation.

In common, with higher plant studies, light stress effects on photosynthesis in macroalgae are often assayed as a function of changes in O_2 evolution and chlorophyll fluorescence parameters (Scheiber *et al.*, 1994). Except for Chlorophyta, very few works were carried out on the fluorescence behaviour of marine algae, which pointed out that they are divergent from the higher plants. The Pulse Amplitude Modulated (PAM) fluorescence techniques are particularly attractive for higher plants and utmost care should be taken while applying the same to macroalgae. Few data are available which explicitly demonstrate correlation between the variable fluorescence, quantum yield and the oxygen evolution in macroalgae, measured under identical conditions (Hanley *et al.*, 1991 a&b; Franklin *et al.*, 1992; Hanelt *et al.*, 1992, 1995). This work is aimed to find out the physiological status of *Gracilaria* when transferred from the natural bed to controlled environmental condition and exposed to different light intensities.

Material and Methods

Geographical conditions

Tamil Nadu, situated on the southeast coast of India and Mandapam, lies between Lat. 09^{0}17' N and Long. 78^{0}08' E, in between Palk Bay and Gulf of Mannar. Considering the location specificity two places namely Thonithurai and Pudumadam were selected for the collection of seaweed. Both the places lie 8 km apart from each other on the Gulf of Mannar but exhibit diverse sea conditions. Thonithurai is sparsely rocky and sea bottom is muddy, covered with seagrass bed. *Gracilaria edulis* and *G. crassa* are found abundantly in the intertidal area of this coast. The chain of islands situated in the Gulf of Mannar protects strong wave action of the sea in this area. Pudumadam is often exposed to strong wave action, has a rocky coast and sandy bottom. *G. corticata* grows very well in this region.

Morphology

These three species of *Gracilaria* exhibit wide variation in their morphology. *G. edulis* is cylindrical, regularly branched either di or polychotomous. *G. corticata* is dichotomously branched, thick, flattened and cartilagenous in nature. *G. crassa* is cylindrical with club shaped or oblong articulation in dichotomously and irregularly branched thallus. It forms a dense cushion on the substratum growing mostly on small pebbles and dead gastropod shells.

Methodology

Fresh algal samples of *G. edulis, G. crassa* and *G. corticata* were collected from respective locations during low tide in the morning. They were thoroughly washed in filtered seawater and transported to the laboratory in enrich medium at an optimal temperature of 25^0C. Samples were maintained in the growth chamber at 25^0C with 16:8 h light and dark cycle to overcome the transportation stress. The algae were exposed separately to different light intensities (3w/m^2, 2w/ m^2 and 0.5w/ m^2) in a growth chamber at 25^0C for 12 days. The algae were kept in small glass aquarium tank of 5 l capacity with 3 l of enriched seawater medium (Walne, 1974). The medium was changed at weekly intervals. Observations on fluorescent transient were taken on 0, 6 and 12 days of treatment.

Fluorescent transient

In vivo chlorophyll a fluorescent transient were followed in intact thallus after a brief period of incubation in dark. The plant samples were excited with broad band blue light (400-620 nm, corning, CS4-96) at a PFD of 100 w/m^2. The photomultiplier (Shimatzu R 376) placed at 90^0 to the excitation beam was protected by interference filter (λ max 690 nm, half band width 12 mm, Schott, Germany). The signal from the photomultiplier was directly displayed either on a recorder (Hitachi model 056) or stored in the digital oscilloscope (Iwatsu SR 100, Japan). The signal was triggered with the help of an electric shutter with an opening time of 10 milliseconds. Thalloid samples were placed diagonally in a 4 ml glass cuvette to face the photomultiplier at 45^0 angle.

Results

PSII is measured in two conditions, *viz.*, after a period of complete darkness when all the primary acceptor of the photosystem II (QA) molecules are fully oxidized (PSII centers open) and then again after all QA molecules are fully reduced (PSII center closed, Fm) by saturating pulse of light. The variable difference between these two fluorescence states, Fv, is compared with maximum fluorescence (Fig.1). Typically in an unstressed green plant, the Fv/Fm value is found to be 0.82 (Buchel and Wilhelms, 1993).

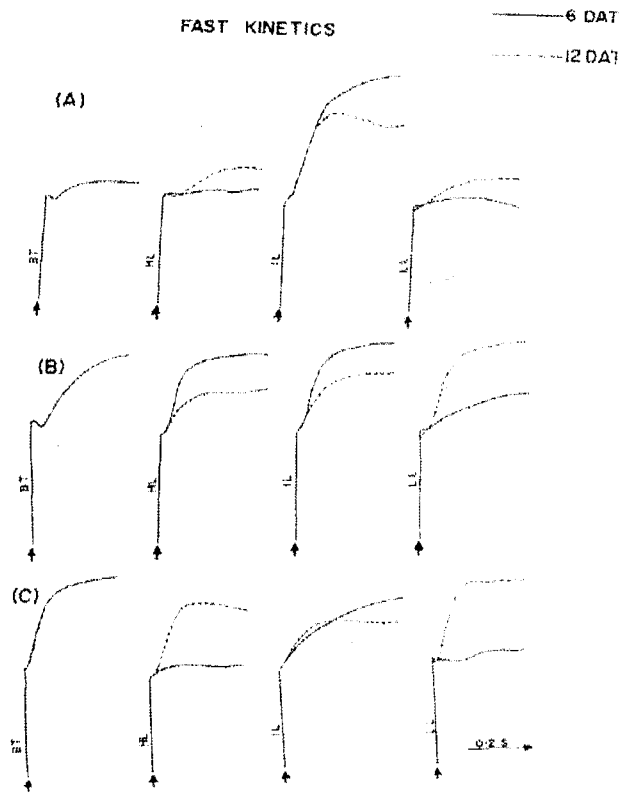

Fig. 1. The fast kinetics of *Gracilaria* spp. (A) *G. edulis*, (B) *G. crassa* and (C) *G. corticata* after exposure to the actinic light for 0.2 second

The magnitude signal and shape are influenced by atleast four different parameters *viz.*, applied intensity of actinic light, ratio of scattered to absorbed light, concentration of chlorophyll and quantum yield.

While comparing the fluorescent transient of all the three different species of *Gracilaria* subjected to a brief period of exposure to darkness (45 minutes), it was observed that the fluorescent yield was maximal in *G. corticata* (the sample before exposed to any treatment) followed by *G. crassa* and *G. edulis*. When the plants were exposed to different light intensities, wide variation was noticed among different species, different treatments and their duration. The variable fluorescence was found to be more under intermediate light in all the species of *Gracilaria*. *G. edulis* possessed the highest value of Fv followed by *G. crassa* and *G. corticata*. The quantum yield also exhibited the similar trend on 6 days of treatment. On 12^{th} day although *G. edulis* maintained to have more variable fluorescence and the quantum yield over 0 day of treatment it declined by 19% over 6^{th} day. In other species the quantum yield declined by 19-26% (Fig.2).

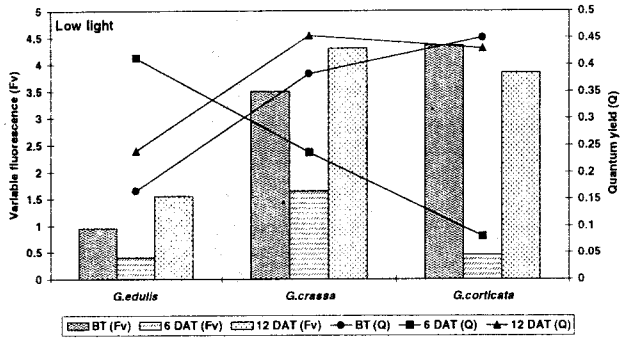

Fig.2. Effect of low light intensity on the fluorescent transient of *Gracilaria* species with reference to variable fluorescence and quantum yield

In *G. crassa*, the variable fluorescence was found to be more under intermediate light and declined by 23.7% on 12th day. Marginal variation was noticed among all the treatment in *G. crassa*. The quantum yield increased by 18% under low light within 0-12 days of treatment (Fig.3). *G. corticata*, which is considered as the highly photosynthesizing species, possessed higher value of Fv and quantum yield on 0 day of treatment and drastically reduced when exposed to high light intensity for 6 days. It showed a recovery on 12th day of treatment but in general there was a reduction of quantum yield by 5%. Under intermediate light there was a gradual decline in the fluorescent transient and quantum yield from 0-6 days and again 6-12 days of treatment. Similarly, under high light, there was a marked decline in the variable fluorescence and quantum yield on 6th day of treatment which recovered to a marked extent on 12th day (Fig.4).

It is understood from the experiment that under stress condition, the photosynthetic signal show an immediate response as observed in *G. edulis* and *G. corticata* under high and low light intensities. Prolonged treatment showed the physiological adaptation of the plant to particular stress and marginal recovery was noticed on long-term exposure to light intensities. The plant exhibited gradual decline in the fluorescent transient value in all the species of *Gracilaria* on 0-6 days and 6-12 days of treatment under intermediate light except in *G. crassa*, where the quantum yield increased to a greater extent on 6 days of observation. The decline may be significant as the plant was kept in an artificial environment, might have affected as physiological stress of dissolved carbon and nutrients.

In slow kinetics, which was subjected for a time span of one minute showed a different trend in the activity. After the onset of light a fast transient in few milliseconds can be observed after the origin 'O' passing though intermediate state 'I' and a dip 'D'. The difference of peak and terminal value after exposing the plants to one minute actinic light explain the efficiency of dark reaction in photosynthesis (Fig.5).

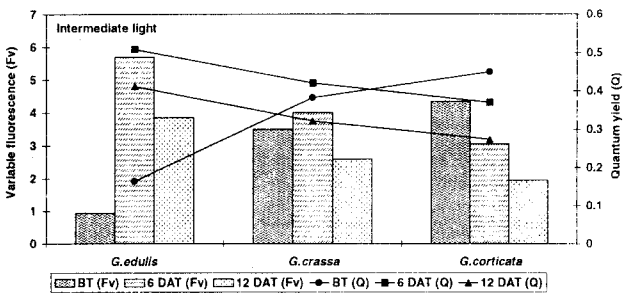

Fig.3. Effect of intermediate light intensity on the fluorescent transient of *Gracilaria* species with reference to variable fluorescence and quantum yield

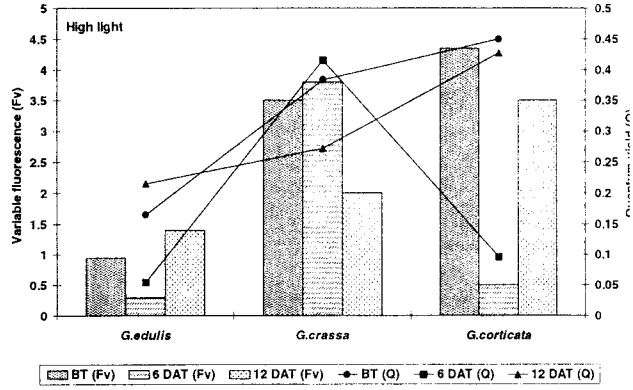

Fig.4. Effect of high light intensity on the fluorescent transient of *Gracilaria* species with reference to variable fluorescence and quantum yield

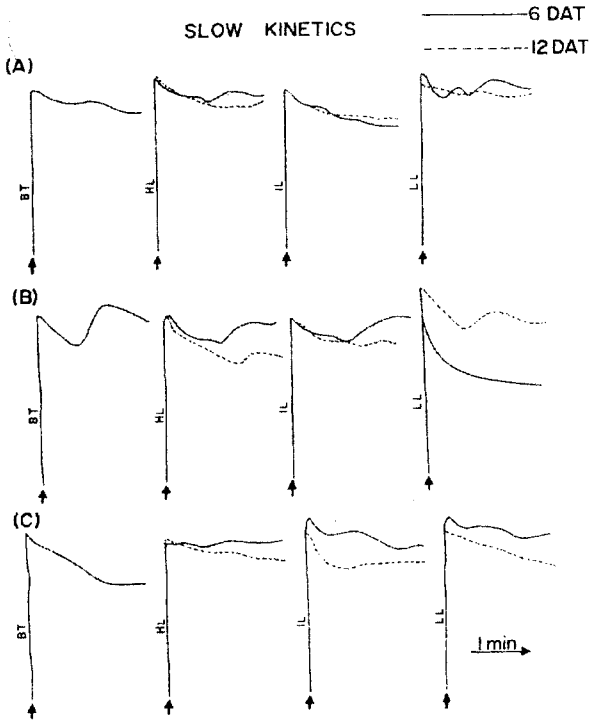

Fig. 5. The slow kinetics of *Gracilaria* spp. (A) *G. edulis*, (B) *G. crassa* and (C) *G. corticata* after exposure to the actinic light for 1 minute

In the present experiment, *G. corticata* showed higher efficiency than *G. edulis* and *G. crassa* on 0 day of treatment. Under exposure to high light intensity, all the species showed drastic reduction in the transient value within 6th day of treatment but recovered marginally on 12th day. Under intermediate light, *G. edulis* showed an increase in the photosynthetic efficiency of the dark reaction and reduced by 18% when exposed continuously for 12 days (Fig.6). *G. crassa*, on the other hand exhibited very less peak and terminal value of Po activity on 6th day and increased drastically on 12th day. Similar is the observation under high light treatment (Fig.7). In *G. corticata* dark reaction was pronounced under intermediate light and increased marginally on 12th day of treatment. Under low light, *G. crassa* possessed a higher Po efficiency than *G. corticata* or *G. edulis* and reduced by 61% when exposed for a prolonged period.

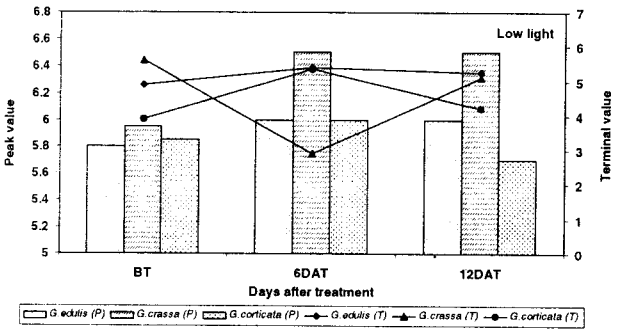

Fig.6. Effect of low light intensities on the slow fluorescent transient of *Gracilaria* spp.

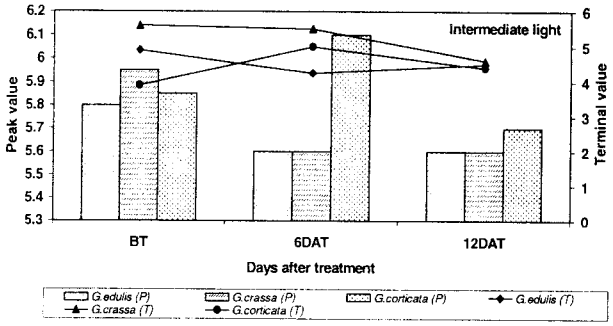

Fig.7. Effect of intermediate light intensities on the slow fluorescent transient of *Gracilaria* spp.

While comparing the fluorescent transient with the pigment composition of *Gracilaria* species, it was observed that the pigment content such as chlorophyll, phycoerythrin, phycocyanin and allophycocyanin were found to be more in *G. corticata* than *G. edulis* and *G. crassa* (Table 1). It is well understood that red algae posses a large extrinsic phycobilisomes mainly connected to PSII whereas small intrinsic antenna for PSI (Morschel and Schatz, 1988). Due to large antennae system, red algae posses a very high Fo value compared to Fm leading to decrease in value of Fv/Fm ratio (Bose *et al.*, 1988; Hanelt *et al.*, 1992). Similar observation was noticed in the present experiment.

From the above experiment, it is well understood that exposure to intermediate light can enhance the fluorescent transient in *G. corticata* and *G. edulis* whereas *G. crassa* shows more efficiency in Po activity under low light than high or intermediate light condition.

Discussion

The physiological status of the plant not only depends on their habitat but also on the morphology and the multiple environmental factors. *G. corticata* being exposed to strong wave action and high light intensities, it is morphologically adapted for higher photosynthetic activity. It is also expected that marine algae in natural environment have no limitation of available dissolved carbon as the thallus is always exposed to continuous exchange of seawater. However, transferring the plants from their natural habitat to the controlled environmental condition may impose severe stress on the plant (Harder *et al.*, 1998). Limitation of dissolved carbon, change in pH and deficiency of nutrients finally affect the photosystems. This type of water stress reduces the Po activity and influence fluorescence behaviour of the plants.

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Table 1. Effect of light intensities on the pigment constituents of *Gracilaria*

Species	Light intensity	Chlorophyll			Phycoerythrin			Phycocyanin			Allophycocyanin		
		BT	6 DAT	12 DAT	BT	6 DAT	12 DAT	BT	6 DAT	12 DAT	BT	6 DAT	12 DAT
G. edulis	HL	0.0982	0.0613	0.1151	0.1278	0.2087	0.1699	0.0501	0.0799	0.0628	0.0473	0.0791	0.0622
	IL		0.0837	0.0845		0.1806	0.2929		0.0781	0.1976		0.0526	0.0958
	LL		0.0956	0.0776		0.2112	0.2273		0.0703	0.0956		0.0743	0.0542
G. crassa	HL	0.0333	0.0641	0.0262	0.1265	0.1858	0.1378	0.06	0.078	0.0515	0.0404	0.125	0.0629
	IL		0.0581	0.0448		0.2058	0.1943		0.071	0.1264		0.1077	0.1048
	LL		0.0789	0.0713		0.1791	0.2658		0.0741	0.156		0.1936	0.1024
G. corticata	HL	0.1161	0.1508	0.1075	0.3122	0.3345	0.1578	0.1932	0.1791	0.0558	0.13	0.2123	0.0915
	IL		0.1595	0.1382		0.3609	0.2005		0.1645	0.0897		0.2674	0.0604
	LL		0.1516	0.0938		0.3719	0.1993		0.0786	0.0675		0.3676	0.0782

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