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Effect of ultra violet radiation on pigments profile of seaweeds *Gracillaria edulis* and *Hypnea musciformis*

P. IYAPPARAJ*, G. IMMANUEL, R. RAMASUBBURAYAN, P. ESAKKIRAJ, S. SANKARALINGAM, M. NAVIN CHANDRAN and A. PALAVESAM

Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629 502 (India).

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ABSTRACT

Effect of different wavebands of ultraviolet radiation (UV–A and UV–B) on pigments profile of south Indian macroalgae *Gracillaria edulis* and *Hypnea musciformis* was assessed under *invitro* conditions. In general, the Chlorophyll – a (Chl a) was higher than the Chlorophyll – b (Chl b) content in the candidate seaweeds. The long wavelength UV–A progressively increased Chl a, Chl b and total chlorophyll content correspond to the increase in exposure duration in both the seaweeds. But the short wavelength UV–B considerably reduced the chlorophyll pigments. The total carotenoid content of the selected seaweed species exposed to UV–A and UV–B irradiation was found to be increased notably as a measure of photo protective function to guard the photosynthetic apparatus from damage and to resist the photo oxidation.

Key words: UV-A, UV–B, Chlorophyll, Carotenoid, Macroalgae.

INTRODUCTION

Seaweeds are the sedentary plant biota of sea, one of the major contributing communities in marine primary producers. Especially, algae are adapted well to survive in intertidal zone where as it has to face the drastic change in the incident solar radiation and variation in climate to the greater extent because algae are uncovered during low tide or floating near the water surface. The above variation in physiological parameters leads to strong photo inhibition in algae (Hanelt, 1992; Henley *et al.,* 1992; and Hanelt *et al.,* 1994a). Intertidal zone is potentially vulnerable zone to photo inhibition or damage by photosynthetically active radiation or UV radiation (Wood, 1987). Mainly the ecology of seaweed is attributed with their ability to absorb and efficiently manage the incident radiant energy (Luning, 1990).

The stratospheric depletion of ozone layer results in increased levels of incident ultraviolet radiation on the crust and aquatic bodies also not excluded to this. Despite of more anthropogenic and natural destruction is leading to larger depletion in ozone layer results in enhanced UV-B radiation on earth. Both UV-A (315-400 nm) and UV-B (280-320 nm) radiation are capable of penetrating the water column to an ecologically significant depth (Calkins and Thordardottir, 1982; Smith *et al.,* 1992). On comparison with other organism, the effects of UV radiation on plants are huge (Bornman and Teramura, 1993; Holm-Hansen *et.al.,* 1993). The intertidal algae may possess photo adaptive mechanism to minimize the damage by solar UV and plants of sub tidal zone are more sensitive than the plant species of intertidal zone (Polne and Gibor, 1982).

Still now, conflicting reports arise about the regulatory effects of UV-A radiation. Hashimoto and Tajima (1980) and Biswal *et al*., (1997) found inhibition of total chlorophyll and carotenoid contents induced by UV-A. Promotory effects of UV-A on the synthesis of Chl and carotenoids were also reported by Senger and Schmidt (1986) and Rau

and Schrott (1984). Photo repair and photo reactivation processes may be stimulated by radiation in the blue and UV-A spectral regions which activate photolyase (Sutherland 1981, Pang and Hays, 1991).

In total solar energy, 1.5% was constituted by UV-B radiation and showed its impacts on biological systems (Teramura *et al.,*1980). Photosynthesis (Bischof *et al.,* 1999; Brouwer *et al.,* 2000) and growth (Aguilera *et al.,* 1999; Altamirano *et al.,* 2000) reflected due to the acclimation of seaweeds to UV-B exposure to a certain extent. Physiologically sensitive indicators can recognize UV-B related stress at an early stage (Cordi *et al.,* 1997, 1999). So far, only scanty data have been collected on effects of UV radiation on macro algae from in situ experiment and few field studies have been done in Arctic region (Hanelt *et al.,* 1997a; Aguilera *et al.,* 1999; Brouwer *et al.,* 2000 and Bischof *et al.,* 2001). In the light of the above, the present work has been carried out to investigate the impact of UV irradiation on pigments profile of marine macroalage *G. edulis* and *H. musciformis.*

MATERIAL AND METHODS

Experimental samples

Marine macroalgae *G. edulis* and *H. musciformis* were collected from the rocky shore with the depth about 1.0 to 1.5 metre at Leepuram, Kanyakumari District, Tamilnadu, India (Lat. $8^0$06' 46.1" and Long. $77^0$33' 21.9"). Immediately after collection, they were brought to the laboratory and were washed thoroughly in sterile seawater to remove the adherent particles and debris etc. Then the seaweeds were kept individually in the enriched seawater medium of Provasoli (1968) for further study.

Experimental setup

The seaweeds in the seawater enriched medium are divided into three groups, one control group and two experimental groups. The control group is kept in normal light of 1000 lux and the experimental groups were subjected to UV–A (320 – 400nm) and UV–B (280 – 320nm) treatments respectively using the UV chamber (ADVANCE, SLW 6W, India) for a period of 8h. The experimental samples were collected at an interval of every 2h.

Estimation of chlorophyll

Determination of chlorophyll a, b and Total chlorophyll

The amount of chlorophyll contents of the seaweeds were estimated by the method of Arnon (1949). 500 mg of experimental sample was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well. The homogenate was centrifuged at 500 x g for 15 minutes and the supernatant was stored. The pellet was re-extracted by repeated washing with 5 ml of 80 % acetone till it become colourless. All the extracts were pooled and utilized for chlorophyll determination and the absorbance was measured at 645 nm and 663 nm in a UV visible spectrophotometer (Techomp 8500, Hongkong).

Estimation of total carotenoids

For carotenoid extraction, a gram of experimental samples were macerated individually with acetone : water (9 : 1). The obtained homogenate was centrifuged at 500 x g for 20 minutes in order to obtain clear supernatant. The extracted carotenoid sample was diluted to appropriate volume so as to obtain the optical density value of 0.8 or less. For that, the same solvent system used for the carotenoid extraction was used. After proper dilution, the sample was centrifuged and the clear supernatant obtained was used to measure carotenoid by taking optical density at 444 nm (Rodriguez-Amaya , 1993).

Absorption spectra (λ max)

The carotenoid samples extracted from the respective experimental samples were also used for absorption spectral analysis. The samples were scanned from 350 nm to 500 nm in a UV-visible Spectrophotometer by follwing the methods of Rodriguez-Amaya (1993).

Statistical analysis

The experiment and control set were repeated at least three times for each pigment analysis and the results were compared to the control through the one way and two way analysis of variance described by Zar (1974).

RESULTS AND DISCUSSION

In the present study, the effect of UV-irradiation on major photosynthetic pigments such

as Chl a, b, total chlorophyll content, carotenoids content and its profile of marine macroalgae *G. edulis* and *H. musciformis* were investigated. The results revealed that, the short wave length UV–B was more adverse than the long wavelength UV–A radiation. Usually UV–A radiation exhibits both positive and negative effects on plant photosynthesis (Wellmann, 1983); whereas, UV–A radiation activates gene expression for photosystem (PS II), reaction centre proteins (Christopher and Mullet, 1994) and also it inflicts damage to photosynthetic apparatus (Joshi *et al.,* 1997; Turcsanyi and Vass, 2000). Yet today, conflicting reports were raised about the regulatory effects of UV–A radiation. Hashimoto and Tajima (1980) and Biswal *et al.,* (1997) found inhibition of total chlorophyll and carotenoids content induced by UV–A radiation. The promoting effects of UV–A radiation on the synthesis of chlorophyll and carotenoids were also reported by Senger and Scmidt (1986) and Rau and Schrott (1984).

In the present study, *G. edulis* during UV–A exposure, chlorophyll a and b contents were

Table 1: Effect of UV irradiation on chlorophyll content of *Gracillaria edulis*

Conditions	Duration of exposure (h)	Chlorophyll pigments (µg/g)		Total Chlorophyll (µg/g)
		a	b	
Control	0	1.25 ± 0.020	0.32 ± 0.016	1.77 ± 0.033
UV-A	2	1.63 ± 0.012	0.75 ± 0.028	2.70 ± 0.020
	4	1.97 ± 0.033	0.91 ± 0.012	3.42 ± 0.045
	6	2.15 ± 0.044	1.18 ± 0.033	4.19 ± 0.012
	8	2.71 ± 0.061	1.47 ± 0.045	5.45 ± 0.033
UV-B	2	1.01 ± 0.016	0.43± 0.020	1.69 ± 0.020
	4	0.96 ± 0.028	0.27 ± 0.028	1.37 ± 0.020
	6	0.72 ± 0.008	0.16 ± 0.044	0.97 ± 0.028
	8	0.54 ± 0.012	ND	0.63 ± 0.012

ND : Not Detected ; Each value is a mean of triplicates

Table 2: Effect of UV irradiation on chlorophyll content of Hypnea musciformis

Conditions	Duration of exposure (h)	Chlorophyll pigments (µg/g)		Total Chlorophyll (µg/g)
		a	b	
Control	0	2.83 ± 0.045	0.94 ± 0.032	3.94 ± 0.008
UV-A	2	3.06 ± 0.044	1.20 ± 0.037	4.53 ± 0.020
	4	3.68 ± 0.020	1.56 ± 0.028	5.57 ± 0.016
	6	3.91 ± 0.033	1.79 ± 0.020	6.19 ± 0.028
	8	4.15 ± 0.061	2.02 ± 0.016	6.78 ± 0.044
UV-B	2	2.53 ± 0.069	0.86± 0.032	3.53± 0.016
	4	2.08 ± 0.020	0.52 ± 0.028	2.83 ± 0.020
	6	1.74 ± 0.033	0.33 ± 0.020	2.28 ± 0.016
	8	1.32 ± 0.045	0.11 ± 0.008	1.47 ± 0.020

Each value is a mean of triplicates

remarkably enhanced and reached maximum at the end of the experiment (8th h). The increase in UV–A exposure duration subsequently increased the chlorophyll content over the control. In general, the Chl a content was higher than the Chl b content. The Chl a content increased form 1.25 ± 0.020 to 2.71 ± 0.061µg/g. Chl b content also enhanced from 0.32 ± 0.016 to 1.47 ± 0.045 µg/g. Like wise the total chlorophyll content also enhanced from 1.77 ± 0.033 to 5.45 ± 0.033 µg/g (Table 1). Influence of UV–A exposure duration was statistically significant ($p<0.05$) for Chl b and it was not statistically significant for Chl a ($p>0.05$) where as it was statistically more significant ($p< 0.0001$) for total chlorophyll content.

In *H. musciformis* also enhanced chlorophyll pigments were noticed during UV–A exposure. The exposure duration, increased the chlorophyll pigments (Chl a, b and total chlorophyll content). Chl a content increased from 2.83 ± 0.045 to 4.15 ± 0.061 µg/g and also Chl b is enhanced from 0.94 ± 0.032 to 2.02 ± 0.016 µg/g. Similarly, total chlorophyll content also increased from 3.94 ± 0.008 to 6.19 ± 0.028 µg/g (Table 2). The influence of UV–A exposure duration on Chl a and Chl b content were not statistically significant ($p> 0.05$) and for total chlorophyll content it was statistically more significant ($p< 0.0001$). For both *G. edulis* and *H. musciformis*, chlorophyll (a, b and total chlorophyll) content registered an increasing trend. Similar result was also reported by Dohler (1998).

UV–B radiation known to affect a wide range of functional aspects including gene variations (Jordan *et al.,* 1996), biochemical and physiological changes (Eswaran *et al.,* 1993), behaviour and ecological (Behrenfeld *et al.,* 1994) systems in photosynthetic organisms. Effect of UV–B radiation in marine plants reported to affect the PS II activity (Worrest, 1983), DNA damage (Karentz *et al.,* 1991a) and inhibition of Rubisco activity (Lassar *et al.,* 1994). Changes in the levels of photosynthetic pigments in (Kappaphycus) seaweed exposed to UV–B radiation was also investigated by Eswaran and Subba Rao (2001).

Table 3: Effect of UV irradiation on carotenoid profile of *Gracillaria edulis* and *Hypnea musciformis*

Conditions	Duration of exposure (h)	*Gracillaria edulis*		*Hypnea musciformis*	
		λmax	Name of the carotenoids	λ max	Name of the carotenoids
Control	0	430	Zeaxanthin	370	Unknown
				442	Violaxathin
UV-A	2	430	Zeaxanthin	370	Unknown
				439	g-carotene
	4	430	Zeaxanthin	370	Unknown
				439	g-carotene
	6	430	Zeaxanthin	370	Unknown
				411	Unknown
	8	430	Zeaxanthin	370	Unknown
				430	Zeaxanthin
UV-B	2	430	Zeaxanthin	370	Unknown
				430	Zeaxanthin
	4	430	Zeaxanthin	430	Zeaxanthin
				470	Neoxanthin
	6	430	Zeaxanthin	430	Zeaxanthin
				470	Neoxanthin
	8	430	Zeaxanthin	370	Unknown
				470	Zeaxanthin

In *G.edulis*, the Chl a, b and total chlorophyll content declined during UV–B exposure and it showed a significant linear trend with exposure duration. The Chl a content significantly ($p<0.05$) decreased from 1.01 ± 0.016 to 0.54 to 0.012 µg/g. Likewise, the Chl b content also significantly ($p<0.05$) decreased from 0.43 ± 0.020 to 0.16 ± 0.044 µg/g at sixth hour of exposure and during 8th hour, no Chl b content was assessed. The total chlorophyll content also expressed a declining trend from 1.69 ± 0.020 to 0.63 ± 0.012 µg/g (Table 1) which was statistically more significantly ($p< 0.0001$).

In *H. musciformis*, the UV–B irradiation reduced the Chl a content from 2.53 ± 0.069 µg/g to 1.32 to 0.045 µg/g and the Chl b content from 0.86 ± 0.032 µg/g to 0.11 to 0.008 µg/g at the end of the experiment. Total chlorophyll content was also decreased remarkably with subsequent increase in UV–B exposure duration from 3.53 ± 0.016 to 1.47 ± 0.020 µg/g (Table 2). Influence of UV–B exposure duration on Chl b was statistically significant ($p<0.05$) and for chlorophyll a it was not significant ($p<0.05$) where as the decrease in total chlorophyll content was statistically more significant ($p< 0.0001$).

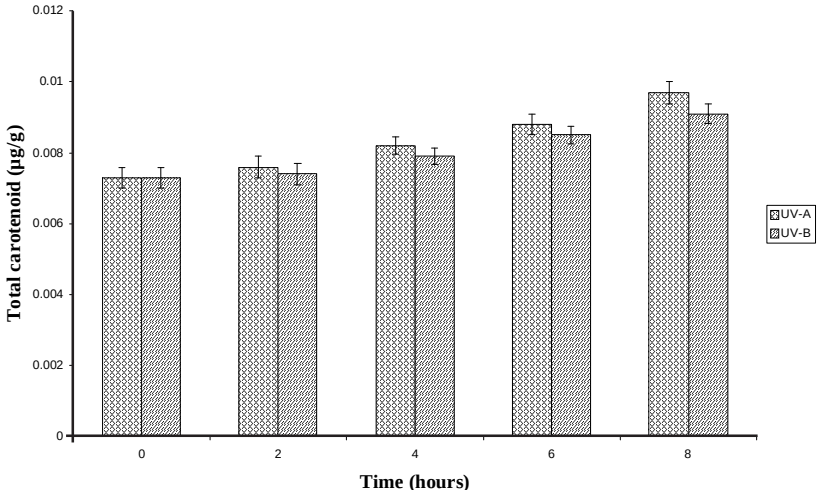

Fig. 1: Effect of UV irradiation on total carotenoid content of *Gracillaria edulis*

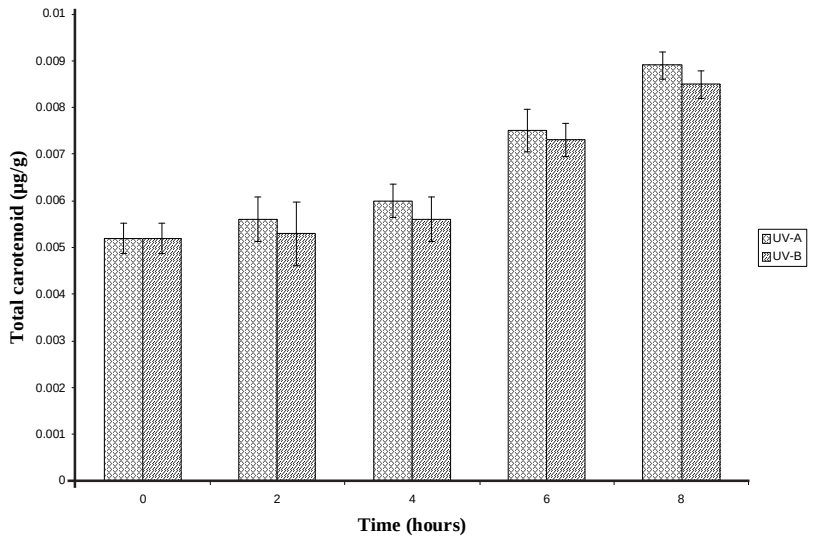

Fig. 2: Effect of UV irradiation on total carotenoid content of *Hypnea musciformis*

Similarly, photosynthetic pigments have been shown to represent critical targets of UV–B radiation (Vass, 1997). Ambient levels of UV–B radiation have already been demonstrated to be effective in reducing the concentration of all major photosynthetic pigments in natural populations of Antartic phytoplankton (Bidigare, 1989) as well as in different macroalgae from the North Sea (Dohler *et al.,* 1995). Further, the report of Lingakumar and Kulandaivelu (1998) was in agreement with the UV–B induced decrease in chlorophyll pigments.

The total carotenoid content of *G. edulis* established an increasing trend for both UV–A and UV–B radiation. The increase was from 0.0076 ± 0.00030 µg/g to 0.0097 ± 0.00033 µg/g in UV–A and from 0.0074 ± 0.00031 to 0.0091 ± 0.00027 µg/g for UV–B exposure respectively over the control value of 0.0073 ± 0.00029 µg/g. Variation in carotenoid content due to exposure duration was statistically significant ($P < 0.001$), similarly the variation in carotenoid content due to UV–A and UV–B radiation was also statistically significant ($P < 0.05$) (Fig.1).

In *H. musciformis* also the carotenoid content enhanced from 0.0056 ± 0.00048 µg/g to 0.0089 ± 0.00030 in UV–A and from 0.0053 ± 0.00068 to 0.0085 ± 0.00029 µg/g in UV–B exposure respectively against the control value of 0.0052 ± 0.00033 µg/g. Variation in carotenoid content due to exposure duration was statistically significant ($P < 0.0001$), similarly the variation in carotenoid content due to UV–A and UV–B radiation was also statistically significant ($P < 0.05$) (Fig.2).

Carotenoids are the accessory photosynthetic pigments (Young, 1991; Yamamoto and Bassi, 1996) present in plants ubiquitously which responds well against the UV radiation to guard the photosynthetic apparatus from damage and resist the photooxidation (Demmig – Adams, 1990; Gilmore, 1997). The observed increase in carotenoid contents was attributed to the protection of photosynthetic apparatus.

The reactivity of carotenoid profile to UV irradiation is species dependent. During spectral analysis, in *G. edulis* both the UV – A and UV–B radiations did not exert any conflicts in carotenoid profile in all the exposures and control. The absorption maxima (λ max) at 430nm confirmed the presence of Zeaxanthin. On the other hand *H. musciformis* was highly reactive with UV exposure and during UV–A exposure, expression of unknown carotenoids, γ-carotene and zeaxanthin were noticed but the control sample concluded the presence of violaxanthin and during UV–B exposure, expression of unknown carotenoids, zeaxanthin and neoxanthin were recorded against the control profile of unknown carotenoid and zeaxanthin (Table. 3)

Protection of photoxydative damage aids by dissipating excessively absorbed light energy in addition to xanthophylls cycle (Schafer *et al.,* 1994; Niyogi *et al.,* 1997). The general contribution of xanthophylls cycle to the protection of marine macroalgae from photodamage by high levels of PAR has previously been demonstrated by several authors such are Vershinin and Kamnev (1996), Hanelt *et al.,* (1997b) and Schofield *et al.,* (1998). Xanthophylls are the carotenoids, accessory pigments of seaweeds. Hence the change in carotenoid profile was in accordance with the earlier findings regarding the photo guarding activity of marine macroalgae.

CONCLUSION

Seaweeds are one of the important marine eco systems responsible for the productivity in marine food web. Adverse impact of UV radiation on seaweed community disturbs the major bio portions of the ocean. The present study revealed that UV–B was more deleterious than UV–A in concern with the photosynthetic and accessory pigments of seaweeds. Hence it's hourly need to channelize further studies in this aspect to protect the seaweed community from the harmful effects of UV radiation.

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