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Influence of irradiation quality on photosynthetic pigments, saccharides, nitrate reductase activity, thylakoid organization and growth of *Ulva pertusa*

B. MUTHUVELAN, K. FUJIMORI, C. MURUGAN*
and G. KULANDAIVELU**

United Graduate School of Agricultural Sciences, Kagoshima, 890, Japan
*School of Energy Sciences, Madurai Kamaraj University, Madurai 625 021, India**
*School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India***

Abstract

A floating green alga *Ulva pertusa* Kjallman was grown in the laboratory under various irradiations: "white light" (as reference, broad spectral band, WLC), red radiation (600-700 nm, RRC) and blue radiation (400-500 nm, BRC). During 15 d of culture, the specific growth rate of WLC varied highly when compared to BRC and RRC. The contents of chlorophyll (Chl) and proteins, and the nitrate reductase (NR) activity were significantly higher in BRC than in RRC while the content of saccharides was slightly higher in RRC than BRC. *U. pertusa* in WLC had the highest contents of saccharides, proteins, and Chl, and the highest NR activity. In the WLC, closely arranged well organized thylakoids were seen whereas in the BRC, although the number of thylakoid layers was similar to WLC, they were widely separated from each other. In contrast to this, in the RRC, the thylakoids were less prominent and were also densely covered with ribosomes.

Additional key words: chlorophyll, green alga, proteins, specific growth rate, ultrastructure.

Introduction

The problem of seawater eutrophication in integrated mariculture, has been effectively reduced by some seaweeds. Among them, *Ulva* is the most effective genus because of its rapid nitrogen uptake and ease of cultivation and harvesting

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Abbreviations: BRC - blue radiation culture; Chl - chlorophyll; DP - dark period; ELP - early light period; MLP - mid light period; NR - nitrate reductase; RRC - red radiation culture; SGR - specific growth rate; TEM - transmission electron microscope; WLC - "white light" culture.
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Fax: (+91) 452 85239

(Neori *et al.* 1991). In Japan, *U. pertusa* is abundant and forms an important biomass in the intertidal zone, along almost the entire coast (Kamiya *et al.* 1993). With a view for future cultivation of this alga in the integrated mariculture system, the irradiance, temperature and salinity requirements for their optimal development were determined (Floreto *et al.* 1993), also in a polyculture system with red sea bream (Hirata and Xu 1990) and *Peanus japonicus* (Danakusumah and Hirata 1991). So far, the polyculture system was designed by floating *U. pertusa* in a net on the surface of the cage, but difficulties arose during the typhoon and monsoon seasons. Since it is a floating alga, it would be better to cultivate it in the bottom level of culture site. Regarding this, the another factor which shows considerable variation in the natural environment of this depth is quality of radiation, and its influence has never been investigated for this species. In marine plants, radiation quality affects growth (Mc Lachlan and Bidwell 1983), photosynthesis (Luning and Dring 1985), and plant distribution (Tremblin *et al.* 1993). The aim of this work was to study the influence of blue and red radiation, in comparison of "white light", on growth, photosynthetic pigments, saccharides, thylakoid organization and on nitrate reductase activity in *U. pertusa* cultivated in the laboratory.

Materials and methods

Algae and culture system: *U. pertusa* Kjallman was collected from Azuma town, Kagoshima, Japan. Only healthy and clean fronds were used in order to avoid wound respiration (Bidwell and McLachlan 1985). The cut sections of the algal thalli were incubated for at least 20 h in the same medium as in the experiment.

A single factor experiment was conducted using completely randomized block design with treatments in triplicate. In each treatments 50 specimens were used and 5 specimens were sampled for each experiment. The experiment was conducted for 15 d and the samplings were made on alternate days. The values were subjected to the single factor *ANOVA* and linear regression analysis. Differences are reported as significant when $P < 0.05$ or $P < 0.01$.

In this experiment 3000 cm^3 Erlenmeyer flasks were used. Each flask was autoclaved with 2500 cm^3 culture medium (seawater with 2 % medium, Provasoli 1968), and pluged with silicon caps. Three openings were made in each silicon cap. The first one was used as an inlet for filtered air, the second one was covered with a cotton plug and used as air outlet, and the third one was used as a node to collect water samples. To avoid the evaporation of medium, air was first passed through a 1000 cm^3 Erlenmeyer flask containing autoclaved seawater. The inlet and outlet were capped with an 0.2 μm filter to avoid the passing of bacteria through air. The thalli and the cut *U. pertusa* discs were cleaned with autoclaved seawater as described by Reddy *et al.* (1989). The *Toshiba 10 LD* fluorescence lamps provided 55 - 60 μmol m^{-2} s^{-1} of either "white" (no filter), red (red cellophane: 600-700 nm) or blue (blue cellophane: 400-500 nm) radiation to the cultures on a 14-h photoperiod. Photosynthetically active radiation was determined with a *Li-Cor* model *LI-185B*

(*Li-Cor*, Lincoln, USA). Continuous aeration was given and the temperature was maintained at 25 °C.

Measurements of growth and Chl, saccharide and protein contents: Growth was measured as a per cent increase in the area of discs (1.8 cm diameter) cut from mature thalli. The discs were cut from the expanded region of the fronds, avoiding the thin areas within 5 cm of the margin and the thicker areas near the centre. Specific growth rate (SGR) was expressed as an increment of area using the equation: $SGR = [\ln (A_f/A_i)/t] \times 100$, where A_i is the initial area and A_f is the area on the day of observation (De Boer *et al.* 1978). The content of saccharides was determined by the phenol sulfuric acid method (Dubois *et al.* 1956). Protein was quantified by the *Bio-Rad* protein assay, using bovine-globulin as a standard. Chl content was determined using the method of Hansmann (1973). All spectrometric analyses were made on a *Hitachi* double beam spectrophotometer model *U-2000*. NR activity was assayed *in situ* by the procedure of Maurino *et al.* (1986), as modified by Corzo and Niell (1991). The incubation mixture consisted of 0.1 M PO_4^{3-} buffer, pH 8.0, 0.05 mM EDTA, pH 8.0; 0.01 mM glucose, 30 mM KNO_3 and 0.1 % (v/v) *n*-propanol. Three samples of plants (0.16 g FM each) were collected (two in a light period and one in a dark period). To understand the difference in NR activity during light and dark periods, sampling was done three times in alternative days, in early light period (ELP), mid light period (MLP) and dark period (DP).

Transmission electron micrographs: For the TEM analysis, the samples were fixed in 4 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The embedding was performed in an *Epon* resin. Sections were cut on the *LKB, Bromma 8800 Ultrome RIII* (*LKB*, location, Sweden) and stained with a saturated solution of 10 % uranyl acetate and followed by lead citrate. Observations were made at 100 kV in a *Hitachi* model *H-700H* electron microscope.

Results

During the 15-d treatment, radiation quality had a significant effect on growth. SGR was very high in WLC when compared with BRC and RRC (Fig. 1). The thallus growth remained almost the same in RRC and BRC during the first 6 d after which the growth of BRC was significantly higher than that of RRC. SGR for BRC and RRC was around 3.1 and 1.2 % d^{-1}, respectively. However, in WLC, it was around 8.5 % d^{-1} ($P < 0.001$). The content of saccharides was about the same in RRC and BRC (Fig. 1). The protein content in BRC was significantly higher than in RRC (Fig. 1). *Ulva* in WLC had the highest amounts of saccharides and proteins. The NR activity was studied in early light phase (ELP), mid light phase (MLP) and in the dark (DP). In all cultures the NR activity gradually increased from DP to MLP (Fig. 2) indicating that the NR activity was limited by radiant energy and varied with time of day since the plants were at the same irradiance during whole day.

The pattern of Chl content followed those of protein and saccharides: the maximum was observed in WLC followed by BRC and RRC. The Chl content increased exponentially after 12 d in RRC, while the changes were almost linear in both BRC and RRC. As much as 4 fold variation existed between WLC and BRC or RRC. Typical transmission electron micrographs of WLC and RRC *Ulva* (Fig. 4) show changes in chloroplast thylakoids in their number and amount of stacking. Well developed structure of cell organelles was observed in WLC and BRC, when compared to the RRC. Enlarged portions of the chloroplasts showed many layers of

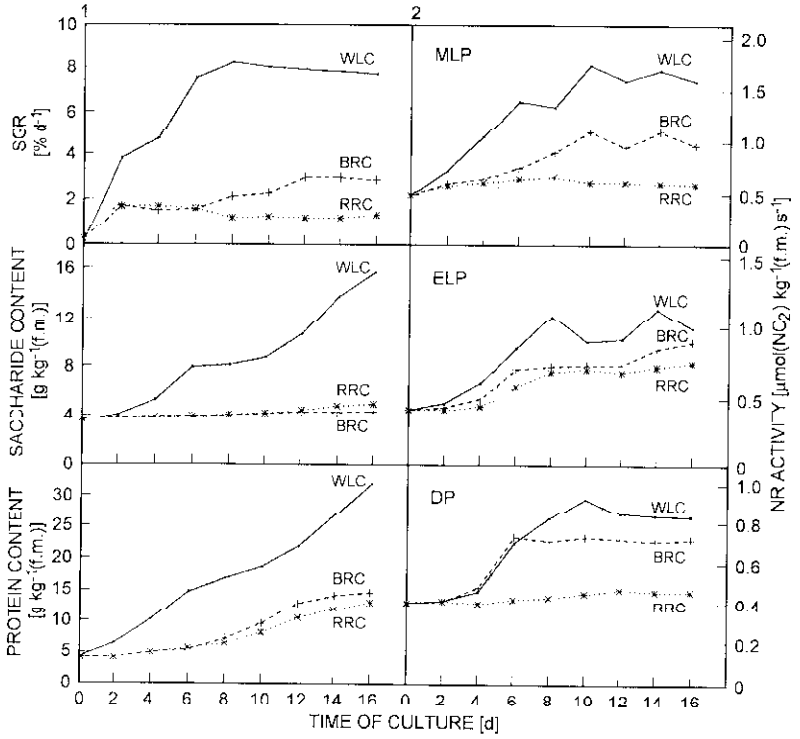

Fig. 1. Changes in protein and saccharide contents [g kg^{-1}(f.m.)], and in specific growth rate, SGR [% d^{-1}] in *Ulva pertusa* grown under "white light" (WLC), blue radiation (BRC) and red radiation (RRC). Mean ± SE, $n = 5$.

Fig. 2. Changes in the nitrate reductase (NR) activity [μmol(NO_2) kg^{-1}(FM) s^{-1}] in *Ulva pertusa* collected at early light period (ELP), medium light period (MLP), and dark period (DP). Mean ± SE, $n = 5$.

thylakoids in the WLC and BRC. On the other hand, in RRC the number of thylakoids was less and they contained large amounts of ribosomes. A great increase in stacks of thylakoids was also found in WLC. In BRC, the thylakoids were not only free and unstacked but also widely separated from each other.

Discussion

More proteins and comparatively less saccharides accumulated when *U. pertusa* thalli were grown in BRC than RRC. The higher relative content of proteins may be ascribed either to a blue radiation activation of protein biosynthesis, that would lead to a decrease in saccharide content by draining of degradation products of the BRC, or to a blue radiation enhancement of saccharides degradation, delivering more intermediates for the synthesis of amino acids and proteins (Kowallik 1987).

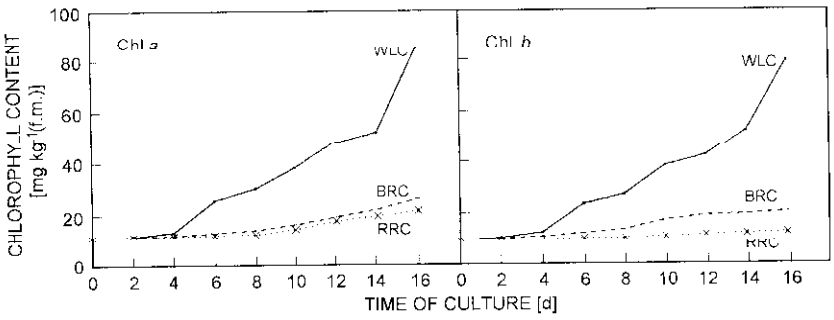

Fig. 3. Changes in the chlorophyll (Chl) *a* and Chl *b* [mg kg^{-1}(f.m.)] contents in *Ulva pertusa* grown under "white light" (WLC), blue radiation (BRC), and red radiation (RRC). Mean ± SE, $n = 5$.

The observation of higher NR activity in BRC with respect to RRC was also consistent with the results of Ninnemann (1987). NR activity is photoregulated *via* a blue radiation receptor and phytochrome in higher plants (Schuster *et al.* 1987). However, continuous blue radiation is more efficient than continuous red radiation at stimulating nitrate uptake and metabolism (Corzo and Niell 1994). Generally, in the MLP higher amounts of nitrate were reduced to nitrite in WLC than in BRC and RRC. This could be interpreted as: *a*) the blue radiation was capable of producing or activating more NR enzyme than the red radiation, *b*) the availability of substrates for NR enzyme in BRC was higher than in RRC, or *c*) changes in growth/photosynthesis, whereby metabolic demand for N was higher in BRC than in RRC.

The photoregulation of Chl synthesis has been studied extensively in higher plants (Kasemir 1983), but much less in lower plants (Senger 1987). In general, photomorphogenesis in lower plants is mainly controlled by a blue radiation photoreceptor (Humbeck and Senger 1984, Kowalick and Schurmann 1984, Lopez-Figueroa and Neil 1989). In *U. pertusa* we observed that the control of Chl *a* synthesis by blue radiation was similar to that observed in other green algae (Kowallik and Schurmann 1984). The effect of radiation quality on chloroplast morphology illustrated that in RRC, the thylakoid organization was not as clear as in BRC. Under prolonged red radiation, the thylakoid membranes become disorganized(Voskresenskaya 1972). Unicellular marine algae contain larger number of thylakoids per chloroplast in blue green radiation compared to "white light" (Vesk

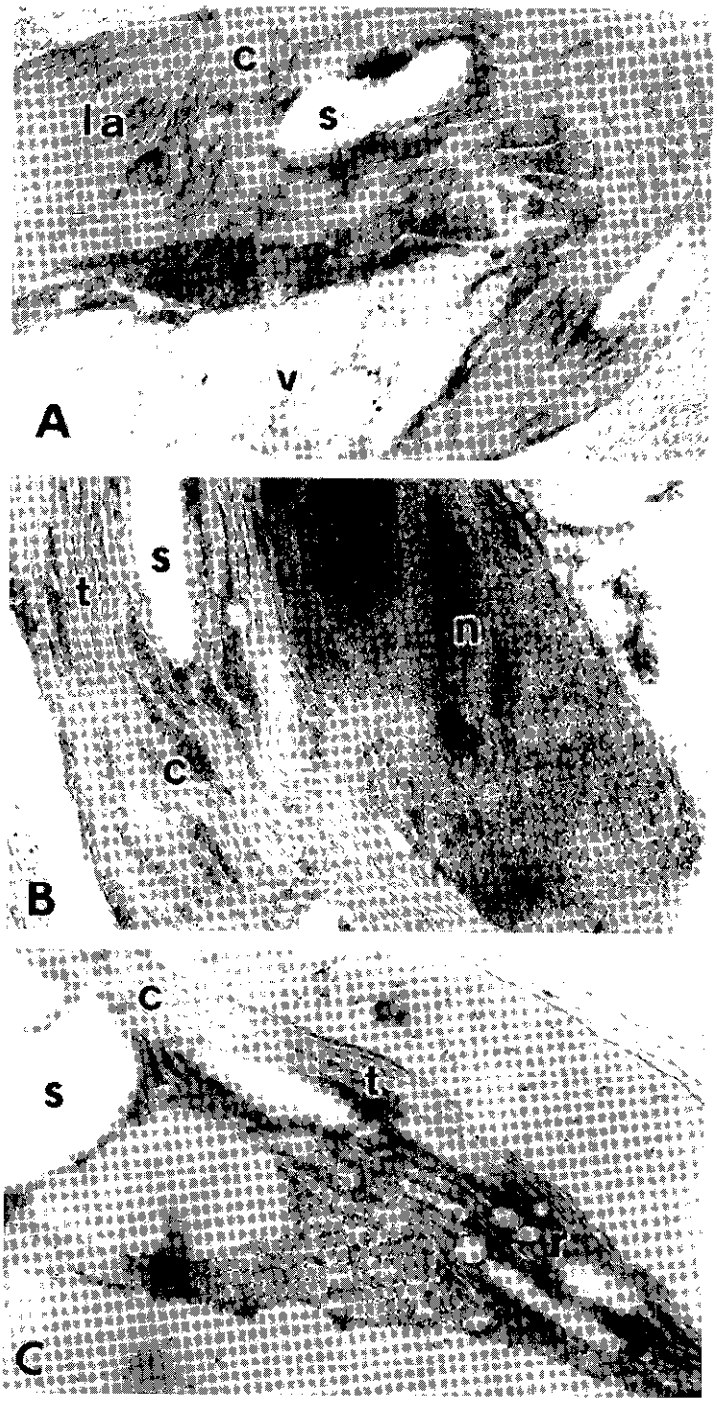

Fig. 4. Typical transmission electron micrographs showing the effect of radiation quality on chloroplast membranes of *Ulva pertusa* (*A* - "white light", *B* - blue radiation, *C* - red radiation; c - chloroplast, la - lamella, n - nucleus; r - ribosomes, s - starch, t - thylakoids, v - vacuole).

and Jeffrey 1977). In blue green algae an exposure to any spectral band (red, blue or green radiation), reduces the number of thylakoids compared to the cultures that are grown in "white light" of same amount of quanta (Albertano 1991). In our study, the enlarged chloroplasts together with greatly increased stalks of thylakoids in WLC, clearly developed thylakoids without any banding like WLC in BRC, and finally less clear thylakoids with dense ribosomes in RRC, were consistent to earlier findinds.

However, only under blue radiation the thalli showed a development and morphology almost similar to those in WLC. Therefore, in the event of application of this floating *U. pertusa* as a biofilter in the bottom level site in an integrated mariculture system (polyculture) with fish, where the radiation regimes fluctuates, irradiance is strongly reduced and also the spectral transmittance modified, results reported in this findings be considered.

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