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CHLOROPHYLL FLUORESCENCE CHARACTERISTICS OF TWO STRAINS OF *PORPHYRA HAITANENSIS* T.J. CHANG & B.F. ZHENG

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Key words: Porphyra haitanensis, Chlorophyll fluorescence, Rapid light curve

Abstract

Two strains of *Porphyra haitanensis* T.J. Chang and B.F. Zheng were used to study the chlorophyll fluorescence characteristics. Results showed that the Platt model can fit the rapid light curve of *P. haitanensis* ($R^2 > 0.95$). The α of the first harvest laver was higher than that of the second, third, and fourth harvest laver. The *r*ETRmax and I_k of the two strains of *P. haitanensis* reached the highest at the time of the second harvest ($P < 0.01$). The α, *r*ETRmax and I_k of the basal parts were higher than that of the top parts of the two strains of *P. haitanensis* ($P > 0.05$). The α was minimal under the combined red and blue light, red light and blue light showed antagonistic effect on α. Whereas the I_k, $r\mathrm{ETR}_{max}$ was minimal under red light. The α and *r*ETRmax reached their maximum under the blue light, the combined red and blue light the I_k was the highest.

Introduction

Porphyra is remarkable for its position in the upper intertidal ecosystem. In addition, *Porphyra* is also the major source of edible seaweed and is mainly cultured in China, Japan, and Korea (Fei 2004). There are two *Porphyra* strains that are cultivated in China, *P. haitanensis* T.J. Chang and B.F. Zheng, which is naturally distributed and farmed in the south (24°30′ - 30°38′N, 117°39′ - 120°32′E), and *P. yezoensis*, which is naturally distributed in the north (35°23′ - 40°39′N, 119°32′ - 122°49′ E; Wang 2011). *P. haitanensis* is an economically important red algae that is widely cultivated in China's Zhejiang, Fujian, and Guangdong Provinces along the coast. Its output accounts for about 75% of the yield of Chinese laver. Additionally, lavers retaining their rhizoid will grow a new laver after harvest. In the farming cycle, lavers can usually be continuously harvested three to five times.

Chlorophyll fluorescence has recently been recognized as an independent method for assessing algal physiology in the aquatic environment (Prasil *et al*. 2008). Pulse amplitude modulated (PAM) fluorometry has been favored by many researchers in investigating photosynthetic properties of algae due to its swiftness, convenience, and nondestructive nature (Andersson *et al*. 2006, Zacher *et al*. 2007), and has a unique advantage for research into PS(II) primary reactions and energy distribution (Enríquez and Borowitzka 2011). Pulse amplitude modulation fluorometry has been used extensively to investigate responses of land plants and algae to environmental stress (Gong *et al*. 2008).

The differing habitats of *Porphyra* strains have resulted in varying temperature adaptations (Zhang *et al*. 2012). Photosynthetic electron flow changed considerably during desiccation and re-hydration of the intertidal macroalgae *P. haitanensis* (Gao 2013). In *P. katadai* the susceptibility of photosynthesis to dehydration depends on the accommodative ability of PS(I), the relatively lower content of phycobiliprotein in male reproductive parts may be the cause for a stronger PS(I) after

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severe dehydration (Lin *et al*. 2009). However, the influence of different activated light quality on fluorescence parameters *Porphyra* chlorophyll fluorescence was not considered in these previous studies. Additionally, previous studies have not considered the chlorophyll fluorescence characteristics of the different periods and different parts of the laver.

Therefore, this experiment was conducted on the chlorophyll fluorescence characteristics from the first to the fourth harvest of two strains of *P. haitanensis* in Wenzhou. The research can lay a theoretical foundation for further understanding of the light energy utilization characteristics of *P. haitanensis* during different periods, in different parts, and different light qualities.

Materials and Methods

Two strains of *Prophyra haitanensis* were collected from the shore of Wenzhou (27°51′ - 27°52′N, 121°4′ -121°5′E), China. The laver was collected four times on the following dates: October 23, 2014, November 15, 2014; December 12, 2014 and January 9, 2015. One of the strains of *P. haitanensis* was the "Dongtou local strain", which is the local traditional breeding strain in Dongtou. Another strain of *P. haitanensis* was "Eastern Zhejiang No. 1". During transportation, the laver was immersed in the original sea water, generally shipped to the laboratory within 2 hrs, and then the tests were immediately conducted. The basic physical and chemical parameters of seawater were determined by the Ap2000 AQUAread water quality analyzer (Table 1).

Table.1 Basic physicochemical characteristics of seawater.

Date	Temp. (ºC)	pH	EC (µS/cm)	DO (mg/l)	SAL (‰)	SSG (σt)
2014.10.23	22.85	8.05	45223.25	8.01	29.26	20.35
2014.11.15	20.48	7.95	51891.75	8.03	34.12	24.73
2014.12.12	14.55	7.81	39099.50	11.62	24.87	18.60
2015.01.09	13.10	7.48	37951.00	12.17	24.06	18.15

EC: Electrical conductivity; DO: Dissolved oxygen; SAL: Salinity; SSG: Seawater specific gravity.

Four to six complete *P. haitanensis* were selected of each strain (Dongtou local strain and Eastern Zhejiang No.1 strain). The rapid light curve was measured with a DUAL-PAM-100 fluorescence spectrometer. In order to prevent rapid water loss in the determination process, *P. haitanensis* was wrapped in cling film. The determination site was at the base, the activated light source was blue light, the light intensity ranged from 0 to 332 µmol/m^2/s. In order to study the rapid light curve under different light intensity gradients, a gradient of 8 - 9 activated light intensities were selected in the determination of the first and the second harvest laver, a gradient of 10 - 11 activated light intensities were selected in the determination of the third and the fourth harvest laver. The rapid light curve of the top part of the second harvest laver was measured at the same time, and the light intensity settings were the same as in the base measurements. The rapid light curve of the activated light as red light and red and blue light were measured at the same time for the fourth harvest laver, red light provided a gradient of 11 activated light intensities from 0 to 1178 µmol/m^2/s, and red and blue provided a gradient of 11 activated light intensities from 0 to 1629 µmol/m^2/s.

The fitting of rapid light curve parameters followed the method described in Platt *et al* (1980): the initial slope (α), the maximum relative electron transfer rate (*r*ETRmax), and the semi saturation light intensity (I_k) were obtained:

$$P = Pm(1-e^{-\alpha PAR/Pm})\cdot e^{-\beta PAR/Pm} \quad (1)$$

In this formula, *P* indicated light intensity under PS(II) relative electron transport rate (*r*ETR); α indicated rapid light curves of the initial slope; *P*m indicated maximal relative electron transport rate (*r*ETRmax); Semi saturated light intensity (I_k) is the ratio of *P*m to α.

Excel 2003 was used for data calculation and drawing. Rapid light curve fitting, calculation of regression coefficients, and all statistical analysis was conducted with SPSS17.0 statistical software. The differences were analyzed via a one-way analysis of variance.

Results and Discussion

Fig. 1 illustrates that the rapid light curve of *P. haitanensis* can be better fit by the Platt model, and the coefficient of determination (R^2) is greater than 0.95. In the low light intensity range, the *r*ETR of two strains of *P. haitanensis* increased rapidly with the increase of light intensity. When the light intensity exceeded a certain intensity, the rise of *r*ETR of the first harvest and the fourth harvest decreased, and the fitting curve had a downward trend. In the second and the third harvest in the set of the light intensity range, the *r*ETR did not show a significant downward trend.

The rapid light curve (RLC) indicated the sample change of *r*ETR under different active light, this not only can be used to analyze the actual photosynthetic capacity, but also reflects the potential photosynthetic capacity of photosynthetic organisms in a certain range of light intensity. The RLC usually has three distinct stages: the low light intensity stage, saturation intensity stage, and the super saturation light intensity stage (Zhang *et al.* 2011). The RLC of the first harvest laver and the fourth harvest laver clearly showed the three stage characteristics, but the three stages for the second harvest laver and the third harvest laver were not obvious. This may be due to the higher light intensity adaptation in the second and the third harvest laver. During the same period, the rapid light curves of two strains of *P. haitanensis* showed a consistent trend.

The Platt *et al.* (1980) method was used to fit the rapid light curve, and calculate the α, *r*ETR$_{max}$, I_k and other fluorescence parameters. Present results showed that there were some differences in the chlorophyll fluorescence parameters at different stages. The *r*ETR$_{max}$ and I_k of Dongtou local strain were higher than that of the Eastern Zhejiang No.1 strain, but there was no significant difference between the two strains ($P > 0.05$).

Table 2 illustrates that the α of the first harvest laver was higher than that of the second, third, and fourth harvest laver. The α was up to 0.26 and 0.25 for the Dongtou local strain and the Eastern Zhejiang No.1 strain, respectively. This showed that the utilization efficiency of light energy was relatively high at lower light intensity. In the second to the fourth harvest laver the α ranged from 0.17 to 0.20 for the Dongtou local strain and was 0.20 for the Eastern Zhejiang No.1 strain, thus was more stable. There were no significant difference ($P > 0.05$) among the second, third, and the fourth harvest for both strains.

The α was the highest in the two strains during the first harvest, which indicated that the first harvest laver had a high photosynthetic capacity at the low light intensity. The growth period of the second harvest at the end of October to mid November, the fourth harvest at the end of December to early January, properly after the first harvest, the second harvest laver in that period with suitable environmental conditions and strong physiological activity. Under natural climate conditions, the physiological activity, including *r*ETRmax, $I_{k,}$ decreased in both *P. haitanensis*

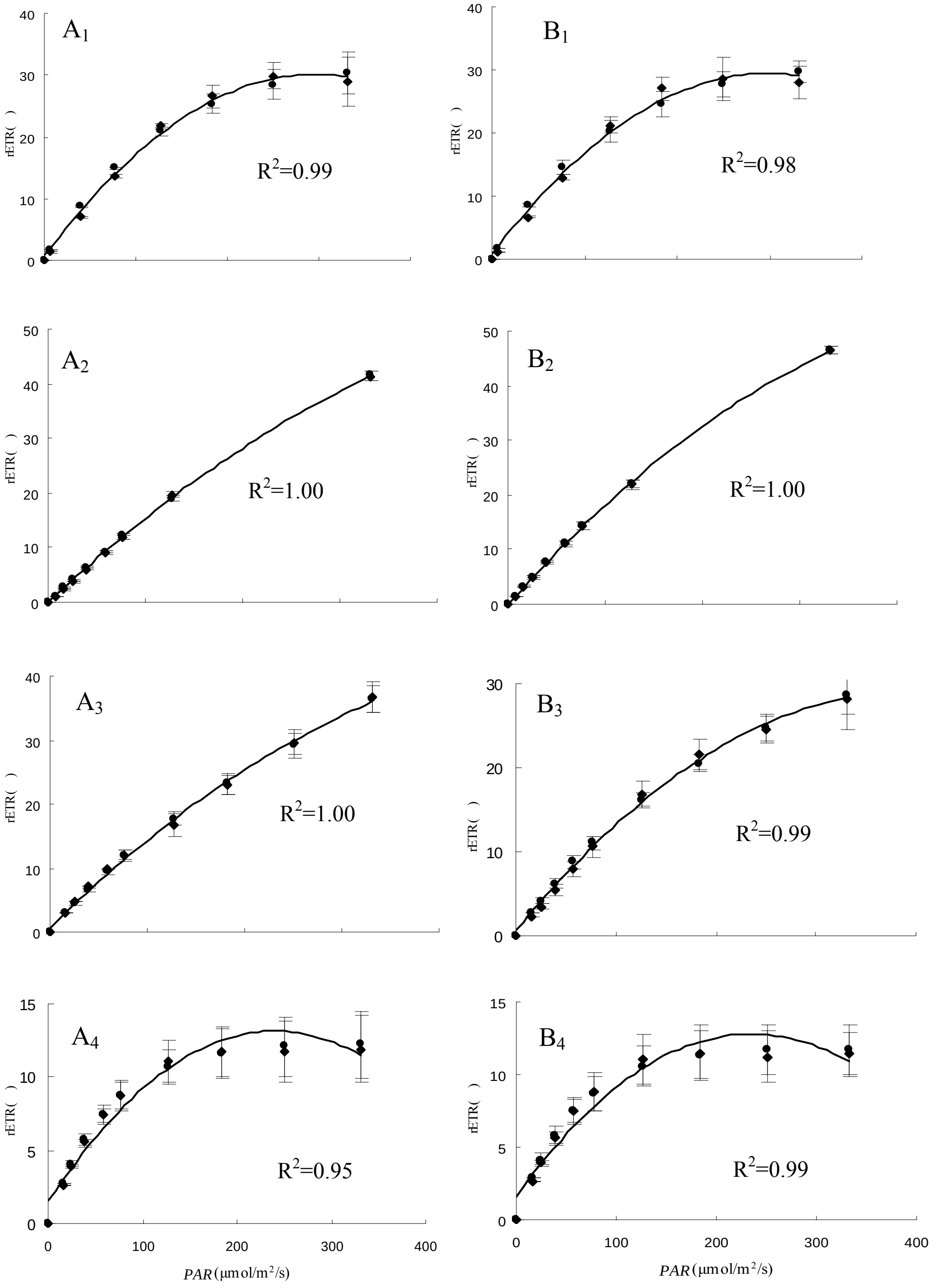

Fig. 1. A_1-A_4, B_1-B_4. The rapid light curve of *r*ETR(II) of the first harvest to the fourth harvest *P. haitanensis.* A: Dongtou local strain; B: Eastern Zhejiang No.1 strain; 1, 2, 3, 4 indicate first, the second, the third and the fourth harvest; □Measured data; ●Fitted data.

strains in January (winter) and was the highest in November (fall). With the continuous harvest of the *P. haitanensis* under natural climate conditions (i.e., winter), the physiological activity of the fourth harvest laver decreased, which led to the two strains of *P. haitanensis* of the *r*ETRmax, I_k reached the highest in the second harvest, lowest in the fourth harvest.

Table 2 illustrates that the *r*ETRmax of the two strains of *P. Haitanensis* reached the highest at the time of the second harvest: the *r*ETRmax of the Dongtou local strain was up to 77.62 and up to 67.46 for the Eastern Zhejiang No.1 strain. The *r*ETRmax performance of the fourth harvest laver was the lowest. The *r*ETRmax of the different periods was very significant ($P < 0.01$). The changing trends of I_k in the two strains were in agreement with *r*ETRmax in different periods, and the difference was very significant ($P < 0.01$). The I_k up to 336.07 µmol/m^2/s in the second harvest of the Eastern Zhejiang No.1 strain, may due to the lower activated light intensity set in the experiment.

Results from a study conducted by Zhang Tao *et al.* (2011) suggested that in both the protonema or thallus of *P. haitanensis*, I_k values were less than 150 µmol/m^2/s at the low level. In the second harvest laver, the I_k of the two strains were basically more than 150 µmol/m^2/s, which showed that the second harvest laver had a strong adaptability to high light intensity, but the first, third, and fourth harvest laver showed a bias to low light intensity adaptability. Research conducted by Häde *et al.* (1999) suggested that most red algae grow abundantly in the deep sea, which is consistent with our research. The RLC and chlorophyll fluorescence parameters of *P. haitanensis* female and male algotrophic vegetative cells showed no significant differences (Li *et al.* 2013). Our experiment of the different stages was conducted on the basal vegetative cells, so the difference between female and male algae was not considered.

Table 2. The rapid light curve parameters of the first to the fourth harvest in PS (II).

Period	Strain	α	$r\mathrm{ETR}_{\max}$	I_k (µmol/m^2/s)
The first harvest	Dongtou local strain	0.26 ± 0.01^{a}	39.13 ± 6.92^{b}	153.75 ± 32.23^{b}
	Eastern Zhejiang No.1 strain	0.25 ± 0.04^{A}	35.97 ± 9.90^{B}	144.38 ± 42.78^{B}
The second harvest	Dongtou local strain	0.17 ± 0.01^{b}	77.62 ± 2.48^{a}	464.40 ± 16.20^{a}
	Eastern Zhejiang No.1 strain	0.20 ± 0.02^{A}	67.46 ± 2.93^{A}	336.07 ± 16.98^{A}
The third harvest	Dongtou local strain	0.19 ± 0.01^{b}	18.72 ± 4.43^{c}	97.53 ± 16.55^{b}
	Eastern Zhejiang No.1 strain	0.20 ± 0.00^{A}	17.96 ± 2.88^{BC}	89.52 ± 15.71^{B}
The fourth harvest	Dongtou local strain	0.20 ± 0.02^{b}	12.53 ± 2.48^{c}	63.50 ± 9.26^{b}
	Eastern Zhejiang No.1 strain	0.20 ± 0.04^{A}	12.33 ± 2.26^{C}	60.98 ± 7.10^{B}

Note: Different letters at the same strain for each parameter indicate significant differences at 0.05 level.

In Fig. 2, the *r*ETR of the basal part was higher than the top part of the two strains of *P. haitanensis* under the same light intensity. In the set of the light intensity range, the *r*ETR of two strains of *P. haitanensis* increased rapidly with the increase of light intensity, did not show a downward trend. The α, *r*ETRmax and I_k of the basal part of the two strains were all higher than the top part, but there was no significant difference between the different parts ($P > 0.05$)(Table 3).

Chlorophyll *a* and chlorophyll protein are important photosynthetic pigments in *Porphyra*. In the presence of chlorophyll *a*, chlorophyll is the photosynthetic reaction center pigment. Phycobiliprotein contains phycoerythrin (PE) and phycocyanin (PC) (Glazer *et al.* 1984). PE in red algae first captures light in the light-harvesting system, then passes light energy to PC, and finally passes it to chlorophyll *a* (Zhao *et al.* 2010). In general, the energy absorption and transmission of algae is directly involved in photosynthesis. The higher the content of the phycobiliprotein, the stronger the photosynthetic ability (Glazer *et al.* 1984). Jiang *et al.* (2010) found that in two strains of *P. haitanensis* the content of PE in the top > base and the content of PC in the base > top. Higher content of PC and chlorophyll in the basal part of *Porphyra*, may be the reason why *r*ETR, *r*ETRmax, and α of two strains of *Porphyra* are higher compared with the top under the same light intensity. During the period of the culture, the basal part was suspended in the upper water layer, where the light intensity is higher, and the top part was in the deeper water layer, where the light intensity was weak. The higher chlorophyll fluorescence activity of the basal part may be an adaptation mechanism for the performance of different parts to the growth environment.

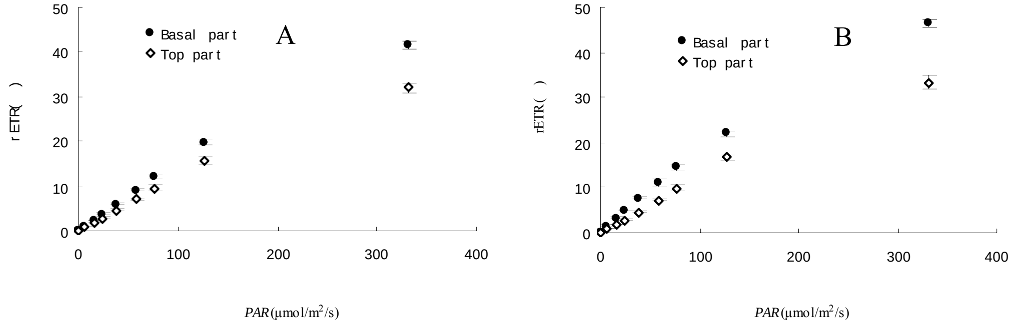

Fig. 2. A-B. The rapid light curve of *r*ETR (II) in different parts of the second harvest. A: Dongtou local strain; B: Eastern Zhejiang No.1 strain.

Table 3. PS (II) rapid light curve parameters of the second harvest in different parts.

Strain	Part	α	*r*ETR$_{max}$	I_k (μmol/m^2/s)
Dongtou local strain	basal	0.17 ± 0.01 ^{a}	77.62 ± 2.48 ^{a}	464.40 ± 16.20 ^{a}
	top	0.15 ± 0.01 ^{a}	39.07 ± 3.02^a	254.75 ± 15.21^a
Eastern Zhejiang No.1 strain	basal	0.20 ± 0.02 ^{A}	67.46 ± 2.93^A	336.07 ± 16.98 ^{A}
	top	0.14 ± 0.01^A	41.58 ± 2.60^A	299.65 ± 16.54^A

Note: Different letters at the same strain for each parameter indicate significant differences at 0.05 level.

Fig. 3 illustrates that the rapid light curve of *P. haitanensis* can be better fitted by the Platt model under different light quality, as the determination coefficients (R^2) were greater than 0.95. Under the conditions of red light and red and blue combined light as activated light of the two strains of *P. haitanensis*, with the increase of the intensity of light, *r*ETR rapidly increased in the low light intensity range, when the light intensity was higher than a certain intensity the *r*ETR gradually decreased. The changing trend of the rapid light curve under the same light quality was consistent in the two strains of *P. haitanensis*.

There was no significant difference of the α, *r*ETR$_{max,}$ and I_k between the two strains of *P. haitanensis* under the same light conditions ($P > 0.05$). Table 4 illustrates that under blue light the α was up to 0.20 in the two strains of *P. haitanensis*. Under the combined red and blue light the α was the lowest, and the α differences between different light qualities is significant ($P < 0.05$). For two strains of *P. haitanensis*, *r*ETRmax also reached its highest under blue light; Dongtou local strain reached 12.53 and the Eastern Zhejiang No.1 strain reached 12.33. The *r*ETRmax was the lowest under the red light, and the difference between different light qualities was significant ($P < 0.05$). The I_k of two strains of *P. haitanensis* was the lowest under red light; the Dongtou local strain was 51.39 μmol/m^2/s and the Eastern Zhejiang No.1 strain was 47.14 μmol/m^2/s. Under the combined red and blue light the I_k was the highest. It was 75.04 μmol/m^2/s and 82.12 μmol/m^2/s for the Dongtou local strain and the Eastern Zhejiang No.1 strain, respectively. The difference of I_k between different light qualities was significant ($P < 0.05$).

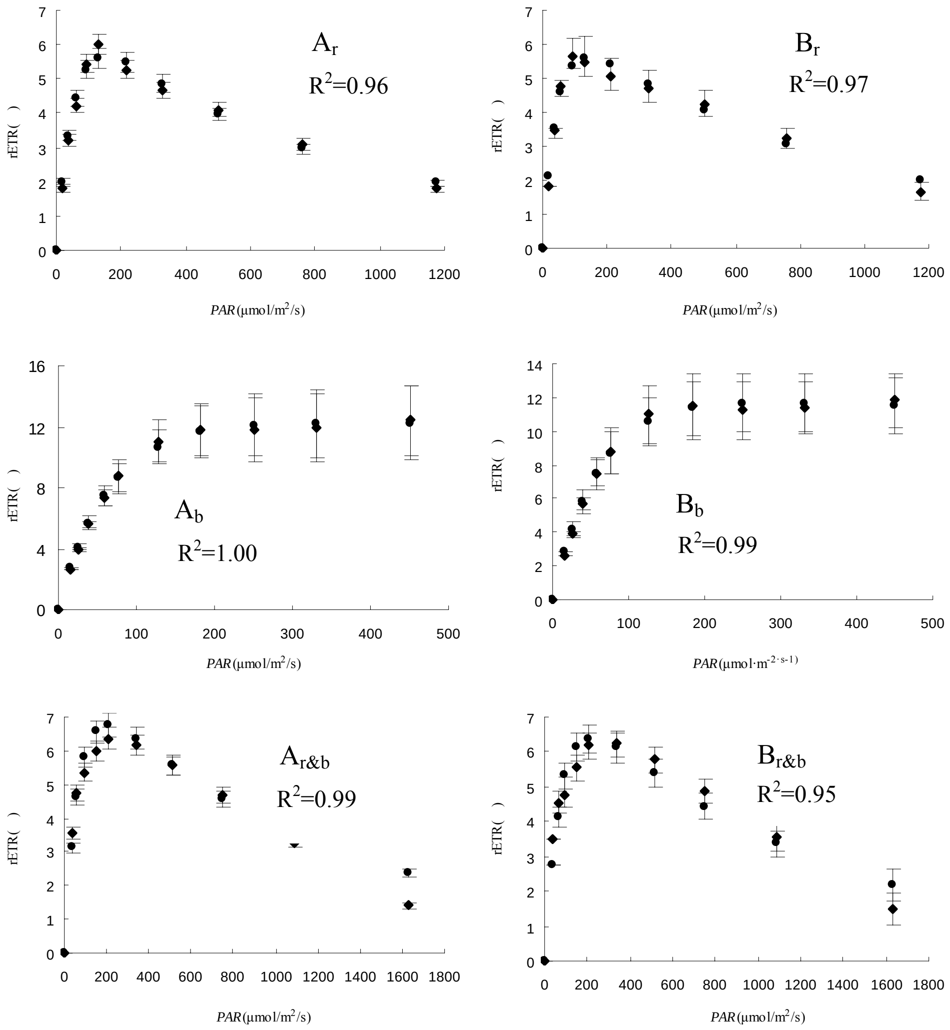

Fig. 3 A_r-A_b-$A_{r\&b}$, B_r-B_b-$B_{r\&b}$. The rapid light curve of *r*ETR (II) under different activated light.

A: Dongtou local strain; B: Eastern Zhejiang No.1 Strain; r: Red light; b: Blue light; r&b: Red and blue light; □Measured data; ●Fitted data.

Light quality is one of the basic factors in the regulation of plant metabolism, plant growth, morphological structure, photosynthesis, and metabolism that has a regulatory role (Zhao *et al.* 2010). Häder *et al.* (1999) proposed that green light has the ability to penetrate the sea stronger than red light and blue light. *Porphyra* can absorb the green light better after a long period of adaptation. Large red algae phycobiliproteins have higher activity than chlorophyll in energy utilization (Haxo *et al.* 1950). The content of PE was significantly higher than that of other types of phycobiliprotein, and there was no significant difference in chlorophyll content of different strains of *Porphyra* (Häder *et al.* 1999). Zhao *et al.* (2010) showed that the photosynthetic efficiency of *Porphyra* was able to utilize light as follows: white light irradiation > green light > red light > blue light and that red light and blue light were mainly absorbed by chlorophyll. According to Yokoya *et al.* (2007) a higher level of phycoerythrin content could also cause higher growth rates under the same light conditions. Light quality affects the ETR relationship in the analyzed macroalgae and α of *P. leucosticta* was minimal under red light (Félix 2003). The α, $r\mathrm{ETR}_{\max}$, and I_k was significantly different ($P < 0.05$) among different light qualities. This showed that light quality has significant effects on the chlorophyll fluorescence of *P. haitanensis*. In this study, α of *P. haitanensis* was minimal under the combined red and blue light, red light and blue light showed antagonistic effect on α. Whereas the I_k, $r\mathrm{ETR}_{\max}$ in two strains of *P. haitanensis* was minimal under red light. Under red light, ETR decreased because a lesser number of photons from other wavelengths are being absorbed through accessory pigments (i.e., blue light in green algae and green light in red algae) (Félix 2003).

Table 4. PS (II) rapid light curve parameters of the fourth harvest under different activated light.

Strain	Active light	α	$r\mathrm{ETR}_{\max}$	I_k (µmol/m^2/s)
Dongtou local strain	Red light	0.15 ± 0.03 ^{b}	7.34 ± 1.66 ^{b}	51.39 ± 17.00 ^{c}
	Blue light	0.20 ± 0.0 2 ^{a}	12.53 ± 2.48^{a}	63.50 ± 9.26 ^{b}
	Red and blue light	0.12 ± 0.03^{b}	8.61 ± 1.51^{b}	75.04 ± 8.63 ^{a}
Eastern Zhejiang No.1 strain	Red light	0.15 ± 0.02 ^{B}	6.83 ± 0.64 ^{B}	47.14 ± 6.98^{C}
	Blue light	0.20 ± 0.04 ^{A}	12.33 ± 2.26 ^{A}	60.98 ± 7.10^{B}
	Red and blue light	0.10 ± 0.01^{C}	8.28 ± 1.11 ^{B}	82.12 ± 17.07^{A}

Note: Different letters at the same strain for each parameter indicate significant differences at 0.05 level.

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