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Comparative studies on the growth and photosynthesis of the pigmentation mutants of *Porphyra yezoensis* in laboratory culture*

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Growth, photosynthesis and respiration were studied with the wild, red and green type fronds of *Porphyra yezoensis* under laboratory conditions. The mean length of the wild (C-13), red (C-22) and green type (C-32) fronds was 4.5, 2.8 and 1.8 mm respectively in 25 days old populations. The mean relative growth rate per day was highest (0.24, 0.22 and 0.18 in the wild, red and green type fronds, respectively) during the earliest growth period and became lower with frond age in all the three strains, and the differences among the strains were almost negligible during the period later than 25 days old. In fronds of the three strains younger than 30-40 days old, the photosynthetic rate was nearly saturated at 15-20 klux but continued to increase slightly up to 90 klux. The saturation light intensity became lower with frond age. The photosynthetic rate on a frond area basis was higher both at low and high light intensity in the wild type fronds than in the red and green type fronds, but the photosynthetic rate on a chlorophyll *a* basis was almost the same in the three types of fronds. The maximum light-saturated photosynthetic rates were observed at 20-25°C in photosynthesis-temperature curves of all the types of fronds. The photosynthetic rate on a frond area basis became lower with age at each temperature in all the three strains. Little difference was obtained in the respiratory rate among the three types of fronds. The light-limited photosynthetic rate was lower in green light than in white light in all the three types of fronds, and the difference was remarkable especially in the green type frond which has very low phycoerythrin content. In another green type strain (C-0), the relative growth rate was slightly lower than that of the wild type strain (C-13) but equal to the red type strain (C-22) at the early growth period, and slightly higher than that of the above three strains at the later stage of growth. The photosynthetic rate on a frond area basis was relatively low in the green type strain (C-0).

Key Index Words: *chlorophyll* a; *culture*; *growth*; *photosynthesis*; *phycocyanin*; *phycoerythrin*; *pigmentation mutants*; Porphyra yezoensis; *respiration*.

The color of *Porphyra* fronds is dependent on the contents and the ratios of such pigments as chlorophyll *a*, phycoerythrin, phycocyanin and carotenoids, and is an important factor which controls the commercial value of the dried "Nori". There is a high correlation between the pigment contents and amino acid contents which flavor the dried Nori (MIURA 1976, SAITO *et al.* 1975). Furthermore, these pigments play a very important role in trapping light energy for photosynthesis. Especially, the existence of phycoerythrin and phycocyanin is very important for the light-harvesting capabilities of

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red algae. Phyccerythrin and phycccyanin fill in, or at least narrow, much of the light energy gap left by chlorophyll *a* and carctenoids, allowing the algae to use the solar radiation much more efficiently in photosynthesis, in a manner much like that of fucoxanthin in the brown algae (GOVINDJEE and BRAUN 1974).

In recent years, color mutants (the red and the green type mutants in reference to the wild type) have been confirmed or established in cultivated populations and in laboratory cultures of *Porphyra yezoensis* and *P. tenera* (ARUGA and MIURA 1984). Various types of variegated chimeral fronds composed of two or more sectors of different colors have been found in cultivated populations and in laboratory cultures of *P. yezoensis* (MIURA 1976 1984). On the other hand, KOBARA *et al.* (1976) have obtained the green type individuals from the green type sector of a variegated chimeral frond of *P. yezoensis* found in a cultivated population, and have succeeded in completing the life cycle of the green type mutant in laboratory culture. The distinction of the wild, red and green type fronds is possible by naked eyes. ARUGA and MIURA (1984) have made clear their characteristics by comparing the *in vivo* absorption spectra. The red type is distinguished from the wild type by clear two absorption maxima due to phycoerythrin and a shift of absorption maximum due to phycocyanin. The green type has remarkably lower absorbance in the wavelength range mainly due to phycoerythrin than the wild and red types. These characteristics are consistently found in each type fronds, in each type sectors of chimeral fronds and in each type conchocelis. In addition to these strains, later, the yellow type strain was newly established by cross breeding of the red and the green type mutant strains under laboratory conditions, the details of which will be published elsewhere.

MIURA (1976) reported the patterns and frequency of occurrence of variegated chimeral fronds in *P. yezoensis* populations both under field and laboratory conditions. He also suggested the possibility and importance of utilizing the color mutants as markers in breeding of *Porphyra* for making clear the genetic pattern. MIURA and KUNIFUJI (1980) summarized their genetic study of *P. yezoensis* utilizing the color mutants. Comparative physiclogical and biochemical studies with these color mutants were reported only by KIKUCHI *et al.* (1979) and MERRILL *et al.* (1983).

The present study deals with the growth, photosynthesis and respiration of the wild, red and green type fronds of *P. yezoensis* under laboratory conditions, and will give a clue which contributes to make clear the role of phycobilin pigments in *Porphyra*.

Material and Methods

The wild type (W, strain number C-13), red type (R, C-22) and green type (G, C-32) strains used in the present study are the strains isolated through carpospore collection from a variegated chimeral frond which was found in a cultivated population of *Porphyra yezoensis* at Shitazu, Futtsu, Chiba Prefecture, in March 1975 and composed of the wild, red and green type sectors. Another green type strain (G, C-0) of *P. yezoensis* used is the strain isolated by KOBARA *et al.* (1976). These strains are kept as free-living conchocelis in laboratory cultures at 20°C under a 14 : 10 LD cycle.

By transferring from stock cultures to the lower temperature and short-day conditions (15°C and 10 : 14 LD), the conchocelis matured and released conchospores, which immediately attached to synthetic fibers (Cremona monofilaments) of about 4 cm long and developed into fronds (leafy thalli). The day of the conchospore attachment was assigned to zero-day for the age of fronds. The culture medium was the natural seawater, which was collected from the Kuroshio off the Izu Oshima Island and filtered through a glass fiber filter (Whatman GF/C), enriched with modified ESP (PROVASOLI 1966) as shown in Table 1. Until 30 days old, the frond cultures were maintained in 1 *l* flat-bottom flasks

Table 1. Composition of the modified ESP medium used in the present study. To obtain ESP add 2 m*l* of ES enrichment to 100 m*l* of filtered seawater.

(A) ES Enrichment	
H_2O	100 m*l*
$NaNO_3$	350 mg
Na_2-glycerophosphate	50 mg
Fe (as EDTA, 1 : 1 molar)	2.5 mg
P II metal mix*	25 m*l*
Vitamin B_{12}	10 μg
Thiamine	0.5 mg
Tris buffer	500 mg
pH	7.8
*(B) P II metal mix	
H_2O	100 m*l*
H_3BO_3	114 mg
$FeCl_3 \cdot 6H_2O$	4.9 mg
$MnCl_2 \cdot 4H_2O$	14.4 mg
$ZnSO_4 \cdot 7H_2O$	2.2 mg
$CoCl_2 \cdot 6H_2O$	0.4 mg
Na_2-EDTA	100 mg

with a branch for aeration at the bottom corner and were kept in the incubator at 15°C and 10 klux with a photoperiod of 10 : 14 LD. The fronds were removed from the synthetic fibers and cultured in 25 *l* culture tanks (Nihon Chisei Sangyo Co., Ltd.) with 2 cool-white fluorescent lamps which supplied illumination of 3, 5 and 10 klux to the lower, middle and upper part of the tanks, respectively. The cultures were aerated with an air pump throughout the experiment. The culture medium was renewed every 5 days.

The growth was determined by measuring of the frond length. During the initial 25 days, a few Cremona monofilaments with fronds were used to measure the length of about 100 fronds at intervals of 5 days. After 25 days old, 30 fronds were used for the growth measurements. The fronds less than 2 mm long were measured under the microscope with a screw micrometer, and those larger than 2 mm long with a slide calipers. Although the release of monospores was observed after 15 days old, the fronds from monospores were not used for the measurements.

The light-and-dark bottle method was employed for the measurements of photosynthesis and respiration. One or more fronds were placed in a D.O. bottle of about 100 m*l* filled with filtered seawater, and incubated for 20 or 40 min for photosynthesis or respiration measurement, respectively. The oxygen concentration in seawater was determined by the Winkler titration technique before and after the incubation. Photosynthesis was measured at 15°C and various light intensities by changing the distance of D.O. bottles from the light source to obtain photosynthesis-light curves. Photosynthesis-temperature curves were obtained at 25 klux by changing temperature at intervals of 5°C in the range 5–30°C. Respiration-temperature curves were obtained in the same temperature range in the dark. A photoreflector lamp (Toshiba 100 V 500 W, Spot) was used for photosynthesis measurements. The light intensity was measured with a Toshiba SPI-5 photometer. In the measurements with fronds of 48 days old, the basal and mature marginal parts of fronds were cut off to exclude the marginal effects partly related to the sexual maturation (cf. OGATA and MATSUI 1963). The measurements were started in the middle of light period to exclude the effect of diurnal rhythm (OOHUSA *et al.* 1977). The photosynthesis under green light was measured by using a colored cellophane filter with a transmittance spectrum as shown in Fig. 1.

Immediately after the end of each experiment, sample fronds were estimated for their area and preserved in a desiccator for the measurements of dry weight and pigment contents. After weighing with a chemical balance, the dry samples were smashed in a mortar with 90% acetone or distilled water to obtain the extracts of pigments. The absorbances of the extracts were measured with a Shimadzu QV-50 spectrophotometer. Chlorophyll *a* concentration of the 90% acetone extract was calculated by the formula of SCOR-UNESCO (1966). Phycoerythrin and phycocyanin contents of the water extract

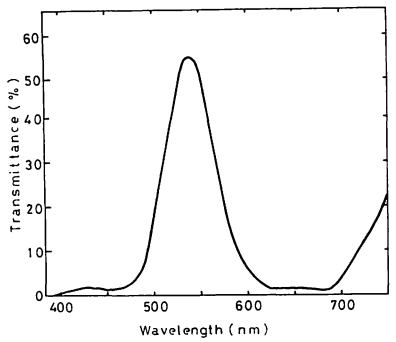

Fig. 1. Transmittance spectrum of a colored cellophane filter used for the measurement of photosynthesis under green light.

were calculated by using the extinction coefficients reported by Ó hEOCHA (1965). *In vivo* absorption spectra of the fronds were obtained with a Shimadzu MPS-50L recording spectrophotometer with the air as reference.

Results

1) Growth

The frond length compositions of the wild (C-13), red (C-22) and green (C-32 and C-0) type fronds of 10, 15, 20 and 25 days old are shown in Fig. 2. The mean lengths (M), standard deviations (SD) and coefficients of variability (CV) were calculated (Table 2). The frond length compositions were almost the same in the wild, red and green type populations of 10 days old. A difference was, however, found among the populations of 15 days old; the wild type population was composed of a great number of fronds 0.4-0.8 mm long, while the red and green type (C-32 and C-0) populations 0.2-0.6 mm long. The wild type population of 20 days old was composed of many fronds larger than 1.5 mm long while the red and green type (C-32

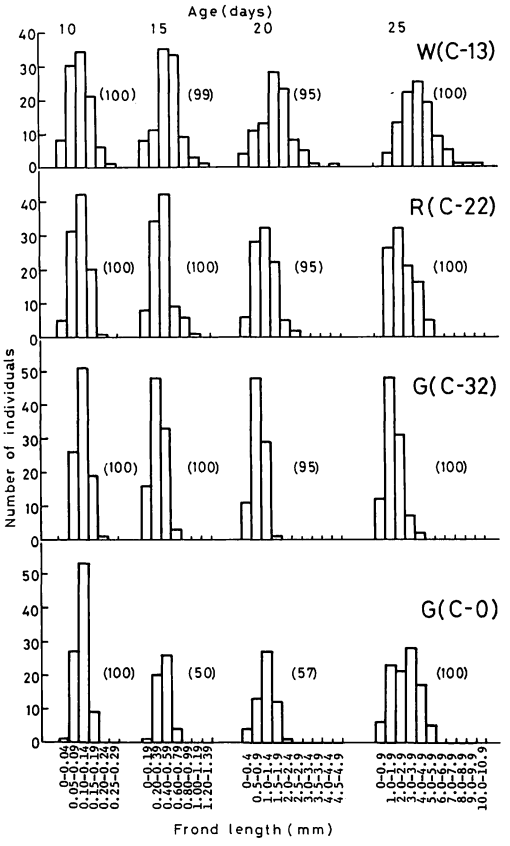

Fig. 2. Fond length compositions of the wild (W), red (R) and green type (G) fronds (10, 15, 20 and 25 days old) of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the number of sample fronds at each measurement.

Table 2. Mean frond length (M, μm), standard deviation (SD) and coefficient of variability (CV) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laborarory.

Strain		Frond age (days) 10			15			20			25		
		M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
W	(C-13)	117	53	45	567	321	41	1825	774	42	4.5×10^3	1.6×10^3	37
R	(C-22)	112	39	35	434	181	42	1240	528	43	2.8×10^3	1.2×10^3	41
G	(C-32)	125	36	29	358	123	34	923	410	44	1.8×10^3	0.7×10^3	44
G	(C-0)	109	27	25	425	118	28	1150	405	35	2.9×10^3	1.3×10^3	44

and C-0) populations many fronds less than 1.5 mm long; the red and green type (C-32) populations were composed of many fronds 1.0-1.5 mm long and the green type (C-32) populations many fronds 0.5-1.0 mm long. Among the populations of 25 days old, the wild type population was composed of many fronds 3-6 mm long, some fronds larger than 6 mm long and a few fronds less than 2 mm long; the red and green type (C-0) populations were composed of many fronds 1-5 mm long and more fronds 1-2 mm long than the wild type; the green type (C-32) population was composed of many fronds 1-3 mm long, of which fronds 1-2 mm long occupied 50% of all the fronds.

The mean frond length was almost the same, about 0.12 mm, in all the populations of 10 days old. However, the difference of the mean length became clear as the fronds aged. The mean frond length of the wild, red and green type (C-32 and C-0) populations of 25 days old was 4.5, 2.8, 1.8 and 2.9 mm, respectively (Table 2).

It is presumed that the differences of the frond length composition and the mean frond length are due to the difference of the growth rate among the strains of different types. Therefore, at each age the fronds were arranged according to the frond length and classified in four groups. The relative growth rate per day was calculated with the mean frond length of each group and the total mean frond length (Table 3) using the following formula:

Table 3. Mean frond length (μm) and relative growth rate per day of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Fronds were classified in four classes according to their frond length. (M) is the relative growth rate per day for the mean frond length in Table 2.

Strain	Age (days)	Frond length 10	15	20	25		Relative growth rate 10-15	15-20	20-25	19-25
W (C-13)		54	280	877	2.6×10^3		0.33	0.23	0.22	0.26
		96	495	1613	3.8×10^3		0.33	0.24	0.17	0.24
		131	645	2058	4.9×10^3		0.32	0.23	0.17	0.24
		188	859	2836	6.7×10^3		0.30	0.24	0.17	0.24
						(M)	0.32	0.23	0.18	0.24
R (C-22)		67	226	587	1.5×10^3		0.24	0.19	0.19	0.21
		99	376	1048	2.3×10^3		0.27	0.20	0.16	0.21
		122	486	1394	3.1×10^3		0.28	0.21	0.16	0.22
		166	676	1961	4.5×10^3		0.28	0.21	0.17	0.22
						(M)	0.27	0.21	0.16	0.22
G (C-32)		82	186	471	0.9×10^3		0.16	0.19	0.13	0.16
		110	333	725	1.5×10^3		0.22	0.16	0.14	0.17
		134	406	1031	2.0×10^3		0.22	0.19	0.13	0.18
		173	511	1489	2.9×10^3		0.22	0.21	0.13	0.19
						(M)	0.21	0.19	0.13	0.18
G (C-0)		76	273	595	1.3×10^3		0.26	0.16	0.15	0.19
		98	397	1043	2.3×10^3		0.28	0.19	0.16	0.21
		117	471	1295	3.5×10^3		0.28	0.20	0.20	0.23
		147	571	1933	4.5×10^3		0.27	0.21	0.20	0.23
						(M)	0.27	0.20	0.18	0.22

$$\text{Relative growth rate} = \frac{\ln l_2 - \ln l_1}{t_2 - t_1},$$

where l_1 and l_2 are the length of frond of t_1 and t_2 days old, respectively. There was little difference in the relative growth rate between the results from the total mean frond length and from the mean frond length of the four groups. Although clear differences were found in the relative growth rate for the total mean frond length among the wild, red and green type (C-32 and C-0) populations at 10-15 days, the differences became smaller as the fronds aged. The relative growth rates of the wild, red and green type (C-0) populations of 20-25 days old were almost the same, whereas that of the green type (C-32) population was lower. The relative growth rate for the total mean frond length was 0.24, 0.22, 0.18 and 0.22 in the wild, red and green type (C-32 and C-0) populations, respectively, for the period of 10-25 days. Thus, there were significant differences in the relative growth rate among the wild, red and green type populations, even though the relative growth rate became lower as the fronds aged in each type population. In order to follow furthermore the growth of each type populations, 30 fronds of 25 days old from each population were classified in 5 groups according to the frond length; <3.0, 3.0-3.9, 4.0-4.9, 5.0-5.9 and >6.0 mm. The relative growth rate for the mean frond length of each group was calculated (Table 4). There was little difference in the relative growth rate for 25-66 days among the wild, red and green type (C-32) populations, whereas the green type (C-0) population showed a slightly higher relative growth rate than the other populations. The reproductive maturation was observed in the wild, red and green type (C-32) fronds of about 45 days old, while in the green type (C-0) fronds it was not observed

Table 4. Mean frond length (mm) and relative growth rate per day of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Selected 30 individuals of 25 days old were classified in 5 classes according to their frond length.

Strain	Age (days)	Frond length							Relative growth rate						
		25	30	37	45	54	66	80	25-30	30-37	37-45	45-54	54-66	66-80	25-66
		2.1	4.8	9.6	—	—	—	—	0.17	0.10	—	—	—	—	—
		3.5	10.5	22.2	45	66	73	—	0.22	0.11	0.09	0.04	0.01	—	0.07
W (C-13)		4.4	13.2	30.4	59	76	98	—	0.22	0.12	0.08	0.03	0.02	—	0.08
		5.4	15.9	35.8	70	90	105	111	0.22	0.12	0.08	0.03	0.01	0.01	0.07
		7.6	22.4	51.9	100	151	189	221	0.22	0.12	0.08	0.05	0.02	0.01	0.08
		2.3	6.1	12.4	—	—	—	—	0.20	0.10	—	—	—	—	—
R (C-22)		3.5	9.4	20.7	40	66	87	100	0.20	0.11	0.08	0.06	0.02	0.01	0.08
		4.5	11.7	27.4	58	90	112	125	0.19	0.12	0.09	0.05	0.02	0.01	0.08
		5.4	15.3	36.0	73	119	156	189	0.21	0.12	0.09	0.05	0.02	0.01	0.08
		2.4	6.1	11.2	—	—	—	—	0.19	0.09	—	—	—	—	—
G (C-32)		3.4	9.6	17.8	35	57	75	78	0.21	0.09	0.08	0.05	0.02	0.01	0.08
		4.8	12.7	26.1	54	73	99	113	0.19	0.10	0.09	0.03	0.03	0.01	0.07
		1.7	4.4	9.0	—	—	—	—	0.19	0.10	—	—	—	—	—
		3.4	10.7	23.5	55	92	122	165	0.23	0.11	0.11	0.06	0.02	0.02	0.09
G (C-0)		4.5	14.0	33.0	70	115	153	196	0.23	0.12	0.09	0.06	0.02	0.02	0.09
		5.4	16.0	36.9	75	123	178	220	0.23	0.12	0.09	0.05	0.03	0.02	0.09
		6.6	19.3	38.9	79	149	211	286	0.23	0.10	0.09	0.07	0.03	0.02	0.08

even at 88 days old. The relative growth rate became successively lower as the fronds aged from 25 to 80 days old. The relative growth rate for 25-30 days was a little higher than that for 20-25 days (Tables 3 and 4). This seems to be related to the removal of fronds from Cremona monofilaments resulting in the decrease of frond density in culture.

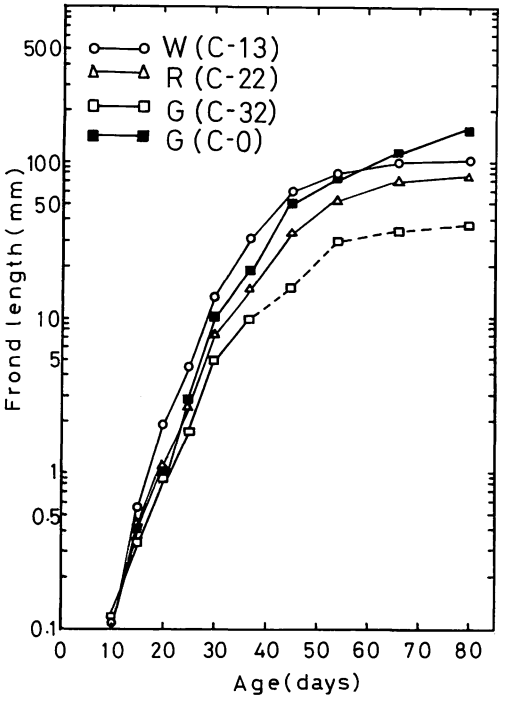

Fig. 3. Growth curves of the wild (W), red (R) and green type (G) fronds of cultured in laboratory.

The growth curves of the wild, red and green type fronds are shown in Fig. 3 as based on the mean frond length. The mean frond length was about 100, 80, 40 and 160 mm in the wild, red and green type (C-32 and C-0) populations, respectively, at the end of culture. These differences could be resulted largely from the differences of the growth rate in younger stage. It is possibly due to the fastest growth rate after 25 days old and the delay of reproductive maturation that the green type (C-0) fronds became longest of the four strains at the end of culture (cf. Table 4).

2) Photosynthesis and respiration

Photosynthesis-light curves of the wild, red and green type fronds obtained at 15°C and 0-90 klux are shown in Fig. 4. Fronds of 32-36 days old were the youngest used to measure photosynthesis and respiration. In these fronds the photosynthetic rates on a frond area basis were nearly saturated at 15-20 klux, but the rate continued to increase slightly with increase in light intensity up to 90 klux; i.e. the light saturation of photo-

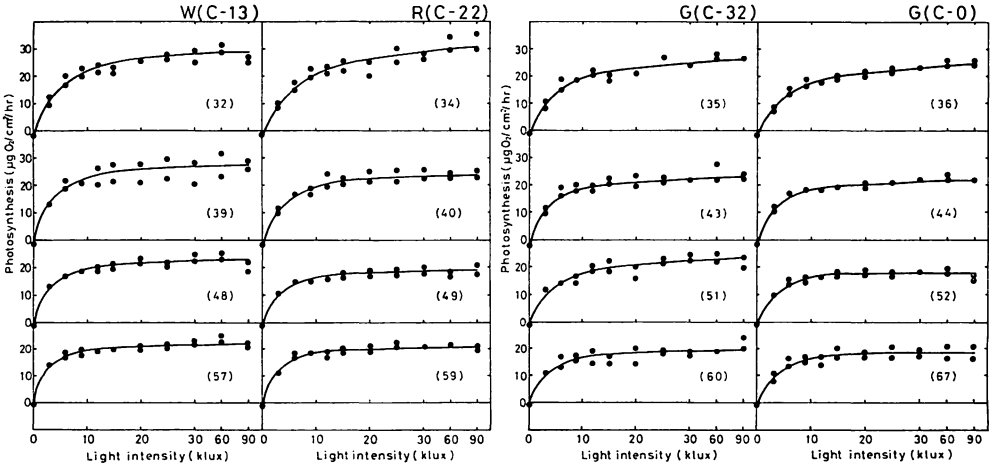

Fig. 4. Photosynthesis-light curves at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

synthesis was not clear. However, the light saturation became clear as fronds aged; the photosynthetic rates were saturated at 5-10 klux in the wild and red type fronds and at 10-15 klux in the green type fronds. No inhibition of the photosynthetic rate by high light intensity was observed in the range employed in the present experiment.

The photosynthetic rates on a frond area basis at 3, 9 and 30 klux obtained from the photosynthesis-light curves are shown in Fig. 5. At 30 and 9 klux, the photosynthetic rate decreased with frond age in the wild and green type (C-32) fronds, while in the green type (C-0) fronds it similarly decreased until 52 days old and was constant after that. In the red type fronds, on the other hand, the photosynthetic rate at 30 and 9 klux decreased rather sharply with age and increased a little at 57 days. The changes of the photosynthetic rates at 30 and 9 klux correspond well to the changes of the relative growth rate in the wild and green type (C-32 and C-0) fronds. The photosynthetic rate at 3 klux indicated no significant changes with age in all of the strains used. The photosynthetic rates at 30 and 9 klux were higher in the wild and red type fronds than in the green type fronds except for the rate of the red type fronds at 49 days. The photosynthetic rate of the green type (C-32) fronds was slightly higher than that of the green type (C-0) fronds at 30 and 9 klux. At 3 klux the photosynthetic rate of the wild type fronds was higher than that of the red and green type (C-32 and C-0) fronds which showed almost the same rates.

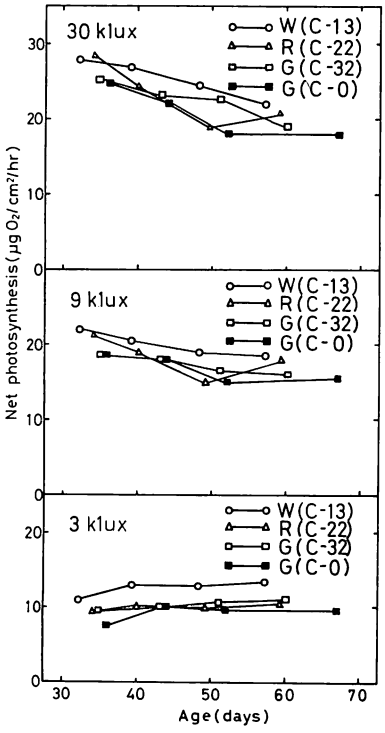

Fig. 5. Changes in the net photosynthetic rates at 3, 9 and 30 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-light curves in Fig. 4.

The changes of chlorophyll *a* content per cm^2 of frond area are shown in Fig. 6. The chlorophyll *a* content decreased with frond age in all the strains. The changes were especially remarkable in the wild, red and green type (C-32) fronds, but slight in the green type (C-0) fronds. The chlorophyll *a* content of the green type (C-0) fronds was lower than that of other type fronds. Although the photosynthetic rate on a frond area basis was different among the strains of different types, the photosynthetic rate on a chlorophyll *a* basis was not so different in all the types of fronds, and its changes with age were not conspicuous as a whole (Fig. 7).

The respiratory rates on a frond area basis

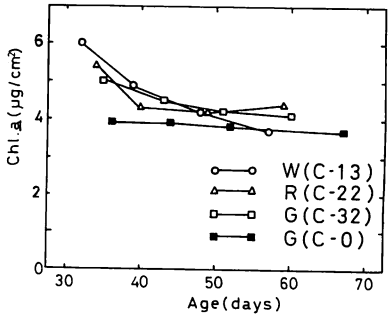

Fig. 6. Changes in chlorophyll *a* content per unit frond area of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory and used for the measurements of photosynthesis.

measured at 15°C in the dark showed great variations, but it is presumed that the rate decreased with frond age (Fig. 8). No remarkable differences were confirmed in the respiratory rate among the wild, red and green type fronds.

Photosynthesis- and respiration-temperature curves of the wild, red and green type fronds obtained at 5-30°C and 25 klux are shown in Fig. 9. The photosynthetic and respiratory rates of the three types of fronds showed the same tendency against temperature. The photosynthetic rate on a frond area basis increased with increase in temperature, attained a maximum at 20-25°C and decreased remarkably at 30°C. The respiratory rate on a frond area basis increased slightly with increase in temperature in the range of 5-30°C.

The changes with frond age of photo-

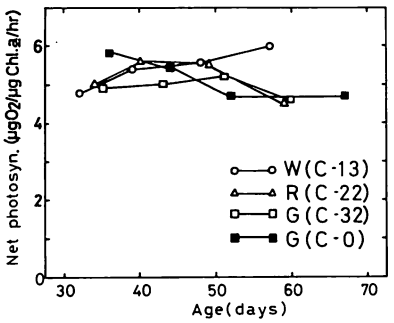

Fig. 7. Changes in the net photosynthetic rate on a chlorophyll *a* basis at 30 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory.

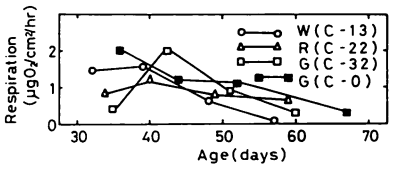

Fig. 8. Changes in respiratory rate at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-light curves in Fig. 4.

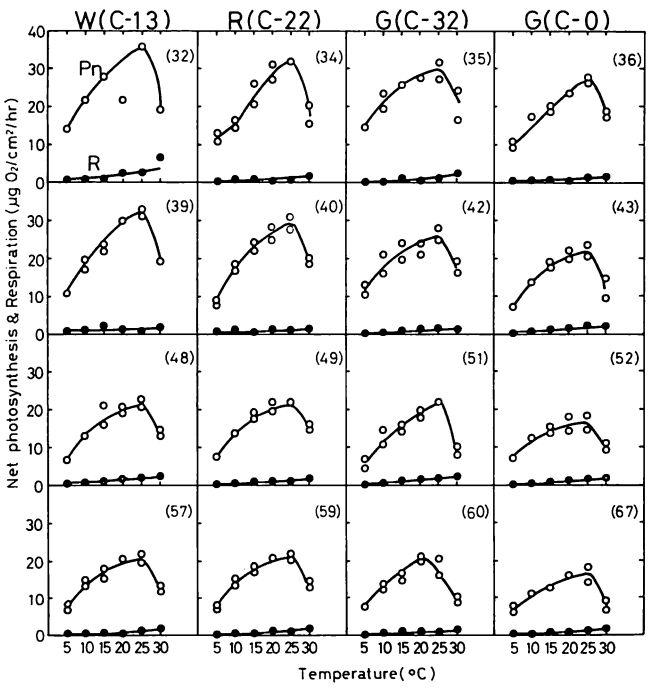

Fig. 9. Photosynthesis-temperature curves (○) at 25 klux and respiration-temperature curves (●) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

synthetic rates at 5, 10, 15, 20, 25 and 30°C are shown in Fig. 10. The photosynthetic rates decreased with frond age at each temperature in all the types of fronds, and the decrease was especially remarkable at 20 and 25°C. These changes were consistent with the changes of the photosynthetic rate at 30 and 9 klux shown in Fig. 5. The maximum photosynthetic rate attained at 25°C in most of the cases and was 35, 32, 30 and 27 μg $O_2/cm^2/hr$ in the wild, red and green type (C-32 and C-0) fronds of 32-36 days old, respectively. The maximum photosynthetic rates of the fronds of about 60 days old were almost the same, 20-21 μg $O_2/cm^2/hr$, in the wild, red and green type (C-32) fronds; but in the green type (C-0) frond it was considerably low, 16 μg $O_2/cm^2/hr$.

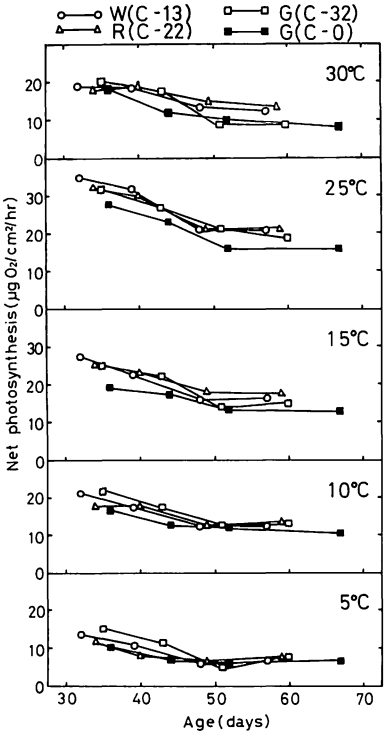

Fig. 10. Changes in the net photosynthetic rate at 5, 10, 15, 20, 25 and 30°C under 25 klux of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the photosynthesis-temperature curves in Fig. 9.

The changes of the respiratory rates at 15°C obtained from the respiration-temperature curves are shown in Fig. 11. The respiratory rates showed considerable variations until about 50 days, thereafter they gradually decreased with frond age. As an accuracy of the respiratory measurements by the present technique is not very good, the results shown in Figs. 9 and 11 would only suggest that the respiratory rate decreased gradually

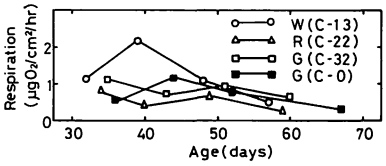

Fig. 11. Changes in the respiratory rate at 15°C of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. Data were from the respiration-temperature curves in Fig. 9.

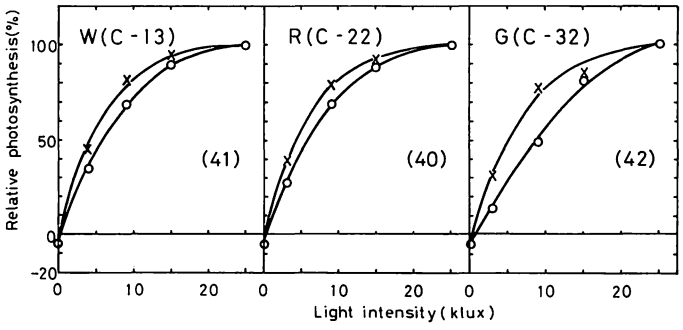

Fig. 12. Relative photosynthesis-light curves under white light (×) and green light (○) of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory. The figures in parentheses indicate the frond age in days.

with frond age.

Photosynthetic rates under white light and green light were compared with the wild (C-13), red (C-22) and green type (C-32) fronds of 40-42 days old. Photosynthetic rates were measured four times at 15°C under 3, 9, 15 and 25 klux, and respiratory rates were also measured at the same temperature. The relative photosynthesis-light curves obtained are shown in Fig. 12. The photosynthetic rates of all the three types of fronds were lower in green light than in white light. The extent of lowering in green light was slight in the wild and red type fronds, but it was remarkable in the green type frond. The result thus indicates that the green type frond is inferior to the wild and red type fronds in using green light for photosynthesis.

Discussion

The four strains of *Porphyra yezoensis* used in the present study have respective characteristic colors which are quite clearly reflected to their *in vivo* absorption spectra (Fig. 13). The spectra show conspicuous differences in the wavelength range where phycoerythrin and phycocyanin mainly take part in the absorption. Detailed comparisons of the spectra and pigment contents of the strains were described by ARUGA and MIURA (1984). Fronds of the red type strain have lower phycocyanin content and higher phycoerythrin/phycocyanin ratio (Table 5), while

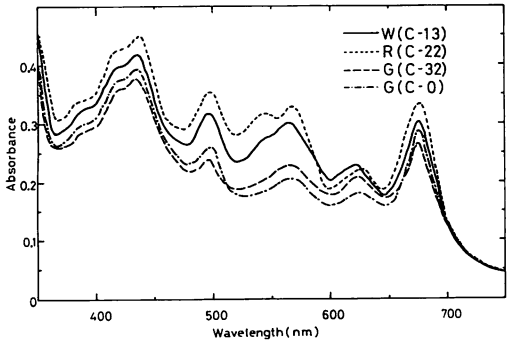

Fig. 13. *In vivo* absorption spectra of the wild (W), red (R) and green type (G) fronds (54 days old) of *P. yezoensis* cultured in laboratory.

Table 5. Phycoerythrin (PE) and phycocyanin (PC) contents of the wild (W), red (R) and green type (G) fronds of *P. yezoensis* cultured in laboratory.

Strain	PE (μg/cm^2)	PC (μg/cm^2)	PE/PC
W (C-13)	33.2	14.0	2.4
R (C-22)	28.3	10.2	2.8
G (C-32)	16.7	9.8	1.7
G (C-0)	17.4	11.4	1.5

fronds of the two green mutant strains show especially lower absorbance in the wavelength of 460-600 nm (Fig. 13) and have lower phycoerythrin content and consequently lower phycoerythrin/phycocyanin ratio (Table 5). As the chlorophyll *a* content of the three types of fronds is almost at the same level except for the green type (C-0) frond (cf. Fig. 6), it is important to compare the growth and photosynthesis in consideration of the contents and the ratios of photosynthetic pigments in the three types of fronds.

There are several reports as to the culture conditions of *Porphyra* fronds. KINOSHITA and TERAMOTO (1958) obtained the highest growth of *P. tenera* at 15°C and 6000 lux. IWASAKI and MATSUDAIRA (1958) and IWASAKI (1965) showed that the most suitable conditions for *P. tenera* were 14-16°C and an illumination by sheltered sunlight of 9 hr/day. The natural seawater from Tokyo Bay was used by KINOSHITA and TERAMOTO (1958) for culturing *P. tenera*. IWASAKI and MATSUDAIRA (1958) and IWASAKI (1965) studied the nutritional requirements of *P. tenera*. TATEWAKI (1971) indicated that the ESP medium was suitable for culturing many marine algae. KOBARA *et al.* (1976) were successful in completing the life cycle of the green type mutant of *P. yezoensis* in the ESP medium. In the present study the modified ESP medium was used to culture the three types of fronds of *P. yezoensis* at 15°C and 3-10 klux (10 hr light/day), and the growth of the fronds was fairly good even though the conditions and the medium were not confirmed to be most suitable.

OOHUSA *et al.* (1977) investigated the

diurnal variations of photosynthesis and respiration in *P. yezoensis*. They found out that the photosynthetic rate attained its maximum in the light period and its minimum in the dark period, and the respiratory rate showed its maximum at the end of the light period and its minimum at the end of the dark period. As the measurements of photosynthesis and respiration were started usually at the same time in the middle of the light period in the present study, comparisons of the results can be properly made without consideration of the diurnal rhythms.

In the present study the highest relative growth rate, 0.32/day, was obtained in the wild type fronds of *P. yezoensis* during the period of 10-15 days old (Table 3). The relative growth rate was high in an earlier period of growth and decreased with frond age in all the three types of fronds. This trend was in agreement with that of cultivated *Monostroma latissimum* (MAEGAWA and ARUGA 1974). However, such difference as observed in *M. latissimum* of the relative growth rate according to the frond length was not obtained in the present study. It was possibly due to the fact that all the fronds used in the present study were removed from Cremona monofilaments and cultured under the same light and nutrient conditions. Although YOSHIDA (1972) reported the highest relative growth rates of 0.27 and 0.46/day in cultivated *P. tenera* and *P. pseudolinealis*, respectively, the direct comparisons cannot be made between the result of laboratory culture and that of field cultivation.

SATOMI *et al.* (1968) showed that the photosynthetic ability of cultivated *P. yezoensis* was initially low, attaining its maximum of 30-35 m*l* O_2/g (d.w.)/hr at about 40 days after the conchospore seeding, and then continuously decreased to about half the maximum rate in 3 months after the seeding. The maximum photosynthetic rate of 30 μg O_2/cm^2/hr obtained with the wild type fronds in the present study was well comparable to the values of SATOMI *et al.* (1968). The photosynthetic rates at higher light intensities and at various temperatures decreased with frond age (Figs. 5 and 10). The same trend was also obtained both in cultivated *P. yezoensis* (SATOMI *et al.* 1968) and in cultivated *M. latissimum* (MAEGAWA and ARUGA 1974) whose environmental conditions were variable. Therefore, the frond age seems to be important in controlling the growth, photosynthesis and respiration.

The pattern of photosynthesis-temperature curves was almost the same in the three types of fronds of *P. yezoensis* (Fig. 9). The photosynthetic rate showed its maximum at 20-25°C, mostly at 25°C, irrespective of the frond age. The same type of photosynthesis-temperature relationships were reported with Rhodophycean seaweeds living in winter (YOKOHAMA 1973a), and with seaweeds in the colder region of Japan (HATA and YOKOHAMA 1976) and in arctic regions (HEALEY 1972). The photosynthetic rate at 15-25°C of the green type (C-0) frond was mostly lower than that of other types of fronds (cf. Fig. 10). No special differences were found in the photosynthesis-temperature relationship among the three types of fronds.

Algae generally have considerable flexibility in responding to surrounding light conditions by changing the contents and the ratios of photosynthetic pigments. The chlorophyll *a*-biliprotein system of the blue-green algae and red algae shows much greater flexibility in changing the pigment ratio than the chlorophyll *a*-carotenoid combination (HALLDAL 1970). YOKOHAMA (1973b) showed that the higher efficiency in utilizing green light, which was observed in the red algae from deeper range, was considered to be due to a high ratio of phycoerythrin content to chlorophyll *a* or phycocyanin content. According to CALABRESE (1972) *Petroglossum nicaeense* living inside of a sea cave adapted themselves to the shade by considerably increasing the phycoerythrin formation for a more efficient utilization of light energy. CALABRESE and FELICINI (1973) showed that the massive accumulation of phycoerythrin and chlorophyll *a* in the red thallus of *Glacilaria compressa* permitted a relatively

high photosynthetic efficiency even at low light intensity. In the present study a significant difference was found in the photosynthetic rate under green light between the green type frond and the wild or red type frond (Fig. 12). The photosynthetic rate was lower in green light than in white light in all of the three types of fronds, but the difference was especially remarkable in the green type frond which had considerably lower phycoerythrin content than the wild and red type fronds. The fact that the photosynthetic rate was lower in green light than in white light is in agreement with the character of the red algae which adapt themselves to the environment in the upper region of the sea (YOKOHAMA 1973b). It is likely that the wild, red and green type fronds cultured in the present study adapted themselves to white light, but details of this point remain to be investigated. The red type frond, which is presumed to be a qualitative mutant in phycobilins, showed almost the same photosynthetic response as the wild type frond under green light. This seems to indicate that phycoerythrin of the red type frond functions to the same extent as that of the wild type in harvesting green light for photosynthesis.

Obvious differences were found of the growth rate in an early period of growth among the wild, red and green type fronds (Table 3), but the differences became small as the fronds aged. In the blue-green alga *Anacystis nidulans*, yellow-green mutants which have normal chlorophyll but only half the phycocyanin of the parent were similar to the parent in the specific growth rate and photosynthetic rate, but blue mutants with somewhat higher phycocyanin but only one-third the chlorophyll of the parents are dissimilar to the parent in the specific growth rate and photosynthesis (STEVENS and MYERS 1976). A mutant of the coccoid blue-green alga *Agmonellum quadruplicatum*, which had a higher content of chlorophyll *a* relative to phycocyanin than the wild type, showed impaired growth on the medium to which NO_3 was added as a nitrogen source (STEVENS and VAN BAALEN 1970). In the present study the photosynthetic rate on a frond area basis was higher in the wild and red type fronds than in the green type frond, but this was mainly due to the difference of chlorophyll *a* content per unit frond area. There was no significant difference in the photosynthetic rate on a chlorophyll *a* basis among the three types of fronds. It is not considered that a decrease of phycoerythrin content had any effect on the photosynthetic rate under white light. Therefore, the difference in the growth rate at an early period of growth among the three types of fronds, especially a considerably inferior growth rate of green type, is considered not to be due to a decrease of phycoerythrin content by mutation.

The green type (C-0) fronds grew up largest of all at 80 days old in spite of their lower growth rate at an early period of growth and lower photosynthetic rate. This seemed to be mainly due to the delayed reproductive maturity. Although the photosynthesis, of course, plays an important part in the growth, other factors, such as reproductive maturity, hormones, vitamins and other growth regulators, which control the growth, should be investigated in the future.

Acknowledgements

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加藤光雄・有賀祐勝：培養によるスサビノリ色彩変異体の生長と光合成に関する比較研究

スサビノリ（*Porphyra yezoensis*）の野生型，赤色型，緑色型について，室内培養条件下で葉状体の生長と光合成に関する比較研究を行なった。葉齢25日の平均葉長は，野生型（C-13）で 4.5 mm，赤色型（C-22）で2.8 mm，緑色型（C-32）で 1.8 mm であった。1日あたりの平均相対生長率は，いずれの色彩型でも生長初期には高く（野生型 0.24，赤色型 0.22，緑色型 0.18），葉齢とともに低下し，葉齢25日以後は色彩型間で差がほとんど認められなくなった。葉齢 30～40 日以前の葉状体では，その光合成速度は 15～20 klux でほとんど光飽和に達したが，90 klux までわずかながら上昇がみられた。光飽和に達する光強度は葉齢とともに低下した。葉面積あたりの光合成速度は，弱光下でも強光下でも，赤色型や緑色型より野生型で高かったが，クロロフィル *a* 量あたりの光合成速度は色彩型間でほとんど差がみられなかった。いずれの色彩型の光合成-温度曲線でも，光飽和光合成速度は20～25°C で最大値を示し，葉面積あたりの光合成速度は葉齢とともに低下した。呼吸速度については，色彩型間でほとんど差異は認められなかった。光強度制限下での光合成速度は，いずれの色彩型でも白色光下より緑色光下で低かったが，フィコエリスリン含量の著しく低い緑色型でその差は特に顕著であった。他の緑色型（C-0）では，生長初期の相対生長率は野生型（C-13）よりわずかに低く，赤色型（C-22）とほぼ同じであったが，後期には上述の3つの色彩型より若干高かった。緑色型（C-0）の葉面積あたりの光合成速度は比較的低かった。

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