Jap. J. Phycol. (Sôrui) 32: 243-250, September 20, 1984

In vivo absorption spectra and pigment contents of the two types of color mutants of *Porphyra**

Yusho ARUGA and Akio MIURA

Laboratory of Phycology, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan

ARUGA, Y. and MIURA, A. 1984. *In vivo* absorption spectra and pigment contents of the two types of color mutants of *Porphyra*. Jap. J. Phycol., 32: 243-250.

Comparative studies were made on the pigmentation of fronds (leafy thallus) and conchocelis of the red and the green type mutants of *Porphyra yezoensis* and *P. tenera*. *In vivo* absorption spectra of fronds and conchocelis consistently showed characteristic differences between the wild type plants and the red and the green type mutants at the wavelengths where absorption is mainly due to phycobilins. The red type mutant showed the spectra having two-peaked absorption in a 530-580 nm region and a slight shift to longer wavelengths of the peak due to phycocyanin. The green type mutant had the spectra with markedly low absorption in a 460-590 nm region. As expected, phycoerythrin content per unit frond area was extremely low in the green type mutant of *P. yezoensis*. On a dry weight basis, chlorophyll *a* content was highest in the green type frond and lowest in the wild type frond, phycoerythrin content was almost the same in the wild and the red type fronds but distinctly low in the green type frond, and phycocyanin content was not so markedly different among the three types of fronds in *P. yezoensis* cultured under the same laboratory conditions. The red type mutant is characterized with lower PC/Chl. *a* ratio and higher PE/PC ratio, while the green type mutant with lower PE/Chl. *a* and PE/PC ratios. It is inferred that the red type mutant resulted from qualitative variation of phycobilin(s), while the green type mutants resulted from quantitative variation of phycoerythrin.

Key Index Words: *chlorophyll* a; *color mutants*; in vivo *absorption spectra*; *phycocyanin*; *phycoerythrin*; *pigment contents*; Porphyra tenera; Porphyra yezoensis.

Studies have been carried out on the pigmentation of *Porphyra*s in our laboratory since 1967. Among them there have been two principal subjects. One is concerned with the fading of color, i.e. the bleaching phenomena, in cultivated *Porphyra* fronds and its recovery (ARUGA and IWAMOTO, 1972). The other is concerned with pigmentation, photosynthetic pigment contents and photo-physiology of color mutants of *Porphyra*. The studies have, however, been published only partly (KOBARA *et al.* 1976, MERRILL *et al.* 1983, MIURA 1984). The red type mutant used in this study was a strain originally established through selection by a Nori farmer, while the green type mutant was obtained in laboratory culture from a green sector of a variegated chimeral frond collected in the Nori farm (KOBARA *et al.* 1976). KIKUCHI *et al.* (1979) reported chemical nature of phycobilins of the color mutants of *Porphyra yezoensis* from our laboratory. Details of variegated chimeras were partly reported on fronds found in cultivated populations of *Porphyra* with several different types of variegations and several types of

* Partly presented at the 9th International Seaweed Symposium, August 1977, Santa Barbara, California, U.S.A.

Financially supported in part by Nori Zoshoku Shinkokai (Nori Cultivation Promotion Association).

combinations of different color sectors (MIURA 1984). As to other species of seaweeds, on the other hand, only VAN DER MEER and his co-workers published a series of important results especially of genetic studies on the pigmentation mutants of *Gracilaria* (VAN DER MEER 1977, 1978, 1979a, b, 1980, VAN DER MEER and BIRD 1977, VAN DER MEER and TODD 1977) and a study of the life history of *Palmaria palmata* with a pigmentation mutant (VAN DER MEER and TODD 1980).

The contents of such photosynthetic pigments as chlorophyll *a*, phycobilins and carotenoids of cultivated *Porphyra* fronds are not only of physiological interest but also of greatest significance to the grading of commercial Nori sheets because of a high correlation between the pigment contents and the quality of dried Nori sheets (SAITOH *et al.* 1980). Especially, the contents of chlorophyll *a* and phycoerythrin are important in determining the color, and therefore the quality, of dried Nori sheets.

In the present paper we describe the characteristics of pigmentation in fronds and conchocelis, which have been confirmed in color mutants of cultivated *Porphyra yezoensis* and *P. tenera* populations since 1973, with special reference to the comparisons of color and pigment contents in the wild (normal) type, red type and green type plants.

Material and Methods

Fronds (leafy thalli) and conchocelis of *Porphyra yezoensis* and *P. tenera* cultivated in Nori cultivation farms and/or cultured in laboratory were analyzed. Laboratory cultures of fronds and conchocelis were carried out using ESP medium (PROVASOLI 1966). Other culture conditions were the same as described by KOBARA *et al.* (1976). *In vivo* visible absorption spectra were recorded with air as reference with a Shimadzu Multipurpose Recording Spectrophotometer MPS-50L.

Chlorophyll *a* was extracted in 90% acetone and absorbances of the extract were determined at 750, 663, 645 and 630 nm with a Hitachi 101 Spectrophotometer. The amount of chlorophyll *a* in the extract was calculated by the equation of SCOR-UNESCO (1966).

Phycobilins were extracted in distilled water. Absorbances of the extract were determined at 568 and 615 nm with a Hitachi 101 Spectrophotometer, and calculations of the amounts of phycoerythrin and phycocyanin were made by using the extinction coefficients reported by Ó hEOCHA (1965).

Results and Discussions

In vivo visible absorption spectra were recorded for the wild, red and green types of fresh *Porphyra* fronds to make clear the characteristics of their frond color. In a strict sense, absorption or absorbance in such cases should be called attenuation or attenuance according to SHIBATA (1974). In this paper, however, the popular term absorption or absorbance is used. As is well-known (cf. HAXO and BLINKS 1950) the *in vivo* absorption spectrum of *Porphyra* frond has in general five representative characteristic peaks; from shorter to longer wavelengths, the first peak is mainly based on the absorption by chlorophyll *a*, the second one mainly by carotenoids and phycoerythrin, the third one mainly by phycoerythrin, the fourth one mainly by phycocyanin, and the fifth one mainly by chlorophyll *a*.

In vivo absorption spectra of the wild type and the red type sectors of a variegated chimeral frond in *P. yezoensis* are shown in Fig. 1. These absorption spectra are clearly different from one the other at wavelengths where the absorption is mainly due to phycoerythrin and phycocyanin. The red type sector showed two apparent absorption maxima approximately at 543 and 568 nm, and a maximum at 622 nm which is slightly shifted to longer wavelengths (a shift of about 3 nm) as compared with the wild type sector which showed absorption maxima at 568 and 619 nm.

Fig. 2 illustrates the *in vivo* absorption spectra of cultivated wild type and red type fronds of *P. yezoensis*. The same charac-

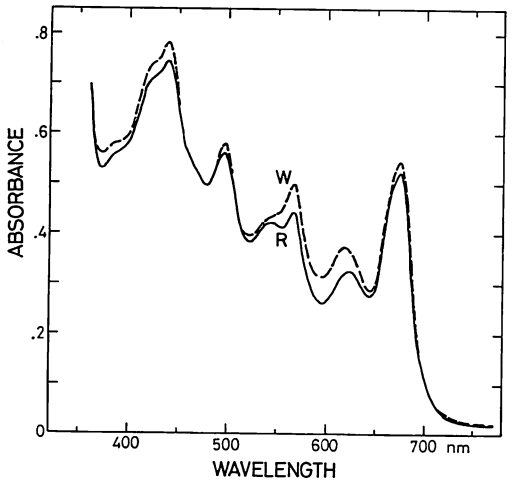

Fig. 1. *In vivo* absorption spectra of the red type (R) and the wild type (W) sectors of a variegated chimeral frond of *Porphyra yezoensis* obtained from a cultivated population.

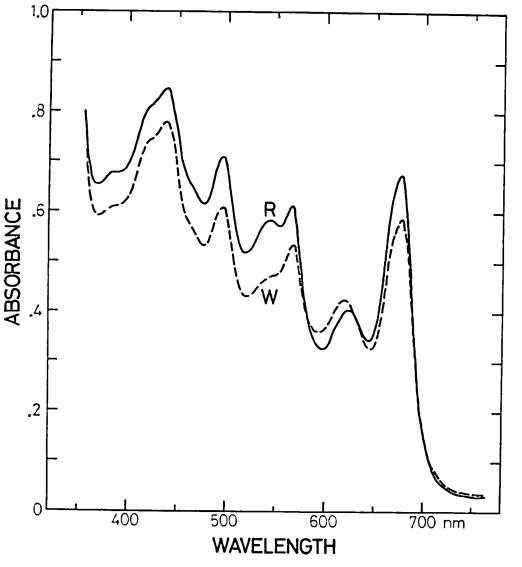

Fig. 2. *In vivo* absorption spectra of the red type (R) and the wild type (W) fronds of *Porphyra yezoensis* obtained from a cultivated population.

teristic features as seen in Fig. 1 can clearly be found in these two spectra. In *P. yezoensis* fronds subtle changes of color can be noticed normally in the basal portion ; the basal portion is light and greenish in color as compared with the central and upper portions of the frond. Therefore, we examined the

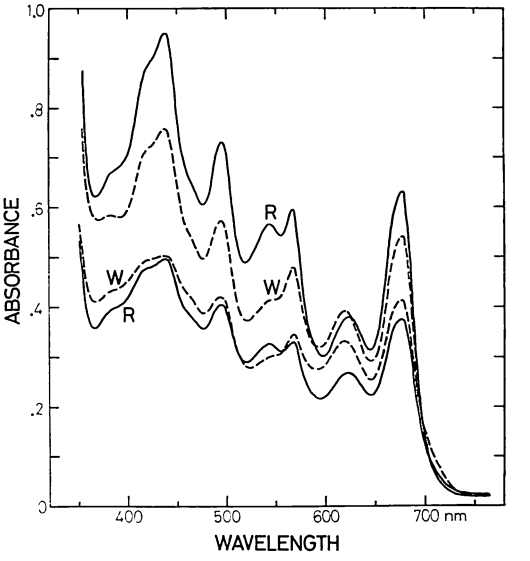

Fig. 3. Comparisons of *in vivo* absorption spectra between the basal portion (lower two spectra) and the upper portion (upper two spectra) of the wild type (W) and the red type (R) fronds of *Porphyra yezoensis* obtained from a cultivated population.

absorption spectra of various portions of a frond both with the wild type and the red type plants and found no difference at all with respect to the characteristic absorption pattern as mentioned above, although the absorbance was usually lower in the basal portion than in the upper portion. Fig. 3 illustrates the comparisons of absorption spectra between the basal and the upper portions of both the same fronds. We also examined more than 200 red type fronds and red type sectors of variegated chimeral fronds of *P. yezoensis* and confirmed that all of them had the same patterns of absorption spectra as the red type shown in Figs. 1 and 2.

In vivo absorption spectra were also compared between the green type sector and the wild type sector of a variegated chimeral frond of *P. yezoensis* (Fig. 4). In the green type sector the wavelenths and patterns of absorption maxima were well in accordance with those of the wild type sector, except that the absorbance was quite low as compared with that of the wild type sector at wavelengths of 460-590 nm where phycoery-

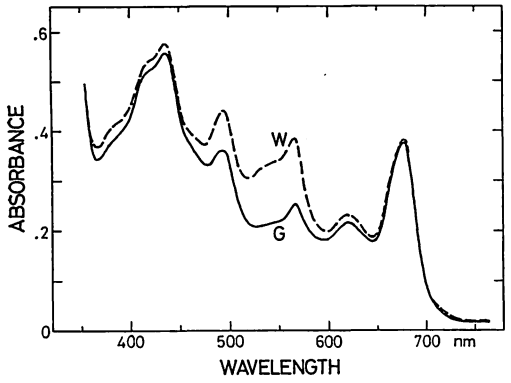

Fig. 4. *In vivo* absorption spectra of the green type (G) and the wild type (W) sectors of a variegated chimeral frond of *Porphyra yezoensis* cultured in laboratory.

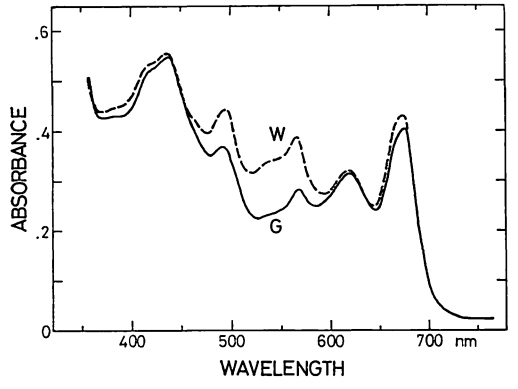

Fig. 5. *In vivo* absorption spectra of the green type (G) and the wild type (W) fronds of *Porphyra yezoensis* cultivated in a Nori farm.

thrin mostly contributes to the absorption. Fig. 5 shows the *in vivo* absorption spectra of the green type mutant frond and the wild type frond of *P. yezoensis* both cultivated in a Nori farm. The absorption spectrum of the green type mutant frond had the same characteristic pattern as that of the green type sector of the variegated chimeral frond shown in Fig. 4. The same type of *in vivo* absorption spectra were consistently obtained with all of the investigated green type fronds both cultivated in the field and cultured in laboratory.

In Fig. 6 are illustrated the *in vivo* absorption spectra of the wild, red and green types of *P. yezoensis* fronds which were cultured

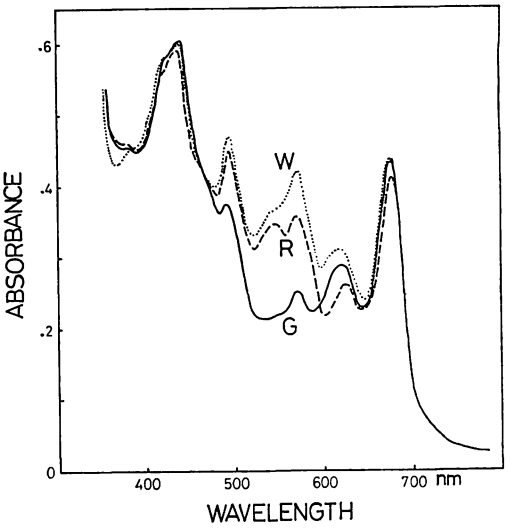

Fig. 6. *In vivo* absorption spectra of the red type (R), the green type (G) and the wild type (W) fronds of *Porphyra yezoensis* cultured under the same laboratory conditions.

under the same laboratory conditions; i. e. the conchospores were collected from free-living conchocelis on the same day and the sporelings were cultured under the same light and temperature conditions using the same culture medium. As can be clearly seen, the pattern of absorption spectrum of each frond was consistent with that of respective fronds (Figs. 2, 3 and 5) obtained from Nori farms at different periods of the cultivation season. Thus, it is established that the difference in color of fronds certainly exists and can be clearly distinguished according to the *in vivo* absorption spectrum characteristic of each type of color mutants.

From the wild, red and green type fronds of *P. yezoensis* we isolated respective carpospores and cultured them in laboratory to obtain respective free-living conchocelis. As the results, we succeeded to establish the wild, red and green types of conchocelis cultures respectively from the wild, red and green types of fronds. We also succeeded to obtain cultures of the wild, red and green types of shell-inhabiting conchocelis in laboratory. Differences in color of both the free-living and the shell-inhabiting conchocelis

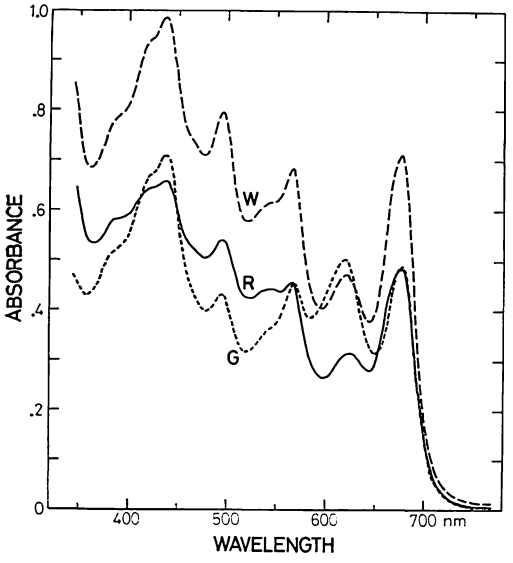

Fig. 7. *In vivo* absorption spectra of the red type (R), the green type (G) and the wild type (W) free-living conchocelis colonies of *Porphyra yezoensis* cultured in laboratory.

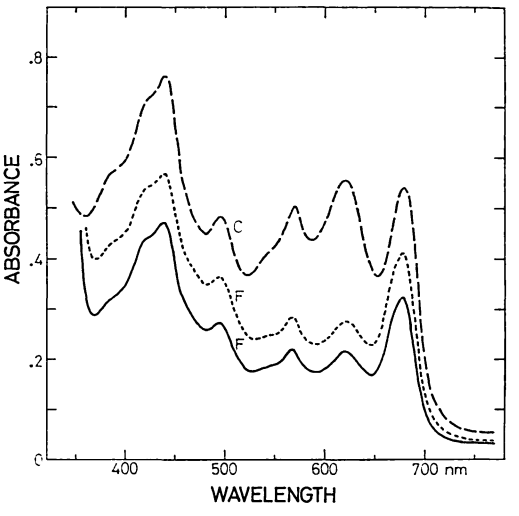

Fig. 8. *In vivo* absorption spectra of the two green type (C and F) conchocelis of *Porphyra yezoensis* cultured in laboratory.

colonies can easily be distinguished by naked eyes when the culture conditions were favorable. With the different types of free-living conchocelis colonies we tried to compare the *in vivo* absorption spectra.

Fig. 7 illustrates the *in vivo* absorption spectra of the wild, red and green types of free-living conchocelis colonies of *P. yezoensis* cultured in the laboratory. In each of the curves, the absorption characteristics of conchocelis are consistently in accordance with those of the respective type of frond described above. Two different green types of mutant strains of *P. yezoensis* are kept in our cultures of conchocelis, of which one shows bright green color and the other dim green color. In the former strain the absorbance at 619 nm was higher than that at 568 nm (Fig. 8, C), while the absorbance at 568 nm was slightly higher than that at 619 nm in the latter strain (Fig. 8, F). The same trends were confirmed to be true in the absorption spectra of fronds of respective strain.

From the above-mentioned characteristics of the *in vivo* absorption spectra of fronds and conchocelis, it is suggested that the red type strain is based mainly on the qualitative genetic variation(s) of phycobilins while the green type strain is based at least on the quantitative genetic variation of phycoerythrin. In this context, KIKUCHI *et al.* (1979) reported that there are qualitative differences of phycobilins among the wild, red and green type fronds of *P. yezoensis*.

With the fronds cultured during the same period under the same laboratory conditons, the contents of chlorophyll *a* and phycobilins were compared among the wild, red and two green type fronds of *P. yezoensis*. Table 1

Table 1. Contents of chlorophyll *a*, phycoerythrin (PE) and phycocyanin (PC), and their ratios in the wild type (W), the red type (R) and the green type (G) fronds of *Porphyra yezoensis* cultured under the same laboratory conditions. Age: 40 days after conchospore attachment.

Frond types*	Chl. *a*	PE	PC	PE	PC	PE
	(μg/cm^2)			Chl. *a*	Chl. *a*	PC
W(C-13)	4.90	33.2	14.0	6.8	2.9	2.4
R(C-22)	4.33	28.3	10.2	6.5	2.4	2.8
G(C-32)	4.48	16.7	9.8	3.7	2.2	1.7
G(C-0)	3.88	17.4	11.4	4.5	2.9	1.5

* Strain numbers in parentheses.

shows the results of quantitative determinations of the pigments with the sample fronds about 40 days after the conchospore attachment. Pigment contents are expressed on a frond area basis in this table. Chlorophyll *a* content differed only slighty, being only a little lower in the red type and in the green type. Phycoerythrin content was appreciably higher in the wild and the red types than in the green type as expected from the pattern of the *in vivo* absorption spectra described above. Phycocyanin content was not so greatly different among the three types, even though it was slightly lower in the red and the green types.

The ratios of contents of chlorophyll *a* (Chl. *a*), phycoerythrin (PE) and phycocyanin (PC) were also compared among the three types of fronds of *P. yezoensis* (Table 1). The PE/Chl. *a* ratio was of course appreciably higher in the wild and the red types than in the green type. Almost no difference was found in the PC/Chl. *a* ratio among the three types, even though the ratio was slightly lower in the red type and in a strain of the green type. The PE/PC ratio was apparently higher in the wild and the red types than in the green type.

In Table 2 are shown the results of another quantitative determinations of the pigments on a dry weight basis of the three types of *P. yezoensis* fronds cultured under the same laboratory conditions. Chlorophyll *a* content of fronds was highest in the green type frond and lowest in the wild type frond within a range of 0.63–0.77%. Phycoerythrin content was almost the same (4.8–5.1%) in the wild type and the red type frond but low (3.9%) in the green type frond. Phycocyanin content was not so markedly different (2.0–2.4%) among the three types of fronds. The PE/Chl. *a* ratio was distinctly lower in the green type frond than in the wild type and the red type fronds. The PC/Chl. *a* ratio was higher in the wild type frond than in the red type and the green type fronds. The PE/PC ratio was relatively high in the red type frond and relatively low in the green type frond. Thus, the trends of the ratios are quite similar to those shown in Table 1.

Table 2. Contents of chlorophyll *a*, phycoerythrin (PE) and phycocyanin (PC), and their ratios of the wild type (W), the red type (R) and the green type (G) fronds of *Porphyra yezoensis* cultured under the same laboratory conditions.

Frond types*	Chl. *a*	PE	PC	PE / Chl. *a*	PC / Chl. *a*	PE / PC
	(% of D. W.)					
W (C-13)	0.63	4.8	2.4	7.6	3.8	2.0
R (C-22)	0.69	5.1	2.0	7.4	2.9	2.5
G (C-32)	0.77	3.9	2.2	5.1	2.9	1.7

* Strain numbers in parentheses.

In consequence, the wild type is characterized with higher PE/Chl. *a* and PC/Chl. *a* ratios, i.e. higher PE and PC contents relative to Chl. *a* content; the red type mutant is characterized with lower PC/Chl. *a* ratio and higher PE/PC ratio, i.e. lower PC content relative to Chl. *a* and PE contents; and the green type mutant is characterized with lower PE/Chl. *a* and PE/PC ratios, i.e. lower PE content relative to Chl. *a* and PC contents.

Fig. 9 shows the *in vivo* absorption spectra of the wild, red and green types of *P. tenera* fronds cultivated in Nori farms. The characteristic features of the spectra were quite well in accordance with those obtained in

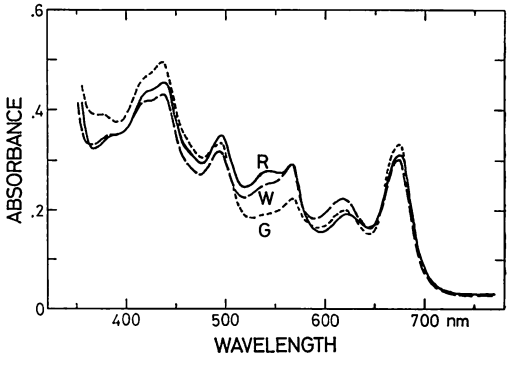

Fig. 9. *In vivo* absorption spectra of the red type (R), the green type (G) and the wild type (W) fronds of *Porphyra tenera* obtained from cultivated populations.

P. yezoensis. This suggests that similar genetic variations occurred in this species of *Porphyra*. Details of the mutant strains of *P. tenera* remain to be investigated.

The red type strains of *P. yezoensis* and *P. tenera* investigated in the present study have been cultivated by Nori cultivators in Nori farms in Japan. The green type strain of *P. yezoensis*, on the other hand, was originally produced in our laboratory from a variegated chimeral frond with wild type and green type sectors cultivated in the Nori farm (KOBARA *et al.* 1976). The green type strain of this species is now cultivated commercially by some Nori cultivators in some districts of Japan. The green type strain of *P. tenera* has also been cultivated by Nori farmers in some districts of Japan. It is quite interesting to note that our results clearly show quantitative and qualitative genetic variations existing in a species of *Porphyra*. The characteristic changes in pigmentation of *Porphyra* described above are essentially different from those reported in *Chondrus crispus* in the natural habitat (RHEE and BRIGGS 1977) in the sense that the latter changes are not genetic. Genetic studies of the mutant strains of *P. yezoensis* have been conducted in our laboratory and will be published elsewhere.

Acknowledgements

We are very grateful to Mr. Kazuhiro TAKAHASHI of Shin-Futtsu Fishermen's Co-operative Association for his continuing co-operation in cultivating and supplying the sample fronds of *Porphyra yezoensis* used in the present study. Financial support by Nori Zoshoku Shinkokai (Nori Cultivation Promotion Association) is also gratefully acknowledged.

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有賀祐勝・三浦昭雄： アマノリ属色彩変異体の生体吸光スペクトルと色素含量

スサビノリ (*Porphyra yezoensis*) およびアサクサノリ (*P. tenera*) にみられる赤色型および緑色型色彩変異体について，色彩と色素含量に関する比較研究を行った。葉状体および糸状体の生体吸光スペクトルで，主としてフィコビリン色素が光吸収に関与する波長域において赤色型，緑色型，野生型でいずれも明瞭に異なる特徴がつねに認められた。すなわち，野生型とは異なり，赤色型変異体では 530-580 nm 域に 2 つのピークをもつ吸光極大があり，フィコシアニンによる 620 nm 付近の吸光極大がわずかに長波長側へシフトしているのに対して，緑色型変異体では 460-590 nm 域の吸光度が著しく低いのが特徴である。スサビノリ葉状体について色素を定量した結果，単位葉面積あたりのフィコエリスリン含量は緑色型で著しく低かった。また，同一条件で室内培養したスサビノリ葉状体の分析結果では，乾燥重量あたりのクロロフィル *a* 含量は緑色型で最も高く，野生型で最も低く，フィコエリスリン含量は野生型と赤色型でほとんど同じであるが，緑色型で明らかに低く，フィコシアニン含量は色彩型間に著しい差異が認められなかった。赤色型変異体ではフィコシアニン/クロロフィル *a* 比が低く，フィコエリスリン/フィコシアニン比が高いのに対して，緑色型変異体ではフィコエリスリン/クロロフィル *a* 比もフィコエリスリン/フィコシアニン比も共に低いのが特徴である。赤色型はフィコビリン色素の質的な変異に，緑色型はフィコエリスリンの量的な変異に基づくものであると推測される。(〒108 東京都港区港南 4-5-7 東京水産大学植物学教室)