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Quality Improvement of Dried Sheets of *Porphyra* Using Recombinant Wild-Type in *P. yezoensis* (Bangiales, Rhodophyta)

Jong-Ahm Shin

Division of Aqualife Science, Yosu National University, Yeosu 550-749, Korea

The green-type and the red-type pigmentation mutant of *Porphyra yezoensis* were used to make the recombinant wild-type (ZGRW) by the cross experiment. This ZGRW was cultivated to determine its characteristic properties. The local cultivar was grown as a control. The quality of dried products was compared with the local cultivar in terms of the photosynthetic pigments content of gametothalli, the color, luster and flavor substances of dried sheets. The contents of chlorophyll *a*, phycoerythrin and phycocyanin of the ZGRW were higher than those of the local cultivars. The color and luster of dried sheets of the ZGRW showed better quality than those of the local cultivars. The concentration of dimethyl sulfide of the ZGRW was higher than that of local cultivars. The quality of the ZGRW was better than that of the local cultivars.

Key Words: color, cross breeding, flavor substances, luster, photosynthetic pigment content, pigmentation mutant, *Porphyra yezoensis*, quality improvement, recombinant wild-type.

INTRODUCTION

Recently, there have been several studies on the genetics of pigmentation mutants in *Porphyra yezoensis* by Miura and others (Miura 1985; Ohme *et al.* 1986; Ohme and Miura 1988; Niwa *et al.* 1993; Hamada *et al.* 1994; Miura and Ohme-Takagi 1994; Shin and Miura 1997; Shin *et al.* 1997; Shin 1999). In addition, the breeding studies on *Porphyra* by crossing between the pigmentation mutants were also conducted by the same group (Miura and Shin 1989; Shin and Miura 1995; Shin *et al.* 1996).

The target of genetic improvement of *Porphyra* may be different depending on the cooking methods and serving types of *Porphyra*. Generally, the lustrous deep pigmentation and higher concentration of volatile sulfur compounds with high yield are the desired characteristics of cultivars of *Porphyra*.

The recombinant wild-type from the crossing between two pigmentation mutants of *P. yezoensis* was investigated to test its properties from the commercial point of view. The quality of *Porphyra* were investigated in terms of the contents of photosynthetic pigments of gametothalli, degree of color and luster in dried sheets of gametothalli, and concentration of volatile sulfur compounds in dried sheets of gametothalli.

*Corresponding author (shin@yosu.ac.kr)

MATERIALS AND METHODS

The recombinant wild-type of *Porphyra yezoensis* (ZGRW) and the local cultivars were investigated by cultivation. A green-type (C-O giant) and a red-type (H-25) pigmentation mutant of *P. yezoensis* were used as parental lines for creating the recombinant wild-type. These two mutants were monogenic, recessive to the wild-type, and belong to the same linkage group, with each gene situated on different loci on a homologous chromosome. Crossbred sporothalli produce sectorially variegated chimeral and single phenotypic gametothalli in color since meiosis occurs at the time of conchospore germination (Ma and Miura 1984; Ohme *et al.* 1986; Ohme and Miura 1988; Tseng and Sun 1989; Shin 1991; Hamada *et al.* 1994; Miura and Ohme-Takagi 1994). A recombinant wild-type gametothallus, produced from the green-type as maternal parent and the red-type as paternal, was selected among single color gametothalli. Fixation of the recombinant wild-type (ZGRW) was performed by successive self-fertilization in a gametothallus *in vitro*. Then the ZGRW was grown to determine its

characteristics and to compare with the local cultivars in Japan.

Chlorophyll *a* (Chl. *a*) was extracted in 90% acetone and the absorbancies of the extracts were determined at 750, 663, 645 and 630 nm with a Shimadzu UV-120-02 Spectrophotometer. The amount of Chl. *a* was calculated by the equation of SCOR-UNESCO Working Group (1966).

Phycobilins were extracted in distilled water. Absorbancies of the extract were determined at 568 and 615 nm with a Shimadzu UV-120-02 Spectrophotometer. The amounts of phycoerythrin (PE) and phycocyanin (PC) were calculated using the extinction coefficients reported by O'Carra and O'hEocha (1976).

The color and luster of dried sheets of gametothalli were determined with a Murakami Color Institute SG-102 Color-checkman (*Porphyra* Quality Tester). The color was determined as black level. The color and luster of six points on a bundle of ten dried sheets were measured automatically, and the marks of them using their respective mean values were appraised automatically with the Color-checkman. The color was measured at 630 nm, and the luster was measured by the reflection ratio of 0° incidence and light-interception. Moreover, considering visual sensation and sensitiveness of the Color-checkman, the ratio of the color and luster was set up at 8.5:1.5. The total marks were automatically calculated by (mark of color × 0.85) + (mark of luster × 0.15). The grade of quality of dried sheets was automatically appraised by grade-discriminant level settled beforehand with the Color-checkman.

The level of dimethyl sulfide (DMS), hydrogen sulfide (H_2S) and methylmercaptan (MM) were determined to test the flavor substances of dried sheets of *Porphyra* among volatile sulfur compounds. These compounds in the head space vapor were analyzed by gas chromatography. Two grams of small pieces of dried sheets of *Porphyra* in a 50 m*l*-vial with 30 m*l* distilled water was kept for 20 minutes at 40°C in a water bath, and then 5 m*l* of the head space vapor was applied to a Shimadzu GC-9A gas chromatograph equipped with a flame photometric detector and a 3×3 mm glass column packed with 25% 1,2,3-tris[2-cyanoethoxy] propane on 60-80 mesh Shimalite. The flow rate of nitrogen as carrier gas was 50 $ml \cdot min^{-1}$ and the column temperature was 40°C (Osumi *et al.* 1990).

RESULTS AND DISCUSSION

Table 1 shows the results of quantitative determinations of Chl. *a*, PE and PC based on a gametothallus area and a dry weight. The ratios of contents between the ZGRW and the local cultivars of *P. yezoensis* cultivated at different sites and collected on specific dates. The contents of Chl. *a*, PE and PC per unit gametothallus area of the ZGRW were higher than those of the local cultivars in the range of 105-128, 102-118 and 100-133%, respectively. The dry weight based contents of Chl. *a*, PE and PC of the ZGRW were higher than those of the local cultivars in the range of 108-153, 104-148 and 103-156%, respectively.

These results show that the color of the ZGRW was much dark in black than that of the local cultivars. The color of dried sheets of *Porphyra*, which is an important factor that controls the commercial value of the dried *Porphyra*, is dependent on the contents and the ratios of photosynthetic pigments (Saitoh *et al.* 1975; Amano and Noda 1978; Oohusa *et al.* 1978; Nisizawa and Oofusa 1990).

The ratios of PC/Chl. *a* of the ZGRW were not significantly different from those of the local cultivars. The ratios of PE/Chl. *a* and PE/PC of the ZGRW were almost lower than those of the local cultivars. These results show that the color of the ZGRW is weakly purple compared to the local cultivars.

The amount of these pigments in ZGRW were always higher than those in the local cultivars tested in the present study in terms of farming years, seasons and sites. The difference between the ZGRW and the local cultivars in the amount and the ratio of pigments, however, varied widely depending upon the farming years, seasons and sites. This suggests that the contents of photosynthetic pigments are governed by polygene, which represents the modification of cultivar caused by environment.

Table 2 shows the comparison of the color and luster in dried sheets of *Porpyhra* between the ZGRW and the local cultivars. Although the grade and mark of the color and luster, and the total grade and mark varied depending upon the farming years, seasons and sites (Noda and Iwata 1983), the grade and mark of the ZGRW were higher than those of the local cultivars except for one case of color and two cases of luster.

The grade of quality of dried sheets of *Porphyra* was classified by the color and luster generally. The color is

Table 1. Comparison of contents ($\mu g/cm^2$, % of D.W.) of chlorophyll *a* (Chl. *a*), phycoerythrin (PE) and phycocyanin (PC), and their ratios between the recombinant wild-type(ZGRW) and the local cultivar of *Porphyra yezoensis* cultivated at different sites and collected on specified dates

Cultivar	Chl. *a*	PE ($\mu g/cm^2$)	PC	Chl. *a*	PE (% of D.W.)	PC	PE / Chl. *a*	PC / Chl. *a*	PE / PC
First year of growing test, spring harvest									
Tokyo Bay, Shin-Futtsu, Apr. 4, 1988									
ZGRW	3.62	44.4	30.7	0.355	4.35	3.01	12.3	8.48	1.45
Local cultivar	3.43	42.5	29.5	0.238	2.95	2.05	12.4	8.60	1.44
Mikawa Bay, Koromozaki, Jun. 7, 1988									
ZGRW	2.83	24.0	13.6	0.337	2.85	1.62	8.48	4.81	1.76
Local cultivar	2.46	20.6	11.5	0.293	2.45	1.37	8.37	4.67	1.79
Second year of growing test, autumn harvest									
Ishinomaki Bay, Hamaichi, Nov, 20, 1988									
ZGRW	3.33	35.3	20.2	0.573	6.08	3.48	10.6	6.07	1.75
Local cultivar	2.97	34.7	20.2	0.463	5.41	3.15	11.7	6.80	1.72
Tokyo Bay, Shin-Futtsu, Nov. 22, 1988									
ZGRW	4.60	39.4	19.5	0.608	5.20	2.58	8.57	4.24	2.20
Local cultivar	4.28	37.8	15.2	0.562	4.97	2.00	8.83	3.55	2.49
Mikawa Bay, Koromozaki, Nov. 29, 1988									
ZGRW	3.91	37.0	16.8	0.592	5.60	2.54	9.46	4.30	2.20
Local cultivar	3.67	35.3	15.5	0.511	4.92	2.16	9.62	4.22	2.28
Second year of growing test, spring harvest									
Ishinomaki Bay, Hamaichi, Jun, 16, 1989									
ZGRW	3.85	31.4	14.1	0.534	4.36	1.96	8.16	3.66	2.23
Local cultivar	3.11	28.0	12.3	0.350	3.15	1.38	9.00	3.95	2.28
Tokyo Bay, Shin-Futtsu, Feb. 12, 1989									
ZGRW	5.17	44.0	22.4	0623	5.30	2.70	8.51	4.33	1.96
Local cultivar	4.57	43.2	22.0	0.541	5.12	2.61	9.45	4.81	1.96
Mikawa Bay, Koromozaki, Feb. 14, 1989									
ZGRW	5.14	39.2	18.5	0.651	4.96	2.34	7.63	3.60	2.12
Local cultivar	4.80	36.7	16.6	0.602	4.80	2.08	7.65	3.46	2.21
Third year of growing test, autumn harvest									
Ishinomaki Bay, Hamaichi, Dec, 9, 1989									
ZGRW	4.18	36.5	15.4	0.596	5.21	2.20	8.73	3.68	2.37
Local cultivar	3.67	32.8	13.8	0.518	4.63	19.5	8.94	3.76	2.38
Tokyo Bay, Shin-Futtsu, Dec. 18, 1989									
ZGRW	4.30	42.7	20.2	0.548	5.44	2.57	9.93	4.70	2.11
Local cultivar	4.11	40.8	18.6	0.428	4.25	19.4	9.93	4.53	2.19
Mikawa Bay, Koromozaki, Nov. 18, 1989									
ZGRW	4.25	44.9	17.0	0.584	6.17	2.34	10.6	4.00	2.64
Local cultivar	3.32	39.0	14.7	0.478	5.62	2.12	11.7	4.43	2.65
Third year of growing test, spring harvest									
Ishinomaki Bay, Hamaichi, Jan, 15, 1990									
ZGRW	3.54	32.8	16.8	0.603	5.59	2.86	9.27	4.75	1.95
Local cultivar	3.18	27.8	12.6	0.462	4.03	1.83	8.74	3.96	2.21
Tokyo Bay, Shin-Futtsu, Mar. 9, 1990									
ZGRW	4.49	36.0	18.2	0.610	4.89	2.47	8.02	4.05	1.98
Local cultivar	4.20	34.6	15.2	0.470	3.87	1.70	8.24	3.62	2.28
Mikawa Bay, Koromozaki, Jan. 9, 1990									
ZGRW	4.49	35.2	18.4	0.542	4.25	2.22	7.84	4.10	1.91
Local cultivar	4.21	33.6	15.3	0.543	3.62	1.65	7.98	3.63	2.20

Table 2. Comparison of the color and luster in dried sheets of *Porphyra* between the ZGRW and the local cultivar of *Porphyra yezoensis* (grade/mark)

Farming site	Harvesting date	Cultivar	Color	Luster	Total
Hamachi	Nov. 15, 1989	ZGRW	2/186	4/142	2/179
		Local cultivar	2/178	6/127	3/170
Nishio	Dec. 26, 1989	ZGRW	1/192	5/137	2/184
		Local cultivar	3/160	5/132	5/156
Koromozaki	Jan. 09, 1990	ZGRW	2/185	4/146	2/179
		Local cultivar	3/172	7/119	4/164
Toushi	Dec. 10, 1989	ZGRW	2/183	6/125	3/174
		Local cultivar	2/177	6/123	3/169
Tsukura(I)	Jan. 10, 1990	ZGRW	2/184	6/126	2/175
		Local cultivar	3/172	8/106	4/162
Tsukura(II)	Jan. 20, 1990	ZGRW	3/173	6/128	3/166
		Local cultivar	3/167	7/117	4/160
Tamatsu	Jan. 10, 1990	ZGRW	4/151	7/115	5/164
		Local cultivar	4/156	6/125	5/151
Takada	Jan. 20, 1990	ZGRW	4/158	8/106	5/150
		Local cultivar	5/137	7/114	7/134
Minamikawazoe	Dec. 08, 1989	ZGRW	3/164	8/103	4/155
		Local cultivar	3/160	9/99	5/151
Bunsei(I)	Dec. 27, 1989	ZGRW	2/176	6/122	3/168
		Local cultivar	2/175	8/106	3/165
Bunsei(II)	Jan. 20, 1990	ZGRW	3/173	5/134	3/167
		Local cultivar	3/160	6/124	4/155

*Hamaichi: Ishinomaki Bay; Nishio, Koromozaki: Mikawa Bay; Toushi: Ise Bay; Tsukura, Tamatsu: Seto Inland Sea; Takada, Minamikawazoe: Ariakekai Bay; Bunsei: Shiranuikai Bay

determined by the contents of photosynthetic pigments of gametothlli and their ratios (Saitoh *et al.* 1975; Oohusa *et al.* 1978; Amano and Noda 1987; Nisizawa and Oofusa 1990). The luster is described by the reflected light from the surface of the dried sheets and the reflected light transmitting cell wall (Ogata and Kitakado 1996; Migita 1979). The color and luster of the dried sheets showed similar trends of the color and luster in the toasted sheets of *Porphyra* (Ohnaka *et al.* 1986, 1987)

These results show that the quality of the color and luster of the ZGRW were better than that of the local cultivars. Although the luster of dried sheets of *Porphyra* is influenced by the size of vacuoles, transmittance of protoplasms, thickness of cell walls, existence of plasmoptysis, the number of dead cells, and so on (Migita 1979; Ohonaka *et al.* 1984; Seko *et al.* 1984), further investigation is required.

Table 3 shows comparison of concentration of volatile sulfur compounds in dried sheets of *Porphyra* between the ZGRW and the local cultivars. The concentration of DMS of the ZGRW was higher than that of the local cultivars in the range of 120-801%, and the concentration of H_2S and MM between the ZGRW and the local cultivars did not show any trends. There are many flavor substances of *Porphyra* (Katayama 1956, 1961; Kasahara and Nishibori 1975; Kasahara *et al.* 1986). DMS is the main ingredient of the flavor substances of dried *Porphyra* among DMS, H_2S and MM (Noda and Horiguchi 1975; Araki *et al.* 1983; Osumi *et al.* 1990). Although H_2S and MM emit stench, it turns to make sweet smell when small amount of them are contained in the dried sheet (Katayama 1961; Iida *et al.* 1986; Kasahara and Nishibori 1987; Osumi *et al.* 1990). Therefore, the flavor of the ZGRW is better than that of the local cultivars.

These substances varied widely depending upon the farming years, seasons and sites; this suggests that the concentrations of DMS, H_2S and MM are governed by polygene, which represents the modification of cultivar caused by environment.

The content of the photosynthetic pigments of a gamotothallus of *Porphyra* is a important character to show the color of a gametothallus and the color and free amino acid content of dried sheets of *Porphyra* (Amano and Noda 1978, 1987; Oohusa *et al.* 1978; Nisizawa and Oofusa 1990). The color and luster are a trait concerning to quality of dried and toasted *Porphyra* (Saitoh *et al.*

Table 3. Comparison of concentration of volatile sulfur compounds in dried sheets of *Porphyra* between the ZGRW and the local cultivar of *Porphyra yezoensis* (ppb)

Farming site	Harvesting date	Cultivar	Dimethyl sulfide	Hydrogen sulfide	Methyl mercaptan
Hamachi	Nov. 15, 1989	ZGRW	31681	678	52
		Local cultivar	23729	601	48
Nishio	Dec. 26, 1989	ZGRW	244	456	35
		Local cultivar	44	465	45
Koromozaki	Jan. 09, 1990	ZGRW	138	529	38
		Local cultivar	71	459	35
Toushi	Dec. 10, 1989	ZGRW	90	898	55
		Local cultivar	39	180	32
Tsukura (I)	Jan. 10, 1990	ZGRW	151	271	23
		Local cultivar	94	176	26
Tsukura (II)	Jan. 20, 1990	ZGRW	314	716	28
		Local cultivar	202	848	42
Tamatsu	Jan. 10, 1990	ZGRW	94	465	18
		Local cultivar	71	598	32
Takada	Jan. 20, 1990	ZGRW	202	550	31
		Local cultivar	149	463	28
Minamikawazoe	Dec. 08, 1989	ZGRW	55	535	29
		Local cultivar	46	365	26
Bunsei (I)	Dec. 27, 1989	ZGRW	13421	550	34
		Local cultivar	1676	980	49
Bunsei (II)	Jan. 20, 1990	ZGRW	5160	1217	68
		Local cultivar	3648	1865	85

*Hamaichi: Ishinomaki Bay; Nishio, Koromozaki: Mikawa Bay; Toushi: Ise Bay; Tsukura, Tamatsu: Seto Inland Sea; Takada, Minamikawazoe: Ariakekai Bay; Bunsei: Shiranuikai Bay

1975; Oohusa *et al.* 1978; Noda and Iwata 1983; Ohnaka *et al.* 1986, 1987; Amano and Noda 1987; Nisizawa and Oofusa 1990). The flavor of *Porphyra* is a character relating to palatability (Katayama 1956; Noda and Horiguchi 1975; Araki *et al.* 1983; Iida *et al.* 1986; Noda and Iwata 1983; Osumi *et al.* 1990). All above characters are quantitative and modification of the cultivar (genotype) caused by the environment. It is a major problem in the study of quantitative traits because it complicates the interpretation of genetic experiments and makes predictions difficult. The study on statistical genetics and quantitative trait loci is required in the future.

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