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Yield Improvement Using Recombinant Wild-Type in *Porphyra yezoensis* (Bangiales, Rhodophyta)

Jong-Ahm Shin

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

The recombinant wild-type (ZGRW) in pigmentation was bred by the cross between the green-type and the red-type pigmentation mutant of *Porphyra yezoensis*. The yield characteristics of the ZGRW were examined by cultivation at farming sites for up to three *Porphyra* years. The ratio of length to width of gametothalli and the frequency of lesions of the red rot disease infected gametothalli were determined to compare the ZGRW with the local cultivars. The mean value of ratio of length to width of gametothalli of the ZGRW was higher than that of the local cultivars in the range of 106-216%. The frequency of lesions of the red rot disease-infected gametothalli of the ZGRW was smaller than that of the local cultivars in the range of 14.3-15.6%. The ZGRW yield was higher than those of the local cultivars.

Key Words: cross breeding, pigmentation mutant, *Porphyra yezoensis*, recombinant wild-type, yield improvement

INTRODUCTION

The red algal genus *Porphyra*, with approximately 133 species (Yoshida *et al.* 1997), is widely distributed in estuaries, bays and open seas worldwide. Only a few species of *Porphyra* have been cultivated as sea vegetables for food and its cultivation is one of the largest aquaculture industries in China, Japan and Korea (Mumford and Miura 1988; Tseng 1993; Oohusa 1993; Sohn 1996; Koh 1997).

Modern techniques for cultivation of *Porphyra* have been remarkably developed such as artificial seeding, nursing by artificial exposure, low-temperature storage of nursing-nets in a freezer, floating system facility of cultivation, high-speed harvesting boat and all-automatic processing machine, and so on. However, despite a great deal of research on life history, ecology, physiology and biochemistry of *Porphyra* in relation to commercial interests, there were few studies on the breeding (Miura 1975, 1979) largely due to lack of knowledge in the genetics of *Porphyra*.

Miura and collaborates have studied the genetics of *Porphyra* (Miura 1985; Ohme *et al.* 1986; Ohme and Miura1988; Niwa *et al.* 1993; Hamada *et al.* 1994; Miura and Ohme-Takagi 1994; Shin and Miura 1997; Shin *et al.* 1997; Shin 1999) and the breeding of *Porphyra* (Miura and Shin 1989; Shin and Miura 1995; Shin et al. 1996).

The recombinant wild-type (ZGRW) in pigmentation was bred by the cross between the green-type and the red-type pigmentation mutant of *Porphyra yezoensis*. The ratio of length to width of gametothalli and the frequency of lesions of the red rot disease infected gametothalli were determined to compare the ZGRW with the local cultivars. This paper describes the yield characteristics of the recombinant wild-type from the crossing between two pigmentation mutants of *P. yezoensis*.

MATERIALS AND METHODS

The recombinant wild-type of *Porphyra yezoensis* (ZGRW) and local cultivars were used as materials in this study. A green-type (strain: C-O giant; ♀) and a red-type (strain: H-25; ♂) pigmentation mutant of *P. yezoensis* were used as parental lines for crossbreeding to create the ZGRW. The two pigmentation mutants are monogenic, recessive to the wild type, and their genes are non-allelic belonging to the same linkage group (Ohme *et al.* 1986; Ohme and Miura 1988; Shin 1991; Hamada *et al.* 1994). Meiosis of *P. yezoensis* occurs at the time of conchospore germination (Ma and Miura 1984; Ohme and Miura 1988; Tseng and Sun 1989). Non-parental wild-

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

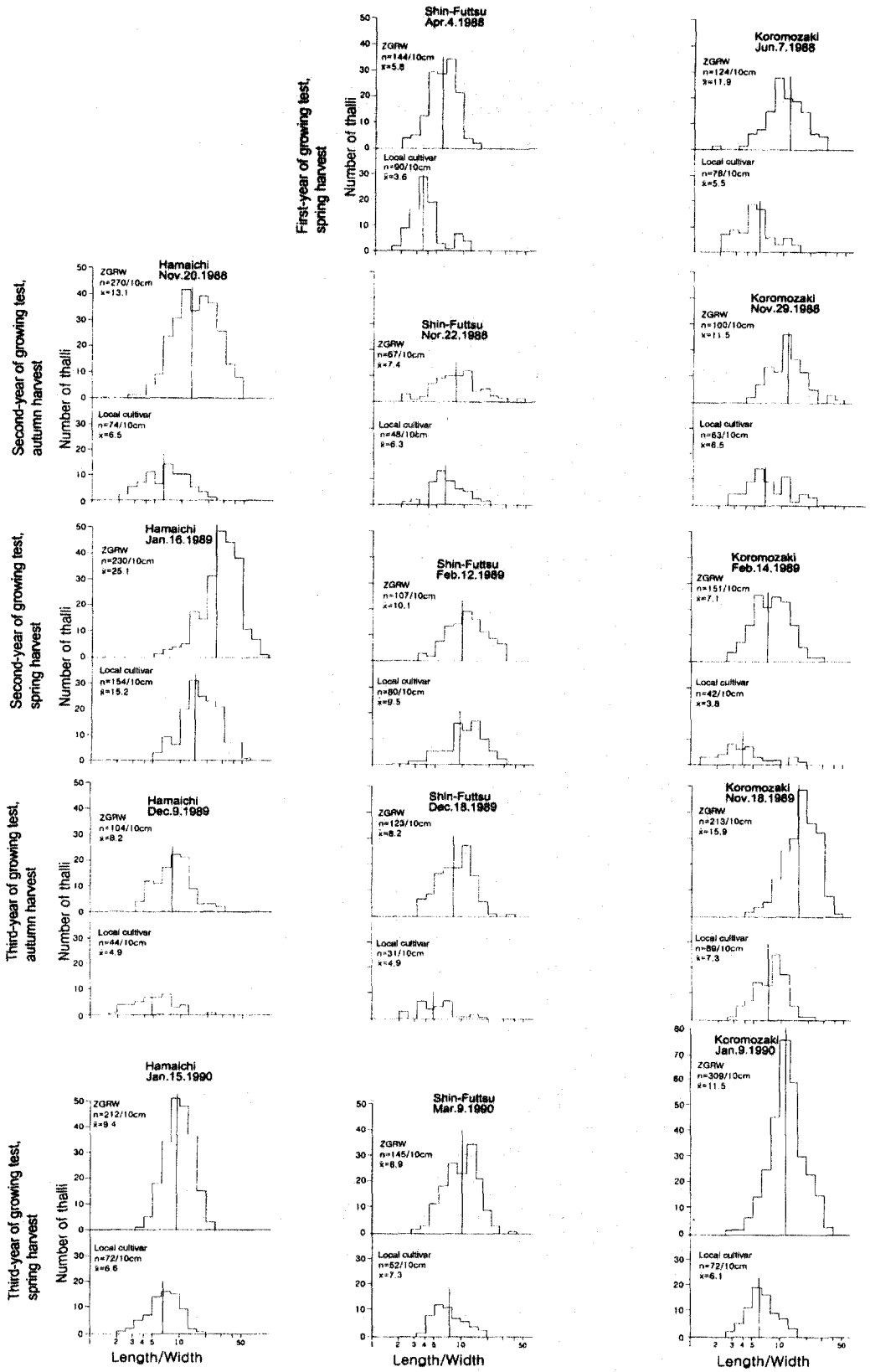

Fig. 1. Comparison of the frequency of ratio of length to width of gametothalli longer than 5 cm per 10 cm long netting twine between the ZGRW and the local cultivars of *Porphyra yezoensis* of 1987, 1988 and 1989 *Porphyra* years, autumn and spring harvesting seasons and farming sites: the Hamaichi aquafarm in Ishinomaki Bay, the Shin-Futtsu in Tokyo Bay and the Koromozaki in Mikawa Bay. Upper histograms show the ZGRW and lower shows the local cultivar. The vertical bars in histograms are mean values of ratio of length to width.

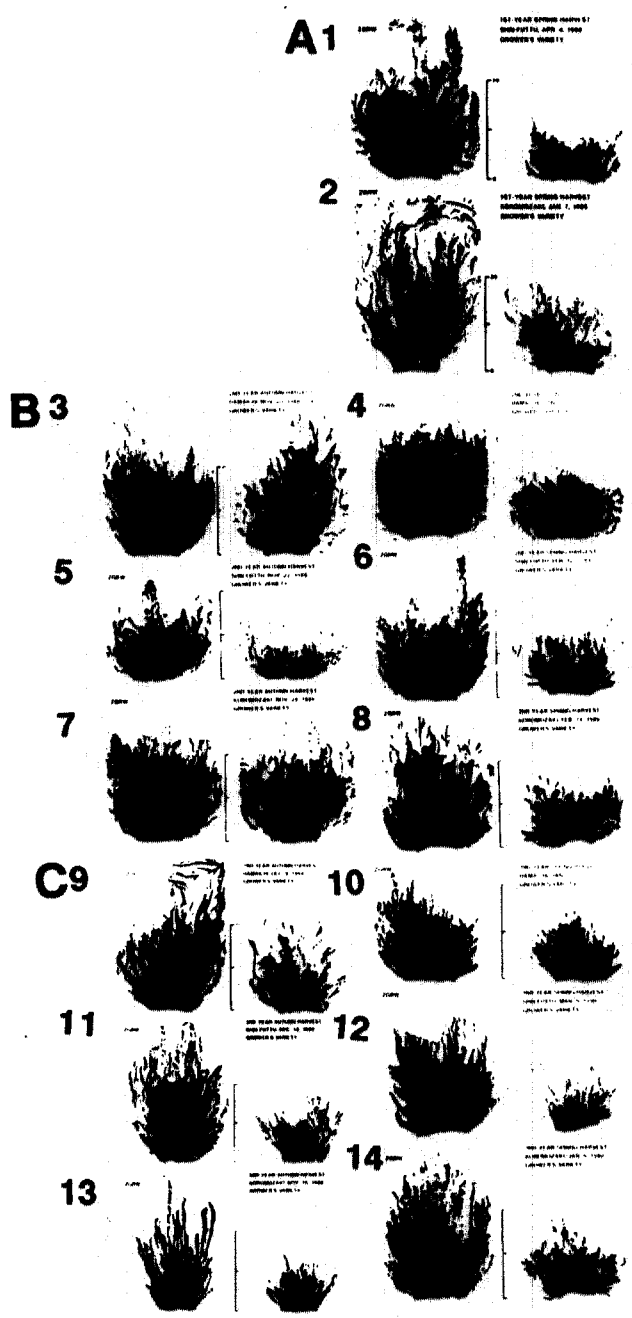

Fig. 2. Comparison of standing crops represented by herbarium sheets between the ZGRW and the local cultivars of *Porphyra yezoensis* of 1987, 1988 and 1989 *Porphyra* years, autumn and spring harvesting seasons and farming sites: the Hamaichi aquafarm in Ishinomaki Bay, the Shin-Futtsu in Tokyo Bay and the Koromozaki in Mikawa Bay. Herbarium sheets were prepared with gametothalli grown on a 10 cm long netting twine of a *Porphyra* net. Both the ZGRW and the local cultivar were grown under the same conditions and collected on the same day. Characters of A, B and C are the first, the second and the third *Porphyra* year, respectively. Numbers of 3, 5, 7, 9, 11 and 13 are herbarium sheets collected in autumn harvest, numbers 1, 2, 4, 6, 8, 10, 12 and 14 are those collected in spring harvest. Herbarium sheets of 1, 5, 6, 11 and 12 collected at the Shin-Futtsu aquafarm, those of 3, 4, 9 and 10 at the Hamaichi, and those of 2, 7, 8, 13 and 14 at the Koromozaki. The left side herbarium sheets of each pair in each site and each farming season are those of the ZGRW, while the right side herbarium sheets the grower's cultivar.

Table 1. Comparison of the number of lesions on 100 randomly collected the *Porphyra yezoensis* (ZGRW) and a local cultivar (Aka-1) thalli infected with red rot disease from Takada aquafarm, Ariakekai Bay

Harvesting date	Cultivar	Total number of lesions in 100 thalli
Nov. 07, 1990	ZGRW	7
	Aka-1	49
Jan. 17, 1991	ZGRW	18
	Aka-1	115

type dihybrid sporothallus (conchocelis), which is derived from the crossing between the green-type and the red-type mutant, produces dihybrid conchospores. They germinate into sectorially variegated chimeral gametothalli and single phenotypic gametothalli of parental ditype, non-parental ditype and tetratype in color. The pattern of the gametothalli development in the ZGRW was similar to those of ordered tetrad pattern of fungi *Neurospora* (Ohme and Miura 1988; Shin 1991; Miura and Ohme-Takagi 1994).

The ZGRW is a homozygous pure line that was bred from one single carpospore, which was fixed by intragametophytic selfing of one specific individual selected among non-parental wild-type gametothalli (Shin 1991). The local cultivars are a kind of cultivar line of *P. yezoensis*.

The ZGRW was cultivated at three farm sites for three *Porphyra* years to study standing crop and at one farm site for one *Porphyra* year to determine the resistance to red rot disease in Japan. Local cultivars were also cultivated as control.

The population of the ZGRW and the local cultivars were the cohort seedlings from their seed pools in each farm site and year, and they were always cultivated in the same way at the respective farm sites.

The standing crop as a marker of harvest yield was shown as the ratio of length to width (L/W ratio) of gametothalli longer than 5 cm occurring on a 10 cm long netting twine (Miura 1975, 1979). The resistance to red rot disease pertaining to yield was shown as the number

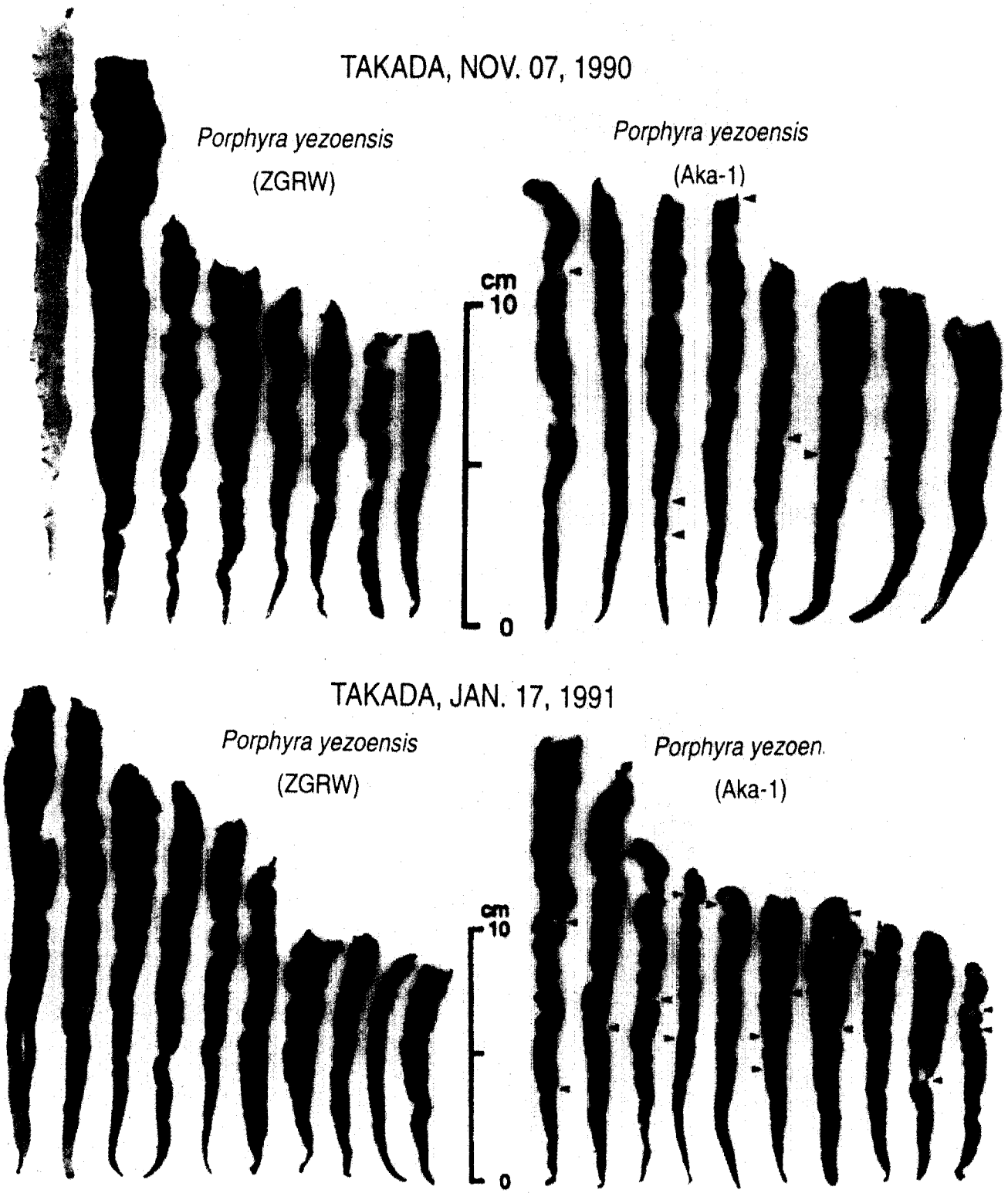

Fig. 3. Herbarium specimens representing the difference of resistance to red rot disease between the ZGRW and the local cultivar (Aka-1) of *Porphyra yezoensis*. The specimens on the left are the ZGRW and on the right the Aka-1. Arrowheads show lesions of red rot infected by *Pythium porphyrae*. Upper plate: Specimens collected at the Takada aquafarm, Ariakekai Bay on Nov. 07, 1990. Lower one: Specimens collected at the Takada, Ariakekai Bay on Jan. 17, 1991.

of lesions on 100 randomly collected individuals.

RESULTS AND DISCUSSION

Fig. 1 shows the comparison of the L/W ratio of gametothalli longer than 5 cm long gametothalli per 10 cm long netting twine between the ZGRW and the local cultivar of *P. yezoensis* of 1987, 1988 and 1989 *Porphyra* years, autumn and spring harvesting seasons, and the Hamaichi aquafarm in Ishinomaki Bay, the Shin-Futtsu in Tokyo Bay and the Koromozaki in Mikawa Bay, Japan.

There were more individuals attached on a 10 cm long netting twine of ZGRW than those of the local cultivars in the range of 134-429%. The mean values of L/W ratio of the ZGRW were higher than those of the local cultivars in range of 106-216%. As the L/W ratio indicates the shape and size of a gametothallus, the correlation between the mean value of L/W ratio and the number of individuals attached on a netting twine is positive. The number of individuals on a 10 cm long netting twine was used as a marker of harvest yield; therefore, the L/W ratio can be regarded as the harvest yield (Miura 1975, 1979). The yield of the ZGRW was different from the local cultivars and was considered as genetically determined.

Although the L/W ratio is genetically stable and governs the number of attached individuals per unit netting twine (Miura 1979), the L/W ratio and the numbers of attached gametothalli varied widely depending upon farming *Porphyra* years, sites and seasons. There are several reasons for the wide variation. The characters are quantitative and their states are affected by environmental factors, which modify the final expression of states. The gametothallus is haploid which does not have alleles masking or enhancing the expression of every gene and the environmental effect is expressed directly on the phenotypes as a result (Shin and Miura 1995).

Fig. 2 shows the comparison of standing crop represented by herbarium sheets between the ZGRW and the local cultivars corresponding to Fig. 1. The length of gametothalli as well as individuals of the ZGRW attached on a 10 cm long netting twine presented a marked contrast to those of the local cultivars.

Table 1 shows the comparison of the frequency of lesions on 100 randomly collected gametothalli of the ZGRW and the local cultivar from the Takada aquafarm in Ariakekai Bay when the gametothalli were infected with the red rot disease judging by the naked eye. Fig. 3 shows herbarium specimens representing the difference of resistance to red rot disease between the ZGRW and the local cultivar. The number of lesions between the ZGRW and the grower's cultivar were significantly different.

The red rot disease of gametothalli of *Porphyra* caused by parasitism of a marine fungus *Pythium porphyrae* (Takahashi *et al.* 1977). It causes decrease in the yield of gametothalli and consequently deteriorates the quality of commercial products. Therefore, breeding a resistant cultivar to red rot disease is important in *Porphyra* cultivation. The number of lesions of the ZGRW is very lower than that of the local cultivar. Although the ZGRW showed the red rot disease-resistant characteristic, this resistance needs further investigation over a long period of years in many farm sites.

The mechanism of the red rot disease-resistance of *Porphyra* was closely related with quantity and ratio of photosynthetic pigments (Yan and Fujita 2000), content of porphyran (Uppalapati and Fujita 2000, 2001) or 3,6-anhydro-L-galactose (Park 2002). The study on the biophylactic mechanism of *Porphyra* including both true resistance and apparent resistance as a part of phycomedicine (algae medicine) is needed in the future.

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