Culture study on the hybrid by interspecific crossing between *Porphyra pseudolinearis* and *P. dentata* (Bangiales, Rhodophyta), two dioecious species in culture

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Physiological studies on the hybrid by crossing between two dioecious species, *Porphyra pseudolinearis* and *P. dentata* from Korea were conducted at constant temperatures (5, 10, 15, 20, and 25°C), at photon flux densities (10, 20, 40, and 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) under photoperiods (14 L : 10 D and 10 L : 14 D). In the hybrid, higher growth of conchocelis was observed at 20 and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D. Conchosporangial branches were produced under 10-80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at only 25°C, and were abundant when the conchocelis was cultured under 10 L : 14 D. Foliose thalli of the hybrid grew well at the conditions of 10-20°C, 10 L : 14 D and 15-20°C, 14 L : 10 D. The foliose thalli grew very slowly at 5°C and 30°C, respectively. No archeospores were observed at any culture conditions. Spermatangial and zygotosporangial sori were formed at the marginal portion of mature thallus. Zygotospores from the hybrid were released at 10-20°C under both photoperiods, and gave rise to form conchocelis filament. Monoecious thalli were observed at 10°C under 14 L : 10 D. Neither monospores nor protothalli were produced from the conchocelis in culture.

Key Words: Bangiales; hybrid; interspecific crossing; physiology; *Porphyra dentata*; *Porphyra pseudolinearis*; Rhodophyta

INTRODUCTION

Porphyra dentata and *P. pseudolinearis*, originally described from Japan (Ueda 1932) were recorded in a checklist of marine algae from Korea by Lee and Kang (1986). *Porphyra dentata* is widely distributed in warm temperate waters (Ueda 1932, Tseng 1948, Tanaka 1952, Kim 1999), whereas *P. pseudolinearis* is found in cold temperate waters of the North Pacific (Ueda 1932, Tanaka 1952, Kang 1966, Wynne 1972, Scagel et al. 1989, Lindstrom and Cole 1992, 1993, Kim 1999). At present, *P. dentata* and *P. pseudolinearis* as the cultivars are crops of the most economical importance to inhabitants along the southern and eastern coast of Korea, respectively and one of the

popular Korean marine products. Especially, they are famous for Dolkim (in Korean, meaning of *Porphyra* growing on the rocky beds in nature). Until now, the cultivated plants are mainly *P. tenera* and *P. yezoensis* in Korea (Kim 1999). However, both species are cheaper than the other cultivars (e.g., *P. dentata*, *P. pseudolinearis* and *P. seriata*). Therefore, the cultivation of *P. dentata* and *P. pseudolinearis* are very important for the seaweed farmer of Korea. But there are some problems in the farming periods of the both species. The cultivation of *P. dentata* is limited in higher temperature periods (from September to November), while that of *P. pseudolinearis* is limited in low-

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er temperature periods (from December to February). Therefore, seaweed farmers has need for prolongation the farming period of both valuable species in Korea.

Studies on the crossing of *Porphyra* have been completed in laboratory culture (Suto 1963, Niwa et al. 1993, Shin and Miura 1995, Shin et al. 1996, Shin 1999).

Suto (1963) attempted artificial cross experiments between 5 species including two monoecious (e.g., *P. tenera* and *P. yezoensis*) and three dioecious (e.g., *P. angusta*, *P. pseudolinearis* and *P. umbilicalis*). Shin and Miura (1995), Shin et al. (1996) and Shin (1999) succeeded in interspecific crossing between *P. tenera* and *P. yezoensis* using their color mutants as genetic markers. Ohme and Miura (1988) and Niwa et al. (1993) reported intraspecific crossing between wild-type and various color mutants of *P. yezoensis*.

I have been conducting cross experiments between *P. dentata* and *P. pseudolinearis* using their field materials as new cultivars for characteristic tests and physiological identification of the hybrid as a part of breeding studies of both species. In present study, the results of interspecific crossing between *P. dentata* and *P. pseudolinearis* are reported.

MATERIALS AND METHODS

Mature foliose thalli of *P. dentata* and *P. pseudolinearis* were collected from the upper intertidal zone at Wando in Chonnam Prefecture, and Pohang in Gyeongbuk prefecture, on 11 and 28 February 1996, respectively (Kim 1999). From these thalli, zygospores were obtained to start conchocelis cultures. Conchospore liberation in both species was induced by reducing the temperature from 20 to 15°C. The resulting foliose thalli were transferred into round flasks. Cultures were separately conducted by the use of one plant per a flask. The artificial crossing was conducted by using the male thalli of *P. pseudolinearis* and female thalli of *P. dentata*. Zygospores from hybrid were cultured at five temperatures (5, 10, 15, 20, and 25°C) and four photon flux densities (10, 20, 40, and 80 $\mu\text{mol m}^{-2}\text{s}^{-1}$) under photoperiods of 14 L : 10 D and 10 L : 14 D (light : dark). The growth of conchocelis was determined by measuring the diameter of colonies at weekly intervals. Conchospore liberation from matured conchosporangial branches was induced by reducing the temperature from 25 to 15°C. The resulting foliose thalli were cultured at five temperatures (5, 10, 15, 20, and 25°C) and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ under photoperiods of 14 L : 10 D and 10 L : 14 D in 300 mL round flask with an air tube. Growth

was determined from measurements of blade length and width. Modified Grund medium (McLachlan 1973) was used in all cultures and was renewed weekly.

RESULTS

Zygospores from the hybrid by interspecific crossing between both species were germinated to the conchocelis filaments at 5-25°C (Fig. 1A & B). Conchocelis filaments grew to a colony of about 1.5 mm diameter at 20°C after 3 weeks (Fig. 1C). The conchosporangial branches were produced only from the conchocelis colonies grown at 25°C under both photoperiods (Fig. 1D). Conchospores liberated from the conchosporangial branch were well developed to four cells sporeling (ca. 40 μm long) after 3 days (Fig. 1E & F). Archeospores did not liberate from foliose thalli. Male and female reproductive organs began to occur in the marginal parts of plants, about 10 mm long after 8 weeks at 15°C and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ under 14 L : 10 D (Fig. 1G & H). Mature spermatangia had a Hus's (1902) formula 64, (a/4, b/4, c/4) or 128, (a/4, b/4, c/8) (Fig. 1G & I). The zygotosporangia contained 8 zygospores formed by the division pattern (a/2, b/2, c/2) (Fig. 1H & J). The marginal portions of fronds were entire or micro and macroscopic spinulate processes (Fig. 1K & L). The basal portion of frond has the more clear spinulate processes (Fig. 1M). The largest thalli were obtained at 10-20°C under both photoperiods after 18 weeks in culture. These foliose thalli reached to a blade length of about 130 mm under the long photoperiod, and of about 190 mm under the short photoperiod after 14 weeks. The shape of foliose thalli were linear or lanceolate types (Fig. 1N & O). The zygospores produced either monoecious thalli (Fig. 1P) or dioecious thalli.

Growth of the Sporophyte (conchocelis) phase

The zygospores of hybrid were germinated into the conchocelis colonies at 5-25°C and four photon flux densities under both photoperiods. Higher growth of conchocelis was observed at 15 and 20°C under both photoperiods (Fig. 2). There were no remarkable differences in the growth of conchocelis according to photon flux densities. The maximum diameter of conchocelis colonies was about 2.5 mm within 10 weeks.

Formation rates of conchosporangial branches at different combinations of temperature and photon flux density are presented in Fig. 3. Conchocelis colonies with conchosporangial branches were produced only at 25°C

Fig. 1. Life history of hybrid by crossing between *Porphyra pseudolinearis* and *P. dentata*. (A) Zygospores liberating from mature hybrid foliose thallus by crossing between *Porphyra pseudolinearis* and *P. dentata* in culture. (B) Germinating spore showing developing conchocelis from zygospore. (C) Free-living conchocelis colony grown at 20°C and 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D. (D) Conchosporangial branch after 3 weeks at 25°C and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under 10 L : 14 D. (E) Conchospore liberated from conchosporangia. (F) Sporlings germinated from conchospore. (G) Surface view of spermatangia. (H) Surface view of zygotosporangia. (I) Spermatangia in cross-section. (J) Zygotosporangia in cross-section. (K) Entire marginal part of hybrid foliose thallus. (L) Marginal portion of frond showing an only microscopic spinulate process. (M) Basal part of young foliose thallus with microscopic spinulate processes. (N & O) Foliose thalli of 15 weeks old grown in culture showing different thallus shapes under 14 L : 10 D (N) and 10 L : 14 D (O), respectively; (a) 5°C, (b) 10°C, (c) 15°C, (d) 20°C, (e) 25°C. (P) Monoecious thalli after 10 weeks at 10°C and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D. Scale bars represent: A, 10 mm; B, D & L, 5 μm ; C, 50 μm ; E & F, 10 μm ; G-K & M, 20 μm ; N & O, 30 mm; P, 10 mm.

and 10–80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under both photoperiods within 3 weeks. Formation rates of conchosporangial branches reached 100% within 10 weeks. Conchospores did not liberate at any culture conditions.

Growth and maturation of the gametophyte phase

Liberation of conchospores in hybrid species was obtained by reducing the temperature from 25 to 15°C. Growth of foliose thalli at various temperatures under both photoperiods are shown in Fig. 4. Germlings of the hybrid reached their largest size, about 19 cm long at 20°C under short photoperiod after 14 weeks in cul-

Fig. 2. Growth of hybrid conchocelis colonies by crossing between *Porphyra pseudolinearis* and *P. dentata* at five temperatures (5-25°C) and photon flux densities (10-80 $\mu\text{mol m}^{-2}\text{s}^{-1}$) under 14 L : 10 D and 10 L : 14 D. Vertical bars are standard deviations.

Fig. 3. Formation rates of conchosporangial branch by crossing between *Porphyra pseudolinearis* and *P. dentata* at 25°C and photon flux densities (10-80 $\mu\text{mol m}^{-2}\text{s}^{-1}$) under 14 L : 10 D and 10 L : 14 D. Percentage of conchocelis colonies with conchosporangial branches.

Fig. 4. Growth of hybrid foliose thallus by crossing between *Porphyra pseudolinearis* and *P. dentata* at five temperatures (5-25°C) and $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D and 10 L : 14 D. Vertical bars are standard deviations.

Fig. 5. Liberation of spermatia and zygotes in relation to the age of cultured hybrid foliose thallus by crossing between *Porphyra pseudolinearis* and *P. dentata* at five temperatures (5-25°C) and $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D and 10 L : 14 D. Open space bars, neither spermatia nor zygotes liberated; horizontal stripe space, spermatia liberated; vertical stripe space, spermatia and zygotes liberated; S and Z refer to spermatia and zygotes, respectively.

ture, whereas initially thalli grew faster 15°C under long photoperiods. At 5 and 25°C, foliose thalli showed almost no growth. They grew to 10 mm or under in blade length during the culture period. The liberation of spermatia and zygotes at various temperatures and both photoperiods is presented in Fig. 5. In this hybrid species, archeospores did not liberate in all culture conditions. Their liberation occurred at 10 and 20°C under both photoperiods within 11 weeks. The linear or lanceolate shape of fronds, similar to that in nature, was observed under culture conditions that produced rapid growth (Fig. 6).

DISCUSSION

Hybrid shows a typical biphasic life history of the *P. lacerata* type (Notoya et al. 1993). Zygotes mostly developed into conchocelis colonies in all culture conditions. Conchosporangial branches were produced at 25°C only and $10\text{--}80 \mu\text{mol m}^{-2} \text{s}^{-1}$ under both photoperiods. Kim (1999) reported that zygotes of *P. dentata* and *P. pseudolinearis* were germinated into conchocelis colonies and conchosporangial branches were produced at 10-25°C, $10\text{--}80 \mu\text{mol m}^{-2} \text{s}^{-1}$ under short photoperiod. Kim (1999) also reported that formation rates of concho-

Fig. 6. Relationship between blade length and width in hybrid by crossing between *Porphyra pseudolinearis* and *P. dentata* at five temperatures (5-25°C) and $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ under 14 L : 10 D and 10 L : 14 D. Solid triangles are 14 L : 10 D (L); open circles are 10 L : 14 D (S).

sporangia in two species were higher at high temperatures than at low temperatures. This result suggests that the abundance of conchosporangial branches in hybrid were also affected by high temperature (Kurogi and Akiyama 1966). Conchospore liberation in this species was induced by reducing temperature from 25 to 15°C (Kurogi and Akiyama 1966, Iwasaki and Sasaki 1971) and changing photoperiod from 14 L : 10 D to 10 L : 14 D (Kim 1999). In other species for *Porphyra* such as, *P. abbottae* and *P. perforata* (Waaland and Dickson 1983) and *P. rosengur-tii* (Kapaun and Luster 1980) require a photoperiodic change to induce spore liberation (Waaland et al. 1990). Our results agree well with those reported on *P. suborbic-ulata* f. *latifolia* by Iwasaki and Sasaki (1971), on *P. colum-bina* by Avila et al. (1986) and on *P. spiralis* var. *amplifolia* by Kapaun and Lemus (1987).

Notoya et al. (1993) studied four species of Japanese *Porphyra*, including *P. dentata*. They reported that conchosporangial branches were formed at a temperature

range of 20 to 25°C, and the fast growth of foliose thalli occurred at 20°C in *P. dentata*. Our results well agree with their results with respect to growth and maturation of both phase which are observed at various temperatures and photon flux densities. *P. dentata* widely distributed on intertidal zone in the warm, temperate and cold waters (Ueda 1932, Tseng 1948, Kim 1999). Kim (1999) reported that in *P. pseudolinearis*, the growth of conchocelis and the formation of conchosporangial branches occurred at 5-25°C and 10-25°C under all the photon flux densities tested, respectively. Kurogi and Akiyama (1966) and Iwasaki and Sasaki (1971) reported that conchosporangial branches were formed at 15 to 25°C or 27°C in all the species studied.

Kim (1999) reported that the growth of foliose thalli, in *P. pseudolinearis* and *P. dentata* was fast at 15°C in early developmental stage. Generally, *P. pseudolinearis* is distributed in temperate and cold water regions (Tanaka 1952, Kang 1966, Wynne 1972, Perestenko 1982, Scagel et

al. 1989, Lindstrom and Cole 1992, 1993).

He also reported that *P. pseudolinearis* showed almost no growth at 25°C while the blade length of *P. dentata* did not increase at 5°C for 10 weeks. Our results suggest that hybrid will grow at wide range (10-20°C) temperatures.

The young thallus with denticulate marginal cell rows was observed at 10°C at first, and it was quickly produced at low temperatures than high temperatures. In this study, the monoecious foliose thalli sectored by horizontal line were produced at 10°C and 10 L : 14 D. Hitherto, some authors (Chang and Zheng 1960, Lindstrom and Cole 1992, 1993) reported dioecious *Porphyra* species with monoecious plants for *P. haitanensis*, *P. lanceolata*, *P. pseudolanceolata*, *P. linearis*, *P. mumfordii*, *P. lanceolata*, *P. pseudolinearis* and *P. purpurea*.

P. dentata and *P. pseudolinearis* are typical dioecious species (Tanaka 1952), but monoecious plants were reported by Hwang (1994) in field and culture observations from Japan and Korea, respectively.

No archeospores were produced under any culture conditions in hybrid species, and neither monospores nor protothalli were produced from the conchocelis phase.

In the anatomical examination of the hybrid, the divisional formulas of spermatangia according to Hus (1902) well agree with those of Tanaka (1952) but zygotosporangia did not.

Based on the results of this study and previous reports of two species in culture (Kurogi and Akiyama 1966, Notoya et al. 1993) and field investigation (Kang 1966, Wynne 1972, Scagel et al. 1989, Lindstrom and Cole 1992), the temperature needed for the growth and maturation of both phases of hybrid falls between low and high temperatures.

In conclusion, these culture studies indicate that the growth and maturation of both the foliose and conchocelis phase of the hybrid are more influenced by temperature than photon irradiance. Furthermore, it is apparent that the hybrid has monoecious and dioecious thalli, and suggests that hybrid more can be eurythermal cultivar than two cultivars, *P. dentata* and *P. pseudolinearis*.

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