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Physiological Responses of Blade and Conchocelis of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho (Bangiales, Rhodophyta) from Thailand in Culture

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The effects of temperature (10-30°C), photon flux density (10-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), salinity (10-40 ppt) and photoperiod (14L: 10D and 10L: 14D) on the growth and maturation for the conchocelis and blade phase of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho from Thailand were examined under laboratory conditions to investigate data of tropical species. Maximum growth rate of the conchocelis showed at 25°C, 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under 35 ppt and 14L: 10D without formation of conchosporangial branch during 6 weeks of culture period. An early formation of conchosporangial branch was observed at 30°C, 20-40 ppt under 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of both photoperiods after 2 weeks in culture. Early conchospore release occurred at 20-35 ppt and 10L: 14D under 30°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 4th weeks of culture. Fast growth of the conchospore germlings was observed at 25°C under the both of photoperiods in 35 ppt and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The blades culture at 30°C produced only archeospores after 2 weeks throughout the culture period. Under the optimum culture conditions of 25 ppt and 14L: 10D at 25°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the blades matured and liberated zygotospores within 6 weeks. In good combinations of conditions at the four factors, the life cycle was completed within 12 weeks in culture.

Key Words: Bangiales, blade and conchocelis phase, life cycle, photon flux density, photoperoid, physiological responses, *Porphyra vietnamensis*, Rhodophyta, salinity, temperature

INTRODUCTION

The tropical species *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho is distributed along the coasts of the Northwest Pacific in the region of Vietnam (Tanaka and Ho 1962), Thailand (Lewmanomont and Ogawa 1978) and China (Tseng 1984). In Thailand, the alga is found on the rocks of upper tidal zone along the coasts of Songkhla. The blade phase occurs during monsoon season in Novem-ber to February (Brohmanonda and Sahavatcharin 1968). *P. vietnamensis* is economically important edible seaweed in Thailand comparable to *Gracilaria*, *Caulerpa* and *Sargassum* (Lewmanomont 1998).

The effect of temperature and salinity on the life cycle of *P. vietnamensis* has been reported by Lewmanomont and Chittpoolkusol (1993). However, the preliminary information was restricted on the small sizes of blades, and by using the conchocelis in shell or free-floating filaments.

In this study, detailed information on the physiological responses of both phases of *P. vietnamensis* will be presented. The effects of various environmental factors (*i.e.* temperature, salinity, photoperiod and photon flux density) on the growth and maturation of the blade and conchocelis phase in the laboratory culture will be discussed. Furthermore, from the results of this study, we intend to develop a sea-farming technology, and feasible seeding technique of this species at the coasts of tropical area due to its high economic potential.

MATERIALS AND METHODS

Mature blades were collected from Leam Son-on Rocky Dam, Amphur Muang, Songkhla Province, Peninsula Thailand (07° 13′ 795″ N and 100° 34′ 983″ E) in January 19, 1999. The surface of the blade pieces was cleaned up several times with a soft artist brush in sterilized seawater to remove epibionts. The pieces of tissue were placed in petri dish filled with sterilized seawater

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and 5 ppm of GeO_2 for diatom control at 25°C and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density under 10L: 14D photoperiod and left overnight. Released zygotospores were isolated and cultured in 50 m*l* under the same as above conditions. The culture medium was renewed every 2 weeks until stock culture of conchocelis was established.

Effects of temperature, photon flux density, salinity and photoperiod on the growth, maturation and spore liberation of conchocelis phase

Tufts of conchocelis filaments were cut with centrifuge blade for two minutes. A suspension medium with the conchocelis fragments was poured into a petri dish containing slide glasses for conchocelis attachment and left overnight under 25°C and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density under 10L: 14D photoperiod. Conchocelis filaments (about 200 μm long) that attached to slide glass were used as initial culture materials.

To determine the effect of temperature and photon flux density on the conchocelis growth and maturation, the glasses were set in 50 m*l* vials and incubated in different temperature conditions (between 10 to 30°C at 5°C intervals) and photon flux densities (10, 20, 40 and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under salinity of 35 ppt and photoperiod of 14L: 10D. Weekly diameter of the fifteen conchocelis tufts and maturation was observed under an inverted microscope. The optimum temperature and photon flux density was used in succeeding experiments.

In an experiment on the effect of salinity and photoperiod on the conchocelis, the new sets of conchocelis on glass were cultured at 30°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density under various salinities (10 to 40 ppt at 5 ppt increment) and two photoperiods (14L: 10D and 10L: 14D). Observation was done similar to first experiment.

Effects of temperature, salinity and photoperiod on the growth, maturation and spore liberation of blade phase

Conchospores were collected on synthetic fibers made of polyvinyl of 1 mm diameter (about 4 cm long) by bubbling air in a 300 m*l* flask contain with mature conchocelis tufts at 25°C, 35 ppt 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 10L: 14D and used for the experiment. To determine the effect of temperature and photoperiods, the conchospore germlings (3-5 germlings $\cdot cm^{-1}$, 4 fibers per flask) that attached to the synthetic fiber were incubated under various temperatures between 10 to 30°C at 5°C intervals and two photoperiods (14L: 10D and 10L: 14D) at 35 ppt and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The cultures were circulated approximately at 25-30 rpm in a 300 m*l* flask. The size of blade (length and width) and maturation of fifteen blades were investigated once a week under a microscope and graphing paper.

In an experiment on the effect of salinity and photoperiod on the blades, a new set of conchospore germlings was cultured at salinity between 10 to 40 ppt at an interval of 5 ppt and two photoperiods as above at 25°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The circulation of culture, the size of blade (length and width) and maturation were investigated as same as the first experiment.

Culture medium was enriched with modified Grund medium (MGM) (McLachlan 1973) completely renewed once a week. Different salinity levels of culture medium were obtained by mixing natural seawater at a double concentration of artificial seawater for hypersaline or diluting with autoclaved ionized water for hyposaline. Salinity was checked using a refractometer. Cool white fluorescent lamps were used to illuminate the plants in temperature-controlled incubators (± 0.5°C).

Data of growth and maturation of the conchocelis and blade were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range tests for mean comparisons using SPSS statistic software. The significant level was at $p < 0.05$.

RESULTS

Effects of temperature, photon flux density, salinity and photoperiod on the growth, maturation and spore liberation of conchocelis phase

Conchocelis colony grew at all combinations of temperature and photon flux density of 10-30°C and 10-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ respectively, within 6 weeks of culture. Growth of the diameter of conchocelis colony is shown in Fig. 1. Faster growth of the conchocelis colonies was observed at higher photon flux densities at 20-25°C. The fastest growth of conchocelis colonies occurred at 25°C and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At photon flux densities of 40 and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under 25°C, conchocelis filaments grew fast and the colonies exhibited hemispherical shape within 2 weeks. In other conditions, it had only creeping filamentous formation. The conchocelis colonies at 30°C did not grow well compared with 20 or 25°C, while the conchocelis at 10-15°C showed slow growth.

Conchosporangial branches were produced only at the temperature of 30°C at all photon flux densities of 10-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as early as 2 weeks. Formation rate of conchosporangial branches is shown in Fig. 2. In the highest photon flux densities of 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the formation

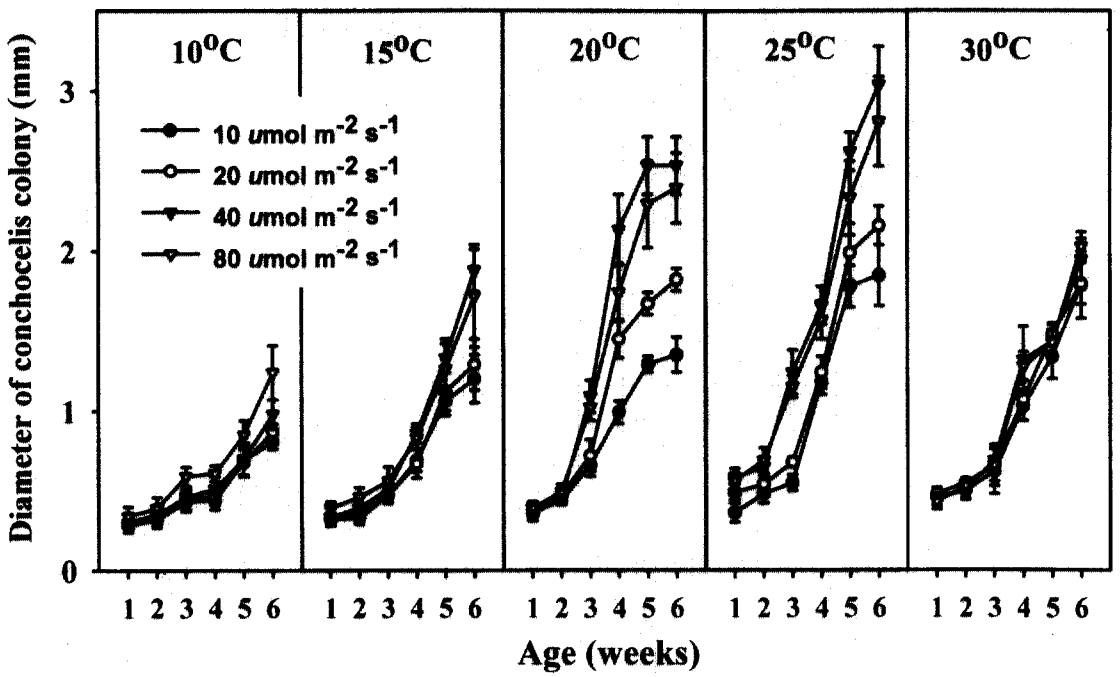

Fig. 1. Effect of temperature and photon flux density on growth of conchocelis colony of *Porphyra vietnamensis* at 35 ppt and 14L: 10D in culture.

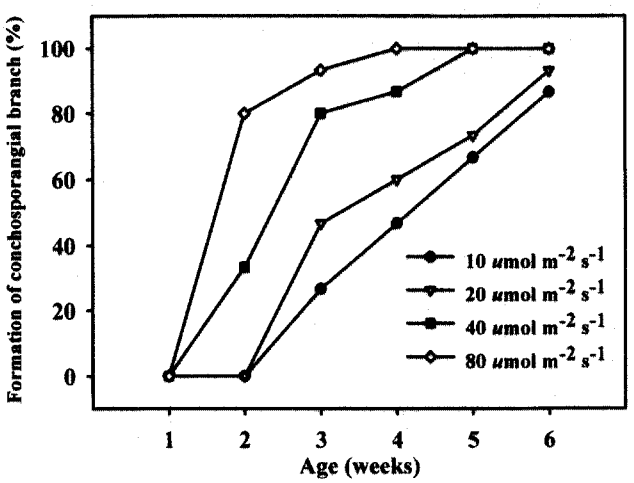

Fig. 2. Effect of photon flux density on the formation of conchosporangial branch of *Porphyra vietnamensis* at 30°C, 14L: 10D and 35 ppt in culture.

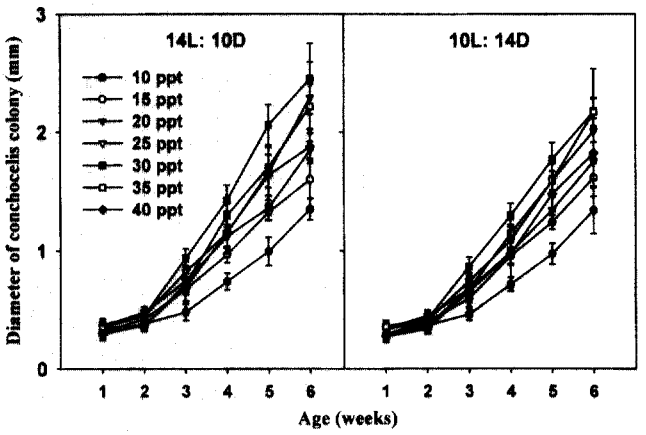

Fig. 3. Effect of salinity and photoperiod on the growth of conchocelis colony of *Porphyra vietnamensis* at 30°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in culture.

rate was 100% within 4 weeks, but maturation was delayed several weeks at lower photon flux densities. The earliest release of conchospores was observed at 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after 6 weeks in culture.

The effects of the salinity and photoperiod on the growth of conchocelis under 30°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ within sixth weeks culture were shown in Fig. 3. Growth of the conchocelis was observed at a salinity of 10-40 ppt in both photoperiods. The highest growth occurred at 25-35 ppt in both photoperiods but low growth rate was observed at lower (10-20 ppt) and highest (40 ppt) salinities. High density of hemispherical formation of conchocelis colonies was observed at 20-35 ppt under both photoperiods after 3 weeks culture. In all other salinity conditions, mostly creeping conchocelis filaments were observed.

Fig. 4 shows the effects of salinity and photoperiod on

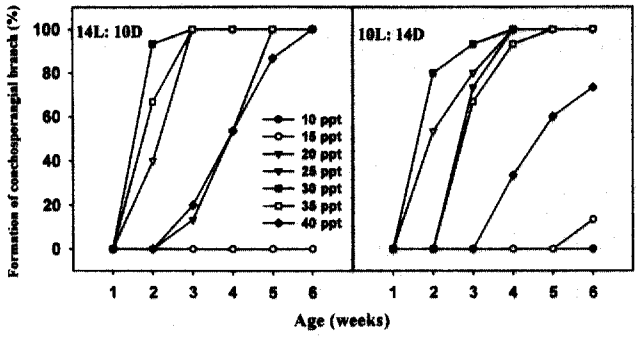

Fig. 4. Effect of salinity and photon flux density on formation of conchosporangial branch of *Porphyra vietnamensis* at 30°C and 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} in culture.

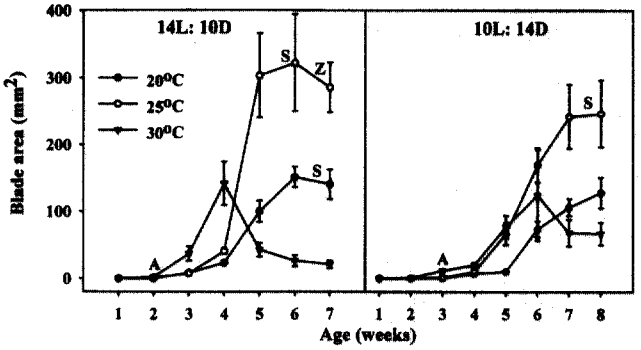

Fig. 5. Effect of temperature and photoperiod on growth of blade area of *Porphyra vietnamensis* at 35 ppt and 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} in culture. A, S and Z indicate first release of archeospores, spermatia and zygotospores, respectively.

the formation rate of conchosporangial branch within 6 weeks culture. Conchosporangial branches were produced at wide range of salinity of 20-40 ppt under 14L: 10D and of 15-40 ppt under 10L: 14D, respectively. Early formation of conchosporangial branch occurred at the salinity range between 25 to 35 ppt at both photoperiods within 2 weeks in culture. Early conchospores release was observed at salinities of 20-35 ppt under 10L: 14D in 4 weeks culture. While under 14L: 10D, the conchospores were released after 6 weeks at 20-40 ppt.

Effects of temperature, salinity and photoperiod on the growth, maturation and spore liberation of blade phase

Conchospore germlings at 10°C and 15°C under both photoperiods died after two to four weeks in culture. The blade germlings grew and matured at 20-30°C under both photoperiods for 7 and 8 weeks, respectively (Fig. 5). Fast growth in early culture period was observed at 30°C under both photoperiods and archeospores were

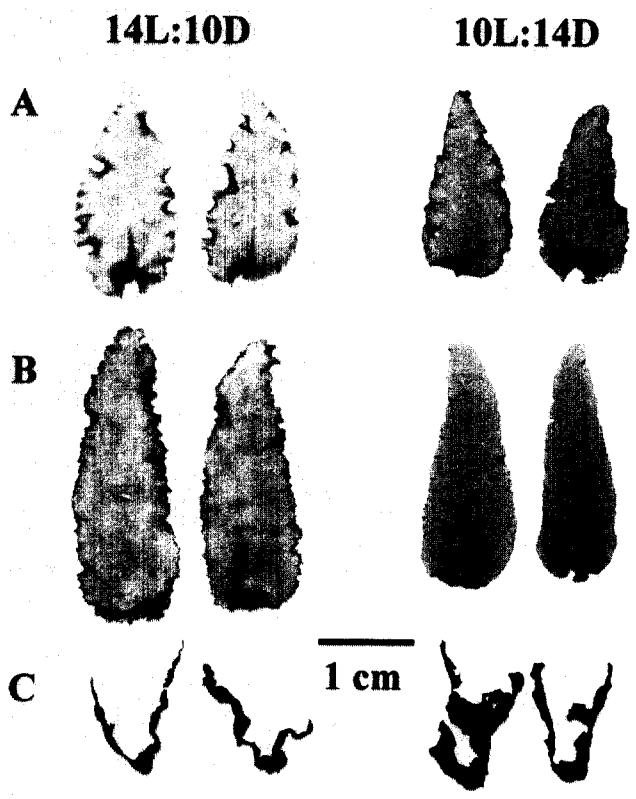

Fig. 6. Effect of temperature and photoperiod on blade shape of *Porphyra vietnamensis* at 35 ppt and 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} after 7 weeks in culture. A, 20°C; B, 25°C and C, 30°C.

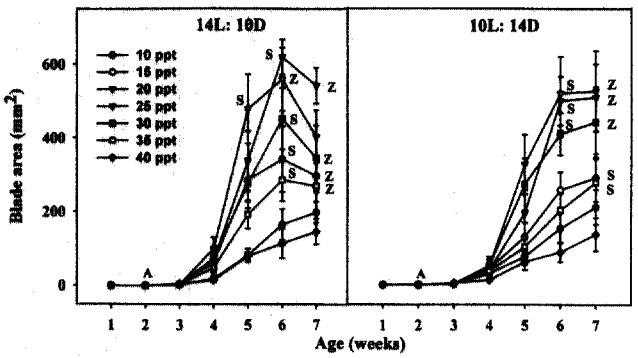

Fig. 7. Effect of salinity and photoperiod on growth of blade of *Porphyra vietnamensis* at 25°C and 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} in culture. A, S and Z indicate first release of archeospores, spermatia and zygotospores, respectively.

released from young blades after 2-3 weeks in culture. The blades grew until 6 and 4 weeks under short and long photoperiod, respectively, and subsequent thallus deterioration after releasing big amount of archeospores from the apical central part of blade. Several lateral branched blades however, remained after 7 and 8 weeks

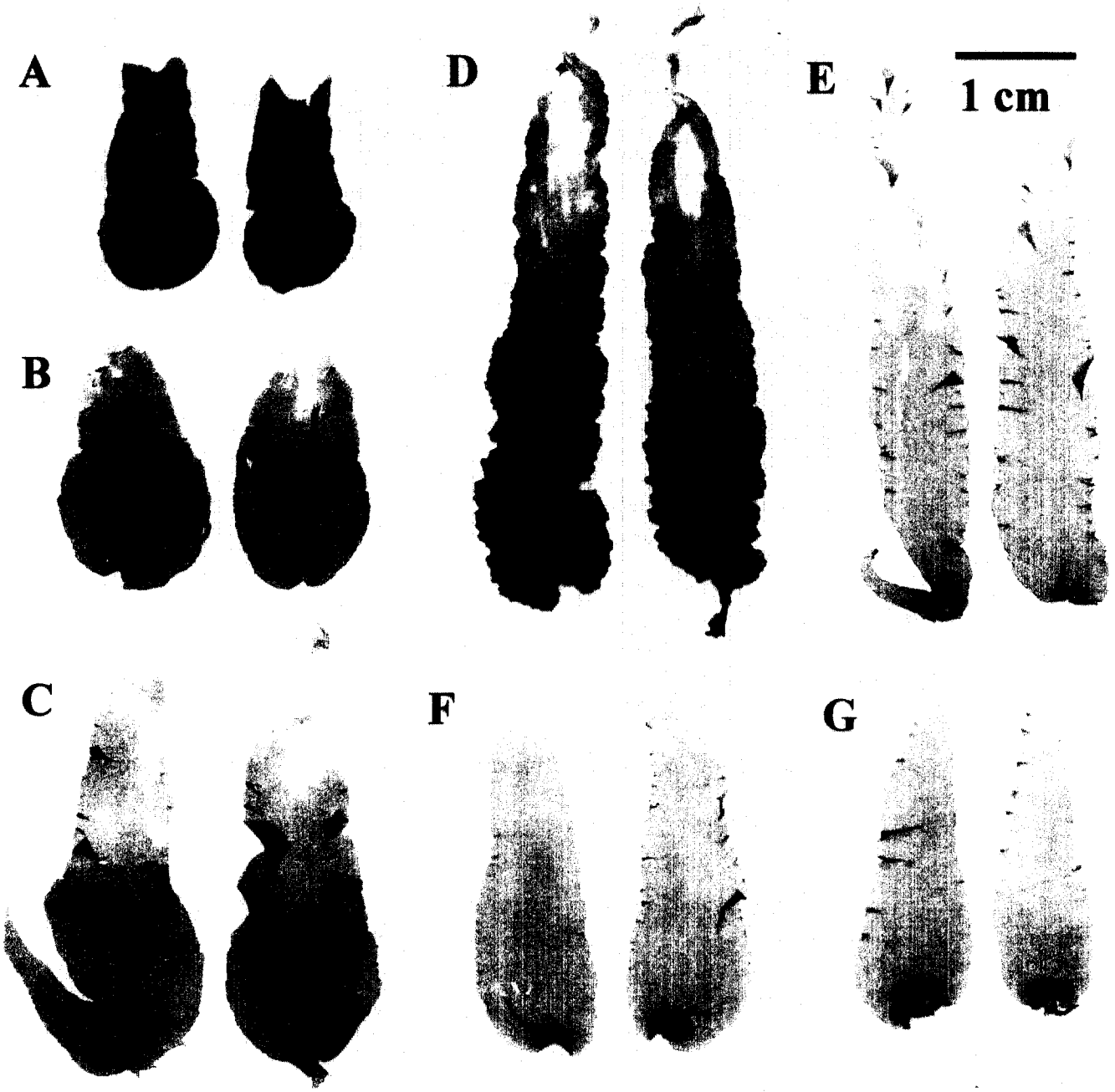

Fig. 8. Effect of salinity and photoperiod on blade shape of *Porphyra vietnamensis* at 25°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after 7 weeks in culture. A, 10 ppt; B, 15 ppt; C, 20 ppt; D, 25 ppt; E, 30 ppt; F, 35 ppt and G, 40 ppt.

(Fig. 6C). The blades in these conditions did not produce any sexual reproductive cells. The largest blade was observed at 25°C after 5-6 and 7 weeks in culture under long and short photoperiod, respectively. The blades at 20°C and 25°C under both photoperiods released spermatia and very small amount of archeospores after 6-8 weeks in culture and zygotospores were liberated one week after in each condition. Linear or linear lanceolate blades were observed at 20°C and 25°C under both photoperiods after 7 weeks in culture. The blade of 20 and 25°C showed linear-lanceolate in shape while the blade shape of 30°C was clearly different from the others due to release of archeospores (Fig. 6).

Fig. 7 illustrates the effects of salinity and photoperiod on growth of the blade area at 25°C under 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. High growth rate was observed at 20-25

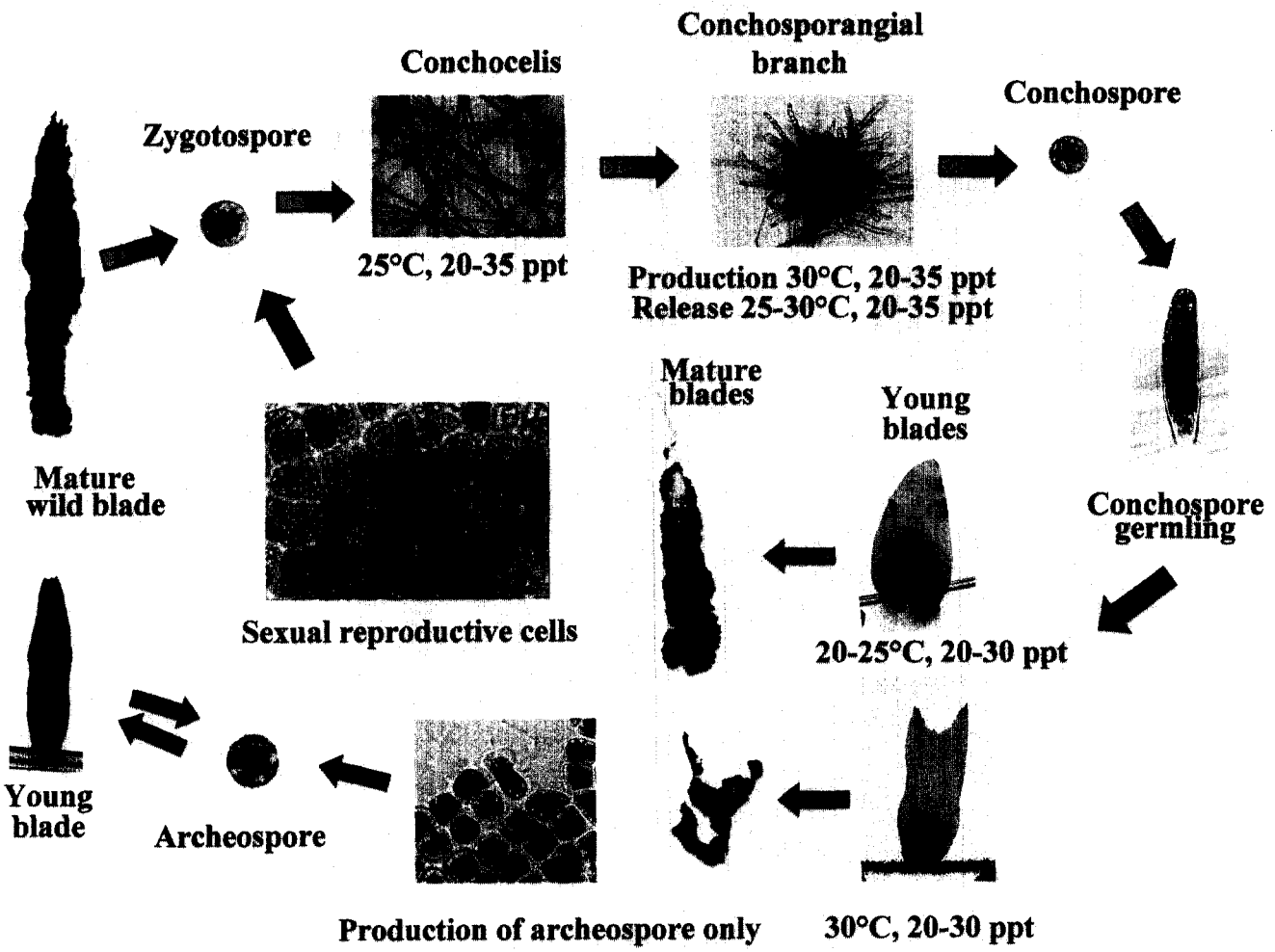

Fig. 9. Life cycle of *Porphyra vietnamensis* at 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 14L: 10D.

ppt, and gradually decreased with higher or lower salinity under each photoperiod. Culture blades at the 10-30 ppt released archeospores at 2 weeks; however, the blades at 35-40 ppt did not release archeospore for 7 weeks in culture under both photoperiod conditions. Spermatia and zygotospores were released at 5 to 7 weeks under 15-35 ppt in both photoperiods. The blades at 10 and 40 ppt did not release spermatia and zygotospores for the 7 weeks culture period under both photoperiods. The shape of blades under the various salinities after 7 weeks culture are shown in Fig. 8. Bigger mature blades were observed at 20-30 ppt and have linear-lanceolate shape similar to wild blades.

The results from one-way analysis of ANOVA indicated significant influence ($p < 0.01$) of temperature and salinity on vegetative growth of the both phases. The life cycle of *P. vietnamensis* and the optimum temperature and salinity conditions for good growth and maturation of the conchocelis and blade phase in culture were summarized in Fig. 9. Released zygotospores from the wild blades germinated into conchocelis phase. The conchocelis grew well at 25°C and 20-35 ppt and conchosporangial branches were produced and released conchospores at higher temperature 30°C and lower salinities of 20-35 ppt. Conchospore germlings grew fast earlier at 30°C and 20-30 ppt and only released archeospores and eventual thallus deterioration. However, at 20-25°C and 20-30 ppt, blades continue to grow bigger producing and releasing sexual cells and archeospores. Spermatangial cells were first observed on the upper marginal edge of the blades and one week later, carposporangial cells were found adjacent to the spermatangial area inside of blades or sometimes in mixed proportion. Furthermore, archeosporangia were also found intermixed with the reproductive cells of the blade cultures, predominantly at 20°C after 6-7 weeks at both photoperiods. The life cycle of *P. vietnamensis* was completed within 12 weeks under these culture condi-

tions.

DISCUSSION

The life history of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho from Songkhla, Thailand was completed under the various conditions of temperature, salinity, photon flux density and photoperiod in laboratory culture. Similar optimum growth temperature of both conchocelis and blade phase was observed at higher temperature of 25°C in this species. Generally, in temperate species the optimum growth temperatures of blade and conchocelis are different. For example, *P. lacerata* (Notoya and Nagaura 1998), *P. moriensis* (Notoya and Miyashita 1999), *P. pseudolinearis* and *P. dentata* (Kim 1999) have lower temperature (5-15°C) requirements for the blades and higher temperatures (20°C) for the conchocelis phase. It may be considered that the adaptation of tropical species of *P. vietnamensis* require higher temperature than that of temperate species, and it has little difference for the maturation of conchocelis at higher temperature in summer season from the blade at lower in winter season.

Although conchocelis colonies of the highest growth and also the largest size were observed at 25°C and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ yet, they could not produce conchosporangial branches within 6 weeks of culture period in this species. Moreover, conchocelis maturation occurred only at 30°C and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. It suggests that the maturation of conchocelis phase in *P. vietnamensis* is not related with the conditions of growth activities. This result agreed with the report of Lewmanomont and Chittpolkusol (1993) in which it needed 10 days incubation at 30°C for the formation of conchosporangial branches of this species. Kapraun and Lemus (1987) have reported that both conchospore formation and release occurred at temperature of 20-30°C and day length of 12h or less in another tropical species, *P. spiralis*. It seems that *P. vietnamensis* has rather restricted temperature conditions than *P. spiralis*.

There have been a few reports on the effect of the salinity for growth or maturation of *Porphyra* species. Lewmanomont and Chittpoolkusol (1993) reported that salinity is a major influence factor on the life history of *P. vietnamensis* in the preliminary information. However, from this study, the growth of the conchocelis and blades required a wide salinity range (20-40 ppt) and the lanceolate blades similar to wild specimen grew well at 25°C at a salinity of 20-30 ppt. Moreover, the maturation of conchocelis at 30°C within 3-4 weeks culture also occurred at wide salinity range and photoperiod at 25-35 ppt under 14L: 10D and 20-35 ppt under 10L: 14D. It is considered that the salinity is an important factor for maturation of conchocelis phase but not restricted to a narrow range.

The effect of photoperiod on the growth and maturation of conchocelis and blade phase of *P. vietnamensis* occurred slightly earlier at long day than short day. It is rather different in other temperate or colder species where conchosporangial formation and conchospore release occurred usually at short day conditions; e.g. *P. columbina* (Avila *et al.* 1986), *P. linearis* (Bird *et al.* 1972; Katz *et al.* 2000), *P. leucosticta* (Gargiulo *et al.* 1994), *P. abbottae* (Hannach and Waaland 1989) and *P. rosengurtii* (Kapraun and Luster 1980). It was considered that physiological effect of photoperiod on tropical species is not highly significant factor on the growth and maturation of conchocelis phase as well as blade phase. *Porphyra vietnamensis* could have developed adaptation natural environmental conditions of the source locality where almost similar day and night lengths (12 hours) during the whole year is observed in Songkhla, Thailand.

From this study, *P. vietnamensis* exhibited a typical biphasic *Porphyra lacerata* type (Notoya 1997) life history alternating between the gametophytic phase and conchocelis phase. Sexual reproduction on the blade phase is manifested by the fusion of male and female reproductive cells producing zygotospores. The zygotospores germinated into conchocelis. The conchocelis produced only conchospores and they germinated into new blades. The blade phase on the other hand, also produced asexual spores (archeospores) that germinate into new blades. In addition, the culture blades of *P. vietnamensis* at highest tolerable temperature for growth (30°C) released only archeospores without producing sexual reproductive cells during 7 weeks of this culture period. After the blades age, it deteriorates due to massive archeospore release. The phenomena of large number of cohort archeospore release at restricted conditions of high temperature at a time for this species presents a suitable technique for the development of commercial cultivation as "seeds" for sea-farming "Nori" nets.

CONCLUSION

The life cycle of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho from Songkhla, Thailand was completed and the detail physiological responses of the reproductive

cells of the conchocelis and blade phase were clearly understood in the present laboratory culture. Optimum temperature of the growth of the conchocelis and blade is under the same conditions of 25°C. However, the maturation of conchocelis and blade occurred at 30°C, 25-35 ppt and 25°C, 20-35 ppt under the same photon flux density (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and photoperiod (14L: 10D), respectively. A large number of archeospores from the blade phase were released only at 30°C without producing sexual reproductive cells. Massive archeospore released at high temperature can be easily used for seeding of *P. vietnamensis* on commercial cultivation.

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