Thesis for the Degree of Master of Fisheries Science

Tissue growth and branch induction of the red seaweed *Gracilariopsis persica* and *Gp. chorda*

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

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홍조류 *Gracilariopsis persica* 및 *Gp. chorda* 의 조직 성장과 잔가지 유도

Advisor: Prof. Yong-Ki Hong

By

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Tissue growth and branch induction of the red seaweed *Gracilariopsis persica* and *Gp. chorda*

A dissertation

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Table of Contents

Table of Figures

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Abstract

Tissue growth and branch induction of the red seaweed *Gracilariopsis persica* from the Persian Gulf have been investigated under various cultural conditions. The *Gp. persica* showed optimal growth in PES medium at 24°C under 60 μmol m^{-2} s^{-1} light intensity on a 12 h light: 12 h dark cycle with 39‰ salinity. Branches were produced the mostly at the cultural conditions of 24°C under 20 μmol m^{-2} s^{-1} light intensity on a 16h light: 8h dark cycle with 39‰ salinity. Optimal size of propagules usable as seeds was 2cm, and less numbers of propagule appeared faster growth of tissues and branch

induction. Apical part of the *Gp. persica* showed as the growing point. As chemical preservatives in cultural medium, p-hydroxybenzoic acid and potassium sorbate might be used with marginal or weakly negative effects on tissue growth and branch induction. Thus, the *Gp. persica* appeared to be a good agarophyte candidate with a fast growing and high salinity-tolerant characteristics.

Tissue growth, branch induction and tolerance of *Gracilariopsis chorda* from Korea were investigated under a variation of temperature (10-35°C), light irradiation (10-80 μmol.m^{-2}.s^{-1}), salinity (20-45‰), light period (0-24L), and medium volume (10-100%) in unialgal culture. *Gp. chorda* shown maximum growth rates with 20°C under 40μmol.m^{-2}.s^{-1} with 16L:8D in 27‰ salinity. The relevance observed between light irradiation as an enhancement factor on branch induction that optimum ranges was 10μmol.m^{-2}.s^{-1} for *Gp. chorda*. Daily growth rates of *Gp. chorda* was 2.1% day^{-1}. To provide axenic unialgal culture, effects of several preservative chemical as contamination inhibitor and influence on relative growth rates of samples examined.

Key words: branch induction, *Gracilariopsis persica*, preservative, Iran, red seaweed, tissue culture, *Gracilariopsis chorda*, Korea, axenic culture, preservative

Chapter *1*. Tissue growth and branch induction of the red seaweed *Gracilariopsis persica*

Abstract

Tissue growth and branch induction of the red seaweed *Gracilariopsis persica* from the Persian Gulf have been investigated under various cultural conditions. The *Gp. persica* showed optimal growth in PES medium at 24°C under 60 μmol m^{-2} s^{-1} light intensity on a 12 h light: 12 h dark cycle with 39‰ salinity. Branches were produced the mostly at the cultural conditions of 24°C under 20 μmol m^{-2} s^{-1} light intensity on a 16h light: 8h dark cycle with 39‰ salinity. Optimal size of propagules usable as seeds was 2cm, and less numbers of propagule appeared faster growth of tissues and branch induction. Apical part of the *Gp. persica* showed as the growing point. As chemical preservatives in cultural medium, p-hydroxybenzoic acid and potassium sorbate might be used with marginal or weakly negative effects on tissue growth and branch induction. Thus, the *Gp. persica* appeared to be

a good agarophyte candidate with a fast growing and high salinity-tolerant characteristics.

Key words*:* branch induction, *Gracilariopsis persica*, preservative, Iran, red seaweed, tissue culture

Introduction

The red seaweed of agarophyte produces the hydrocolloid agar in its cell walls. Agarophytes are harvested commercially for use as a laxative, a vegetarian gelatin substitute, a thickener for soups, in jellies, ice cream and other desserts, as a clarifying agent in brewing, and for sizing paper and fabrics (Phillips and Williams 2009). In some countries especially in the developing world, the harvesting of agarophytes, either as natural stocks or a cultivated crop is of considerable economic importance. Notable genera of commercially exploited agarophytes include *Gracilaria*, *Gracilariopsis* and *Gelidium*. The red seaweeds belonging to the genus *Gracilaria* are very important as a food source for humans and marine animals, and as a source of industrial agar (Zemke-White and Ohno 1999). *Gracilaria* is now the most important agarophyte producing approximately 60% of the agar in the world (FAO 2010). The genus *Gracilariopsis* was segregated from *Gracilaria* by Dawson (1949) based on features of the cystocarps. Further compelling morphological evidence for considering *Gracilariopsis* as a

distinct genus was presented by Fredericq and Hommersand (1989a; 1989b), based on detailed morphological and developmental studies of the genotype species of both genera (Bellorin, Buriyo et al. 2008). The genus *Gracilariopsis* of red seaweed is distributed throughout the tropical and warm-water regions of the world and known as an agarophyte similar to the *Gracilaria*. The species *Gracilariopsis persica* was first described by Bellorin et al. (2008) based on cellular and molecular analysis. *Gp. persica* began to grow from late September to July and showed high growth rate from January to May in the Persian Gulf (Salehia, Dashtia et al. 2010). The overexploitation of the wild biomass of economically important agarophytes has led to the development of techniques for cultivation of the seaweed to meet industrial demands (Raikar, Iima et al. 2001). If this fast growing species of *Gp. persica* is available to produce in a mass scale by aquaculture, it will be also usable as an alternative agarophyte source in warm and high salty water region. However, there are no data about the conditions of growth and reproduction of the *Gp. persica* so far. Vegetative clones of *Gracilaria* and *Gracilariopsis* proved to grow indefinitely (Dawes 1995; Hernández, Pérez-Pastor et al. 2005). Thus, aquacultural farming of *Gracilaria* and *Gracilariopsis* can mainly base on the regenerative capacity

of thallus fragments regeneration. It need not go through sexual cycles or spore production to propagate the seaweed crops. The initial fragment size and the original position of thalli along an axis are also parts of the few factors that the farmer can effectively handle in the field, improving production if dealt with properly (Santelices and Varela 1995). Therefore, for tissue growth and branch induction of the *Gp. persica*, besides determining optimal culturing conditions of temperature, light intensity, light period and salinity, we compared propagule size, density, and growing point to be used as seeds. Chemical preservatives are also selected for bacteriostatic cultivation of the propagules.

Materials and Methods

Tissue materials

Vegetative plants of *Gracilariopsis persica* were collected at the intertidal zone of Bandar-e-Abbas (27°18'N, 56°30'E), Hormozgan Province, Iran in February 2010. The plants were transported to the laboratory, rinsed several times with filtered seawater to remove epiphytes and detritus, and kept in seawater tank for maintenance.

Species identification

Morphologically, the red seaweed *Gp. persica* seems to be the only cylindrical species with a slender and freely ramified thallus in Iran, which makes its identification simple (Bellorin et al., 2008). To confirm the species, cross section of main axis was prepared on microscope slide and observed under 400-fold magnitute. Thin sections are maden by slicing the tissue with different angles of razor blade

Cutural condition

Middle part of vegetative tissues was used as materials for tissue culture and branch induction. Clean tissues of seven fragments cut by 5 cm were used as propagules. The propagules were attached on rope and submerged in 500 mL beaker containing 200 mL of PES medium (Provasoli 1968) for 10 days. The standard cultural condition was at 24°C under 60 μmol m^{-2} s^{-1} light intensity on a 12 h light: 12 h dark cycle with 35‰ salinity. Light was provided by white fluorescent lamps and measured by a photometer (Sekonic Lumimeter 246, Japan). Different salinities were prepared by adding distilled water or solar salt (Daesang Co., Korea) to filtered seawater to get the desired salinity, which checked using Salinometer (Atago Master-S28M, Japan) (Raikar et al., 2001). The seawaters were autoclaved and enriched for PES medium. After 10 days culture, wet weight of the tissues was measured after removing extra water from tissues by centrifugation at 1000 x G for 5 min. Relative growth rate (RGR; % day^{-1}) was calculated using the formula: RGR = $\{[(W_t - W_0)/W_0]x100\}/10$ days, where W_0 is the

initial weight, W_t is the weight after 10 days culture. Number of lateral branches was counted at the initial time and after 10 days culture.

Chemical preservatives

For tissue growth and branch induction in a bacteria-free or bacteriostatic condition, several chemical preservatives using in food preservation were tested. Each preservative agent was added to PES medium with a permissible amount as food additives (KFDA. 2004); such as menadione sodium bisulfate with 10 ppm, sodium propionate with 600 ppm, potasium sorbate with 270 ppm, sodium dehydroacetate with 230 ppm, sodium benzoate with 500 ppm, sodium salicylate with 370 ppm, chitosan with 200 ppm, germanium oxide with 100 ppm, and p-hydroxybenzoic acid with 250 ppm.

Statistical Analyses

Statistical analyses carried out using SPSS software. Oneway ANOVA used to determine the differences in relative growth rate and branch number.

Results

Species identification

Morphologically, the seaweed collected from the Persian Gulf looks like cylindrical species with a slender and freely ramified thallus (Figure 1). To confirm the species, cross section of main axis was observed under 400-fold magnitude. It has characters of abrupt transition in cell size from medulla to cortex typical to Gracilariopsis genus, and the slender branch is known as a unique shape to the species of *Gp. persica* Bellorin.

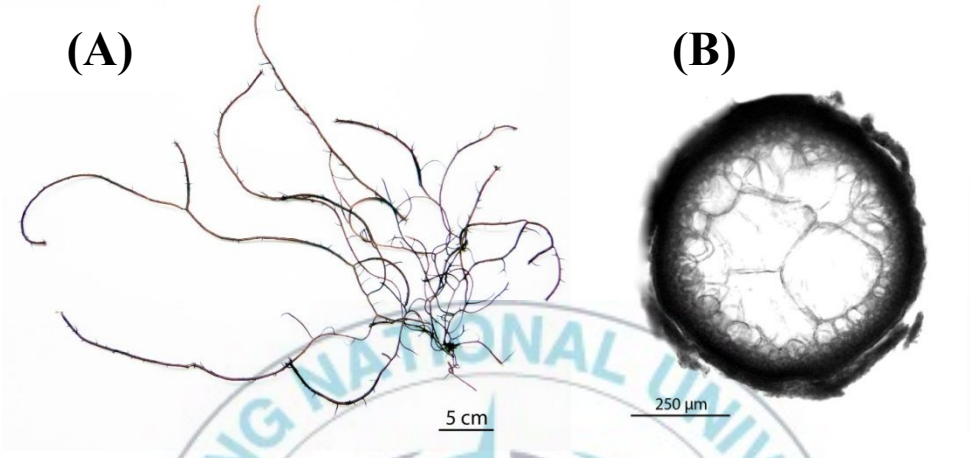

Figure 1. Morphological shape (A) and cross section of main axis (B) from the seaweed sample collected at the intertidal zone of Bandar-e-Abbas in Iran.

Temperature

To determine the optimal temperature of the *Gp. persica* growth, tissues were cultured at wide range of temperatures from 10°C to 35 °C in PES medium under standard cultural condition. The *Gp. persica* showed optimal growth at 24°C with the average growth rate of 3.7% day^{-1} (Figure 2). It

grew well in a range of 20 °C to 32°C, but tissues became completely discolored after 3 days at 35°C. New branches were also produced the most at 24°C with average branches of 8.5 per cm of tissues. Many branches were also produced at 28 °C and 32 °C.

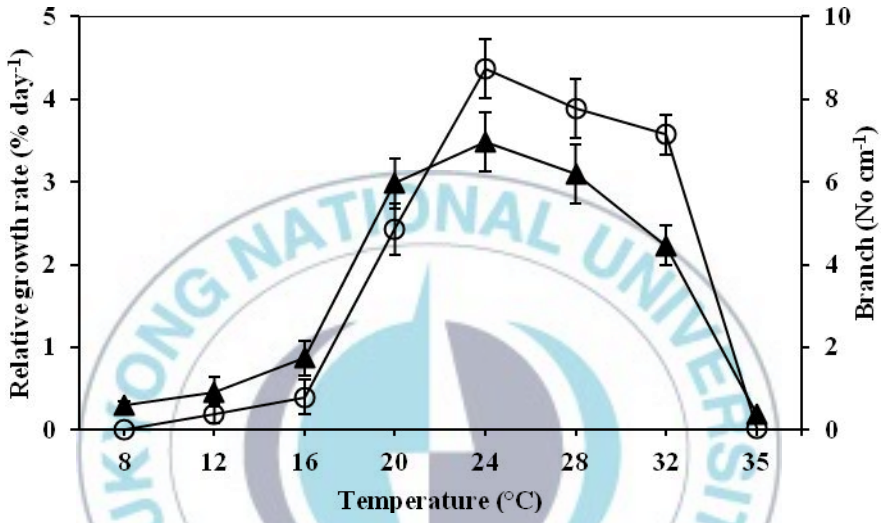

Figure 2. Effects of different temperatures on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p < 0.05$).

Light

To determine the optimal light of the *Gp. persica* growth, tissues were cultured at a range of light intensity from 10 to 80 μmol m^{-2} s^{-1} in PES under standard cultural condition. The tissues showed optimal growth at 60 μmol m^{-2} s^{-1} with the average growth rate of 3.5% day^{-1} (Figure 3). It grew slowly at 20 and 40 μmol m^{-2} s^{-1}. New branches were produced the most at 20 μmol m^{-2} s^{-1} with average branches of 8.3 per cm of tissues. Many branches were also produced at 40 μmol m^{-2} s^{-1}.

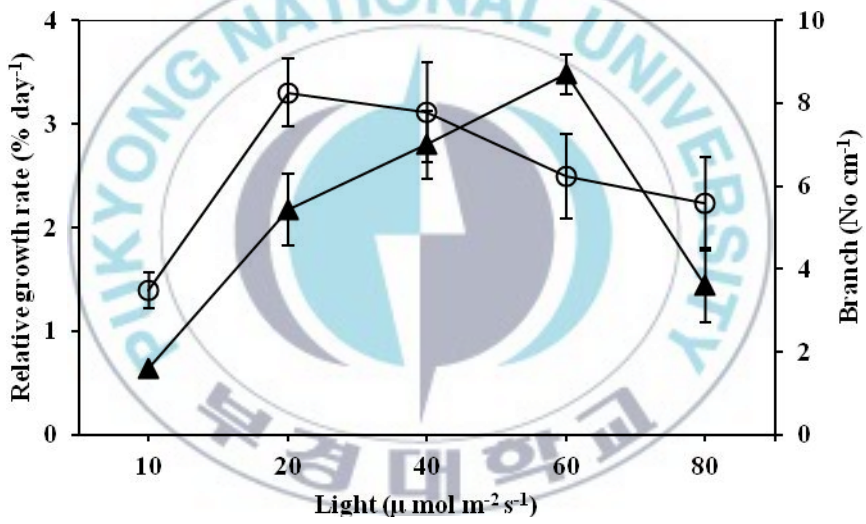

Figure 3. Effects of different light on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p < 0.05$).

Light period

To determine the optimal light period of the *Gp. persica* growth, tissues were cultured at different photoperiods under standard cultural condition. The tissues showed optimal growth on a 12 h light: 12 h dark cycle at 60 μmol m^{-2} s^{-1} with the average growth rate of 3.9% day^{-1} (Figure 4). New branches were produced the most on a 16 h light: 8 h dark cycle with average branches of 10.3 per cm of tissues.

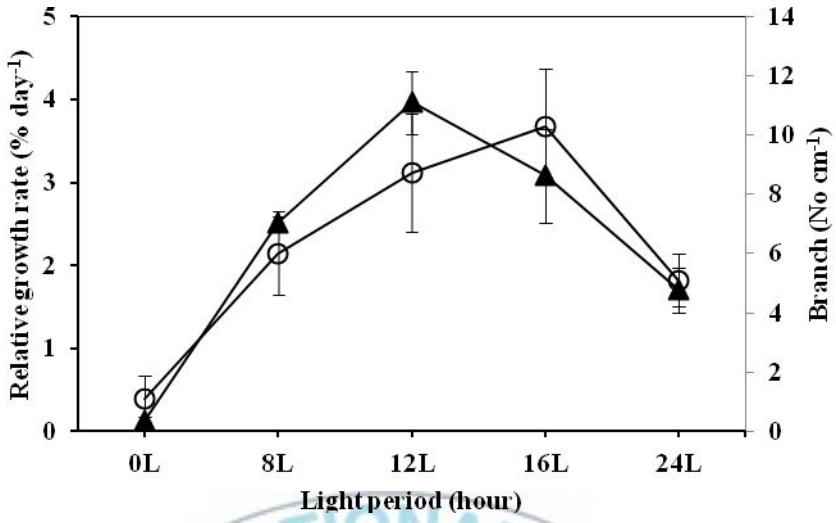

Figure 4. Effects of different light period on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p > 0.05$).

Salinity

To determine the optimal salinity of the *Gp. persica* growth, tissues were cultured at a range of salinity from 24 to 42 ‰ under standard cultural condition. The tissues showed optimal growth at 39 ‰ with the average growth rate of 2.2% day^{-1} (Figure 5). It grew 2.0% day^{-1} at normal seawater salinity of 35‰. New branches were produced the most at 39 ‰ with average branches of 7.4 per cm of tissues. It induced 5.8 branches per cm at

normal seawater salinity of 35‰. More tissue growth and branch induction were occurred in high salinities (up to 39‰), which is indicating that the seaweed is adapted to high salinity condition and can be cultivated in closed seawater ponds.

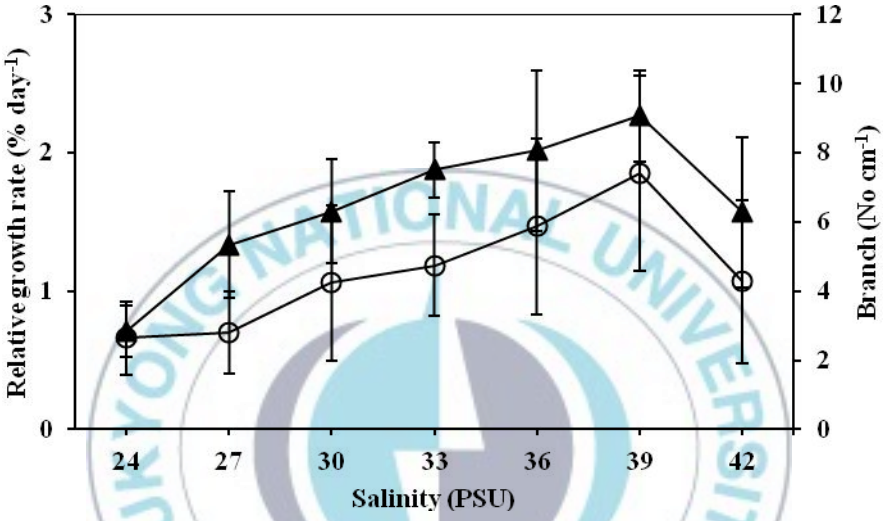

Figure 5. Effects of different salinity on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p > 0.05$).

Propagule size

To determine the optimal size of propagules usable as seeds of the *Gp. persica* growth, tissues were cultured by different length of fragments from 1 cm to 20 cm under standard cultural condition. The tissues of 1 or 2 cm showed the highest growth rate with the average growth rate of 5.4 or 5.2 % day^{-1} (Figure 6). New branches were induced the most from the 2 cm size of tissues with average branches of 17.6 per cm. The short 2 cm tissues are usable for seed maintenance or initial in-door cultivation.

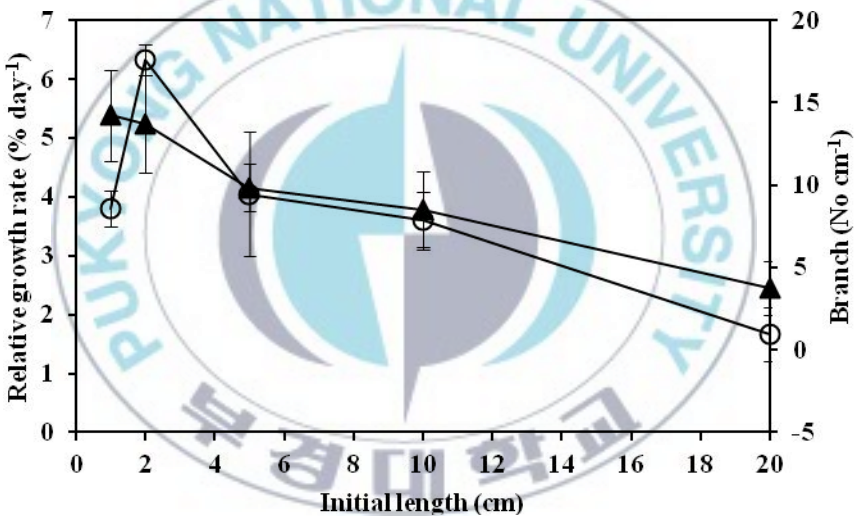

Figure 6. Effects of propagule size on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p < 0.05$).

Propagule density

To determine the optimal propagule density of *Gp. persica* thalli in a closed culture system, tissues of 1 to 30 pieces of 5 cm fragment size were cultured in 200 mL medium under standard cultural condition. Less numbers of propagule appeared faster growth. When a single piece of propagule was cultured, it showed a high growth rate of 6.6 % day^{-1} that is almost double comparing to 7 pieces-culture of standard condition (Figure 7). New branches from the single-piece culture were also induced almost double with average branches of 13.7 per cm comparing to 7 pieces culture. By increasing numbers of propagule, growth rates and branch induction were decreased to 1.1% day^{-1} and 0.7 branches per cm, respectively, with 20 or more pieces of propagule.

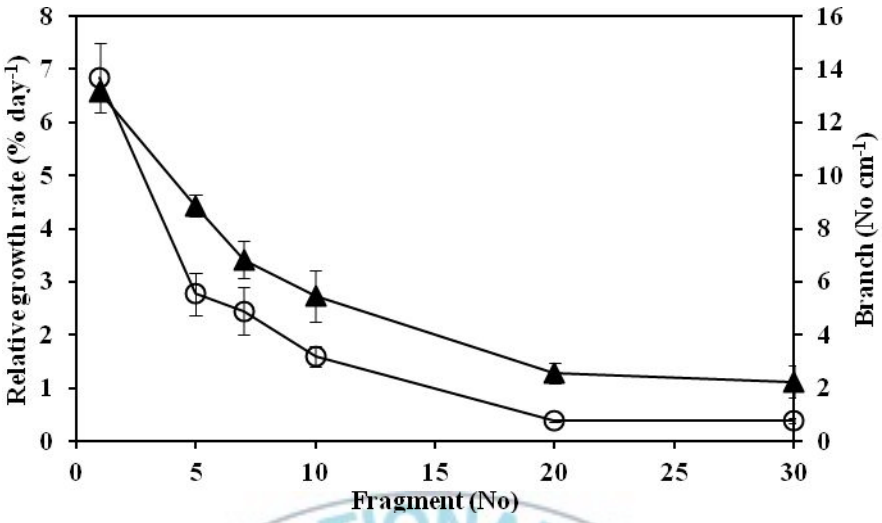

Figure 7. Effects of propagule density on tissue growth (▲) and branch induction (○) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p < 0.05$).

Growing point

To determine the growing point of *Gp. persica* thalli, tissues of apical fragment (5 cm) and subapical fragment (5 cm) were cultured under standard cultural condition. Elongation of thalli occurred faster at apical part with the average growth rate of 6.6 % day^{-1} (Figure 8). New branches were induced more from the same apical part with average branches of 9.9 per cm. Thus, the apical part of *Gp. persica* appears as the growing point.

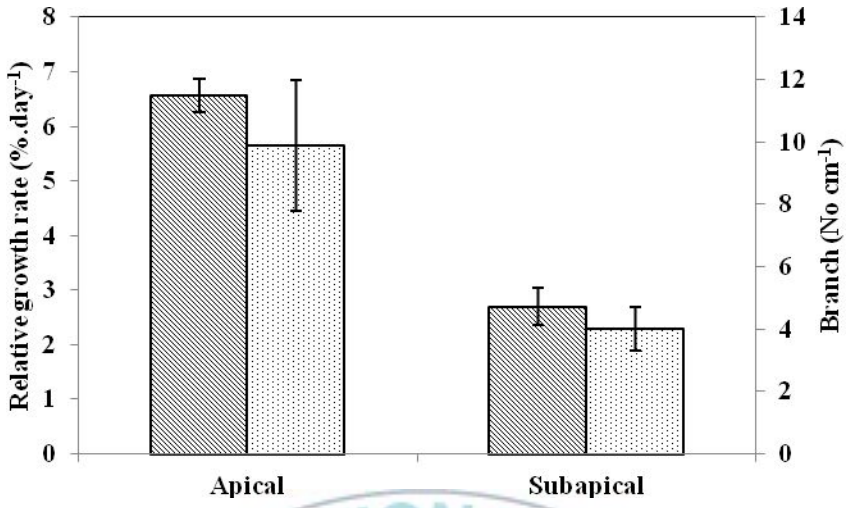

Figure 8. Comparison of apical and subapical parts on tissue growth (▨) and branch induction (⁙) of *Gp. persica* under standard cultural condition. Values represent the mean ± SE (n ≥ 7).

Treatment of chemical preservatives

For stock maintenance and propagule cultivation, it is necessary to culture propagules in a bacteria-free or bacteriostatic condition. When chemical preservatives using for food preservation are added to PES culture medium, propagule tissues showed different effects on growth and branching. Compared to control without adding preservatives, potassium sorbate, sodium dehydroacetate, sodium benzoate and p-hydroxybenzoic

acid had marginal negative effects on tissue growth (Figure 9). When branch induction is also required during stock cultivation, p-hydroxybenzoic acid and potassium sorbate are recommended to be used. Sodium benzoate at 500 ppm produced no branches but main stems were elongated normally.

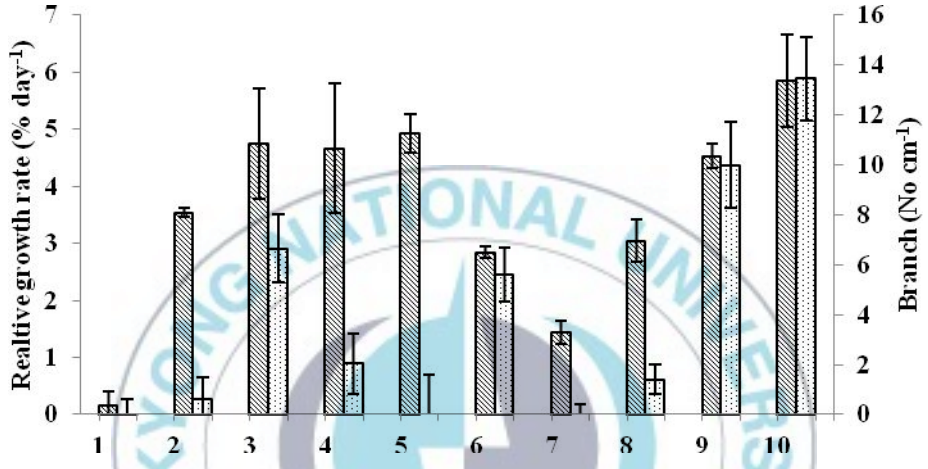

Figure 9. Effects of chemical preservatives on tissue growth (▨) and branch induction (⬚) of *Gp. persica* under standard cultural condition. **1**, menadione sodium bisulfate (10 ppm). **2**, sodium propionate (600 ppm). **3**, potasium sorbate (270 ppm). **4**, sodium dehydroacetate (230 ppm). **5**, sodium benzoate (500 ppm). **6**, sodium salicylate (370 ppm). **7**, chitosan (200 ppm). **8**, germanium oxide (100 ppm). **9**, p-hydroxybenzoic acid (250 ppm). **10**, control. Values represent the mean ± SE ($n \geq 7$).

Discussion

The fast growing and tall agarophyte species of *Gp. persica* is abundant in the Persian Gulf area. Collection site of the species is sand beach located in Bandar-e-Abbas city on the southern coast of Iran. *Gp .persica* grows fast after attaching to pebbles or artificial substrates on sandy-loam or sandy bottoms, at the intertidal or sublittoral regions of coasts not exposed to strong wave actions or currents. The fronds are abundant from mid-October to May in the area. The plants are growing caespitose up to 250 cm long (Bellorin et al., 2008). The average sea surface temperature ranges from 20 to 27°C throughout the year, and the salinity ranges from 37‰ in winter to 38‰ in summer (Bidokhti and Ezam 2009).

Our results show that the growth of *Gp. persica* was optimal at 24°C, 60 μmol m^{-2} s^{-1} light intensity and 39‰ salinity, those are similar to the environmental condition of the collection site. The temperature, light and salinity are the most critical factors determining the growth and distribution of benthic marine algae (Raikar et al., 2001). *Gp. persica* tolerated at relatively high temperature of 32°C and high salinity of 42‰, those can be used as a thermo- and halo-tolerant gene resource. Higher growth rate of

apical part indicates the growing point of the *Gp. persica*, same like *G. chilensis* (Santelices and Varela 1995) and *G. gracilis* (Smit and Bolton 1999). To enhance biomass of the seaweed, branches may be induced at early culture period and set out to sea as main cultivation. In case of *Gp. persica*, we found that dim light at 20 μmol m^{-2} s^{-1} light intensity on a 16 h light: 8 h dark cycle was a critical condition to induce more branches. To provide yearlong seedstock for seaweed cultivation, it is necessary to keep the seedstock in a condition of good viability and no bacterial contamination. Food preservatives are relatively cheap and safe in mass use.

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Chapter *2*. Tissue growth and branch induction of the red seaweed *Gracilariopsis chorda*

Abstract

Tissue growth, branch induction and tolerance of *Gracilariopsis chorda* from Korea were investigated under a variation of temperature (10-35℃), light irradiation (10-80 μmol.m^{-2}.s^{-1}), salinity (20-45‰), light period (0-24L), and medium volume (10-100%) in unialgal culture. *Gp. chorda* shown maximum growth rates with 20°C under 40μmol.m^{-2}.s^{-1} with 16L:8D in 27‰ salinity. The relevance observed between light irradiation as an enhancement factor on branch induction that optimum ranges was 10μmol.m^{-2}.s^{-1} for *Gp. chorda*. Daily growth rates of *Gp. chorda* was 2.1% day^{-1}. To provide axenic unialgal culture, effects of several preservative chemical as contamination inhibitor and influence on relative growth rates of samples examined.

Key words: *Gracilariopsis chorda*, Branch Induction, Korea, axenic culture, preservative

Introduction

Seaweeds belonging to the genus Gracilaria are very important as a food for humans and marine animals, and as a source of industrial agars (Zemke-White WL 1999) and harvested and cultured on a commercial scale in many countries because it has considerable economic importance as an agarophyte. *Gracilaria* is now the most important agarophyte, producing approximately 60% of the agar in the world (Tseng 2001), and commercial cultivation is performed on a very large scale in several countries such as Chile, China and Taiwan (Dawes 1995). The total annual *Gracilaria* production in the world increased to more than 89,000t, including 50,000t of cultured production, in 1995. *Gracilaria* plants are also used as sources of traditional seaweed salad in Japan and feed for shellfish (abalone) in many countries. There is increasing demand for industrial agars for use as materials in electrophoresis and as a culture medium for microbes. For obtaining *Gracilaria* biopolymers of constant quality and quantity, a cultured strain is likely to be more suitable than a wild one. In Korea, industrial agars extracted from *Gracilaria* spp. are imported from Chile and Brazil.

Gracilariopsis chorda occurs in the lower intertidal zone of the southeast coast of Korea. A few studies have been performed on the commercial cultivation of *Gracilariopsis chorda* to develop cultivation techniques. Environmental factors including temperature, salinity and light play an important role in the growth, reproduction and distribution of marine algae (Lobban 1994). Temperature requirements for survival and growth of *Gracilaria* species have been extensively studied. Some *Gracilaria* species require less than 100 μmol m^{-2} s^{-1} for optimal growth, while others require higher irradiance (Kaktia and Kamishima 2006).

However, there are few data on the growth and reproduction of *Gracilariopsis chorda*, even though the alga is of commercial interest as an agarophyte and foodstuff due to its higher production. The aim of this study is to characterize the physiological responses of *Gp. chorda* to temperature, salinity, irradiance and photoperiod, assessing tolerance and optimal conditions for tissue growth and branch induction in unialgal cultures.

In addition, data on the growth and reproduction of natural populations of *Gp. chorda* are needed. Accordingly, the aims of this study were to examine the effects of salinity and temperature on the growth of *Gracilariopsis chorda* in laboratory culture.

Materials and Methods

Study site **-** Studies were conducted at Ilkwang beach, on the northeast Busan, Korea. Rock beds lie along the shoreline and a large amount of gravel and vast mud flats are found in the intertidal zone in the study area. The seawater movement is relatively low and the transparency of seawater is approximately 0.8–2.0 m. The average sea surface temperature ranges from 7 to 24°C throughout the year, and the salinity ranges from 27‰ in summer to 34‰ in winter. Stock unialgal cultures were incubated with aeration at seawater tank.

For culture, vegetative plants of *Gracilariopsis chorda* were collected from the intertidal zone of Ilkwang, Korea in September 2010. The plants were transported to the laboratory and the apical parts of fronds were rinsed several times with filtered seawater to remove diatoms and detritus attached to the fronds and kept in seawater tank for 2 weeks. The healthy apical fronds, 10mm in length, were excised from the vegetative fronds.

Unialgal cultures of *Gp. chorda* were obtained from vegetative thalli under laboratory conditions, and grown in 500ml beaker with 200ml PES under controlled laboratory conditions of temperature (5-35°C), photon

irradiance (10-80μmol.$m^{-2}s^{-1}$), and photoperiod (0, 8:16, 16:8 and 24h L:D). Algae from the culture were incubated 7 days before each experimental. The fresh weight of algae was determined 10 days after each treatment. Relative daily growth rate (R = % d^{-1}) was calculated as $R = (W_t - W_0)/W_0$ where W_0 is the initial biomass and W_t the biomass at day t (Evans, 1972). Three replicates were used for each tested condition (Robledo 1999).

Light & temperature and salinity- Algal growth rates in long-day (16:8h light:dark) regime were compared with short-day (8:16h light:dark) regime at optimal growth temperatures (20°C). Irradiances were measured with a Sekonic Lumi 246 (Japan). The controlled conditions were the same as described above for stock unialgal cultures except that irradiance set to from no light to 80 μmol m^{-2} s^{-1}. For each experiment, five replicates of seven apical segments (10cm) cut from the stock unialgal culture strains were inoculated into 500mL beaker containing 200mL of PES. The data on algal growth rate, which were measured after 10 days of cultivation, were analyzed by two-way ANOVA for temperature and irradiance experiments. Relative growth rates R were calculated using the formula: $R = (W_t - W_0)/W_0$, where W_0 is the initial fresh weight, W_t is the fresh weight after t

days and t is the number of days (Kain 1987). Growth rate (%) was defined as R × 100. Salinity experiments were carried out at 20 to 40‰. Different salinities were made by adding distilled water to filtered seawater and fine solar salt (Daesang Co. Korea) to get the desired salinity, which checked using salinometer (Atago, Master-S28M, Japan), then autoclaved and enriched with PES before being used for the culture experiments.

Growth rates and branch induction of *Gracilariopsis* was investigated at different light intensities (0 to 80 μmol m^{-2} s^{-1}) at their optimum temperatures and salinities. The light intensity was measured using a photometer (Sekonic Lumi 246, Japan) (Raikar, Iima et al. 2001).

Results

The growth was significantly affected by salinity and temperature in culture. *Gracilariopsis chorda* grew in a wide range of temperatures (10-30°C) and salinities between 20-40‰. However, within few days at 35°C, thalli became completely discolored. Therefore, *Gp. chorda* grew well at higher temperatures (15 to 25°C) than at 10°C. However, the fronds of *Gp. chorda* grown at 30°C showed lowest RGR within 10 days (Figure 10). The relative growth rates ranged from 0.8 to 2.1% for *Gp. chorda*. There were significant differences in the relative growth rates among the temperature treatments. The optimal temperature for growth of was between 15-25°C and the maximal growth was observed at 20°C. *Gracilariopsis chorda* grew in salinity rage of 20 to 35‰. The growth was more temperature dependent than salinity dependent. The optimal salinity for growth was 25‰ for *Gp. chorda* (Figure 11).

The difference between the growth rates at different light intensities was significant, their growth rates increased with increasing light intensities and

as a summer seaweed showed maximum growth rates at 16:8h L:D light period (Figure 12). In fact the species studied showed an increase in growth rate with increasing light intensity implying that *Gracilariopsis* species grew well at higher light intensities, but at light intensity higher than 80 μmol m^{-2} s^{-1} frond discolored. *Gp. chorda* showed the highest optimal RGR of 2.1% at 40 μmol m^{-2} s^{-1} (Figure 13). But light intensities have conversely effects on branches induction. The difference between the branches inducting at different light intensities was significant, branching increased with decreasing light intensities. *Gp. chorda* showed the highest new branches number at 10 μmol m^{-2} s^{-1}.

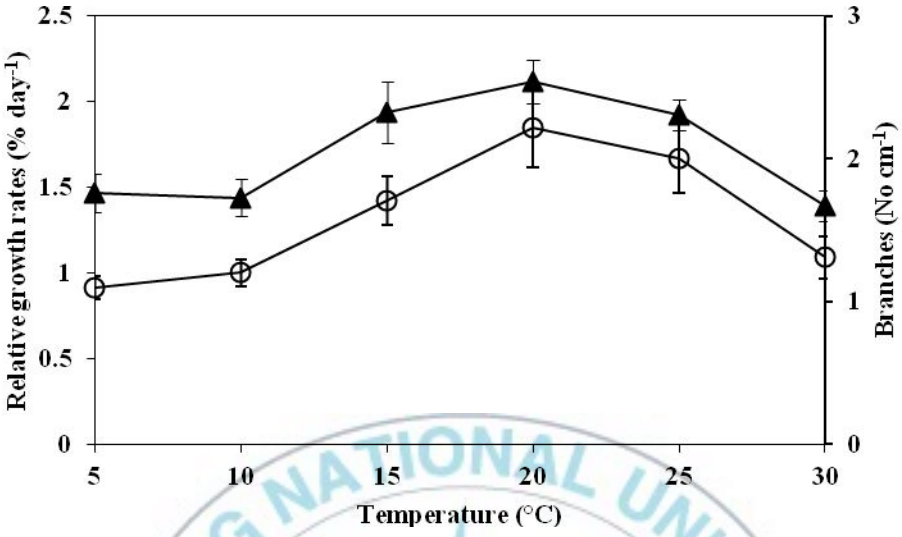

Figure 10. Effects of different temperatures on tissue growth (▲) and branch induction (○) of *Gp. chorda* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p < 0.05$).

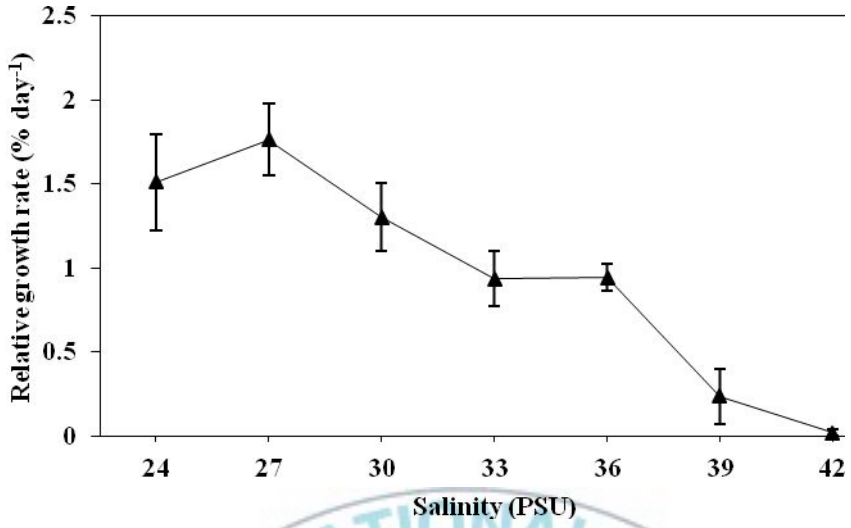

Figure 11. Effects of different salinity on tissue growth (▲)of *Gp. chorda* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p > 0.05$).

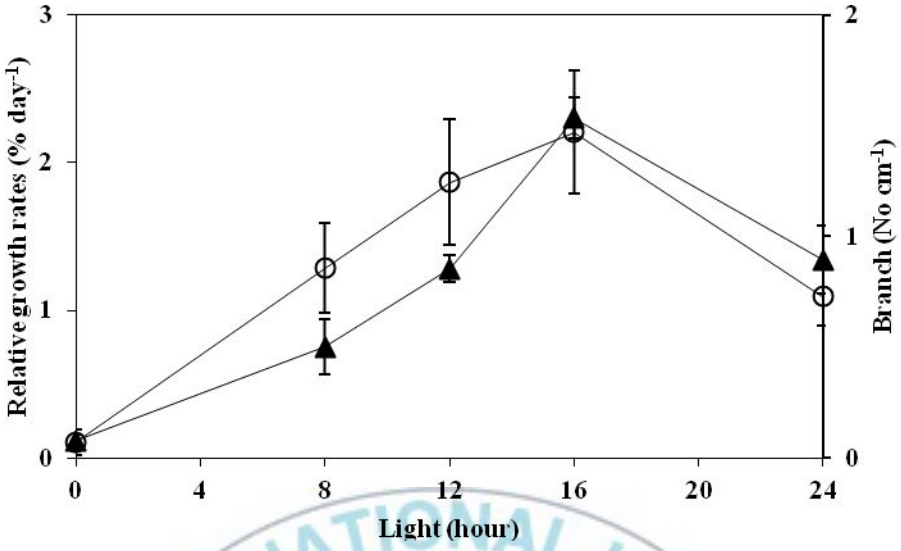

Figure 12. Effects of different light period on tissue growth (▲) and branch induction (○) of *Gp. chorda* under standard cultural condition. Values represent the mean ± SE ($n \geq 7$, $p > 0.05$).

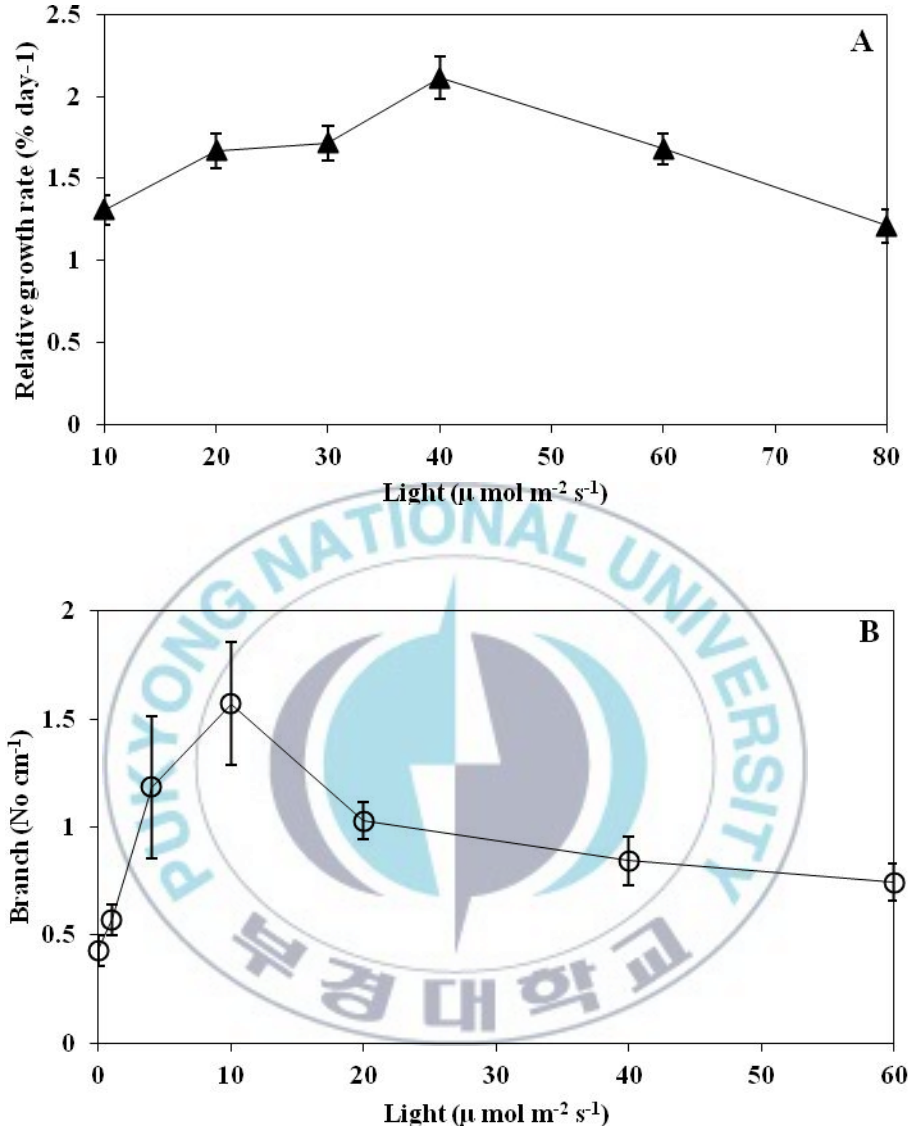

Figure 13. Effects of different light on tissue growth (A) and branch induction (B) of *Gp. chorda* under standard cultural condition. Values represent the mean ± SE (n ≥ 7, $p < 0.05$).

Discussion

However, the relative growth rate of *Gp. chorda* is low compared to *Gracilariopsis*, *Grasilaria* species. In laboratory culture, the growth rate of *Gp. chorda* reached a maximum of 2.1% at 20°C and 25‰ salinity. *Gracilariopsis chorda* grew well at the higher temperatures (25–30°C) in culture, and the growth of the species reached a peak in summer in the field population. In addition, the growth rate of *Gp. chorda* was higher at high salinities (20 and 35‰) than at the low salinities (5 and 15‰) indicating that the seaweed is adapted to oceanic conditions and can be cultivated in open seawater (Choi, Kim et al. 2006).

According to resource allocation theory, reproduction imposes a cost (Mathieson & Guo, 1992). Thus, it is known that the presence of reproductive structures in *Gracilariopsis* spp. affects the growth of the vegetative plants. This is supported by the results of Santelices & Varela (1995), who reported that vegetative plants grew faster than cystocarpic and tetrasporic plants of *G. chilensis*. Thus, we believe that mass culture of vegetative plants is likely to be preferable, even though the growth rates of

Gp. chorda in the various reproductive states was not compared in the present study (Choi, Kim et al. 2006).

On the basis of optimal temperature and salinity for growth, we suggest that the cultivation of *Gracilariopsis chorda* could begin in the spring, when the seawater temperature is approximately 13℃ and after harvesting of *Undaria pinnatifida* and *Laminaria japonica*, which are cultivated in winter. *Gp. chorda* should then be harvested during the reproductive period in summer. In conclusion, *Gp. chorda* appears to be a good agarophyte with a fast growth rate and has the potential for commercial cultivation (Choi, Kim et al. 2006).

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