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Temperature and Light Responses in Growth of *Gracilaria verrucosa* (Rhodophyta) and Its Potential for Mariculture in Korea

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Apical and subapical fragments of *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta) were cultured on a temperature-light gradient table with four temperature (15, 20, 25 and 30℃) and five light intensity (20, 40, 60, 80 and 100 μmol photons $m^{-2}s^{-1}$) regimes to examine growth responses. Their growth was measured weekly. Plant weight and lateral branch formation were affected by temperature and light intensity. As compared with other reports, relative growth rate (RGR) in both fragments was more or less high with 6.27 to 11.95% day^{-1}. It was lowest at 15℃ with 20 μmol photons $m^{-2}s^{-1}$, whereas, the highest value was recorded at 25℃ with 100 μmol photons $m^{-2}s^{-1}$. During the experimental period, the growth pattern in *G. verrucosa* was significantly different between apical and subapical fragments, even though RGR based on weight was similar. Growth in apical fragments depended on elongation by apical growth. By contrast, subapical fragments mainly increased their weights by proliferation of lateral branch. This suggests that intercalary activity (e.g. lateral branch formation) is also an important means for growth of the thallus in Korean *G. verrucosa*. In conclusion, the relatively high growth potential with the intercalary activity in *G. verrucosa* will be helpful for mariculture in Korea.

Key words: Apical and subapical fragments, Intercalary activity, Mariculture, Temperature, Light, Growth, *Gracilaria verrucosa* (Rhodophyta)

Introduction

Gracilaria, which has been used for agar production and for food of both human (Abbott, 1988) and marine animals (Critchley, 1993), contains over 100 species with a worldwide distribution in tropical and temperate waters (Bird and McLachlan, 1986; Oliveira and Plastino, 1994). This useful red alga has been harvested from natural populations in a number of countries (Critechley, 1993). However, over-harvesting from the wild can cause a decline in natural production (Gunanzon and De Castro, 1992). Therefore, in order to maintain steady supply of *Gracilaria*, commercial cultivation has been practised in many countries such as Chile, China, Taiwan, Namibia and South Africa (Tseng, 1981; Dawes, 1995; Friedlander and Levy, 1995; Smit et al., 1997).

It is well known that the growth of *Gracilaria* spp. depends on various environmental factors such as temperature (McLachlan and Bird, 1984; Penniman et al., 1986; Friedlander et al., 1987), light intensity (Laing et al., 1989; Levy and Friedlander, 1990), salinity (Bird and McLachlan, 1986; Haglund and Pedersen, 1992) and availability of nutrients (Lapointe, 1985; Haglund and Pedersen, 1992). Especially, temperature and light are major physical factors determining their growth in the marine en-

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vironment (Lapointe et al., 1984).

Gracilaria verrucosa (Hudson) Papenfuss is one of eight species reported in Korea, and often occurs in the intertidal zone (Kang, 1968; Lee and Kang, 1986). In spite of its economical importance, basic biological studies have been little made for this Korean alga (Koh, 1969; Kim et al., 1993; Lee et al., 1995; Kim et al., 1998; 2001). In this study, therefore, the effects of temperature and light on the growth of *G. verrucosa* are examined to obtain basic information for *Gracilaria* mariculture in Korea. Particularly, growth responses of apical and subapical fragments of *G. verrucosa* are studied, considering the regenerative ability of *Gracilaria* (Raju and Thomas, 1971; Goldstein, 1973).

Materials and Methods

Sporophytes of *Gracilaria verrucosa* were collected at the intertidal region of Cheongsapo near Busan, Korea in August 1995. The collected plants were immediately transported to the laboratory in plastic boxes filled with natural seawater. Plants of *G. verrucosa* were rinsed several times with a brush in autoclaved seawater to remove epiphytic algae and diatoms, and cut into apical and subapical fragments (10 mm long, 0.56 ± 0.27 mg).

For each part of *G. verrucosa* fronds, ten beakers with each ten fronds and 150 mL of culture medium were prepared. The culture medium was made according to the Provasoli's formulation (Starr and Zeikus, 1993). Germanium dioxide (GeO_2 0.5 mg L^{-1}) was added into the culture medium when necessary to inhibit the growth of diatoms (Lewin, 1966). Plants were cultured on a temperature-light gradient table with four temperature (15, 20, 25 and 30℃) and five light intensity (20, 40, 60, 80 and 100 μmol photons $m^{-2}s^{-1}$) regimes for 35 days. Photoperiod was kept constant in a 12:12h L:D and culture medium was renewed weekly during the experimental period. This experiment was replicated twice.

Growth was determined weekly in fresh weight after blotting water with paper tissue in an accuracy of 0.01 mg. The number of lateral branches was counted at the end of experiments. The relative growth rate (RGR) using fresh weight data was calculated for each replicate according to the following formula (Smit and Bolton, 1999):

$$RGR = \ln (W_t/W_0)t^{-1} \times 100$$

where W_0 is initial wet weight and W_t is the wet weight after t days.

Three-way ANOVA was applied to test the effects of temperature and light intensity on the relative growth rate for each culture experiment.

Results

Apical and subapical fragments of *G. verrucosa* grew continuously over a period of 35 days in all experimental treatments, but their growth was influenced by temperature and light intensity (Fig. 1). The growth in fresh weight was better at higher

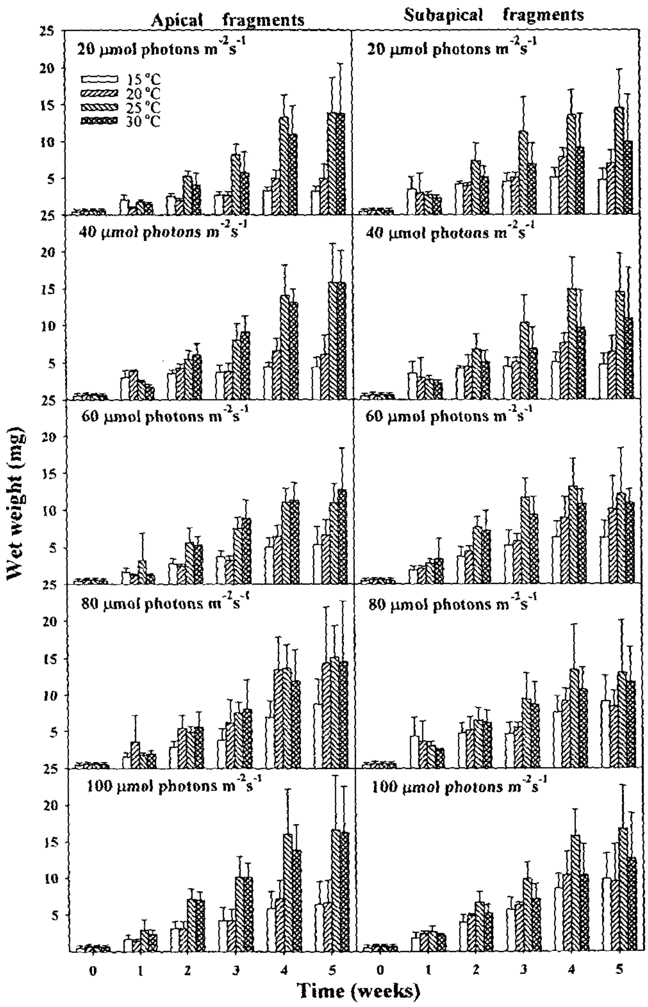

Fig. 1. The effect of temperature on the growth of apical and subapical fragments of *G. verrucosa* under the five lighty intensity regimes.

temperatures and light intensities. In the range of light intensities tested, the growth of *G. verrucosa* was remarkably better at 100 μmol photons $m^{-2}s^{-1}$ than at 20 μmol photons $m^{-2}s^{-1}$. Maximal growth occurred at 25℃, whereas its minimal value recorded at 15℃. These growth response pattern was found in both fragments of *G. verrucosa*.

RGR based on fresh weight was significantly increased when temperatures and light intensity increased (Fig. 2 and Table 1). For apical and subapical fragments, maximal growth rates of *G. verrucosa* were found at 25℃ and 100 μmol photons m^{-2} s^{-1}. In all treatments, the growth rate was lowest at 15℃ with 20 μmol photons $m^{-2}s^{-1}$ (6.27 ± 0.29% day^{-1}, mean ± SE), while highest value was recorded at 25℃ with 100 μmol photons $m^{-2}s^{-1}$ (11.95 ± 0.28% day^{-1}). Even though some variations of RGR to the factors are observed between both fragments, significant difference was not recognized (Table 1).

During the experiment, growth patterns between apical and subapical fragments were different in the

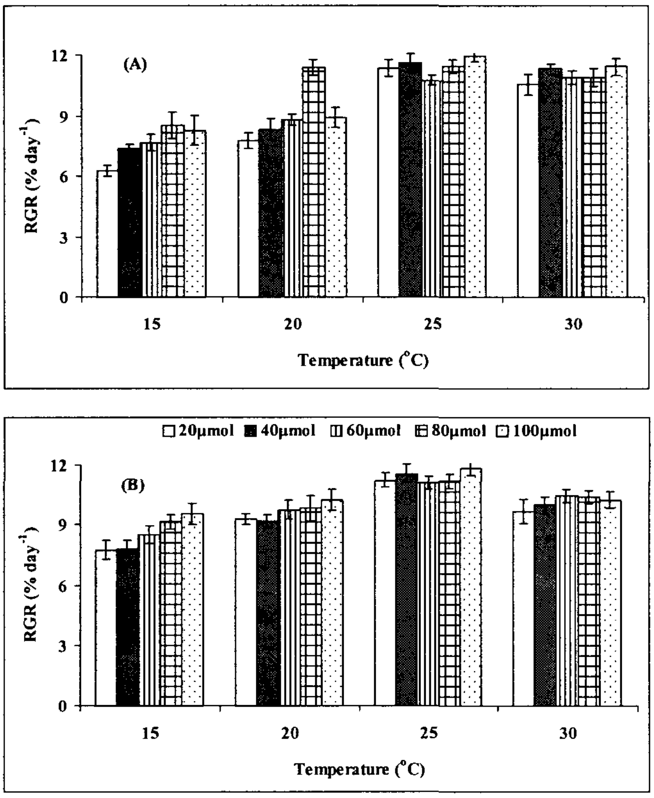

Fig. 2. **Relative growth rate of apical (A) and subapical (B) fragments of *G. verrucosa* under different temperatures and light intensities.**

Table 1. **Analysis of variance (ANOVA) for the effects of the different environmental factors on the growth of *Gracilaria verrucosa***

Source of variation	Degrees of freedom	*F* value	Significant probability
T	3	29.837	<0.001*
L	4	7.553	<0.001*
F	1	0.297	0.586
T×L	12	3.323	<0.001*
T×F	3	4.526	<0.01*
L×F	4	0.513	0.726
T×L×F	12	0.700	0.751

T, temperature (15, 20, 25 and 30℃); L, light intensity (20, 40, 60 80 and 100 μmol photons m^{-1} s^{-}); F, number of branches (apical and subapical fragments)
*p<0.05

examined temperature regimes, though RGR was similar between both fragments (Fig. 2). Growth in apical fragments depended on elongation of thallus, whereas subapical fragments produced many lateral branches. At most temperature except for 20℃, subapical fragments formed more lateral branches than apical fragments (Fig. 3). However, positive correlation between temperature and formation of lateral branches was not observed in the experimental regimes (Fig. 3). The production of lateral branch in apical fragments showed a peak at 20℃, and then rapidly declined with increasing of temperature. This variation pattern was more sharply found in apical fragments than subapical ones (Fig. 3). Mean number of lateral branches in both fragments ranged from 0.7 ± 0.1 to 6.7 ± 2.7 (ind./fragment). Light intensity did not significantly influence the formation of lateral branches (data were not shown).

Discussion

Our results show that the growth of *G. verrucosa* is positively correlated with temperature and that optimal temperature and light intensity for the growth of *G. verrucosa* are 25℃ and 100 μmol photons m^{-2} s^{-1}, respectively. These relationships between temperature and growth are easily found in several literatures on *G. verrucosa* (Causey et al., 1946; Whyte et al., 1981; Hurtado-Ponce and Umezaki, 1987) and *G. tikvahiae* (Lapointe and Ryther, 1978; Lapointe,

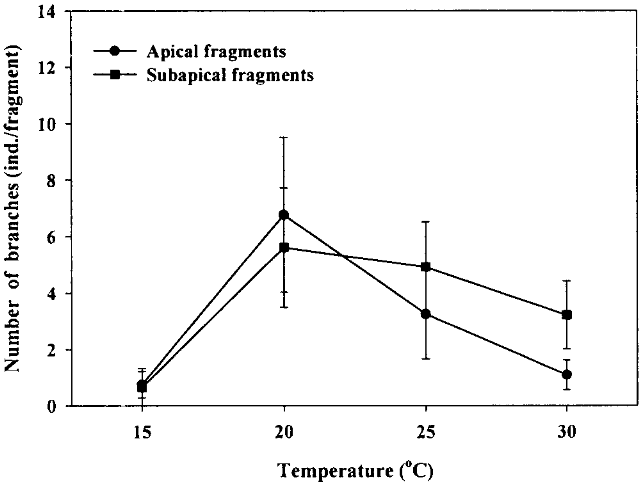

Fig. 3. Number of lateral branches produced in apical and subapical fragments of *G. verrucosa* at different temperatures.

1981). However, optimal temperature for growth seems to be different between populations of *G. verrucosa*, as based on some previous reports (Chen, 1976; Nhoung, 1981; Ren et al., 1984). They commented that maximal growth found in 12~20℃ (Li et al., 1984), or 15~20℃ (Ren et al., 1984) in field cultivation. By contrast, it has been reported to be 20~25℃ in polyculture (Chen, 1976) and swamp culture (Nhoung, 1981).

RGR of *G. verrucosa* obtained from this study ranged 6.27 to 11.95% day^{-1}. This represents more or less higher value than that of other growth measurements in the species from Norway and the Philippines (Hurtado-Ponce and Umezaki, 1987; Rueness et al., 1987; Largo et al., 1989). However, this value is very low in comparison with that (47% day^{-1}) of *G. secundata* reported in culture of plexiglass cylinder with enriched and recirculated seawater (Lignell et al., 1987). In same species, Lignell et al. (1987) reported relatively low RGR in a tank cultivation (3% day^{-1}) and a spray system (6% day^{-1}). These reports may suggest that some kinds of enhancement in culture, such as water flow, are very effective for RGR of *Gracilaria*. As considering this result, maximal growth potential of Korean *G. verrucosa* may be more large.

Although RGR of *G. verrucosa* is similar between apical and subapical fragments, their growth patterns are different from each other. As mentioned above, growth of apical fragments depends on elongation by apical growth. By contrast, subapical fragments mainly increase their weights by proliferation of lateral branch. Unlike in Hurtado-Ponce and Umezaki (1987)'s report, similar RGR based on weight between both fragments was consequently found in this study. This suggests that intercalary activity (e.g. lateral branch formation) is also an important means for growth of the thallus in Korean *G. verrucosa*. Similar growth patterns have been also reported in *G. chilensis* (Santelices and Varela, 1995) and *G. gracilis* (Smit and Bolton, 1999). This growth pattern with the intercalary activity has some beneficial points in seedlings for field cultivation, because seedlings can be done regardless of presence or absence of apical growing fragments in *G. verrucosa*. In addition to this feature, relatively high growth potential in Korean *G. verrucosa* will be helpful for mariculture. With these beneficial points, development of culture system that can induce more favorable growth condition is also required for establishment of the mariculture in Korea.

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