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Development and Morphology of *Pelvetia siliquosa* Tseng et Chang (Phaeophyta) in Culture

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The present study reports morphology and developmental pattern of *P. siliquosa* cultured in a laboratory condition. The zygote was spherical with a diameter of 85μm. During development the polarized zygote divided horizontally and the lower daughter cell divided horizontally into 2 cells. The upper cell was divided repeatedlyin horizonal and vertical directions to form a cylinder-like structure, which subsequently developed into secondaryand tertiary dichotomous branches. Optimum temperature for zygote release and fertilization was 25C. Injury inflicted by slicing was cured by epidermal differentiation, and adventitious branches; the branches emerging from the pith cells, however, developed no rhizoid. Adventitious branch formation rate was over 88% in all plates supplemented with 0.5 mg/L IAA and peaked at 98% under 0.5 mg/L IAA plus 0.5-5.0 mg/L NAA treatment. NAA stimulated the differentiation of adventitious branches at a wide range of concentrations, while IAA, 2,4-D and kinetin exhibited dose-dependent stimulation.

Keywords: *Pelvetia siliquosa*, Phaeophyta, Morphology, Adventitious branches, Growth regulators

Introduction

Phaeophyte *Pelvetia siliquosa* Tseng et Chang is commonly found on the warm coasts of the south and west of Korea. In Korea, *P. siliquosa* has been utilized as an important source of food and alginic acid. With increasing coastal pollution and over-exploitation, the algae have been decreased rapidly hence artificial breeding technology for propagating *P. siliquosa* is required.

Although seaweed thalli growing in intertidal environments, including *P. siliquosa*, are susceptible to desiccation, extreme temperature changes, storms, surges or grazing, their population density is maintained through the regeneration of adventitious branches (Yoon and Soh, 1998). Growth regulators such as auxin, gibberellin, and cytokinin are responsible for the regeneration and growth of the algal body (Cha et al., 1981). Growth regulators are present in marine algae as well as in terrestrial plants, albeit in different forms and at different concentrations (Du Buy and Olson, 1937). According to Kentzer et al. (1980), cytokinin is more abundant in algae distributed in deeper water than the one in surface water where *Fucus vesiculosus* thrives. In addition, 6-(3-methyl-2-butenylamino) purine was also detected in the thalli extract. They concluded that cytokinin is thus responsible for the number of adventitious branches developing from the thalli of *F. vesiculosus*. On the other hand, Du Buy and Olson (1937) reported the presence of auxin in several parts of *F. vesiculosus*, especially at high concentrations in eggs and sperm cells and lower concentrations in fronds. Similarly, Moss (1967a, b, 1968) reported that culturing the receptacles of *F. vesiculosus* in media containing NAA and kinetin affects gamete formation and regeneration.

Although extensive studies have been conducted on the effect of growth stimulators, their effect particularly on the tissue regeneration of *P. siliquosa* has not yet been documented. This study reports therefore aims to: (1) examine the life cycle of *P. siliquosa* through indoor culture of fertilized eggs and (2) determine appropriate culture conditions and concentrations of plant growth regulators affecting the regeneration of *P. siliquosa*.

Materials and Methods

Samples were collected from the Baek-ya island in Hwajeong-myon Yosu-city, Jeollanamdo in April 1998. After transferred to the laboratory, organisms attached on the algae were removed with a brush and sterilized for one minute in 50% ethanol.

To study the development of *P. siliquosa*, mature recepta-

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cles were cut and cultured in 150 cm diameter petri dishes (Abe, 1969, 1970). Number of zygotes (fertilized eggs) released was examined at selected temperatures (10, 15, 20, 25 and 30°C) with 2,500 lux light intensity and 12L:12D photoperiod. The zygotes were then cultured at 15°C constant water temperature, 2,500 lux light intensity and 14L:10D photoperiod. Development of the zygotes was observed at each cell division and growth stage.

To examine the life cycle of *P. siliquosa*, the zygotes were attached to 1.2 m×20 m laver nets which were placed in a FRP circular water tank (156 cm×62 cm) and stored in a polyethylene-covered chamber to allow light to pass through the ceiling. They were exposed into air for 3 hr twice a day. Water temperature and photoperiod were set to simulate natural conditions. The culture was continued for 2 years and 8 months (from 12 April 1998 to 6 December 2000) from the zygote to the disappearance of the algal body.

To elucidate the regeneration behavior of *P. siliquosa*, the algal bodies were pre-cultured for 2 d under 15°C, 2,500 lux light intensity and 14L:10D photoperiod. Subsequently, explants of 2 mm length (Fig. 4A, B) were obtained from the thallus area of constant width downward from the point of the dichotomous branching at the upper part of each thallus. One liter of 0.45 membrane (Whatman, E298) filtered and autoclaved seawater containing 20 ml PESI solution (Tatewaki, 1966) was used as the basal medium for culture.

To find the optimal condition for adventitious branch formation, culture was made under different conditions such as water temperatures (5, 10, 15, 20 and 25°C), light intensities (500, 1,000, 2,000, 4,000 and 6,000 lux) and salinities (15, 20, 25 and 30 ppt). Four kinds of plant growth regulators - indole-3-acetic acid (IAA), 1-naphthalene acetic acid (NAA), 6-furfurylaminopurine (kinetin) and 2,4-dichlorophenoxyacetic acid (2,4-D) - were used in culture at 7 concentrations (0.01, 0.1, 0.5, 1, 5, 10 and 50 mg/L). For the control, PESI medium without growth regulator was used under 15°C, 2,500 lux light intensity, 14L:10D photoperiod and 30‰. The 100 tissue sections were cultured in 5 identical plates (90×25 mm) containing 20 tissue sections each. The media was renewed every 5 days. The rate of adventitious branch development was expressed as average percentage of 3 repeated experiments, which lasted for the 2-year culture period.

Results

Growth

Quantity of zygotes released after one day culture of *P. siliquosa* was the highest at 25~30°C. On the other hand, its release in the culture at 15~20°C gradually increased over time (Fig. 1). For the 15°C plate in particular, the release of zygotes increased after 3 days in contrast to the other plates, all of which exhibited decreases. In the 30°C plate, a large number of eggs were released immediately, although most of them were unfertilized. Similar trend was observed from the plated cultivated at 25°C. However, the plate yield much less unfertilized eggs. On the other hand, the receptacles did not release any zygotes with a greenish-brown thalli in the 10°C plate. All other plates had yellowish-brown thalli.

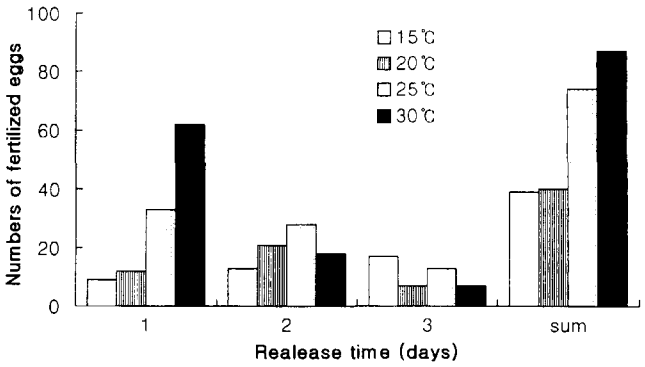

Fig. 1. Effect of temperature on egg production (per g wet weight) of the receptacle of *Pelvetia siliquosa*.

The zygote of *P. siliquosa* (Fig. 2A) is spherical with average diameter of 85 μm. It was polarized within 24 hours after being released from the receptacle. It then divided horizontally into 2 cells, with the upper part dividing vertically (Fig. 2B) and the lower part dividing horizontally. The resulting lower cell elongated to become the primary rhizoid cell. They were then imbedded into the substrate (Fig. 2C). The upper part of the germling divided continuously and developed into a cylinder. On the other hand, the primary rhizoid was branched into many secondary rhizoids (Fig. 2D, E). 5~12 hair strands emerged at the cylinder tip (Fig. 2F). The rhizoids combined to develop into a discoidal holdfast (Fig. 2G), and the cylindrical germling branched dichotomously into young thalli (Fig. 2H). The germling continued to grow with secondary and tertiary branchings. Surface of the mature receptacles of *P. siliquosa* was pitted with extruded conceptacles, each of which had an opening at the center, through which eggs and sperms were released. Strands of 2~5 mm long hair developed around the openings of conceptacles, appearing as white rectangular cells connected in line. Eggs released out of the conceptacle were attatched to the hair strands around the opening before finally precipitating and adhering to the substrate. The

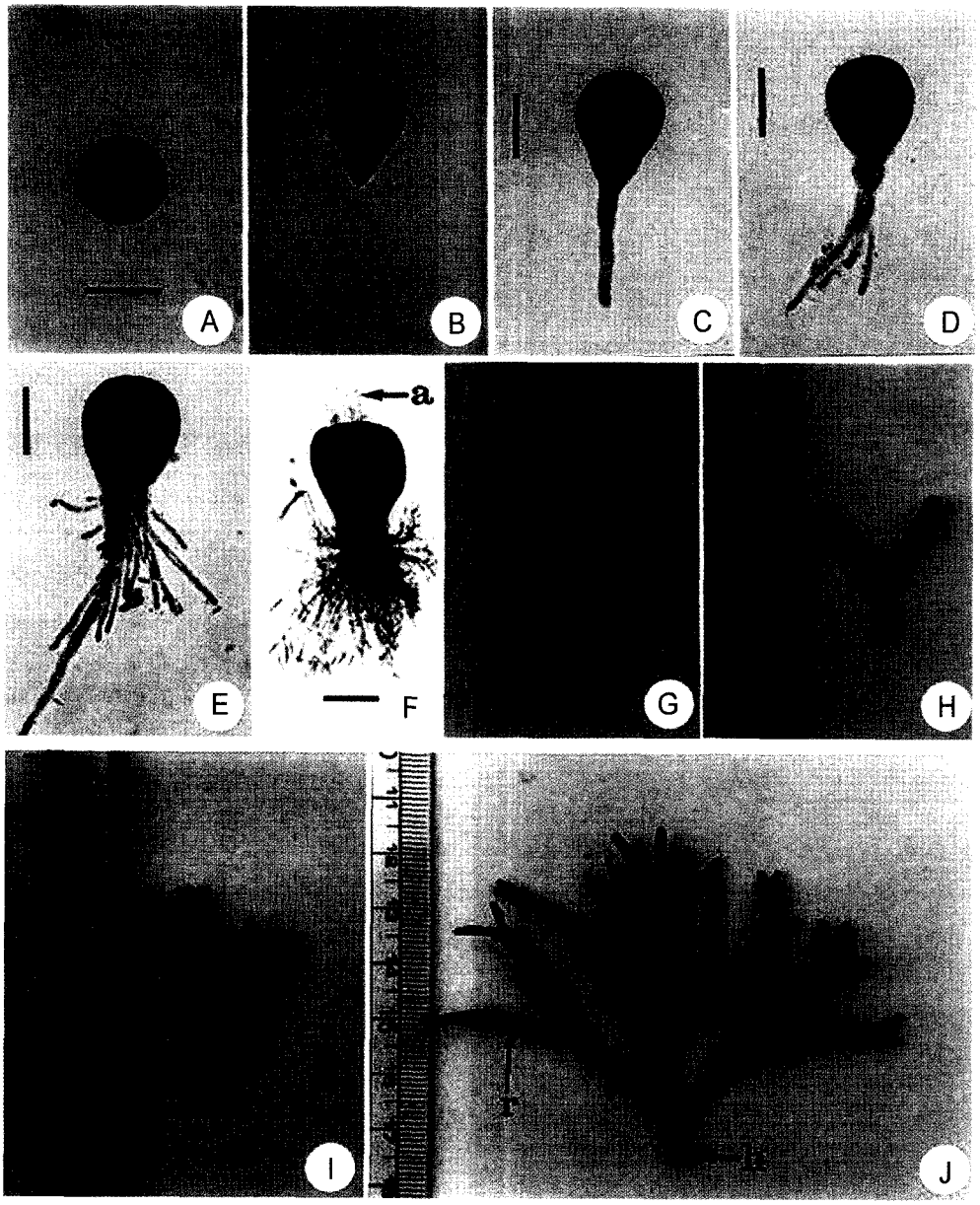

Fig. 2. Growth patterns of *Pelvetia siliquosa*. A. Fertilized egg; B. Germling - the developmental polarity; C. Primary rhizoid germinated from the germling; D. Secondary rhizoids diverged from a primary rhizoid; E. Germling 15 days after germination; F. Germling 20 days after germination; G. Young thallus 60 days after germination dichotomous stipe; H and I. Further development; J. A mature thallus with receptacles. Scale bar: 80 μm for A; 100 μm for B-E; 200 μm for F-G. a. apical hairs; r. receptacle; h. holdfast.

precipitating eggs, that failed to adhere to the substrate, withered up and discolored. On the other hand, the receptacles were formed on all the upper branches of thalli, and matured in accordance with the upper branches development.

The zygotes released in the spring grew rapidly during summer. Receptacles then emerged and matured to release new zygotes in fall. Some matured receptacles were found in winter, however. The zygotes released in summer or fall remained as young thalli during the winter. Then they rapidly grew in the spring of the following year. The receptacles released zygotes fell off the stalk. From February 1999, adventitious branches developed from the thalli in place of the receptacles (Fig. 3A). Biennial thalli grew more rapidly to become 1.5 times bigger than those developed from zygotes. Biennial thalli released zygotes from their receptacles that matured in the spring and summer, with only the main branch at the lower part of the thalli that passed the winter. The triennial thalli that passed the winter were light-

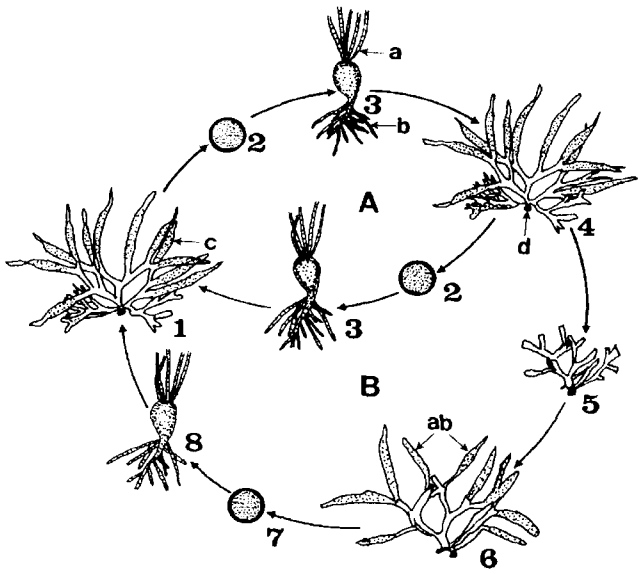

Fig. 3. Life history of *Pelvetia siliquosa*. A. 2 - years life cycle. B. 3 - years life cycle; 1. Thallus; 2. Fertilized egg; 3. Germling; 4. Thallus grown for 2 - years; 5. Thallus for winter; 6. Thallus grown for 3 - years; 7. Fertilized egg; 8. Germling. a. apical hairs; b. rhizoids; c. receptacle; d. holdfast; ab. adventitious branches.

brown and surfaced with pits. The adventitious branches regenerated only partly, and no other dichotomous branches were detected except for the regenerated ones. These triennial thalli broke off from the substrate in the fall or winter, after the zygotes were released from the adventitious branches regenerated at the main branch (Fig. 3B). The thalli matured at unfixed times; zygotes were continuously released from some mature receptacles as the thalli developed. The maturing rate of the thalli reached the peak from June to August.

Formation and regeneration of adventitious branches

Immediately after being cut, the tissue slice of *P. siliquosa* turned greenish-brown at the epidermis, light brown at the endodermis, and colorless at the pith. Upon culturing, however, the epidermis turned dark brown and the pith light brown. Small brown oval cells appeared in the cut portion of the tissue that were cultured for 3 days in the PESI media. After 7 days of culture, new adventitious branches developed at several spots of the wound surface (Fig. 4C). Adventitious branch formation in the sections gradually occurred one by one. After 40 days of culture, only 37 adventitious buds formed; none emerged thereafter. These adventitious buds grew and developed into a cylinder (Fig. 4D). Their development was similar to that of a zygotic germling except that no rhizoids were developeded. The growth of adventitious branches proceeded relatively rapidly compared to that of the germlings from zygotes.

Figure 5 shows the rate of adventitious branch formation from the cultures under different environmental conditions. Temperature-wise, the 15°C and 20°C plates showed 96% and 98% branch formation, respectively. No adventitious branches were formed in the 5°C plate, however. On the other hand, plates condition with light intensity of 2,000 and 4,000 lux plates showed 96% and 94% rates, respectively. Salinity-wise, adventitious branches formed at the rate of 92% at 30 ppt plate. For the plate with less than 15 ppt salinity the body withered to death, while for the 20 ppt plate the growth was weak and sluggish.

Relationship between the rate of adventitious branch formation and the concentration of plant growth regulators is shown in Fig. 6. For the IAA-treated plates, the rate at 0.5 mg/L was the highest after 40 days of culture (98%). It decreased with increasing IAA concentration up to 5 mg/L. At 10 mg/L, however, growth was rather inhibited with the tissue disintegrating after 40 days of culture. Plates treated with 50 mg/L showed tissue sections hardening and turning into reddish brown after 5 days, but no adventitious branch formation was observed. On the other hand, the rate was highest at NAA at 0.5-5 mg/L (98%). Plates with NAA higher than 10 mg/L still showed adventitious branch formation. The tissue sections were dark greenish-brown color and exhibited faster growth compared to the other plates. For the plates treated with 2,4-D, the rate was highest at 0.51 mg/L (91%). Plates with lower concentrations showed rates of 82%, but those with concentration higher than 10 mg/L died. On the other hand, plates with 0.5~5 mg/L kinetin concentrations showed the rates higher than 83% after 30 days of culture. In the plate with 10 mg/L, new adventitious branches developed after 40 days of culture, while the tissue sections in the 50 mg/L plate all withered away after 5 days. In general, for high rate of adventitious branch formation of *P. siliquosa*, 0.5~1 mg/L was the optimal concentration range of the plant growth regulators. NAA could induce adventitious branch development over a wide range of concentration. On the other hand, IAA, 2, 4-D and kinetin exhibited dose-dependent stimulating effects.

Discussion

Early development of *P. siliquosa* was consistent with the results reported by Inoh (1933). He defined the stage, at

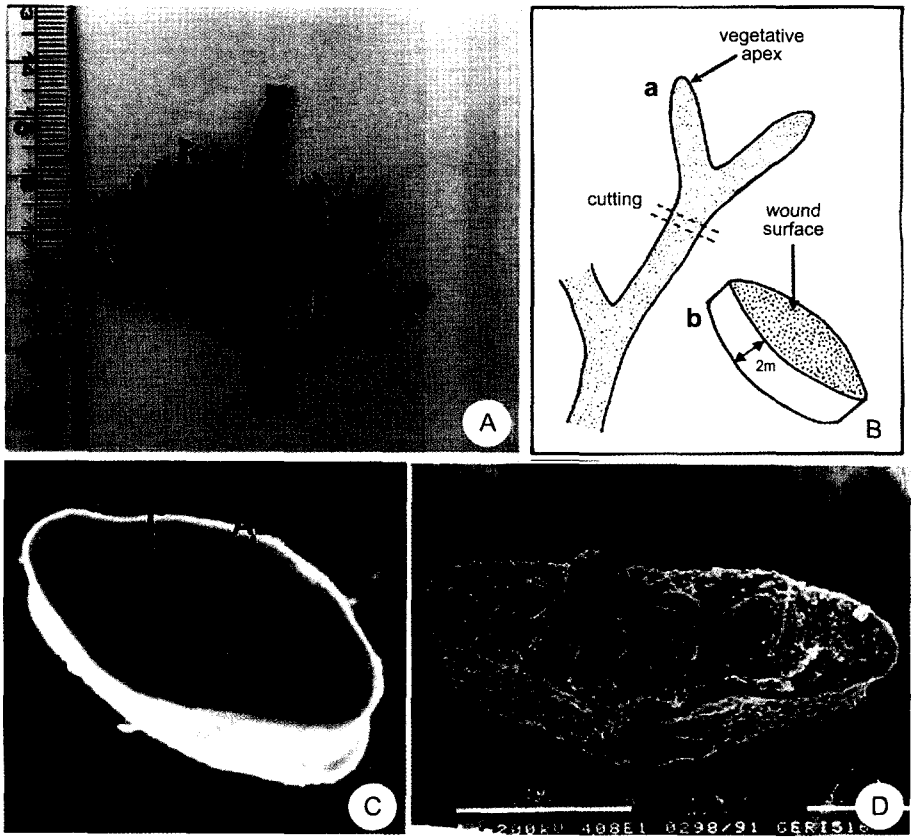

Fig. 4. A. Mature plant of *Pelvetia siliquosa*; B-a. Diagram of a portion on the vegetative thallus of *Pelvetia* indicating the region from which segments were cut for culture (broken line) ×2; B-b. Enlarged drawing of a freshly cut segment showing the wound surface ×4; C. Wound surface (WS) of a segment cultured in PESI medium for 30 days ×20. Notice adventitious branches (AB) germinating from the wounded surface; D. Adventitious branches germinated from the wounded surface of a segment cultured for 40 days.

which the primary rhizoid develops from a zygote and branches into 2 as an embryo. He also reported that an abnormal development of *P. siliquosa* embryo could result in 2 individual rhizoids. Our investigation, however, showed the formation of a normal primary rhizoid that was followed by that of secondary rhizoids. It has been reported that the polarity of Fucales zygotes are determined by: (1) light or UV, (2) centrifuge, (3) electricity, (4) pH, and (5) K-ion (Nakazawa 1969). In this study, the most crutial factor was light intensity.

P. siliquosa zygotes were temporarily attached to the hairs developed around the conceptacle opening before precipitating and adhering to the substrate. Presumably, *P. siliquosa* conceptacle contains both an antheridium and an carpogonium, so sperms and eggs were released from the same opening. Since the eggs released from the oogonium were temporarily attached to the hairs, the sperms from the antheridium could easily be fertilized the eggs. Some eggs that fell onto the substrate discolored and died; apparently, they were all unfertilized eggs.

The experiment on the zygote released at different temperatures indicates that 25°C is the optimum for planting, since a large number of zygotes including a relatively few unfertilized cells were obtained. Such condition is identical to the habitat temperature in July and August. To induce the release of *P. siliquosa* zygotes in large quantities in short time, it was effective to stimulate the growth by drying the mother algae in the air for about 2~3 hrs before releasing zygotes.

Physiological effects of various environmental conditions on regeneration and adventitious branch formation of *P. siliquosa* are as following: the rate of the adventitious branch formation was higher than 96% at 15~20°C, higher than 90% at light intensity of 2,000~6,000 lux and salinity of 30 ppt. Expectedly this new physiological description on the adventitious branch formation from *P. siliquosa* is applicable to the artificial planting.

Rate of the adventitious branch formation by adding plant growth regulators decreased in the order of NAA-IAA-2, 4-D - kinetin at concentrations of 0.5~5 mg/L. IAA at a lower

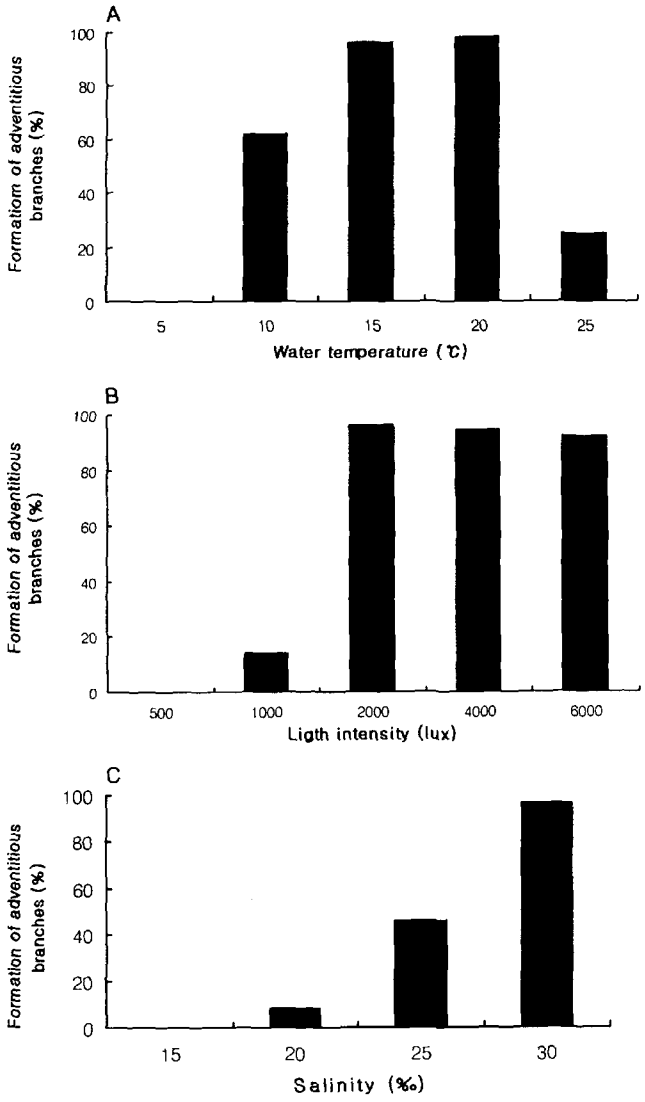

Fig. 5. Advetitious branche formation on segments of *Pelvetia siliquosa* cultured in PESI media under various culture conditions. A. The culture condition of 2,500 lux, 14L:10D, and 30 ppt; B. 15°C, 14L:10D, and 30 ppt; C. 15°C, 2,500 lux, and 14L:10D.

concentration than 1 mg/L, 2, 4-D in a relatively narrow range of concentration from 0.1 to 1 mg/L, NAA and kinetin at a higher concentration than 0.5 mg/L stimulated the adventitious branch formation of the sections. However, IAA and 2,4-D toxically withered the section at concentration higher than 10 mg/L. Therefore, our examination by culturing *P. siliquosa* tissue sections in the media treated with selected plant growth factors showed that the growth and adventitious branch formation differs according to the kind and concentration of the growth regulators.

Our experiments can be applied to increase the natural source of *P. siliquosa* through a good number of adventi-

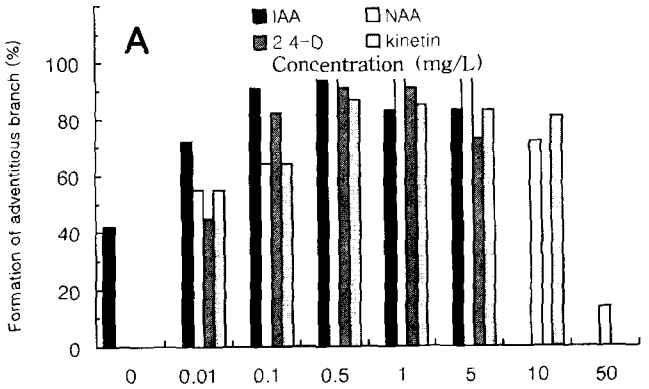

Fig. 6. Formation of adventitious branches on the segments cultured in PESI media containing the different concentrations of IAA, NAA, 2, 4-D and kinetin.

tious branches developed from its small tissues, and new individuals developed from them of the same form with those from zygotes. *P. siliquosa*, which was a dominant species in the intertidal zone of south and west coasts of Korea until 1980, are now rarely found because of the "whitening" phenomena. This situation makes it necessary to use tissue culturing for the source increase and to study the physiological characteristics using plant growth regulators. Although the Korean brown alga *P. siliquosa* is an economical edible seaweed, its extinction is imminent due to the destruction of habitats by reclamation and to the ecological change by the environmental pollution. *P. siliquosa,* as an intertidal alga which is exposed to air, can serve as an indicator of environmental pollution, so the relation should be examined between environmental changes and the growth of *P. siliquosa.*

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