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Tissue culture and the developmental condition of callus from young sporophytes of *Eisenia bicyclis* (Kjellman) Setchell (Laminariales, Phaeophyta)

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Rectangular pieces (1–4 mm^2) excised from young sporophytes (3–5 mm long) of *Eisenia bicyclis* (Kjellman) Setchell were cultured at different temperatures (10–25°C) and light intensities (1000–8000 lux), and the development and differentiation of callus were observed. A line of one or two callus-like cells were produced on the cut edge of explants from the sporophyte after four days in liquid culture. These cells were hemispherical and pigmented only a very little. After three weeks the callus was cut off from the original sporophyte piece and cultured in liquid medium. In about two weeks, a part of the callus cells differentiated and grew up to blade-like plantlets. The growth of callus was optimal at 20°C and 1000 lux. Higher temperatures and light intensities were effective in the differentiation of callus cells to blades.

Key Index Words: callus—development—Eisenia bicyclis—*Laminariales—Phaeophyta—tissue culture—young sporophyte.*

We reported that dedifferentiated young sporophyte cells of *Laminaria japonica* developed into filamentous male and female gametophytes, which produced sporophytes after fertilization (Notoya and Aruga 1990a). We also reported in *Ecklonia cava* that the callus developed spontaneously on the young blade which was formed parthenogenetically from female gametophyte and small cut pieces of normal young sporophyte differentiated to blade-like thalli (Notoya and Aruga 1991a). In these experiments cultures were not axenic, however the culture technique is easy and within a very short period of time the callus was formed from the excised pieces of young sporophytes and the blade differentiated from the callus. In this paper we report the result of cultures of small pieces of tissue cut from young sporophytes in *Eisenia bicyclis*.

Material and Methods

A mature sporophyte of *Eisenia bicyclis* (Kjellman) Setchell was collected at Ohsaki in Zushi, Kanagawa Prefecture, on April 10, 1990. A part of the blade surface with mature sori was cleaned up with a paper towel and excised. The excised pieces of about 4 cm^2 were further cleaned several times by sterilized seawater and a paper towel. They were put into Petri dishes filled with sterilized seawater for an hour. Liberated zoospores were collected by a glass pipet and transferred into a new Petri dish with sterilized seawater. They were washed three times repeatedly in the same manner. The zoospores attached on the slide glass were cultured at 15°C and 4000 lux (14 L : 10 D). Modified Grund medium (McLachlan 1973) was used and renewed every week. From the attached zoospores, gametophytes developed and matured within three weeks. In eight weeks young sporophytes developed to 3–5 mm long (Fig. 1A), from which rectangular blade pieces 1–4 mm^2 were cut out for tissue culture.

The artificial seawater "Jamarin S" (Jamarin Laboratory) enriched with PESI (Tatewaki 1966) was used for culture of the sporophyte pieces. The cultures were incubated at 15°C and 4000 lux (14 L : 10 D). Separated calluses were cultured in 40 m*l* flasks with liquid medium at 15°C and 12000 lux (14 L : 10 D). The culture medium was renewed once a week. The illumination was supplied by cool white fluorescent lamps.

Callus formation was examined at different temperatures (10, 15, 20, 25°C) and light intensities (1000, 2000, 4000, 8000 lux) with a photoperiod of 14 L : 10 D. Eight pieces of 4 mm^2 from the central part of young blades were used. The height of callus was measured with a micrometer under the microscope.

Results and Discussion

Callus-like cells were produced in 1-3 cell lines on the cut edge of a piece of single-cell-layered blade after 4 days in culture (Fig. 1B). They were hemispherical and larger than the original blade cell. They had a few small pigments and were transparent and pale yellow (Fig. 1C). These callus-like cells were similar to the callus cells just before differentiation from explants of large natural plants in *Ecklonia cava* (Notoya and Aruga 1989, 1991a, Notoya 1990), *Eisenia bicyclis* (Notoya and

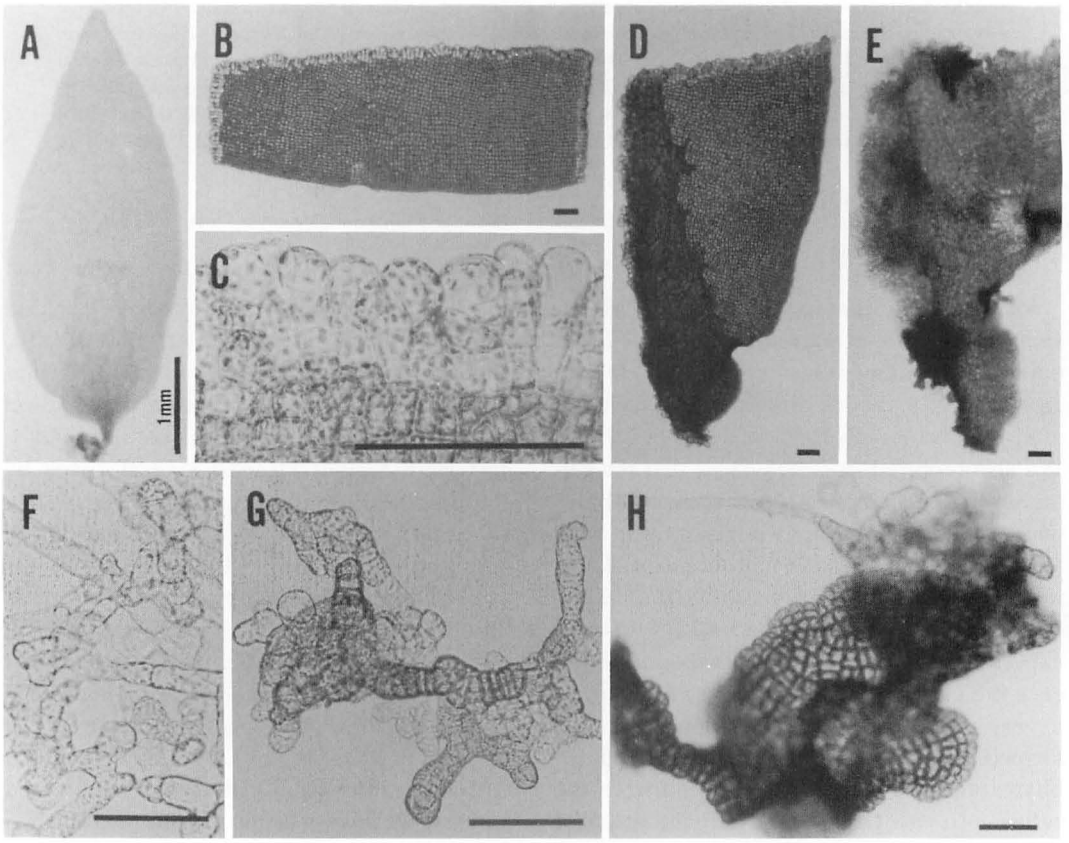

Fig. 1. Tissue culture from young sporophyte explants of *Eisenia bicyclis* (Kjellman) Setchell. (A) A young sporophyte of *Eisenia bicyclis* cultured for three weeks after fertilization at 15°C and 4000 lux (14 L : 10 D). (B) A piece of a single-layered part of young sporophyte after four days in culture. (C) Enlarged callus-like cells developed on the cut edge of the explant. (D) A piece of the lower part of a sporophyte cultured for a week. (E) A piece of the lower part of a sporophyte cultured for three weeks. (F) Mass of callus cells separated from the sporophyte piece and cultured for a week. (G) Primordia of blade-like sporophytes. (H) Developed young sporophytes three weeks after the separation of callus from the sporophyte piece. Bars, 100 μm in (B)–(H).

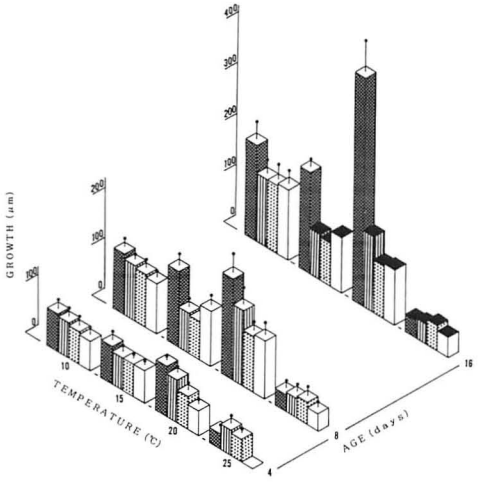

Fig. 2. Growth of callus at different temperatures and light intensities (from left to right 1000, 2000, 4000 and 8000 lux at each temperature). Columns shaded on the top indicate the formation of blade-like sporophytes from callus. Vertical lines for half the standard deviation.

Aruga 1990b), *Laminaria japonica* (Kirihara *et al.* 1991) and *Undaria pinnatifida* (Notoya and Aruga 1991b).

In the lower part of a blade of young sporophyte tissues were partly single- and partly multi-cell-layered. After eight days it was observed that the callus-like cells were arranged in single-cell-layer on the single-cell-layered part and in multi-cell-layer on the multi-cell-layered part of the blade piece (Fig. 1D). After two weeks, massive callus cells were observed to have propagated vigorously from the multi-cell-layered part of the blade, but not many callus cells were produced from the single-cell-layered part of the blade (Fig. 1E). After three weeks, these clumps of callus cells were separated from the original piece of blade explant and transferred to the culture under a high light intensity of 12000 lux (Fig. 1F). Two weeks after separation from the original explant, primordia of blade-like sporophyte differentiated from the callus (Fig. 1G). In addition, irregularly shaped blade-like plantlets with transparent rhizoidal cells were observed on the mass of callus (Fig. 1H).

The growth of callus at various temperatures and light intensities was compared in Fig. 2. After 4 days the height of callus was not greatly different at each light intensity from 10 to 20°C, while at 25°C it was smaller than that at 10-20°C. After eight days, the size of callus increased at 15 and 20°C, especially at 1000 lux. The highest growth of callus was attained at 20°C and 1000 lux after sixteen days. The calluses grew well also at 10°C under all the light intensities and at 15°C and 1000 lux. The differentiation of callus cells to blade-like plantlets was observed at 15°C under 2000-8000 lux, at 20°C under 2000-8000 lux and at 25°C under all the light intensities. Thus, it is clear that the growth of callus was fast at lower temperatures and lower light intensities, and the callus differentiated to blade-like plantlets at higher temperatures and higher light intensities in this species. Similar results were obtained with explants from the natural material in *Eisenia bicyclis* (Notoya and Aruga 1990b).

Two types of differentiation have been reported in the tissue culture from young sporophytes of Laminariales; sporophytes were produced directly from the callus (Saga *et al.* 1978, Notoya and Aruga 1991a) and by fertilization of aposporously formed male and female gametophytes (Notoya and Aruga 1990a). In the present culture experiment of young sporophyte explants in *Eisenia bicyclis* the callus differentiated directly to young sporophytes.

The manner of differentiation of callus cells produced from the young sporophyte explant in the present experiment was similar to that of callus cells produced from the explant of natural materials of *Ecklonia cava* and *Eisenia bicyclis* (Notoya and Aruga 1989, 1990b). In such a way like this the callus can be produced easily and differentiate to sporophytes in a very short period of culture. Thus, it is suggested that the present procedure for tissue culture is useful for seed production of Lamiariales plants.

References

Kirihara, S., Notoya, M. and Aruga, Y. 1991. Tissue

culture from the explant of stipe of *Laminaria japonica* Areschoug (Laminariales, Phaeophyta). Abstracts of papers presented in the Japanese Society of Scientific Fisheries spring meeting: 168. (in Japanese)

McLachlan, J. 1973. Growth media—marine. p. 25–51. *In* J. R. Stein (ed.), Handbook of phycological methods. Cambridge Univ. Press, New York.

Notoya, M. 1990. Tissue culture of macro-seaweeds—Laminariales, Phaeophyta. Kaiyo Monthly **22:** 728–736. (in Japanese)

Notoya, M. and Aruga, Y. 1989. Tissue culture from the explant of *Ecklonia cava* Kjellman (Laminariales, Phaeophyta). Jpn. J. Phycol. **37:** 302–304.

Notoya, M. and Aruga, Y. 1990a. Formation of aposporous gametophyte from dedifferentiated young sporophyte cell of *Laminaria japonica* Areschoug (Laminariales, Phaeophyta). Abstracts of papers presented in the Japanese Society of Scientific Fisheries autumn meeting: 88. (in Japanese)

Notoya, M. and Aruga, Y. 1990b. Tissue culture from the explant of stipe of *Eisenia bicyclis* (Kjellman) Setchell (Laminariales, Phaeophyta). Jpn. J. Phycol. **38:** 387–390.

Notoya, M. and Aruga, Y. 1991a. Tissue culture of parthenogenetic and normal young sporophytes of *Ecklonia cava* Kjellman (Laminariales, Phaeophyta). Jpn. J. Phycol. **39:** 102. (in Japanese)

Notoya, M. and Aruga, Y. 1991b. Tissue culture of *Undaria pinnatifida* (Harvey) Suringar (Laminariales, Phaeophyta). Abstracts of papers presented in the Japanese Society of Scientific Fisheries spring meeting: 168. (in Japanese)

Saga, N., Uchida, T. and Sakai, Y. 1978. Clone *Laminaria* from single isolated cell. Bull. Jap. Soc. Sci. Fish. **44:** 87.

Tatewaki, M. 1966. Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. Phycologia **6:** 62–66.

能登谷正浩・長嶋美香子・有賀祐勝：褐藻アラメ幼胞子体からの組織培養とカルス形成条件

アラメ *Eisenia bicyclis* (Kjellman) Setchell の遊走子を培養して得られた 3–5 mm の幼胞子体の葉片を用いて組織培養を試み，カルスの形成から葉状体への分化を観察するとともに，カルス形成におよぼす温度と照度の影響を調べた。幼胞子体を 1–2 mm 角の葉片に切断して 15°C, 1000 lux, 14 L : 10 D の条件で培養した結果，4 日目に切断面から 1 層の半球状で色素体の少ないカルス様細胞の成長が認められた。培養 3 週間後，直径約 1 mm に成長したカルスの塊を分離して培養を続けたところ，5 週目にはカルス塊の一部に色素体の多い細胞が生じ，それらの細胞は葉状体へ分化した。また，温度 10, 15, 20, 25°C と照度 1000, 2000, 4000, 8000 lux を組み合わせた条件下で幼胞子体葉片を培養してカルスの成長を観察した結果，成長は 20°C, 1000 lux で最も速く，高温，高照度ほど葉状体への分化が早いことが分かった。（108 東京都港区港南4–5–7　東京水産大学藻類学研究室）