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Masahiro Notoya and Yusho Aruga: Tissue culture of parthenogenetic and normal young sporophytes of *Ecklonia cava* Kjellman (Laminariales, Phaeophyta)

Key Index Words: Ecklonia cava—*Laminariales—parthenogenetic sporophyte—Phaeophyta—tissue culture—young sporophyte.*
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Large blades of field materials have usually been used for tissue cultures of Laminariales seaweeds. Techniques of sterilization of explants and the results of cultures were reported by Fries (1980), Fang *et al.* (1983), Saga and Sakai (1983), Yan (1984), Lee (1985), Polne-Fuller *et al.* (1986), Polne-Fuller and Gibor (1987), Notoya (1988), Heather *et al.* (1989) and Notoya and Aruga (1989, 1990).

This paper reports the results of tissue cultures of explants from parthenogenetic and normal young sporophytes of *Ecklonia cava* Kjellman cultured xenically in laboratory.

Male and female gametophytes of *E. cava* were separately cultured at 20°C under a photoperiod of 10 L : 14 D illuminated by cool white fluorescent lamps at 10–30 μmol $m^{-2} s^{-1}$. Parthenogenetic sporophytes were obtained in separate cultures of female gametophytes, whereas normal sporophytes were obtained by fertilization in culture of female gametophytes after being mixed with male gametophytes.

After 16 days parthenogenetic sporophytes grew to 3–5 mm long and callus-like mass of cells were found at the tip of the parthenogenetic sporophyte (Fig. 1A, arrowhead, and B). The cells were very similar to the callus-like cells in tissue cultures from field materials of *Ecklonia cava* (Notoya and Aruga 1989), *Eisenia bicyclis* (Notoya and Aruga 1990) and other species of Laminariales (Lee 1985, Polne-Fuller *et al*, 1986, Polne-Fuller and Gibor 1987). They were unpigmented and spherical, and bigger than the sporophyte cells. A clump of the cells was separated from the parthenosporophyte and cultured in the liquid medium (PESI) at 15°C and 300–320 μmol $m^{-2} s^{-1}$. After two weeks, the clump multiplied and some of the cells became gradually pigmented to pale yellow (Fig. 1C). During the next two weeks, these cells became more pigmented to become brownish and after transverse cell divisions they looked like they were in an early stage of the sporophyte development (Fig. 1D, arrowheads). These pigmented cells developed to blade-like plantlets of irregular shape within 6 weeks after inoculation of the callus-like cells.

In the experiments with normal sporophytes, blades of 3–5 mm long were used which were obtained by fertilization in co-culture of female and male gametophytes for a month under the same conditions as for the gametophyte stock culture.

Rectangular blade tissue fragments of 1–2 mm long were excised from the normal young sporophyte. These fragments were cultured in plastic Petri dishes with the liquid medium at 15°C and 300–320 μmol $m^{-2} s^{-1}$. In 4-day-old culture, newly regenerated callus-like cells were observed on cut margins of the excised tissue (Fig. 1E). The regenerated cells were white in color with only a little pigmentation. One-cell-layered blades developed from the regenerated cells. On the other hand, multi-cell-layered blades developed from the regenerated callus-like clump. After a week in culture, they grew up to about 200 μm high on the cut margin of multi-cell-layered fragment of the original blade (Fig. 1F). Then, a part of the regenerated callus-like clump was separated from the original

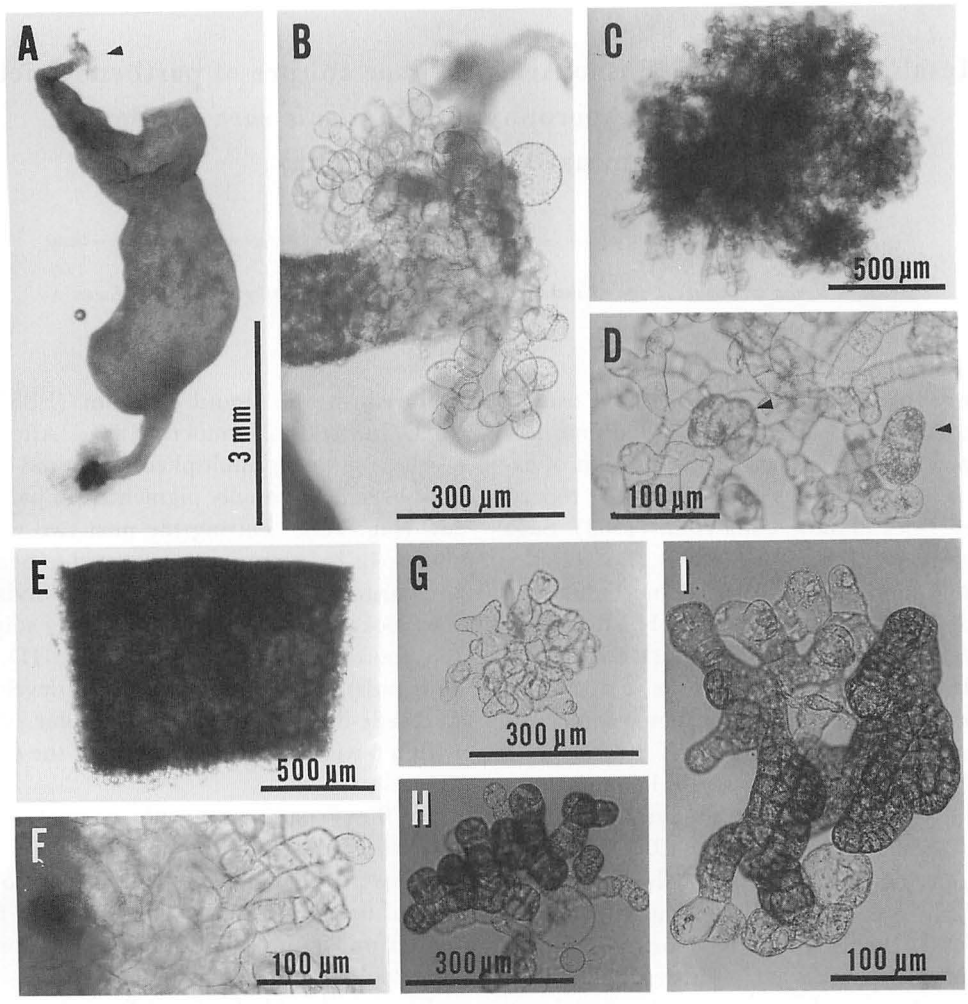

Fig. 1. Tissue culture of parthenogenetic sporophyte (A–D) and normal young sporophyte (E–I) of *Ecklonia cava* Kjellman. (A) Callus-like cells (arrowheads) developed at the tip of the parthenogenetic sporophyte from a female gametophyte. (B) Enlargement of callus-like cells developed on the parthenogenetic sporophyte. (C) A clump of callus-like cells cultured at 15°C and 300–320 μmol m^{-2} s^{-1} in the liquid medium. (D) Initial stage of blade-like plantlet (arrowheads) after transverse cell divisions. (E) A blade fragment from a normal young sporophyte cultured for four days. Regenerated cells appeared from the cut edge. (F) In one-week-old culture, the callus-like cells were seen growing at the cut edge of the blade fragment. (G) A clump of the callus-like cells separated from the sporophyte. (H) In two-week-old culture, the clump of callus-like cells differentiated to a new blade-like plantlet. (I) A more developed irregular plantlet.

sporophyte fragments and cultured under the same conditions (Fig. 1G). In about a week, some cells of the callus-like clump became colored from faint yellow to brown, and in some of these cells transverse divisions were observed similar to the initial stage in the development of the sporophyte (Fig. 1G). Irregular sporophyte-like plantlets were formed from these cells in a week (Fig. 1H & I).

In this experiment, the excised tissues from normal as well as from parthenogenetic young sporophytes produced the callus-like cells. Their growth rate was very fast as compared with that of the excised tissue from large natural material. In general, it takes about one year or more for tissues from natural material to differentiate to sporophytic plantlets, whereas for tissues from cultured young sporophytes it

takes only 1-1.5 months to develop to the sporophytic plantlets. The regeneration ability of cultured young sporophyte tissues would be useful for micropropagation of this species.

Although the sporophytic plantlets obtained from parthenogenetic tissues were not cytologically examined, it is clear that callus-like cells from the excised tissues differentiated directly to the sporophytic plantlet without formation of male and female gametophytes. These results were the same as those in tissue cultures from large natural materials of *Ecklonia cava* and *Eisenia bicyclis* (Notoya and Aruga 1989, 1990).

Unpigmented cells of the callus-like tissue became pigmented and differentiated to the sporophytic plantlet at a high light intensity of 300-320 μmol m^{-2} s^{-1} in the same manner as in the tissue culture of *Eisenia bicyclis* (Notoya and Aruga 1990). Thus, the high light intensity seems to be an important factor for differentiation of unpigmented callus cells to pigmented cells.

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能登谷正浩・有賀祐勝：カジメの単為発生体および幼胞子体からの組織培養

カジメの雌配偶体からの単為発生体（葉長 3–5 mm）を培養していたところ，葉状体先端部に色素体の少ないカルス様細胞塊を形成した藻体が認められた。このカルス様細胞塊を分離・培養した結果，6 週間後には一部の細胞に色素体の増加が認められ，急速に細胞分裂を繰り返して不定形の葉状体へと分化した。また，雌雄配偶体を用いて受精させ，生じた幼胞子体（葉長 3–5 mm）の葉状部を切断して 0.5–1 mm の長方形葉片とし，培養すると，葉片の切断面から色素体の少ない細胞が生じて次第にカルス様細胞塊を形成した。このカルス様細胞塊を分離・培養したところ，不定形の葉状体へと分化した。これらの組織培養に要した期間は 4 – 6 週間で，天然の大型の藻体から組織培養した場合に比べて非常に速く葉状体へ分化させることができた。従って，このような微小藻体を用いる組織培養はコンブ目植物のクローン増殖に有効であると考えられる。（108 東京都港区港南4–5–7 東京水産大学藻類学研究室）