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Masahiro Notoya and Yusho Aruga: Tissue culture of *Undaria pinnatifida* (Harvey) Suringar (Laminariales, Phaeophyta)

Key Index Words: blade midrib—Laminariales—Phaeophyta—stipe—tissue culture—Undaria pinnatifida.

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There have been two reports of tissue culture in *Undaria pinnatifida* (Fang *et al.* 1983, Yan 1984), in which parts of blade and stipe were cultured in the medium containing a synthetic plant growth regulator, sodium naphthenate, and sporophytes were formed directly from the cultured tissues. We reported results of tissue cultures in three species of Laminariales, *Ecklonia stolonifera* (Notoya 1988), *Ecklonia cava* (Notoya and Aruga 1989) and *Eisenia bicyclis* (Notoya and Aruga 1990). In *Ecklonia cava* and *Eisenia bicyclis* bladelets were formed directly from cultured tissues in the medium without any synthetic plant growth regulators. In this paper we report the result of tissue culture from the meristematic zone of stipe of *U. pinnatifida*, in which male and female gametophyte-like filamentous thalli and bladelets were directly formed from the cultured tissues in the medium without any synthetic plant growth regulators.

A young sporophyte about 20 cm in blade length of *U. pinnatifida* was collected at Banda, Chiba Prefecture, on January 11, 1990. The surface of midrib, blade and stipe was cleaned with paper towels. Tissues for culture were excised from various parts of the sporophyte (Fig. 1). The sterilizing procedures of the explants were the same as described in a previous report (Notoya 1988).

Solid (1.5% bacto-agar, Difco Laboratories) and liquid (artificial seawater "Jamarin S", Jamarin Laboratory) culture media enriched with PESI (Tatewaki 1966) were used. The cultures on solid media in 60 × 10 mm Petri dishes were incubated at 15°C and 1000 lux. The cultures in liquid media in 40 ml culture flasks were incubated at 15°C and 12000 lux. The illumination was supplied by cool white fluorescent lamps under a photoperiod of 14 L : 10 D. The solid medium was not renewed until tissues were transferred into liquid medium, which was renewed at two-week intervals.

Tissues from various parts of the sporophyte (Fig. 1) were cultured for regeneration of cells on the solid medium for a month. The tissues were greenish brown on the solid medium within a day after sterilized, and thereafter they gradually changed to brown. Color of the tissues from blade (E and F) did not change to brown within a month on the solid medium and their cultures were taken

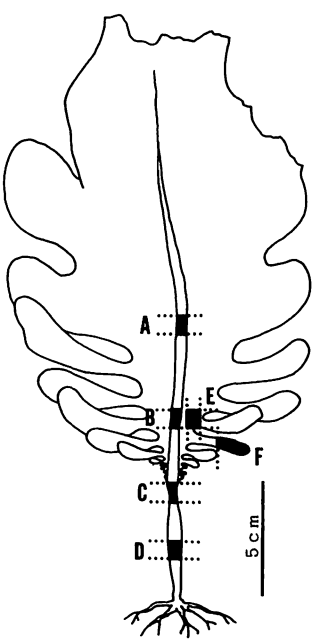

Fig. 1. A young sporophyte of *Undaria pinnatifida* (Harvey) Suringar showing the excised portions of thallus in this experiment. A and B, midrib; C and D, stipe; E and F, blade.

off. The tissues from midrib (A) and stipe (D) were contaminated with endophytic fungi and bacteria. Good outgrowth of regenerated cells was observed on the explants both from midrib (B) and stipe (C). In these explants regenerated cells grew out from cut portions (Fig. 2, A). These outgrown cells were round with callus-like structure and unlike the filamentous structure as observed in *Ecklonia stolonifera*, *Ecklonia cava* and *Eisenia bicyclis* (Notoya 1988, Notoya and Aruga 1989, 1990).

After a month, the outgrown tissues were cut in small pieces and transferred into culture flasks with the liquid medium at 15°C and 12000 lux. After a month, the tissues produced narrow filamentous thalli, the cells of which had many chloroplasts and were brown (Fig. 2, B). When the tissues with filamentous thalli were maintained for

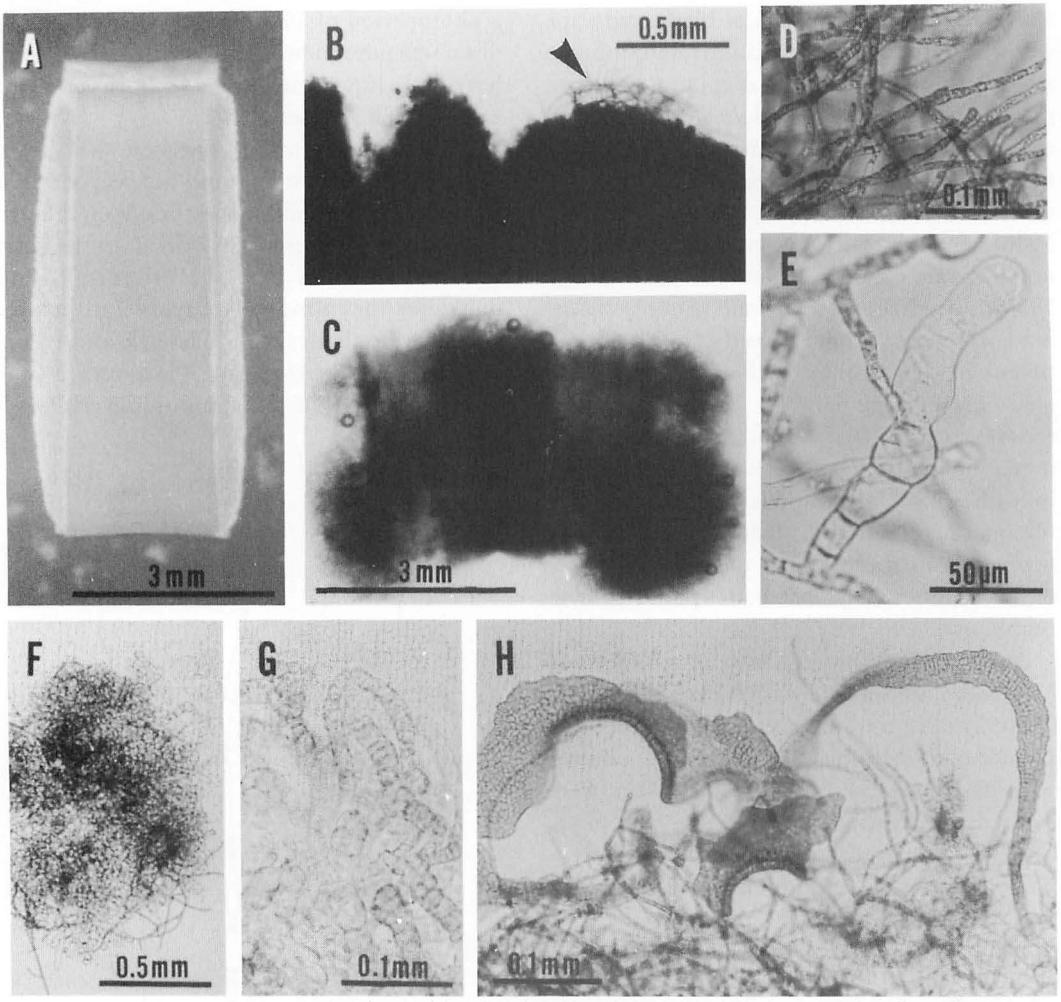

Fig. 2. Tissue culture from the stipe explant of *Undaria pinnatifida* (Harvey) Suringar. (A) Callus cells grown out at the cut end of the explant from stipe after a month on agar plate. (B) An enlarged part of initial filamentous thalli (arrowhead) developed from outgrown callus in the liquid medium after a month. (C) Further developed filamentous thalli clustered around the explant tissue two months after transferred in the liquid medium. (D) An enlarged part of male gameophyte-like filaments. (E) Female gametophyte-like filament developed from male gametophyte-like filament. (F) Mass of unpigmented callus developed from female gametophyte-like filaments one and half months after separated from the explant tissue. (G) Initial growth of bladelets. (H) Developed bladelets after four months in the liquid medium.

another month in the liquid medium under the same conditions, the filamentous thalli progressively grew and covered the whole tissue (Fig. 2, C). Color and shape of the filamentous thalli (Fig. 2, D) were very similar to those of the male gametophyte of *Undaria pinnatifida*. The clustered filamentous thalli were removed to the new liquid medium in a flask. After two weeks, a part of them attached to the bottom of flask. Subsequently, broad and unpigmented filaments were produced from the narrow filaments (Fig. 2, E). Although the shape of narrow pigmented or broad unpigmented filaments was similar respectively to the male or female gametophyte, they did not produce spermatangia or eggs. After a month, broad unpigmented filaments grew gradually into masses about 0.5–1 mm in diameter (Fig. 2, F). These masses were similar to the callus just before differentiation into bladelets of *Ecklonia cava* and *Eisenia bicyclis* (Notoya and Aruga 1989, 1990). After two weeks, blade-like tissues were observed outgrowing from the masses (Fig. 2, G). After longitudinal or transverse cell divisions occurred in two-week culture, the blade-like tissues became young plantlets like sporophytes (Fig. 2, H).

The two types of differentiation from callus have been reported in the tissue culture of Laminariales plants; the formation of sporophytes directly from callus (Fang *et al.* 1983, Yan 1984, Notoya and Aruga 1989, 1990) and the formation of aposporous male and female gametophytes from which sporophytes were fortmed by fertilization (Fries 1980, Lee 1985). In the present tissue culture from stipe of *Undaria pinnatifida*, however, bladelets differentiated directly from the male and female gametophyte-like filamentous callus without fertilization. This is the third type of differentiation from callus.

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能登谷正浩・有賀祐勝：褐藻ワカメの組織培養

ワカメ *Undaria pinnatifida* (Harvey) Suringar の茎状部，中肋，葉状部を用いて組織培養を行なった。寒天培地上で 15°C・1000 lux (14 L : 10 D) で約 1 か月間培養したところ，中肋および葉状部直下の茎状部からの組織でカルス様細胞の増殖がよく認められたが，糸状のカルス様細胞の増殖は認められなかった。葉状部直下の茎状部の組織から形成されたカルス様組織を切り離し，液体培地に移して 15°C・12000 lux (14 L : 10 D) で培養したところ，雄性配偶体様の糸状体の発出が認められ，更にこの糸状体から雌性配偶体様のカルスが形成された。この雌性配偶体様のカルス細胞は分化して葉状体（胞子体）となった。これまでコンブ目植物の組織培養ではカルスが直接葉状体へ分化する場合と，配偶体に分化した後，受精によって葉状体が形成される場合が知られているが，本研究のワカメの組織培養の結果は，上記 2 型とは異なる第 3 の型である。（108 東京都港区港南4–5–7　東京水産大学藻類学研究室）