

Seed production and cultivation of *Grateloupia turuturu* (Cryptonemiales, Rhodophyta) by callus culture

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Abstract: Application of tissue culture techniques to seed production and cultivation of the red alga *Grateloupia turuturu* Yamada was examined. Callus masses excised from explants of *G. turuturu* exhibited an undifferentiated growth on solid ASP12NTA (1.2% agar) medium supplemented with 0.1 mg/L of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP). Growth of the callus was maintained for over five years by subculture every 2-3 months. The callus cells were able to attach to twines of Nori-net and oyster shell, following which they developed into crusts in PES liquid medium. The seeds were observed from upright development of the crusts. The young thalli grew to 3-5 mm in length after 6-8 weeks in room culture. For cultivation in sea, the Nori-nets were suspended from experimental rafts at Nagasaki and Kochi coast. The thalli grew most rapidly from January to April. After five months growth, the thalli attained a length of approximately which is similar to naturally growing thalli.

Key words: callus, cultivation, *Grateloupia turuturu*, red alga, seed production, Rhodophyta, tissue culture

INTRODUCTION

Many benefits to both basic genetics, physiology and biochemistry have been derived from tissue culture techniques applied to high plants (Green *et al.*, 1987; Vasil and Thorpe, 1994). For similar means, callus induction has been reported from a number of phycocolloid-producing and edible red algae, such as *Pteroladia* (Liu *et al.*, 1987), *Porphyra* (Polne-Fuller and Gibor, 1987; Liu and Kloareg, 1991), *Eucheuma* (Polne-Fuller and Gibor, 1987; Dawes and Koch, 1991), *Gelidium* (Gusev *et al.*, 1987), *Agardhiella* (Bradley and Cheney, 1990), *Grateloupia* (Robaina *et al.*, 1990; Yokoya *et al.*, 1993; Yokoya and Handro 1996; Huang and Fujita, 1997a), *Gracilaria* (Kaczyna and Megnet, 1993) and *Meristotheca* (Huang and Fujita, 1997b).

Some species of *Grateloupia* has been commercially used for "seaweed salad" in Japan. So, there is a great need for increasing their production. Studies for seed production and outdoor cultivation of some species of *Grateloupia* have been reported using the method of spore development in *G. filicina* (Ishikawa, 1984), and regeneration from cut fragments of basal crusts from the carpospores and tetraspores in *G. filicina* (Migita, 1988) and *G. acuminata* (Iima *et al.*, 1995). However, there are no reports on the seed production and cultivation by callus culture techniques in the *Grateloupia* species.

Huang and Fujita (1997a) reported on callus induction and thallus regeneration from axenic explants of *G. turuturu*. This paper reports on the seed production and outdoor cultivation of *G. turuturu*, which is a carrageenan - producing and edible red alga through callus culture.

MATERIAL AND METHODS

Immature thalli of *G. turuturu* were collected from Nagasaki and Ehime coasts of Japan. Detailed methods for the preparation of axenic explants and callus induction were followed as described by Huang and Fujita (1997a). The excised callus masses were cultured on solid ASP12NTA (1.2% agar) medium (Provasoli, 1963), supplemented with 0.1 mg/L of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) at 20°C under a photon flux density of 20 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ with a 12h light: 12h dark cycle. The callus masses were cut into 2-3 mm diameter of approximately (about 10-20 mg in weight) and transferred to fresh medium every 2-3 months for callus growth.

For seed production, the callus masses were cutted into pieces of 50-200 μm using a blender, and then scattered onto a cut Nori-net (1.0 \times 1.0 m) in PES liquid medium (Provasoli, 1968). The callus of about 50 mg fresh weight was used for the one cut Nori-net. Oyster shells also were used for attachment test of callus pieces. After 6-8 weeks of culture, the regenerated thalli (seeds) from the callus pieces attached to the Nori-net were used for cultivation.

The Nori-nets were finally fixed to a frame by polyvinyl chloride resin pipe (1.0 \times 0.5 m) and transferred into the sea. The nets were hung on the experimental rafts in Nomozaki Fisheries Station of the Faculty of Fisheries, Nagasaki University, Nagasaki Pref., southern Japan from November 1996 to January 1997, and Usa Marine Biological Institute of Kochi University, Kochi Pref. eastern Japan at December 1997. The growth of the transferred thalli were observed every month. The callus culture and seed production process is summarized schematically in Fig. 1.

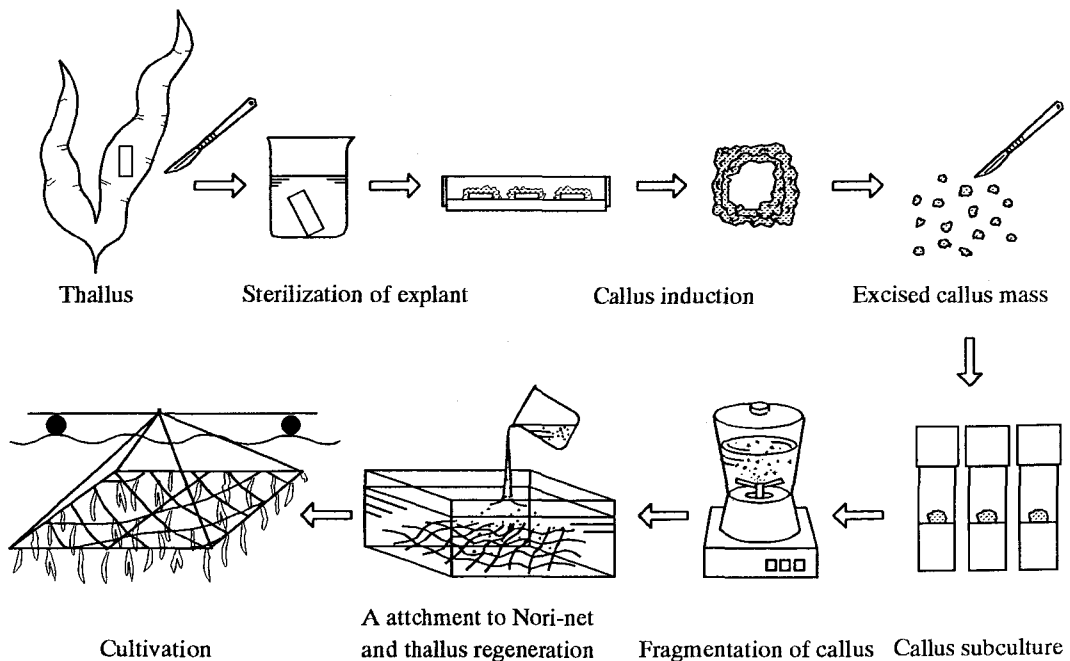


Fig.1. A schematic representation of seed production by the tissue culture technique.

RESULTS

The callus masses of *Grateloupia turuturu* showed irregular growth on the surface of solid ASP12NTA medium (1.2% agar) with the addition of IAA and BAP (0.1 mg/L) (Fig. 2A). The callus cells were initially spherical or oval cell chains of 10-25 μm diameter and brownish red in color (Fig. 2B). In this work, undifferentiated growth of the callus was maintained for over 5

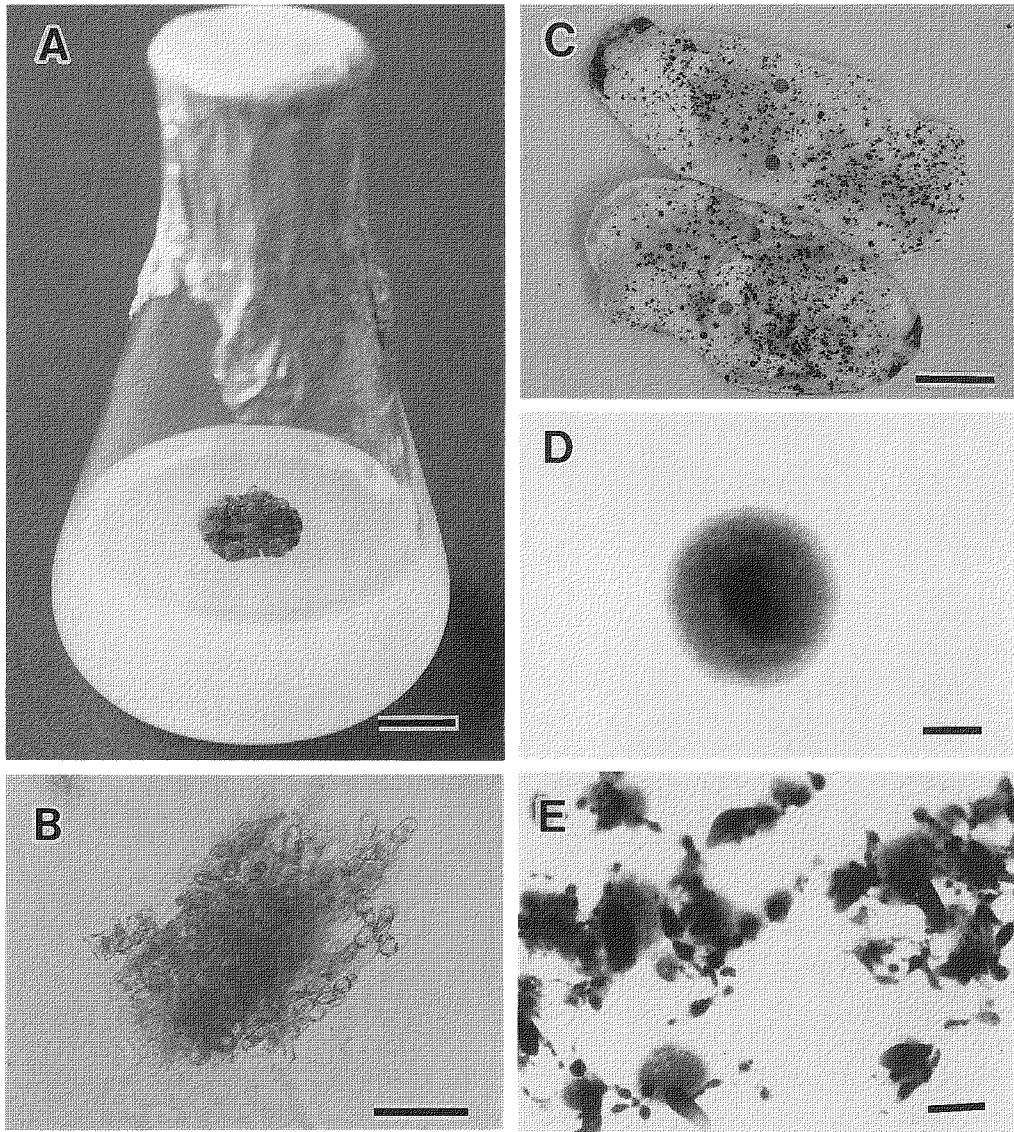


Fig.2. Callus culture, callus attachment to substratum and thallus development from the calli of *Grateloupia turuturu*. (A) A callus mass of *G. turuturu* (about 100 mg in weight) (bar = 1 cm). (B) Callus cells showing spherical or oval cell chains (bar = 100 μm). (C) Attachment to oyster shells and crust formation (bar = 1 cm). (D) A crust from callus cells after 4 weeks in culture (bar = 100 μm). (E) Upright thalli (seeds) development from the crusts on the oyster shell after 8 weeks in culture (bar = 1 mm).

years through subculture at 2-3 months intervals. The callus mass initially weighed about 10 mg and grew to 30-40 mg after 1 month in culture.

The callus pieces showed direct thallus regeneration in PES medium. While crust development much on that which normal spore settlement was observed after the callus cells attachment to twines or oyster shells (Fig. 2C). The crusts showed a rapid growth and reached 300-500 μm in diameter after 4-5 weeks culture (Fig. 2D). After this the upright thalli were been to develop from the surface of the crusts. After 8 weeks, the upright thalli (seed) from the crusts grew up to 3-5 mm in length (Fig. 2E).

In the open sea, the transplanted thalli of *G. turuturu* grew to adult thalli similar to plants from naturally. The thalli showed different growth rates depending on the time of outplanting. The thalli outplanted in November had the maximum growth and it reached 113 cm after 5 months cultivation (Fig. 3). While the thalli outplanted in January did not exceed 25 cm. Some seeds could not develop into adult thalli and died due to overgrowth of epiphytes (e. g. *Ulva* etc.) and animals. Irrespective of the areas of cultivation (Nagasaki or Kochi), thalli grew most rapidly from January to April, and the thalli matured in May (Fig. 4).

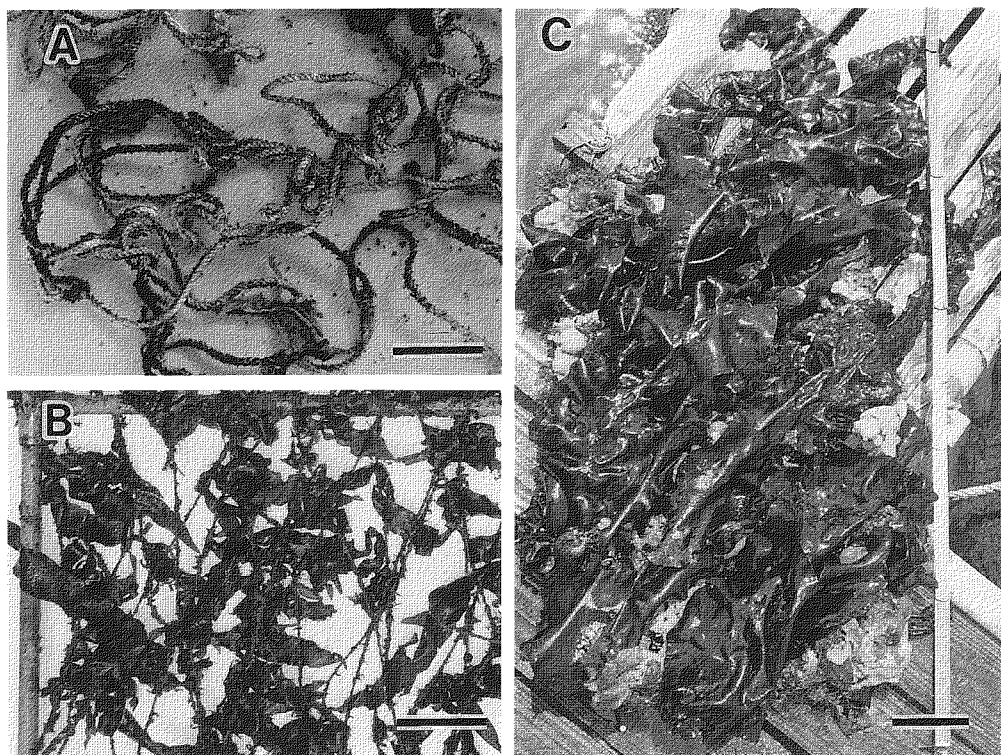


Fig. 3. Results of outdoor cultivation on the Nori-net. (A) Crust formation and upright development on the cut Nori-net after 2 months in culture (bar=1 cm). (B) Cultivated thalli on the Nori-net for 2 months (bar=10 cm). (C) Cultivated thalli after 5 months (bar=10 cm).

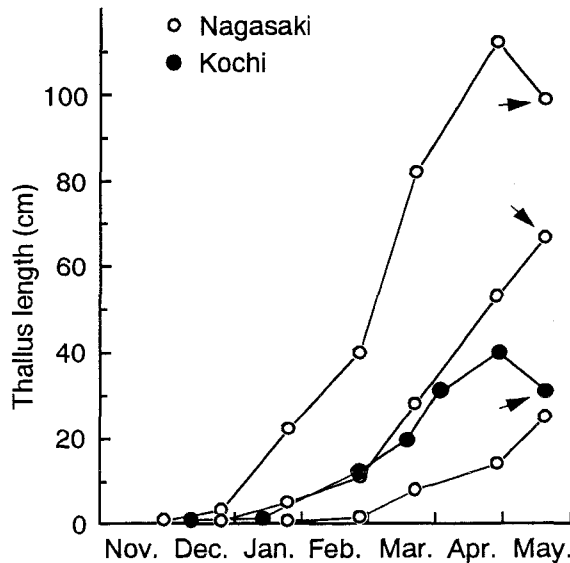


Fig. 4. Growth of cultivation thalli of *G. turuturu* in Nagasaki (1996-1997) and Kochi (1997-1998) (arrow showing thallus maturation).

DISCUSSION

Mass culture of callus application to seed production or to basic use as material for physiological and biochemical purposes is a major problem. Previous knowledge, however, has focused too much on callus induction and plant regeneration, but hardly on the application of callus culture. Among a few exceptions, Liu *et al.* (1987) successfully mass cultured the red alga *Pterocladia capillacea* in PES liquid medium for developmental stages from single cells of callus. In this study, the callus of *G. turuturu* showed undifferentiated growth on an agar medium, and regeneration in liquid medium. In order to apply callus cells to large-scale mass culture, the invention of both more effective culture medium and procedures should be very important.

Another important problem depends on attachment of callus cell at the seed production. Kawashima and Tokuda (1993) reported that callus cells of *Undaria pinnatifida* are attached to a substratum easily (vinyl string), when and regenerated to the frond stage, but the mechanism was not explained. The callus cells of *G. turuturu* are known to form a crust, if a suitable substratum is available. Such a crust is so much strong as comparable with developed spores, and may allow for upright thalli to developed well. If any callus cells of species can not attach to substratum, chemical or physical means may be give them some help for attachment of callus cells.

The thalli of *G. turuturu* showed optimum growth during the period January to April. Earlier operation is more favourite for growth of the thalli. According to available evidence, thalli grew favourably in November outplanting, but not in January. This is apparently from an adverse effect due to epiphytes. Developing young thalli suffered from such factors as the blooming of epiphytes and the increasing of suspended matter in seawate especially from February. This is why they must be reared in the open sea as early as possible to grow up to a suitable size by January. This is effective for reducing the epiphytes and for promoting thallus growth.

Seed production and cultivation can be much more profitable if application of callus culture in some algae, a conventional application of spores, because of the following factors. (1) Callus stage is independent the quality of the mother thalli, maturity of which controls usually results of seedling production in the spore stage. (2) Cultivation may allow seedling production at any desired time, because long-term preservation of callus can be maintained by means of subculture. (3) A considerably short-term production and easy management can be obtained by callus stage. It results from about a 2-month completion of developmental stages from thalli regeneration of the callus. Accordingly, the tissue culture technology should furnish a useful means for masspropagation of some economically important seaweeds.

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