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Tissue culture and regeneration of thallus from callus of *Gelidiella acerosa* (Gelidiales, Rhodophyta)

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The tissue culture of an economically important red alga *Gelidiella acerosa* (Gelidiales, Rhodophyta) included preparation of axenic material, culture of explants, subculture of excised callus and regeneration of *de novo* plants from callus in the laboratory. Sequential treatment of explants with sterile seawater consisting of 0.1% liquid detergent for 10 min, 2% betadine (with 0.5% w/v available iodine) for 5 min and 3.5% broad-spectrum antibiotic mixture with nystatin for 2 days enabled yields as high as 90% of viable and axenic explants. A prolific and rapid growth of filamentous callus on explants was observed on cut surfaces during the first month of culture. The highest level of callus induction occurred in Provasoli enriched seawater (PES) medium solidified with 1.5% agar incubated at 20–22°C and a photon flux density of 5 µmol photons m^{-2} s^{-1} with a 12:12 light–dark photoperiod. Up to 90% of the explants cultured at 5 µmol photons m^{-2} s^{-1} produced callus, whereas at 30 and 70 µmol photons m^{-2} s^{-1}, 70% and 9% produced callus, respectively. The explant culture medium with 0.5% agar content stimulated bud development in all explants, whereas higher agar concentrations (0.8%, 1.0%, 1.5%, 2.0% and 3.0%) resulted in a filamentous type of callus growth. Addition of the plant growth regulators naphthalene acetic acid and indole 3-acetic acid (auxins), and benzyl amino purine and kinetin (cytokinins) and different organic carbon supplements (glycerol, sucrose, sorbitol and mannitol) to the culture medium had no effect on callus growth or induction rate. All carbon supplies at 0.5 and 1.0 M concentration showed an inhibitory effect and most of the explants perished gradually after 2 weeks in culture. The callus mass with bud or shoot developments continued to grow when transferred to semisolid PES medium (0.2% agar w/v) on a rotary shaker. In 4 months, these shoots gave rise to 2–3 cm long plantlets of *G. acerosa*. The tissue-cultured *Gelidiella* germlings were successfully grown into full plants in the field on coral stones in 6 months.

INTRODUCTION

Tissue culture techniques have increasingly been applied to seaweeds because they facilitate development and propagation of genotypes of commercial importance (Dawes & Koch 1991; Dawes *et al.* 1993; Reddy *et al.* 2003). Furthermore, tissue culture systems have also been studied for *in vitro* production of value-added products like tocopherols and tocotrienols (Lawlor *et al.* 1990) and halogenated monoterpenes (Maliakal *et al.* 2001). Nevertheless, the successful application of tissue culture to seaweeds for either genetic improvement or applied phycology studies has been limited and lags far behind that of higher plants. The poor response of seaweeds to tissue culture techniques has largely been attributed to the fact that algal thallus structure lacks the high degree of tissue differentiation found in higher plants (Aguirre-Lipperheide *et al.* 1995). Nevertheless, recent studies on tissue culture of *Kappaphycus alvarezii* (Doty) Doty demonstrated mass production of micropropagules clonally from pigmented filamentous callus through somatic embryogenesis (Reddy *et al.* 2002; Reddy *et al.* 2003). Furthermore, the plants regenerated from tissue culture had a higher growth rate than the cultivated strain. This finding has provided the impetus for intensifying seaweed tissue culture work in our laboratory on other economically important algae to select and propagate possible elite clones as seed stock for commercial farming purpose.

Gelidiella acerosa (Forsskål) Feldmann & Hamel is considered to be one of the more important sources of raw material for industrial agar production (Armisen & Galatas 1987; Roleda *et al.* 1997). In India, *G. acerosa* is the preferred source of raw material for agar production because it has higher yields of high gel strength agar (i.e. > 600 g cm^{-2}; A.K. Siddhanta, personal communication). Annually, 200–300 dry tonnes of *G. acerosa* material is harvested from natural stocks and processed for indigenous agar production (Kaliaperumal & Kalimuthu 1997). Due to the emergence of biotech industries in the country, the demand for agar has increased recently, resulting in the use of other agarophytes [*Gracilaria edulis* (S. Gmelin) P.C. Silva, *G. crassa* Harvey *ex* J. Agardh and *G. folifera* (Forsskål) Børgesen] and has also led to overharvesting of *Gelidiella acerosa* to complement the growing market demands (Kaliaperumal & Kalimuthu 1997). The efforts to restore and conserve the overexploited natural beds of *Gelidiella* have been impeded by its restricted distribution and inherently slow growth and reproduction. Consequently, the cultivation of *Gelidiella* on artificial substrata has been initiated in coastal seawaters with the aim of developing industrially viable indigenous cultivation technology for large-scale farming of *G. acerosa*.

This study was undertaken to help increase the overall pro-

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