

Plantlet regeneration of *Kappaphycus alvarezii* var. adik-adik by tissue culture

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Abstract Three color morphotypes of *Kappaphycus alvarezii* var. adik-adik (brown, green and red) collected from a farming area in Tictauan Is., Zamboanga City, Philippines were used as explants in the study in order to micropropagate ‘new’ plants. Individual sections of sterile *Kappaphycus alvarezii* var. adik-adik, initially cultured in a 48-well culture plate containing ESS/2 + E3 + PGR, released callus cells after 4–5 days of incubation at 23–25°C, 13:11H LD cycle and 10–15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity. True calli were formed after 29–35 days following dense formation of filaments or undifferentiated round cells at the medullary and inner cortical layers of the section. Plantlets (2–3 mm long) of *Kappaphycus alvarezii* var. adik-adik were able to regenerate after 98, 150 and 177 days *in-vitro* among the reds, greens, and browns, respectively. This study established successful methods for the production and regeneration of tissue explants of *Kappaphycus alvarezii* var. adik-adik which can possibly be used to mass produce ‘new’ cultivars for

land- and sea-based nurseries as sources for commercial farming.

Keywords Callus · *Kappaphycus* · Plantlet regeneration · Tissue culture

Introduction

The commercial farming of the different strains of *Kappaphycus alvarezii* and *Euclidean denticulatum* made use of vegetative thalli since its introduction in the early 1970s. The repeated propagation of vegetative thalli of these seaweeds in the Philippines for more than 35 years has brought problems to the industry. These include: (1) declining quality of the harvested crop and poor quality of carrageenan due to the use of inferior cultivar, (2) unstable production due to the persistent occurrence of ‘ice-ice’ and harmful endophytes, (3) poor post-harvest management.

Dawes and Koch (1991) demonstrated the first successful branch micropropagation and tissue culture of *Euclidean denticulatum* and *Kappaphycus alvarezii* from the Philippines using modified enriched ESS (Saga 1986) and combinations of auxins and cytokinins as plant growth regulators. The results of this study were further substantiated in Dawes et al. (1993, 1994), wherein the same cultivars were tested for growth under laboratory and field conditions. The results of their study showed promising results in sourcing ‘seedstocks’ for commercial farming, and as further demonstrated by the works of Reddy et al. (2000, 2003) in *K. alvarezii* and Hurtado and Cheney (2003) in *E. denticulatum*.

Seaweed production in the Philippines, notably *Kappaphycus*, plateaued from 2000 to 2004 and started to decline in 2005 (Personal Comm., Neish) due to the reasons stated

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above plus the occurrence of typhoons in non-traditional farming areas. If no remedial measures are undertaken by the industry and the concerned government and non-government agencies, the production of the Philippines will be overtaken by the neighboring countries like Indonesia and Malaysia in the next 5–10 years.

The study was undertaken to micropropagate *Kappaphycus alvarezii* var. *adik-adik* using tissue culture to develop by mass production ‘new’ plants as source of cultivars for land and sea-based nursery purposes and consequently for commercial farming.

Materials and methods

Kappaphycus alvarezii var. *adik-adik* (brown, green and red) specimens were collected from a farming area in Tictauan Is., Zamboanga City, Philippines (6°9' N latitude and 123°2' E longitude) and used as explants in the study. The explants were acclimatized in tanks for three days prior to the selection of apical segments. Selected apical segments, 3 cm long were cut using a sterile blade, wiped, shaken with

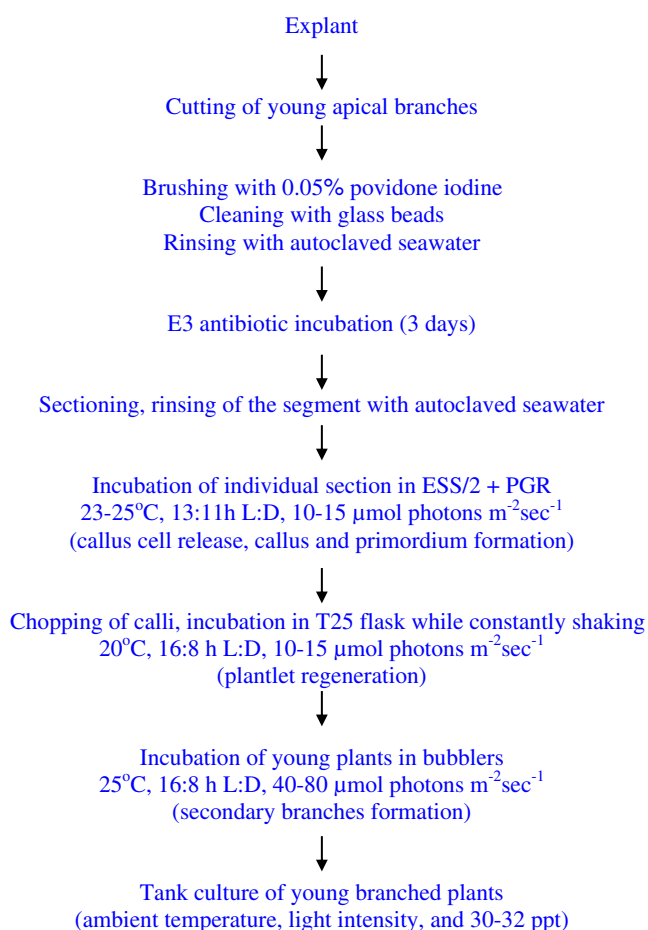


Fig. 1 Schematic diagram of the tissue culture procedure

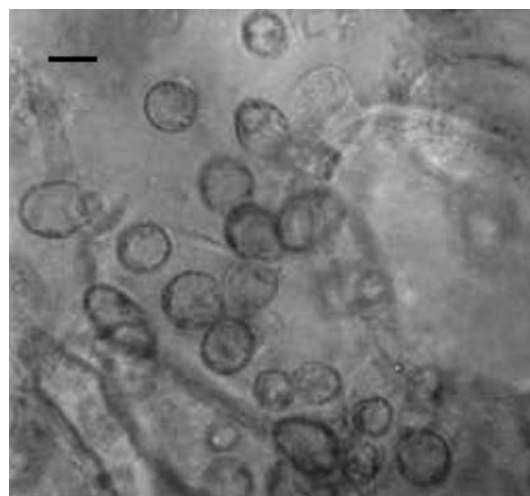


Fig. 2 Newly released cells (bar=20 μm)

glass beads (1 mm size) brushed with 0.05% povidone iodine using soft camel hair brush, and finally rinsed 3–4 times in autoclaved seawater. The segments were placed in an E3 antibiotic solution and incubated for three days at 23–25°C, 13:11h L:D cycle and 10–15 μmol photons m⁻² s⁻¹ light intensity. The segment was cut into sections 2 mm thick, rinsed 3–4× with autoclaved seawater, individually placed in a 48-well culture plate containing ESS/2 + E3 + PGR (PAA + zeatin at 1 mgL⁻¹) and incubated at 23–25°C, 13:11h L:D cycle and 10–15 μmol photons m⁻² sec⁻¹ light intensity. Figure 1 shows the procedure in the tissue culture process.

Results

The release of new cells (Fig. 2) from the cut surfaces was observed 2–3 days after the section when grown with ESS/

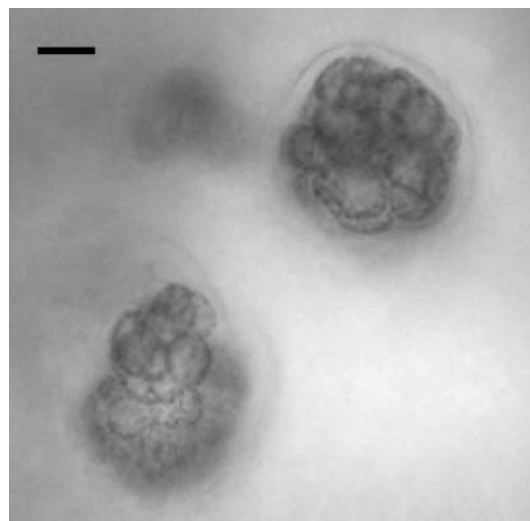


Fig. 3 Ball formation of meristematic cells (bar=2 μm)

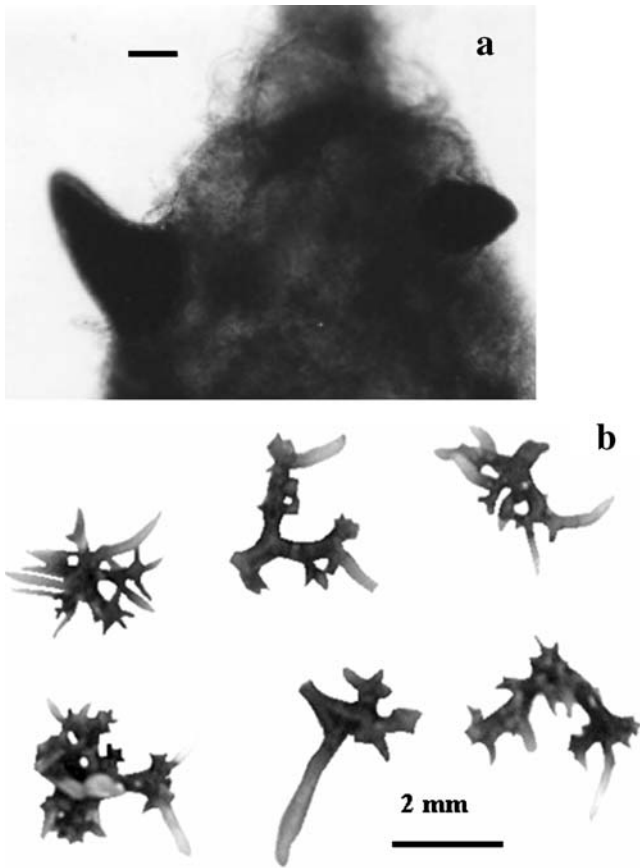


Fig. 4 a, b Newly emerged shoots (a) and very young shoots developed from calli (b)

2 + E3 + PGR at 25°C, 10 $\mu\text{mol photons m}^{-2}\text{sec}^{-1}$, 13:11h L:D cycle. Cell division was either anticlinal or periclinal, leading to the formation of filamentous or meristematic cells (Fig. 3). Callus formation was observed, originating

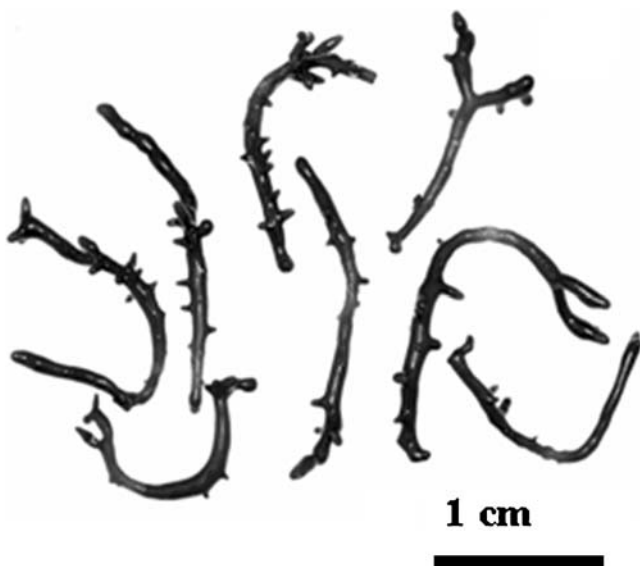


Fig. 5 Very young plantlets of *K. alvarezii* var. *adik-adik* with emerging secondary branches

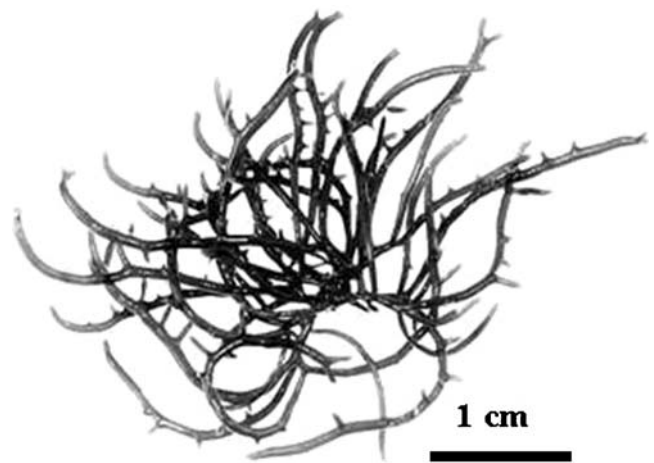


Fig. 6 Young plantlets of *K. alvarezii* var. *adik-adik* with secondary branches

from the medullary layer after 25–30 days. Chopping the calli into fine pieces and placing them in a T25 plastic culture flask containing 25 ml ESS/2 + E3 + PGR and incubated at 20°C, 5 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 13:11h L:D cycle in rotary shaker led to the formation of a shoot primordium. Further growth of the shoot primordium led to the formation of young shoots (Fig. 4a). These young shoots were cut individually and cultured in bubblers at 25°C, 40–60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 13:11h L:D cycle. After one week of culture, the emergence of secondary branches was observed (Fig. 4b). Further growth of these young plants under the same conditions led to the formation of tertiary branches, so that a ‘new’ young plant of *Kappaphycus alvarezii* var. *adik-adik* (Fig. 5) was observed. These ‘new’ young plants originating from the same callus were placed inside a net bag (38 cm \times 32 cm), hanged from a bamboo support inside a concrete tank under ambient light at 32 ppt, and 24 h aeration conditions. After 3–4 weeks, the ‘new’ plants were hanged individually from a bamboo support for further growth (Fig. 6). A month after, robust plants similar to the plants being commercially farmed were obtained (Fig. 7).



Fig. 7 Young plant of *K. alvarezii* var. *adik-adik* resembling the commercially farmed variety

Discussion

The present study demonstrated techniques for the production of ‘new’ plants of *Kappaphycus alvarezii* var. *adik-adik* by tissue culture through the regeneration of a true callus. Most of the callus appeared to develop from the medullary tissue, as observed in previous studies like *Kappaphycus alvarezii* (Dawes and Koch 1991; Wang 1993), *Euचेuma* (Hurtado and Cheney 2003) and other carreegonphytes like *Chondrus crispus* (Chen and Taylor 1978) and *Agardhiella subulata* (Bradley and Cheney 1990; Huang et al. 1998). The work of Reddy et al. (2003) on *Kappahycus alvarezii* demonstrated the regeneration of somatic embryos to whole plants from a pigmented uniseriate filamentous callus, similar to the results obtained by Cheney et al. (1987) in *Agardhiella subulata* and Polne-Fuller and Gibor (1986, 1987) in two species of *Euचेuma*.

Plant growth regulators (PGR) in the form of auxins and cytokinins induce callus development in higher plants (Reinert and Bajaj 1977), but this was refuted by Polne-Fuller and Gibor (1987), since their work did not affect growth morphology in any noticeable way. The findings of Dawes and Koch (1991), Bradley (1991), and Bradley and Cheney (1990), however, proved otherwise. The right combination of auxin and cytokinin (type and concentration) to be used is critical in callus induction and development (Dawes and Koch 1991).

The PGR used in the present study was a result of an earlier study reported by Bradley (1991); Bradley and Cheney (1990). The combination of PAA and zeatin at 1 mgL^{-1} proved to be sufficient in callus induction and development, and consequently for plantlet regeneration as demonstrated in *Euचेuma denticulatum* (Hurtado and Cheney 2003).

In order to obtain a greater number of ‘new’ plants, established calli are chopped into fine sections (Dawes and Koch 1991; Hurtado and Cheney 2003); however, Wang (1993) claimed that he obtained a greater number of new plants from subcultures of chopped stock pieces than from shoots.

Methods of tissue culture described in the present study are useful tools for the production of ‘new’ plants that can serve as propagules in land and sea-based nursery and consequently for commercial farming. The concept of culturing the newly regenerated plantlets in bioreactors to

mass produce individual plantlets in the future is worth exploring (Rorrer and Cheney 2004).

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