

IN VITRO GROWTH RATE OF *Kappaphycus alvarezii* MICROPROPAGULE AND EMBRYO BY ENRICHMENT MEDIUM WITH SEAWEED EXTRACT

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ABSTRACT

The development of micropropagule and embryo of seaweed depend on nutrient and fertilizer used. Seaweed has been reported contain hormone regulators such as auxine, cytokinine, gibberelins, and various minerals applied in stimulating the growth oca plant and wheat culture. The objectives of this study were to determine the potential of *Kappaphycus alvarezii* extract and its optimal concentration in accelerating of *Kappaphycus alvarezii* micropropagule and embryo growth. Micropropagule and embryo produced through callus induction were planted into PES 1/20 liquid medium supplemented with seaweed extract at the concentrations of 0 (control), 25, 50, 75, and 100 µL in 10 mL of medium. The results showed that medium enrichment with 50 µL of seaweed extract had the highest survival rate and growth of thallus. In addition, this concentration was also resulted in a good performance of *K. alvarezii* thallus with the lighter color. The advantage of this study for seaweed cultivation in Indonesia, among others, seaweed can be used as fertilizer, especially in the maintenance of seaweed seed, so that cultivation can be better develop.

KEYWORDS: callus induction, filamentous callus, micropropagule, embryo, *Kappaphycus alvarezii*

INTRODUCTION

Improvement the genetic quality of seaweed particularly *Kappaphycus alvarezii* has been demonstrated by using several methods such as hybrid izationin protoplasts or spores (Cheney & Emily, 1986) and genetic engineering carrageenan controller gene transfer or growth gene inserted in thallus, organ or embryo through mediation with agrobacterium (Hallman, 2007). The use of embryo as a medium for gene transfer is easier than the use of thallus or organ because it is a living cell resulted from callus induction and somatic embryo (Reddy *et al.*, 2003). Gene transfer to the embryo can be implemented by using electroporator or gene pulser (Rajamuddin *et al.*, 2010). Several promoters have been succesfully used in genetic engineering of seaweed such as cauliflower mosaic virus (CaMV), *cytomegalovirus* (CMV) and *green fluorescent protein* (GFP). Callus induction is one tehniques for finding out of seaweed embryo *in vitro*. The embryo development to be seedlings with callus induction through several phases and stages in which at the early stage is induction of explant grown to

produce filaments. The callus induction method has been used by Reddy *et al.* (2003) on *Kappaphycus alvarezii* using a mixture of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) with several comparisons in culture medium enriched with *Provasoli's enriched Seawater* (PES) 1/20. Moreover, Suryati *et al.* (2006) have demonstrated callus induction and embryo by using Conwy medium enriched with plant growth regulators (PGR) of a combination of IAA and kinetinas the inductor. Embryos produced by using these media have been rudimentary, in which filaments produced have been not able to develop to be propagules and seedlings completely. Therefore, it is needed hormone regulators which can stimulate the development filaments to be intact and perfect seedlings.

Seaweed particularly *Kappaphycus alvarezii* has been reported contain PGR such as IAA, gyberelin GA3, kinetin, and zeatin, suspected to has the ability in accelerating of thallus forming on seaweed embryo (Prasad *et al.*, 2010). PGR isolated from seaweed extract were used in the multiplication of oca plant and wheat culture (Zodape *et al.*, 2008; 2009). In this study, the potential of *K. alvarezii* extract as growth regulator in accelerating the growth of *K. alvarezii* filaments and embryo was investigated.

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MATERIALS AND METHODS

Seaweed Extract Preparation

Seaweed, *Kappaphycus alvarezii* used in this study was collected from Takalar Regency, South Sulawesi. Seaweeds were cleaned from dirt and epiphyte before washing with fresh water. The dirt was gently cleaned using a soft brush. Clean and salt free seaweeds were then ground to be slurry form and extracted with 90% methanol for 3 x 24 hours. Extract obtained was evaporated by using a rotary evaporator (Eyela type) to get dried methanol extract. The methanol extract was added with HCL 0.1 N to reach pH 2.5 and partitioned with ethyl acetate to yield ethyl acetate extract containing plant growth stimulators (PGR) of cytokinins and gibberelins derivative (Chapman & Chapman, 1980). Ethyl acetate extract resulted was then evaporated until reach 1 mL ethyl acetate extract equivalent to 100 g fresh weight.

Collection of Materials and Explant Preparation for Callus Induction

Kappaphycus alvarezii was collected from the farmer's in Barru Regency. The sample was stored in the container covered by cloth moistened with sea water for further analysis. The analysis was conducted in the tissue culture laboratory of Research and Development Institute for Coastal Aquaculture (RDICA) Maros, South Sulawesi. The healthy and clean *Kappaphycus alvarezii* thallus were selected and cut with the size of 5 cm to be explants. The explants were then washed with sea water filtered with membrane filter and cultured for two weeks in sterile sea water enriched with PES 1/20 by using a shaker (IKA WERKE) at temperature of 22°C-25°C and illuminated with fluorescent lamps at 1,500 lux of light intensity with a 12:12 hours light and dark cycle. During two weeks of rearing time period, 10 mg/L GeO₂ was mixed into culture medium to remove diatoms. The cultured explants for callus induction were sterilized by using surface sterilization method as described by Polne & Gibor (1984) and Huang & Fujita (1997) with some modification. The explants were gently cleaned using a soft brush under a stereoscopic microscope. The explants sterilization was carried out by washing the explants using 0.5% liquid detergent in sterile sea water for 10 minutes. The explants were then treated with 2% betadine in the sterile sea water for three minutes and continued to be sterilized with 3% broad spectrum antibiotic mixture in PES culture medium for two days. To confirm the sterilization, the culture medium was inoculated on agar medium and incubated.

Callus Induction

The sterile explants were washed with sterile sea water and cut into pieces with 4-5 mm length. Each explant was wiped gently on sterile filter papers to remove moisture and any mucilaginous substances exuded from the cut ends. Dried explants were planted on 20 mL of 0.8% (w/v) Bacto-Agar-solidified PES 1/20 medium enriched with seaweed extract at the concentrations of 0 (control), 25, 50, 75, and 100 µL with the density of 15 explants/petri dish and cultured in the glass cupboard at the temperature of 20°C using a neon lamp at 1,500 lux with photoperiod of dark and light (12 hours). Each treatment was applied with 10 replications. After 30 days of callus induction, the transparent filaments and brown filaments formed on the explant were counted their number and growth. Subsequently, all callus out growth were excised from the explants and subcultured separately on 20 mL of 0.5% (w/v) Bacto-Agar-solidified PES 1/20 medium enriched with seaweed extract at the concentrations of 0, 25, 50, 75, and 100 µL. After 30 days of rearing time periods, all callus separately cultured resulted in micropropagules. During the rearing of callus into micropropagules, the development of filamentous callus was observed under a microscope (Bausch & Lomb).

Embryo Differentiation

Embryo differentiation was carried out by subculturing of micropropagules produced into PES 1/20 liquid medium supplemented with seaweed extract containing plant growth regulators (PGR) from sitokinin and gibberelin derivative at the concentration of 0 (control), 25, 50, 75, and 100 µL. The cultures were shaken with a shaker for 60 days at room temperature of 22°C-25°C and illuminated with fluorescent lamps at 1,500 lux of light intensity with a 12 hours light and dark cycle. All treatments were carried out in 10 replicates. The micropropagules were reared for 60 days or until yielded thallus with medium change interval of two weeks. The data were collected during the observation such as the condition, mortality, and growth of thallus. The data were analyzed using ANOVA statistical method.

RESULTS AND DISCUSSIONS

To stimulate the differentiation of embryonic seaweed *K. alvarezii* to the talus, in addition used of commercial PGR, in this study also used *K. alvarezii* seaweed extract to stimulate the growth and differentiation of the talus filament to be perfect. Prasad *et al.* (2010) have demonstrated on detection the presence of a class of plant growth regulator auxin and

cytokinin in the seaweed extract. Zodape *et al.* (2008; 2009) have taken advantage of this seaweed extract on okra plants and cultivation of wheat. In addition, some researcher have conducted assessments of fertilizer derived from seaweed *Ascophyllum nodosum*, among others, which can be used as fertilizer in cultivation of seaweed *K. alvarezii* (Loureiro *et al.*, 2010) and to test the animal manure livestock, as well as fertilizer in higher plants (Khan *et al.*, 2011).

At embryonic differentiation of seaweed, seaweed extract was used at concentrations ranging from 0-100 μL containing 1 mL of extract equivalent to 100 grams of fresh weight. The results show that the growth and survival of embryos seaweed *K. alvarezii* on 1/20 PES culture medium enriched with seaweed extracts 50 μL in 10 mL of culture medium had the highest survival of about 82%, while in the control (no addition of extract) had the lowest survival and tend to blanch which eventually die (Figure 1).

Figure 1 shows that the highest survival rate (82%) of *K. alvarezii* embryo was obtain from the concentration of 50 μL in 10 mL culture medium (1 mL extract equivalent with 100 g fresh weight of seaweed), followed by 75, 100, 25, and 0 μL with the survival rate of 60%, 58%, 42%, and 20%, respectively. Compared to the control, (without seaweed extract addition), survival rate exhibited by *K. alvarezii* embryo planted into PES 1/20 medium enriched with 50 μL of seaweed extract was indicated much higher. At the control, survival rate had the lowest value. It was caused by mostly embryo which found tend to blanch and finally they were obtained to be dye. This result indicated that seaweed particularly *K. alvarezii* could be a promising plant growth regulators source for

stimulating the growth and differentiation embryo of *K. alvarezii* to become thallus.

Utilization of seaweed extract *K. alvarezii* as fertilizer in higher plants has been carried out by Zodape *et al.* (2008; 2009), namely the okra plants and cultivation of wheat. Prasad *et al.* (2010) have analyzed and detected the presence of hormones regulating growth in seaweed *K. alvarezii* among others giberellin, cytokinins, and auxin; in addition to a variety of nutrient elements which can be used as fertilizer.

In this study, *K. alvarezii* extract used as plant growth regulators in embryonic *K. alvarezii* into shoots. Shoot length growth of seaweed for observation is shown in Figure 2.

That the embryos planted into PES 1/20 liquid medium supplemented with 50 μL of seaweed extract was showed an optimal growth with the shoot length average of 50 mm after eight weeks of rearing time period (Figure 2). While, the lowest growth was found at the embryos cultured into PES 1/20 liquid medium without medium enrichment with seaweed extract (control) with the shoot length average of 25 mm. The growth rate of embryos planted into the liquid medium enriched with seaweed extract at all concentrations was indicated a similar pattern in which the growth rate drastically increased after a week of rearing time periods and continue to gradually increased after two weeks of rearing time period until eight weeks of rearing time period. The statistical analysis showed that treatment 50 $\mu\text{L/L}$ provides the highest value is 82.30% significantly different from the control (not given the extract) with a value of 21%; and 25 $\mu\text{L/L}$ with a value of 42%, but not significantly different from the treatment 75 $\mu\text{L/L}$ with a

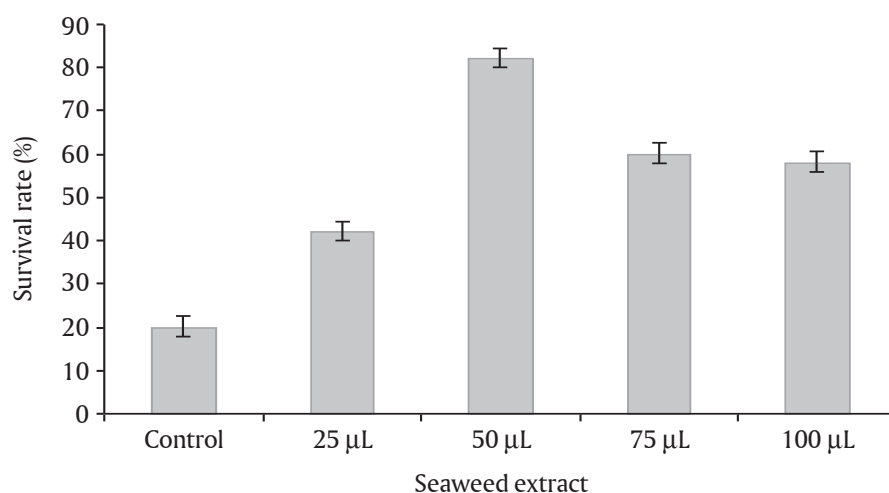


Figure 1. Survival rate of *K. alvarezii* embryo cultured using PES 1/20 liquid medium enriched with seaweed extract at the concentrations of 0 (control), 25, 50, 75, and 100 μL

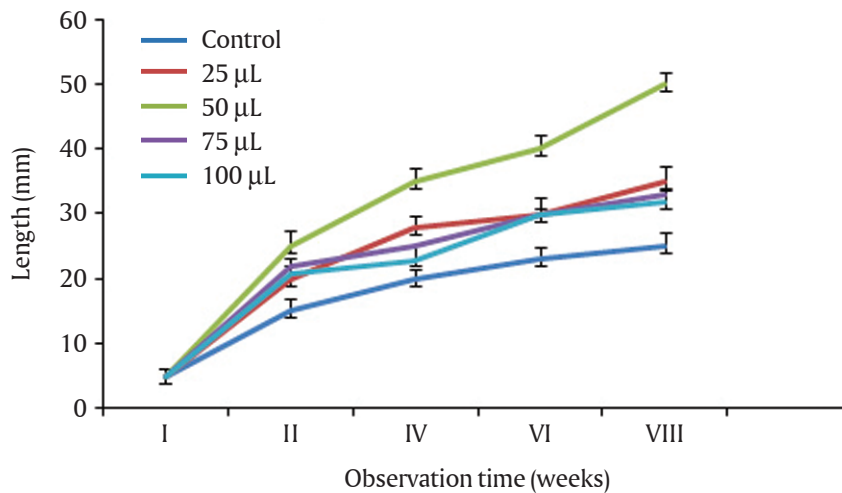


Figure 2. The shoots length produced by *K. alvarezii* embryo planted into PES 1/20 liquid medium enriched with seaweed extract at the concentrations of 0 (control), 25, 50, 75, and 100 µL

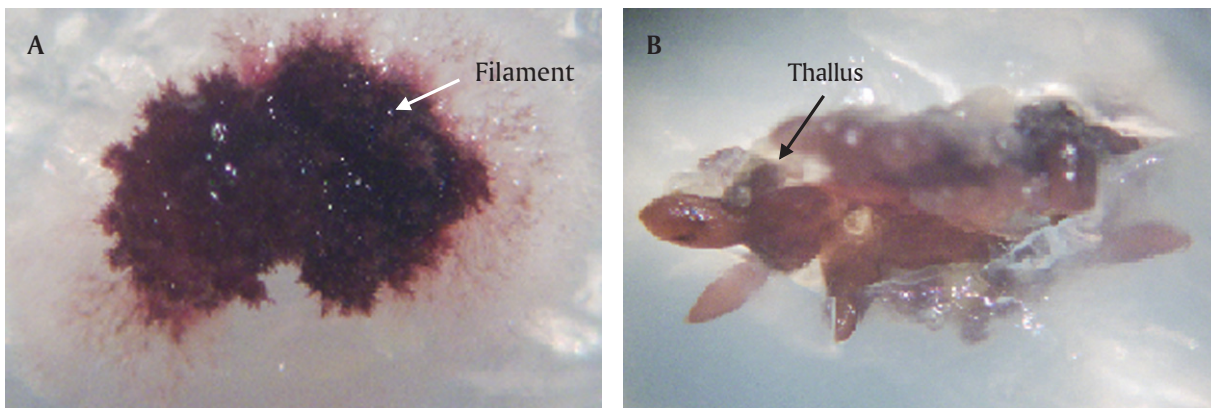


Figure 3. Performance of filaments (A) and thallus (B) cultured in PES 1/20 liquid medium enriched with 50 µL of seaweed extract

value of 60.80% and 100 µL/L with a value of 59.00% (Figure 1).

The enrichment of PES medium with 50 µL of seaweed extract was also indicated a good response on the filament and embryos growth in which the phase of filament into callus, callus into thallus grew faster with lighter colour (Figure 3).

The filament growth was also influenced by culture media and nutrient content in the culture media. Medium enrichment with seaweed extract in this study was able to stimulate the filament growth due to the presence of hormones and nutrients in seaweed extract as reported by Prasad *et al.* (2010). *K. alvarezii* produced nutrients and hormones which have a function in the growth and survival rate of seaweed. Seaweed extract was used as growth regulators for oca plant and wheat culture. Nevertheless, seaweed extract used in stimulating the growth of *K. alvarezii* was reported for the first time in this study.

CONCLUSIONS

Kappaphycus alvarezii extract has the potential to be developed as growth hormone to stimulate the growth of filament into callus and callus into thallus. PES 1/20 culture medium enriched with seaweed extract at the concentration of 50 µL/10 mL culture medium was indicate the highest survival rate and growth of *Kappaphycus alvarezii* filaments and embryos and also produced filaments with a good and lighter performance. It is suggested to isolate and identify hormone regulators used from *Kappaphycus alvarezii*.

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