In vitro micropropagation of Eucheuma seaweeds

Article · April 2011

CITATIONS
6

READS
469

5 authors, including:

Wilson Thau Lym Yong
Universiti Malaysia Sabah (UMS)
36 PUBLICATIONS **188** CITATIONS
SEE PROFILE

Grace Joy Wei Lie Chin
Universiti Malaysia Sabah (UMS)
15 PUBLICATIONS **42** CITATIONS
SEE PROFILE

K. F. Rodrigues
Universiti Malaysia Sabah (UMS)
19 PUBLICATIONS **115** CITATIONS
SEE PROFILE

Ann Anton
Universiti Malaysia Sabah (UMS)
28 PUBLICATIONS **383** CITATIONS
SEE PROFILE

2011 2nd International Conference on Biotechnology and Food Science
IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

In vitro Micropropagation of Eucheuma Seaweeds

Wilson Thau Lym Yong*, Siew Hoo Ting, Wei Lie Chin, Kenneth Francis Rodrigues, Ann Anton
Biotechnology Research Institute, Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
*e-mail: wilsonyg@ums.edu.my

***Abstract*—The cultivation of seaweeds contributes significantly to the national economy and provides employment to the economically backward communities of Sabah, Malaysia. Cultivation of seaweeds on a commercial scale requires a large number of propagules with desirable phenotypic traits which include high growth rates and resistance to diseases. Thus, seaweed tissue culture can be considered as one of the best methods to provide a large amount of seedlings for commercial cultivation. The parameters which have been determined to have a significant effect on the growth of *in vitro* propagated Eucheuma seaweeds are the sterilization techniques, media composition, light intensity and aeration. Eucheuma seaweeds were surface sterilized by washing with optimized concentration of disinfectants and antibiotics. The appropriate medium was determined to be Provasoli's Enriched Seawater (PES) while optimum light intensity was in the range of 6,000 lux. Continuous aeration was important to provide enough carbon dioxide for carbon fixation. Hormone IAA:BAP was provided with concentration ratio of 5:1 mg/l. Temperature was maintained in the range of 25 to 30°C while salinity of the seawater was kept constant at 32 ppt.**

Keywords-Eucheuma; micropropagation; tissue culture

I. INTRODUCTION

The fisheries and aquaculture sector in Sabah has proven to be a significant contributor to the state's economy. Aquaculture has expanded tremendously in the past decade, witnessing an increased number of prawn producers, cage culture operators and seaweed farmers. Under the Tenth Malaysian Economic Plan, Sabah has been given the mandate to increase the production of seaweed for the purpose of commercialization. Researchers are, therefore, looking for application of fine technology for the production of high-quality seaweed seedlings which produce agar with high gel strength, faster growth rate and resistance to disease.

II. LITERATURE REVIEW

The development of tissue culture techniques has led to several advanced opportunities in plant biotechnology such as mass propagation, genetic engineering and the production of bioactive compounds [1, 2]. Following the success achieved in various biotechnological applications of higher plants, seaweeds are increasingly viewed as a potential source of compounds with industrial, pharmaceutical and nutraceutical importance [3]. Seaweeds have been reported to be a source of bioactive compounds as they are able to produce a variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal, and antibacterial activities have been detected in green, brown and red algae [4, 5].

In today's market, the demand for seaweed based-products has exceeded the supply of seaweed raw material from natural stock. In Sabah, the logistical problems faced by conventional seaweed farmers include identification of appropriate sites, labor intensive tasks such as inspection, disease and losses resulting from extreme weather conditions. Therefore, the development of an effective tissue culture system will overcome the problem, leading to savings in time and cost. The development of tissue culture systems, bioprocess engineering of tissue cultures including bioreactor design and identification of strategies for secondary metabolite production can expedite the production of valuable compounds from the seaweeds [6]. Additionally, new approaches in understanding seaweed physiology, biochemistry and molecular biology should contribute to new insights into human nutrition and enable genetic engineering of favorable agronomic traits, such as disease resistance, to improve the quality as well as the production of commercially important seaweeds [7].

III. MATERIALS AND METHODS

A. Samples Collection and Explants Preparation

The Eucheuma species which are cultivated commercially by local farmers in Semporna were collected. After cleaning with seawater in the field, all the samples were wrapped in moistened towels and brought to the laboratory. Healthy plants were selected, surface sterilized and cultured according to the methods described in [8].

B. Explants Cultivation

Following sterilization, the explants were thoroughly rinsed with autoclaved seawater to remove sterilants and then excised into 1-2 cm length sections. Each explant was then wiped gently with sterile filter paper to remove excessive moisture and mucilage from the cut ends prior to transferring to the culture medium supplemented with different concentration of plant growth regulators [9].

C. Thallus Regeneration

The effects of media composition (PES, VS or f/2) supplemented with 5:1 mg/l of IAA:BAP, light intensity, and aeration on thallus regeneration of seaweeds were optimized following the procedures in [3]. The explants were cultured at 20-22°C under cool white fluorescent tube light with

12:12 light and dark photoperiod. In order to ensure sustainable growth of explants, subcultures were carried out regularly to transfer the explants to fresh medium at 2-week intervals.

D. Data Collection and Statistical Analysis

The experimental design was fully randomized and three replicates were carried out for each parameter. All data were analyzed statistically by single factorial analysis of variance (ANOVA) followed by the Turkey multiple comparison tests at 95% significance.

IV. Results and Discussion

A. Optimization of Aseptic Culture Techniques

Explants have to be surface sterilized in order to obtain the axenic cultures for propagation [8]. Tab. I and II show the results of surface sterilization of Eucheuma seaweeds using different concentrations of disinfectants and antibiotics, respectively.

TABLE I. SURFACE STERILIZATION OF EXPLANTS WITH DIFFERENT CONCENTRATION OF DISINFECTANTS

Disinfectants		**Time (sec.)**	**Observation***
Povidone Iodine (g/l)	*Ethanol (%)*		
1.0	0	15	b
2.0	0	15	b
3.0	0	15	b
4.0	0	15	c
0	5	15	b
0	10	15	b
0	15	15	b
0	20	15	c
3.0	15	15	b
3.0	15	30	a
3.0	15	45	c

* a = axenic cultures obtained; b = contamination observed; c = explants died

TABLE II. SURFACE STERILIZATION OF EXPLANTS WITH DIFFERENT CONCENTRATION OF ANTIBIOTICS

Antibiotics		**Observation***
Penicillin G (mg/l)	*Streptomycin sulphate (mg/l)*	
25.0	25.0	b
50.0	50.0	b
75.0	75.0	b
100.0	100.0	a

* a = axenic cultures obtained; b = contamination observed

The addition of antibiotics to the growth medium did not result in a reduction in the contamination levels, this is likely to be the result of antibiotic degrading *in vivo* during long periods of culture under high light intensity. Povidone iodine and alcohol are general disinfectants which were found to be much more effective as they have a localized activity, on the other hand Penicillin and Streptomycin are narrow spectrum antibiotics and their functionality is limited to specific classes of microbes. Samples collected from the wild are associated with a significant level of biological contamination which is likely to be commensal or symbiotic, therefore in the case of Eucheuma spp. it is necessary to surface sterilize the explants with a general disinfectant.

B. Optimization of Media Composition

The culture medium found to be suitable for Eucheuma seaweeds was PES medium whereas f/2 and VS media were found not effective for Eucheuma cultures. Fig. 1 shows the daily growth rate (%) of Eucheuma seaweeds cultured in different types of media composition. PES medium has low concentration of nutrients whereas f/2 and VS media have a higher concentration of salts which interfere with the growth *in vitro*. It is interesting to note that the control exhibited a higher growth rate as compared to the f/2 and VS media clearly implicating that some of the salts in these two media were contributing to a lower rate of growth.

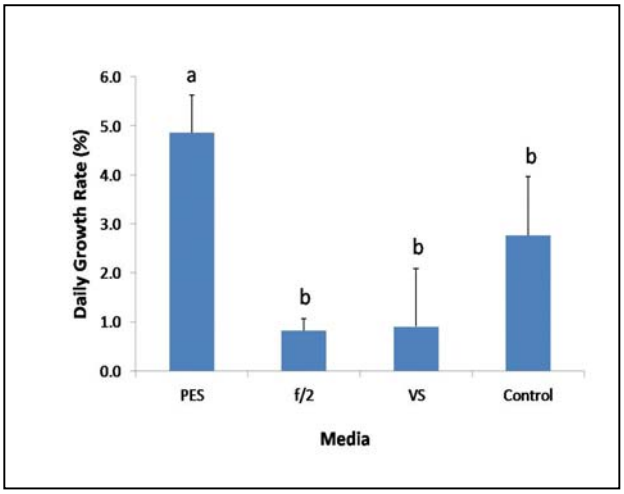

Figure 1. Daily growth rate (%) of Eucheuma seaweeds cultured in different types of media composition.

C. Optimization of Light Intensity

Light is one of the most important parameters for the culture of seaweeds. The intensity, wavelength and spectral quality of light, all influence photosynthetic productivity. Explants responded to higher light intensity by producing more buds and subsequently leading to an increase in biomass. Optimum light intensity for Eucheuma seaweeds was determined in the range of 6,000 lux. Further increase of light intensity was found to be a detriment to the growth of seaweed and this might be due to the photoinhibition. Fig. 2

shows the daily growth rate (%) of Eucheuma seaweeds cultured under different light irradiance.

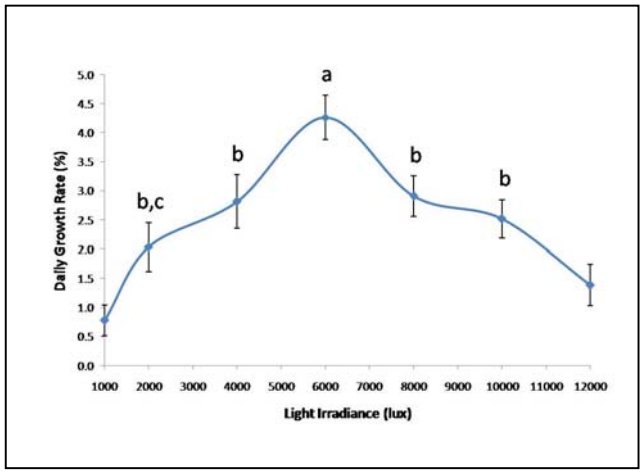

Figure 2. Daily growth rate (%) of Eucheuma seaweeds cultured under different light irradiance.

D. Optimization of Aeration

Aeration was found to significantly enhance the growth of Eucheuma seaweeds. Aeration is the mechanism whereby, atmospheric carbon dioxide is diffused into the culture medium. An enhanced rate of aeration had a significant impact on the growth of Eucheuma seaweeds. Meanwhile, carbon source can be provided in the form of carbon dioxide or in organic form such as sucrose or glycerol. Glycerol was used for induction of callus in previous literature as glycerol-containing medium reduced the morphogenetic capacity of the explants [10]. Therefore the addition of glycerol in the medium is counter to our objective which was to ensure that explants were totipotent. In order to ensure this feeding with carbon dioxide is the ideal method of energy transfer to the explants.

V. CONCLUSION

Eucheuma species was found to have optimal growth when cultured in PES medium with 6,000 lux of light intensity and continuous aeration. With the development of tissue culture system, *in vitro* mass propagation and production of genetically modified good quality seedlings can be achieved.

ACKNOWLEDGMENT

The authors wish to thank the Ministry of Higher Education Malaysia for funding the research under the project FRG0201-SG-1/2010.

REFERENCES

[1] P. Zhao, Y. Iwamoto, I. Kouno, Y. Egami, and H. Yamamoto, "Stimulating the production of homoisoflavonoids in cell suspension cultures of Caesalpinia pulcherrima using cork tissue," Phytochemistry, vol. 65, Sept. 2004, pp. 2455-2461, doi:10.1016/j.phytochem.2004.08.004.

[2] G. R. Rout, A. Mohapatra, and S. M. Jain, "Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects," Biotechnology Advances, vol. 24, Nov. 2006, pp. 531-560, doi:10.1016/j.biotechadv.2006.05.001.

[3] G. R. Kumar, C. R. K. Reddy, and B. Jha, "Callus induction and thallus regeneration from callus of phycocolloid yielding seaweeds from the Indian coast," Journal of Applied Phycology, vol. 19, Feb. 2007, pp. 15-25, doi:10.1007/s10811-006-9104-0.

[4] U. Lindequist and T. Schweder, "Marine biotechnology," in Biotechnology, vol. 10, H. J. Rehm and G. Reed, Eds. Weinheim: Wiley-VCH, 2001, pp. 441-484.

[5] D. J. Newman, G. M. Cragg, and K. M. Snader, "Natural products as sources of new drugs over the period 1981-2002," Journal of Natural Products, vol. 66, June 2003, pp. 1022-1037, doi:10.1021/np0300961.

[6] G. L. Rorrer and D. P. Cheney, "Bioprocess engineering of cell and tissue cultures for marine seaweeds," Aquacultural Engineering, vol. 32, Dec. 2004, pp. 11-41, doi:10.1016/j.aquaeng.2004.03.007.

[7] C. X. Chan, C. L. Ho, and S. M. Phang, "Trends in seaweed research," Trends in Plant Science, vol. 11, Apr. 2006, pp. 165-166, doi:10.1016/j.tplants.2006.02.003.

[8] G. R. Kumar, C. R. K. Reddy, M. Ganesan, S. Tiruppathi, S. Dippakore, K. Eswaran, P. V. Subba Rao, and B. Jha, "Tissue culture and regeneration of thallus from callus of Gelidiella acerosa (Gelidiaies, Rodophyta)," Phycologia, vol. 43, Sept. 2004, pp. 596-602, doi:10.2216/i0031-8884-43-5-596.1.

[9] A. Q. Hurtado and A. B. Biter, "Plantlet regeneration of Kappaphycus alvarezii var. adik-adik by tissue culture," Journal of Applied Phycology, vol. 19, Dec. 2007, pp. 783-786, doi:10.1007/s10811-007-9269-1.

[10] R. R. Robaina, P. Garcia, G. Garcia-Reina, and A. Luque, "Morphogenetic effect of glycerol on tissue cultures of the red seaweed Grateloupia doryphora," Journal of Applied Phycology, vol. 2, June 1990, pp. 137-143, doi:10.1007/BF00023375.