# *IN VITRO* PROPAGATION AND PHOTOBIOREACTOR CULTIVATION OF COMMERCIALLY IMPORTANT SEAWEED, *Kappaphycus alverazii* IN SABAH.

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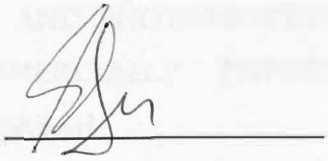

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## CERTIFICATION

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TITLE : ***IN VITRO* PROPAGATION AND PHOTOBIOREACTOR CULTIVATION OF COMMERCIALLY IMPORTANT *Kappaphycus alverazii* IN SABAH**

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VIVA DATE : **17 OCTOBER 2014**

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## ABSTRACT

In Sabah, there wasa problemfor conventional farming of *Kappaphycusalverazii*, where after seasonal variations and disease infestations, there was lack of healthy seedlings for next cultivation.*In vitro* tissue culture techniques can solve this problemand facilitate the propagation of commercially important genotypes which are healthy and fast growing. This research was carried out to optimize the suitable conditions for the propagation of *Kappaphycusalverazii*. Culture media, explants weight over volume concentration, aeration activity and light illuminescence were found to be the critical factors that affect the growth of *Kappaphycusalverazii.* The optimum conditions for culturing seaweeds were found to be initial explants of 0.5 to 1.0 of weight in gram over 100ml media, 50 % PES media enrichment and supplemented with 2.5 g/l BAP and 1.0g/l IAA, light intensity of 6,000 lux, continuous aeration and temperature of 24 to 27°C. In order to achieve mass cultivation of seedlings, a customized airlift photobioreactor was constructed to provide the optimal culture conditions to the seaweedcultivation. The percentage ofincrease infresh culturesper day for 60 days in the photobioreactor (6.0 %(g $g^{-1}d^{-1}$)) was found to be higher than the growth rate obtained from the culturing flask (4.26%(g $g^{-1}d^{-1}$)) and the cultivation in sea (5.5%(g $g^{-1}d^{-1}$)). A study involving the profiling of protein expression between sea cultivated seaweed and clonally propagated seaweed revealed that short peptides with a molecular mass of 5-10 kDA were produced in significant amounts in naturally cultivated seaweeds but stop production during the first 12 week of laboratory culture. These peptides were revealed to be in the class of Lectin by MALTIDOF method. The reappearance of this peptide after 12 weeks of cultivation may indicate that the propagules are ready to be transferred to the natural environment as they possess defence systems for adaptation to the wild. Thus, Lectins can be applied as a biomarker to monitor the quality of seaweed cultures *in vitro* and this biomarker can be highly beneficial to the seaweed farming industry.

## *ABSTRAK*

### *IN VITRO PROPAGASI DAN KULTIVASI FOTOBIOREAKTOR Kappaphycus alverazii YANG BERKEPENTINGAN KOMERSIAL DI SABAH*

*Di Sabah, terdapat satu masalah untuk perladangan konvensional bagi Kappaphycus alverazii di mana selepas variasi musim dan serangan penyakit, terdapat kekurangan benih yang sihat untuk kultivasi seterusnya. Teknik tisu kultur in vitro boleh mengatasi masalah ini dan memudahkan propagasi genotip berkepentingan komersial yang sihat dan pertumbuhan pantas. Penyelidikan ini telah dilakukan untuk mengoptimumkan keadaan yang sesuai untuk propagasi Kappaphycus alverazii. Media kultur, kepekatan berat kepada isipadu eksplan, kaedah pengudaraan, dan iluminasi cahaya telah dikenal pasti sebagai factor kritikal yang mempengaruhi tumbesaran Kappaphycus alverazii. Keadaan optimum untuk pengkulturan rumpai laut ini telah dikenal pasti adalah dengan eksplan awal antara 0.5 kepada 1.0 daripada jisim dalam gram untuk 100 ml media, dikayakan dengan media PES 50 % dan ditambah dengan 2.5 g/l BAP dan 1.0 g/l IAA, intensiti cahaya pada 6,000 lux, pengudaraan berterusan dan suhu di antara 24 dan 27 °C. Untuk mencapai pengeluaran benih rumpai laut secara besar-besaran, satu fotobioreaktor pengudaraan dibina supaya keadaan kultur optimum dapat disesuaikankan untuk pengkulturan rumpai laut ini. Kadar tumbesaran di dalam photobioreaktor (6.0% (W/d)) didapati adalah lebih tinggi daripada kadar tumbesaran di dalam kelalang kon (4.2% (W/d)) dan penanaman di tapak semaian laut (5.5 % (W/d)). Selain itu, satu kajian melibatkan profil ekspresi protein antara rumpai laut dikultivasi di laut dengan rumpai laut yang dipropagasi secara klonal mendapati terdapat peptida yang pendek dengan berat molekul antara 5-10 kDA ditemui dengan banyak di rumpai laut yang dikultivasi secara semula jadi tetapi didapati hilang pada 12 minggu pertama untuk rumpai laut yang dipropagasi di makmal. Peptida ini telah dikenalpasti sebagai Lectin dengan kaedah MALTIDOF. Kemunculan peptida ini selepas minggu ke-12 kultivasi menunjukkan propagul berkenaan boleh dipindahkan ke tapak semaian semula jadi kerana memiliki sistem pertahanan untuk mengadaptasi keadaan semula jadi. Maka dengan itu Lectin boleh diaplikasikan sebagai biopenanda untuk mengawasi kualiti kultur rumpai laut in vitro dan biopenanda ini boleh memberi manfaat kepada industri perladangan rumpai laut ini.*

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## LIST OF SYMBOLS AND ABBREVIATION

| | |
|---|---|
| **%** | percentage |
| **>** | greater than |
| **<** | less than |
| **≤** | less than or equal to |
| **≈** | almost equal to |
| **°C** | degree Celsius |
| **µg** | microgram |
| **µl** | microliter |
| **µM** | micromolar |
| **µmol** | micromol |
| **cm** | centimetre |
| **BAP** | benzylaminopurine |
| **BSA** | bovine serum albumin |
| **$CO_2$** | carbon dioxide |
| **d** | day |
| **DGR** | daily growth rate |
| **E** | east |
| **HDL** | high density lipoprotein |
| **g** | gram |
| **$H_2O$** | water |
| **HP** | horse power |
| **IAA** | indole acetic acid |
| **hr** | hour |
| **kb** | kilobase |
| **kDa** | kilodalton |
| **L** | litre |
| **LDL** | low density lipoprotein |
| **m** | meter |
| **M** | molar |
| **$MgCl_2$** | magnesium chloride |
| **Mpa** | megapascal |

| | |
|---|---|
| **mg** | milligram |
| **min** | minute |
| **ml** | milliliter |
| **mm** | milimeter |
| **mM** | millimolar |
| **nm** | nanometer |
| **N** | north |
| **$O_2$** | oxygen |
| **rpm** | revolution per minute |
| **PUFA** | polyunsaturated fatty acid |
| **ppm** | parts per million |
| **sec** | second |
| **spp.** | species |
| **U** | unit |
| **v** | volume |
| **V** | voltage |
| **vs** | versus |
| **W** | weight |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background

In Malaysia, the seaweed farming sector has developed enormously over the past few years with the help of government's subsidy. *Kappaphycus spp.* have been successfully established and cultivated in farm around Sabah, Malaysia. However the growth rate of the number of seaweed's farm is not satisfactory in order to help to curb the poverty among the fishermen in the East Sabah coastal region. Department Of Fishery Sabah has been providing many incentives including materials, seedlings, suitable site identifications and trainings to gain the interest of more fishermen to be involves in the seaweed farming. Workshops and discussions are conducted on a continuous basis to the local farmer for the development of seaweed industry (Department of Fisheries (DOF) Sabah, 2010).

In Sabah, the long line system is more widely practiced due to the economical and practically easy to handle as compared to other systems. This method was known as tie-tie cultivation method using raffia string elongated along two floats or poles. Seedlings are planted by tying up on the raffia string and harvesting as done by cutting of the branches that have grown. Poor post-harvest handling methods were known to cause the degradation of carrageenan content in the raw materials. Meanwhile the old thallus that was left to regenerate has the issue of genes degeneration, easily infested by disease from the cut area and indirectly affects the quality of seaweeds produced.

In order for Sabah to develop the capacity of increased seaweed production, researchers are trying to apply modern biotechnological processes in order to develop novel technology for the production of high quality seaweed seedlings, which feature high gel strength, faster growth rate and resistant to disease. Thus development of more advance technology, such as micropropagation and bioreactors may be one of the solutions to enhance the

seaweed production in Malaysia.

## 1.2 Problem Statement

The cultivation of *Kappaphycus* and *Eucheuma* seaweed in Sabah depends greatly on suitable monsoon seasons. The threat increases as seaweeds cultured in Sabah faces diseases such as 'ice-ice' and endophytes. The total production for both seaweed species are of 111,298 tonnes wet weight in 2008, however the national target of 250,000 tonnes wet weight is yet to be achieved by 2010 (Department of Fisheries (DOF) Sabah, 2010). Due to the axenic growth conditions of these seedlings in tissue culture, diseases such as 'ice-ice' and endophytes can be prevented. The micropropagation of seaweed in tissue culture offers an alternative to provide healthy and fast growing seedlings. This project, therefore, aims to develop a protocol for successful micropropagation of the commercially important *Kappaphycus alverazii.* From this protocol, mass production of seedlings for the seaweed can be done in more controlled environment with photobioreactor.

## 1.3 Objectives

The research objectives of this research are as follows:

a. To establish the sterilization method for preparation of axenic *Kappaphycus spp.* explants for *in vitro* micropropagation.
b. To optimize parameters such as explants size, basal media, phytoregulators concentrations, light intensity, temperature and aeration for thallus regeneration of *Kappaphycus spp.*
c. To evaluate and compare the growth performance of *in vitro* micropropagated *Kappaphycus spp.* in tissue culture flasks and customized photobioreactor.
d. To analyse and compare the proteins profiles generated from different ages of micropropagated seaweed cultures and farm cultivated species using SDS-PAGE.

## 1.4 Significance of Study

The problem in seaweed micropropagation was lack of optimized protocols to obtain axenic cultures and propagation in appropriate media and plant growth regulators (Collantes and Melo, 1995). This study aims to obtain axenic explants and optimize parameters for thallus regeneration. *Kappaphycus* spp. propagation will be studied and the focus of the research is to find a way for mass production of seedlings. Conditions of the propagation provided are simulated by the Sabah's sea and weather conditions which are favourable to this species. Thus initial studies on the culture conditions of this species are required. Through *in vitro* micropropagation, the bulk of seedlings will be cultivated in the rich media and consequently acclimatized in the open sea. Reddy *et al.* (2003) reported that seaweeds from tissue culture can achieve growth rates of 1.5 to 1.8 times higher than the field plants when cultivated in sea farms. Tissue culture of seaweeds is thus viable as healthy and fast growing strains and can be selected for farming and subsequently contribute towards the development of seaweed farming in Sabah. In addition, protein profiles will be determined and compared between different ages of micropropagated seaweeds to search for the indicator that can determined the quality of the seaweeds whether they are suitable to be planted to the sea.

# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Seaweed Background

The use of seaweed as food has been traced back to the 4th century in Japan and the 6th century in China. The seaweed aquaculture production which consists of 92% of the world seaweed supplies is estimated at 11.3 million tonnes and US$5.7 billion whereas 99.7% being provided by Asian countries (Chopin and Sawhney, 2008). China is the largest producer of edible seaweeds and most of them are *Laminaria japonica* which contributes to the production of kombu. The Republic of Korea produces about 800,000 tonnes of various species with 50 % of *Undaria pinnatifida* which contribute to the production of wakame. Japanese production is around 600,000 tonnes with 75 % of *Porphyra* species which contribute to the production of nori (Dennis, 2003).

Seaweed cultivation started during the Tokugawa (or Edo) Era (AD 1600–1868) in Japan (Chopin and Sawhney, 2008). As the demand for raw material increases, natural seaweeds populations became over exploited. Many wild seaweed populations were lost from its natural habitat and the need for farm cultivation was required. Nowadays, about 90 % of the world seaweed supply is harvested from the cultivated species (Dennis, 2003). Depending on the species and their life cycle, the cultivation technology can be done with a lower cost or highly advanced and mechanized with on-land cultivation of seedlings prior to transfer into the open-sea aquaculture sites (Chopin and Sawhney, 2008).

Besides food, alginate, agar and carrageenan are thickening and gelling agents extracted from seaweeds and widely used in food production and pharmaceutical industries. Culturing seaweeds as a source of hydrocolloids started in 1658 when the agar with gelling properties was first extracted from red seaweed in Japan. Carrageenan extracted from Irish moss, red seaweed was popular in 19th century as thickening agents. Meanwhile, alginate was extracted

from brown algae and sold as thickening and gelling agents since 1930s. Industrial uses of these three seaweed extracts expanded rapidly after World War II but were sometimes limited by the availability of raw materials (Dennis, 2003).

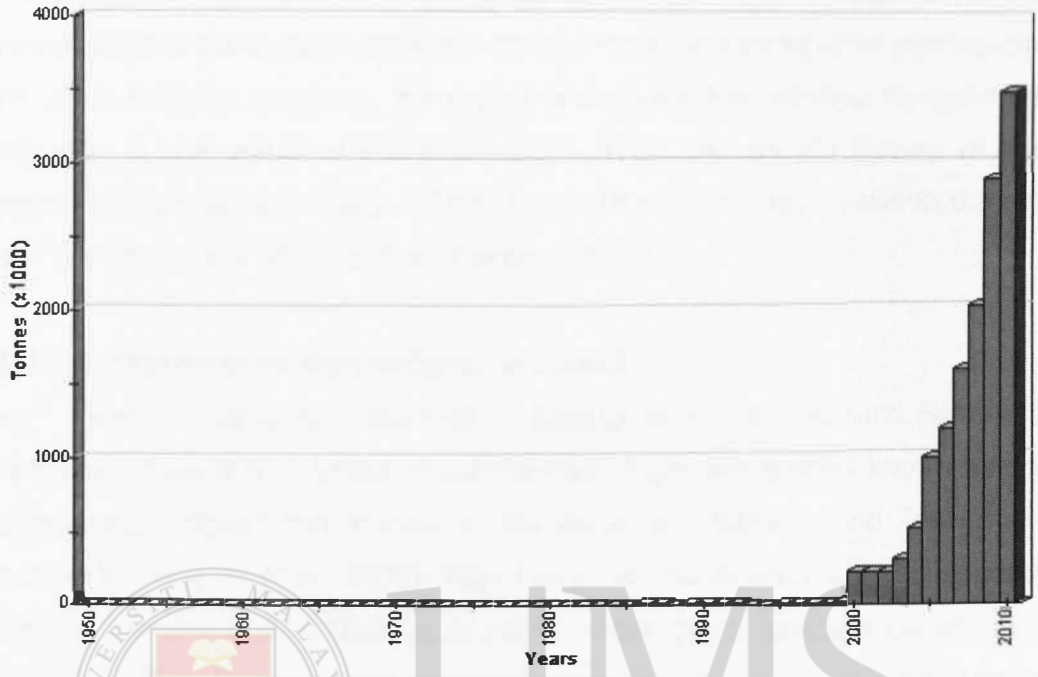


**Figure 2.1: Global aquaculture production of *Eucheuma* spp.**

Source: FAO Fishery Statistic (2010)



According to FAO Fishery Statistic in 2010 (Figure 1.1), aquaculture production for *Eucheuma* spp. started since the 1950's but in insignificant amounts. Only until 2000 when the government policy in the Asia pacific regions allocate funds to increase the production through research and development of more sustainable farming. Today approximately one million tonnes of wet seaweed are harvested annually and extracted to produce 55,000 tonnes hydrocolloids with the total value of US$ 585 million (Dennis, 2003). Alginate which valued US$ 213 million in 2010 is extracted from wild brown seaweeds as the cultivation of brown seaweeds is too expensive to provide raw material for industrial uses (Dennis, 2003). Agar valued amount of US$ 132 million in 2010 is produced from red seaweed, which has been cultivated since 1960-70s (Dennis, 2003). Larger scale cultivation since 1990 has thus allowed the expansion of the agar industry. Carrageenan production valued amount of US$ 240 million in 2010 was originally dependent on wild seaweeds, especially Irish moss, a small alga growing in cold