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Executive Summary.

Two red algal species, Eucheuma denticulatum and Kappaphycus alvarezii, are farmed in over 10,000 family owned farms as well as in over 10 company-owned marine ranches in the Philippines. The farmed seaweeds presently account for over 90% of the carrageenan processed in the Philippines and the Philippines is the main source of the phycocolloid to western industry. Because of declining natural populations and reduction of yields in farmed varieties we proposed to develop culture methods to insure quality seed stock and maintenance of high yield strains of the two species. The goal of the study was to develop culture techniques that would make high yielding seed stock available to the farmers and companies.

During our four year study, we developed selection, shipment, and propagation procedures using 9 varieties of the two species of seaweeds. Laboratory cultivars produced from branch, micropropagation, and callus production were successfully grown on rafts along with typical farmed material in the Philippines. Based on our success culturing the farmed strains and in the Tampa and Quezon City culture facilities we submitted a proposal to set up an run a seed stock facility in the seaweed farming area in Zamboanga City to the Seaweed Industry Association of the Philippines in January, 1992.

The studies carried out in the Tampa and Quezon City laboratories indicate that seaweed micropropagation is economically feasible and the propagules are very aggressive in growth when returned to seaweed farms in the field. The culture facility at

the Marine Science Institute, University of the Philippines, Quezon City (UP-MSI) is now being used in another A.I.D. grant dealing with species of the red alga, Gracilaria. The Philippine Research Assistant, Mr. Lluisma completed his M. S. program at MSI and is in a doctoral program in molecular biology at Dalhousie University.

Research Objectives.

Because of the declining quality of the farmed seed stock on the Philippine farms we proposed to develop culture techniques for the maintenance of seed stock of varieties of the two red seaweeds. Five research objectives were established and accomplished during the research program, first in Tampa and then in Quezon City laboratory.

1. Development of shipping, and culturing of over 9 varieties of the two red seaweed species.

2. Creation of micropropagation procedures that allow rapid production of a large number of branches for seed stock.

3. Creation of long term retention of selected strains though tissue culture and callus production.

4. Testing of laboratory cultivars in the field, side by side with typical farmed plants.

5. Demonstration that a culture facility can be run at low cost and produce sufficient propagules of seed stock on a yearly basis to be economically feasible.

The research was initiated in the PI's Laboratory in Tampa Florida using 9 varieties of the two farmed species of red seaweeds

shipped to us by Professor Trono. Once we were successful in basic branch culture, culturing was also begun in a culture facility Dr. Trono and Mr. Lluisma and I established at UP-MSI. Dr. Edgardo Gomez, Director of the MSI, was very helpful in supplying a room in the new Phase I MSI building that was opened in the fall of 1991. He also made available the field station at Bolanoe, norther Luzon where we carried out our field testing of laboratory cultivars.

Methods and Results.

The proposed research was initiated in the Tampa laboratory of the PI in August, 1989 and then begun in January, 1991 in a culture facility established in the Marine Science Institute of the University of the Philippines (MSI, UP; Diliman, Quezon City, Philippines). Professor Gavino Trono Jr. (MSI, UP) and a Philippine graduate student, Mr. Arturo Lluisma carried out the studies in the Philippines. Some of the grant funds were used to establish a pilot culture facility at MSI, UP and Mr. Lluisma was supported as the Research Assistant on the grant while he completed his M. S. degree in Marine Science.

All of the techniques for this project have been published (see Project Activities/Outputs). A special techniques report was published by the Marine Science Institute of the University of the Philippines in which details of culture techniques are given in a "how to" format. Rather than repeat the results of our studies, copies are enclosed with this report which includes all of the culture procedures.

Appendix I. Dawes, C. J., Trono Jr., G. C. and Lluisma, A. O. 1992. Laboratory culture of Eucheuma denticulatum and Kappaphycus alvarezii. SICEN Leaflet 3, Mar. Sci. Inst., Univ. Phil., 5 pp.).

Impact, relevance and Technology Transfer.

A "how to culture" leaflet was published by Seaweeds and Invertebrates Information Center (SICEN) of UP-MSI and is included as Appendix I of this final report. The culture facility established at UP-MSI through funds of this grant is presently operational and is being used to demonstrate culture techniques. The MSI Eucheuma culture facility is now being used in another A.I.D. grant by Dr. Kimon Bird and Dr. Don Kapran (Univ. North Carolina, Wilmington).

Mr. Arturo Lluisma, a graduate student at the University of the Philippines, was the research assistant and carried out the laboratory and field studies at the Marine Science Center. He was trained in the Tampa Laboratory in the fall of 1990. He completed his M. S. degree at the Marine Science Institute, Univ. Philippines in December, 1992 and has begun his doctoral studies at Dalhouise University. I served as a co-major professor on his committee.

Project Activities/Output.

The techniques developed for the culture of the farmed varieties of the two red Philippine speces were presented in international meetings, published in refereed journals, and presented in a workshop in the seaweed farming region of the

Philippines, Cebu City. Philippine scientists also visited the PI's laboratory in Tampa, Florida, Dr. Gavino Trono in May, 1990 and Mr. Arturo Lluisma in October, 1990. More importantly, a culture facility was operational at the Quezon City campus of the University of the Philippines by January, 1991 and used as a demonstration facility during 1991 and 1992.

Talks. Invited talks were given at international meetings (* denotes speaker).

1. Fourth International Phycological Congress, Duke University, Durham, U.S. A. August, 1991. Dawes, C. J.* and E. W. Koch. Branch, micropropagule, and tissue culture of the red algae Eucheuma denticulatum and Kappaphycus alvarezii farmed in the Philippines.

2. Second RP-USA Phycology Symposium/Workshop, Cebu City, Philippiines, January, 1992. Dawes, C. J., Trono, G. C. and Lluisma*, A. O. Eucheuma and Kappaphycus branch and tissue culture at UPMSI.

3. Ninth International Seaweed Symposium, Brest, France. August, 1993. Dawes*, C. J., Trono Jr., G. C. and Lluisma, A. O. 1993. Clonal propagation of Eucheuma denticulatum and Kappaphycus alvarezii for Philippine seaweed farms.

Publications. The following articles have been published in

refereed scientific journals dealing with the grant research. Reprints of papers 1, 2, and 3 were submitted with previous reports. Page proofs of paper 4 is included with this report (Appendix II).

1. Dawes, C. J., Koch, E. W. 1991. Branch, micropropagule and tissue culture of the red algae Eucheuma denticulatum and Kappaphycus alvarezii farmed in the Philippines. J. Applied Phycology. 3: 247-258.

2. Dawes, C. J. 1992. Irradiance acclimation of the cultured Philippine seaweeds Kappaphycus alvarezii and Eucheuma denticulatum. Bot. Mar. 35: 189-195.

3. Dawes, C. J., Trono Jr., G. C. and Lluisma. A. O. 1993. Clonal propagation of Eucheuma denticulatum and Kappaphycus alvarezii for Philippine seaweed farms. Hydrobiologia 260/261: 379-383.

4. Dawes, C. J., Lluisma, A. O. and Trono, G. C. 1994. Laboratory and field growth studies of commercial strains of Eucheuma denticulatum and Kappaphycus alvarezii in the Philippines. J. Applied Phycology.

Project Productivity.

The project accomplished all of the proposed goals. In addition, we have a culture facility at UPMSI and have proposed the

establishment of a commercial facility at Zamboanga City.

Future Work.

We are now studying strains of the two farmed species using physiological methods (photosynthesis-irradiance responses; growth responses, carrageenan content) with the intention of selecting high yield plants. These studies are being carried out on our own. We hope to convince the Seaweed Industry Association of the Philippines to establish a culture facility at Zamboanga City that would make available seed stock cultivars to the farmers.

Appendix I. SICEN Leaflet 3. (1992).

Appendix II. Proofs of final paper to J. Applied Phycology (1994).

Appendix I
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SICEN Leaflet 3

May 1992 ISSN : 0117-3820

Published by the Seaweeds and Invertebrates Information Center (SICEN), Marine Science Institute, C.S.,University of the Philippines, Diliman, Quezon City, through a grant from the International Development Research Centre (IDRC) of Canada.

Laboratory Culture of Eucheuma denticulatum and Kappaphycus alvarezii

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INTRODUCTION

Branch, micropropagule, and callus cultures have been accomplished for three forms of the farmed *Eucheuma denticulatum* (Brunan) Collins *et* Hervey and four forms of *Kappaphycus alvarezii* (Doty) Doty (Dawes &Koch 1991). The objectives of this study are three fold: (1) To produce standard inexpensive culture procedures that will permit the maintenance of seed stocks that were selected for optimal seasonal growth and yield of carrageenan from farmed seaweeds (branch culture, micropropagation); (2) To produce callus tissue for propagation of a large number of selected strains so that clonal material can be made available for field mariculture; (3) To demonstrate that a culture facility could supply seed stock to the seaweed farms in the Philippines. The techniques reported here were developed with funding from the U.S. Agency for International Development (USAID) and have resulted in the establishment of a pilot culture facility in the new Marine Science Institute Building at the campus of the University of the Philippines, Diliman, Quezon City.

Eucheuma and **Kappaphycus** in (1) large Erlenmeyer flasks, (2) deep culture dishes, and (3) multiwell trays in culture shelf.

HANDLING AND CULTURE METHODS

Initial processing

Before shipping, the branches up to 30 cm long are gently cleaned in filtered seawater, with the epiphytes and debris removed. These are shipped up to 4 days, packed between layers of dried rewetted *Sargassum* seaweed or wet paper towels. The branches are kept moist and do not touch each other during shipment. There are no air spaces between the layers.

After arrival, the branches are rinsed in filtered seawater and placed in large flasks such as Fernbach (2800 ml) or Erlenmeyer (2000 ml) flasks containing filtered seawater overnight at 25°C and 10-12 hour photoperiod, and irradiance of ca 25 uE $m^{-2}s^{-1}$ (measured by LICOR quantameter with cosine collector). The next day, the branches are gently damp dried in clean paper towels and dipped in a **cleaning antibiotic mixture** for 30 seconds to 10 minutes (Table 1). The branches are returned to the Fernbach/Erlenmeyer flasks and placed

in modified ESS media (Saga 1986; half strength, Table 2) with **standard antibiotic mixture** (Table 3). These procedures are repeated over the next 2 to 5 days. Myo-inositol can be added (1 mg L^{-1}) to improve wound healing (in addition to that in standard ESS). After the branches are established in culture (1 to 2 weeks) they should be pulse fed for not more than 24 hours per week in ESS media if maintained in Fernbach/ Erlenmeyer flasks to avoid bacterial contamination. Irradiance levels can vary but usually are effective from 25 to 50 uE $m^{-2}s^{-1}$.

Culture procedures

A number of culture media can be used to culture the two species including ESS (Table 2), SWMD-1 (Chen &Taylor 1978), VS media (Guiry & Cunningham 1984) and Erdschreibers soil extract (McLachlan 1973). We selected a modified version of ESS enriched autoclaved seawater (Table 2) and found that epiphyte is lowest if enriched seawater is alternated with sterile seawater on a weekly basis. Myo-inositol is added to the media because this appears to be important in wound healing of the cut branches (Azanza-Corrales & Dawes 1989). All stock solutions are stored at 10°C or lower. Seawater is autoclaved (twice in 30 min at 121°C) as well as all glasswares (once in 20 min at 121° C). Media is prepared as needed in Fernbach/ Erlenmeyer flasks using autoclaved pipettor tips because precipitation or contamination may occur if larger amounts of media are left standing at room temperature.

Salinity of the culture media is be 34 to 36 ppt because both species are stenohaline. However, during cleaning, brief (30 sec to 1 min) rinses in tap water will not damage the plants. Philippine species grow best in temperatures of 22 to 26°C while higher (30°C) temperatures usually result in bacterial contamination and require more frequent changes of media.

Table 2. *ESS-1 seawater enrichment* modified from Saga (1986). One ml of the stock solution is added to each liter of autoclaved seawater.

Metals and Salts Stocks	
$NaNO_3$	15.0 g
Na glycerophosphate	2.0 g (26 uM P)
Fe sequestrene (EDTA)	1.0 g
KI	0.0025 g
$ZnCl_2$	0.03407 g
$MnCl_2.4H_2O$	0.04948 g
$CoCl_2.6H_2O$	0.05946 g
$FeSO_4.7H_2O$	0.06948 g
$EDTA.2H_2O$	0.09305 g
H_3BO_3	0.01546 g
Vitamins and Minerals Stocks	
Vitamin B_{12}	0.00250 g
Biotin	0.00250 g
Thiamine HCl	0.25000 g
Nicotinic acid	0.25000 g
D-panthotenic acid	0.25000 g
p-aminobenzoic acid (hemicalcium salt)	0.02500 g
Myo-inositol	2.50000g
Thymine	0.25000 g
***Auxin Stock**	
Alpha-NAA or IBA	0.00250 g
***Cytokinin Stock**	
2iP or kinetin	0.00250 g

* PGR (Plant Growth Regulator)

Table 1. *Cleaning antibiotic mixture* * - to be used full strength for cleaning of fresh branches for short periods of 30 seconds to 30 minutes (dissolve chemicals in autoclaved seawater).

38 mg/l Polymixin B sulfate
15 mg/l Erythromycin
30 mg/l Neomycin
10 mg/l Gentamycin
36 mg/l Rifampin
300 ug/l Naldixic acid

*This mixture can, also, be added to either ESS or SWMD-1 culture media for long period use (1 wk) by adding 1 ml of stock solution to each liter of culture media. To prepare stock solution, dissolve mixture in distilled water.

Temperatures lower than 20°C are avoided (Dawes 1989). Irradiance levels of 50 uE $m^{-2}s^{-1}$ are sufficient for normal culture and growth rates are higher (1 to 3 % day^{-1}) in irradiance of 100 to 160 uE $m^{-2}s^{-1}$ if branches are acclimated. At least one half of the fluorescent bulbs are"plant grow" type and the photoperiod is about 12 h D and 12 h L. The pH of the media is 7.5 to 8.0. Aeration is not effective.

1. *Branch culture*

Clean segments that are 5 to 9 cm long are transferred into deep culture dishes (200 to 500 ml) containing modified ESS media (Table 2). Plants are rinsed and brushed under tap water for about 1 minute. If contamination is visible, the branches can be cleaned with a toothbrush or ultrasonicated to remove the epiphytes and "ice-ice " white spots, then dipped for 30 seconds in **cleaning antibiotic mixture** (Table 1) every 2 to 3 weeks. A dilute (5 ug L^{-1}) solution of GeO_2 can be added to the culture media to inhibit diatom growth. The addition should not be used longer than 2 weeks or should the concentration be above 5 ug L^{-1} as the seaweeds are sensitive to the compound.

Table 3. *Standard antibiotic mixture* * to be added to ESS or SWMD-1 culture media by adding 1 ml of the stock solution to each liter of culture media; (dissolve chemicals in autoclaved seawater).

0.3 g/l Penicillin G
0.1 g/l Streptomycin
0.05 g/l Neomycin
0.20 g/l Kanamycin

* This mixture can, also, be used full strength as a rinse (for 30 seconds to 30 minutes); dissolve chemicals in seawater.

Antibiotics can be used both for initial cleaning (Table 1 or 3) and during continuous culturing adding 1 ml of stock solution (Table 3) to each liter of media. If bacterial or blue-green algal contamination becomes visible, polymixin B sulfate (stock = 38.5 mg/L = 300 units/L; Sigma P - 1004, 7800 units/g) is effective. Penicillin G (Stock = 75 mg/L; Sigma 7794) and Naldixic Acid (Stock = 75 mg/200 ml; Sigma N 8878) are also useful alternatives during continuous culture (add 1 ml stock solution L^{-1} media). The cleaning procedure is repeated weekly until the branches are algae-free. At this point, the media can be changed, alternating a week with ESS enriched seawater with up to 2 or 3 weeks in sterilized seawater.

2. *Micropropagation*

A rapid (2 months) method of clonal propagation of branches utilizes explants that are 0.5 cm long. Falcon Multiwell Tissue Culture Plates (24 x 3 and 6 x 10 ml wells) are used. These plates are sterilized in 10 % bleach solution overnight, then soaked in distilled water for 24 h and after thorough drying, sterilized in a microwave oven (full strength, 4 x 4 min exposure).

To start the micropropagation procedure, epiphyte-free branches from enriched seawater culture are cut into 0.5 cm explants under sterile conditions (using laminar flow hood, flamed tweezers and scalpels, sterilized glasswares and liquids). The explants are soaked for 30 min to 1 h in full strength **antibiotic cleaning mixture** (Table 1). Each explant is placed in a 3 ml well with 2 ml of sterilized seawater enriched with modified ESS without the standard plant growth regulators (PGRs) given in Table 2, and 10 mg L^{-1} of the auxin alpha naphthalenacetic acid (NAA) or indol-3-butyric acid (IBA) and 1 mg L^{-1} of the cytokinins 2iP (N^6 [isopentenyl] adenine) or kinetin (Stock: 1 mg L^{-1}). The stock solutions of all PGRs can be stored frozen. Lower levels of the **standard antibiotic solution** (1 ml of stock L^{-1} of media; Table 3) can be added. Multiwell plates with explants are incubated at 25°C, 25 uE $m^{-2}s^{-1}$ and 12:12 L/D photoperiod.

During the first week, 1 to 2 mm growth is observed at the medullary area (cut area) as well as elongation of the spines. After four weeks, the explants are transferred into new multiwells with media (ESS + NAA or IBA + 2iP or kinetin). After 6 to 8 weeks, the explants are transferred to larger (10 ml) multiwell trays or deep culture dishes with ESS enriched seawater (Table 2) without addition of cytokinins. The auxins can be eliminated from the culture media as soon as the plant reaches 1 to 2 cm in size. After 4 months in culture, the micropropagules will reach 3 to 4 g (wet weight) filling up a 300 ml petri dish. At this point, these plants can be used to generate new explants.

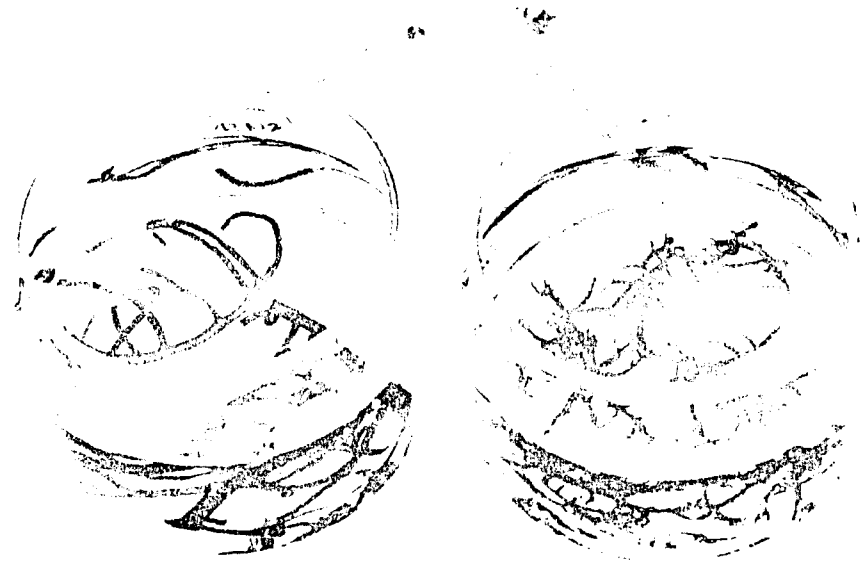
3. *Callus production and regeneration*

Callus production

Callus will develop at the cut ends of explants of both species. The callus is of two types: (1) crystalline hairs which will not regenerate into new plants; and, (2) brown to white undifferentiated tissue that regenerates. Plants with active growth and no evident contamination are ideal for callus production, thus micropropagules are good sources.

The micropropagules are cut into 0.4 to 0.5 cm sections (explants) under sterile conditions and transferred to antibiotic solution (Table 1) or 300 ml units $100ml^{-1}$ of polymixin B, prepared with autoclaved seawater. These explants are soaked for 1 hour with agitation every 3 to 5 minutes.

Callus production has been successful (50 to 75% of explants) in 3 to 8% agar (very hard) containing ESS enriched autoclaved seawater (Table 2) with the addition of PGRs. The viscous agar mixture must be poured while hot to the test tubes or plates. These tubes and plants are then autoclaved and allowed to cool on a slant. Added to the media is a concentration of 1 mg L^{-1} each of the auxin IBA and either cytokinin BAP or



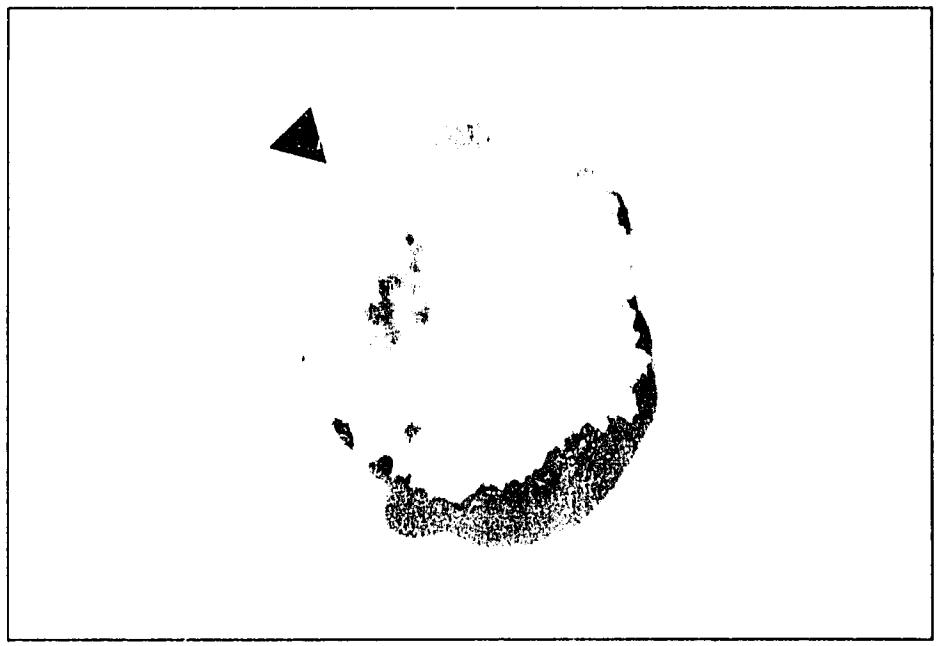
Closer view of **Kappaphycus** and **Eucheuma** in deep culture dishes.

2iP, although different forms of each species respond to other PGRs as well.

The axenic explants are transferred to the tissue culture media, and inserted into the agar so that one cut end of the explant is exposed to air, which appears to be crucial for callus production. Within the first two weeks, fuzzy crystalline hairs or an amorphous brown to white to pink callus develops on the cut end. Development of the callus may take 2 to 10 weeks, depending on the species form.

Regeneration

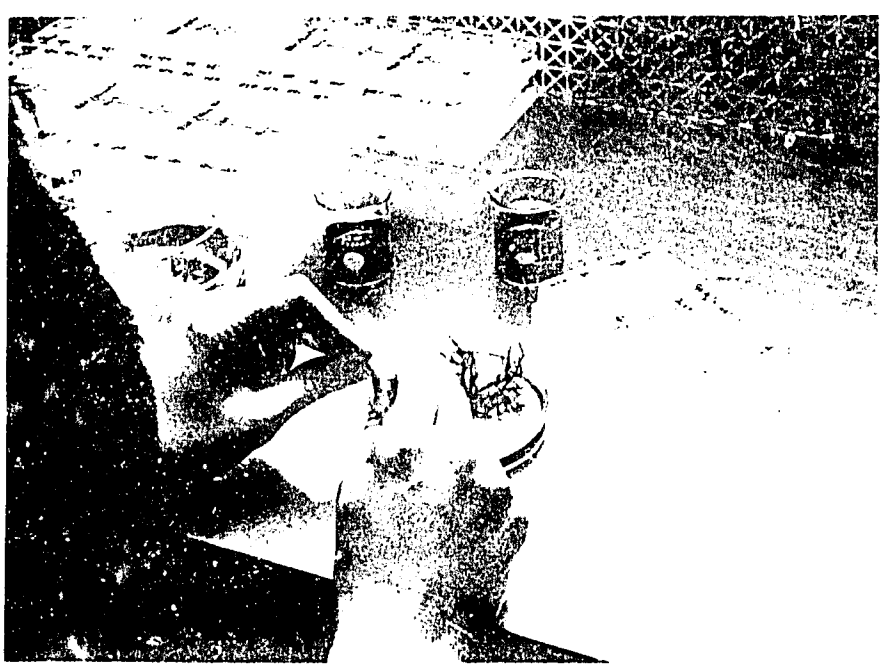
Once the callus has reached 0.2 to 1.0 cm in diameter, this is excised from the explant and transferred to sterile liquid media (ESS enriched autoclaved seawater with 10 mg L-1 NAA or IBA and 1 mg L-1 2iP or BAP) using sterile techniques. The pieces will regenerate into one or more branches with doubling times of 1 to 2 weeks.



Callus formed on 0.5 cm explant of **Kappaphycus alvarezii.**

CONCLUSION

The culture techniques developed for *Eucheuma denticulatum* and *Kappaphycus alvarezii* will allow the production of seed stock for the seaweed farms in the Philippines. Cost analysis and the modification of culture methods are now being tested to reduce the expenses of seaweed culture in the laboratory. A culture facility is being designed to function in the seaweed farming regions to test the usefulness of the culture procedures.



Processing explants for micropropagation (using multiwell trays).

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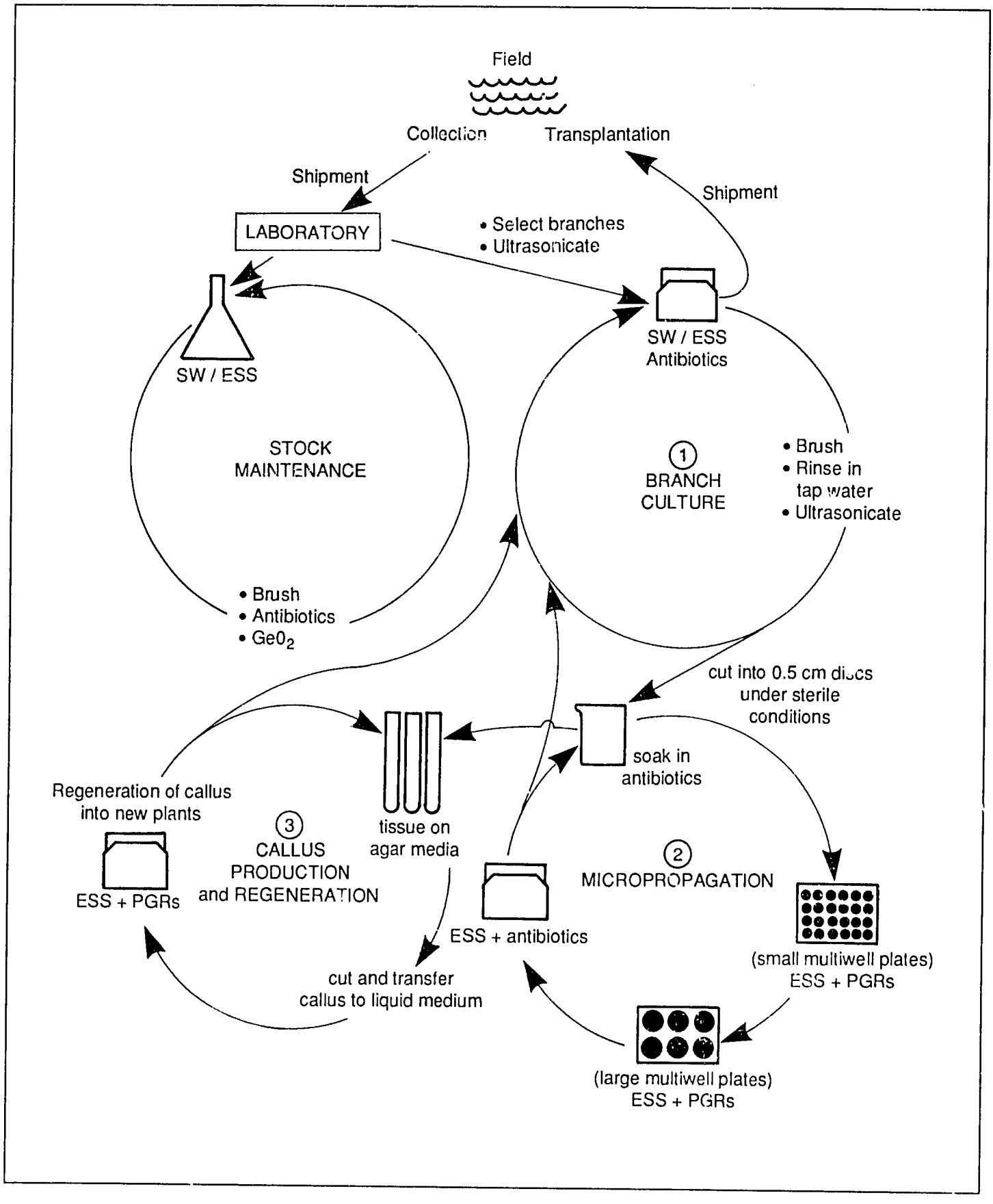
Schematic overview of the procedures for the laboratory culture of **Eucheuma** and **Kappaphycus**.



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Laboratory and field growth studies of commercial strains of *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the Philippines

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Key words: Eucheuma, Kappaphycus, culture media, field growth

Abstract

Daily growth rates of 0.1 to 8.4% d^{-1} for the brown form and 0.2 to 6.3% d^{-1} of the green form were measured for 3 to 5-cm long branches of the tropical red seaweed *Kappaphycus alvarezii* cultured in the laboratory. Highest growth rates were found using inexpensive enrichments such as soil water and coconut water supplemented with 0.7 mM N and 13 μM P and with a liquid fertilizer, Algafer, produced from seaweeds in the Philippines. Laboratory grown branches of both *K. alvarezii* and *Eucheuma denticulatum* transplanted to rafts in the field showed daily growth rates of 4.4 to 8.9% d^{-1}, as high or higher than other reported growth rates. The studies, carried out in the Philippines, demonstrate the viability and high yield of laboratory cultivars and methods to keep laboratory culture costs low.

Introduction

Two tropical red seaweeds, *Eucheuma denticulatum* (Burm.) Collins and Hervey and *Kappaphycus alvarezii* (Doty) Doty, account for over 90% of all seaweed exports from the Philippines and 70% of the world's supply of semirefined carrageenan (Llana, 1991). Based on the importance and size of the Philippine carrageenan industry the establishment of culture facilities were proposed to supply new material to the farmers as well as be a site for seed stock selection (Dawes *et al.*, 1993). Culture procedures have been developed to maintain and select seed stock through laboratory cultivation (Polne-Fuller & Gibor, 1987; Dawes & Koch, 1991) using branch, micropropagules, and callus production.

If laboratory cultivars are to be successful as a seed stock source, it must be demonstrated that these cultivars show high growth rates when utilized in the farms. This paper presents a summary of our field tests of laboratory cultivars of both species and a comparision of inexpensive laboratory media for maintaining seed stock of *Kappaphycus alvarezii*.

Materials and methods

The studies were carried out in the culture facility of the Marina Science Institute, University of the Philippines (UPMSI), Quezon City. Shipping, cleaning and culturing of both species have been described (Dawes & Koch, 1991). The standard laboratory medium that was selected for culture of branches and 0.5-cm micropropagules of 8 forms of the two species was autoclaved seawater enriched with modified ESS (Dawes & Koch,

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1991). However this medium is expensive, and includes plant growth regulators difficult to obtain in the seaweed farming areas of the Philippines.

In order to reduce the cost of branch culture, autoclaved seawater was enriched with a number of inexpensive sources of nutrients and tested on *Kappaphycus alvarezii*, as had been done on *Eucheuma denticulatum* (Dawes *et al.*, 1993). The enrichments included two lots of a commercial Philippine liquid fertilizer made from seaweed extract, Algafer (MCPI, Cebu City), addition of 0.7 mM N ($NaNO_3$) and 13 μM P (KH_2PO_4), modified ESS, 0.1% coconut water or soil extract (10 ml L^{-1}) with 0.7 mM N and 13 μM P. Daily growth rates (DRG = percent growth d^{-1}) were calculated using a linear growth rate formula because growth was observed to be linear in the laboratory cultures:

$$DGR = W_f - W_i / W_i \times 100 / \# d,$$

where W_f = final weight, W_i = initial weight, and d = number of days between W_f and W_i.

Branches (3 to 5 cm long) were grown in 500 ml autoclavable jelly jars (350 ml medium) similar to the 300 ml storage dishes (Dawes and Koch, 1991). The cultures were maintained at 25, ±2 °C under 60 μmol photon $m^{-2}s^{-1}$ using 240W fluorescent tubes (12L:12D photoperiod).

The field viability of laboratory cultivars of both brown and green forms of each species was tested at the Marine Science Institute's field station at Bolinao, Pangasinan on Luzon Island (16°24′ N, 119°54′ E). The cultivars were layered between wet paper towels in coolers and transported (6 h) from Quezon City to Bolinao. Individuals were weighed, tagged and tied to rafts using standard farming procedures (Doty, 1973; Parker, 1974). The rafts were enclosed in netting to minimize grazing. Daily growth rates (DGR = percent d^{-1} were calculated using a formula that takes into account exponential growth as shown for other field studies (Dawes *et al.*, 1993):

$$DGR = \ln (W_f / W_i) \times 100 / \# d,$$

where ln = normal log of the quotient of final over initial wt.

Table 1. Percent daily growth of branches of *Kappaphycus alvarezii* (KAB = brown form; KAG = green form) cultured for 63 and then 71 d in the laboratory using (1) autoclaved seawater only, (2) + ESS (Dawes & Koch, 1191), (3) + 0.7 mM N and 13 μM P, (4) + 0.01 lot 1 Algafer every other 7 d, (5) + 0.01% lot 1 Algafer continuous, (6) + 0.01% lot 2 Algafer continuous, (7) + 10 ml soil extract l^{-1} with 0.7 mM N, 13 μ M P, (8) + 0.1% coconut water with 0.7 mM N, 13 μM P. Initial wts = 1g, n = 3, ±1 SD.

Medium	First run (63d)		Second run (71d)	
	KAB	KAG	KAB	KAG
1	0.6±0.2	0.5±0.1	0.1±0.0[1]	0.2[2]
2	2.8±0.1	1.6±0.1	6.6±1.3	3.9±0.7
3	1.7±0.3	0.9±0.1	5.7±0.2	3.7±0.8
4	1.7±0.1	1.0±0.3	2.9±0.9	3.0±1.0
5	2.0±0.6	0.8±0.1	5.7±1.3	3.3±0.5
6	2.0±0.7	0.6±0.1	6.1±0.6	2.4±0.5
7	1.8±0.5	0.9±0.2	7.7±0.7	6.2±0.1
8	1.9±0.0	1.1±0.1	8.4±0.8	6.3±0.6

[1] n=2 [2] n=1

Results

Daily growth rates of branches of both the green and brown forms of *Kappaphycus alvarezii* were generally lower during the first 63 d regardless of the media used when compared with the last 71 d except for plants held continuously in autoclaved seawater (Medium 1, Table 1).

High growth rates occured with branches of the brown form in the standard ESS media (Medium 2, Table 1), using 0.01% of lot 2 Algafer and the two enrichments using coconut water and soil extract supplemented with N and P (Media 6, 7, 8, Table 1). These last two enrichments also resulted in the highest growth rates for branches of the green form of *K. alvarezii*.Four experiments tested the viability and growth rates of laboratory cultivars of the green and brown forms of both species after transplantation to the field at Bolinao from June to Nov 1992 (Table 2).

The branches had been in laboratory culture from 90 to 550 d and were allowed to grow in the field from 33 to 145 d. Although breakage due to

Table 2. Growth (% G d^{-1}) of transplanted *Kappaphycus alvarezii* (KAB = brown form. KAG = green form) and *Eucheuma denticulatum* (EDB = brown form. EDG = green form) from laboratory culture (Quezon City) to field rafts (Bolinao) on Luzon Island. Philippines.

Pl.	Expt no.	Lab. days	*n*	Mean g.wet wt	Field days	*n*	Mean g wet wt	Mean %G d^{-1}
EDB	6/92	154	10	1.2	114	6	307	4.5±0.9
EDG	6/92	304	4	0.9	145	3	502	4.4±0.5
KAB	6/92	154	6	1.0	74	5	18	7.1±0.4
KAG	6/92	160	10	1.1	114	8	233	5.4±0.8
KAG[1]	6/92	550	7	0.7	72	6	239	8.2±0.8
KAB	7/92	184	7	1.4	43	5	34	7.4±0.2
KAG	7/92	190	6	1.2	43	5	40	8.0±0.5
EDB	9/92	222	6	1.0	43	4	16	6.3±1.3
KAB[2]	9/92	90	45	0.8	105	42	377	5 9±0.5
KAG	9/92	374	6	1.0	43	5	19	6.4±1.8
KAG	9/92	231	6	1.1	43	6	8	4.4±1.0
KAG[3]	9/92	459	5	0.7	43	5	10	6.1±0.9
KAG	11/92	263	11	2.4	33	11	53	8.7±2.2
KAB	11/92	257	16	3.3	33	13	77	8.9±3.2

[1]Forms C_1 of *K. alvarezii* shipped to Tampa. Jan 1991. in culture 2 yr. cultivars shipped back to UPMSI. Jan 1992; in culture 180 d.
[2]Micropropagules (2–3 cm) grown to transplant size at UPMSI.
[3]Form C_1 of *K. Alvarezii* regenrated from callus (Dawes and Koch. 1991) of plants shipped to Tampa. Nov 1990. returned to UPMSCI Jan 1992; in culture 270 d at UPMSI.

storm damage and grazing occured, survival was high (60 to 100%) for all transplantations. More importantly, DGR were uniformly high, from 4.4 to 8.9%, even over longer periode of raft culture (e.g. EDG, 6/92. 145 d = 4.4%). Cultivars of *Kappaphycus alvarezii* (green forms) grown in Tampa and shipped back to Quezon City showed also showed high growth rates (KAG[1/3]; Table 2) after transfer to raft culture.

Discussion

One of the goals in the development of culture techniques for *Kappaphycus alvarezii* and *Eucheuma denticulatum* is to select inexpensive procedures. Soil extract (+ N,P). coconut water (+ N,P), and a commercial liquid fertilizer were all effective, inexpensive media for culturing of branches of *Kappaphycus alvarezii* at the UPMSI culture facility in Quezon City. The findings parallel the branch culture studies of forms of *Eucheuma denticulatum* (Dawes *et al* ., 1993) also conducted at the UPMSI facility and previous studies in Tampa. In both cases coconut water (+ N,P) and a locally produced liquid fertilizer made from seaweeds were effective enrichments for culturing. The use of different production lots of Algafer resulted in similar DGRs. The growth rates were higher for *K. alvarezii* (both green and brown forms) than the two forms of *E. denticulatum*. This may be due in part to the supplement of coconut water and soil extract with N and P and the higher growth rate shown by the former species (Dawes & Koch, 1991). Acclimation was evident as the branches of both forms

showed higher growth rates during the second phase of the media experiment except for those continued in sterile seawater where starvation was evident (pigment loss, thinning of branches) The transplantation experiments demonstrate the viability and high growth rates of laboratory cultivars transplanted to typical farm rafts at the Bolinao fiels station of the Marine Science Institute. Growth rates were similar to those reported from the central Philippines (3.7 to 7.2% d^{-1}, Hurtado-Ponce, 1992) and generally higher than the growth rates summarized by Doty (1987) for earlier studies (1 to 5% d^{-1}). The period of raft growth ranged from 33 to 145 days, the longer periods of 114 to 145 days being shorter than periods required for harvesting by farmers. Thus these transplantations demonstrate the longevity of the cultivars exceeding our preliminary experiment of 84 days (Dawes *et al.*, 1993).Because of the small size (0.7 to 3.3 g wet wt) of the laboratory cultivars being transplanted to the field, the final weights were only beginning to approach harvestable size. The farmers could break up the plants into 2 5 to 50 g wet wt after 50 to 100 d to increase the number of propagules and shorten the time to harvestable size (0.5 to 1 kg).In conclusion the present and previous studies indicate that both green and brown forms of *Kappaphycus alvarezii* and *Eucheuma denticulatum* can be propagated in laboratory culture easily and inexpensively. Also all cultivars presently produced via callus regeneration, micropropagation, or simple branch culture are as productive in the field as farmed plants after transplantation.

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