Seaweed Tissue Culture Technique in Balai Perikanan Budidaya Air Payau (BPBAP) Situbondo

Lusiana BR. Ritonga[1]*, Muh. Hery Riyadi Alauddin[1]*Dini Wahyu Suryandini[1]*

[1]Fisheries Cultivation Engineering Study Program, Marine and Fisheries Polytechnic Sidoarjo
*Email: lusi.poltekkpsda@gmail.com

Abstract

Kappapyhcus alvarezii seaweed or more commonly known as Eucheuma cottonii is a potential commodity to be developed because seaweed has many benefits. The problem found in the development of seaweed in Indonesia is the number of pests and diseases in seaweed, especially ice-ice. In vitro culture techniques have become an alternative to help overcome the problems that are often faced in the provision of seeds through conventional means. There are 6 stages in seaweed tissue culture, namely broodstock acclimatization, callus induction, callus/micropropagule regeneration, micropropagule regeneration, plantlet acclimatization and seed propagation in the sea. During the observation, water quality was still in the normal range, namely temperatures ranging from 25-30 0C, pH between 8.16-8.35 and salinity 34-36 ppt.

Keyword *: Kappapychus alvarezii, plant tissue isolation, acclimatization, regeneration*

Introduction

Indonesia has various types of seaweed including *Gracilaria*, *Gelidium*, *Eucheuma*, *Hypnea*, *Sargasum* and *Tubrinaria*. Of the various types of seaweed, which are cultivated, developed and traded widely in Indonesia are the karaginophyte species (including *Eucheuma spinosium*, *Eucheuma edule*, *Eucheuma serra*, *Eucheuma cottonii*, and *Eucheuma* spp), agarophytes (*Gracilaria* spp, *Gelidium* spp and *Gelidiella* spp), as well as alginophytes (*Sargassum* spp, *Laminaria* spp, *Ascophyllum* spp and *Macrocystis* spp), which are raw materials for various industries because they are a source of carrageenan (seaweed flour), agar and alginate (Directorate General of National Export Development, 2013). Priono (2013), stated that *Eucheuma cottonii* seaweed is one of the potential commodities and can be used as a mainstay for small and medium-scale business development efforts.

E. cottoni seaweed has the potential to be developed because it has a fairly good nutritional content, with low calories. *E. cottoni* also contains a variety of minerals that are quite high which can be used for making gelatin. *E. cottoni* has an important role in international trade as a producer of carrageenan extract. Carrageenan levels in *E. cottoni* species ranged from 64 - 73% depending on the type and location. In Indonesia, the levels of carrageenan in *E. cottoni* ranged from 61.5 to 67.5%. In addition to carrageenan in *E. cottoni*, there are other organic substances such as fat, coarse fibers, ash and water.

The development of *E. cottoni* seaweed cultivation encountered several obstacles, one of which was ice-ice disease. Ice-ice disease predominantly attacks the seaweed *E. cottoni* with early clinical symptoms such as increased mucus production, rough thallus surface, wilted thallus, formation of white spots, and whitening of the tip of the thallus. A more severe attack of ice-ice disease can cause the thallus to become porous and eventually the infected thallus becomes broken (falls out). Several research results show that the spread of ice-ice disease is caused by pathogenic bacteria attack (Situmorang *et al*., 2016).

Tissue culture technique is an alternative to overcome this problem and has several advantages, including providing seeds that have good plant genetic stability, high multiplication rate, and can produce healthy seeds free of disease, especially viruses (Sukmadjaja *et al*., 2014).

Dwiyani (2015), said plant tissue culture is a technique for growing cells, tissues or slices of plant organs in the laboratory on an artificial medium containing aseptic (sterile) nutrients to become a whole plant. Sterile conditions are an absolute requirement for the successful implementation of tissue culture, so

this condition must be maintained throughout the culture process.

Methods

The research was carried out at the Situbondo Brackish Water Cultivation Fisheries Center. March 2-30 2020. Prior to culture of seaweed tissue, parent acclimatization was carried out. Seaweed that will be used as broodstock must be healthy, branched thick, bright in color, hard stems, no organisms attached. Acclimatization was carried out in a wet laboratory for 3-4 weeks. Healthy seaweed will be acclimatized in the culture laboratory for 1 month.

Result and Discussion

a) Parent Acclimatization

Acclimatization is carried out for 3-4 weeks. Seaweed that will be used as broodstock must be healthy, branched thick, bright in color, hard stems, no organisms attached. This statement is in accordance with the opinion of Pong-masak et al. (2011), seaweed that has good quality has the characteristics of a large thallus and many branches, thick with pointed ends, no wounds, no spots, bright colors, hard stems, not slimy, fresh and free of disease and organisms attached.

Taking seaweed to be used as broodstock as much as 5-10 kg. In acclimatization activities, seaweed will first be rinsed using a detergent solution for 1-2 minutes, then rinsed using sea water. The rinsed seaweed will be brushed using a soft toothbrush one by one slowly to remove mucus and adhering dirt. The acclimatized seaweed was kept in an aquarium container and circulated for 24 hours non-stop. Acclimatization can be seen in image 1 below:

a b

Image 1. Parent acclimatization in a wet laboratory a. stressed seaweed b. healthy seaweed (Personal documentation, 2020)

Seaweed that survives and is healthy will be acclimatized in the culture laboratory. Treatments carried out during the acclimatization process were changing the water for maintenance media once a week and brushing the seaweed one by one using a soft toothbrush slowly. Seaweeds that fail to adapt will usually secrete mucus, the color will turn pale, and will produce foam. This statement is in accordance with the opinion of Rima *et al.* (2016), seaweeds that cannot adapt to an aquatic environment usually experience a change in color to pale and increased mucus production. Handling that must be done is to replace the media water as soon as possible, and the seaweed is rinsed using a diluted iodine solution with a concentration of 0.1%.

Seaweed that was acclimatized in the culture laboratory was the broodstock that managed to survive during acclimatization in the wet laboratory. Wet laboratories and culture laboratories have very significant differences, where the wet laboratory has an uncontrolled environment while the culture laboratory has a controlled environment where the room temperature is set with a temperature constant of 20 – 22˚C and lighting using lamps with a irradiation time of 12 hours and 12 hours. dead clock. The aim of acclimatization in the culture laboratory is that the seaweed can survive in controlled environmental conditions. The acclimatization of the broodstock in the culture laboratory can be seen in image 2 below:

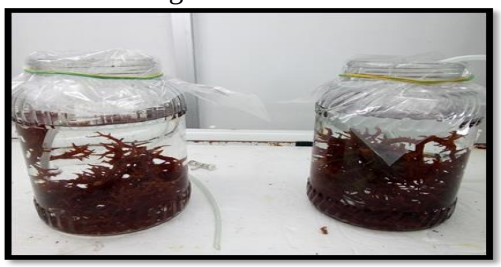

Image 2. Acclimatization in the culture laboratory (Personal documentation, 2020)

Seaweed that was acclimatized in the culture laboratory was maintained using a glass jar with a volume of 2.5 liters of water as shown (Image 2). The media water used in the acclimatization process is semi-sterile seawater. Semi-sterile

seawater is a mixture of seawater that has been sterilized by autoclaving mixed with seawater that has been filtered using cotton. Making water for maintenance media, namely 1 liter of sterile seawater mixed with 1 liter of seawater that has been filtered using cotton. To meet the seaweed nutrition during the water acclimatization process, the maintenance media was given 10 ml of *Provasoli Encriched Seawater* (PES) fertilizer. This statement is in accordance with the opinion of Listiani (2014), fertilizer *Provasoli Enriched Seawater* (PES) serves to provide additional nutrients to seaweed.

The acclimatization of the broodstock in the culture laboratory lasted for 1 month, the treatment carried out during the acclimatization process was changing the water of the maintenance medium once a week. When changing the media water, the seaweed is rubbed one by one by hand gently to remove the mucus that is attached to the seaweed thallus. The seaweed rearing container is covered with plastic which aims to avoid contamination and is given aeration as oxygen supply during maintenance. During the acclimatization process, stressed seaweed will secrete mucus, over time the color will turn pale and white and then begin to fall off. Stressed seaweed must be removed so as not to infect healthy seaweed. Seaweed that is stressed is usually caused by differences in media water temperature, which is different from before, in acclimatization activities, really healthy seaweed will survive, and can be used as seaweed brooders to produce good and quality seaweed seeds.

b) Callus Induction

Explanation Selection

Seaweed that managed to survive the acclimatization process was the best seaweed. The criteria for selecting explants were that there was no mucus in the thallus and a bright color. This statement is in accordance with the opinion of Dwiyani (2015), the criteria for sorting seaweed explants are a brightly colored thallus, no mucus, and a diameter ranging from 0.5 cm. The seaweed selected as explant candidates was cut into smaller sizes to facilitate the explant cutting process. Explant candidates are some of the seaweed selected and cut to be used as explants in the induction process. explant candidates can be seen in image 3.

Image 3. Explant candidate (Personal Documentation, 2020)

Explant Cutting

The purpose of cutting explants is to prepare callus filaments, where callus will later grow on the cut scars. The size of the explant cutting thickness is 4-5 mm, in contrast to the opinion of Rajamuddin *et al*. (2010), explants were cut 2 - 3 mm thick. The cuts were made on a sterile tissue sheet to reduce moisture. In this cutting activity, it must still be in sterile conditions for the success of tissue culture activities. Contaminated explants will not grow callus but will brown or grow fungus. Explant cutting activities can be seen in image 4.

Image 4. Explant cutting (Personal Documentation, 2020).

Thallus Sterilization

Thallus sterilization is done by soaking the pieces of thallus using an antiseptic solution in the form of a soap solution and an iodine solution. The first immersion using a soap solution (image 5a) with a ratio of 3 drops of liquid

soap/50 ml of sterile seawater, wait for 3 minutes while gently shaking. The soaked thallus was rinsed using sterile sea water until it was completely clean. The second immersion using an iodine solution (image 5b) which has been diluted with a concentration of 0.1%, wait for 3 minutes while gently shaking. Furthermore, the thallus was rinsed using sterile sea water until it was completely clean.

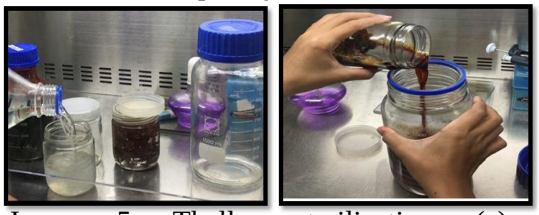

Image 5. Thallus sterilization; (a) sterilization using soapy water (b) sterilization using iodine (Personal Documentation, 2020).

The sterilized thallus was soaked in a hormone solution and incubated for 24 hours. Immersion using a hormone solution aims to stimulate growth in the thallus. In this activity, the hormones used are Indole-3-Acetic Acid (IAA) at a dose of 2.5 ppm, and the hormone 6-Benzylaminopurine (BAP) at a dose of 1 ppm. According to Prasetyo (2016), the function of the hormone Indole-3-Acetic Acid (IAA) is to promote elongation of the thallus that has been cut. The function of the hormone 6 Benzylaminopurine (BAP) is to stimulate shoot growth (Muliati *et al*., 2017).

c) Callus Induction

Making Planting Media

The planting medium used in tissue culture activities is solid Provasoli Encriched Seawater (PES). Solid PES media is obtained by producing independently. The materials needed in the manufacture of media can be seen in Table 1.

Table 1. Media Making Materials

No	Material	Requirement
1	*Bacto agar*	24 gram
2	Sterile Seawater	400 ml
3	PES fertilizer	13 ml
4	IAA hormone	2,5 ppm
5	BAP hormone	1 ppm

Source: (Primary Data, 2020).

Making a media solution is done by mixing all the ingredients listed in table 1. Homogenize the solution on the hot plate using a magic stirrer until it is completely dissolved. Furthermore, the PES media solution was autoclaved at 121oC for 15 minutes. The cold media solution is stored on the petridisk, wait for the media to solidify and then isolate the petridisk using a plastic warmp so that it is not contaminated. The process of making the media solution can be seen in image 6.

Image 6. Process of making media solution (Personal documentation, 2020).

Thallus Cultivation Process

Thallus that has been soaked in hormone solution for 24 hours is placed on a bed of tissue to reduce the moisture of the thallus. The dried thallus was removed using tweezers, the whole process was carried out aseptically in a laminar air flow. In each petridisk contains as many as 5 pieces of explants and arranged neatly. To keep from contamination the petridisk is wrapped using plastic warp. The process of planting the thallus can be seen in image 7.

Image 7. Thallus planting process (Personal documentation, 2020).

d) Callus Regeneration/ Micropropagules Production

Callus regeneration is the process of forming new tissue from pieces of induced thallus grown on *Provasoli Encriched Seawater* (PES) solid media containing PGR. The function of ZPT is to accelerate growth. The formation of callus on the injured explants is caused by

cell autolysis, and from these damaged cells compounds will be produced that will stimulate cell division in the next layer (Lina *et al*., 2013)

The callus induction process was said to be successful, indicated by the appearance of fine white fibers like cotton on the surface of the explant. Fine fibers began to be separated from the explants and planted in new dense *Provasoli Encriched Seawater* (PES) media after reaching a maximum growth of about 2 months in the induction medium. These fine fibers (calus) will grow or begin to regenerate into micropropagules. The callus regeneration process into micropropagules took 3 weeks after the callus was separated from the explants and planted in new solid PES media. Callus sub culture on new media was carried out every 1 month which served to accelerate the growth of micropropagules. Micropropagules can be seen in image 8.

Image 8. Micropropagules (Personal Documentation, 2020).

The replacement of solid media was carried out by moving the callus on a sterile tissue to reduce moisture, then one by one the callus were replanted on the new planting medium. Petridisk is wrapped in plastic warp to avoid contamination. Petridisk was stored in the subculture room for 24 hours at 22^{o}C for temperature acclimatization. The acclimatized culture media were stored in a culture room at a temperature of 20-21^{o}C and irradiated with a light intensity of 1,500 lux and an irradiation time of 12 light and 12 hours dark. The growth of regenerated micropropagules in *E. cottoni* seaweed tissue culture for 4 weeks can be seen in Table 2.

Table 2. Weight of micropropagules

Week	Mikropropagule Weight(gr)
0	0.28
1	0.38
2	0.61
3	0.88
4	1.08

Source: (Primary Data, 2019).

According to Ode (2018), the nutrients provided in the form of nitrogen in large quantities will not be optimally absorbed on the thallus walls, on the contrary in small amounts can limit the growth rate of micropropagules, thus requiring nitrogen in sufficient and balanced quantities. PES media contains NaNO3: 3.5 g/l which supports optimal growth of micropropagules.

e) Micropropagules Regeneration/ Planlet Production

This micropropagule regeneration is an activity where the callus which was originally in the form of fine white fibers has grown into young shoots with a length ranging from 1 to 2 mm. According to Ode (2018), micropropagule regeneration is increasing plant production from selected plant strains through tissue culture techniques. Micropropagules in general can be interpreted as an effort to grow plant parts in aseptic media and then multiply the perfect plant parts in large quantities and in a short time. The next treatment was subculture of micropropagules from solid *Provasoli Encriched Seawater* (PES) medium transferred to liquid *Provasoli Encriched Seawater* (PES) media to accelerate the growth of micropropagules into young plantlets. In this phase it takes 3 months and usually the media water changes will be done once every 1 week. The goal is that the growth of plantlets is not disturbed and to remove mucus and dirt attached to the plantlets that can affect the intake of nutrients for growth. Pictures of plantlets (young seaweed) can be seen in image 9.

Image 9.Planlet (Personal Documentation, 2020).

Plantlets were maintained using 1 liter culture bottles, filled with 980 ml of sterile seawater and added 20 ml of *Provasoli Encriched Seawater* (PES) fertilizer. Sub-culture of young plantlets was carried out once a week by immersing plantlets in a solution of 3 ppm of the hormone 6-Benzylaminopurine (BAP). During the immersion treatment with Benzylaminopurine hormone (BAP), the culture bottles were shaken using a shaker for 12 hours in order to maximize the mixing of the media water solution with the hormone evenly.

After soaking, rinse using sterile seawater, then sorting for grouping the plantlets according to size. This grouping aims to maximize plantlet growth. Plantlets were reared in fresh medium water with 1960 ml of sterile seawater plus 40 ml of *Provasoli Encriched Seawater* (PES) fertilizer. The culture bottles were closed using plastic and given aeration. The culture bottles were stored on a culture rack in a culture room at a temperature of 20-22^{o}C, light intensity 1,500 lux, irradiation time of 12 light and 12 hours dark.

f) Plantlet Acclimatization (in Aquarium/tub)

Plantlets that have been kept in the culture laboratory for 3 months will then be acclimatized in a wet laboratory. Acclimatization aims to adapt plantlets to the new environment. According to Fadilah (2016), the acclimatization stage is an important stage in the propagation of seaweed seedlings because the process of transferring from controlled culture conditions to uncontrolled field conditions requires adaptation. Plantlets from the culture laboratory were first acclimatized to temperature by leaving the culture bottle open so that the water temperature in the culture bottle was the same as the temperature of the new media water. Plantlet acclimatization activities can be seen in image 10.

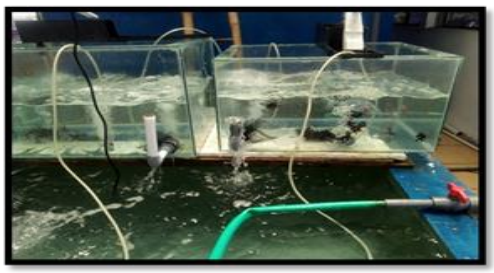

Image 10. Acclimatization of plantlets (Personal Documentation, 2020).

Maintenance of plantlets in a wet laboratory is carried out once a week, by cleaning so that moss and dirt do not stick to the plantlets. The plantlet maintenance media water is always circulated continuously to maintain the oxygen supply of the maintenance media water. Plantlet acclimatization in a wet laboratory takes ± 1 month.

Water quality during maintenance shows the value is still in the good range. The temperature of the media in the morning during this study ranged from 25 - 25.6^{0}C, during the day it ranged from 27 - 30 ^{0}C and in the afternoon it ranged from 26 - 27.5^{0}C. According to Mariska (2013), the optimal temperature for the growth of E. cottoni seaweed is in the range of 25 – 30^{0}C.

The salinity of seawater media in the morning during maintenance ranged from 34 ppt, during the day it ranged from 34 - 36 ppt, and in the afternoon it ranged from 34 - 35 ppt. Mariska (2013), that seaweed can grow well in waters with a salinity of 30-37 ppt.

The pH of seawater media during maintenance ranged from 8.16 to 8.35. This range is still in good condition for seaweed, because according to Marisca (2013), seaweed can live and grow well in the pH range of 7.3 - 8.8 alkaline waters can more quickly encourage the process of disassembling organic matter into mineral salts. such as ammonia, nitrate and phosphate which will be used by aquatic plants as food

g) Seed Propagation in the Sea

Seaweed seeds from tissue culture produced by the seaweed tissue culture laboratory of BPBAP Situbondo were cultivated in Banyuwangi waters, Wongsorejo sub-district. The planting method used is the long-line method, the longline method or commonly referred to as the verticulture method is a method of planting seaweed vertically at a certain depth, adjusted to the penetration ability of sunlight (Widowati et al., 2015). Preparations made for propagation of seeds at sea are preparing a ris rope with a length of 25 m. Every 1 ris rope there are 250 points and each point there is a pair of small ropes that function to tie seaweed seeds. The distance from one seed to another is ± 10 cm. Seaweed planting can be seen in image 11.

Image 11. Seaweed planting (Personal Documentation, 2020)

According to Haryasakti (2017), environmental factors that support the growth of seaweed include elements of phosphate and nitrate as well as water quality (temperature, salinity, brightness, current waves and pH). Seaweed generally requires large amounts of N and P, elements of N and P are needed for reproductive growth and for the formation of food reserves in the form of organic substances such as carbohydrates, proteins, and fats. The results of observations of water quality in the waters of wonsorejo, Banyuwangi can be seen in table 3.

Table 3. Water quality parameters

No	Parameter	Unit	Result	Optimal
1	pH		8,29	6,8 – 9,6
2	Temperature	^{0}C	28	26 – 33
3	Salinity	ppt	32	15 – 38
4	Brightness	m	2	2 – 5
5	Depth	cm	500	60 – 200
6	Current	cm/d	2,22	1, 2 – 2,0
7	Wave	cm	5 – 30	30
8	Nitrite (NO_2)	mg/l	0,52	0,01 – 0,7
9	Phosphat (PO_4-P)	mg/l	0,023	0,003

Source: (Primary Data, 2020).

Based on the measurements, the phosphate content at the location where the phosphate content was observed was 0.023 mg/l. According to Haryasakti (2017), phosphate levels that exceed 0.003 mg/l can be a limiting factor. What is meant by limiting factor is the condition of the minimum phosphate content that can still support seaweed cultivation. However, in the conditions found in the field, phosphate levels in the water can support the growth of *Kappaphycus alvarezii* seaweed.

Conclusion

From the results of research on "Seaweed Tissue Culture in ", the following conclusions are obtained:

- The time required to obtain tissue culture seedlings is ± 1 year.
- Acclimatization of broodstock, acclimatization activities are divided into 2, namely acclimatization of broodstock in the wet laboratory and acclimatization of broodstock in the culture laboratory. The length of time required for the acclimatization process is 1 month.
- Callus induction, the stages of activity starting from the selection of explants until the treatment of thallus becomes callus. Callus treatment takes 4 months.
- Callus/micropropagules regeneration is characterized by white cotton-like fibers that have started to grow new shoots. This callus regeneration process takes 4 months.
- Regeneration of micropropagules, the period in which new shoots develop from micropropagules to a size of ± 2 mm, this

micropropagule regeneration process takes 3 months.

- The plantlet acclimatization process aims to adapt plantlets that were previously in solid media, transferred to liquid media to adjust to the media.
- Seed propagation at sea is carried out in the waters of Banyuwangi, Wongsorejo District, where the seeds from tissue culture are given as seed assistance to farmers.

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