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The Development and Potential of Seaweed Tissue Culture

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Abstract

Seaweed is widely used in many industries such as cosmetic foods, and drugs resulted in its increasing demand. To complete the market demand, it is necessary to escalate the seaweed cultivation. One of the problems of cultivation is the availability of the seed. The conventional and vegetative system of seaweed seed reproduction in the form of direct fragmentation or thallus cutting affects the degradation of seed quality. Therefore, another system of reproduction must be performed. One of the best ways to multiply the plants is tissue culture technique. Tissue culture is a plant multiplication technique using the artificial medium in a controlled and sterilized environmental condition. The advantage of this technique is the seeds will be all even, pathogen-free, numerous in one single production, and not influenced by any season. Some methods used in seaweed tissue culture are protoplasm culture, spore culture, and somatic embryogenesis. The government takes a serious action in seaweed industry by releasing the regulation of seaweed research and development through Presidential Regulation No. 33 of 2019 about the roadmap of National seaweed industry development 2018-2021. Therefore, the research and development of seaweed tissue culture is still widely open for its underexplored potential. On the other plants, this technique has reached the utilization stage to produce bioactive compounds e.g. the increasing production of saponin in Javanese ginseng callus culture. Based on that reason, the tissue culture technique can be implemented to search seaweed's bioactive compound potential.

Introduction

Seaweed is one of well-known marine commodities in the society. Many people in coastal communities cultivate and earn it for living. Due to its beneficial use, such as an ingredient in producing jelly, cosmetics, and food containing high fiber, the demand of seaweed remains increasing. The increasing demand occurs not only in Indonesia but also in Europe, America, Africa, and Pacific Asia as much as 2.8% increasing number every year (Bixler&Porse, 2011). In order to fulfill the increasing market demand, the production of seaweed is also being upgraded. The volume of seaweed production increased from 3.8 ton in 1990 to 19 ton in 2010 (FAO, 2012).

One of the most frequent problems found in seaweed cultivation is the availability of seed. The seaweed seed is usually obtained from vegetative reproduction such as direct fragmentation and thallus cutting. However, the repetitively vegetative multiplication in seaweed cultivation affects its qualities such as the degradation of genetic variation, growth, and quality of jelly. The vegetative cultivation also allows the epiphyte organism, parasite, and pathogen from the parent plant to be carried away in the seed affecting the degradation of cultivation product due to that contaminated seed (Mulyaningrum, 2018)

One of the mechanisms to overcome the problem is the seed multiplication by using plant tissue culture method. It is a technique of plant multiplication by using artificial medium in aseptic environment. The multiplication by using in vitro culture method is applicable for seaweed based on several advantages such as; 1) being able to produce high quality seed, 2) producing the pathogen-free plant 3) not depending on any season and the condition of natural environments (Purita *et al*., 2018). The method used in seaweed plant tissue culture is basically similar with that in vascular plant. It is generally applied by preparing accent explants which is put in the solid medium enriched with various macro and micro-nutrients, vitamins, and sugar (as a carbohydrate source), and plant growth regulator substance (ZPT) (Reddy, 2008). Basically, all seaweed variants can be multiplied by the plant tissue culture method. There are several in vitro techniques of plant tissue culture method such as 1) spore culture 2) protoplasm culture 3) somatic embryo genesis (Mulyaningrum, 2018). The research related to the plant tissue culture method, especially those of seaweed, is found to be rarely performed roundly including the advantages and potencies. Furthermore, this research presents the development of plant tissue culture method in Indonesia.

Methods

The method in this research is literature studies. The method of literature studies is implemented by reading from relevant sources related to the data to be obtained. Activities in literature studies include collecting library data, reading and writing, and processing research data. Data collection techniques using literature, integrated and presenting data (Danandjaja, 2014). Kartiningsih (2015), this literature study was conducted by researchers to obtain a rationale and determine hypotheses. The data obtained comes from articles, books, magazines, documents, news, etc.

In this study, researchers used more reference articles containing national journals and international journals. Articles that use as sources related to the potential of seaweed tissue.

Results and Discussion

Explant For Tissue Culture

Seaweed tissue culture requires healthy explants identified by the healthy appearance, being disease-free, containing talus, good holdfast, having bright colors, having numbers of branch, and young age (Yong et al., 2011). The explants used in the culture are the talus of the seaweed coming from natural environment. Several methods had been developed by researchers to acquire the high-quality explants. The morning and the evening were the recommended time to take the sample of the plant. The fresh sample was cleaned from the dirt agent in the form of epiphyte and other microorganisms (Mo *et. al*., 2020). The cleaned sample was kept in the poly bag and put in the cool box based with ice bag and covered by used newspaper on top. This mechanism could anticipate the damage caused by the distanced place of the sample taking to the laboratory (Lumbessy, 2019). The sample arrived in the laboratory was then scrubbed to clean the dirt agent left. The next step would be the sample acclimatization so that the sample could easily adapt with the laboratory environment. The adaptation was performed by preserving sample in a 50L tub which was continuously water-irrigated for 1 month. The Seaweed with the optimal growth and healthy characteristic was ready to be the source of explants (Mo *et. al*., 2020).

Aseptic Culture Condition

Tissue culture is plant multiplication technique by using artificial medium in an aseptic environtment (Purita, *et al*., 2018). Aseptic technique in the seaweed tissue culture refers to the vascular plant tissue culture. Aseptic condition in seaweed tissue culture is more difficult to achieve than that in the vascular plants culture. However, it must be performed nevertheless since it is a basic factor in in vitro culture.

Explant's sterilization became the important factor in seaweed tissue culture. Several methods had been reported by researchers to create aseptic explants. The regular methods used in the research were presented in table 1.

Table 1. Explants sterilization in some variant of Seaweed explants

No	Explant's variant/ Species	Active subtance	Method	Reference
1	Thalus/ *Eucheuma striatus*	AgNPs solution	Immersion	Mo *et al*., 2020
2	Thalus/ *Kappaphycus*sp.	E3 anti-bacterial solution	Immersion	Yunque *et al*., 2011
3	Tetrasporophytes growing/ *Gracilariatenuistipitata and Gracilariaperplexa*	solution of sterile seawater (filter-sterilized and autoclaved for 30 min at 121°C), with sodium hypochlorite (0.5%) and detergent (200 µL)	Immersion	Yokoya *et al*., 2004
4	Tetrasporophytes/ *Grateloupiasubpectinata*	1% germanium dioxide	Immersion	Adharini and Kim, 2016
5	Thalus / *Kappaphycusalvarezii*	0.5% povidone iodine (Betadine) and CW medium containing 3% antibiotics mixture (penicillin G 1 g, streptomycin sulphate 2 g, kanamycin 1 g, nystatin 25 mg, Neomycin 200 mg)	Immersion	Sulistiani *et al*., 2012
6	Thalus/ *Kappaphycusalvarezii*	iodine 1% and larutanantibiotik	Immersion	Wiwien *et al*., 2019

The sterilized explants influenced the result of seaweed tissue culture. The contaminant removal process started with physical cleaning (using hands or brush). The removal of microscopic contaminant was performed by using several chemical substances such as AgNPs solution, anti bacteria to remove the bacteria, Anti fungus to remove fungi, and germanium oxide to remove diatomic algae. Beside explants, medium used in in vitro culture process also needed sterilization. The common medium used in seaweed tissue culture is including Provasoli enriched seawater/PES (Provasoli 1968) and Conwy/CW (Liao, 1983). Those mediums contained several components such as macronutrient and micronutrient. The sterilization for the heat and pressure resistant medium uses autoclave (with temperature 121 °C and pressure 1 atm for 15-30 minutes) (Sulistiani et al., 2012). Substances like sea water, vitamin, and hormone (NAA, BAP) were sterilized by using 0.22-micron filter (Mo et al., 2020). Aseptic mechanism was applied to avoid the contamination by using 70% percent alcohol to clean the location of subculture. The usage of equipment e.g., laminar air flow would support the aseptic environment in the activity of seaweed tissue culture. The usage of Bunsen to burn the equipment e.g., tweezers and other fireproof tools also created the more aseptic environment.

Seaweed Tissue Culture Method

a. Protoplasm Culture Method

Protoplasm is a living cell of the plant which has been separated from its wall. The cell wall removal process in protoplasm culture is both mechanically (cell plasmolysis and cell wall cutting) and enzymatic (using several enzymes e.g., Cellulose, Pectin and Hemicelluloses). The challenges of this method are the problem of cell's osmosis pressure and cell's regeneration capability. The cell's wall removal resulted in the low cell defense mechanism toward the environment and being easily lyses. It also took effect to the viability and cell regeneration capability. To maintain the osmosis pressure, it is important to create isotonic external condition and keep the cell viability for a long time. This condition could be created by using anti blasting substance such as mannitol/ sorbitol. In the other hand, cell choice was the key for cell's viability and regeneration matters. The usage of young cell or cell in the exponential phase would boost the success factor of this protoplasm culture method because the young cell in the exponential phase still split actively and it has high regeneration capability. This protoplasm culture technique developed because this protoplasm cell can also be used in biotechnology such as metabolic engineering and genetic transformation. The protoplasm culture method also allowed the amalgamation of two cells called fuse-protoplasm. By applying fuse-protoplasm, the new characteristic would be created and it became the new candidate of high-quality seaweed seed. Several kinds of seaweed that has been successfully multiplied by using protoplasm culture method are Chlorophyta, Phaeophyta dan Rhodophyta (Mulyaningrum, 2018).

b. Spore Culture Method

Spore used to multiply the seed by using spore culture is carpospores type. This type of spore is large in size making it visible without microscope. Carpospore is located in a spore bag (cytocarp). Carposporhyte that must be prepared for the seaweed seed multiplication should have several cytocarp characteristics such as light brownish colour, large diameter, clean thallus, yellowish color, brown cytocarp with relatively big diameter.

Figure 1. Thalus Gracilaria sp which contain Carpospore (Carposporophyte) (Source: Lideman et al, 2016)

The selected Carposporhyte was then cut into 1-1.5 cm containing 3-4 cytocarp in each cut. After the right size was acquired, it was sterilized by immersing it in 1% iodine solution for 2-3 minutes. The next step was setting the carposporhyte piece in the filter soaked in the preservation medium with 2-3 cm depth above the medium surface (Lideman et al., 2016).

The preservation medium used in this research was Pavasolls Enrich Seat water (PES) which has nitrogen and phosphate structure needed for seaweed growth. After 5-7 days, carphosporhyte was taken from the preservation medium. The spore attached to the string was preserved until having thalus and hold fast (young Gracilaria).the environment condition was set to temperature 25℃, 500-1000 lux lighting and 30 ppt salinity. Periodic checking was performed by using microscope to ensure whether the spore could attach and growth or not. Medium PES could grow the spore into Gracilariasp with thalus in 30-40 days. Young Gracilaria sp. grew and could be planted as seed after 2 months old and acclimatized in the cultivation location (Lideman et al., 2016). According to Mulyaningrum (2018), this spore culture method was mostly applied in seaweed cultivation of trhodophyta dan gracilariaceae.

c. Somatic Embryogenesis Method

Somatic Embryogenesis is a forming process of new plant bipolar embryo inducted from the somatic tissue or strait. This seaweed multiplication method needed several explants from young tissue which actively splits. Explants would be planted in culture medium with the addition of plant regulatory substance (ZPT). There were two ways of Somatic Embryogenesis, direct and indirect (through the callus forming). ZPT combination, ZPT concentration, medium and other cultures factors would determine whether the seaweed Somatic Embryogenesis would overtake through the direct or indirect ways.

Rajamudin *et al,* (2016), reported the successful callus induction of *Kappaphycusalvarezii* as the first step of *K.alvarezii* multiplication with Somatic Embryogenesis method. The best result from that research with the highest percent of callus parent (90%) was acquired from callus induction by using ZPT ratio in the form of IAA and kinetin with ratio 1.0:1.0 mg/L in jelly concentration 0.8% and 1.0%. At the next stage, callus was inducted to regenerate into somatic embryo cell mass by using solid semi-jelly medium. It was reported that in this stage, ZPT did not have any visible influence o the growth of somatic embryo cell mass. Cell mass from semi solid medium was cultured in the liquid medium to form new filaments (seaweed shot). A 3-4 mm will experience a new cell elongation somatic embryo cell for average 0.5 mm in a month of culture process. The development stages of embryogenic cell from one cell into one filament were presented in picture 2. Somatic embryogenesis method was widely applied in Chlorophyta, Rhodophyta, dan Phaeophyta (Mulyaningrum, 2018).

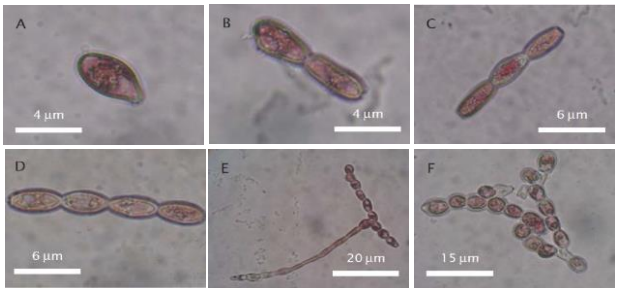

Figure 2. The development of Cell Somatic Embryogenesis *K. alvarezii* : (A) single cell, (B) double cell, (C) three cell, (D) four cell, (E & F) filament (Source: Rajamudin et al, 2016)

Conclusions and Recommendations

The regulation of the research and development related to seaweed acquired many attentions from the government. By releasing Presidential Regulation No. 33 off 2019 containing the roadmap of national seaweed industrial development in 2018-2021, the government of Indonesia signified serious efforts in the development of seaweed industry. The establishment of fishery industry through seaweed cultivation using tissue culture provides some hopes in the seaweed development in potential coastal area. The fishery development program is supposed to bring economic, social, and cultural changes of the seaweed cultivator. Therefore, the seaweed cultivation by using tissue culture is expected to be an instrument to increase community's income in the coastal, small islands, outskirts, or borders area.

The research and development of seaweed tissue culture is still widely open. Most of researchers are still focusing on red algae because recently Indonesia is recognized as one of five biggest macro algae producers in the world. Most macro sea algae species exported from Indonesia comes from red algae (Rhodopyceae) such as: Kappaphycusalvarezii, Eucheuma spinosum, Gracilariasp, and Gelidiumsp (Pangestuti et al., 2019). In the other hand, Indonesia has 903 seaweed species, with the composition of green seaweed / Chlorophyta for about 201 species, red seaweed 564 species and brown seaweed 138 species (Atmaja and Prud'homme van Reine, 2012; Atmaja and Prud'homme van Reine, 2014). Many seaweed species are not optimally exploited, in the other words; it is under-exploited resources. Meanwhile, those species are potential for food and pharmacy industry.

Beside many under-exploited seaweed species, the production of metabolite compounds coming from the seaweed which is beneficial for functional foods and pharmacy can be upgraded by using tissue culture. The potential compounds inside seaweed are hydrocolloid which is utilized as industrial ingredients for jelly, yeasts, alginate, and secondary metabolite compounds in the form of bioactive compounds such as alkaloid, fenolik, flavonoid (Julyasih and widiyanti, 2020). The production of metabolite compounds in nature is influenced by the environment condition. The existence of the stress factor will define the metabolite level especially the secondary one. By applying tissue culture method, the environmental condition including the stress factor can be raised by adding elicitor. A research performed the increasing saponin production in callus culture of Javanese ginseng (*Talinumpaniculatum*Gaertn) by adding some elicitor in the form of yeast extract and cytosine (Wijaya *et al*, 2020; Dena et al, 2021). Based on that fact, it is possible to increase both primary and secondary metabolite production of the seaweed by using tissue culture technique.

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