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Producing New Variety of *Gracilaria* Sp. Through Cross Breeding

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Abstract: In recent years the quality of *Gracilaria* sp. cultivation in some cluster areas has been decreased because of environmental problems, like high temperature, sewage contamination, and Indonesian water-quality deterioration that have constrained the growth. Breeding technology of *Gracilaria* sp. is an effective option that can stimulate the development of cultivation technology for Indonesian *Gracilaria* sp. like gamet induction. As a result, it then can improve either the quantity or quality of *Gracilaria* sp. production that is economically potential. Through cross breeding and free living technique programs, moreover, super variety with good and stable quality is obtained. This study, therefore, is divided into 3 stages involving (i) isolation and identification of *Gracilaria* sp. obtained either from natural environment (wild environment) or from cultivation process, (ii) cross breeding and free living technique for improving the rate of *Gracilaria* sp. gamet induction, (iii) technology engineering of breeding medium for improving the growth, the number of chlorophyll *a,* and the moisture level of *Gracilaria* sp. As a result, a new variety of *Gracilaria* sp. created has average ability in secreting gamet at pH 7 about 6666.67 ± 2886.75 cells/ml, with the growth rate of thallus mass about 2.56%/day, with the number of chlorophyll *a* about 0.001948 μg/ml, and with the water content of thallus about 28.48%. Thus, it is considered to be better than *Gracilaria* sp. either from natural environment or from cultivation process.

Key words: *Gracilaria* sp., cross breeding, free living technique, variety

INTRODUCTION

Seaweed is a well-known commodity and has a lot of advantages for industries of food and drink materials, personal care, cosmetics, animal food, steels, ceramics, polish, ink, paint, mining, coal briquettes and asphalt, papers and pulp, textiles, fertilize, and medicines (pharmaceutical) as dietary fibers, antihypertensive substances, hypercholesterolemic activity, heparinoid activity, antihelmintic effect, immunity activation, anti tumor, anti cancer, antivirus, antibacterial, algicidal, and others[6]. However, from 782 species of seaweeds in Indonesian waters, there are only 18 species of 5 genus newly marketed, while from five of those genus there are only *Euchema* dan *Gracilaria* newly cultivated.

Therefore, until now Indonesian entrepreneurs of seaweeds still compete in exporting dried seaweed to China, Hongkong, Philipina, Vietnam, South Korea, and Japan with the selling value about US$ 0.5/kg. Actually, the price of seaweed flour categorized as industrial grade about US$ 3.30-3.80/kg, categorized as food grade about US$ 12-14/kg, and categorized as research media and pharmacy materials about US$ 55-110/kg. Unfortunately, according to Istini *et al.*[18], Indonesia still imports more than 150 MT/year of karaginan, 260 MT/year of gelatin, and 3100 MT/year of alginat, while for export Indonesia export 14000 tons/ year of dried seaweed.

The low selling price of seaweeds in Indonesia as well as the competition of seaweed polysaccharide products (gelatin, karaginan and alginate) is actually caused by the quality of seaweed (e.g. *Gracilaria* sp. as the gelatin polysaccharide source) that is still various and often has many growth problems as the environment changing[33]. On the other side, the high and stable growth rate and moisture content become absolute requirements determined by every importer of seaweed. Therefore, the breeding engineering of *Gracillaria* sp. is important to be implemented in all cultivation groups of *Gracillaria* sp. in order to obtain the super variety with the low moisture content and the resistance to environmental problems.

In Indonesia, the technology developing nowadays still focuses on how to create the high quality seaweed by using mass cultivation (outdoor cultivation). Alamsjah *et al.*[3] also mention that the cultivation of seaweed, *Gracilaria* sp., in Indonesia still uses vegetative reproduction model (cutting) by using bottom method, off-bottom method, and floating

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method. Moreover, the harvesting process of *Gracilaria* sp. is generally conducted after the seaweeds are in the age of 6 – 8 weeks, and then they are dried under the sun for about 2 – 3 days. And, the dried seaweeds considered to meet trading standardization are those containing strange materials like sands / corals that must not be more than 5% and moisture content that must maximally be 30%[47]. However, the quality in many cluster areas of *Gracilaria* sp. cultivation in Indonesia has been decreasing recently because of the environmental problems like the increasing temperature, the sewage contamination, and the deteriorating Indonesian water quality.

Thus, technology engineering through the use of genetic principles is used in breeding plants from high and low levels, and the new variety of cultivated species then can be created from the genetic ordering either specifically or communally derived from many character combinations. In classical plant breeding, furthermore, the deliberative use of interbreeding (crossing) is good for closest or furthest characters of the species in order to create the new expected plan variety. Plants that got crossbreeding then basically get genetic test from one variety to another genetic series. Some experiments using the cross breeding even are often conducted to improve the quality and the quantity of harvest, to improve the ability of adapting to environmental problems (salinity and temperature), to improve the resistance to virus, fungi, and bacteria, to improve the sustainability over pesticides and herbicides[22,45,23,37].

Free living technique, moreover, is a breeding technique of seaweeds by using generative reproduction process that has been implemented in cluster areas of *Undaria pinnatifida* cultivation in France (Kaas, 1998). In this technique, the combination of the best character of male and female gametophyte is conducted in order to be able to be secreted from thallus and to fertilize, thus, the new variety with the superiority of high polysaccharide content and the ability of adapting to the extreme environmental problems is created[29].

In this experiment, furthermore, *Gracillaria* sp. growing wildly in natural environment with characteristic of strain that is more endurable over the environmental problems is tested with that derived from cultivation process with better growth rate through cross breeding and free living technique. The aim of this experiment is to obtain the new variety of *Gracilaria* sp. with good quality, especially for the gamet induction rate and the growth rate, as well as to enrich plasma nutfah and to improve the selling value of seaweeds produced as the commodity of export.

MATERIALS AND METHODS

***Gracilaria* sp. Collection:** The collection of seaweeds from intertidal area was conducted for the first month of the experiment period. The damage of ecology during taking samples was possibly minimized by maintaining algal stem. All samples then were taken to laboratory by putting them into plastic bags containing sea water for preventing evaporation, and were cleaned afterwards with distilled water for separating potential contaminant away[4]. Furthermore, the areas of *Gracilaria* sp. cultivation group have been determined by Bureau of East Java Fishery and Marine in order to make those which are wildly living in natural environment and are growing as the product of cultivation become priority in collecting samples that will be engineered, thus, the super variety with the high rate of growth will be obtained. The morphology and anatomy characteristics of the species then must be checked by using optical microscope in order to isolate and identify expected specimen[1].

Preparation of Thalli Selected Through Indoor Culture: The method as done by Jin and Dong[19] was conducted in order to evaluate sporophyte and gametophyte of the tissue of seaweed. The specific reproductive stage of the seaweed then was identified based on Seavy[39]. Next, twenty fragments of the seaweed, were cultured in Enriched Seawater Medium (ESM)[34] by using 500 ml flat bottom aeration flasks (pre-culture treatment), and the medium then was replaced once three days. The Pre-culture tissue, finally, was cultured in the main culture in ESM for about 7 days by using 1000 ml flat bottom aeration flasks, and the medium then was replaced once three days[2].

Axenic Culture of Selected Seaweeds: Tissue of seaweeds produced from main culture was similar with the analysis result of the best gelatin polysaccharide content conducted by the cultivation with axenic culture. The selected tissue and cleaned with 0.02% detergent liquid (Joy, Procter & Gambler) by using autoclaved seawater (ASW) for about 1 minute. Afterwards, the segment was soaked in 20 ml ASW containing 2% Isodine (Meiji) for about 1 minute. The segment, furthermore, was cleaned with ASW and treated in 100 ml ASW containing antibiotic mixture (penicillin G 1 g, streptomycin sulfate 2 g, kanamycin 1 g, nystatin 0.025 g, and neomycin 0.2 g in 100 ml distilled water; Lipperheide dan Evans,[28] for about 24 hours. The tissue was cleaned in ASW and checked for its axenicity level in ZoBell 2216E of gelatin medium [47] for more than 5 days. Finally, the tissue of seaweed were cultured.

Cross Breeding and Free Living Technique: Cross breeding was conducted based on the superiority of selected *Gracilaria* sp. growing in natural environment

and in cultivation process. The following process was the application of free living technique started with isolation and identification of fertile plants obtained from the previous selection result. Fertile pieces of thalli obtained then were put into beaker containing NaOCl solution with 90 ppm for 1 minute. The repetitive cleaning and cutting of thalli were actually expected to make the secreting of male and female gametophyte fast. Thalli of sori were then incubated for 1 day at the temperature 18ºC, so it got dehydration. On the next day, furthermore, thalli were soaked into sterile sea water, and checked for the secretion of gamete. The gamete induction was also conducted with the treatment on medium pH 5, 6, 7, 8, 9, and 10 (for about 6 days).

In this stage, the mobility of gamete then was checked under optical microscope. After 20 minutes, the mature thallus or ripe fragments of thallus were moved. Next, the remained solution was filtered with mesh size 30, 20, and 10 µ. 10 – 20 ml of the filtered solution then was poured into flask containing sterile nutrient solution (f/2 medium). During the experiment, moreover, the temperature was set between 15 – 17ºC; the intensity of light was also set at 40 µmol photons $m^{-2}s^{-1}$; and oxygen was given for 24 hours. The temperature, however, was increased gradually, from 0.5ºC per day to 22ºC. The reason is because at the temperature, 20ºC, the intensity of light was decreased into 20 – 30 µmol photons m^{-2} s^{-1}. The substitution of medium then was conducted periodically for about once a week. The emergence of thallus in this stage was considered as the indication of new variety as the result of gamete fertilization in selected *Gracilaria* sp. with their superiorities.

Test for Selected *Gracilaria* sp. Variety: Test of selected *Gracilaria* sp. variety was conducted by using medium completed with organic fertilizer (compost) and inorganic fertilizer (NPK) for about 35 days. The combination of organic fertilizer (compost) and inorganic fertilizer (NPK) was as the following (0 g/L : 2 g/L), (0.5 g/L : 1.5 g/L), (1 g/L : 1 g/L), (1.5 g/L : 0.5 g/L), and (2 g/L : 0 g/L). Moreover, the fertilizer was put into cloth, tied, and hung above the experiment container. The circulation of sea water then was conducted once three days and for about 35 days of maintenance. Relative Growth Rate (RGR) was also measured based on Raikar *et al*.[36], while the growth of relative length of thallus produced was measured based on Effendie[11]. Procedure of chlorophyll *a* measurement was conducted based on Mackinney[30] and Hoffman and Werner[15], meanwhile the analysis of water content was conducted based on the procedures of Sudarmadji *et al.*[41].

Data Analysis: Data obtained were divided into two types, which are qualitative and quantitative data. Qualitative data derived from the isolation and identification results of *Gracilaria* sp. that were from natural environment group and cultivation group, including data about the origin of specimen and the ability of adaptation to environment. Meanwhile, quantitative data derived from the measuring result of *Gracilaria* sp., created through cross breeding and free living technique, that was then related with the number of gamete as the induction result, the RGR, chlorophyll *a*, and water content, as well as the ability of adaptation to the extreme environmental problems. All of the experiments in this study then were done separately in a least triplicate and tested by ANOVA test ($p<0.05$). Thus, if there was any difference among the treatments, it would be tested by Duncan Multiple Range Test with significance degree 0.05 in order to analyze the difference among all of those treatments [24].

RESULTS AND DISCUSSION

Identification of *Gracilaria* Sp. From Natural Environment and Cultivation Process:

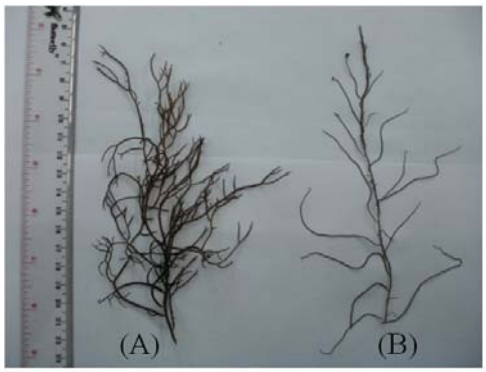


Fig. 1: Morphology of Gracilaria sp. obtained from cultivation process (A) and natural environment (B)

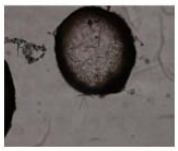
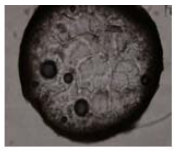
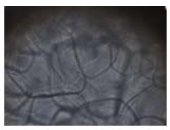
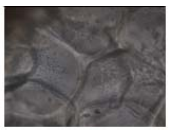
Gracilaria sp. obtained from natural environment could be able to survive in the extreme condition of environment affected by higher wave and temperature. The reason is because *Gracilaria* sp. actually grows in intertidal area with relatively lower water level (< 60 cm during low tide) than that grows in the condition of fishpond environment where *Gracilaria* sp. is cultivated. However, the cultivation of *Gracilaria* sp. in Indonesia is generally conducted in fishpond area with sandy mud base, about 1 km from beach, with the water level of fishpond about 60 – 80 cm, closed to freshwater reservoir, and at pH of fishpond water 8.2

– 8.7. The other advantage of *Gracilaria* sp. cultivation in fishpond is that it is relatively safe from wave attack, strong sea current, and predator animal attack. The basic difference among both *Gracilaria* sp samples used in this experiment can be seen in Table 1.

Gamet induction of *Gracilaria* sp: The result of cross breeding and free living techniques of *Gracilaria* sp. variety selected shows that the active gamete mobility had normal size (Figure 2).

ANOVA measurement shows that gamete induction with pH manipulation of media influenced significantly ($p < 0.05$) on the number of *Gracilaria* sp. gamete. The result tested with Duncan Multiple Range Test then shows that the best average number of gamete *Gracilaria* sp. was at D treatment (pH 7) that was significantly different from the other treatments ($p < 0.05$).

Table 1: The difference of *Gracilaria* sp. obtained in natural environment and in cultivation process

Identification	*Gracilaria* sp.	
	Natural Environment	Cultivation Process
Color and morphology of thallus	Brownish and smother thallus	Blackish brown and harder thallus
The adaptation ability towards environmental problems	High	Low
The rate of growth	Slow	Fast
The bud branching for thallus with length 10 cm (mean ± SD)	21.33 ± 2.89	56.67 ± 7.02
The number of thallus / grams of wet mass (mean ± SD)	98.33 ± 17.10	59.67 ± 8.33
The vertical cutting of thallus (zoom in 100 x)		
The horizontal cutting of thallus (zoom in 100 x)		
The picture of cell histology (zoom in 400 x)		

Note: the line scale of seaweed shown was about10 µm

Fig. 2. Gracilaria sp. gamete

Table 2: The average number of *Gracilaria* sp. gamete in every treatment

Treatment	The average number of gamete ± SD (cell/ml)
A (control)	$2500^{d} \pm 0$
B (pH 5)	$3333.33^{c} \pm 1443.38$
C (pH 6)	$5000^{ab} \pm 0$
D (pH 7)	$6666.67^{a} \pm 2886.75$
E (pH 8)	$5833.33^{ab} \pm 1443.38$
F (pH 9)	$4166.67^{bc} \pm 1443.38$
G (pH 10)	$3333.33^{c} \pm 1443.38$

Note: the different superscripts show significant difference ($p < 0.05$)

Table 3: The result of Duncan Multiple Range Test on the Relative Growth Rate (RGR) of *Gracilaria* sp. for 35 days

Treatment	RGR (%/day)	RGR (transformation √y)
A (Compost : NPK = 0 g/L : 2 g/L)	1.36	1.16^{c}
B (Compost : NPK = 0.5 g/L : 1.5 g/L)	1.86	1.36^{b}
C (Compost : NPK = 1 g/L : 1 g/L)	2.56	1.60^{a}
D (Compost : NPK = 1.5 g/L : 0.5 g/L)	1.82	1.35^{b}
E (Compost : NPK = 2 g/L : 0 g/L)	1.19	1.09^{c}
F (without fertilizer)	1.12	1.06^{c}

Note: the different superscripts show the significant difference ($p < 0.05$)

Table 4: The result of Duncan Multiple Range Test on the concentration of chlorophyll *a Gracilaria* sp.

Treatment	The amount of chlorophyll *a* at final average of research (*μ*g/ml)	The amount of chlorophyll *a* in average (Transformation √Y)
A (Compost : NPK = 0 g/L : 2 g/L)	0,001599	$0,0156^{c}$
B (Compost : NPK = 0,5 g/L : 1,5 g/L)	0,001908	$0,0235^{a}$
C (Compost : NPK = 1 g/L : 1 g/L)	0,001948	$0,0243^{a}$
D (Compost : NPK = 1,5 g/L : 0,5 g/L)	0,001874	$0,0227^{ab}$
E (Compost : NPK = 2 g/L : 0 g/L)	0,001667	$0,0177^{bc}$
F (without fertilizer)	0,001440	$0,0075^{d}$

Note: the different superscripts show the significant difference ($p < 0.05$)

Table 5: The result of Duncan Multiple Range Test on the water content of *Gracilaria* sp.

Treatment	Water content in final average of research (%)	Water content in average (Transformation √Y)
A (Compost : NPK = 0 g/L : 2 g/L)	29,70	$1,55^{bc}$
B (Compost : NPK = 0.5 g/L : 1.5 g/L)	28,99	$1,76^{ab}$
C (Compost : NPK = 1 g/L : 1 g/L)	28,48	$1,90^{a}$
D (Compost : NPK = 1.5 g/L : 0.5 g/L)	29,91	$1,47^{bc}$
E (Compost : NPK = 2 g/L : 0 g/L)	30,28	$1,33^{c}$
F (without fertilizer)	30,39	$1,31^{c}$

Note: the different superscripts show the significant difference ($p < 0.05$)

Relative Growth Rate (RGR): In the growing stage of thallus, the result of cross breeding and free living technique selected shows that the combination between compost and NPK significantly influenced ($p < 0.05$) on the rate of daily mass growth of *Gracilaria* sp. The result tested with Duncan Multiple Range Test then shows that the combination between compost and NPK gave the best rate of daily mass growth at treatment C

(compost and NPK = 1 g/L: 1 g/L) that was significantly different from other treatments ($p < 0.05$).

The Concentration of Chlorophyll *A Gracilaria* sp: The result of ANOVA calculation shows that combination between compost and NPK fertilizer has definitely affected the amount of chlorophyll *a* with selected variety *Gracilaria* sp. This is followed by Duncan multiple range test showing that combination between compost and NPK fertilizer which gives the best amount of chlorophyll *a Gracilaria* sp. is on the treatment C (Compost : NPK = 1 g/L : 1 g/L), but it does not have definite difference from treatment B, D and E.

The Water Content of *Gracilaria* sp: The result of ANOVA calculation shows that combination between compost and NPK fertilizer has definite effect ($p < 0.05$) on water content of selected variety *Gracilaria* sp. This is followed by Duncan Multiple Range Test showing that combination of compost and NPK which can give the best water content of *Gracilaria* sp. is on the treatment C (Compost : NPK = 1 g/L : 1 g/L), but it does not have definite difference from treatment B, D and A.

Discussion: The difference of *Gracilaria* sp. growing in cultivation is the result of adjustment in a long period in which both groups of seaweed have positive and negative sides in their breeding. Through a research which involves seeding *Gracilaria* sp. with cross breeding and free living technique on wild seaweed either living in nature or cultivation, new variety for the species of *Gracilaria* sp. in a good quality can be obtained. Furthermore, ability to adjust with the environment, the rapid growth and low water content can also be obtained.

This is shown in the gamete induction where new variety can easily be inducted, so the production of gamete can rapidly be obtained. Huang *et al.*[16] states that the gamete induction in seaweed can be applied by changing the adult seaweed to be stress. Consequently, the seaweed will easily produce gametes. The gamete induction in new variety of *Gracilaria* sp. shows the highest result on the treatment pH 7. Factor determining greater gamete induction depends on the condition of selected seaweed and the method that is assumed to cause stress signaling. As the reason, when the seaweed is stress due to enumeration and pressure from the environment, the seaweed *Gracilari* sp. leads to release cell of gametes as signal to respond any change in its environment. According to Utama[46], stress is an external factor in which particular condition will lead organelle activity to balance its mechanism known as one model called stress signaling. The condition outside the cell (physical, chemical condition and energy) will affect the activity inside the cell. The cell must be able to survive or adapt through homeostasis process. In this case, stress can be assumed as hindrance or disruption but in the other sides it can be stimulation or induction.

The seaweed which is able to adjust to its environment can yield components of hormone that physiologically trigger reproduction and growth as signal response. Gardner[12] reports that stimulation from the environment can lead to the production of growth hormone. The plants which are stress due to stimulation will produce phytohormone or other growth hormones such as auxin, gibberallin, cytokinin and brassinolide which function to survive[35]. In addition, stress can influence cytokinin that affects cell diffusion. One function of cytokinin is to enhance the plant resistance towards the stress from the environment, stimulate its growth, induce cell diffusion, differentiate mitosis and stimulate the growth in general. If the surface on the cell of the seaweed is open, its surface will be wider and easier to release gametes even in extreme condition. Gardner[12] mentions that brassinolide and brasinosteroid function as stimulation hormone to generate the resistance of bud towards the stress from the environment.

The change in the media of culture applying treatment of pH manipulation is not only to maintain the activity or the movement of gametes but also to support the gamete induction. Huang *et al.*[16] and Raikar *et al.*[36] state that the change from the environment, such as pH, is considered supportive factor for the induction and the activity of gametes. Besides, the change on external pH (culture media) leads to the change of internal pH. This is the same as Masithah's opinion[32] stating that the change of external pH will cause the change of internal pH of algae. The change of intracellular to be alkaline is caused by the exchange of ion H^+ and Na^+ in the gametes. The increase of intracellular pH will influence the motility activity of gametes. Consequently, gametes will be mobile by moving their tail or flagella. Finally, the change of pH will be significant to control the movement of gametes[32].

The condition in the environment actually affects the algae growth. As it is mentioned by Kamlasi[20], internal factors that affect the growth are type of algae, part of thallus and age, while external factors are physical and chemical condition of waters. Ilknur and Cirik[17] mention that the physical and chemical factors in waters include water movement, temperature, salinity, nutrient and light.

On the treatment C (Compost : NPK = 1 g/L : 1 g/L) the growth of daily weight and relative length of *Gracilaria* sp. is 2.56% /day. This definitely differs

from the treatment B (Compost : NPK = 0.5 g/L : 1.5 g/L) as much as 1.86 %/day and 1.65% with ($p < 0.05$) the growth of daily weight and relative length of *Gracilaria* sp. This is caused by the difference of the numbers of macro and micro nutrients found in fertilizer. It is assumed that combination between compost and NPK = 1 g/L : 1 g/L is effective to absorb nutrients. It has been known that all kinds of plants need nutrients both macro and micro. In the combination between compost and NPK there are eight nutrients (macro and micro) needed in the plant growth (data not shown). Silea and Masitha[40] say that if one of the nutrients is not available, it makes the plant growth and its productivity hindered. New variety of seaweed has daily growth as much as 2.56%/day in average and the growth of relative length as much as 2.21%. This indicates that there is more increase of seaweed growth than that which is cultivated in embankment. Sulitijo[42] claims that the best seaweed of *Gracilaria* sp. has the growth as much as 2%/day in 35 days. In addition to this, the stable result can be obtained.

The presence of organic and inorganic supplies the nitrogen content (N) and phosphor (P) that are sufficient to the growth of new variety of *Gracilaria* sp. Hanisak[14] and Anggadiredja *et al.*[2] state that N is the partition factor for macro algae growth. Latif[25] adds that N fertilizer in waters cause the plants fertile so their productivity is increased. In accordance with Latif's opinion, Hanisak[14] mentions that the micro algae growth can be stimulated by adding N into media with circulation system of sea water. Nitrogen functions to help the forming of chlorophyll, photosynthesis, protein, fat and other organic substances[38]. Besides nitrogen, seaweed needs phosphor (P) in its growth. Phosphor is the source of nutrients for the seaweed growth which can easily be scattered and absorbed by the plants[25]. Phosphor functions to induce the growth and accelerate the form of seaweed spores[5]. Susanto *et al.*[44] adds as well that P addition is necessary to form ATP and absorb ion by algae.

The addition of combination between compost and NPK has not given different effect on the concentration of chlorophyll *a Gracilaria* sp. because new variety of *Gracilaria* sp. has relatively obtained enough light on all treatments. So, this activates the chlorophyll. The light coming to the waters will be captured by the chlorophyll found on the chloroplast of the plant for photosynthesis. In the other hands, the lack of lights in the waters will cause the photosynthesis obstructed[26]. The N test indicates that the concentration of chlorophyll *a* is increased in accordance with the increase of N content in combination between fertilizers (data not shown). This case is supported by a research [10] mentioning that the addition of N on seaweed of *Laminaria japonica* can enhance the concentration of chlorophyll *a.* Lobban and Harison[29] assert that synthesis of chlorophyll *a* and phycoerythrin needs N. Denault *et al.*[9] reports that the amount of chlorophyll *a* is decreased in accordance with the decrease of nutrients on maintenance media.

In this study, the addition of fertilizer can cause the absorption of nutrients by seaweed emerge. As consequence, the growth of cell takes place. This growth will lead to the increase of primary and secondary metabolism result which is shown by enlargement of cell and thickening process on the cell's surface. Primary metabolite yielded by the seaweed is polysaccharide (in this case it is used to form the cell wall); secondary metabolism is the most important chemical process for the existence of an organism to live and survive. The thickening process of polysaccharide in seaweed will trigger internal pressure in the cell so the solution in the cell will come out. This process aims to maintain the balance of metabolism in the cell or homeostasis and it will cause the water content in the cell decreased[31]. The water content in the plant has interaction with the turgor pressure in the plant tissue and affects the photosynthesis.

In the other hands, Hafara[13] claims that ion K^+ has important role in photosynthesis and sugar distribution, efficiency of water use, metabolism of carbon and protein, and activation of enzyme. K^+ is derived from NPK fertilizer because potassium (K) is absorbed in the form of ion K^+ [27]. Ion K^+ with low concentration is distributed through energy transfer by ATP-ase, while ion K^+ with high concentration will hinder mechanism of active transport. The speed of K^+ absorption is controlled by ion K^+ which is found in the cell and it will affect the cell turgor. On the day the ongoing photosynthesis produces energy to support the absorption of K^+ and finally it will increase the K^+ concentration and turgor pressure. That the turgor pressure is decreased in stressful condition will cause the plant flabby while the young tissue needs K^+ to maintain the turgor pressure for cell enlargement. In contrary, the decrease of K^+ will cause the growth decreased and it increases the plant resistance towards disease[43].

From the result above, it can be concluded that the success of cross breeding method and free living technique on selected variety of *Gracilaria* sp. from the group living in the wild nature and area which is cultivated relates to the presence of media in the environment. Consequently, new variety that has ability to induce more gametes, high growth, better thickening process on the cell wall and low water content will be obtained.

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