Growth and carrageenan content of local and tissue culture seed of *Kappaphycus alvarezii* cultivated in floating cage

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Abstract. *Kappahycus alvarezii* seaweed cultivation is continuously developing in coastal area of Indonesia. A wide range of efforts to trigger seaweed growth has been conducted. One of them is by conducting plant tissue culture. This research analyzed the difference between tissue culture seed and local seed. The present research findings showed that the growth is different between local seed and tissue culture seed. Natural growth from initial weight 20 g became 42.4 g and 57.8 for local seed and tissue culture seed, respectively. Specific growth rate (SGR) had around 1.7%/day^{-1} differences for local seed and 2.6%/day^{-1} for tissue culture seed. The growth of each part of thallus had shown that base thallus has a quite good growth compared to middle part thallus and peak thallus. From the initial weight 10 g after cultivation, it turned to 36.8 g and 56.8 g for local seed and tissue culture, respectively. SGR appeared between 3.7%/day and 4.3%/day for local seed and tissue culture, respectively. Pearson analysis showed that there is no significant correlation between local seed and tissue culture. However, there was weight difference from every growth from both.
Key Words: thallus, specific growth rate, seaweed, cultivation.

Introduction. *Kappaphycus alvarezii* is one of macroalgae species that is still developing throughout Asia Pacific. Not only in Indonesia, but many countries around the world has cultivated this species of seaweed because it's decent economic value (Kasim et al 2016). Philippines has been developing cultivation of *Eucheuma* sp. since 1971, Fiji has been developing since 1970, Tuvalu, Malaysia, Maldives, and Indonesia has been developing since around 1975 (Doty & Alvarez 1973; Doty 1973; Parker 1974; Prakash 1990; Luxton et al 1987, 1993; Adnan & Porse 1987; Luxton 1993). While some other countries has started developing this cultivation after 1980 such as India, Tanzania, Vietnam, Brazil, Venezuela (Smith 1990; Lirasan & Twide 1993; Ohno et al 1996; de Paula et al 1999; Rincones & Rubio 1999).

Munoz et al (2004) stated that *K. alvarezii* cultivation has developed quite well and gives pretty good growth which related enough with several environment physics and chemicals factors especially in Yucatan Mexico area. *K. alvarezii* cultivation it is developing quite well but growth is fluctuated seasonally especially in Northwest of India coastal area (Kumar et al 2015). Production value of *K. alvarezii* also depends on its cultivation method. In a good method by protecting *K. alvarezii* from various herbivore attacks will show better result compared to other methods with open characteristics (Kasim & Ahmad 2017). These situations occur in South beaches of Rio de Jeneiro, Brazil as well, where it is describe that cultivation technique with tubular netting is better than longline method (Geromel de Goes & Reis 2011).

Quality and quantity of seaweed cultivation production are influenced significantly by the seaweed seed and the method used. A good seed has high growth level. Superior seaweed seed usage is expected to gain a decent harvest yield and big production. One of the methods that can be used to trigger sea algae growth is by improving seed quality by using tissue culture method (Dawes et al 1994). Tissue culture is a method that isolates part of the plant (in seaweed it is the thallus) and foster it in aseptic condition in closed container, so that the part can multiply and generated becoming complete plant likewise its parent (Dawes et al 1994). A good method is the one that is able to protect the seaweed from variety of pest attack and dirt attachment in the sea. Floating Raft Net is one of protected seaweed cultivation method in preserving seaweed from pest attack as well as reducing potential disease (Kasim et al 2016, 2017 a,b).

In the present research tested of seed growth difference between local seed and tissue culture seed of *K. alvarezii*. To reassure, the researcher conducted growth test of every thallus to see the growth differences of either on base thallus, middle thallus, and peak thallus. The test process would be very important to present argument of best thallus part growth for seaweed cultivation farmers.

Material and Method. The research was conducted in one of seaweed development center in Buton District Coastal area, South East Sulawesi Province, Indonesia (L 5°29'26.78"S, L 122°33'41.77"E). Research location was one of the locations used for seaweed cultivation with sandy base topography which is perfectly suitable for seaweed type *K. alvarezii.* Bottom side of the waters in the study area was dominated by sand and coral ridge. Research location water was mostly utilized by local people as seaweed cultivation location. To avoid herbivore pest attack during the research, researcher used floating cages in preserving the seaweed. Floating cages had rectangular shape with height, width, and depth size in consecutive order of 200 x 100 x 64 cm. Raft framework was made of PVC pipe with 2.5 inch size and a net made of multifilament with net eye size of 1.5 cm (Kasim & Mustafa 2017; Kasim et al 2017 a,b). To make easy the growth observation, every floating cage was divided onto 4 plots, based on the height of the raft by using net. Net plots were used to preserve the tested seaweed (Kasim et al 2017a).

Tissue culture of *K. alvarezii* seed was obtained from natural breeding that has been done for 2 years. The development of tissue culture was done in previously unpublished studies (Kasim et al 2016). Seaweed tissue culture has been cultivated and developed in nature for 2 years of cultivation and separated from non-tissue source seeds. Local seed was collected from farmers around the research location. Seaweed seed had been already prepared to cleanse from dirt or attaching organism. The chosen *K. alvarezii* seaweed condition was: young, fresh, clean, and free from other seaweed type. After that the initial weight had been measured for 20 g each thallus by using analytic scale. Each seed type was categorized and spread by using floating cages. For each thallus observation from the seed used, there was a cutting for each thallus part. Each seaweed (*K. alvarezii*) thallus clump was divided into 3 parts based on its position (base, middle, and peak). Each part was measured with weight of 10 g using analytic scale. Each seed part was labeled with different color and categorized based on the similar initial weight. The sample spread in floating cages had been prepared.

Carrageenan concentration as an extraction result was calculated based on ratio between carrageenan weight produced and seaweed dry weight by utilizing carrageenan concentration analysis following the laboratory work procedure, which was, before the test seaweed had been cleansed from sands, dirt, and foreign substance then dry rested. Carrageenan measurement was conducted using carrageenan extraction method (Munoz et al 2004). Water quality was also very impactful towards the growth rate. To give good information from this research finding, there had been done water quality observation including physical factor, such as: temperature, salinity, transparency, and current velocity. While for chemical factors the observation included nitrate concentration and phosphate in the water. Water chemical factor was measured by using water sample collection and nitrate phosphate observation was conducted in Laboratory of Fisheries and Marine Science, Halu Oleo University. To analyze specific growth rate, the formula of Luhan & Solesta (2010) were used.

$$SGR = (\ln Wt - lnWo)/t)x\ 100\ \%$$

SGR : Specific Growth Rate
Wt : Weight in the end of measurement
Wo : Initial weight
t : time

To understand the correlation between every tested sample and environmental factor, it was used the Pearson correlation analysis. Data analysis used SPSS 22.0.

Results and Discussion. Growth of *K. alvarezii* in every station, performed using floating cages, can be seen in Figure 1. From the research findings can be seen that the total growth was different between the local and tissue culture seeds. The highest mark on growth from initial weight of 20 g became 42.4 g and 57.8 for local seed and tissue culture seed, respectively (Figure 1).

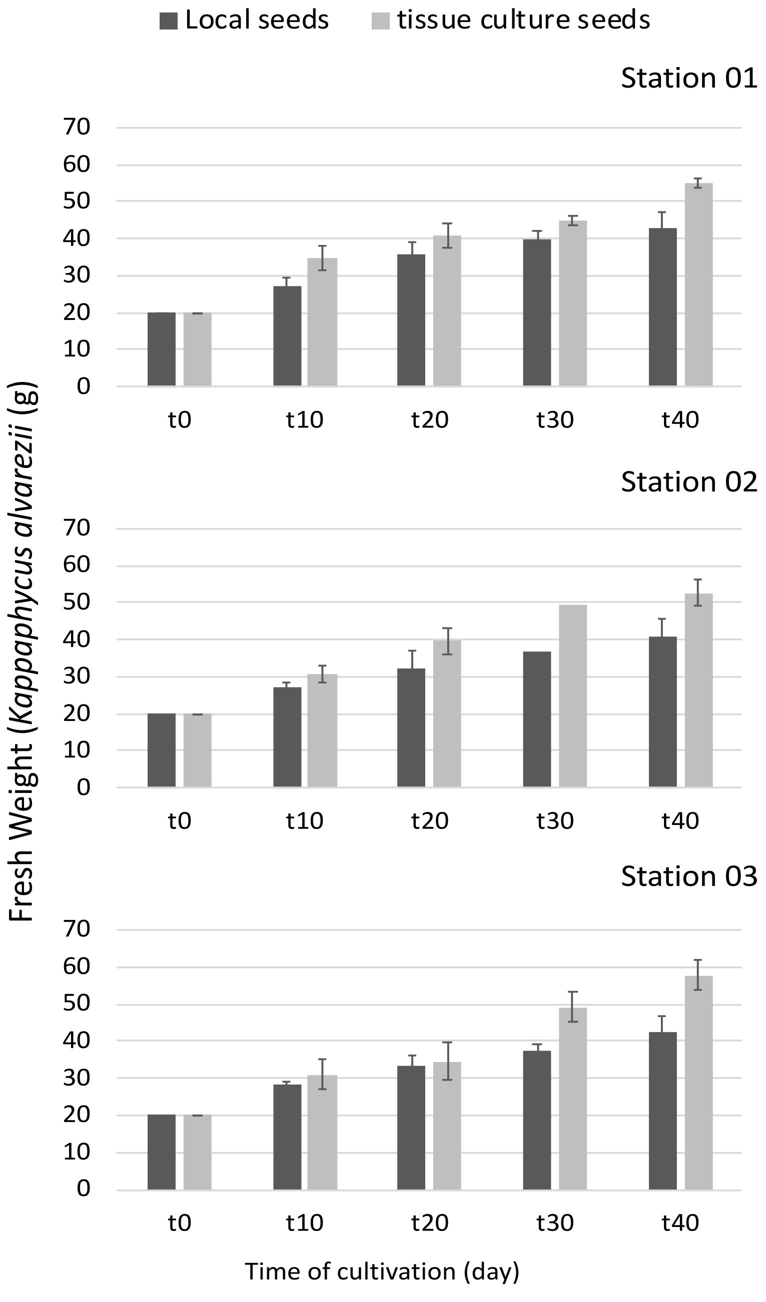

Figure 1. Growth of *Kappahycus alvarezii* thallus from local and tissue culture seed (t0 = day 0, t10 = days 10, t20 = days 20, t30 = days 30 and t40 = days 40).

According to the growth on every thallus part which were taken for cultivation, it can be seen that thallus growth in the base part is better than the middle and peak part. Total growth of the base part from 10 g of preservation during 40 days turned up becomes 36.8 g for local seed weight (Figure 2) and 56.8 g for tissue culture seed (Figure 3).

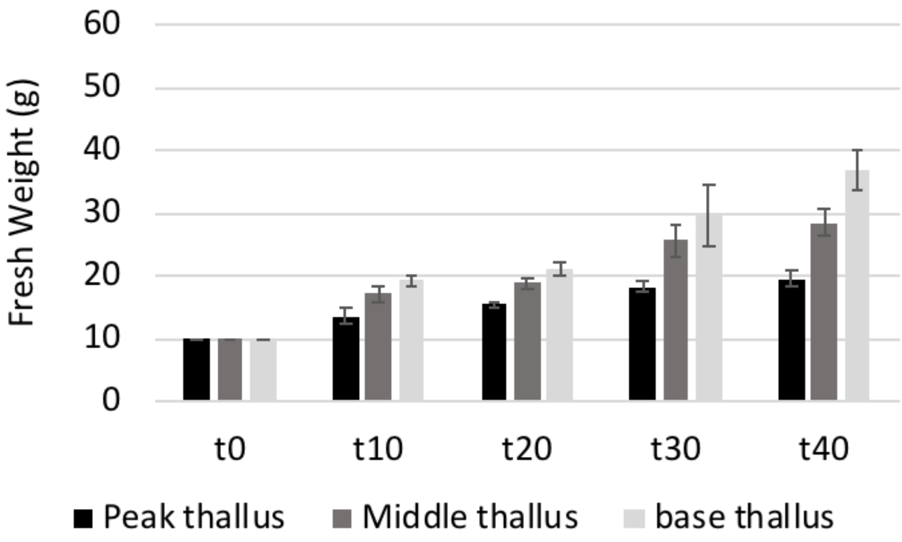

Figure 2. Growth comparison of thallus part of *Kappahycus alvarezii* from local seeds.

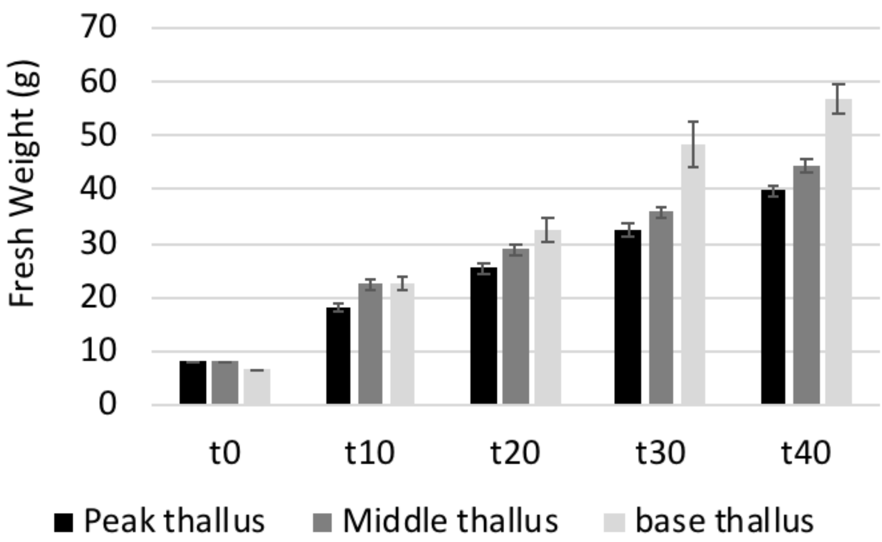

Figure 3. Growth comparison of thallus part of *Kappahycus alvarezii* from tissue culture seeds.

Specific Growth Rate (SGR) appeared quite low for both seed sources either from the local and tissue culture. However, the differences were obvious. Seed obtained from tissue culture had SGR around 1.7% day^{-1} while tissue culture seed showed around 2.6% day^{-1} (Figure 4).

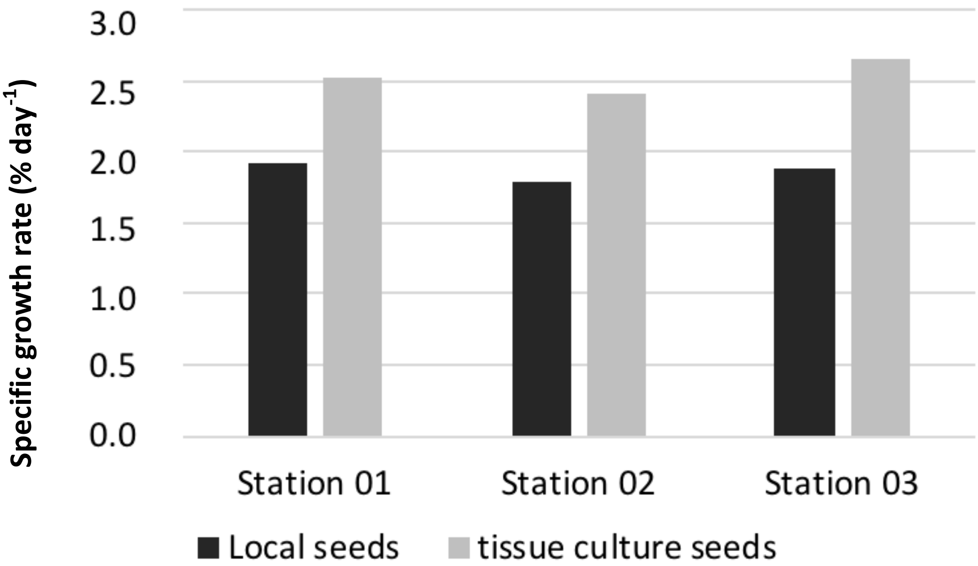

Figure 4. Specific growth rate in each observation station.

Research findings for the growth in every thallus part tested had shown that the base part had SGR 3.7%/day for local seed and 4.3%/day for tissue culture seed (Figure 6).

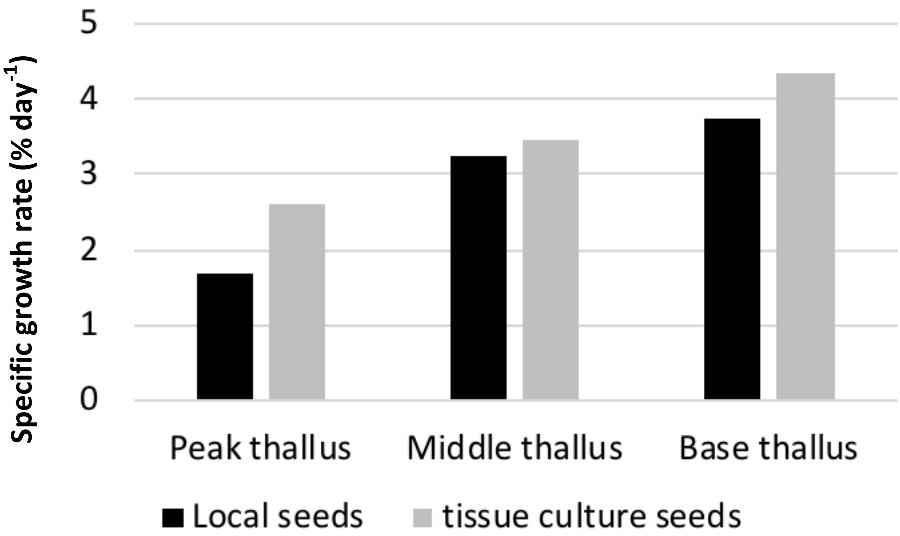

Figure 5. Specific growth rate of every part of thallus.

From the carrageenan content analysis, it can be seen that in two stations the local seed appeared with higher content, namely 49.13% compared to tissue culture seed with 40.52%. However, in station 1 it appeared that carrageenan level reached 54.48% and 48.68% for local and tissue culture seeds, respectively (Figure 6).

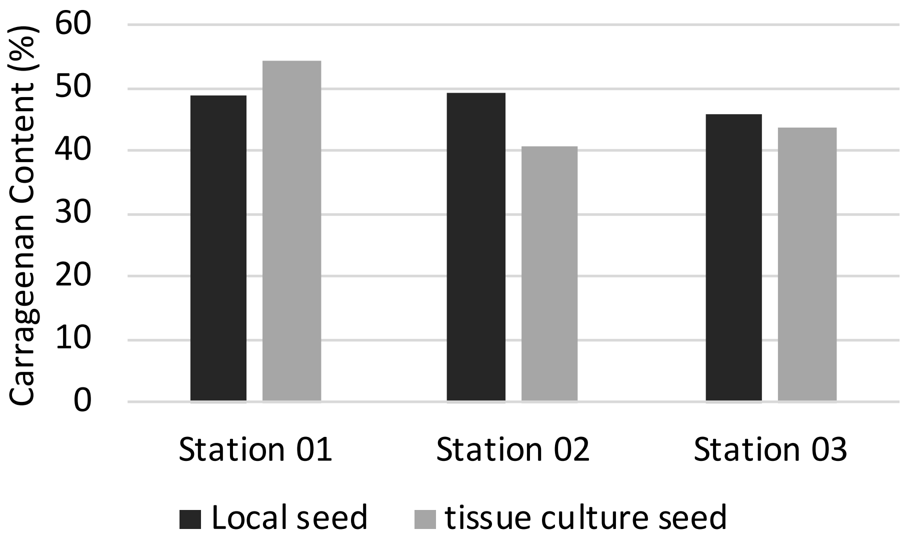

Figure 6. Carrageenan content of *Kappahycus alvarezii* during observation.

Environmental factor is very important to determine the seaweed growth. From the research findings, it can be seen that in all of the observation stations, the temperature was around 25–29°C which occurred on August, stream velocity was around 0.017 and 0.16 m/sec on May. While for nitrate concentration we found values of 0.014–0.047 mg/L and phosphate between 0.0012–0.0099 mg/L which occurred on May (Table 1).

Table 1

Environmental factors parameter during research

Measurement time (day/month)	*Temperature (ºC)*	*Current velocity (m/sec)*	*Salinity (‰)*	*Nitrate (mg/L)*	*Phosphate (mg/L)*
10/05	27	0.017	32	0.0134	0.0099
20/05	26	0.035	31	0.0474	0.0061
30/05	25	0.072	31	0.0638	0.0057
10/06	27	0.078	31	0.0164	0.0086
20/06	26	0.163	32	0.0345	0.0084
30/06	27	0.051	32	0.0150	0.0051
10/07	26	0.135	33	0.0342	0.0085
20/07	27	0.167	31	0.0396	0.0079
30/07	29	0.087	29	0.0275	0.0079
10/08	29	0.067	29	0.0241	0.0087
20/08	26	0.018	31	0.0136	0.0012
30/08	27	0.031	30	0.0291	0.0079

From the Pearson analysis result, it can be seen that there was no significant correlation between local seed and tissue culture for trust level $p>0.005$ (Table 2).

Table 2
Pearson analysis for correlation between growth of local seed and tissue culture seed to environmental condition

	Tissue Seed	*Temperature*	*Current*	*Salinity*	*Nitrate*	*Phosphate*
Local seed	.172	-.153	-.356	-.109	-.192	-.287
Tissue seed		-.401	.389	.030	.349	-.312
Temperature			-.025	-.522	-.536	.409
Current				.236	.306	.373
Salinity					-.078	.011
Nitrate						-.039

*. Correlation is significant at the 0.05 level (2-tailed).

Growth rate. Growth of *K. alvarezii* is the difference between final biomass weight and initial biomass during the research. Factors that influenced the growth consisted of external and internal components. External factor includes environment such as water quality and light intensity while internal factor includes age, thallus type, and genetics. Research findings during 40 days show that growth of *K. alvarezii* in each treatment gives varied results along with its aging and seaweed weight increase for both local and tissue culture seeds. Tissue culture seaweed seed growth is higher than of the local seed. It is possibly caused by branching of tissue culture thallus that is a lot more compared to local seed, so that the thallus surface absorbs more nutrients in water environment, also local seed is the seed that is cut continuously until the quality is reduced. Bujang (2013) stated that seaweed from tissue culture has higher growth compared to local seaweed seed. Moreover, Hurtado & Cheney (2003) stated that seaweed seed is generally obtained from vegetative method that occurs repeatedly and causes reduced growth. Reddy et al (2003) also explained that tissue culture plant has growth rate 1.5 to 1.8 higher than other plants that are cultivated in Indian Sea. Sudjiharno (2001) stated that the highest growth level could be occurred between ages of 23-35 days while seed weight which has good growth level is around 50-100 g. While Sapitri et al (2016) stated that absolute growth of tissue culture seed was much higher compared to the control which is seed that does not come from tissue culture where the weight increase 224.73 g during preservation for one month. Then Arisandi et al (2011) stated that the high of *K. alvarezii* growth from tissue culture seed was caused by the inside of the Conway media, growth booster substances, so the results were higher than cultivated seaweed in nature. Thallus from tissue culture has higher growth rate than its natural growth process, because inside the tissue culture media there was growth booster substance such as auxin hormone. Stable environment condition and set appropriately according to life range and seaweed growth could also result in a relatively good growth. Besides, environment factor cannot be separated from seaweed growth supporting factor like light intensity. This research was preserved in floating raft with 50 cm depth where in such condition the seaweed is very good to support its growth because sun light can penetrate the water to support seaweed needs for photosynthesis. Pratiwi & Ismail (2004) stated that in its growth seaweed requires sun light to perform photosynthesis, so seaweed only could only grow in water that has certain depth with sun light that penetrates to the bottom of the water area. Ilalqisny et al (2013) explained that high or low chance of the growth is caused by cultivation system related with growth space and sun light absorbance as photosynthesis process controls. The low growth of local seeds is caused by lower local seed quality compared to tissue culture seeds. Seed quality greatly affects the growth rate of seeds. Indriani & Sumiarsih (2003) stated that seaweed growth is influenced not only by environmental factors but also by the plant seed quality. Other factor that causes a low absolute growth of local seed seaweed is the use of seed over and over again so it reduces in quality and quantity and it is vulnerable towards disease. Parenrengi et al (2011) stated that *K. alvarezii* and *Eucheuma denticulatum* seaweed cultivation in Indonesia, especially South Sulawesi has been experienced a vigorous development in order to fulfill the increasing demands of carrageenan and gelatin. Even though, problems had been reported at farmer level, especially issues concerning seed quality. Decent seaweed seed are provided from nature. One of the serious problems is the continuously use of vegetative seedlings will lead to quality and quantity decrease.

Carrageenan content. The results showed that carrageenan content at station 1 was higher than station 2. However, the difference in carrageenan content was not too high in each station. This can occur because seaweed has benefited of a relatively equal supply of nutrients, sunlight, and temperature (Kasim et al 2018). Hayashi et al (2007) explained that carrageenan content varied due to extraction method and ecological factors like sun light, nutrition, temperature, and water content during dry out. The highest carrageenan concentration came from variant of seed weight 75 g which was 44.24% followed by the 50 g seed weight variant which was 42.81% and the lowest in method of 100 g seed weight variant which was 42.54%. Thus were presumably caused by thallus diameter differences, 75 g seaweed seed has initial average thallus diameter at base part of 0.88 cm and final diameter average of 1 cm, followed by 50 g seaweed seed which has initial average thallus diameter at base part of 0.83 cm and final average diameter of 0.99 cm, and 100 g seaweed weight with initial average thallus diameter at base part of 0.79 cm and average final diameter of 1 cm. This showed that thallus diameter affected carrageenan content. This statement was supported by Mendoza et al (2006) that in young thallus carrageenan percentage are lower than that of old thallus. Considering the carrageenan data attained was still considered as good. Syahputra (2005) stated that good carrageenan standard is 40% while carrageenan content of 30% is considered low. Seaweed growth is inversely proportional with its carrageenan content. When the growth is high, the carrageenan content decrease (carrageenan content decrease when the seaweed aged >45 days). It is because the high of carrageenan content in 75 g seed is affected by thallus diameter that has large size and nutrient distribution absorbed by thallus is used to form carrageenan and young thallus for growth. Carrageenan concentration level difference is also caused by pigment difference in seaweed that plays important roles in photosynthesis process to form carrageenan. Munaeni (2011) stated that photosynthesis is sun energy absorbance process by plant cell's that support seaweed optimal growth including carrageenan content formation. It is because *K. alvarezii* has 2 life cycle phases which are vegetative phase and generative phase. In vegetative phase, energy is distributed for growth and carrageenan formation. Then the generative phase is where the energy for carrageenan formation is reduced for generative process so that its concentration decreases while the growth still continues until it reaches maximum point. The high and low carrageenan level could be influenced by preservation duration and varieties. Hayashi et al (2007) stated that the best carrageenan condition could be reached if the seaweed is cultivated for 45 days. In addition, Hayashi et al (2007) also stated that carrageenan content differences could be caused by extraction method.

Water quality. Temperature is an important indicator to show environment condition changes, and very important in seaweed photosynthesis process. Based on the measurement results during 45 days, the temperature at the research location ranged between 28 and 31^{o}C. It showed that water temperature was relatively stable. That temperature level was considered as a tolerable borderline by *K. alvarezii* seaweed. Afrianto & Liviawaty (1993) stated that seaweed can grow well in the water with temperature of 26-30^{o}C. Rahim (2009) stated that temperature between 27-32^{o}C is still in the borderline that can be tolerable by *K. alvarezii* seaweed. These reports confirm that water temperature condition in our research location was suitable for *K. alvarezii* growth.

Current velocity has big impact towards aeration, nutrient transportation as food sources and water mixture, quickly rinse soft dirt that attached on thallus. Indriani & Sumarsih (2001) stated that current movement functioned to supply nutrient substance and also help seaweed in absorbing nutrient and cleaning attached dirt. Current velocity attained during the research in our research sites water range between 8.77 and 11.49

cm/s. Indriani & Sumiarsih (2003), found that good current velocity for seaweed is between 20 and 40 cm/s.

Water transparency is deeply related with sun light penetration for photosynthesis process, because murky water could hinder the sun light to penetrate the water and photosynthesis process is disturbed. Light is one of determining factors of water plant life development which may directly or indirectly determine other organisms to become its sustenance. According to Yusuf (2004), sun light is a factor that seaweed requires. Water transparency in our research sites was 100% clearance in depth of 3–7 m. Pong-Masak et al (2011), good water transparency for seaweed growth is more than 1 m. Sunarto (2008) stated that photosynthesis would increase along with the increasing light intensity on field. Light intensity was also related directly to primer productivity of water, the higher the light intensity the higher is the primer productivity on certain boundaries. Kune (2007) found that an important factor that can affect the growth rate of seaweed is the intensity of light received by seaweed. The difference in light intensity will have an impact on cell activity in carrying out development and growth. Water depth is one of the determining factors toward growth rate of seaweed (Sulistijo 2002). The lower of seaweed growth rate along with the depth within, the lower is the sun light penetration and the lower is the oxygen circulation in the water column. Depth measurement result on research location during the highest tide and the lowest during 45 days of research was 8 m and 6 m, respectively. Sulistijo (2002) stated that generally seaweed cultivation was conducted in the water with 5-15 m depth. Moreover, Poncomulyo et al (2006) stated that when water in cultivation location had its lowest tide, the seaweed still could grow well in 4-17 m depth of water, the nutrition absorbance could still occurred and seaweed did not dry out because of the direct sun light and still received sun light during tide. Based on depth measurement, at our research location, the depth was 3-7 m and it proved to be suitable for *K. alvarezii* seaweed growth. Seaweed growth in cultivation environment could be influenced by salinity. Salinity measurement results showed that the concentration in our research site was appropriate for *K. alvarezii* cultivation. Salinity measurement results showed that the concentration in our research site was appropriate for *K. alvarezii* cultivation. Nitrate is one of nutrient substance required for seaweed growth. Nitrate concentration analysis result ranged between 0.0210 and 0.0377 mg/L. Aslan (1998) stated that good water nitrate concentration for seaweed growth is between 0.0071 and 0.0169 mg/L. It is possibly because current brings organic substance element so it affects water condition. Nitrogen-nitrate level which exceeds 0.2 mg/L could cause water eutrophication that stimulates algae and water plant growth afterward rapidly (blooming) (Effendi 2003). Nitrate content measurement in our research site was adequately appropriate for *K. alvarezii* seaweed cultivation. Phosphate is one of the important parameter that is required for seaweed growth and commonly in the form of orthophosphate. Phosphate analysis result during the research ranged between 0.0016 and 0.0022 mg/L, that level could be categorized as good for seaweed growth. According to Aslan (1998) the appropriate phosphate level for seaweed cultivation is 0.0071-0.0169 mg/L.

Conclusions. *K. alvarezii* thallus from tissue culture showed better growth than those from local seeds. However, Pearson analysis showed that tissue culture seed growth was not really different from local seed. Research findings concluded that thallus from the base part appeared to have better growth than those from the middle and peak part of the thallus. Carrageenan level of tissue culture seeds was not different from those of the local seeds during the research.

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