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## The effects of planting distances and seedling sources on *Kappaphycus alvarezii* growth

L O Aslin<sup>1,2\*</sup>, E Supryiono<sup>3</sup>, K Nirmala<sup>3</sup>, Nurjanah<sup>4</sup>, D T Soelistyowati<sup>3</sup>

<sup>1</sup>Aquaculture Doctoral Program, Graduate School, Bogor Agricultural University, Bogor, Indonesia;

<sup>2</sup>Department of Aquaculture, Halu Oleo University, Kendari, Indonesia;

<sup>3</sup>Department of Aquaculture, Bogor Agricultural University, Bogor, Indonesia;

<sup>4</sup>Department of Aquatic Product Technology, Bogor Agricultural University, Bogor, Indonesia.

\*E-mail: laodeaslin@gmail.com

**Abstract.** This paper describes the effects of planting distance and seedling sources on the performance growth of *K. alvarezii* cultured by using a long line method. The research was conducted in Bungin Permai Village, Southeast Sulawesi Province, Indonesia. A factorially experimental design consisted of four seedling sources, namely tissue-cultured with nutrient enrichment (KJR-P), tissue-cultured without any nutrient enrichment (KJR-TP), conventional seedling with nutrient enrichment (KV-P), and conventional seedling without any nutrient enrichment (KV-TP). Three planting distances (10 cm, 20 cm, and 30 cm) with three replicates. The parameters like biomass growth, daily growth rate, and pigment content, were paid in attention during the research. Results of this research reveal that the planting distance has significant effects on biomass growth, daily growth rate, and chlorophyll content. It proves that the 20 cm long of binding distance conduces the highest biomass growth, daily growth rate, and chlorophyll content in each source of seaweed *K.alvarezii* seedling. The Duncan test analysis for the 20 cm of planting distance indicates that the KJR-P seedling source yields the highest biomass growth (464 grams), daily growth rate (7.24%), and chlorophyll content (6.21 mg/g), and it is significantly different ( $p < 0.05$ ) with three other seedling sources.

**Keywords:** aquaculture, *Kappaphycus alvarezii*, planting distance, seaweed

### 1. Introduction

Seaweed are multicellular algae that can be grouped into *Thallophyta* division where these plants do not possess any root, trunk, and leaf. However, their entire body consists of thallus that is able to expand naturally in seawater [1]. *Kappaphycus alvarezii* is one of the most cultivated seaweeds due to this species being the main source of carrageenan or caraginoifit property [2]. An achievement of cultivating production will be met when it is supported by developing quality seeds. Selecting seedlings in cultivating seaweeds is carried out carefully. Common propagation and multiplication of *K. alvarezii* are carried out by fishermen generatively through stem cuttings where the cut thallus will then grow to be a new plant [3]. The seaweed seedlings originating from previous cultivation that have been used many times will result in sluggish growth and be more susceptible to diseases [4]. This matter will lead to an increase in the production of the seaweeds.

Various attempts have been taken in order to scale up the quality and quantity of the seaweed seedlings namely promoting tissue-cultured seedlings, for instance. This type of seedling pledges to



enhance qualified seaweeds continuously. Anyhow, a successful cultivation of seaweed from tissue-cultured seedlings is strongly influenced by environmental conditions. Seaweed in general have a high growth rate when they are in a nitrogen and phosphate-rich environment [5]. Natural water as growing media of *K. alvarezii* seedlings comprises of less solved nutrients that are useful for seaweed to grow and it even has a low concentration of nitrogen, phosphate, and potassium. Consequently, seaweed is not able to expand optimally [6]. To upgrade the seaweed production, it is not enough for both conventional and tissue cultured seedling sources to just rely on the natural environment. This condition drives an attempt to utilize seaweed *K. alvarezii* seedlings that have been enriched by nutrients before they are planted in order to scale up their growth optimally.

Furthermore, another effort to escalate production of seaweed is the suitability of cultivation system. A method selection often becomes an obstacle of farmers in utilizing water condition and quality of seaweed seedlings, especially in setting a binding distance of seedlings. The common method for cultivating seaweeds is a long line system that is able to be applied in either relatively deep or shallow waters, and it has other advantages than other methods [7]. To use this method for any kinds of seedlings, attention needs to be paid to the binding distance of seedlings in order to bring in an optimal production.

The planting distance is a technical factor that affects nutrient absorption in the water and photosynthesis process which will result in the growth of cultured seaweeds. This is in line with a statement argued by Harrison and Hurd [8] that setting seaweed seedlings at a certain distance is highly related to the nutrition and light which are environmental parameters that affect biomass and productivity of seaweeds. The wider the planting distance, the larger seawater circulation that brings nutrients in order for the seaweed's growth to be increased. Furthermore, the different amounts of obtained nutrition based on planting distance are going to influence the difference of morphology, chlorophyll content, and phycoerythrin [9]. A linear correlation between nutrients (especially total nitrogen and total phosphor) and chlorophyll has been evidenced by previous researchers [6]. Among nutrients that are important for seaweeds are N and P [10]. Hence, an arrangement of binding distance needs to be adjusted with the environmental condition and the quality of used seaweed seedlings. A narrow planting distance is going to intensify competition among thalli that may interfere with the growth process. However, a distance that is too wide will also result in space for phytoplankton to grow. A lack proper information relating to an optimal planting distance from tissue-cultured and conventional seedling sources in escalating *K. alvarezii* production means that it was important for this research to be carried out. The built hypothesis was how the planting distance and seedling sources are able to increase the growth of *K. alvarezii* cultured using an experimental design. Furthermore, this research will offer a technical suggestion for stakeholders, especially fishermen, in cultivating seaweed in natural waters.

## 2. Material and Methods

### 2.1. Description of the study

The field work of this research was carried out in September 2016 in Bungin Permai Village, Tinanggea Sub-district, South Konawe Regency, Southeast Sulawesi Province, Indonesia. This research consisted of two steps, namely laboratory scale growing and fieldwork, and laboratory analysis. The laboratory analysis was conducted in the Laboratory of Mathematics and Natural Science Faculty, University of Halu Oleo, Kendari.

### 2.2. Test plants and seedling preparation

The plants used in this research are seedlings of *K. alvarezii* that had been categorized into four groups, which are tissue-cultured seedlings with nutrient enrichment (KJR-P), tissue-culture seedlings without any nutrient enrichment (KJR-TP), conventional seedlings with nutrient enrichment (KV-P), and local seedling without any nutrient enrichment (KV-TP). The last was selected by specific characteristics namely young, fresh, abundance of thalli branches, and bright colors. The preparation of nutrient enrichment for the two mentioned seedling groups of *K. alvarezii* above had been taken for

15 days of cultivating in a laboratory scale by using NPP (Nitrogen, Phosphate, and Potassium) fertilizers. This process used seawater as media that had been filtered by using a filter bag.

Initial weight for each *K. alvarezii* seedling was 20 g. Furthermore, each seedling was put in a raising container that had been fertilized. Nutrient enrichment was administered every three days after replacing the water media as many as 100% [11]. This procedure referred to a previous research that the selected frequencies of enrichment in the preliminary treatment (a treatment for determining a frequency of enrichment) were 1, 2, 3, 4, and 5 times of enrichment. After 15 days of raising in the laboratory, these seaweed were then harvested and weighted. The fresh seaweed were cut into as much as 20 g of each for the next treatments at field cultivation in the open waters.

### 2.3. Cultivation process

Cultivating the *K. alvarezii* in this research utilized a long line method. In the shallow waters, the main rope was set as many as 40 meters long. There were four other small strings with 20 m long that were bound perpendicularly at the main rope and distance among those strings were 2 m length. The already prepared *K. alvarezii* seedlings that weight about 20 g each were tied to an open twist. This was intended to be easy in harvesting. These seedlings were tied at the small string serially and arranged horizontally based on the treatment's requirement of binding distance namely 10, 20, and 30 cm long and repeated three times. Every string had nine stocks of seaweed seedlings and a total in all strings was thirty-six seedlings. A routine control was done every two days, specifically to wipe out unwanted algae and barnacles that attached along the strings and the seaweeds. The parameters of water quality were measured during the research. Wet weight of the culturing seaweed was measured every nine days. The cultivation was scheduled for one cycle period namely forty-five days of raising that was based on the Indonesia National Standard (SNI) No. 7579.2:2010 and according to the fishermen habits in the research location.

### 2.4. Experimental design

This research applied a completely randomized factorial design that consisted of two factors: seedling sources and binding distance. Treatments of seedling sources were composed of four grades namely the tissue culture seedlings with nutrient enrichment (KJR-P), the tissue culture seedlings without any nutrient enrichment (KJR-TP), the conventional seedlings with nutrient enrichment (KV-P), and the local seedling without any nutrient enrichment (KV-TP). The second factor was divided into three different distances which are 10, 20, and 30 cm in length. Overall, there were 36 units of treatment that were composed of 12 combinations and three replicates in each combination.

### 2.5. Observed parameters

The observed data were absolute growth, daily growth rate, chlorophyll pigment content and phycoerythrin in the thallus of the cultured seaweed that were then analyzed descriptively. The absolute growth of the seaweed was gained by the final weight of the seaweed at the end of cultivation subtracted by the initial weight at the beginning of cultivation. There were 12 spots of seaweed observation that had been sampled by weighing for each treatment. This sampling was set three times. The weight data of the culturing seaweeds were then calculated by using an absolute growth as below.

$$H = W_t - W_o \quad (1)$$

Notes: H = an absolute growth (g);  $W_t$  = an average weight at harvesting (g);  $W_o$  = an average weight at the beginning of cultivation (g). The specific growth rate (SGR) is able to be calculated by using the formula [12]:

$$SGR = [(L_n W_t - L_n W_o)/T] \times 100\% \quad (2)$$

Notes: SGR = Specific growth rate (%);  $W_t$  = Weight after t day (gram);  $W_o$  = initial weight (gram); T = observation time (day).

### 2.6. Pigment content test

One of the methods that is able to know the photosynthesis activities is through a measurement of pigment content of photosynthesis which consists of chlorophyll-a and phycoerythrin. Testing the quality of cultured seaweed that was composed of chlorophyll and phycoerythrin in thallus was conducted at the beginning and the end of cultivation by using the Kjeldahl Method in the Laboratory of Biology, Mathematics and Natural Science Faculty, Halu Oleo University, Kendari. The chlorophyll measurement was taken by weighing samples of 500 mg (wet weight) of the cultured seaweed, drained, and then pulverized using mortar in a phosphate buffer solution (pH 6.5) to demolish the cell wall. The extract was poured into a centrifuge tube until it became 10 mL and then it centrifugated in 20 minutes with 2500 rpm in order to yield supernatant granules. As much as 5 mL of supernatant was re-extracted in an 80% of acetone (reagent analysis) and mixed by utilizing homogenizer until the solution lost color. The pigment concentration was read by using a spectrophotometer according to an absorption rate. The analysis was sized based on absorption peak, where a chlorophyll-a in  $\lambda$  (wavelength) was 665 nm, a carotenoid in was  $\lambda$  460 nm, and phycoerythrin in  $\lambda$  were 455 nm, 564 nm, and 592 nm, respectively.

The method used for analyzing pigment content was referred to Naguit, et al. [13]. Both contents were counted based on the formula:

$$\text{Chlorophyll } a \left( \frac{mg}{L} \right) = 11.93 (A664) - 1.93 (A647) \quad (3)$$

$$\text{Phycoerythrin} \left( \frac{mg}{L} \right) = [(A564 - A592) - (A455 - A592) 0.20] * 0.12 \quad (4)$$

The pigment content per gram of the cultured seaweed (mg/g) was calculated by this formula as follows

$$\text{Pigment content} \left( \frac{mg}{g} \right) = \frac{\text{Concentration} \left( \frac{mg}{g} \right) \times \text{solvent volume (mL)}}{\text{Weight of seaweed samples (g)}} \quad (5)$$

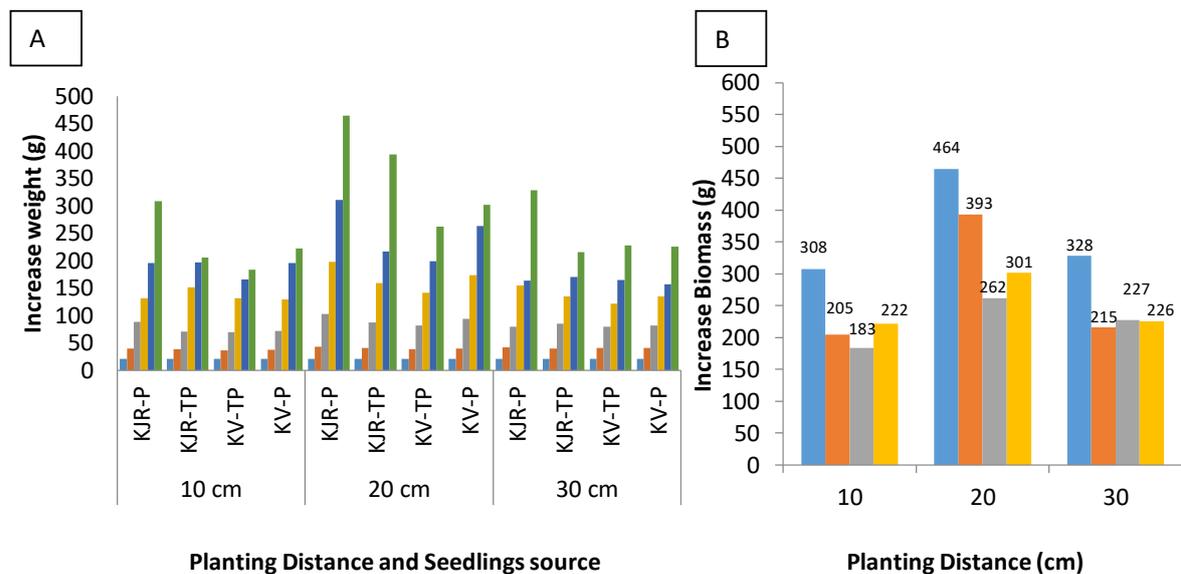
### 2.7. Statistical analysis

The four types of data gathered and mentioned above were biomass growth, daily specific growth rate and the content of chlorophyll and phycoerythrin. Before analyzing the variance, those data had to pass three different tests such as normality, homogeneity, and additivity. The normality test was applied to signify that those data were distributed normally. The two other tests were then conducted in order to exhibit that they were homogenous and additive as required for an ANOVA test. This test was conducted to signify the interaction influence among treatments with 95% confidence level. When there is a significant effect in each treatment, the Duncan test was enforced. These data were processed through a statistical software, SPSS Version 16, to notice the highest median in each treatment in order to decide the best treatment combination.

## 3. Results

### 3.1. The absolute growth rate

The weighing and measuring results of the biomass growth of *K. alvarezii* in each planting distance during 45 days of cultivation indicate that those biomasses grow over time. This is due to the growth of the photosynthesis process during the cultivation. The average weights of the cultured seaweed that were measured every nine days are described in histograms at figure 1.

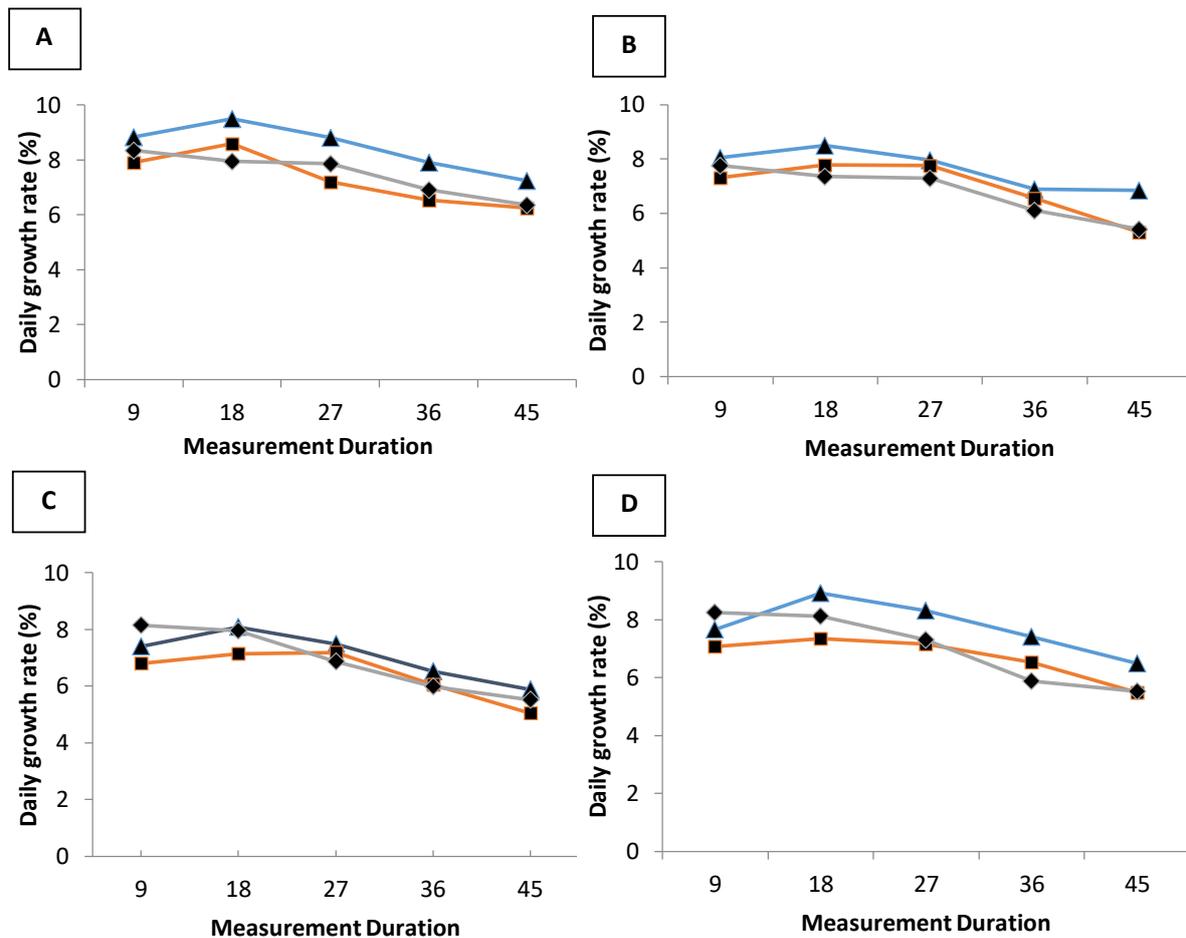


**Figure 1.** The biomass growth of *Kappaphycus alvarezii* seedlings using a long lines system in each treatment during 45 days of cultivation (A) and biomass growth in the end of cultivation (B), left: ■ H-0 ■ H-9 ■ H-18 ■ H-27 ■ H-36 ■ H-45, right: ■ KJR-P ■ KJR-TP ■ KV-TP ■ KV-P.

Figure 1 denotes that the difference of seedling sources of *K. alvarezii* delivers various growth rates. The results of the observation from day nine signify that all treated seedlings have the same pattern of growing which means that there is no difference in each treatment. They are different in growth rate clearly at day eighteen until the end of the cultivation process. This research reveals the binding distance effects ( $p < 0.05$ ) in the biomass growth of *K. alvarezii*. The biomass *K. alvarezii* from the 20 cm binding distance is the highest (301-464 g) compared to the two other binding distances (222 – 308 grams and 227 – 328 grams). The further test results of the 20 cm binding distance above show that the seedlings from the KJR-P treatment generate the highest biomass growth rate and more significant ( $p < 0.05$ ) than the three other treatments of seedling sources. The average biomass weight of all treatments (KJR-P, KJR-TP, KV-P, and KV-TP) were 464 g, 393 g, 262 g, 301 g, respectively.

### 3.2. Daily growth rate

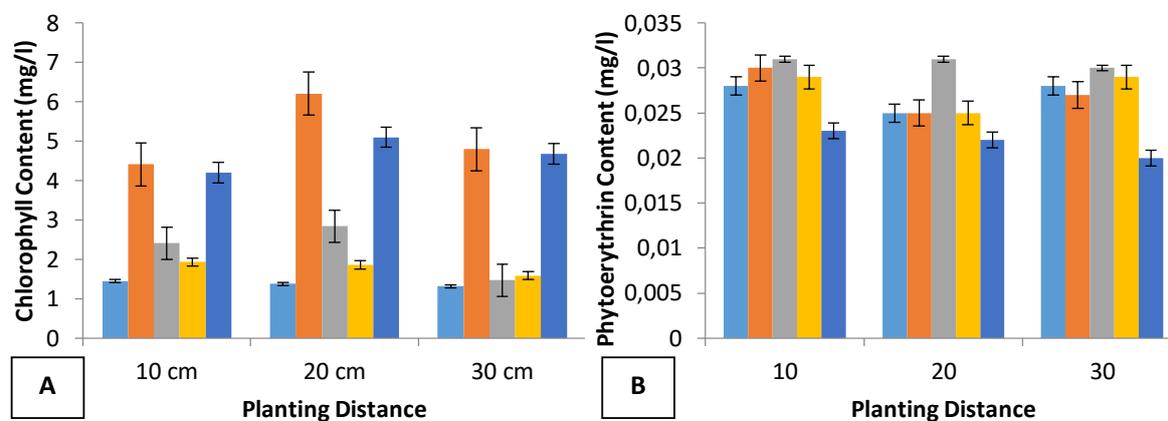
The daily growth rate of *K. alvarezii* seedlings using long line method in each treatment during the cultivation period is graphed in figure 2. The average percentages of daily growth rate *K. alvarezii* exhibit different results for every source of seedling and binding distance. Based on figure 2, the binding distance in 20 cm long for four seedling sources of *K. alvarezii* increased from day nine to day 18. While the 10 cm binding distance represents an increase of growth until day 27 and decreases until day 45 except in the treatment KJR-P which escalated until day 18 and then down until the end of the cultivation. Moreover, the last planting distance, 30 cm in length, indicates that all sources of seedling experienced a decrease during day nine to the end of the cultivation period. The figure 2 also shows that the planting distances influence the daily growth rate of *K. alvarezii* ( $p < 0.05$ ). The 20 cm length of planting distance yields the highest daily growth rate and significantly in every seedling source of *K. alvarezii* (5.87 – 7.24%) than two others planting distances. The percentage growth rates of two other planting distances are in a range of 5.87 - 7.24% for 10 cm length and 5.52 - 6.37% for 30 cm length. Based on the Duncan analysis result of the 20 cm length of binding distance signifies that the daily growth rate of KJR-P is the highest percentage (7.24%) and the most significant ( $p < 0.05$ ) than other treatments. The percentages of those three other treatments namely KJR-TP, KV-P, and KV-TP, are 6.40%, 6.19%, and 5.87%, respectively.



**Figure 2.** Graphics of daily growth rate in the three binding distances for four source seedlings of *Kappaphycus alvarezii*. KJR-P (A), KJR-TP (B), KV-P (C), and KV-TP(D),  
 ▲ 20 cm ■ 10 cm ◆ 30 cm.

### 3.3. The contents of chlorophyll and phytoerythrin of *K. alvarezii*

The pigment content for both chlorophyll and phytoerythrin of *K. alvarezii* seaweed that have been cultivated by using long line method, in general, indicates an increase from the beginning until the end of cultivation process for 45 days long. This increased condition is figured out in the histograms at figure 3. The content of chlorophyll in the cultured seaweed increases during the cultivation process in all treatments (figure 3). The 20 cm length of planting distance generates the highest chlorophyll contents for all *K. alvarezii* seedling sources that are in a range of 1.86 mg/L to 6.21 mg/L. Furthermore, Duncan test for this planting distance points out that the KJR-P produces the highest chlorophyll content (6.21 mg/L) compared to other treatments. Conversely, the planting distance do not deliver a significant influence ( $p < 0.05$ ) on phytoerythrin content. However, the compound of phytoerythrin is observed to have increased than before the cultivating process.



**Figure 3.** Histograms of chlorophyll content (A) and phytoerythrin content (B) of *Kappaphycus alvarezii* seaweed in three different binding distances (10, 20, and 30 cm length) using long line method of cultivation, left: ■ PR-BD ■ KJR-P ■ KJR-TP ■ KV-TP ■ KV-P, right: ■ KJR-P ■ KJR-TP ■ KV-TP ■ KV-P ■ PR-BD.

#### 4. Discussion

In this research we found that the 20 cm-long planting distance is the best distance for growing *Kappaphycus alvarezii* seaweed in the natural waters. The suitable planting distance will provide wider water circulation which takes nutrients leading to enhance diffusion process in upgrading the metabolic and growth rate [3]. The 20cm-long planting distance in the current research is supposed to be proper for receiving the intensity of sunlight in all thalli parts maximally, while the second (10) and third one (30) is either too tight or too wide which implicates thalli's surface cover each other and also covered by filamentous epiphyte algae and barnacles that hinder sunlight absorption. Further, the enriched seedlings of nutrient of the seaweeds also produced higher biomass than the conventional seedlings. These findings are in line with a report of Reddy et al [14] that tissue-cultured seaweed seedling has growth rates in a range of 1.5 to 1.8 times higher than conventional seedlings. In addition, the high growth is affected by a source of the seaweed seedlings that had been enriched in the early growth stages which had a proper supply of nutrient in the seaweeds [15]. This situation influenced the physiological process to escalate assimilation and accelerate growth. This difference rises the biomass of those *K. alvarezii* seedling sources is presumed by an ability of the seaweeds in absorbing nutrients in the waters. The capability is characterized by a morphological character in each seedling source. The enriched seedlings had more thallus branches which composed of young cells in enabling their surface to absorb much nutrition in the water environment. Furthermore, all treatments have positive growths of specific growth rates (SGR) and occur normally in the same patterns during the cultivation process. From day nine to day eighteen, the SGRs of the culturing seaweeds were increasing and getting decrease with increasing ages. This is in line with the result of Villanueva et al [16], the cultured *K. alvarezii* has a high optimization index in the first three weeks and then it declines in the fourth and fifth weeks. Moreover, this research also reveals that the chlorophyll content in the cultured seaweeds were increased during the cultivation process in all treatments and there was no significant influence of planting distance on phytoerythrin content. Forming chlorophyll is not only influenced by light but also affected by nutrients that are components of the chlorophyll. The enriched seedlings had enough nutrients in forming chlorophyll at the beginning of cultivation in the waters. The chlorophyll is useful for photosynthesis where phytoerythrin plays an import role to the process. Phytoerythrin is a protein that works as a complement pigment in red and green-blue algae-like phycobilin that works in the algae cells to assist chlorophyll-a in absorbing sunlight for photosynthesis process [17].

## 5. Conclusion

It can be inferred that the difference in planting distance has a significant influence ( $p < 0.05$ ) towards biomass growth, daily specific growth, and content of chlorophyll pigment of *K. alvarezii*. The best planting distance is 20 cm in length which signifies that the treatment KJR-P (tissue-cultured with nutrient enrichment) possesses the highest biomass value (464 grams), daily specific growth rate (7.24%), and chlorophyll content (6.21 mg/g). Conversely, the binding distance does not affect significantly the phytoerythrin compound. The measured results of water quality in the research location is in a tolerable range for growing *K. alvarezii* seaweed seedlings. This research specifies new ways for cultivating *K. alvarezii* in selecting planting technique and seedling sources.

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