

Short Communication

Study of Seaweed *Kappaphycus alvarezii* Explants Growth in the Different Salinity Concentrations

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Abstract

In term of tissue culture techniques method, the most important thing to note is environmental parameters. An environmental parameter that changes suddenly such as salinity can inhibit the growth of seaweed. Thus, this work is attempting the different salinity treatment on seaweed (*Kappaphycus alvarezii*) explants. This study aimed to determine the explants of *K. alvarezii* with a comparison of the different salinity levels in the in vitro tissue culture method. The method used in this study was Completely Randomized Design (CRD) with different salinity treatments namely 30, 31, 32, 33, and 34 mg/L. The results showed that different salinities influenced the growth rate of *K. alvarezii* seaweed explants with the highest explant growth rate was 31 mg/L and the lowest was 34 mg/L.

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1. Introduction

Kappaphycus alvarezii is one species of seaweeds that produces carrageenan with high economic value (Bixler and Porse, 2010; Valderrama et al., 2015). Seaweed seeds are generally obtained from nature by cutting it from natural seaweed. Vegetative reproduction is strongly influenced by the season. The use of seeds from nature continuously causes a deterioration in both quality and quantity of the seaweed seeds (Mendoza et al., 2002; Hurtado et al., 2006; Vairappan, 2006). The deterioration of quality in seaweed seeds originated from nature can be reduced by several techniques (Yong et al., 2014a). One technique is by multiplying seeds through tissue culture methods in vitro that has been successfully carried out by Titlyanov and Titlyanova, 2010.

Environmental quality is one of the determining factors in the success of tissue culture (Yunque et al., 2011; Terada et al., 2015). Salinity is one of the environmental parameters that can inhibit the growth of seaweed (Yong et al., 2014b). The optimal salinities range for *K. alvarezii* seaweed cultivation was 28–35 mg/L (Doty, 1973; Reis et al., 2011; Harwinda et al., 2018). This is closely related to the physiology of absorption and circulation of nutrients in seaweed so that lower and higher salinity will result in seaweed death (Bindu and Levine, 2011).

According to Lobban and Harrison (1994), salinity greatly contributes to growth, thallus formation, and morphogenetic development of seaweed. Salinity is directly related to osmoregulation that occurs within cells (Hayashi et al., 2010b; Araújo et al., 2014). The difference in concentration between the fluid inside and outside the cell pushes the Golgi apparatus to balance it to become isotonic. This has an impact on greater energy utilization so that it affects the low growth and the development of seaweed (Hayashi et al., 2010a). According to Hayashi et al. (2010b); Araújo et al. (2014); and Castro et al. (2018), those differences in salinity affect physiological and biochemical mechanisms, since the process of osmotic pressure changing is closely related to the role of cell membranes in nutrient transport processes. This study aimed to determine the growth of *K. alvarezii* seaweed explants by comparing the different salinity values in the in vitro tissue culture method.

2. Materials and Methods

2.1 Seaweed collection

Seaweed explants were used as research objects.

The seaweed might be fresh, clean, bright in colors, and free of epiphytes such as moss or dirt attached to seaweed. Seaweed samples were cleaned using seawater, then 1–1.5 cm seaweed thallus explants were cut with an initial weight of 0.029 grams. This work was conducted at the Seaweed Culture Laboratory BBAP Takalar, South Galesong District, Takalar Regency.

2.2 Treatment of salinity

This study used Completely Randomized Design (CRD) with 5 different salinity treatments. Each treatment was repeated 3 times. The treatments done were as follows; Treatment A: Salinity 31 mg/L, Treatment B: Salinity 32 mg/L, Treatment C: Salinity 33 mg/L, Treatment D: Salinity 34 mg/L, Treatment E: Salinity 30 mg/L (control).

Seaweed seeds were taken from the farmers of Sandrobone Village. Then the seeds were acclimatized ± 1 week to prevent stress due to different environmental parameters, subsequently, the seeds were cleaned for cutting preparation. The container used for the maintenance of explants was a multi-chamber that had been previously sterilized using an oven with a temperature of 30–40°C. The device was stored in a culture rack so that the container would not be contaminated. Sea water used was sterile sea water. The sterilization of sea water was carried out by filtering it with a cotton filter on the funnel, then put into erlenmeyer and added distilled water, after that it was sterilized in the autoclave at 121°C and pressure of 1 ATM for 15 minutes. Dilution was carried out based on the salinity values of each treatment, referred to dilution formula in Lansida (2003).

2.3 The observation of explants

The explants that had been prepared was dried using tissues. The next step was to prepare 980 ml of seawater with 30, 31, 32, 33, and 34 ± 20 millimeters of PES fertilizer then inserted into the multi-chamber and stored in a storage or culture rack. The growth of seaweed explants was observed weekly for 35 days. The observations were made to measure the weight gain of explants every week using analytical scales. Observations in this study were to see explant color changes at the beginning until the end of the maintenance. Measuring and calculating the weights of seaweed explants were very important because they were closely related to the growth rate that was used as the main parameter in this study. In order to determine the growth rate of seaweed explants, the formula used was as follows (Dawes, 1981):

$$SGR = \frac{(\ln Wt - \ln Wo)}{t} \times 100 \%$$

Where :

SGR = Average specific growth rate (%)

Wt = The average growth explants in (g) (i = week I, week II ... t)

Wo = Average explants weight at 1 (g)

T = Observation period (days)

2.4 analysis data

The effect of each treatment on the variables was determined by the analysis of variance (ANOVA). If the results of the analysis showed significance then proceeded with a least significant difference test (LSD).

3. Results and Discussion

It can be seen that there was a variation in growth in seaweed explants every week, with the treatment provided (Figure 1). In the first week, the explants underwent a new tissue formation process, and there was an increasing weight in treatment (A) 31 mg/L salinity, treatment (B) 32 mg/L salinity, treatment (C) 33 mg/L salinity, and treatment (E) 30 mg/L salinity (control), except for treatment (D) 34 mg/L salinity which showed decrease. The high growth of explant weights in the treatment A, B, C and E was due to the nutrient content utilized by the explants, at the optimal salinity conditions. Meanwhile, in treatment D (salinity 34 mg/L), it decreased in explant weight because this nutrient content was not utilized properly due to salinity beyond optimum value. High salinity value affected the osmoregulation process that occurred in the cell, causing explants of seaweed to grow less optimally. This trend was continued on the second and third weeks of experiment period. Furthermore, in the fourth week of experiment, seaweed explants on treatment (B) 32 mg/L and (C) 33 mg/L were not able to grow. Nevertheless, on treatment (A) 31 mg/L and (E / control) 30 mg/L the growth of the explants was able to increase due to the optimal salinity. Treatment (D) 34 mg/L showed that there was a very little increase in weight. In the end of the week (the fifth week), it showed that the best growth was on treatment (A) 31 mg/L salinity, then followed by treatment (E/control) 30 mg/L salinity and treatment (B) 32 mg/L salinity. Furthermore, in the treatment (C) the salinity of 33 mg/L was still growing although in a very little gain. In the treatment (D) 34 mg/L salinity,

there was a decline again, so it was not recommended to cultivate seaweed with high salinity. Overall, the difference in explants weight that occurred was due to the effect of salinity. According to Lobban and Harisson (1994), the use of salinity that is appropriate to the life of seaweed explants will have an impact on the use of suitable energy so that, it can increase the growth rate. This indicated that in treatment A (31 mg/L salinity) gave the best results compared to other treatments because the use of energy obtained through salinity was very suitable. This was supported by Doty (1973) and Hayashi *et al.* (2010b) who stated that optimal salinity could increase both the addition of cell numbers and explants weight that was in the range of 25, 30 and 35 mg/L. Furthermore, Reis *et al.* (2011) and Harwinda *et al.* (2018) stated that *K. alvarezii* could grow well in salinity ranged from 28 to 34 mg/L.

According to Lobban and Harrison (1994), if the salinity range of seawater has exceeded the optimal range of the algae, the growth and development of algal cells are linear and inverse (negative) compared to the increase in salinity. Salinity decreases the growth rate through reducing the rate of cell enlargement in parts of the thallus. The effect of high salinity on growth and alga structural changes, among others, is the smaller size of the stomata. As consequence, nutrient and water absorption decrease and it inhibits the growth of algae at the level of organs, tissues and cells. Same result can be seen in the specific growth rate parameter (Figure 2). The data showed that the best growth among all treatments was treatment A (31 mg/L salinity), followed by treatment E (30 mg/L salinity / control), treatment B (32 mg/L salinity), treatment C (33 mg/L salinity), and treatment D (34 mg/L salinity) (Figure 2).

The highest rate of specific growth rate was obtained in treatment A (31 mg/L salinity) with a rate of 1.15% per day, then followed by treatment E (30 mg/L salinity) of 1.10% per day, treatment B (32 mg/L salinity) of 0.99% per day, with salinity range that was still suitable for supporting the growth of *K. alvarezii* explants (Figure 2). From the results obtained on treatment (A), (E), and (B) explants growth weight was still within the appropriate growth range. This is supported by Amin *et al.* (2005) that stated the growth of seaweed ranges from 2% - 3% per day. This depends on the supply of sunlight, climate, and the geographical conditions of the waters that are measured by somatic growth and are expressed as growth, weight growth and length growth of the thallus.

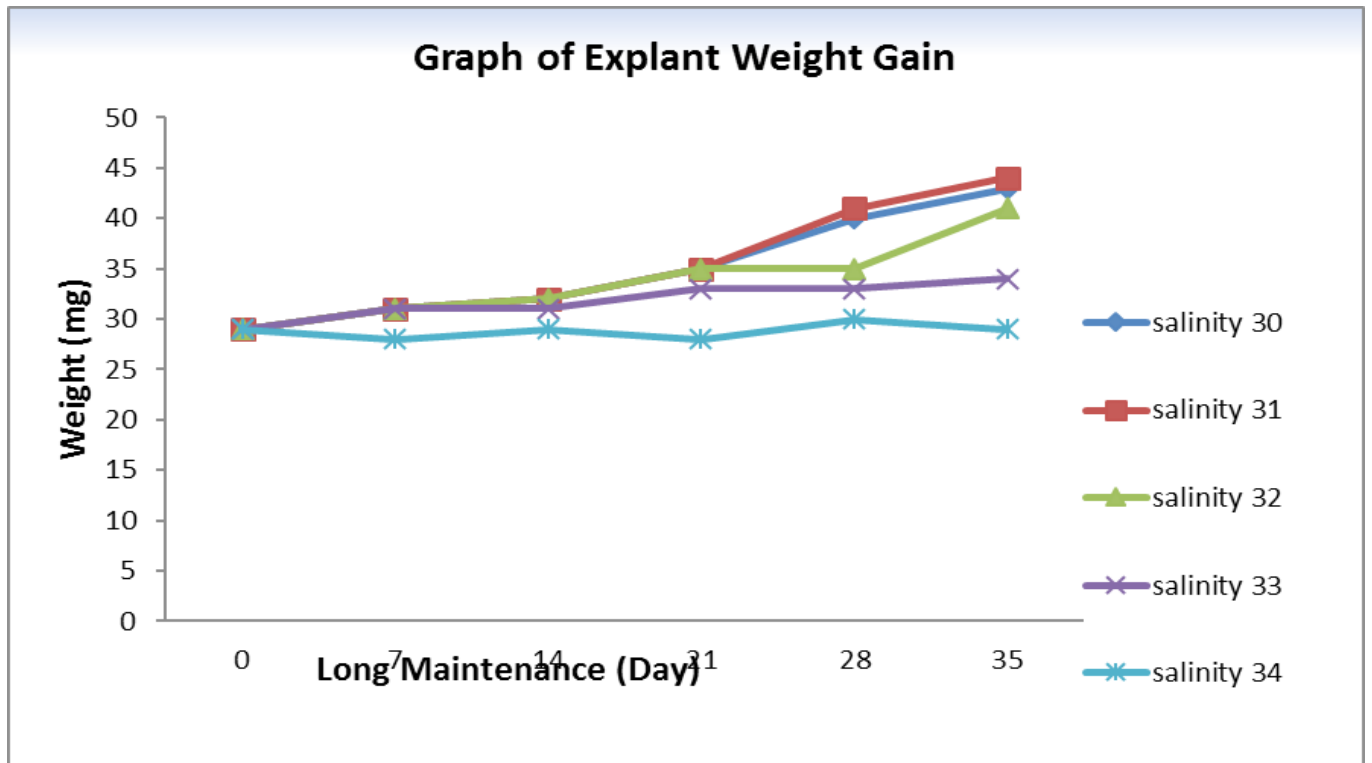


Figure 1. Graph of Weight Growth of Seaweed Explants of *Kappaphycus alvarezii* for 35 Days with Different Salinities.

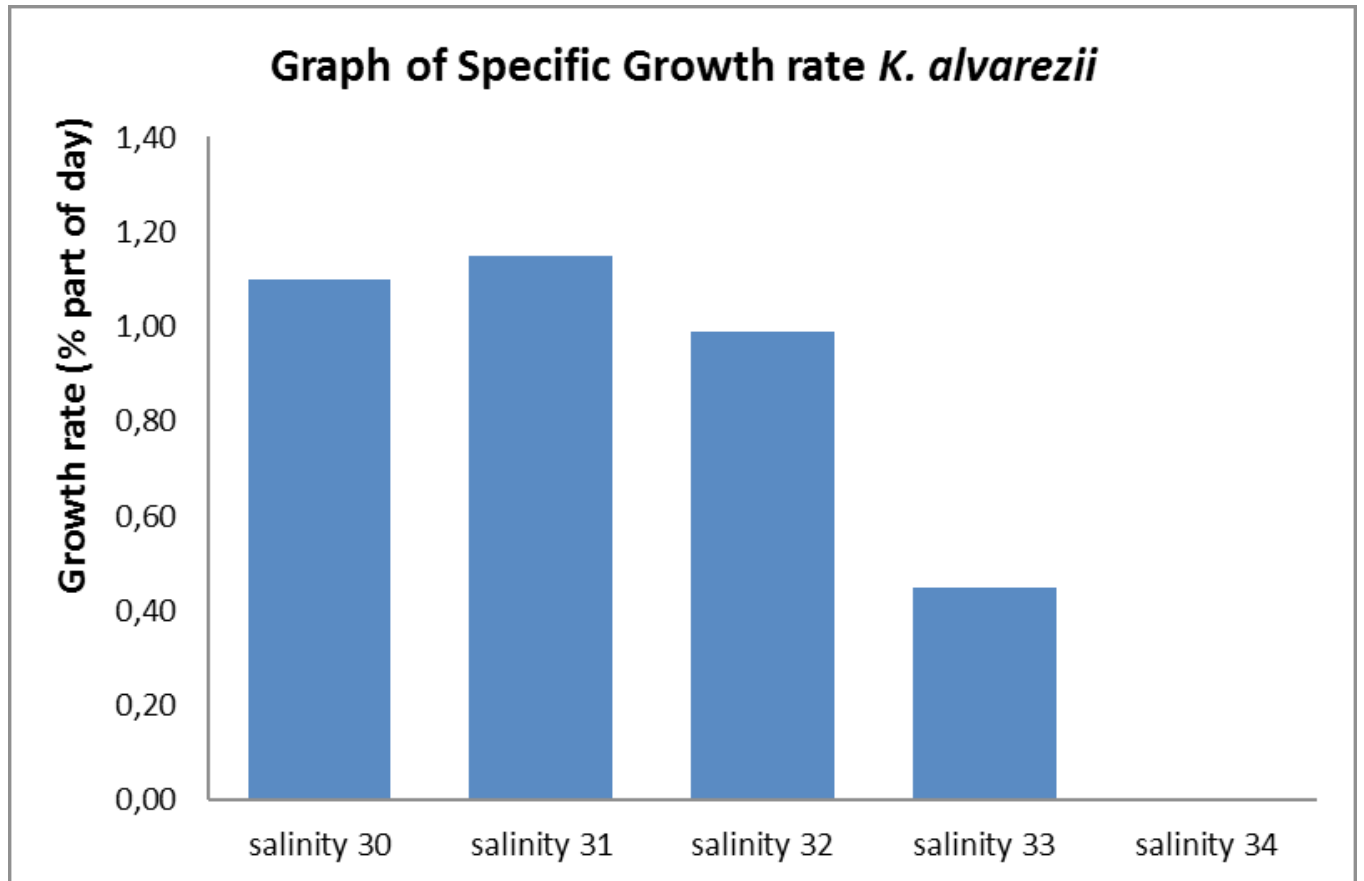


Figure 2. Specific growth rate (% part of day) all treatment with different salinity

Table 1. Observation results of water quality parameters

Parameters	Range Value Parametr
Temperature of	22°C
Salinity	30, 31, 32, 33, 34 mg/L
Light intensity	500 lux

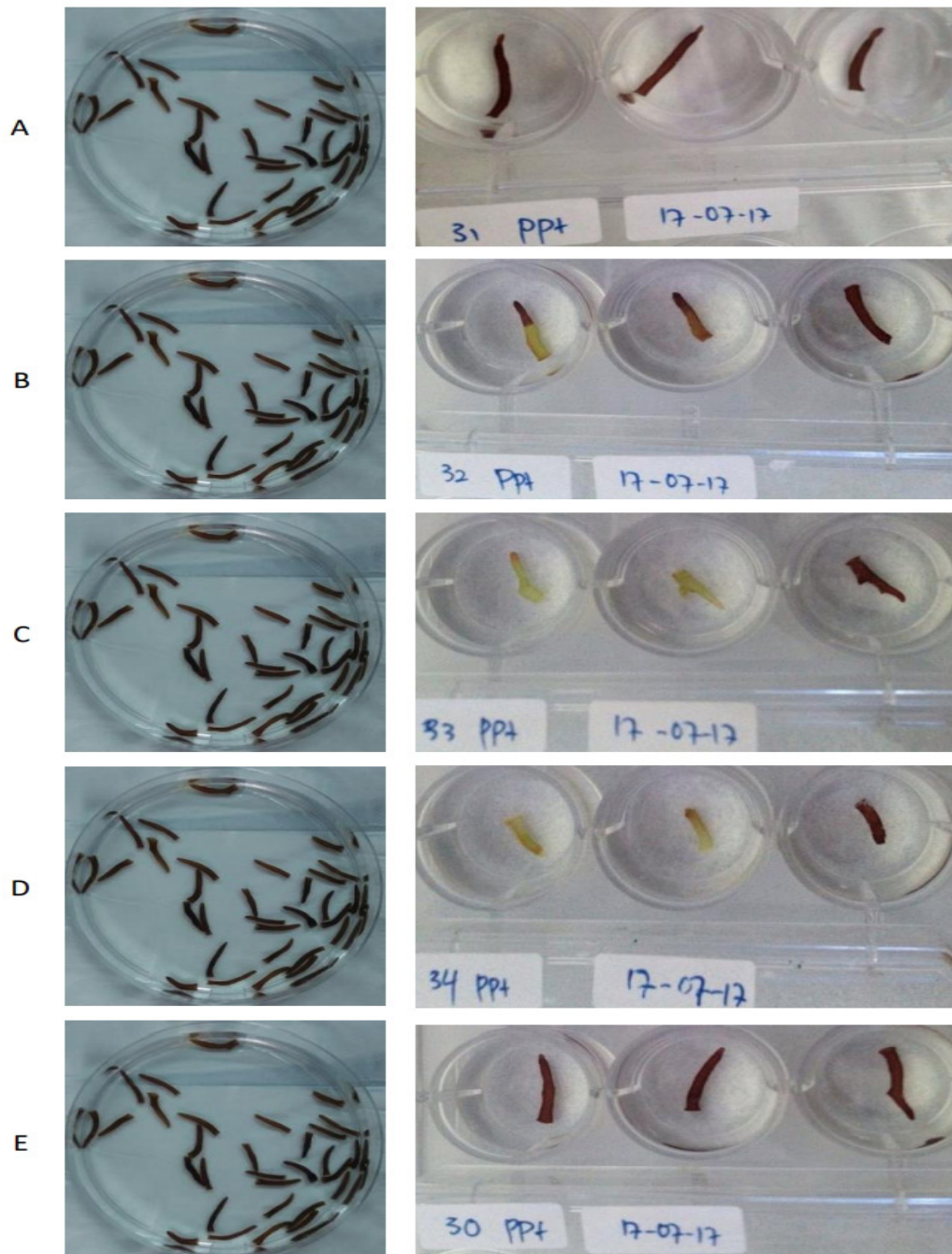


Figure 3. At the beginning and the end of Seaweed *Kappaphycus alvarezii* cultivation for each different salinity treatment (A) 31 mg/L salinity, (B) 32 mg/L salinity, (C) 33 mg/L salinity, (D) 34 mg/L salinity, (E) 30 salinity mg/L.

Furthermore, treatment C (33 mg/L salinity) and treatment D (34 mg/L salinity) underwent a weight increase of 0.45%, and 0.00% per day, less than the range reported by [Amin et al. \(2005\)](#). The osmoregulation process of the explant body is disrupted because the salinity is too high. It has difficulty of absorbing the nutrients that are around them, resulting in the slow growth of the explants. According to [Yokoya et al. \(1999\)](#) and [González et al. \(2013\)](#) the high salinity causes the Golgi body unable to balance the concentration of fluid in the cell with the concentration of fluid outside the cell. In the end, many cell fluids are lysis into the environment, so that the cell shrinks from its previous size. Moreover, the salinities that are too high or too low will cause interference in macroalgae physiological processes ([Yokoya et al., 1999](#); [Paradas et al., 2016](#)).

If the salinity is in accordance with the habitat of the algae, the absorption of nutrients and the growth and development of cells will run well. The slow growth and development of explants due to absorption of nutrients is not optimal because the condition of culture media is not suitable for growth. The condition of inappropriate culture media can interfere enzyme work, decrease turgor pressure in cells, and inhibit cell division. Salinity affects the mechanism of physiology and biochemistry, especially osmosis pressure. This osmosis pressure is closely related to the role of cell membranes in the process of nutrient transport and stimulation that effects on the growth of seaweed ([Yokoya et al., 1999](#); [Reddy et al., 2003](#); [Ortega, 2010](#)).

The Specific Growth Rates (SGR) showed that different salinities had a very significant effect on *K. alvarezii* seaweed explants. The SGR data of *K. alvarezii* (% per day) showed a difference between the treatment with one another. The difference in salinity gave a different SGR response. Based on the results of the LSD test, it was found that treatment E (30 mg/L salinity) was significantly different ($p < 0.05$) with treatment C (33 mg/L salinity) and D (34 mg/L salinity), while treatment A (31 mg/L salinity) and treatment B (32 mg/L) were not significantly different. Therefore, the optimal growth for *K. alvarezii* was at the salinities of 30 mg/L, 31 mg/L and 32 mg/L.

The results of the study on color changes showed there was no change in color from the beginning of cultivation to the end at the salinities of 30 mg/L (control) and 31 mg/L. On the other hand, the salinities of 32, 33 and 34 mg/L could change the colors (Figure 3). In general, *K. alvarezii* has a brownish green color ([Doty, 1973](#); [Hayashi et al., 2010](#)). Observations during the study showed that treatment (E) 30 mg/L salinity

and (A) 31 mg/L salinity did not change colors.

Furthermore, the salinities treatments of B (32 mg/L), C (33 mg/L), and D (34 mg/L) had undergone a change of the explants' colors. Although the explant had a color change, but it still could grow. Higher salinity made the explants difficult to develop because the osmoregulation process in cells will be inhibited and will affect the color of explants. According to [Yong et al. \(2014b\)](#) water quality parameter that played a major role in growth and the color of seaweed was salinity.

Stomata will shrink so that nutrients are poorly absorbed and inhibit growth ([Marroig et al., 2015](#)). Thus, it shows that the growth of seaweed explants is less optimal. Color changes are also related to the wavelength of light received by seaweed ([Borlongan et al., 2016](#)). This is influenced by the spectrum of colors and light that can cause changes in color pigments in seaweed, so it is closely related between wavelength and light ([Reddy et al., 2008](#)). The treatment D (34 mg/L salinity) has very low growth rate of 0.00% per day, so it is not strongly recommended to cultivate seaweed at high salinity.

Water quality parameters observed during the study were temperature, salinity, and light intensity ([Table 1](#)). Temperature is one of the most important factors in regulating life processes and spreading organisms in waters ([Dekić et al., 2013](#); [Banerjee and Ray, 2018](#)). Water temperature controls the growth of seaweed explants. The initial temperature of this study was 29°C and the optimal temperature for seaweed seeds acclimatization was 26°C. The temperature obtained in the process of explants maintenance was around 22°C. The temperature range obtained during the study is still quite good for the growth of *K. alvarezii* seaweed explants. According to [Doty \(1973\)](#) and [Li et al. \(2019\)](#), greenhouse temperature has a temperature range between 22.5-30.5°C. [Doty \(1973\)](#) stated that water temperature is very important in the process of seaweed photosynthesis. The optimal temperature for *K. alvarezii* seaweed growth was in the range of 25-30°C.

However, *Eucheuma* sp. has a temperature tolerance between 24-36°C. Temperature is closely related to the intensity of sunlight, because the higher the sunlight intensity is, the higher the temperature of water will be. Salinity is an oceanographic factor that is easily measured and plays an important role in physical, chemical, and biological processes such as the concentration of dissolved oxygen and the distribution of aquatic organisms ([Odulate et al., 2014](#); [Muchlisin et al., 2017](#)).

Additionally, the light intensity is also needed in the growth and survival of seaweed explants. The light intensity used during the study was 500 lux, especially in the process of photosynthesis and induction of thallus to form an embryo. The results of observations of light intensity start from 570.5-1,119.5 lux of 500 lux showing good growth and survival. This is as stated by Guan et al. (2013) stated that good light intensity for growth and good induction of seaweed thallus is 1500 lux.

4. Conclusion

It can be concluded that different salinity treatments have an effect on the growth rate of *K. alvarezii* seaweed explants with the best explant growth was observed in treatment A (31 mg/L salinity) and the lowest was treatment D (34 mg/L salinity). These results can be tested on other economically important seaweed species with the same treatment.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follow, Fery; collected the data, drafted the manuscript and designed the figures. Ria and Ery; devised the main conceptual ideas and critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests

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