Cultivation of seaweed *Kappaphycus alvarezii* (Doty) doty ex silva using tissue–cultured seedlings in encircling tank culture system

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Abstract. The seaweed, *Kappaphycus alvarezii*, is the most widely cultivated commodity in Indonesia. The problem currently faced is seedling availability due annual seasonal variations such as heavy flooding. This paper presents a study aimed at solving seedling scarcity: the first based on cultivating tissue-cultured seedlings in encircling tanks. The aim of the study was to determine the effect of different initial weight on growth and carrageenan yield of *K. alvarezii* using tissue–cultured seedlings. This research was conducted from March to June 2019 in the Shrimp Hatchery, Kendari, Indonesia. This study applied three treatments based on different initial weight and five replications. The treatments were 5g, B: 10g and 15 g initial weights (IW). The results showed that the daily growth rate (DGR) of all treatments tended not to differ significantly but the DGR of 5-g IW tended to be higher than those of the other two treatments. The means of DGR were 3.76±0.54%/day, 3.30±0.46%/day, and 2.84±0.27%/day for 5-, 10-, and 15-g IW, respectively. Healthy rates of all treatments were 90.28%, 94.45% and 87.47% for 5-, 10- and 15-g IW. The ratio of fresh weight (FW) and dry weight (DW of 5-, 10-, and 15-g IW were 9.51:1, 9.54:1, and 9.56:1, respectively. The ratio showed no significant differences among treatments. Furthermore, for carrageenan yield, 15-g IW treatment showed the highest yield (37.05±2.18%), but had no significant differences from the other two treatments. Furthermore, all water quality parameters, temperature, salinity, DO, pH, nitrat, phosphate and turbidity were within the normal range for cultivating the seedlings. In conclusion, 5-g initial weight is a prominent weight suitable to be used in cultivating the seedlings in the encircling tank.

1. Introduction

The red seaweed farming of *K. alvarezii* is widely considered as the most important mariculture activity in Indonesia, especially in Southeast (SE) Sulawesi [1,2]. This farming could provide a continuing income and requires low costs, minimum technology or expertise and a short period to harvesting [2–4].

In recent years, seaweed production in Indonesia tended to decrease. Seaweed production reached 11,269,342 tons in 2015, while in 2016 and 2017 the production reached 11,631,586 tons and 10,456,043 tons, respectively [5]. This decrease was influenced by the annual heavy flooding that occurs from March to June, low salinity, epiphytic infections and attacks [3,4]. Heavy flood events destroy seedlings which results in a seedling scarcity. Seedling scarcities are regularly found, for instance, in Langere coastal
waters, North Buton Regency SE Sulawesi [6]. This seasonal scarcity means farming is only done for 4-5 months to maximize harvested yield. If the scarcity continues for a longer period it hampers seaweed farming activities for the next planting season. Therefore, it is necessary to overcome the scarcity though innovation; investigating better methods of producing seaweed seedlings. Land-based culture methods, for instance, can be a promising solution to solve the scarcity.

Very few studies have already been done on producing seedlings using land-based culture methods [7] [8]. In the previous studies, encircling tanks were used as part of the cultivation method. The encircling tank for seaweed seedling might provide a better method for solving the scarcity than cultivating them in the open sea, especially during heavy flooding periods. Additionally, an encircling tank importantly provides a continuous, easily produced supply of high-quality seedlings. Two previous studies used a small raceway tank for the cultivation of brown seaweed (*Sargassum horneri*) in indoor tanks [7] and in outdoor tanks for local strain *K. alvarezii* seedlings [8]. From both studies, the seaweeds were reported to have the ability to grow and thrive successfully in the tanks. Although many studies have already reported that, compared to local strain seedlings, tissue-cultured seedlings have some advantages (higher growth rates and carrageenan yield plus disease resistance [3,4,9]) examine the feasibility of tissue-cultured seedlings, an experimental design should also be applied using *K. alvarezii* tissue-cultured seedlings to examine their ability to grow and survive in an encircling tank culture system.

2. Materials and Methods

2.1. Seedling collection

This research was done from March to June, 2019. The tissue-cultured seedlings of red seaweed species (*K. alvarezii*) were selected based on their abundance, morphological performance and quality. Sampling was done at Bungin Permai coastal waters, South Konawe, SE Sulawesi, (S4°29'24.03, E122°13'26.60) where the tissue-cultured seedlings were already used and cultivated by the local farmers. The seedlings were then translocated from the farm to Shrimp Hatchery Center in Kendari. A large number of young, healthy thalli were selected, initially weighing about 5-15g. The seedlings were washed thoroughly using fresh seawater before being cultivated in the encircling tanks located in the Shrimp Hatchery Center figure 1.

![Figure 1. The seedlings cultivated in the encircling tanks](image-url)
2.2. Planting of Seaweed Seedlings
Prepared seaweed seedlings were cut and weighed with different initial fresh weight (W₀): 5g, 10g and 15-g initial weight (IW) as treatments. Seaweed thalli of each seedlings treatment were tied on the tied onto the vertical culture ropes with a 10-cm planting distance.

2.3. Growing of the seedlings
Seedlings were grown for 30 days. The seaweed seedlings were tied across the 1.0 m vertically-hanging polyethylene ropes in quadruplicates (four lines) in the tank. The encircling tank culture system was designed using four PVC (Polyvinylchloride) encircling tanks in 1000 liters of filtered seawater with 1.2 m depth. The tanks were placed in the outdoor area of the hatchery. The encircling tank culture applied running seawater using a flow-through (raceways) system with a filtered seawater source supply and also equipped with continuous aeration. The flow-through occurred when the seawater was added and distributed evenly into the tank from the inlet pipe of the filtered seawater source supply and discharged back to the drain through the outlet pipe. During the growing period, silt and dirt was cleaned from the seedlings. Water quality parameters were measured every 3 days for temperature, salinity, DO and pH, while for nitrate (NO₃), phosphate (PO₄), and turbidity were measured every 10 days.

2.4. Parameters observed
Daily growth rate (DGR) for each seaweed. The weight for each seaweed seedling was measured and recorded every 6 days. The equation used to calculate DGR was calculated based on the equation DGR (%) = [(Wt/W₀)¹/t–¹] ×100% where W₀ is the initial fresh weight, and Wt is the final fresh weight of the seedlings after t days of culture [10].

2.4.1. Healthy rate (HR). The rate was measured by calculating the number of seedlings that failed to resist ice-ice disease divided by the total number of all seedlings that successfully resisted from ice-ice disease at the final day of the culture trial (Day 30) multiplied by 100. Epiphytes and disease found during the culture period were also recorded. Observation of ice-ice disease occurrence was detected by physically examining the conditions of the seedling.

2.4.2. Ratio of dry weight to fresh weight (DW: FW). All harvested plants were cleaned by removing dirt and sand using fresh filtered seawater. All samples of each treatment were weighed as fresh dried FW (g). After dried for 3-4 days using hanging method, all dried samples were weighed for final dried weight/DW (g). Ratio of DW: FW was then calculated and are expressed as mean ± SD for all the harvested seaweed.

2.4.3. Carrageenan yield. After washing the 5-g dried seaweed samples with filtered, fresh water, the seaweed samples were soaked with aquadest for 12 hours. Using an autoclave, the seaweed samples were then sterilized for 30 minutes at 121 °C. After that a blender was used to smooth the sample size and they were then filtered. Precipitation was done by diluting the samples in 100 ml iso-propanol and then drying them in the oven for 24 hours. The carrageenan yield was determined using the formula: Yield (%)=Wc/Wm x100; where Wc is weight of carrageenan extract (g) and Wm is the dry seaweed weight (g) used for extraction [11]. The data were presented as mean± SD, obtained from three treatments.
2.4.4. **Sea water quality.** The water quality parameters in each culture tank were monitored in-situ. Salinity was measured by hand refraktometer, while Dissolved Oxygen (DO), pH, temperature, concentration of nitrate, phosphate and turbidity were measured with the HANNA™ instrument HI98703-11 (HANNA™, Woonsocket, RI, USA) immediately after samples were collected from 6 to 8 a.m.

2.5. **Data analysis**

Data were analysed using ANOVA and a Post-hoc test using SPSS software version 21 (SPSS Inc.) with a 95% confidence level.

3. **Results and Discussion**

3.1. **Daily Growth Rate (DGR)**

Mean DGRs of tissue-cultured seaweed seedlings showed that 5-g IW showed the highest (3.76±0.54%/day) followed by 10-g IW (3.30±0.46%/day) and 15-g IW (2.84±0.27%/day). The results showed that the daily growth rate (DGR) of all treatments tended not to differ significantly but the DGR of 5-g IW tended to be higher than those of other two treatments (Figure 2; Table 1).

![Figure 2. DGRs of seaweed (K. alvarezii) using different initial weight (IW) of tissue-cultured seedlings cultivated in the encircling tank](image)

The DGRs obtained in this encircling tank study were significantly higher than those of the previous study done in outdoor tanks [8]. Although their seedlings were treated with the organic fertilizer enrichment of AMPEP (Acadian marine Plant Extract Powder), their DGRs were still lower 0.41±0.33%/day-1.46±0.06%/day). The DGRs of 5-g IW found in this study are comparable with a previous study done in India where the DGR at 30 days was 3.76±0.08% [12]. In addition, the DGR of this study fulfilled the international specification for commercial DGR (3.50%) [13]. From this study, it was found that 5-g initial weight of tissue-cultured seedling of *K. alvarezii* could be applied to to increase the growth more efficiently in the encircling tank than the heavier weight (10- and 15-g initial weight).
Table 1. DGR and Tukey Test of tissue-cultured seedlings of *K. alvarezii* using different initial weight (IW) cultivated in the encircling tank

| Days | IW (g) | DGR (%/days) ± SD | Tukey Test | p=Value |
|------|--------|-------------------|------------|---------|
|      |        |                   | 1          | 2       | 3       |  |
| 5    | 4.68±1.39 | 4.68<sup>a</sup>  | 0.01       |         |         |  |
| 6    | 4.10±1.25 | 4.10<sup>a</sup>  | 0.52       |         |         |  |
| 15   | 2.76±2.26 | 2.76<sup>b</sup>  | 0.06       |         |         |  |
| 5    | 3.76±1.39 | 3.76<sup>a</sup>  | 0.00       |         |         |  |
| 12   | 2.90±0.52 | 2.90              | 0.00       |         |         |  |
| 15   | 2.50±1.15 | 2.50              | 0.18       |         |         |  |
| 5    | 3.64±2.26 | 3.64<sup>a</sup>  | 0.02       |         |         |  |
| 18   | 3.22±1.25 | 3.22<sup>a</sup>  | 0.05       |         |         |  |
| 15   | 3.12±1.39 | 3.12<sup>a</sup>  | 0.81       |         |         |  |
| 5    | 3.38±0.34 | 3.38<sup>a</sup>  | 0.42       |         |         |  |
| 24   | 3.18±0.16 | 3.18<sup>a</sup>  | 0.20       |         |         |  |
| 15   | 3.10±0.29 | 3.10<sup>a</sup>  | 0.86       |         |         |  |
| 5    | 3.35±0.34 | 3.35<sup>a</sup>  | 0.00       |         |         |  |
| 30   | 3.12±0.16 | 3.12<sup>b</sup>  | 0.00       |         |         |  |
| 15   | 2.71±0.29 | 2.71<sup>c</sup>  | 0.00       |         |         |  |

* values followed by different letters are significantly different at *p* < 0.05

3.2. Healthy rate (%) of seaweed Seedlings

High percentages of healthy rate obtained from this study were 87.47-94.45%. (Table 2). This indicates that the encircling tank system for cultivating the tissue-cultured seedling is very suitable and could be developed for seaweed seedling propagation program.

Table 2. Healthy rate (%) of tissue-cultured seedlings of *K. alvarezii* using different initial weight (IW), cultivated in the encircling tank

| Initial Weight (g) | Total seedlings cultivated | Total seedlings infected with ice-ice | Healthy rate (%) |
|--------------------|----------------------------|--------------------------------------|------------------|
| 5                  | 72                         | 7                                    | 90.28            |
| 10                 | 72                         | 4                                    | 94.45            |
| 15                 | 72                         | 9                                    | 87.47            |

3.3. Ratio of Dry Weight (DW) and Fresh Weight (FW)

The ratio of FW: DW of 5-g, 10-g, and 15-g IW were 9.51:1, 9.54:1, and 9.56:1, respectively (Table 3). The ratios FW: DW obtained during this study were comparatively similar to the previous using tissue-cultured seedling done at open sea in Marobo, Indonesia (9.88:1) [14] and better than those of the previous studies using local strain conducted for 30 days in Indian waters (11.36:1).
Table 3. Ratio of Dry Weight (DW) and Fresh Weight (FW) of tissue-cultured seedlings of *K. alvarezii* Using Different Initial Weight (IW), Cultivated in the Encircling Tank

| Initial Weight (g) | Fresh weight | Dry weight | DW:FW ratio |
|-------------------|--------------|------------|-------------|
| 5                 | 14.73 ± 2.57 | 1.55 ± 0.26| 9.51 ±0.26a |
| 10                | 24.71 ±3.03  | 2.59 ± 0.30| 9.54 ± 0.25a |
| 15                | 33.68 ± 4.66 | 3.53 ± 0.54| 9.56 ± 0.33a |

3.4. Carrageenan yield
No significant differences were found among the treatments. Carrageenan yield of 15-g initial weight (IW) showed the highest (37.05±2.18) followed by 5 g IW and 10-g IW respectively (Table 4).

Table 4. Carrageenan Yield of tissue-cultured seedlings of *K. alvarezii* Using Different Initial Weight (IW) Cultivated in the Encircling Tank

| Initial Weight (g) | Carrageenan Yield (Mean±SD) | p-value |
|-------------------|----------------------------|---------|
| 5                 | 36.92±1.36a                | 0.279   |
| 10                | 34.74±2.01a                | 0.242   |
| 15                | 37.05±2.18a                | 0.994   |

The carrageenan yields found from this study are higher than those reported from previous studies using local strain seedlings done in India for 30 days (27.88 ± 0.98% [12] and using tissue-culture seedlings in Marobo, Indonesia (35.35±2.79) [14]. However, the yield obtained in this study was lower than 40% since the seedlings were cultivated for only 30 days. In addition, carrageenan yield found in this study clearly fulfills internationally defined specifications (minimum 27%) [12]. Therefore, the encircling tank system for cultivating the seedlings is very suitable for seedling propagation.

3.5. Water quality
During the 30-days study the temperature ranged from 25.5–28.6°C, salinity 25–35 ppt, turbidity 0.49-0.87 NTU, nitrates 0.41-0.61 mg/L and phosphate 0.1-0.5 mg/L (table 5). All water quality parameters fall within the range of optimum requirements for the growth of *K. alvarezii* cultivated at open sea. According to [15], the optimum ranges of water quality for *K. alvarezii* cultivated at open sea are: salinity 30-33 ppt, temperature 27-30°C, DO 5-6, pH 7-9. The ranges of nitrate, phosphate and turbidity are 0.008 mg/L, 0.015mg/L and < 5.0 NTU, respectively [16]. Therefore, all tissue-cultured seedlings cultivated in the encircling tanks show better growth due to the optimum water quality.
### Table 5. Water quality parameters in encircling tanks used for cultivating the tissue cultured seedling of *K. alvarezii*

| Water Quality Parameters | Range   | Mean ± SD     |
|--------------------------|---------|---------------|
| a. Temperature (ppt)     | 25.5-28.6 | 26.6 ± 0.97   |
| b. Salinity (°C)        | 25- 35  | 29.18 ± 2.72  |
| c. DO (%)                | 7.47- 8.92 | 8.29 ± 0.07   |
| d. pH                    | 8.174- 8.447 | 8.3769 ± 0.37 |
| f. Nitrate (mg/L)       | 0.41- 0.61 | 0.50 ± 0.08   |
| g. Phosphate (mg/L)     | 0.1- 0.5 | 0.29 ± 0.11   |
| h. Turbidity (NTU)      | 0.49- 0.87 | 0.64 ± 0.21   |

### 4. Conclusion

An encircling tank system, using 5-g initial weight, is most promising as a means to solving a scarcity of seaweed seedlings of *K. alvarezii*. Indeed, this first study of the encircling tanks cultivation method might also create new opportunities for the industry to produce high quality seedlings in the near future.

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