Field cultivation of *Kappaphycus alvarezii* (DOTY) doty ex silva using tissue-cultured seedlings at bungin permai costal waters, south konawe, Southeast (SE) Sulawesi: the third year of seaweed growth monitoring

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Abstract. Monitoring program is very important to evaluate the growth and performance of the seaweed *K. alvarezii cultivated* in the coastal areas. However, the program are rarely to be done in Indonesia continuously. In SE Sulawesi, tissue-cultured seedling are already been used by the seaweed farmers. The aims of this study were to evaluate the third period of growth monitoring program based on daily growth rate (DGR) and ratio of final wet weight and dry weight using tissue-cultured seedlings of the seaweed. This study was conducted in Bungin Coastal Waters, South Konawe. The result showed that the mean DGR of the seedlings were 7.01±0.63% day⁻¹ and significantly higher (*p*<0.05) than the two previous monitoring years (2017 and 2018). Ratio of final dry weight: wet weight was 9.42:1 and this ratio was also higher than the two previous years. In the present study, the epiphyte infection of *Sargassum polycystum* and an unidentified moss were also found. In conclusion, tissue-cultured seedling show good quality even though they have been used as seedlings for four years continuously.

1. Introduction

Indonesia is the largest producer of seaweed in the world [1]. Total production of Indonesian seaweed in 2017 was 10,456,043 ton [2]. Five provinces in Indonesia, South Sulawesi, East Nusa Tenggara, Central Sulawesi, West Nusa Tenggara, and Southeast (SE) Sulawesi, have become the largest producers of seaweed in 2018 [2]. In SE Sulawesi, total area of seaweed farming is 9,825.9 ha and its production in 2017 was 1,004,008.32 MT [3]. High production of seaweed in SE Sulawesi is supported by huge numbers of seaweed farming that has developed in every district/city. Until now, there are 10 seaweed farming centers in this region: South Konawe, Muna, Muna Barat, Wakatobi, Buton, Buton Selatan, Buton Tengah, Buton Utara, Konawe Kepulauan and Bombana [3].

The red seaweed, *Kappaphycus alvarezii*, is currently dominant to be cultivated in SE-Sulawesi. The high demand for this type of seaweed causes many farmers to focus on growing this seaweed throughout the coast of SE Sulawesi [4,5]. In addition, the high selling price of dried seaweed of this
species (IDR 22,000-23,000 per kg) adds to the motivation of farmers to continue their farming activities of this seaweed species.

The district of South Konawe has still low production. The production ranged from 540-2,160 kg/3,000 m² [6] and certainly it needs a new technology of seedlings since all farmers always used seedlings produced by vegetative reproduction method. This method done by harvesting the cultivars, cutting the whole thallus of the cultivars and replanting the new cuttings to the farm. This vegetative method has decreased the quality of seaweed cultivars in terms of low growth rate, carrageenan yield as well as easy to be infected by the ice-ice disease.

One method to solve the above-mentioned problem is the use of seaweed seedlings produced from tissue culture method. The use of tissue-cultured seedlings since 2015 in the village of Bungin Permai, South Konawe District, Sulawesi has showed a positive impact on seaweed production. On the other hand, high growth resulting from the use of tissue culture seedlings requires continuous monitoring of the results of the use of these seedlings in the area. This monitoring is necessary because there are some preliminary studies showed that seaweed seedlings produced by tissue-culture method have an optimum growth rate of up to only 20 times of plantings as stated by Dr E. Sulistiani ([Laboratory of tissue-culture, Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP), Bogor, Indonesia] 2019, pers. comm., 13 July). She also suggested that after 20 times used for farming, the seedlings should be replaced with the new seedlings. To prove whether the seedling are getting lower from time to time, since 2017, we therefore have begun continuous field monitoring program to examine the growth rate of these seedlings using similar method, period and location of cultivation. Long line method was applied and the cultivation period conducted from March/April to May/June annually was applied in Bungin Permai coastal waters, Tinanggea District, South Konawe District to support the monitoring.

The first and second monitoring of the K. alvarezii cultivars using tissue-cultured seedlings in 2017 and 2018 showed that the Daily Growth Rates (DGRs) were 4.62±0.66%.day⁻¹ [7] and 5.68±0.28%.day⁻¹ [8,9], respectively. During the monitoring period, they found also various epiphytes attached to ropes and ice-ice disease on seaweed thalli. To observe the prospects of the tissue-cultured seedling as the very high quality of seedling and to maintain the monitoring program, we reported in this paper the third consecutive years of monitoring program of K. alvarezii cultivars using tissue-cultured seedlings.

2. Materials and Methods
Field cultivation area of this study was done in Bungin Permai coastal waters at South Konawe district, Southeast (SE) Sulawesi from March to May 2019.

2.1 Seedling preparation
The younger and healthier thalli of tissue-cultured seaweed was selected from local farmers in Bungin Permai coastal waters as seedlings for planting purposes (Figure 1). All seedlings were washed with seawaters and cleaned from sediments, silt and all attached organisms before being used in this cultivation.

![Figure 1. Tissue-cultured Seedling of the seaweed K. alvarezii used in this study](image-url)
2.2 Planting of seaweed seedlings
The selected seedlings were then tied on the 2-mm in diameter of rope with a 10-cm planting distance using long-line method. Before planting, all the tied seedlings were then soaked in a chamber to prevent them from desiccation. The seedlings were cultivated for 35 days.

2.3 Seaweed cultivation and maintenance
During the 35 days cultivation period, periodical maintenance were done to ensure good optimum growth by visiting seaweed farm at least 2-3 times a week. Water quality parameters, salinity and temperature were also measured every week.

2.4 Parameters observed
There are two parameters observed during this study. (1). The daily growth rate (DGR) for all the treatments at 35-day using the formula: DGR (%.day\(^{-1}\) = [(W\(_{t}\)/W\(_0\))\(^{1/t}\) - 1] \times 100%, where W\(_0\) is the initial wet weight, W\(_{t}\) is the final wet weight (g) after t days [10]. Data of ten samples of cultivars were measured of each period of monitoring year. Data were expressed as mean \pm SD, and (2). Ratio of fresh weight to dry weight (FW: DW). The ratio was obtained after cleaning of harvested cultivars from silt, sand and other attached organisms. All harvested cultivars were weighed in gram (g). After dried using hanging method for 2-3 days in open sunlight for 2 to 3 days, final dry weight in gram (g) was then measured. Ratio of fresh weight to dry weight was then calculated. Data of ten samples of cultivars of each period of monitoring year were also used and expressed as mean±SD. In addition, epiphytes and ice-ice disease found during the culture period were also recorded.

2.5 Data analysis
Statistical analyses of all data from two previous period of study (2017 and 2018) and this current study were determined using ANOVA. The means were compared by a Tukey post hoc test. Subsequent analysis with Tukey's HSD test was done when there were significant differences among treatments with level of significance p<0.05.

3. Result and Discussion

3.1. Daily growth rates (DGRs)
The DGR of the 3\(^{rd}\) year of the monitoring program done in 2019 was continuously increased and significantly higher than those of the other two previous year (p<0.05; Table 1). In addition, the DGR of the 2\(^{nd}\) year was also significantly higher than the DGRs of the 1\(^{st}\). The DGR of the 3\(^{rd}\) year was 7.01\(\pm\)0.63%.day\(^{-1}\) followed by the 2\(^{nd}\) (5.68\(\pm\)0.28%.day\(^{-1}\)) and the 1\(^{st}\) year (4.62\(\pm\)0.66% .day\(^{-1}\)). In addition, the means final wet weight after 35 days in cultivation of the cultivar in 2019 were significantly higher than those of other previous year (p<0.05). The mean of 2019-final wet weight was 108.50\(\pm\)21.67 g followed by 2018-final wet weight (66.80\(\pm\)7.45 g) and 2017-final wet weight (49.80\(\pm\)11.61 g). It means that the cultivars of 2019-final wet weight had a 1.62- and 2.18-fold increase than 2018- and 2017-final wet weight cultivars, respectively.

Table 1. Daily Growth Rates (DGRs) of tissue-cultured seedlings of K. alvarezii monitored for 3 consecutive years (2017-2019)

| Monitoring Period | Final Wet weight range (Mean±SD) in g | Dry weight range (Mean±SD) in g | DGRs range (Mean±SD) in %.day\(^{-1}\) | References |
|-------------------|-------------------------------------|----------------------------------|------------------------------------------|------------|
| 2017              | 35.00-75.30 (49.8±11.61)\(^{c}\)     | 5.70-12.90 (8.23±2.06)           | 3.60-5.30 (4.62±0.66)\(^{c}\)          | [7]        |
| 2018              | 51.00-76.00 (66.80±7.45)\(^{b}\)    | 6.00-8.00 (7.20±0.92)           | 5.30-6.20 (5.68±0.28)\(^{b}\)          | [8,9]      |
The growth rates using tissue-cultured seedlings recorded at the present monitoring program done in March-May 2019 were comparatively higher than 3.5 % day⁻¹ for commercial seaweed farming [10]. These DGRs were comparatively higher than the other DGRs of Kappaphycus species recorded from many countries, for instance, in India the DGRs were 3.76±0.07 % day⁻¹ [11], in Brazil, 4.07 % day⁻¹ [12], and in Madagascar 5.46±0.09 % day⁻¹ [13]. Comparing to tissue-cultured seedling used in previously seaweed farming, the DGRs found in this study were slightly higher than those found in Malaysia (6.3 ± 0.1 % day⁻¹) [14] but comparatively similar the DGRs in Philippines (5.8-7.2 % day⁻¹) [15]. Lower DGRs found in 2017 was presumably caused by nickel mining activities heavily occurred in South Konawe. Waste resulting from mining activities in the form of sediments was widely discharged into the sea so that a lot of planted seaweed had lower growth and even a few failed crops. In 2018-2019, the activities has decreased due to moratorium mining program done by the Indonesian government.

The higher DGRs recorded in this study were found at an optimum seawater temperature and salinity. Seawater temperature (28-31°C) has an important role influencing the DGR as similarly also found by [16] in India and in Brazil [17]. Moreover, salinity (30-31 ppt) at the study site was also within the required levels for Kappaphycus farming [18]. Based on farmer’s experience in Bungin Permai coastal waters, productive season of seaweed K. alvarezi in the South Konawe district waters was from November to August especially in April–May annually.

This present study of the third year of monitoring program using the tissue-cultured seedlings of K. alvarezi strongly show that the seedlings still viable to be used for the farmers even though the seedlings have been used for more than 4 years in same area in Bungin Permai coastal waters. If the farmers cultivated them 6-7 times a year then the seedlings are already being used for more 24-28 times. In short, the seedlings are able to exceed the predictions of seaweed experts that the seedlings are only capable of constant growth if planted around 20 times.

3.2. Ratio of final wet weight (FW) and dry weight (DW)
The ratio of FW: DW of all seedlings from three consecutive years showed significant difference among the monitoring period (Table 2). The FW: DW ratio of 2018- and 2019-cultivars was significantly different from the ratio of 2017- cultivars (p<0.05) but no significant differences were found between the ratio of the last two years cultivars (p>0.05). The mean of ratios of FW: DW of 2019-, 2018-, and 2107- cultivars were 9.42:1, 9.32:1 and 6.11:1, respectively.

| Monitoring Period | Ratio FW:DW range (Mean±SD) | References |
|-------------------|-----------------------------|------------|
| 2017              | 5.21:1 - 7.81:1 (6.11: 1±0.74)³ | [11]       |
| 2018              | 8.00:1 - 10.83:1 (9.32: 1±0.94)⁴ | [8,9]      |
| 2019              | 8.12:1 - 10.35:1 (9.42:1±0.88)⁵ | This study |

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

The ratio of FW: DW obtained during this study was comparatively similar to a previous study conducted for 30 days in Indian waters where the ratio mean was 9.89 ± 0.13 [10]. The ratio of FW:DW found in these three years studies was tended to more heavier (1:6) than those of the ratio using non-tissue-cultured cultivars (commonly 1:10). It could be due to tissue-culture K. alvarezi
yielded from high nutrient process in a tissue-cultured laboratory using high level of nutrients such as calcium, magnesium, cobalt, copper, lithium, beryllium, manganese, and zinc compared to farm-propagated seedlings [19]. Therefore, using tissue-cultured seedlings, the ratio FW: DW could clearly increase dry weight.

3.3. Epiphytes and disease

During this study, the ropes and the seaweed thalli were also covered with epiphyte of filamentous red algae as well as unidentified mosses (Figure 2).

![Figure 2](image)

**Figure 2.** Epiphyte and an unidentified moss found at seaweed thalli. A, heavily attached epiphyte on seaweed thalli; B, the epiphyte, *S. polycystum*; C, unidentified species of moss

The epiphytes, *Sargassum polycystum* and unidentified algae as well as mosses outbreaks occurred mostly on April-May 2019. Period of the outbreaks was also similar to other previous study in other areas where they regularly occurs in May-June and/or in October-November [21-23]. In addition, the outbreaks resulted in a heavily biomass reduction and certainly reduced the seaweed production. During this study, some parts of branches and tips of the cultivated seaweed was also attacked by ice disease. It is indicated by tips and branches whitening (Figure 3). The disease makes stress on the thalli which makes in biomass and production loss [20–22]. Moreover, the ‘ice-ice’ disease is triggered by an environmental combination (such as high salinity and as well as low water motion) and biological factors such as epiphytes [23,24].
Figure 3. Ice-ice isease on seaweed *K. alvarezii* thalli (an arrow)

4. Conclusion

The third year of growth monitoring program using tissue-cultured seedlings of *K. alvarezii* showed stable and better growth and ratio of fresh weight and dry weight even though the seedlings had been used for four years continuously. This monitoring result also confirms that the seedlings are very feasible to be used to support continuously high seaweed production in Indonesia, especially in SE Sulawesi.

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