Effect of different seedling sources on growth and carrageenan yield of seaweed *kappaphycus alvarezii* cultivated in Marobo Waters, Muna Regency, Southeast (Se) Sulawesi, Indonesia

Odi Nur Aeni¹, La Ode Muhammad Aslan¹*, Wa Iba¹, Andi Besse Patadjai², Manat Rahim¹ and Muis Balubi¹

¹Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia  
²Department of Fish Processing Technology, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232, Indonesia  
³ Department of Economics Science and Development Study, Faculty of Economics and Business, Halu Oleo University, Kendari 93232, Indonesia

*Email: aslaod1966@gmail.com

Abstract. The purpose of this study was to determine the effect of different seedling sources on growth and carrageenan yield of *K. alvarezii*. There were three various seedlings sources used in this study: local strain, tissue-cultured seedlings, and seedlings resulting from mass selection combined with tissue-cultured method called “prof”. This research was over three months (August-October 2018) in Marobo coastal waters, Bone District, Muna Regency SE Sulawesi using the longline method. The results showed that the daily growth rate (DGR) during this study tended to fluctuate among treatments “prof” and tissue-cultured seedlings had higher the DGRs than local strain seedlings. Ratio fresh weight (FW) and dry weight (DW) showed no significant differences among different seedling sources. The mean FW: DW ratio showed the local strain had the highest ratio (10.10:1) followed by tissue-cultured seedlings (9.88:1) and prof (9.06:1). Furthermore, the local strain showed a significant difference in carrageenan yield from the “prof” and tissue-culture seedlings. Local strain seedlings showed the highest carrageenan yield (40.58±2.51%) followed by tissue-cultured seedlings (35.35±2.79%) and prof seedlings (33.88±2.09%). Ice-ice disease and epiphyte (*Sargassum polychystum*) were found during this study. This study showed that prof and tissue-cultured seedlings greater potential for improved seaweed farming.

1. Introduction

Indonesia is the world's largest producer of *Kappaphycus* species with 10,456,043 tons total yield of wet seaweed in 2017. High production of this seaweed is due to two factors. Firstly, the Indonesia government has already prioritized seaweed farming as one of the important commodities to increase the livelihoods of coastal communities [2, 3]. Secondly, intensification seaweed farming programs are being stimulated by the Indonesian government by distributing and developing seaweed farming in all
suitable coastal areas of this country. Farms are mainly located in South, Southeast (SE) and Central Sulawesi, West and East Nusa Tenggara, Northern Kalimantan and East Java. The programs also include providing better quality seedlings, improving farming techniques, developing zonation or culture areas, and enhancing post-harvest and processing technologies. Unfortunately, domestic consumption is relatively low, and most of the harvested product, which consists of dried seaweed is exported to China, Chile, the Philippines, and other Asian markets [4].

Southeast (SE) Sulawesi is one of the primary seaweed cultivation regions in Indonesia. Seaweed cultivation occurs in every district and city of the Province with of 73,247.1 tons of dried seaweed produced in 2017 [5]. The Muna Regency of SE Sulawesi has about 79,258 ha of the sea and 337 km of coastline available for potential seaweed cultivation. Production of seaweed in Muna Regency in 2017 reached 4,361.90 tons of dried seaweed [5].

One of the factors that influence the growth and yield of seaweed carrageenan is the quality of seedling sources. Seaweed farmers always use local strain seedlings obtained by a vegetative production method. However, this method causes a decrease in the quality of seedlings characterized by a decrease in growth rate, low carrageenan yield, and low gel strength [6,7]. To support the continuity of high-quality seaweed production, necessary efforts are needed to develop high-quality seedlings.

The use of better quality seedlings sources can certainly increase the growth and production of seaweed [7]. Seaweed seedling development in Indonesia has focused solely on tissue-cultured methods. Tissue-cultured seedlings are readily available and have a higher growth rate compared to vegetatively-produced seedlings [8]. On the other hand, there has been limited research on a new method, developed in 2013, that combines mass selection with the tissue-cultured method [9]. Seedlings produced from the combination of these two methods are morphologically different from tissue-cultured seedlings and commonly called by local farmers as “Prof”. The thalli “prof” seedlings tend to be shorter and have more branching than tissue-cultured seedlings.

Until recently, studies into growth, and carrageenan yield using different seedling sources (tissue-cultured seedlings, prof seedlings, and local strain seedlings) in Indonesia have never been done. This study is also certainly very important in supporting the government’s program to improve the seaweed production in Indonesia. Therefore, we conducted a study on the effects of different seedlings sources on the growth and carrageenan yield of the seaweed Kappaphycus alvarezi.

2. Materials and Methods
This study was done in the waters of Marobo Village, Marobo District, Muna Regency, SE Sulawesi Province (508°9.06" S and 122018'48.49" E). Carrageenan yield analysis was done in the laboratory of the Faculty of Fisheries and Marine Sciences of Halu Oleo University, Kendari.

2.1 Seedling Preparation
Three different sources of K. alvarezi seaweed seedlings were prepared and used in this study: tissue-cultured, prof, and local strain seedlings. Tissue-cultured and prof seedlings used in this study were derived from a previous study [6] while local strain seedlings were obtained from the seaweed farmers of Marobo village. All seedlings were cleaned to remove dirt and attached organisms.

2.2 Planting of Seaweed Seedlings
Seaweed seedlings of different seedling sources were collected from the rope then trimmed to initial weight (W0) of 10 g. Seaweed thalli were then bound on the prepared rope with a 10-cm planting distance. All the tied seedlings were then soaked to prevent them from dehydration.

2.3 Seaweed cultivation
All seedlings were cultivated using the longline method. Cultivation was carried out for 45 days. During the cultivation period, ropes and seaweed cleaned of dirt, epiphytes and other organisms twice a
week. Temperature and salinity were measured every three days, while nitrate, phosphate, and turbidity were measured every nine days.

2.4 Parameters Observed
The parameters observed during the study were:
- 1. The daily growth rate (DGR) for all the harvests cultivated for 45 days was calculated for different growth periods using the formula: $DGR (%) = \left( \frac{W_t}{W_0} \right)^{1/t} - 1 \times 100\%$, where $W_0$ was the initial fresh weight and $W_t$ was the final fresh weight (g) of the seedlings, and $t$ was the number of culture days [10]. DGR values were expressed as mean ± standard deviation (SD) for all the harvests of the respective growth periods for the study period.
- The ratio of fresh weight to dry weight (FW: DW). After cleaning of harvested seaweed in the laboratory by removing sand and other attached organisms, all harvested and fresh, dried seaweeds for each of the seedling sources were weighed (g). After drying using a hanging method for 2-3 days, final dried weight (g) was also measured. The ratio of fresh weight to dry weight was then calculated. Data were expressed as mean ± SD for all the harvested seaweed [11].
- Epiphytes and disease found during the culture period were also recorded.

2.5 Analysis of carrageenan yield
Dried seaweed was prepared in the laboratory and weighed for 5 g of each sample then washed with fresh water. The samples were then soaked in distilled water for 12 hours, followed by sterilized in an autoclave for 30 minutes at 121 °C. After that, the samples were smoothed in a blender and filtered with filter. Samples were then precipitated with 100 ml Iso-propanol. Carrageenan yield was analyzed by drying the samples dried in the oven for 24 hours. The carrageenan yield (%) was calculated based on the formula: $Yield (%) = \frac{W_c}{W_m} \times 100$ where $W_c$ was the weight of carrageenan extract (g) and $W_m$ was the dry seaweed weight (g) used for extraction [12].

2.6 Data Analysis
Statistical analyses were determined using ANOVA. If the analysis showed a significant effect, it was continued with the Tukey test using SPSS version 16 statistical software at the $p<0.05$ level.

3. Results And Discussion
3.1 Daily Growth Rate (DGR)
During the culture period, DGRs from different seedling sources tended to fluctuate (Figure 1; Table 1) but prof and tissue-cultured seedlings tended to have higher DGRs than seedlings from local strain. DGR on the 9th day was very high and ranged from 9.88-14.17%/day. On the 9th day, local strain seedlings had the highest DGR (14.17%/day) and was significantly different from prof seedlings (10.09%/day) and tissue-cultured seedlings (9.88%/day). On the 18th day, the seedlings of prof and tissue-cultured were higher and significantly different from the local seedlings. On the 26th day, DGRs were significantly different for all seeding sources with prof being the highest. On the 27th and 45th day DGRs were not significantly different from each other.
Figure 1. DGR of seaweed (*K. alvarezii*) from different seedling sources

The higher DGR of prof seedlings were caused by the formation process of these seedlings which combined two stages of selection: the mass selection process that lasts for nine months then followed by seedling formation using tissue culture methods [9]. This formation process of this prof seedling was more selective than those of tissue-cultured seedlings alone so that DGR of prof seedlings was higher than those of tissue-cultured seedlings. The DGRs obtained during this study were 3.06 ± 0.22 to 14.17 ± 2.26%.

**Table 1.** Daily growth rates (DGRs) of seaweed (*K. alvarezii*) from different seedling sources

| Days | Sources of seedlings | DGR (%/day) | Tukey test | p=Value |
|------|---------------------|-------------|------------|---------|
| 9    | Prof                | 10.09       | 10.09±1.39 | 0.869   |
|      | Tissue-cultured     | 9.88±1.25   |            | 0.002   |
|      | Local strain        | 14.17±2.26  |            | 0.003   |
| 18   | Prof                | 6.64±0.52   |            | 0.012   |
|      | Tissue-cultured     | 4.08±1.15   |            | 0.008   |
|      | Local strain        | 4.24±1.39   |            | 0.971   |
| 27   | Prof                | 5.42±0.27   |            | 0.745   |
|      | Tissue-cultured     | 5.51±0.20   |            | 0.090   |
|      | Local strain        | 5.39±0.53   |            | 0.050   |
| 36   | Prof                | 4.58±0.22   | 4.58±0.22  | 0.000   |
|      | Tissue-cultured     | 3.51±0.14   | 3.15±0.14  | 0.016   |
|      | Local strain        | 3.06±0.22   | 3.06±0.22  | 0.000   |
| 45   | Prof                | 5.98±0.29   |            | 0.243   |
|      | Tissue-cultured     | 6.18±0.16   |            | 0.005   |
|      | Local strain        | 5.82±0.34   |            | 0.378   |

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

The variations found in the present DGRs might be mainly due to the culture site in Marobo coastal waters and environmental parameters. These DGRs were comparatively higher than the other DGRs recorded from elsewhere for the similar *Kappaphycus* species: in India, DGRs were 3.76 ± 0.07 and 3.69 ± 0.11% [13] while in Madagascar DGR was 5.46 ± 0.09%/day [14]. In addition, the DGRs...
found in this study was comparable with the previous studies using tissue-cultured seedlings reported in the Philippines (5.8-7.2%/day) [15] and Malaysia (6.3 ± 0.1%/day) [16]. The DGR using prof seedling from this study was also comparable to a previous study using similar prof seedlings, which found 6.27± 0.31%/day [17]. All different seedlings in this study had mean DGRs mostly more than 3.5%, which may be appropriate for commercial seaweed farming [18]. Therefore, all seedlings especially prof and tissue-cultured seedlings, are very suitable for seaweed farming.

The high DGRs were found during August-October 2018. These coincided with the study in the Philippines at September [19] and in India in October [18]. The results suggested that the growth rates are site and season specific. Furthermore, the maximum DGRs recorded in this present study were found at a higher seawater temperature 29-31°C, salinity 30-31 ppt, and turbidity 0.11 -2.12 NTU. Also, the high DGRs were found when the nitrate and phosphate were in an optimum range of 0.65-3.36 µmol L-1 and 0.3-0.43 µmol L-1, respectively. It revealed that the water in Marobo areas was relatively rich in nutrients.

3.2 The ratio of Fresh Weigh and Dry Weight (FW: DW)
The ratio of fresh weigh and dry weight (FW: DW) showed no significant difference amongst seedling sources. The ratio of fresh weight and dry weight of treatment of seedling sources: tissue-cultured, prof and local strain were 9.88:1, 9.06:1 and10.10:1, respectively (Table 2).

Table 2. The ratio of fresh weight and dry weight (FW:DW) of seaweed K. alvarezi from different seedling sources

| Sources of Seedlings | Initial Weight (g) | Fresh Weight (g) | Dry Weight (g) | Dry weight ratio | Tukey test | p=Value |
|---------------------|--------------------|------------------|----------------|-----------------|------------|---------|
| Prof                | 10                 | 137.0±2.34       | 12.4           | 1: 9.06         | 9.06a      | 0.913   |
| Tissue-cultured     | 10                 | 146.0±2.54       | 15.4           | 1: 9.88         | 9.88a      | 0.993   |
| Local strain        | 10                 | 128.0± 4.72      | 12.4           | 1: 10.10        | 10.10a     | 0.863   |
| Mean                | 137± 3.2           | 13               | 1: 9.69        |                 |            |         |

The ratio of fresh weight and dry weight (FW: DW) of prof seaweed, tissue-cultured, and local strain were 9.06:1, 9.88:1, and 10.10:1, respectively. These ratios were nearly similar to the results of a previous study conducted in Indian waters (9.89 ± 0.13) [11].

3.3 Carrageenan yield
The carrageenan yield of seaweed from local strain was significantly higher than those of the other two seedling sources (Figure 2; Table 3). Mean carrageenan yield of local strain was 40.58%, followed by prof (35.29%) and tissue-cultured (33.85%) seedlings. In contrast, carrageenan yield from prof and tissue-cultured seedlings were not significantly different.
The carrageenan yield obtained for *K. alvarezii* in the present study were comparable with those reported from other places, for instance, in Brazil 31 to 43 % [20]; in Mexico 30.3 to 40.7 % [21]; and in India 42.42 to 58.36 % [18]. However, carrageenan yields in the present study were higher than that found from previous studies done in India during April 2011 to March 2012 (24.52±0.65 to 31.10±0.71 %) [13] and 33.30 ± 1.23 to 38.50 ± 0.20 % [22]. Differences in carrageenan yield among different seedling sources could be influenced by environmental parameters, such as culture site and growth conditions (salinity, depth, and nutrients) [20] as well as stress. From this study, stress effects should be considered as the main factor affecting carrageenan yield differences since prof and tissue-cultured seedlings used in this study were transported from North Buton regency and directly planted in Marobo, Muna regency without acclimatization. Fortunately, the seedlings of prof and tissue-cultured obtained in this present study still fulfilled the international specifications (minimum 27 %) [11]. To obtain more detailed information regarding the carrageenan yield as well as growth rate, further studies focusing on the presence of seasonal variation in growth and carrageenan yield by cultivating of these different seedlings at least one year are importantly needed.

| Seedling Sources     | Carrageenan Yield (%) | Tukey Test | p-Value |
|----------------------|-----------------------|------------|---------|
| Prof                 | 33.88±2.09^a          | 1          | 0.650   |
| Tissue-cultured      | 35.35±2.79^b          | 2          | 0.014   |
| Local strain         | 40.58±2.51^c          |            | 0.003   |

^avalues followed by different letters are significantly different at *p* < 0.05

### 3.4 Epiphyte and ice-ice disease

There were epiphytes and ice-ice disease found in this study (Figure 3). The most common epiphyte was *Sargassum polychystum* which attached to the seaweed thalli while ice-ice disease affected the tip of the seaweed thalli. Outbreaks of ice-ice were found on 18th and 36th days of the culture period.
Production of the seaweed in Marobo coastal waters has declined significantly in recent years due to increases of ice-ice disease infection and epiphyte infestation. Epiphytes cover the surface of the seaweed thalli, which significantly inhibits the absorption of nutrients [23, 24]. In addition, the ice-ice disease is generally caused by stress due to drastic changes in temperatures, salinity, culture periods [14], and epiphyte growth [21]. Therefore, it appeared that combined effects of ice-ice disease and epiphyte growth also promoted of lower DGR at 18th and 36th of culture periods as suggested by [24].

4. Conclusion
The present study indicates good prospects and feasibility for commercial cultivation of prof and tissue-cultured seedlings. However, it is apparent that further studies focusing on growth, carrageenan yield, epiphyte infection, and ice-ice disease to obtain the possibility of seasonal variation presence for at least one year are required. Moreover, a survey should be undertaken to identify potential sites for commercial eucheumoid farming of these two different seedling sources along the entire SE Sulawesi coasts.

5. References
[1] Kementerian Kelautan dan Perikanan (2018). Refleksi 2018 dan Outlook 2019. Jakarta, 17 Desember. 64 Hal
[2] Aslan LOM, Iba W, Bolu, LR, Ingram BA, Gooley GJ, Silva, SSD (2015). Mariculture in SE Sulawesi Indonesia: Culture Practices and The Socioeconomic Aspects of The Major Commodities. Ocean &Coastal Management 116: 44 – 57
[3] Aslan LOM, Hafid H, Supendy R, Taridala SAA, Sifatu WO, Sailan Z, Niampel (2018). Income of Seaweed Farming Households: A Case Study From Lemo of Indonesia. IOP Conf. Series: Earth and Environmental Science 175 012221 DOI :10.1088/1755-1315/175/1/012221
[4] FAO (2018). The global status of seaweed production, trade and utilization, by Fatima Ferdouse, Zhengyong Yang, Susan Lovstad Holdt, Pedro Murua and Rohan Smith, FAO Consultants. Globefish Research Programme Volume 124. Rome, Italy
[5] Rasyid A (2018). Muna Produser Terbesar Udang Vaname dan Rumput Laut di Sultra. Sultrakini.com.http://sultrakini.com/berita/muna-produsen-terbesar-udang-vaname-dan-rumput-laut-di-sultra. Accessed on August 2018
[6] Rama R, Aslan LOM, Iba W, Rahman A, Armin A, Yusnaeni Y (2018). SeaweedCultivation ofMicropropagatedSeaweed (Kappaphycus alvarezii)in Bungin PermaiCoastal
Waters, Tinanggea SubDistrict, South Konawe Regency, South East Sulawesi. IOP Conf. Series: Earth and Environmental Science 175 012219 doi :10.1088/1755-1315/175/1/012219

[7] Hurtado AQ, Neish IC, Critchley AT (2015). Developments in production technology of Kappaphycus in the Philippines: more than four decades of farming. J Appl Phycol DOI 10.1007/s10811-014-0510-4

[8] Yong WTL, Chin JYY, Yasir S (2014). Evaluation of Growth Rate and Semi-refined Carrageenan Properties of Tissue-cultured Kappaphycus alvarezii (Rhodophyta, Gigartinales). Phycological Research: 62 : 316-321

[9] Aslan LOM, Iba W, Patadjaib AB, Rahim M. (2018). Pengembangan Kawasan Desa Rumput Laut Kappaphycus alvarezii Hasil Kultur Jaringan Dalam Mendukung Peningkatan Pendapatan Masyarakat Pesisir di Sulawesi Tenggara. Laporan Penelitian Unggulan Strategis Nasional. Universitas Halu Oleo. 233 Hal.

[10] Yong YS, Young WTL, Thien. VY, Ng SE, Anton A(2013). Analysis of Formulae for Determination of Seaweed Growth Rate. J Appl Phycol 25:1831-1824.

[11] Periyasamy C, Subba Rao PV, Anantharaman P (2016) Spatial and temporal variation in carrageenan yield and gel strength of cultivated Kappaphycus alvarezii (Doty) Doty in

[12] Hung LD, Kanji Hori K, Nang HQ, Kha T, Hoa LT (2009) Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga Kappaphycus alvarezii cultivated in Camranh Bay, Vietnam. J Appl Phycol (2009) 21:265–272 DOI 10.1007/s10811-008-9360-2

[13] Periyasamy C, Anantharaman P, Balasubramanian T, Subba Rao PV (2014). Seasonal variation in growth and carrageenan yield in cultivated Kappaphycus alvarezii (Doty) Doty on the coastal waters of Ramanathapuram district, Tamil Nadu. J Appl Phycol DOI 10.1007/s10811-014-0256-z

[14] Ateweberhan M, Rougier A, Rakotomahazo C (2014). Influence of environmental factors and farming technique on growth and health of farmed Kappaphycus alvarezii (cottoni) in

[15] Hurtado AQ, Gerung GS, Yasir S, Critchley AT (2014). Cultivation of tropical red seaweeds in the BIMP-EAGA region. J Appl Phycol 26: 707–718

[16] Yong WTL, Chin JYY, Yasir S (2014). Evaluation of Growth Rate and Semi-refined Carrageenan Properties of Tissue-cultured Kappaphycus alvarezii (Rhodophyta, Gigartinales). Phycological Research: 62 : 316-321

[17] Goa S (2018). Budidaya Rumput Laut Kappaphycus alvarezii (Doty) Doty ex Silva (Soliericeae, Gigartinales, Rhodophyta) Menggunakan Bibit Hasil Seleksi Klon yang telah di Kultur Jaringankan di Perairan Desa Bungin Permai Kecamatan Tinanggea Kabupaten Konawe Selatan Sulawesi Tenggara. Laporan praktikum lapangan, Fakultas Perikanan dan Ilmu Kelautan, Universitas Halu Oleo, Kendari

[18] Subba Rao PV, Suress Kumar K, Ganesan K, Mukund CT (2008) Feasibility of cultivation of Kappaphycus alvarezii (Doty) Doty at different localities on the northwest coast of India. Aquaculture 39: 1107–1114

[19] Hurtado-Ponce AQ, Agbayani RF, Sanares R, de Castro-Mallare TR (2001) The seasonality and economic feasibility of cultivating Kappaphycus alvarezii in Panagatan Cays, Caluya, Antique, Philippines. Aquaculture 199:295–310

[20] Hayashi L, Paula EJD, Chow F(2007) Growth rate and carrageenan analyses in four strains of Kappaphycus alvarezii (Rhodophyta, Gigartinales) farmed in the subtropical waters of Sao Paulo state, Brazil. J Appl Phycol 19:505-511

[21] Munoz J, Freile-Pelegrin Y, Robledo D (2004) Mariculture ofKappaphycus alvarezii ((Rhodophyta, Soliericeae) color strains intropical waters of Yucatan, Mexico. Aquaculture 239:161–177

[22] Periyasamy C, Subba Rao PV, Anantharaman P (2016) Spatial and temporal variation in carrageenan yield and gel strength of cultivatedKappaphycus alvarezii (Doty) Doty in
relation to environmental parameters in Palk Bay waters, Tamil Nadu, southeast coast of India. J Appl Phycol 28:525–532

[23] Vairappan CS, Chong CS, Hurtado AQ, Soya FE, Lhonner GB, Critchley A (2008). Distribution and symptoms of epiphyte infection in major carrageenophyte producing farms. J Appl Phycol 20:477–483

[24] Wakibia JG, Bolton JJ, Keats DW, Raitt LM (2006). Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. J Appl Phycol 18: 565–573 DOI: 10.1007/s10811-006-9058-2

Acknowledgements
The second to five authors (L.O.M.A., W. I, A.B.P and M.R.) want to express their gratitude for the financial support and facilities provided by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Kemenristek-Dikti) of the research scheme Penelitian Unggulan Strategis Nasional (Pusnas) under grant no 478/UN29.20/PPM/2018 and SP DIPA-042.06.1.401516/2018. We would also like to thank Prof. La Sara, Dr. Agus Kurnia Dr. Wellem H. Muskita from Halu Oleo University for their encouragements and facilities. Thanks are also due to Dr. Brett Ingram, Senior Research Scientist of the Fisheries Management and Science Branch, Fisheries Victoria, Department of Primary Industries, Australia, Ms Elizabeth Wright and Mr. Idul Male for helping and assisting us in preparing this manuscript and Mr. Armin, Mr. Syukur, Ms. Erika and Ms Asma for their helping in laboratory and field works.