Seaweed Cultivation of Micropropagated Seaweed *(Kappaphycus alvarezii)* in Bungin Permai Coastal Waters, Tinanggea Sub-District, South Konawe Regency, South East Sulawesi.

Rama Rama[1], La Ode Muhammad Aslan[2], Wa Iba[3], Abdul Rahman Nurdin[4]., Armin Armin, Yusnaeni Yusnaeni[5]
[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232
Email : rama1705@yahoo.com
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232
Email : aslaod66@yahoo.com
[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232
Email : icharyr@yahoo.com
[4]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232
Email : rahman_uh@yahoo.c.id
[5]Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari 93232
Email : yusyusnaini@ymail.com

Abstract : The *Kappaphycus alvarezii* or commonly called as cottonii is the most economically seaweed species in Indonesia. Farming of this seaweed is expanding widely as a main livelihood source for coastal communities in South East (SE) Sulawesi. This study was done in Bungin Permai coastal waters for 3 months from April–June 2017. One cycle of 35-day culture trials were conducted to describe the cultivation of micropropagated seedlings of *K alvarezii* and their daily growth rate (DGR) using long-line farming technique. Cultivation process was started from preparation of materials, tying of seedlings, planting, monitoring, and harvesting. This study found that DGR of *K. alvarezii* were 4.6%.day^{-1}, ratio of dried weight: wet weight of the harvested seaweed was 1:6. In addition, epiphytes such as *Sargassum polychystum* and *Hypnea musciformis* were also found. This DGR result was comparable with those cultured from other countries. In conclusion, the micropropagated *K. alvarezii* seedlings can be used to increase seaweed production in Indonesia.

Keywords: *Kappaphycus alvarezii*, Micropropagated, Epiphytes, DGR

1. Introduction

Seaweed is one of economically important commodities of Indonesia. In recent years, Indonesia had been acknowledged as the biggest producer of seaweed in the world [1]. Indonesia in 2015 already produced 10,335,000 ton of seaweed. It was caused by the Indonesian national policy that had placed seaweed as the important commodity for fishery's sectors [2]. KKP already targetted to achieve the production of seaweed to 13.4 million tons in 2017. The seaweed culture in Indonesia has been distributed throughout the provinces especially in east Indonesia. There are five provinces which have recently become the biggest producers of seaweed: South Sulawesi, Southeast Sulawesi, Central Sulawesi, East and West Nusa Tenggara [2]. Among them, Southeast (SE) Sulawesi is a province in

Indonesia that has the potential for the development of seaweed culture because of its marine area of ± 114 879 km^{2,} with a coastline of 1,740 km [3]. These marine areas are potential for cultivation of seaweed with huge potential to be developed in all districts/cities in SE-Sulawesi [4-5] and due to the easiness to culture the seaweed with low technology input and low production costs [3-6]. Species of seaweed that is currently developed in SE-Sulawesi is *Kappaphycus alvarezii*. [3] stated that the main cultured species of seaweed in the region was *K. alvarezii* and *Eucheuma denticulatum* that supplied most of the global market. One district in SE-Sulawesi which has great potential in the development of seaweed farming is South Konawe. In 2014, the seaweed cultivation area in this district was about 3,210 ha with production reached 275,256.41 tons [3].

Increasing demand and sustainable supply and availability of high quality seaweed seedlings for cultivation is still experiencing many obstacles in SE-Sulawesi. Currently, procurement of seaweed seedlings is mainly from previous culture season which is highly dependent on the local climate and seasonal monsoon. In addition, the use of natural seedlings by vegetative reproduction means that the farmers harvest are the whole thallus and to replant them to the farm with new cuttings. The new cuttings were used by the farmers as seedling stock for the next crop repeatedly. This seedlings stock method has resulted decreasing quality of seaweed products such as low carrageenan yield and growth and eventually seaweed becomes more susceptible to disease. One solution to produce high quality seaweed seedlings is through tissue culture or micropropagated methods. Some benefits of using micropropagated seedlings are constant availability and supply thus procurement can be done at any time and growth rate was higher than the natural or vegetative seedlings [7-8].

There is very little information on seaweed cultivation and growth analysis using micropropagated seedlings in Indonesia, especially in SE-Sulawesi [8]. Therefore, research on the cultivation of seaweed using seedlings from tissue culture which aimed to determine the growth rate of seaweed is needed.

This research was carried out for 3 (three) months from April to June 2017. Preparation of seedlings and culture tools was conducted at the Laboratory of Faculty of Fisheries and Marine Sciences, University of Halu Oleo Kendari before they were cultivated at Bungin Permai coastal waters, Tinanggea District, South Konawe.

2. Method

Before planting the seeds, polyethylene (PE) ropes were prepared with diameter of 8 mm, 4 mm and 2 mm respectively as the main material used for longline. 50 m long line was prepared to be a binding rope. Micropropagated seedlings of *K. alvarezii*, which weighed 10±5 g each using a balance with 0.5 g precision scales were obtained from a seaweed farm located in Bungin Permai coastal waters, Tinanggea district, South Konawe Regency, SE Sulawesi using the long-line method at 26-28 days culture age. Seaweed from micropropagated seedlings showed a denser and larger thallus, blackish brown color and tend to be darker than the local seaweed (Fig. 1). After weighing the seedlings, the seedlings were tied to the rope and after binding were completed, they were then immediately brought to the planting location to maintain fresh seedling conditions.

Figure 1. Comparison of morphology between local seedlings (left) and micropropagated seedlings (right)

The seedlings was planted in 10 cm distance between the seedlings as suggested by [9; Fig. 2] so that each rope has 20 seedlings, and 20 ropes were tied parallel to each other at 1.0 m spacing between the ropes.

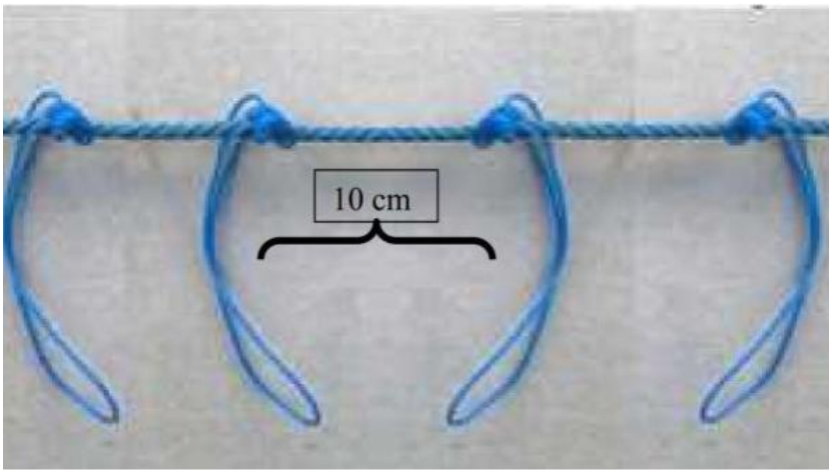

Figure 2. The method of using 1.5 mm diameter nylon rope and a 10 cm spacing between seedlings which recommended to increase production [9]

Regular maintenance of the lines and seedlings was conducted twice a week by removing the unwanted weeds, epiphytes, other marine debris and repairing and /or cleaning the lines. The seaweed material was then harvested in the morning after 35-day growth period by lifting the long line from the seawater then loaded onto a boat and brought ashore. The seaweed then dried using hanging method for 2-3 days to standard level of dryness when the color turned to dark red.

The parameters observed during the study were:
1) Daily growth rate (DGR) of seaweed cultivated for 35 days was calculated using the formula recommended by [10] as follows:
2) 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.
3) Ratio of dry : wet weight was conducted to determine the shrinkage ratio of wet and dried seaweed
4) Epiphytes found during culture period were recorded. In addition, seawater temperature and salinity was also measured every week during the study.

3. Result and Discussion

3.1 Daily growth rate (DGR) and dry: wet weight ratio

The average DGR of the micropropagated seaweed cultured for 35 days reached was 4.6%.day^{-1} whilst the ratio of dry and wet weight was 1: 6 (Table 1). Seawater temperature during culture period was in the range of 28-31 °C and salinity was in the range of 31-33 ppt.

Table 1. DGR data during the study

Clumps	W_0 ial fresh weight)	Wt ıl fresh weight)	Wt (dry weight)	DGR
1	10	38.0	7.3	3.9
2	10	75.3	12.9	5.9
3	10	47.3	7.5	4.5
4	10	48.2	7.8	4.6
5	10	45.5	7.2	4.4
6	10	60.5	9.9	5.3
7	10	48.2	9.1	4.6
8	10	44.5	5.7	4.4
9	10	55.5	8.6	5.0
10	10	35.0	6.3	3.6
Average		**49.8**	**8.23**	**4. 6**

Note: W_0 = initial fresh weight of seed (g); Wt = final fresh after harvest (g). Dry: wet weight was 49.9 /8.23 = 6.04 or equal to 1: 6.0

The growth rates or DGR (4.6%.day^{-1}) using micropropagated seedlings recorded at the present experimental site in April-May 2017 (Table. 1) were comparable with that (3.5 %.day^{-1}) for commercial seaweed farming [11]. In addition, this growth rate were also comparable with other previous study using non-micropropagated seedlings. For example, [12] recorded 1.4–5.3 %.day^{-1} in the subtropical waters of Brazil, while [13] reported a DGR of 2.00–7.10 %.day^{-1} in the tropical waters of Mexico. In India, [14] recorded DGR of 3.76 ± 4.92 %.day^{-1} while in Madagascar was 5.46 %.day^{-1} [15]. However, for comparison with DGR using tissue-cultured seedlings, the DGR found in this study had showed lower growth rates than the previous study done in Malaysia (6.3 %.day^{-1}) [16].

DGR can be seasonally influenced by temperature and salinity. Low DGR found in this study (April-June) was similarly also found in India [14]. The low DGRs recorded in this study were found at a high seawater temperature 28-31°C. Seawater temperature tended to play an important role affecting the DGR of this seaweed as observed in this study and also found by [17] in India. The low growth observed in this study could be attributed to high seawater temperature. In addition, [18] showed that temperature was the main factor influencing growth of *K. alvarezii* grown in Brazil. High temperatures have presumably been observed to reduce the growth rates of micropropagated Kappaphycus in this study. Moreover, salinity at the study site was within the required levels for *Kappapphycus* farming [19], however, the salinity patterns tended to follow the temperature and the growth rate were low. Ratio of dry weight to wet weight found in this study was higher (1:6) than those of the ratio using non-microporpoagated seedlings (commonly 1:8 to 1: 10). From this study, the micropropagated *Kappphycus* has presumably showed heavier weight. It could be due to micropropagated K. alvarezii yielded significantly higher calcium, magnesium, beryllium, cobalt, copper, lithium, manganese, and zinc compared to farm-propagated K. alvarezii [20]. Therefore, the rationale of using micropropagated seedlings to increase production is clearly defined.

3.2 Epiphytes

The ropes and the seaweed thallus were heavily covered with epiphytes of filamentous red algae *Sargassum polychystum* (Fig. 3 A) and *Hypnea musciformis* (Fig. 3 B) and other unidentified epiphytes (Figure 3 C, D). Epiphyte outbreaks occurred on May-June 2017 was similarly also found in other areas. [21] reported that epiphyte outbreak periods: (1) May and June and (2) October and November. This data are nearly similar with data of the outbreaks of epiphytes in Borneo [21-23]. The outbreaks could be possibly by the drastic changes in seawater temperature and salinity usually occur at the onset of monsoon, especially during the month of May – June, which reduced the defense mechanism potential of the seaweed [22]. Physical damage caused by epiphytes has resulted in a serious reduction of biomass production and decline in carrageenan quality [22-24]. In short, low DGR resulted from this study could be possibly caused by epiphytes outbreak

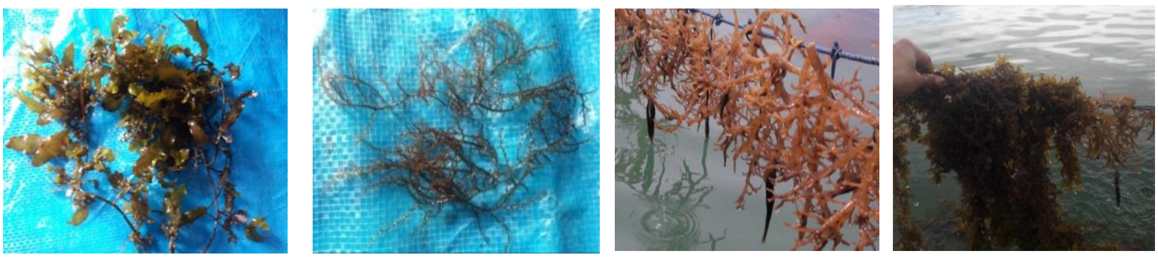

Figure 3. Epiphytes and moss found during the study. Epiphytes *Sargassum polychystum* (A) and *Hypnea musciformis* (B); and unidentified epiphytes (C and D). Bars represent 15 cm

3.3 Ice Ice Disease

During culture period, the seaweed was also attacked by ice ice disease on the branches softening tips whitening (Figure 4). The disease is generally caused by low salinity high temperature, light intensity and coinciding with the period of high temperature and salinity [26]. From this study, DGR was low when ice-ice disease occurred on April-May 2017. The disease makes the thallus stress which results in biomass loss [27-28]. This information agrees well with data reported from Wakibia et al [26] who reported that DGR was low during ice ice disease. In addition, 'ice-ice' could be promoted by a combination of environmental (high temperature/salinity and low water motion) and biological factors such as epiphytes [22-23]. Therefore, "ice–ice" disease and epiphyte outbreaks simulataneoulsy occurs in a commercial seaweed farming.

Figure 4. Ice ice disease on seaweed thallus

4. Conclusion

Cultivation of this micropropagated seaweed is found to be commercially feasible in SE Sulawesi. It may be taken up by the Indonesian government especially for Ministry of Marine Affairs and Fisheries/KKP in order to create rural employment for the seaweed farmers to improve their living standards. However, it is apparent that a first priority should be given to a survey of other potential and suitable areas for commercial farming scale along the coastal areas in Sulawesi Island . Moreover, better farm management with the use of micropropagated seedlings and cultivation procedure should be continuosly disseminated to the farmers groups.

5. Acknowledgments

The authors are grateful to Prof. La Sara, Dean of Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia, for his support and encouragement, and to colleagues at the Laboratory of Fisheries, Halu Oleo University, Kendari, Indonesia for providing expert technical assistance. The authors wish also to thanks to many of the farmers in Bungin Permai, South Konawe supported this study, especially Ms. Siti for their helps from time to time in the execution of the Research and Community Development work.

6. References

[1] FAO (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

[2] Ministry of Marine Affairs and Fisheries of the Republic of Indonesia / KKP-RI, 2016. Statistics of Aquaculture of Indonesia. General Directorate of Aquaculture. Jakarta.

[3] Marine and Fisheries Agency of SE-Sulawesi/DKP-Sultra 2014. Laporan Tahunan Statistik

Perikanan Budidaya Tahun 2013. Prov. Sultra. Kendari.

[4] Sahrir WI, Aslan LOM, Bolu LOR, Gooley, GJ, Ingram BA, Silva SSD (2014) Recent Trends in Mariculture in S.E. Sulawesi, Indonesia. General Considerations. Aquac. Asia 19 (1): 14-19.

[5] Aslan LOM, Iba W, Bolu, LR, Ingram BA, Gooley GJ, Silva, SSD (2015) Mariculture in SE Sulawesi Indonesia: Culture Practices and The Sosioeconomic Aspects of The Major Commodities. Ocean & Coastal Management: 116: 44 – 57.

[6] Albasri, H, Iba, W, Aslan, LOM, Geoley, G, Silva, DS 2010. Mapping of Existing Mariculture Activities in South-East Sulawesi “Potential, Current and Future Status”. Indonesian Aquaculture Journal. 5: 173-185.

[7] Kumar GR, Reddy CRK, Jha B, (2007) Callus Induction and Thallus Regeneration from Callus of Phycocolloid Yielding Seaweeds from the Indian Coast. J. Appl. Phycol 19: 15-25.

[8] Aslan LOM, Sulistiani E, Legit D, Yusnaeni (2014) Growth and Carrageenan Yield of *K. alvarezii* from Tissue Culture Seedlings Using Diffrerent Planting Distance AOAIS 2014. 17-20 Nov. 2014, Daejeon, Korea.

[9] Muñoz J, Freile-Pelegrín Y, Robledo D (2004) Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México. Aquaculture 239:161–177.

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

[11] Ask EI, Azanza RV (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. Aquaculture 206:257–277.

[12] Goés HG, Reis RP (2012) Temporal variation of the growth, carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated at Sepetiba Bay, south eastern Brazilian coast.J Appl Phycol 24:173–180.

[13] Aslan LOM, Ruslaini, Iba, W, Armin, Sitti (2017) Culture of *Kappaphycus alvarezii* using tissue-cultured seedlings. Practical Guidance of seaweed Culture No.1. FPIK-UHO . Kendari. https://laodeaslan.wordpress.com/2017/06/29/ accessed on 9 July. 4p.

[14] 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

[15] Ateweberhan M, Rougier A, Rakotomahazo C (2014) Influence of environmental factors and farming technique on growth and health of farmed *Kappaphycus alvarezii* (cottonii) in south-west Madagascar J Appl Phycol.

[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] Subba Rao PV, Suresh 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.

[18] Paula EJ, Pereira RTL (2003) Factors affecting growth rates of *Kappaphycus alvarezii* (Doty) Doty ex P. Silva (Rhodophyta, Solieriaceae) in subtropical waters of São Paulo State, Brazil. In: Chapman ARO, Anderson RJ, Vreedland VJ, Davison IR (eds) Proceedings of the 17th International Seaweed Symposium. Oxford University Press, Oxford

[19] Ohno M, Largo DB, Ikumoto T (1994) Growth rate, carrageenan yield and gel properties of cultured kappa-carrageenan producing red alga *Kappaphycus alvarezii* (Doty) Doty in the subtropical waters of Shikoku, Japan. J Appl Phycol 6:1–5.

[20] Yong YS, Yong WTL, Ng SE, Anton A, Yassir S (2015) Chemical composition of farmed and micropropagated *Kappaphycus alvarezii* (Rhodophyta, Gigartinales), a commercially important seaweed in Malaysia J Appl Phycol.

[21] Vairappan CS, Chung CS, Matsunaga, S (2014). Effect of epiphyte infection on physical and chemical properties of carrageenan produced by *Kappaphycus alvarezii* Doty (Soliericeae, Gigartinales, Rhodophyta). J Appl Phycol 26:923–931

[22] 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.

[23] Vairappan CS (2006) Seasonal occurrences of epiphytic algae on the commercially cultivated red alga *Kappaphycus alvarezii* (Solieriaceae, Gigartinales, Rhodophyta). J Appl Phycol 18: 611–617.

[24] Vairappan CS, Sangeetha PA, Tan KL, Matsunaga S (2010) Role of secondary metabolites as defense chemicals against ice–ice disease bacteria in biofoulers at carrageenophyte farm. J Appl Phycol 22: 305–311.

[25] Hurtado AQ, Critchley AT, Trespoey A, Bleicher LG (2006) Occurrence of *Polysiphonia* epiphytes in *Kappaphycus* farms at Calaguas Is., Camarines Norte, Phillippines. J Appl Phycol 18:301–306.

[26] Wakibia JG, Bolton JJ, Keats, DW, Raitt, LM (2006) Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. Journal of Applied Phycology (2006) 18: 565–573

[27] Largo DB, Fukami F, Nishijima T, Olino M (1995a) Laboratory induced development of the ice-ice disease of the farmed red algae *Kappaphycus alvarezii* and Eucheuma denticulatum (Solieriaceae, Gigartinales, Rhodophyta). J Appl Phycol 7:539–543.

[28] Largo DB, Fukami F, Nishijima T (1995b) Occasional bacteria promoting ice-ice disease of the farmed red algae *Kappaphycus alvarezii* and Eucheuma denticulatum (Solieriaceae, Gigartinales, Rhodophyta). J Appl Phycol 7:545–554.