Effect of various planting distances on growth and carrageenan yield of *Kappaphycus alvarezii* (doty) using seedlings produced from mass selection combined with tissue-cultured method

Effect of various planting distances on growth and carrageenan yield of *Kappaphycus alvarezii* (doty) using seedlings produced from mass selection combined with tissue-cultured method

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Abstract. *Kappaphycus alvarezii* (Doty), a red seaweed, is the most widely cultivated commodity in Indonesia. However, there is a decline in the cultivation production in the last three years. One way to overcome this problem is by improving the quality of seedlings through a development of farming. Efforts made from these are to develop farming techniques using different planting distances (PD) treatments. This study aimed to determine the effect of different PD on growth and carrageenan yield of *K. alvarezii* using seedlings produced from mass selection combined with tissue-cultured method. This research was conducted in Marobo coastal waters, Bone District, Muna Regency, SE Sulawesi, Indonesia. This study used a Completely Randomized Design (CRD) with three treatments based on different PD and five replications. The treatments were A: 10 cm PD, B: 15 cm PD, and C: 20 cm PD. The results show that the daily growth rate (DGR) of all treatments tended not to differ significantly. For 10 cm PD, the DGR tended to be higher and significantly different from the other two treatments, 15 cm and 20 cm. DGR averagely were 6.00±1.19%/day, 5.36±1.17%/day, and 5.27±1.62%/day for 10, 15, and 20 cm PD, respectively. The ratio of fresh weight (FW) and dry weight (DW) showed no significant differences among treatments. Ratio of FW:DW treatments of 10 cm, 15 cm, and 20 cm were 8.34:1, 9.58:1, and 8.98:1, respectively. For carrageenan yield, the treatment of 20 cm PD showed highest yield and it was significantly different from other treatments. Seaweed diseases and epiphytes found during this study were ice-ice and epiphytes (*Sargassum polychystum*).

Keywords: mass selection, seaweed, tissue-cultured, planting distance

1. Introduction

Seaweed, *K. alvarezii*, is an economically important commodity in Indonesia, especially in Southeast (SE) Sulawesi. This seaweed is widely cultivated because of its ease in cultivating a plant with relatively low cost required [1], short period of cultivation and broad market share with a large volume of needs [2]. Seaweed production in Indonesia has decreased in the last 3 years. In 2015, seaweed production reached 11.27 million tons, but in 2016 and 2017, it was 11.05 million ton and 10.81 million ton, respectively [3]. Therefore, necessary action needs to be taken so that the production can increase again.

One solution that can be done is by improving seedlings quality. Generally, many farmers use seedlings repeatedly [2], as a result, it reduces the daily growth rate (DGR) of cultivated seaweed.

Mass selection combined with tissue-cultured is one method to support the production of qualified seedlings throughout Indonesia. This method is a new method and has firstly introduced and developed since 2013 [4]. The combination of these two methods have produced seedlings which morphologically different from tissue-cultured seedlings and commonly called by local farmers as "Prof*"* (figure 1). Main thalli of the seedling from tissue-cultured method are longer than Prof. Despite the irregular branching patterns, Prof tend to exhibit higher branching frequency than tissue-cultured seedlings.

Figure 1. *K. alvarezii* seedlings from tissue-cultured methods (left) and from mass selection combined with tissue-cultured methods as called by local farmers as "Prof*"* (right).

Previous research showed that DGR tissue-cultured *K. alvarezii* seedlings cultivated in the waters of Bungin Permai coastal waters, Konawe Selatan District, Southeast Sulawesi Province in 2017 for 35 days was 4.6±0.66%/day [2]. On the other hand, for getting better result during cultivation period, the seedling should be cultivated using suitable planting distance (PD). PD is one of the technical factors that can affect the growth of seaweed because of its relationship with nutrient absorption. Research on the effect of different PD on growth and carrageenan yield *of K. alvarezii* seaweed using seedlings produced from mass selection combined with tissue-cultured method are still limited. Therefore, the purpose of this study was to determine the effect of different PD on growth and carrageenan yield of seaweed *K. alvarezii* as result of mass selection combined with tissue-cultured.

2. Materials and Methods

This research was conducted from August to October 2018. Field activities were done at Marobo coastal waters, Muna Regency, South East Sulawesi (5°8'9.06" S and 122°18'48.47" E) and continued with the analysis of carrageenan yield in the laboratory of the Faculty of Fisheries and Marine Sciences of Halu Oleo University, Kendari.

2.1. Seedling preparation

The seedlings used in this study were a continuation from previous study using the combination of mass selection and tissue-cultured methods. Mass selection was undertaken in 2013 for nine months. Seedlings from the mass selection had DGR >3.00 %/d. Selected seedlings produced from mass selection were then brought to SEAMEO-BIOTROP Laboratory, Bogor in 2014. In the laboratory, all the seedlings were proceeded to produce new plantlets through tissue-culture method. After they attained plantlets (2-3 mm long) in the laboratory, they were sent to Kendari, South East Sulawesi for

further field cultivation at an experimental farm in Bungin Permai, South Konawe, South East Sulawesi, Indonesia, since 2016 [5]. *K. alvarezii* seaweed seedlings were collected from seaweed seedlings produced from combination of mass selection combined with tissue-cultured method called "Prof' by local farmers. The seedlings were out planted to an experimental seaweed farm located in Koepisino coastal waters, North Buton regency, SE Sulawesi. The seedlings were then transported by a ship to the farm site at Marobo coastal waters, Muna regency.

2.2. Planting of seaweed seedlings

a. Seaweed seedlings were cut from the rope and the early seaweed thalli were collected.
b. The collected seedlings were then weighed with initial fresh weight of (W_0) 10g.
c. Seaweed thalli of each seedlings were bound on the prepared rope with a 10 cm, 15 cm, and 20 cm PD for each treatment.
d. After the seaweed seedlings were tied, seaweed were soaked in order to prevent from dehydration
e. Seaweed seedlings were planted using long line method

2.3. Growing of the seedlings

Seedlings were grown for 45 days. During the growing period, the seedlings were cleaned from dirt and attached epiphytes on seaweed. Water quality parameters such as temperature and salinity were measured every 3 days while current velocity, nitrate (NO3), phosphate (PO4), and turbidity were measured every 9 days.

2.4. Experimental design

This study used a completely randomized design (CRD) with three treatments and five replications. The treatments were A: 10 cm PD, B: 15 cm PD, and C: 20 cm PD.

2.5. Parameters observed

The parameters observed during the study were:

a. The daily growth rate (DGR) of seaweed cultivated for 45 days was calculated using the formula recommended by [6]: DGR (%)= $[(W_t/W_0)1/t- 1] \times 100\%$
where W_0 is the initial fresh weight, and W_t is the final fresh weight of the seedlings after t days of culture.
b. Ratio of fresh weight to dry weight (FW:DW). In the laboratory, cleaning of harvested was done by removing sand and dirt, and by handpicking other organisms. All harvested seaweed of each treatment was weighed as fresh dried (g) and after dried using hanging method for 2-3 days final dried weight (g) was recorded for each sample. Ratio of fresh weight to dry weight was then calculated for all the samples. Data were expressed as mean ± SD for all the harvested seaweed [7].
c. Epiphytes and disease found during culture period were also recorded.

2.6 Analysis of carrageenan yield

Five grams of dried seaweed was prepared then washed with fresh water. After that, the seaweeds were soaked with distilled water (*aquadest*) for 12 hours followed by sterilization on an autoclave for 30 minutes at 121^{o}C. It was then smoothed the size in a blender. After filtered by using filters, the samples were precipitated with 100 ml of Isopropanol and then dried in the oven for 24 hours, and then finally we analyzed the carrageenan yield. The product of semi refined carrageenan (SRC) was then dried in sunlight and the weight was recorded and expressed as percentage (%) of the dry weight. Triplicate samples were analyzed for each harvested sample of all growth periods. Data are expressed as mean ± SD for all the harvests of the treatments.

2.7. Data analysis

Data were analysed using ANOVA. If the analysis showed significant effect then was continued with Tukey test with a 95% confidence level.

3. Results and Discussion

3.1. Daily growth rate (DGR)

In general, daily growth rate (DGR) of all treatments tended not to differ significantly (Tukey test; $p > 0.05$; figure 2 and table 1).

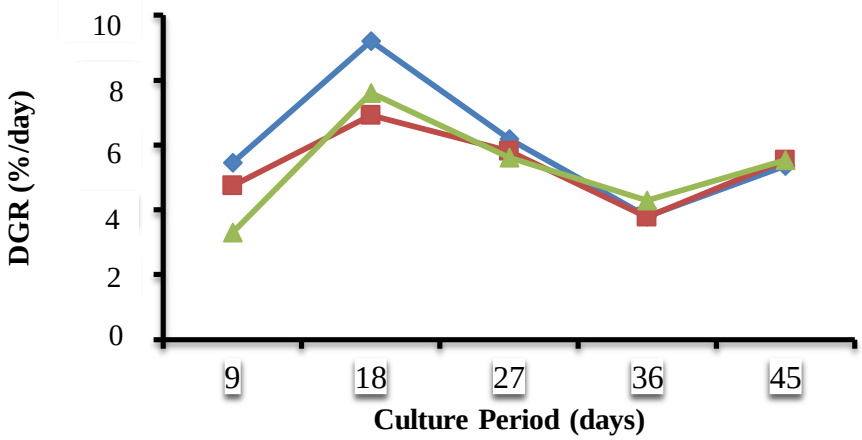

Figure 2. DGR of *K. alvarezii* seaweed seedlings of Prof cultivated using different planting distance (PD)

Mean DGRs obtained in the present study was 6.00±1.19%/day for 10 cm PD, 5.36±1.17%/day for a 15 cm PD, and 5.27±1.62%/day for a 20 cm PD. The range of DGR for 10 cm PD was 3.95±0.37%/day – 8.75±1.13%/day, while for the 15 cm PD and 20 cm PD were 4.05±0.39%/day – 7.00±0.22%/day, and 4.24±1.16%/day – 7.31±0.4%/day, respectively. The mean DGRs obtained in this study was significantly higher than those of internationally recommended for commercial DGR (3.50%).

Table 1. Daily Growth rate (DGR) of *K. alvarezii* seaweed seedlings of Prof cultivated using different planting distances (PD).

Days	Planting distance (cm)	DGR (%/Day) ± SDV	Tukey test		*p*-value
			1	2	
	10	6.21±1.32	6.21[a]		0.089
9	15	4.39±1.38	4.39[a]		0.120
	20	4.24±1.16	4.24[a]		0.983
18	10	8.75±1.13		8.75[a]	0.021
	15	7.00±0.22	7.00[b]		0.006
	20	7.31±0.40	7.31[b]		0.782
27	10	6.17±0.37	6.17[a]		0.977
	15	6.12±0.45	6.12[a]		0.652
	20	5.86±0.41	5.86[a]		0.445
36	10	3.95±0.37	3.96[a]		0.089
	15	4.05±0.39	4.05[a]		0.920
	20	4.56±0.46	4.56[a]		0.169
45	10	5.27±0.29		5.54[a]	0.005
	15	5.70±0.22	4.05[b]		0.000
	20	5.70±0.28	4.56[b]		0.141

*values represent means for three replicates in each column, values followed by different letters are significantly different at $p < 0.05$

DGR from all treatments ranged from 5.27 – 6.00%/day (above 3%). This DGR showed seaweed produced from Prof seedlings had higher growth and very suitable to be cultivated by seaweed farmers. In addition, this DGR was higher than DGR of using seedlings from local strains found in several

countries, such as: in Vietnam DGR ranges from 3.5 – 4.6%/day [8]; India DGR 3.76±0.07 and 3.69±0.11% [9]; and Madagascar DGR 5.46±0.09%/day [10]. Meanwhile, when compared with DGR of seedlings from tissue-cultured seedling, the DGR from this study was comparable to the previous studies reported by different researchers elsewhere such as: in Malaysia DGR 6.3±0.1%/day [11]; Philippines DGR 5.8 – 7.2%/day [12]; Indonesia DGR 4.6%/day [4] 3.91±0.52% /day [13]. DGR from this study is also comparable to DGR from seedlings of Prof obtained by [14] who found the DGR of 6.27±0.31%/day. Therefore, although 10 cm PD tended to be not significantly different from the other two treatments, this treatment is very suitable and more efficient for cultivating the seaweed in terms of water column use. This planting distance (10 cm) is more efficient to be applied in seaweed farming in terms of space between seedlings in a rope because more seedlings could be planted than those of 15 and 20 cm PD.

3.2. Ratio of fresh weight and dry weight (FW:DW)

The ratio of fresh weight and dry weight (FW:DW) showed no significant differences among treatments (Tukey test; $p > 0.05$; table 2). The ratio of FW:DW of 10 cm, 15 cm, and 20 cm PD were 8.34: 1, 9.58: 1, and 8.98: 1, respectively (table 2).

Table 2. Ratio of fresh weight and dry weight (FW:DW) of *K. alvarezii* seaweed seedlings of Prof cultivated using different planting distances (PD).

Planting distance (cm)	W_0		W_t	FW:DW ratio	Tukey test	*p*-value
	Initial Weight (g)	Fresh Weight (g)	Dry Weight (g)			
	1	2	3	4	5	6
10	10	113.00±13.62	14.00±3.24	8.84: 1	8.34[a]	0.078
15	10	101.00±9.24	11.00±1.14	9.58: 1	9.58[a]	0.492
20	10	121.00±14.25	14.00±2.00	8.98: 1	8.98[a]	0.452
Mean		112.00±12.37	13.00±1.09	8.97: 1		

*values represent means for three replicates in each column, values followed by different letters are significantly different at $p < 0.05$

The ratio of (FW:DW) obtained in this study tended to be proportional to the ratio (FW:DW) obtained in India at 9.89±0.13 [7]. This ratio, moreover, supports 10 cm PD as a better standard for applying space use between seedlings in a rope because more seedlings could be planted than those of 15 and 20 cm PD.

3.3 Carrageenan yield

Seedlings planted using 20 cm PD produced the highest yield of carrageenan and significantly different from the other two treatments (10 cm and 15 cm PD) (Tukey test; $p<0.05$; table 3 and figure 3). Carrageenan yield of 20 cm PD had carrageenan was 43.37 ± 1.67 % followed by 15 cm PD (37.60 ± 1.34%) and 10 cm PD (34.94 ± 3.26%).

Table 3. Carrageenan Yield of *K. alvarezii* seaweed seedlings of Prof cultivated using different planting distances (PD).

Planting Distance (cm)	Tukey Test and STDEV		*p*-value
	1	2	
10	34.94 ± 3.26[b]		0.000
15	37.60 ±1.34[b]		0.192
20		43.37± 1.67[a]	0.004

*values represent means for three replicates in each column, values followed by different letters are significantly different at $p < 0.05$

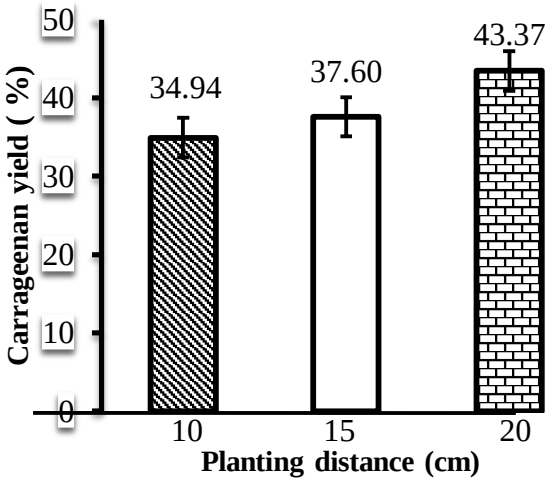

Figure 3. Carrageenan yield of of *K. alvarezii* seaweed seedlings of Prof cultivated using different planting distances (PD). JT.10 cm JT. 15 cm JT. 20 cm

The carrageenan yield produced in this study fulfills internationally defined specifications (minimum 27%) [15]. This is in line with findings of [16, 17] that carrageenan yield are very diverse due to extraction methods and ecological factors, such as light, nutrition, temperature and water content when drying. The carrageenan yield produced tended to be proportional to the carrageenan yield produced in Bengal India, which are 33.33±1.12% and 30.36±1.00% [17]. Harvest age and water quality determine the growth of seaweed and can affect the yields of seaweed carrageenan.

3.4 Epiphyte and "Ice-ice" disease

Seaweed pests and diseases found during this study were epiphyte and *ice-ice* attached to seaweed during culture period (figure 4). Epiphyte species that found attach to seaweed thalli was *Sargassum polychystum* (figure 4 A). Epiphytes were found to be attached to seaweed on the 18th day of culture period, while *ice-ice* disease was found on the 36th day of culture period (figure 4B).

Figure 4. Epiphytes and diseases found during the study. A) *S. polychystum*; B) *Ice-ice* disease on seaweed Prof.

Epiphytes that grow on seaweed ropes can indirectly affect the growth of seaweed due to competition in getting sunlight for photosynthesis. This epiphyte is a competitor for seaweed growth because it is a competitor for seaweed growth. This is in accordance with the statement of [18], which states that epiphytes are one of the factors that affect the growth of seaweed and as a competitor in absorbing nutrients for growth. As a competitor, this epiphyte is also one of the initial causes of infection with the bacteria that causes "*ice-ice"* disease. "*Ice-ice"* is usually characterized by white color on the thalli. This disease can result in a serious problems to decrease biomass and carrageenan yield [19, 20]. Epiphytic outbreak occurred in August to October is common phenomenom [19, 20]. Seasonality in epiphytic

infections has been observed in other areas and is often associated with changes in salinity or high temperatures [19,21].

3.5 Water Quality Parameters

The range of water quality measured during this study was 29-31^{o}C for temperature, salinity of 30-35 ppt, nitrates of 0.65-3.36 mg/L, phosphate of 0.03-0.043 mg/L, turbidity of 0.11 to 2.12 of NTU, current velocity of 0.26-0.51 m/s. All parameters were in normal range for supporting growth of the seaweed in Marobo coastal waters except turbidity which was higher than normal range [22].

4. Conclusion

All treatments tended to show no significant differences on daily growth rate and ratio of fresh weight and dried weight except carrageenan yield. However, to get a high production, 10 cm planting distance was very suitable and more efficient to be used in order to cultivate the seaweed *K. alvarezii* produced from mass selection combined with tissue-cultured method (Prof) in terms of distance between seedlings planted in a rope.

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