Seasonal variation in growth and carrageenan yield of *Kappaphycus alvarezii* (Doty) Doty farmed using mass selection in Bungin Permai Coastal Waters, South Konawe District, Southeast (SE) Sulawesi, Indonesia

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Abstract. Two methods, tissue culture, and mass selection are generally used to improve the quality of the seedlings of the red algae *K. alvarezii*. Mass selection is relatively new to be adapted to the Indonesian seaweed farming. In this study we attempted to cultivate the seaweed for 10 months (February-November 2013) in Bungin Permai coastal waters, South Konawe, Indonesia. Cultivation was performed for 30 days of each cycle, using long-line method. Each rope contained had distance between clumps of 15-, 20-, 25- and 30-cm. The selection was carried out until 9th generation. The results showed that the daily growth rates (DGRs) among treatments were found to vary and tended to have seasonal variation. Minimum DGRs occurred in October while the maximum was in April/May. The DGRs range was from 3.81±1.80%/day to 8.32±1.12%/day while mean annual DGRs of 15-, 20-, 25- and 30- cm PD were 6.46±1.98, 6.62±2.00, 6.74±2.01, and 6.90±2.03%/day, respectively. No significant differences was found among four different planting distance. The mean carrageenan (semi-refined carrageenan/SRC) yields were varied among treatments and tended also to have seasonal variation. Minimum SRC of all treatments was varied and occurred in March/June while the maximum SRC were in May/October/November. The carrageenan yields range were from 31.53±2.04% to 48.91±5.05%. The highest mean annual SRC was recorded for 15-cm planting distance (PD) (42.02 ± 3.38%) followed by 30-cm PD (41.58 ± 2.95%), 20-cm PD (41.56 ± 6.03%) and 25-cm PD (41.45 ± 4.09%). The quality of cultivars produced from the selection met international specifications suggesting that mass selection for longer period could be “a new method” to produce better quality of seedlings.

1. Introduction

The red algae *Kappaphycus alvarezii* is one of major sources of carrageenan, an important hydrocolloid which has widely applied for food and non-food industries. This species along with *Eucheuma* spp had produced approximately 11.63 million tonnes in 2016 [1]. In recent years, *K. alvarezii* has been cultivated throughout Indonesia. The rapid distribution and production of this seaweed species have positively contributed to the income of the seaweed farmers [2,3]. Increasing production of this species needs a continuous supply of high-quality seaweed seedlings. Unfortunately, seaweed seedlings stock is mainly practiced by farmers using vegetative propagation. It means that the farmers use whole harvested
thallus and cut them as new seedling stock for the next crop repeatedly (vegetative propagation). This repeated vegetative method of seedlings stock has led to a lower quality of seaweed products such as lower growth and carrageenan yield and it makes the susceptibility to disease [3,4].

There are two techniques frequently used for supplying a higher quality of seedling stock either tissue-culture methods [5–9] or mass selection [10–12]. Some advantages of using tissue-cultured seedlings are constant supply of seedlings at any time and growth rate was higher than using the vegetative seedlings. The use of tissue culture techniques in the propagation of K. alvarezi has proven to be effective in solving cultivation problems and produced high-quality seedling and providing a sustainable source of better-quality carrageenan. However, to obtain the seedlings ready to be planted from this tissue culture method, it needs around 1.5-2 years. In contrast, although mass selection methods have been commonly used to increase yield and growth in higher plants in a short time [13,14] and could also produce better quality of seaweed seedlings [12]. This method is relatively new to be adapted to the Indonesian seaweed industry [15].

Mass selection to produce a high quality of K. alvarezi seedlings has been firstly done in Indonesia [12–15]. From these two previous works, they had not described yet the possibility of seasonal variation of seedling growth and carrageenan yield since they did the mass selection only until 4th generations. Mass selection suitability should consider the possibility of seasonal variation during the period of seaweed farming. From the study of seaweed growth done in India, it was found that the growth and carrageenan yield could be influenced by seasonal variation due to environmental change [16,17]. To get the detailed information regarding the possibility of seasonal fluctuation of the seedling cultivated using mass selection, longer period of seaweed farming is really required. Therefore, we conducted longer period for mass selection using K. alvarezi with the aim to examine the seasonal variation in growth and carrageenan yield of the seaweed.

2. Materials and methods

2.1. Seaweed seedlings source and mass selection procedure

This study was done in Bungin Permai coastal waters, South Konawe Regency, SE Sulawesi, Indonesia (4°29′24.03 S/122°13′26.60 E). Carrageenan yield analysis was conducted in the Analysis Laboratory of the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia.

The brown strain of K. alvarezi for seedling source was collected from local farmers in Bungin Permai coastal waters, South Konawe Regency, SE Sulawesi, Indonesia. Healthy, clean, highly branched and 25 to 30 days old thalli were selected for the mass selection. The selection was conducted from February to November 2013.

The selection was conducted as described in seaweed selection protocols (RICA 2011) with slight modification. Cultivation was performed for 30 days of each cycle, using long-line method (Figure 1). Planting distances (PD) as treatment used in this study were 15-, 20-, 25- and 30-cm. Seaweed seedlings (G0) with initial clump weight of 40 g was tied to the rope. The seedlings were placed at ± 20-25 cm from water surface. Each clump was weighed at the end of each cultivation cycle. Seedlings had >3.5%/day of highest Daily Growth Rate (DGR) value were selected except in 8th period where seedlings had the DGRs>2.3 5/day were used due to the lower growth during the culture period. The selected seedling clumps were then cut into new seedlings and tied to the new rope, and they were cultivated with similar protocols, processes and period as previous cycles. Selected seedlings resulted from each cycle of mass selection was referred to a generation. Selection was conducted until 9th generation. In short, 1st to 9th period correspond with March-November, respectively. The Daily growth rate (DGR) of seaweed cultivated for 30 days was calculated using the formula recommended by Yong and Anton (2013) [18]:

\[ \text{DGR}(\%) = \left( \frac{W_t}{W_0} \right)^{\frac{1}{t}} - 1 \] ×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. All data were expressed as mean ± SD.
Figure 1. Schematic diagram of mass selection using *K. alvarezii* done for 9 cultivation period. Black clump represented the selected clumps (the DGR > 2.3%/day or > 3.5%/day) used for further period.

2.2. Planting, Monitoring, and harvesting of seaweed seedlings

Seaweed seedlings were planted using a long line method. During culture period, monitoring and maintenance of the ropes and seedlings were done regularly at least twice a week by removing epiphytes, silt, and other marine debris and cleaning the ropes and lines. Every 30 days period, the seaweed was
harvested and were then dried using hanging method for 2-3 days. The dried material was transported to Fisheries Laboratory at Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, Indonesia for the extraction of semi-refined carrageenan. The remaining seaweed was again reseeded and used as new seedlings (if their DGRs >2.3%/day or >3.5%/day; see figure 1) and planted in the cultivation site on the day of harvest. Morphological performances of cultivars produced from mass selection were also observed especially on their thalli and branches.

2.3. Extraction of semi-refined carrageenan (SRC)

Semi-Refined Carrageenan (SRC) was produced by processing the dried materials of seaweed of K. alvarezii. Prior to the extraction in the laboratory, approximately 5 g of all the dried materials of each treatment were chopped using scissors into small pieces. The materials were washed thoroughly with fresh water for 24 h to remove all contaminants such salt, silt, sand, and other impurities. The process was repeated twice. After removing excess contaminant, the seaweed was soaked with aquadest for 12 hours followed by sterilizing the samples into an autoclave using 250 ml aquadest at 121°C for 30 min. Samples were then minced by using a blender resulting in the pulp of the seaweed. The pulp was then poured into the plastic plates along with 100 ml isopropanol solution until the materials coagulated. The coagulated pulp of each sample was dried by sun drying methods for 2-3 days. The weight of semi-refined carrageenan (SRC) was then recorded and expressed as percentage (%) of the dry weight according to the formula [19]:

\[ \text{Yield (\%)} = \frac{W_c}{W_m} \times 100 \]

Where, \( W_c \) = the weight of extracted carrageenan (g) and \( W_m \) = the weight of dried seaweed weight used for extraction (g). Triplicate samples were analyzed for each harvested sample of all periods. Data are expressed as mean ± SD for all the samples of the treatments.

2.4. Environmental parameters

During the experimental period, surface seawater temperature, salinity, nitrate, and phosphate were recorded from the experimental site at 5-days intervals throughout the study period. The temperature was recorded using a thermometer. Salinity was measured by a refractometer (Atago K.K., Tokyo, accuracy = ±1). Nitrate and phosphate were determined following the methods described by [20]. Each parameter was recorded in triplicate. From the recorded data, the mean value with standard deviation for every month was finally calculated.

2.5. Statistical analysis

All data are expressed as the means standard deviations of triplicate measurements. One-way analysis of variance (ANOVA) at the 5% significance level was used to determine significant differences (p < 0.05) between means followed by the Duncan Multiple Range Test. Statistical analysis was performed using SPSS for Windows (version 19).

3. Results and discussion

3.1 Daily Growth Rate (DGR)

The daily growth rates (DGRs) among treatments of this seaweed using mass selection were found to vary and tended to have seasonal variation (Figure 2). Minimum DGRs of all treatments occurred in October while the maximum the DGRs were in April or May. The DGRs range during this study was from 3.81±1.80%/day to 8.32±1.12%/day. The minimum growth rate for 15-cm planting distance/PD (3.81 ± 1.80%/day) was recorded in October with a maximum was in May (8.07 ± 1.01 %/day). The minimum growth rate for 20-cm PD (3.92 ±1.84 %) was obtained in October with a maximum (8.15 ± 1.04 %/day) was in May. In 25 and 30-cm PD, the minimum growth rate (4.06 ± 1.94 %/day and 4.17± 2.00 %/day) was found in October with a maximum (8.24 ± 1.08 %/day and 8.32± 1.12 %/day)) in April. During the entire cultivation period, mean annual DGRs of 15-, 20-, 25- and 30- cm PD were 6.46±1.98, 6.62±2.00, 6.74±2.01, and 6.90±2.03%/day, respectively. In short, among these four treatments, although no significant differences were found (P> 0.05) the maximum growth rate was recorded at 30-cm PD followed by 25, 20 and 15-cm PD.
The DGR seasonal variations found in this study could be influenced by environmental parameters coupled with culture periods done for 10 months (February to November). The mean growth rate obtained in this study for 30 days growth period was comparatively higher than those of other previous studies. Previous studies done for 30 days in India showed the DGRs were only 3.76 ± 0.08%/day [21] while a similar study using mass selection method done in Gorontalo, Indonesia showed the DGR was 2.22%–5.21%/day. Moreover, DGRs of this study were comparable to the findings in 45-days culture period from different countries: in Mexico, from 2.0 to 7.1 % [22], in India, 1.89 to 4.92%, [23–25] to 3.80% [26], and in Brazil, from 4.5 to 8.2 % [27].

The highest DGRs of all the four treatments were recorded in April/May in the present study was nearly in agreement with the result of an earlier study in India undertook by [28] where the highest DGRs were in October/ March at Okha, Gujarat, India. The results indicated that the growth rates are influenced specifically by study site and season. The maximum growth rates in the present study were mostly found from April-May at relatively lower temperature (29.5±1.05°C), high salinity (31.33±1.75 ppt), higher nitrate (0.31±0.32 μmol L−1) and phosphate (0.045±0.017 μmol L−1) while lowest growth rate in October coincidentally occurred at high temperature (31.67±0.52°C) and salinity (34.83± 0.41 ppt) but low nitrate (0.006±0.004 μmol L −1) and phosphate (0.003±0.001μmol L−1) content. Therefore, environmental parameters especially temperature seemingly to be the main factor influencing the growth rates of this seaweed as similarly also noticed by Munoz et al. (2004) in Mexico and Periyasamy et al. in India, and [29] in Brazil. In contrast, favorable condition found in this study was not consistent with the findings of. They found productive season of seaweed K. alvarezii in the North Gorontalo, Indonesia was November to April, while less productive season in May to October annually. Based on this DGRs data, to gain optimum production we should consider to cultivate the seaweed in the region from March to August especially in April –May annually.

3.2. Carrageenan (SRC) yield
Carrageenan yields were varied among treatments and also tended to have seasonal variation (figure 3). Minimum of all treatments was varied and occurred in March or June while maximum the DGRs were in

![Figure 2.](image-url)
May or October or November. The carrageenan yields range during this study were from 31.53±2.04% to 48.91±5.05%. The minimum CYs for 15-cm PD (36.08±2.75%) was recorded in June with a maximum was in May (47.19±0.42 %). The minimum carrageenan yields for 20-cm PD (36.53±2.04%) was obtained in March with a maximum (48.91±5.05 %) was in November. In 25- and 30-cm PD, the minimum carrageenan yields (36.32±5.91% and 38.21±1.97 %) was found in March and June while maximum (45.47±1.03% and 46.13±0.30%) was recorded in May and October, respectively.

Among the four treatments, the highest mean annual carrageenan yield was recorded for 15-cm planting distance (PD) (42.02 ± 3.38%) followed by 30-cm PD (41.58 ± 2.95%), 20-cm PD (41.56 ± 6.03%) and 25-cm PD (41.45 ± 4.09%). The mean carrageenan (SRC) yield of all treatments (41.70 ± 4.23%) obtained in this study was higher than those of the similar 30-days previous study done in India by [21] where the SRC yield was 27.88±0.98% [21]. The different of carrageenan yield presumably to be dependent on environmental parameters, site selection/region, growth conditions (salinity and nutrients) and harvest time [29,30] and process of extraction [31,32]. Furthermore, the SRC yield obtained in this study was comparable to 45-days previous studies in other regions such as Mexico—30.3 to 40.7 % [22]; Brazil—31 to 43 % [32] and 35.3 to 46.1 % and India—(24.52±0.65 to 31.10± 0.71 %) [24].

The maximum carrageenan yield during this study period was found when the water parameters (temperature, salinity, nitrate, and phosphate) are similar for supporting high DGRs. Therefore, based on the present study (DGRs, SRC yield, and environmental parameters), the best period for obtaining optimum SRC yields was from March to August during the year.

Figure 3. Seasonal variation in carrageenan yields (CYs) (%) of K. alvarezi cultivated using different planting distance (15-, 20-, 25-, 30-cm), for 9 periods at Bungin Permai coastal waters (March to November 2013)

3.3. Morphological performances of seedlings
The thalli length of cultivars produced from this mass selection has longer thalli than those of the farmed seaweed (local strain) produced by local farmers. Moreover, thalli produced from mass-selection method tend to have more branch number than local-strain seedlings (figure 4). This performance of the cultivars produced from this study is similar to those of previous study done by [12].
3.4. Future perspectives
We already did mass selection to select high growth rate cultivars from *K. alvarezii*, and we successfully produced cultivars having favorable characteristics such as higher daily growth rates (DGRs) and carrageenan yield, longer thallus with a numerous number of branches as compared to the local strain. However, to produce more numbers of better quality of seedlings/cultivars for future needs, the authors recommended applying a combination the mass selection and tissue-cultured methods to produce a better quality of seedling for future needs. Therefore, all selected cultivars produced after 9 periods of mass selection were collected and then brought in 2014 to SEAMEO-BIOTROP Laboratory, Bogor. In the laboratory, all the cultivars were proceeded to produce new plantlets through tissue-culture method.

On the other hand, to address the concern for future days, a comprehensive and participative collaboration among the various levels of stakeholders: districts, provinces and national government along with the private sectors, academia, seaweed-related stakeholders and other non-government organizations (NGOs) would certainly a guarantee to sustain the implementation of better quality seaweed seedling production.

4. Conclusion
Mass selection could produce higher daily growth rate and carrageenan yield. Cultivars produced from the selection showed longer thalli and more numerous number of branches. The authors recommended applying a combination the mass selection and tissue-cultured methods for producing better quality of seedling for future needs.

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