The Effect of Different Oblique Incision Length in Slide-Slipped Grafting using Tissue Cultured and Local Seedlings on the Growth of Seaweed (Kappaphycus alvarezii) in Sasara Coastal Waters, Kulisusu Bay, Buton Utara, SE Sulawesi, Indonesia

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Abstract. This research was conducted for four months from January to May, 2019 in Sasara coastal Waters, Kulisusu Bay, Buton Utara, SE Sulawesi, Indonesia. The aim of this research was to determine the best oblique incision length (OIL) to use in slide-slipped grafting using tissue cultured and local seedlings in terms of the growth and carrageenan yield of the seaweed Kappaphycus alvarezii. The treatments used in this study were A: 25-mm OIL, B: 30-mm OIL and C: 35-mm OIL. After 18 days, grafts using all OILs were successfully attached together. The new seedlings were then cultivated for 45 days. The results showed that the daily growth rate (DGR) did not differ significantly between treatments. The 25-mm OIL treatment had the highest DGR (3.98±0.97 %.day⁻¹) followed by 30-mm OIL (3.90 ± 1.32%.day⁻¹) and 35-mm OIL (3.69 ± 0.91 %.day⁻¹).The ratio of fresh weight (FW) to dry weight (DW) also showed no significant differences among treatments. FW:DW ratios of treatments 25 mm OIL, 30 mm OIL, and 35 mm OIL were 8.13:1, 7.42:1, and 5.78:1, respectively. For carrageenan content, 30-mm OIL seedlings of showed higher content but it was not significantly different from the other treatments. Water quality parameters were: salinity 24-32 ppt; temperature 29-31°C; nitrate 0.336-0.432 mg.l⁻¹; phosphate 0.195-0.542 ppm; and turbidity 3,124-9,331 NTU.

1. Introduction
Farming the seaweed Kappaphycus alvarezii is an economically important activity in Indonesian coastal waters especially in Southeast (SE) Sulawesi [1–3]. This is because it is easy to plant and harvest, it requires low financial outlay, has an established global market share and is an accessible economic activity for many farmers and their families [4]. For some time now, Indonesia has been the biggest producer of seaweed in the world. In 2017 the seaweed production was 10.81 million ton [5].

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However, to maintain high levels of production, a constant supply of raw materials in the form of high quality seedlings is urgently needed. There have been numerous studies about cultivation methods to increase the seaweed production, but very few efforts have focused on the improvement of the quality of carrageenan by using seedlings produced from tissue-culture method [6,7].

Tissue-cultured seedlings have a higher growth than those from vegetative-produced seedlings, commonly known as 'local strain' [8]. The tissue-cultured seedlings can easily be differentiated from local strain seedlings based on their morphological performances (Figure 1). Local strain seedlings have shorter thalli and have fewer irregular branches at both primary and secondary base of thalli. Moreover, tissue-culture seedlings have bigger thalli than local strain.

![Figure 1. Seaweed Kappaphycus alvarezii: A. Local strain; B. Tissue-cultured](image)

Grafting is one increasingly common method used to improve seaweed growth and carrageenan yield. This method was first initiated by [9,10] using different coloured-local strains. From these studies, it was found that daily growth rate and carrageenan content of grafted strains were higher than un-grafted strains. Furthermore, in Indonesia [11] has already introduced the grafting method using a new combination of tissue-cultured and local strain seedlings. They found that there is a great promise in using tissue-cultured and local seedlings through grafting methods using both side-slipped and straight-slipped grafting positions. However information regarding the effect of these grafting methods on growth rate and carrageenan content were lacking.

Since tissue-cultured and local strain thalli have different morphological characters, we needed to make some modifications to apply the grafting method used previously by [9,10]. A modification used in this study was oblique inserted length (OIL). [10] used 8-10 cm OIL for grafting the local strains while [11] used 1.5-2.5 cm. Shorter OIL was used in the latest study because cultured-seedlings have more numerous thalli so that some difficulties were found when the seedlings of the two different sources were bound. In summary, using shorter OIL as reported was not fully successful in “mating” the two seedling sources. Therefore, the aim of this study was to examine the effects of an oblique insertion length OIL grafting method on the growth and carrageenan content of seedlings produced using tissue-cultured seedlings and local strain seedlings.

2. Materials and Methods
This field farming study was conducted in Sasara coastal waters (SCW), Kulissusu Bay, North Buton Regency, SE Sulawesi (4°49’9.04”S and 123°1’49.89”E) from January to May 2019. Carrageenan content analysis was carried out in the laboratory of the Faculty of Fisheries and Marine Sciences of Halu Oleo University, Kendari. Healthy plants were collected from the local farm at SCW and the plants were also farmed at SCW.
2.1. Grafting
Side-slipped grafting was used in this study (Figure 2). The process of seaweed grafting followed [9,10] with some modifications. The processes were: 1) selected plants were washed thoroughly with clean seawater to remove epiphytes and dirt from the thalli; 2) selected 10-g fronds from TC and LOC seedlings were cut obliquely by a blade into small pieces (with 25 mm, 30 mm and 35 mm of OIL as the treatments in this study); 3) the fronds of each TC and LOC were separated from the base of seaweed thalli; 4) the thalli were grafted by placing the two fronds together using a side-slipped position; 5) the two straws were mated tightly using a plastic ice strap; 6) After being tied tightly, the grafted plants were planted into the cultivation farm using the long line method at SCW until the two strains were successfully attached.

**Figure 2.** Side-slipped grafting using tissue-cultured seedlings (TC) and local strain seedlings (LOC). A. 25-mm Oblique Incision Length (OIL); B. 30-mm OIL; C. 35-mm OIL; D. Oblique incision of tied seedlings (tissue-cultured seeding; arrow no. 1 and local strain; arrow no 2); E. Seedlings were completely “mated” and tied tightly.
2.2. Monitoring of grafted seedlings
Monitoring and maintenance of grafted seedlings were conducted every two days for 24 days. Development of grafted seedlings and the percentage of attachment (grafting rate) of all treatments were monitored every two days.

2.3. Planting Seaweed Seedlings
All successfully attached seedlings (TC and TOC) produced from the grafting method were then tied to a 1.5 mm polyethylene (PE) rope with a 10 cm planting distance. The long line method was used for the cultivation to produce biomass of these grafted TC and LOC seedlings. All seedlings were cultivated for 45 days. During the cultivation period, monitoring was done every 2 days to observe changes in the condition of seedlings and to clean mud, bryophytes and epiphytes from the seedlings.

2.4. Parameters Observed

2.4.1. Grafting rate: grafting rate was the number of seedlings attached, divided by the total number of seedlings x 100, recorded over the 24 day initial post-grafting period.

2.4.2. Daily growth rate (DGR). The DGR was expressed as mean ± SD (standard deviation) for all samples from each growth period of the study period and was calculated over different growth periods using the formula [6]:

\[
DGR \, (\%) = \left[ \frac{W_t}{W_0} \right]^{1/(t-1)} \times 100\%
\]

where

- \(W_0\) = initial seedling weight (g)
- \(W_t\) = final seedling weight (g)
- \(t\) = number of cultivation days

2.4.3. The ratio of fresh weight to dry weight (FW:DW). Harvested seaweed was cleaned in the laboratory to remove sand and any attached organisms. Then all dried seaweed harvested from each seaweed treatment was weighed (FW, in g). After drying using a hanging method for 2-3 days, the final dry weight (DW, in g) was also measured. The ratio of fresh weight to dry weight (FW:DW) was then calculated and the data expressed as mean ± SD for all seaweed harvested.

2.4.4. Carrageenan content. Small pieces of dried seaweed (±5 g) were cut and washed with freshwater. The dried samples were soaked in distilled water for 12 hours. The samples were then sterilized in an autoclave at 121°C for 30 minutes. After that, the samples were ground to a smooth paste using a blender. The samples were filtered using filters, then precipitated with 100 ml of Isopropanol and then dried for 24 hours in the oven. Finally, semi refined carrageenan (SRC) was obtained and dried in sunlight. The weight of dried SRC was recorded and expressed as a percentage (%). The carrageenan content was determined using the formula: Yield (%) = Wc/Wm.100 where Wc is weight of carrageenan extract (g) and Wm is the weight (g) of the dry seaweed used for extraction [12]. Triplicate samples were analysed for each harvested sample in all growth periods. The data obtained from the three treatments were presented as mean±SD.

2.4.5. Seawater quality. Water quality parameters measured in the field were salinity and water temperature, measured in-situ at 6 to 8 a.m. Salinity was measured using a hand refractometer and a thermometer was used for temperature. Nitrate and phosphate concentrations as well as turbidity were measured using a HANNA™ instrument HI98703-11 (HANNA™, Woonsocket, RI, USA) immediately after samples were collected.
2.5. Data analysis
Statistical analysis of all treatment parameters was by analysis of variance (ANOVA). If the ANOVA showed a significant effect, then the Tukey post-hoc test was applied with a 95% confidence level. All statistical analyses were implemented using SPSS version 16 statistical software.

3. Results and Discussion

3.1. Grafting Rate.
The grafting rates of all seedlings cultivated are shown in Table 1.

Table 1. Grafting rate (%) of seedlings grafted using three treatments during the cultivation period

| Day | No | OIL (mm) | No. of seedlings grafted | Seedlings failed to attach | % | Final no. of seedlings attached | % | Mean grafting rate (%) |
|-----|----|----------|--------------------------|---------------------------|---|-------------------------------|---|-----------------------|
| 6   | 1  | 25       | 20                       | 2                         | 10| 18                           | 90| 91.67                 |
|     | 2  | 30       | 20                       | 2                         | 10| 18                           | 90|                       |
|     | 3  | 35       | 20                       | 1                         | 5 | 19                           | 95|                       |
| 8   | 1  | 25       | 20                       | 3                         | 15| 17                           | 85|                       |
|     | 2  | 30       | 20                       | 2                         | 10| 18                           | 90| 90.00                 |
|     | 3  | 35       | 20                       | 1                         | 5 | 19                           | 95|                       |
| 12  | 1  | 25       | 20                       | 6                         | 30| 14                           | 70|                       |
|     | 2  | 30       | 20                       | 6                         | 30| 14                           | 70| 76.67                 |
|     | 3  | 35       | 20                       | 2                         | 10| 18                           | 90|                       |
| 14  | 1  | 25       | 20                       | 9                         | 45| 11                           | 45|                       |
|     | 2  | 30       | 20                       | 7                         | 35| 13                           | 55| 60.00                 |
|     | 3  | 35       | 20                       | 4                         | 20| 16                           | 80|                       |
| 16  | 1  | 25       | 20                       | 13                        | 65| 7                            | 35|                       |
|     | 2  | 30       | 20                       | 10                        | 50| 10                           | 50| 53.33                 |
|     | 3  | 35       | 20                       | 5                         | 25| 15                           | 75|                       |
| 18  | 1  | 25       | 20                       | 15                        | 75| 5                            | 25|                       |
|     | 2  | 30       | 20                       | 12                        | 60| 8                            | 40| 43.33                 |
|     | 3  | 35       | 20                       | 7                         | 35| 13                           | 65|                       |

By the 18th day of the culture period, all remaining grafted seedlings had fully attached with a mean grafting success rate of 43% (Figure 3).
The daily growth rate (DGR) of all treatments (Figure 4 and Table 2) did not differ significantly (Tukey test; $p > 0.05$).

The average daily growth rate (DGR) during 45 days of the grafted seaweed seedlings was $3.98 \pm 0.97\%$/day for 25-mm OIL followed by 35-mm OIL ($3.90 \pm 1.32\%$/day) and 30-mm OIL ($3.69 \pm 0.91\%$/day) (Table 2).
The 2nd International Symposium on Marine Science and Fisheries (ISMF2) – 2019

Table 2. DGR and Tukey Test of seaweed (*K. alvarezii*) from grafting

| Days | ITL(mm) | DGR(%/day ± SD) | Tukey Test | p-value |
|------|---------|-----------------|------------|---------|
| 25   | 5.54±1.49 | 5.17^a        | 0.47       |
| 30   | 5.08±1.07 | 5.33^a        | 0.94       |
| 35   | 6.02±1.38 | 5.95^a        | 0.30       |

The mean DGRs obtained in this study were higher than the DGR internationally recommended as attainable in commercial seaweed cultivation (3.50%) [13] and showed that seaweed produced from grafting seedlings had high growth rates and was very suitable to be cultivated by seaweed farmers. However, the DGRs found in this study were lower than those from a previous grafting study by [10] where DGRs of 6.4%-8.19%/day were obtained. Lower DGRs in this study were most likely owing to the lower salinity (reaching a minimum value of 24.0 ppt) and higher turbidity (9.33 NTU) during this study (see Figure 5), related to heavy flooding. The DGRs obtained were higher than the reported DGR of seedlings from local strains found in India (3.76±0.07 %/day and 3.69±0.11%/day) [6] and comparable to that of tissue-cultured seedlings in Indonesia (3.91±0.52%/day) [14].

3.3. *The ratio of Fresh Weight (FW) to Dry weight (DW)*

The ratio of fresh weight to dry weight (FW:DW) showed no significant difference between treatments (Table 3). The ratio (FW: DW) of 25-mm, 30-mm, and 35-mm OIL were 8.13:1, 7.42:1 and 5.78:1, respectively. The ratios of FW:DW obtained were higher than those of tissue-cultured seedlings cultivated in Marobo coastal waters [7]. It seems that the grafting of tissue-cultured and local strain seedlings could produce a heavier weight of dried biomass for each kilogram of fresh weight.

Table 3. Ratio of Dry Weight (DW) to Fresh Weight (FW) of grafted *K. alvarezii* seedlings

| ITL (mm) | W0 Initial weight (g) | Wt Fresh weight (g) | Wt Dry weight (g) | DW:FW ratio | Tukey Test | p-value |
|----------|-----------------------|---------------------|-------------------|-------------|------------|---------|
| 25       | 10                    | 49.06±15.61         | 6.04±0.47         | 8.13 : 1    | 6.04^a     | 0.409   |
| 30       | 10                    | 44.54±9.34          | 6.00±2.41         | 7.42 : 1    | 6.01^a     | 1.000   |
| 35       | 10                    | 45.06±10.87         | 7.79±0.78         | 5.78 : 1    | 7.79^a     | 0.618   |
| Mean     |                       | 44.22±11.39         | 7.05±1.49         | 6.48 : 1    |            |         |

3.4. Carrageenan content

The carrageenan content from grafted *K. alvarezii* seedlings showed no significant difference among treatments (Table 4). Carrageenan content of 30-mm OIL was 48.54±1.08% followed by 35-mm OIL (47.32 ± 2.72%) and 25-mm OIL (46.67 ± 2.86%).
Table 4. Carrageenan content of grafted *K. alvarezii* seedlings after 45 days cultivation

| ITL (mm) | Tukey Test ± SD | p-value |
|----------|-----------------|---------|
| 25       | 46.67±2.86a     | 0.593   |
| 30       | 48.54±1.05a     | 0.992   |
| 35       | 47.32±2.72a     | 0.987   |

Carrageenan content in this grafting study was higher than those reported from grafting studies in India (31-39%) [15]. Carrageenan content produced from this study meets international specifications (minimum 27%) [13]. This is in line with the research of [12,13] that ecological factors, such as light, nutrients, temperature, and other factors can affect carrageenan content. The results show all the treatments used to prepare grafted strains in this study could be utilized for improving the biomass of the commercially important seaweed *K. alvarezii* production [11].

3.5. Water quality parameters

All water quality parameters remained within the range of optimum requirements for *K. alvarezii* cultured in the sea except for the salinity and turbidity (Figure 5). The temperature obtained was 29.0-31.0°C, salinity 24.0-32.0 ppt, turbidity 3.124-9.331 NTU, nitrates 0.336-0.432 mg/l and phosphate 0.195-0.542 mg/l. The optimum mariculture conditions for *K. alvarezii* farming in the open sea include temperature of about 27-30°C and salinity 30-33 ppt [16]. Phosphate levels for optimal seaweed growth being 0.02-1.0 ppm [13], while nitrate levels should be below 1.0 mg L⁻¹ [17].

Figure 5. Water quality parameters measured during this study. A Temperature; B, Salinity; C, Nitrate; D, Phosphate; and E, Turbidity
4. Conclusion
Side-slipped grafting of the red seaweed *Kappaphycus alvarezi* using the combination of tissue-cultured with local strain thalli is suitable for adoption by seaweed farmers. Due to the greater ease for grafting the seedlings in the field, we recommend 35-mm OIL to be used in grafting techniques. The grafting of tissue-cultured seedlings and local strain seaweeds might also create new opportunities for the seaweed industry to produce high quality seedlings in the future.

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