Grafting of *Kappaphycus alvarezii* seedlings using different seedling sources in Sasara Coastal Waters, Buton Utara, Southeast (SE) Sulawesi, Indonesia

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Abstract. Combining local seaweed thalli with tissue culture seedlings through grafting has been suggested as way to improve seaweed *Kappaphycus alvarezii* seedling quality. The purpose of this study was to investigate the growth of *K. alvarezii* seedlings produced from a combination of three seedling sources using a straight connection grafting method. We applied the grafting method using local and tissue-cultured seedlings. The trials took place in the coastal waters of Sasara, Buton Utara, SE Sulawesi, Indonesia from January to April 2019. The grafting combinations were: tissue culture and local; tissue culture and tissue culture; and local with local. The bonding period for all combinations was 9 days after the initial insertion and the success rate was 91.66-93.33%. The grafted seedlings were then cultured for 45 days using a longline technique. The water temperature, salinity, and nitrate and phosphate concentrations affected grafting success. The salinity range was 29-33 ppt and water temperature was 28-32°C. Nitrate and phosphate ranges were 0.263-0.463 mg.L⁻¹ and 0.112-0.218 mg.L⁻¹, respectively; turbidity was 1.59-2.35 NTU. The highest daily growth rate was obtained from the grafting of tissue cultured and local strain seedlings (6.51 ± 0.340%.day⁻¹) whereas growth rate was lowest for the local with local grafts (5.76 ± 0.378%.day⁻¹).

1. Introduction

Indonesian seaweed production has become distributed across almost all provinces of Indonesia. However, there are five major seaweed producing provinces in Indonesia, namely South Sulawesi, Southeast (SE) Sulawesi, Central Sulawesi, East Nusa Tenggara and West Nusa Tenggara, according to figures obtained from the Indonesian Directorate General of Aquaculture, ministry of Marine Affairs and Fisheries (DGA-MMAF) in 2016. According to the Provincial Marine and Fisheries Service of SE Sulawesi, in 2014 this province produced 956,495 tons of seaweed.

One major challenge which needs to be overcome in order to increase the development of seaweed cultivation in Indonesia is the generally poor quality of seaweed produced by Indonesian seaweed farmers [1] and [2]. Low seaweed quality is often due to three factors: (i) poor quality seedlings, (ii) insufficient cultivation time before harvest, and (iii) the use of inadequate drying processes. In addition, low seedling quality can result from the continuous propagation of seaweed from the same
original plants, due to the widespread practice of farmers planting seedlings taken from the previous harvest.

One initiative considered necessary to support the improvement of seaweed seed quality in Indonesia generally, and in SE Sulawesi Province in particular, is the development of good quality seedlings through tissue culture [3]. Several studies have been carried out on the use of seaweed seedlings from tissue culture [4,5]. Research on seedlings from tissue culture conducted by [4] only examined the feasibility of growing the seedlings in the field, while the scope of [5] was limited to growth and carrageenan yields.

In order to ensure a sustainable supply of good quality seedlings, then tissue culture is one solution which can overcome the problems of low seedling quality [6,7]. Seaweed seedlings originating from tissue culture generally have higher growth rates compared to local strain seedlings. In India, seedlings from tissue culture had a growth rate 1.5 - 1.8 times higher than other seedlings [8], and similar results were also found in Malaysia [3]. A previous study by [9] on the use of seaweed seedlings from tissue culture in SE Sulawesi showed that these seedlings were advantageous in terms of growth, over a range of temperatures, especially in the dry season. At present, other cultivation methods being used for obtaining superior seedlings are grafting methods [10], mass selection methods [11], and mass selection combined with tissue-culture [12].

Grafting using a side-slipped position method was first initiated [10] in India, using two different local colour strains. This study showed the potential to improve growth and carrageenan content through grafting. In addition, [12] also developed grafting methods using both side-slipped and straight-slipped positions; however, there was no information regarding the growth and carrageenan content of the seedlings produced through straight slipped position reported in that study. Therefore, data on the advantages of seedlings produced using this grafting method are still limited. This study aimed to evaluate seedlings produced by grafting tissue-cultured and local strain seaweeds, using the straight-slipped position.

2. Materials and Methods
This research was conducted from January to April 2019. Field activities were carried out in Koepisino Village, Bonegunu District, North Buton Regency, Southeast Sulawesi Province, Indonesia. Analysis of carrageenan content took place in the laboratory of the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Southeast Sulawesi Province.

2.1. Seedling preparation
Two different Kappaphycus Alvarezii seedling types were used. Tissue cultured seedlings were derived from seaweed seedlings produced from previous studies [9]. The local strain seedlings were obtained from the seaweed farmers in Koepisino Village. Seedlings from both sources were selected according to the general criteria for good seedling condition.

2.2. Grafting of the seedlings
The grafting was used on the selected tissue-culture seedlings (TC) and local strain seedlings (LOC) in three combinations: TC x LOC; LOC x OC; and TC x TC. The process of seaweed grafting consisted of two stages. First, the two K. alvarezii seedlings to be joined were each cut in a straight line from the base to the tip of the main thallus. Then the cut edges of the two main thalli were brought together, and the joint was bound and tied using plastic straps (Figure 1). The grafting process shown in Figure 1 was applied for all combinations.

2.3. Planting of Seaweed Seedlings
The grafted seedlings (all combinations) were then weighed to obtain the initial wet weight ($W_0$) which was $\approx 10g$. The bound grafted seaweed seedlings were soaked in order to prevent dehydration, and planted using a long line method. Initial culture period for grafted seedlings was 9 days until the
two thalli had become completely attached to each other. Thereafter, all fully attached seedlings were cultivated for 25 days.

Figure 1. Grafting process of tissue-culture (TC) seedlings with local strain (LOC): A. Preparation of LOC (left) and TC (right) seedlings; B. Seaweed thallus of LOC about to be cut in a straight line; C. TC thallus being cut in a straight line; D. The cut portion of the thallus was removed; E) the cut faces of the thalli of the two seedlings were slipped together and bound using plastic ice ties; F) The seedlings were then physically attached and were placed in seawater and brought to the planting location

2.4. Growing of the Seedlings
The *K. alvarezi* seedlings used in this stage were obtained from seedling grafting and culture processes as described above. The seedlings were cultivated for 45 days. During the growing period, the seedlings were cleaned from dirt, bryophytes and epiphytes. Water quality parameters measured were temperature and salinity (every 3 days) as well as current velocity, nitrate (NO$_3$), phosphate (PO$_4$), and turbidity (every 9 days).

2.5. Parameters Observed
The parameters observed during the study were:
a. The Grafting Rate. The grafting rate (%) was measured by calculating the number of seedlings that successfully attached divided by the number of seedlings initially grafted, multiplied by 100. All planted seedlings were initially cultivated for 10 days. Observation on changes in the condition of seedlings and cleaning the seedlings from mud and epiphytes was done every 2 days. After they had successfully attached, the seedlings were cultivated for 25 days for the propagation process. After that, the seedlings obtained were cultivated for 45 days.
b. The Daily growth rate (DGR) of seaweed after 45 days cultivation was calculated using the formula recommended by [13]:

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

where:
- \( W_0 \) is the initial fresh weight
- \( W_t \) is the final fresh weight of the seedlings after \( t \) days of culture

c. Ratio of dry weight to fresh weight (DW:FW). In the laboratory, harvested seaweeds were cleaned to remove sand and dirt, including hand removal of other organisms. All harvested seaweeds from each treatment were weighed fresh (wet weight) (g) and after drying using a hanging method for 2-3 days (dried weight) (g). Wet and dried weights were recorded for each sample and the ratio DW:FW was calculated for all the samples. Data were expressed as mean ± standard deviation (SD) for all the harvested seaweed [14].

d. Disease and Epiphytes found during the culture period were also recorded.

2.6. Statistical analysis
The between treatment differences were analysed using analysis of variance (ANOVA), followed where appropriate by a post-hoc Tukey test with a 95% confidence level. All statistical analyses were performed using SPSS version 16 statistical software.

3. Results and Discussion

3.1. Grafting rate
The grafting rate of all combinations was high, and the seedlings developed well (Figure 2).

**Figure 2.** Performance of *K. alvarezzi* seedlings during the post-grafting period (days):
- (A) day 0
- (B) 2<sup>nd</sup> day
- (C) 4<sup>th</sup> day
- (D) 6<sup>th</sup> day
- (E) 8<sup>th</sup> day
- (F) 10<sup>th</sup> day
The grafting rate of all combination was 93.33% on the 8th day (Table 1). The grafting rate was high and faster than grafting using a side-slipped position [12]. Based on this study, the straight slipped position is easier to apply and more effective than the side-slipped position.

Table 1. Grafting Success (%) during cultivation period of tissue-cultured (TC) and local strain (LOC) K. alvarezii seedlings

| Days | No | Grafting combination | Initial Number of seedlings | Number of seedlings failed to attach | Final Number of seedlings | Mean of grafting rate (%) |
|------|----|-----------------------|-----------------------------|--------------------------------------|--------------------------|--------------------------|
| 2    | 1  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 2    | 2  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 3    | 1  | LOC x LOC             | 20                          | 0                                    | 20                       | 0                        |
| 4    | 1  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 2    | 2  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 3    | 1  | LOC x LOC             | 20                          | 0                                    | 20                       | 0                        |
| 6    | 1  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 2    | 2  | TC x LOC              | 20                          | 0                                    | 20                       | 0                        |
| 3    | 1  | LOC x LOC             | 20                          | 0                                    | 20                       | 0                        |
| 8    | 1  | TC x LOC              | 20                          | 1                                    | 5                        | 19                       | 95                       |
| 2    | 2  | TC x LOC              | 20                          | 2                                    | 10                       | 18                       | 90                       |
| 3    | 1  | LOC x LOC             | 20                          | 1                                    | 5                        | 19                       | 95                       |

3.2. Daily Growth Rate (DGR)

During the cultivation period, the daily growth rate (DGR) of grafted K. alvarezii seedlings from different sources of tended to be different (Figure 3). The DGR of TC x LOC was higher and significantly different (p <0.05) compared to the DGRs of the TC x TC and LOC x LOC.

Figure 3. DGR of grafted seaweed K. alvarezii using three grafting combinations of local strain and tissue-cultured seedlings

The range of DGR of TC x LOC was 6.49-10.92%/day followed by LOC x TC at 5.84-9.94%/day and LOC x LOC at 5.77-8.61%/day (Table 2). These differences can be attributed to the high growth of tissue-culture seedlings making a contribution to the grafting combinations.
Table 2. Daily growth rate (DGR) and Tukey Test results for three grafting combinations of local grown (LOC), and tissue cultured (TC) K. alvarezii seedlings

| Day | Grafting combination | DGR (%/day±SD) | Tukey Test | p=|Value |
|-----|----------------------|----------------|------------|---|-------|
|     |                      |                | 1          | 2 |       |
| 9   | LOC + LOC            | 8.61±2.237     | 8.61±2.237 | 0.060 |       |
|     | TC + TC              | 9.94±0.949     | 9.94±0.949 | 0.204 |       |
|     | TC + LOC             | 10.92±1.122    | 10.92±1.122| 0.001 |       |
| 18  | LOC + LOC            | 8.30±0.799     | 8.30±0.799 | 0.321 |       |
|     | TC + TC              | 8.83±0.887     | 8.83±0.887 | 0.377 |       |
|     | TC + LOC             | 9.31±1.233     | 9.31±1.233 | 0.020 |       |
| 27  | LOC + LOC            | 8.03±0.339     | 8.03±0.339 | 0.030 |       |
|     | TC + TC              | 8.17±0.472     | 8.17±0.472 | 0.211 |       |
|     | TC + LOC             | 8.43±0.415     | 8.43±0.415 | 0.628 |       |
| 36  | LOC + LOC            | 6.57±0.259     | 6.57±0.259 | 0.463 |       |
|     | TC + TC              | 6.77±0.647     | 6.77±0.647 | 0.005 |       |
|     | TC + LOC             | 7.33±0.407     | 7.33±0.407 | 0.000 |       |
| 45  | LOC + LOC            | 5.77±0.378     | 5.77±0.378 | 0.982 |       |
|     | TC + TC              | 5.84±0.568     | 5.84±0.568 | 0.001 |       |
|     | TC + LOC             | 6.49±0.340     | 6.49±0.340 | 0.000 |       |

1 values followed by different letters were significantly different at p < 0.05

The DGR values obtained under all treatments were comparatively high compared to DGR values reported for K. alvarezii and similar Kappaphycus species, for example in Madagascar (DGR 5.46±0.09%/day) [15], in India (3.76 ± 0.07%/day) [16], and in Gorontalo, Indonesia (2.22-5.21%/day) [11]. In contrast, comparing to tissue-cultured seedlings used previously in seaweed farming, the DGRs found in this study were comparable to those reported from Malaysia (6.3 ± 0.1%/day) [13] and the Philippines (5.8-7.2%/day) [17]. Furthermore, comparing to a previous study using “prof” tissue-cultured seedlings, the DGRs found from this study were lower than those from a previous study by [18] (DGR 6.27± 0.31%/day). DGR from this study was also higher than DGR from seedlings combined by mass selection and a tissue–cultured method called ”prof” obtained by [14]. Overall, the results indicate that grafting using the straight-slipped position is very feasible and should be developed for the improvement of the quality of seaweed seedlings at the farm level.

3.3. The ratio of Dry weight to Fresh Weight

The dry weight to fresh weight ratio DW:FW was significantly different between all treatments (Tukey test; p <0.05). The DW:FW ratio was highest for the LOC x LOC grafts, despite lower biomass produced, while the second highest DW:DF and the highest dry biomass were produced by the LOC x TC grafts (Table 3).

Table 3. Ratio of Dry Weight to Fresh Weight (DW:FW) of K. alvarezii cultivated from grafting combination of Local strain (LOC), and tissue-cultured (TC) seedlings

| Grafting combination | W0 Initial weight (g) | Wf Fresh weight (g) | Wd Dry weight (g) | DW:FW ratio | Tukey Test | p=|Value |
|----------------------|-----------------------|---------------------|------------------|--------------|------------|---|-------|
|                      | 1                     | 2                   | 3                 | 4           | 5          | 6 |
| TC x LOC             | 10                    | 143.4±4.99          | 19.5              | 1 : 7.35    | 7.35b      | 0.000 |
| TC x TC              | 10                    | 132.7±3.80          | 19.0              | 1 : 6.99    | 6.99b      | 0.001 |
| LOC x LOC            | 10                    | 125.7±3.45          | 15.8              | 1 : 7.97    | 7.97b      | 0.000 |
| Mean                 | 137±3.2               | 13.0                | 1: 9.69          |              |            |    |

1 values followed by different letters are significantly different at p < 0.05
The DW:FW ratios obtained in this study were higher than those found in India (1:9.89) [7] and in a previous study in Indonesia (1:8.97) [8].

### 3.4. Disease and Epiphytes

Ice-ice, bryophytes and epiphytes were found during the study (Figure 4). The bryophytes were found attached to all parts of the seaweed, while ice-ice disease occurred close to the base of the thallus. Many bryophytes were found from the 9th day until the 18th day so that they could have reduced seaweed growth, while ice-ice disease was found on the 45th day.

![Figure 4. Examples of disease and epiphytes found during this study. A) ice-ice disease; B) epiphytes](image)

Epiphytes compete with the seaweed for light and nutrients. According to [19], if the epiphytes are not removed immediately, they will grow and spread, eventually covering the entire surface of seaweed thalli. Furthermore, [19] stated that epiphytic infestation can inhibit growth and reduce the productivity of seaweed. Ice-ice and epiphytes are generally caused by high temperatures, low salinity, light intensity and high prevalence coincides with periods of high temperature and salinity [20,21].

### 4. Water Quality parameters

During the study, the temperature ranged from 28–32°C, salinity 29–33 ppt, turbidity 1.59-2.35 NTU, nitrates 0.263-0.463 mg/L and phosphate 0.112-0.218 mg/L. In general, all water quality parameters were in the range generally considered suitable for the growth of the seaweed *K. alvarezii*.

### 5. Conclusion

Straight slipped position grafting using the combination of tissue-cultured x local strain had significantly higher growth, with a high ratio of dry weight to wet weight and good resistance to disease. The straight slipped grafting method can be applied easily because it was faster (full attachment occurred in 8-9 days) and the success rate was more than 90%. The results were superior compared to grafting using a side-slipped position.

### References

[1] Aslan L O M, Iba W, Bolu L R, Ingram B A, Gooley G J and Silva S S D 2015 Mariculture in SE Sulawesi Indonesia: Culture Practices and the Socio economic Aspects of the MajorOcean & Commod. Coast. Manag. 116 44–57

[2] Aslan L O M, Ruslaini, Iba W, Armin and Sitti 2016 Seaweed, Kappaphycus alvarezii, Culture Using Micropropagated Seedlings Pract. Guid. Seaweed Cultiv. No.1 Fac. Fish. Mar. Sci. Univ. Hala Oleo

[3] Yong W T L, Chin J Y Y and Yasir S 2014 Evaluation of growth rate and semi-refined
carrageenan properties of tissue cultured Kappaphycus alvarezii (Rhodophyta, Gigartinales) Phycol. Res. 62 316–21

[4] Yunke D A T, Tibubos K R, Hurtado A Q and Critchley A T 2011 Optimatization of Culture Conditions for Tissue Culture Production of Young Plantlets of Carrageenophyte Kappaphycus alvarezii J Appl Phycol. 23 433–8

[5] Sulistiani E, Soelistyowati D T, Alimuddin and Yani S A 2012 Callus Induction and Filaments Regeneration from Callus of Cottoni Seaweed (Kappaphycus alvarezii Doty) Collected from Natural Islands, Riau Islands Province Biotropia (Bogor). 19 103–14

[6] Rama R, Aslan L O M, Iba W, Rahman A, Armin A and Yusnaeni 2018 Seaweed cultivation of micropropagated seaweed (Kappaphycus alvarezii) in Bungin Permai Coastal Waters, Tinanggea Sub District, South Konawe Regency, South East Sulawesi IOP Conf. Series: Earth and Environmental Science

[7] Febriyanti, Aslan L O M, Iba W, Patadjai A B and Nurdin A R 2019 Effect of Various Planting Distances on Growth and Carrageenan Yield of Kappaphycus alvarezii (Doty) Doty using Seedlings Produced from Mass Selection Combined vs Tissue–Cultured Method IOP Conference Series: Earth and Environmental Science

[8] Reddy C R K, Raja K K G, Siddhanta A K and Tewari A 2003 In Vitro Somatic Embryogenesis and Regeneration of Somatic Embryos from Pigmented Callus of Kappaphycus alvarezii (Doty) Doty (Rhodophyta, Gigarti-nales) J. Phycol. 39 610–616

[9] Aslan, L.O.M. R 2015. Pengembangan Bibit Unggul Rumput Laut Kappahycus alvarezii hasil kultur Jaringan dalam Mendukung Peningkatan Produksi Perikanan Nasional di Sultra (Universitas Haluoleo)

[10] Sahu N, Meena R and Ganesan M 2011 Effect of grafting on the properties of kappa-carrageenan of the red seaweed Kappaphycus alvarezii (Doty) Doty ex Silva Carbohydr. Polym. 84 584–592

[11] Fadilah S, Alimuddin, Pong-Masak P R, Santoso J and Parenrengi A 2016 Growth, Morphology and Growth Related Hormone Level in Kappaphycus alvarezii Produced by Mass Selection in Gorontalo Waters, Indonesia HAYATI J. Biosci. 23

[12] Aslan L O M, Hafid H, Supendy R, Taridala S A A, Sifatu W O, Sailan Z and Niampe L 2018 Income of Seaweed Farming Households: A case Study From Lemo of Indonesia IOP Conf. Ser.: Earth Environ. Sci. Series: Earth and Environmental Science

[13] Yong Y S, Yong W T L, Thien V Y, Ng S N and Anton 2013 Analysis of formulae for determination of seaweed growth rate J. Appl. Phycol. 25 1831–1824

[14] Periyasamy C, Subba-Rao P V and Anantharaman P 2019 Harvest optimization to assess sustainable growth and carrageenan yield of cultivated Kappaphycus alvarezii (Doty) Doty in Indian waters J. Appl. Phycol. 31 587–97

[15] Ateweberhan M, Rougier A and Rakotomahazo C 2015 Influence of environmental factors and farming technique on growth and health of farmed Kappaphycus alvarezii (cottonii) in south-west Madagascar J. Appl. Phycol. 27 923–34

[16] Periyasamy, C Anantharaman, P Balasubramanian, T SubbaRao P V 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. 26 803–10

[17] Hurtado A, Critchley A T, Trespoe A and Bleicher-Lhonneur G 2008 Growth and Carrageenan Quality of Cappaphycus striatum Far. Sacchol Grown at Different Stocking Densities, Duration of Culture and Depth J Appl Phycol 20 551–5

[18] Goa S 2018 Budidaya rumput laut Kappaphycus alvarezii (Doty) Doty exsilva (Soliericeae, Gigartinales, Rhodophyta) menggunakan bibit hasil seleksi klon yang telah dikultur jaringankan di Perairan Desa Bungin Permai Kecamatan Tinanggea Kabupaten Konawe Selatan, Sulawe (Kendari)

[19] Largo D B 2006 Diseases in cultivated in the Philippines: Is it an issue among Seaweed Industry Players. Advances in Seaweed Cultivation and Utilisation In Asia. Phang, Critchley &
Angeds Proc. of a workshop held in conjunction with the 7th Asian Fisheries Forum, Penang (Malaysia: University of Malaysia Research Centre)

[20] Vairappan C S 2006 Seasonal occurrences of epiphytic algae on the commercially cultivated red alga Kappaphycus alvarezii (Solieriaeae, Gigartinales, Rhodophyta) J. Appl. Phycol. 18 611–7

[21] Hurtado A Q, Critchley A T, Trespoey A and Bleicher L G 2006 Occurrence of Polysiphonia epiphytes in Kappaphycus farms at Calaguas Is., Camarines Norte, Phillippines J. Appl. Phycol. 18 301–6