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SHORT COMMUNICATION

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Grafting of the seaweed *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) in SE-Sulawesi, Indonesia

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Abstract

The seaweed *Kappaphycus alvarezii*, commonly known as cottonii is the most important seaweed species cultured in Indonesia. This seaweed is widely cultivated as a main income source for communities in coastal areas of Southeast (SE) Sulawesi. In general, grafting has been rarely used in the seaweed *K. alvarezii*. An attempt was made to graft the *K. alvarezii* in the shallow waters at Bungin Permai, South Konawe, SE-Sulawesi of Indonesia. Tissue-cultured and local strains were selected for intra-generic grafting. The methods of graft combinations were side- and straight-slipped positions. Each combination contained 30 grafting plants. Between intra-generic grafted plants, straight position was found to attach after 9 days. For side-slipped position, it took 21 days to attach. In conclusion, due to easy and fast vegetative potential, high intra-generic grafting success using straight-slipped position method for grafting is achievable.

KEYWORDS
grafting, local strain, tissue culture

Indonesia has been the biggest producer of seaweed since 2008 (Hurtado et al., 2014). In 2016, seaweed production in Indonesia reached 11.6 million tons (FAO, 2018) and accounted for 38.7% of global seaweed production. Although increasing seaweed production has been found to occur throughout all provinces in Indonesia, only 5 (five) provinces are considered as the main producers, Southeast Sulawesi, South Sulawesi, Central Sulawesi, West Nusa Tenggara and East Nusa Tenggara (Badan Pusat Statistik, 2016). In 2017, Southeast (SE) Sulawesi was reported to produce up to 1,000,010 t that was mainly of red seaweed *Kappaphycus alvarezii* (Marine & Fisheries Agency of SE-Sulawesi, 2014). This level of production is expected to increase in the future considering the potential of SE-Sulawesi to extend seaweed farms across marine and coastal areas along its 1,740 km coastal line (Aslan et al., 2015, 2018).

Low seedling quality is still one of the main obstacles to accelerate seaweed production in SE-Sulawesi (Febriyanti et al., 2019; Rama et al., 2018). Using the same source of seaweed broodstock for seedlings is reported to contribute to lowering quality (Kumar et al., 2007), and therefore, micropropagated seaweed seed has been suggested to be used to boost production growth (Aeni et al., 2019). However, access to micropropagated seedlings is not readily available for local farmers in SE-Sulawesi. They have been using local strains of *K. alvarezii* for decades that has contributed to steady increase in seaweed production in this region despite expanding farm area.

Grafting two different coloured local strains was considered to improve seedling quality as reported by Sahu et al. (2010) and Sahu et al. (2011). These two studies found that grafting method using side-slipped position using different coloured strains took around 23 days. It was indicated that seaweed farmers need around one month to obtain results of seedling from this grafting method. Therefore, a new method is required to get the seedlings to be available faster with maximum growth rate to increase seaweed production. Currently, there is no information on methods of suitable seaweed grafting using micropropagated or tissue-cultured seedlings in Indonesia, especially in SE-Sulawesi. In this paper, we present the initial findings on the method of a novel grafting technique of micropropagated and local strains of *K. alvarezii* in Indonesian seawaters.

This research was conducted for 3 (three) months from March to July 2018. The plants of tissue-cultured seedlings and local strain of *K. alvarezii* were collected from a seaweed farm located in Bungin Permai coastal waters, Tinanggea District, South Konawe, SE-Sulawesi. Tissue-cultured seedlings and local strain of *K. alvarezii*, which weighed 10 ± 5 g each using a balance with 0.5-g precision scale, were obtained at 26–28 days of culture. The initial condition of seaweed from tissue-cultured seedlings used for this grafting experiment was that they had a denser and larger thallus, blackish brown colour and tended to be darker than the local seaweed (Figure 1). The seedlings were then tied to the rope, and after binding was completed, they were then immediately brought to the planting location to maintain fresh seedling conditions.

The experiment was conducted in two sets. In the first set, plants of *K. alvarezii* from tissue-cultured seedling were combined with local strains. The selection criteria for plants used for grafting were daily growth rate more than 3%.day^{-1}, disease free particularly from ice-ice and clean and fresh with no epiphyte and apparent wound or broken thallus. The processes were as follows: (a) after selection, the plants were washed thoroughly with clean seawater to remove dirt and epiphytes; (b) the selected plants were then cut obliquely by a cutter into small pieces; (c) the straws were separated from the base of seaweed thalli; (d) the graft combinations were done by putting two straws together; a portion of the seaweed thallus to be propagated was slipped on to the thallus of another, that is forming corresponding 'mates'; thus, the thalli portion of the two straws was slipped from side position. For the second set, the portion was slipped from straight position (Figure 2a, b); and (e) finally, the two straws were combined and tied tightly using plastic strap. These combined plants were then transferred to the government hatchery at Purirano, SE-Sulawesi. Description of grafting method of these two combinations is shown in Figures 3 and 4.

Samples of seawater were collected for analysing the hydrobiological parameters. Seawater temperature was recorded using standard centigrade thermometer. Salinity was measured using Atago Refractometer, and PO4–P and NO2–N were estimated using the standard methods. During the experiment, regular maintenance of the seedlings was conducted three times a week. The seedlings were cleaned from dirt, epiphytes and other unwanted weeds.

The attachment using the different source of seedlings during this grafting experiment strongly confirmed the feasibility of these two techniques (Figure 5). The duration of attachment in the blends depended on the method of the slipped position, and varied significantly. In the side-slipped position, the physical attachment was observed after 21–23 days from the date of initial plantation, and for straight-slipped position, it takes only 9–10 days. During this preliminary study, the range of temperature, salinity, and phosphate and nitrate salinity were 29.5–30.0°C, 28.5–33.0 ppt, and 0.10–0.20 and 0.01–0.06 mg/L respectively.

The result of this study indicates that the grafting protocol of straight-slipped position is simpler to be adopted by farmers than the earlier study done by Sahu et al. (2010), Sahu et al. (2011). Moreover, the attachment of side-slipped position groups found after 21–23 days from the date of initial plantation is in a good agreement with the previous studies of Sahu et al. (2010), Sahu et al. (2011). The present results indicated that physical attachment using straight-slipped position of *K. alvarezii* could be produced more easily and faster under the field conditions of SE-Sulawesi, Indonesia waters.

This result indicated that all of the grafted seedlings especially produced by straight-slipped position could be easily utilized for improving the survival rate of seedlings above 80%. It is clearly showed that the factors affecting graft survival rates mainly depend on grafting protocols. This study provides a new seaweed thallus grafting protocol that simplifies and reduces the attachment time from previous method done by Sahu et al. (2010), Sahu et al. (2011) or mass selection (Aslan et al., 2019). From our field observation, the grafted seedlings are stronger and more resistant in high salinity

FIGURE 1 Comparison of morphology between local seedlings (right) and micropropagated seedlings (left) of *Kappaphycus alvarezii*

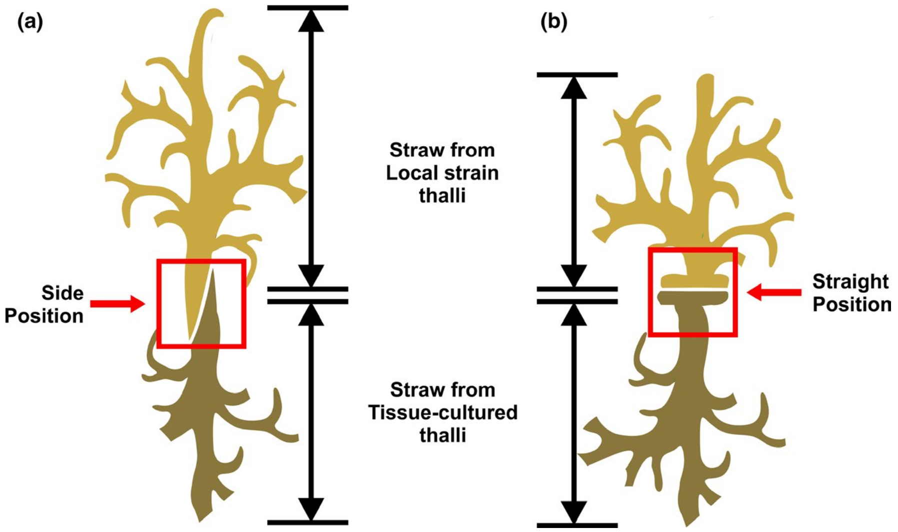

FIGURE 2 Experimental set of grafting methods using different positions. (a) Side position; (b) straight position. Arrows showing the grafting zone

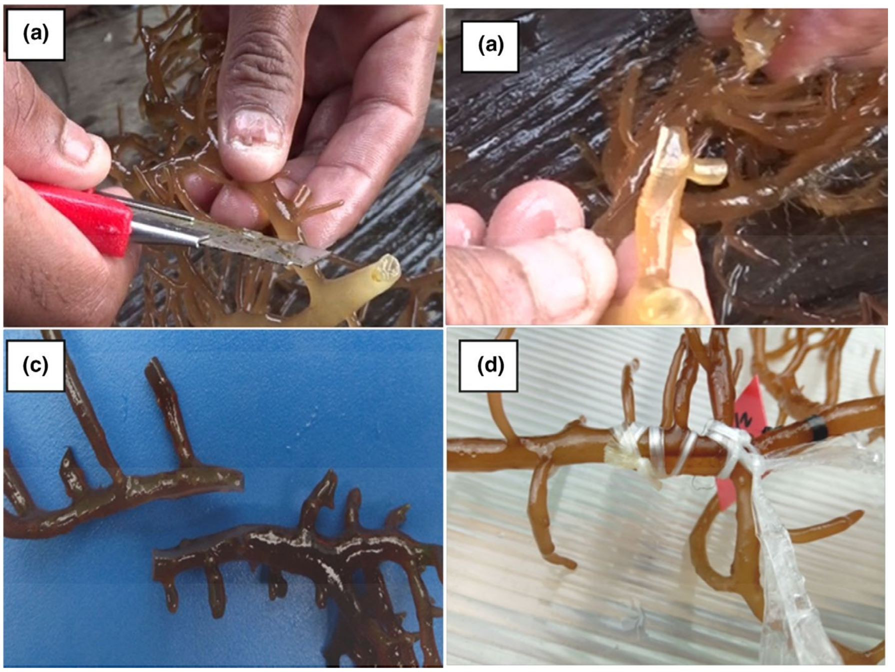

FIGURE 3 Steps of grafting process of side-slipped method. (a, b) Oblique incision and cut of the seaweed thalli; (c) portion of thalli of one seaweed to be propagated was slipped from the side onto the thalli of another; (d) corresponding mates tied tightly

and temperature, which indirectly improves yield. Also, the phenotypic of the grafted seaweed was a blend between the local and tissue culture seedlings. We observed that they have long but dense thallus and darker brown colour although paler than tissue culture seedlings. In this context, the use of adequate high quality of seedlings through grafting provides an important strategy to reduce the losses in seaweed production caused by environmental stresses. Also, further genetic research of the grafted seaweed will determine whether or not they are still in the same genetic strains to their parent plant. Therefore, additional research is needed to support the development of phycocolloid industries by producing high quality of seaweeds through grafting method such as how grafted seedlings could increase the daily growth rate (DGR), carrageenan yield and gel strength, and withstand to epiphyte outbreaks and the ice-ice disease.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

La Ode Muhammad Aslan, Rahmat Hasriah, Aldi La Embi and Ardi Ansa designed study. Wa Iba and Andi Besse Patadjai drafted paper. Manat Rahim analysed data.

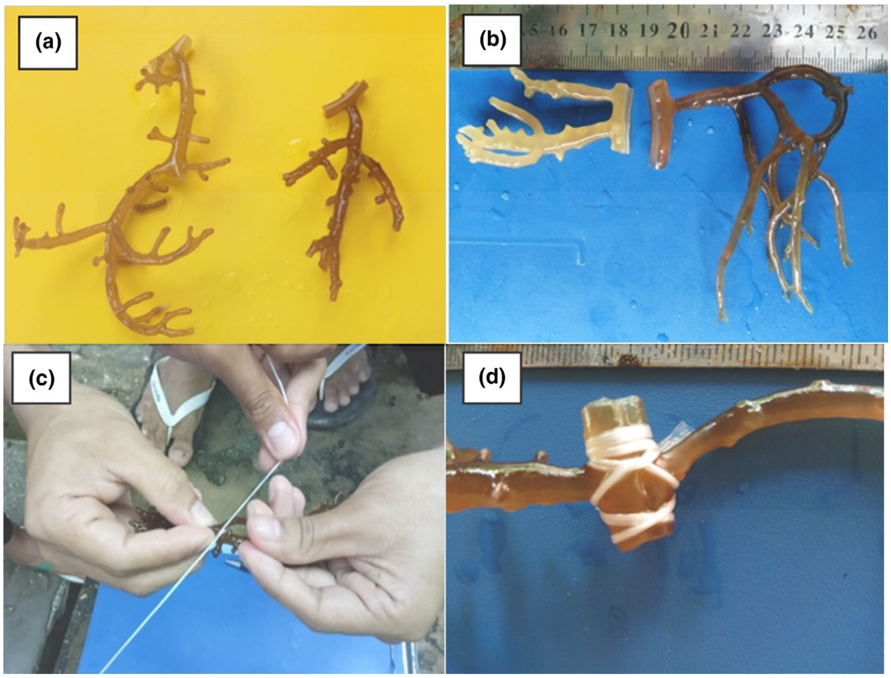

FIGURE 4 Steps of grafting process of straight-slipped method. (a, b) Oblique incision and cut of the seaweed thalli; (c) portion of thalli of one seaweed to be propagated was slipped from the side onto the other thalli; (d) corresponding mates tied tightly

FIGURE 5 The physical attachment of the differently sourced seedlings using two positions of grafting in the cultivation. Side (a) and straight-slipped (b) position. Arrows showing the grafting zone

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available.

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