Growth optimization of *Kappaphycus alvarezii* with Basmingro-nutrient technology through mass tissue culture

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Abstract. This study aimed to apply a prototype of Basmingro technology to optimize seaweed growth through the mass tissue culture method. An immersion treatment using Basmingro with a dose of 0.1 mL L^{-1} of seawater prior to the planting step was tested on *Kappaphycus alvarezii* seedlings from the nursery of the Takalar Marine Cultivation Center. The rearing process was carried out in the coastal waters of Popalo Village, Gorontalo Utara, Indonesia. Measurement of total wet-weight, sample weight, thallus diameter, and the number of branches was performed in pre and post rearing process. The experiment revealed that the growth-weight-specific rate of *K. alvarezii* rises from 2.7% in the first 20 rearing days to 4.5% in the second 20 rearing days. There is a significant (α=0.05; n=25 seedling knots) increase in wet weight, first-branch base diameter, and number of branches of *K. alvarezii* for 40 days of nurturing through the mass tissue with Basmingro solution immersion treatment.

Key Words: biotechnology, growth rate, marine algae, Rhodophyta, seaweed cultivation.

Introduction. Seaweeds are multicellular eukaryotic photosynthetic organisms that potentially produce many essential compounds (Raja et al 2013; Asni & Najamuddin 2021; Kalasariya et al 2021), becoming one of the marine resources with a high economic value. World trade activities of seaweed resources among countries, both in fresh and processed forms and extracts, contribute to a country's income. The potential for development and use of seaweed is diverse globally, for example in the human diet (Brown et al 2014; Palmieri & Forleo 2020; Nielsen et al 2021), as animal feed ingredients (Kinley et al 2020; Morais et al 2020), biostimulants for improving soil and plant health (Nanda et al 2021), and bioethanol source (Meinita et at 2012; Khambhaty et al 2012). In some areas, the utilization is not ideal (Pratama et al 2021).

The global seaweed market involves production from capture and culture (Najamuddin et al 2020; Muawanah et al 2021). Seaweed aquaculture uprooted more than half of the global mariculture production from 2000 to 2018 (Chopin & Tacon 2020; Duarte et al 2022). *Kappaphycus* is one of the red seaweed groups that belong to the phylum Rhodophyta, which can be cultivated (Hung & Trinh 2020). One of the species of this genus is *Kappaphycus alvarezii*, which is commonly cultivated in Indonesia (Kasim & Mustafa 2017; Rama et al 2018; Ratnawati et al 2020) and worldwide (Bindu & Levine 2011; Hayashi & Reis 2012; Valderrama et al 2015; Rudke et al 2020).

Decreased production due to the limited production of tissue culture products and the emergence of ice-ice disease are the main obstacles to the success of *K. alvarezii* cultivation during the planting and rearing stages. This condition encourages us to determine a method to increase seaweed production, without it being susceptible to ice-ice disease. The technology recommended in this experiment is using the Basmingro-nutrient, by soaking seaweed seed stocks for several hours before the planting and rearing stages. This study aimed to apply the Basmingro-nutrient technology prototype to optimize seaweed growth through the mass tissue culture method.

Material and Method

Duration and experimental area. The cultivation of the seaweed was carried out for 40 days starting from June 28 to August 7, 2021, at the Popalo Village, Anggrek District, North Gorontalo Regency, Indonesia (coordinates: 0.826399° latitude; 122.817199° longitude). The field experiment location was in the coastal waters (salinity of 25 ppm), without river water intrusion altering the salinity constancy.

Seedling selection and preparation. The seaweed species used in this research was *K. alvarezii* from the Takalar Marine Cultivation Center Nursery, South Sulawesi, Indonesia. Collecting and cutting the tips of the shoots was done using a knife. The selected seedlings for the experiment had 8 kg of fresh weight. *K. alvarezii* seedlings were cleaned and rinsed with seawater from the coastal area of Popalo Village to remove attached materials, potentially a source of disease. The seedlings were all unraveled, separated, and tied with raffia string into 250 knots.

Initial measurements and treatments. The measurement of weight, thallus diameter, and the number of branches of 25 out of 250 bands was calculated as initial data of samples. All seedling ties were held together and hanged along a long nylon slap rope with a space of ±10 cm amongst the knots (Figure 1). The total weight was determined. The seedling bundles were placed in a box-shaped styrofoam container (100 cm length, 90 cm width, 75 cm height), filled with seawater. Moreover, the Basmingro solution (0.1 mL L^{-1}) was dripped into the container, and the bundles were left to soak for 16-18 h.

Planting, rearing, and harvesting. Seaweed seedlings were planted by spreading them out in the experimental area using a long-line rope method. On the 20th day of rearing, all the tested seedlings were transplanted to quantify the total weight. Then they were put into a container and given the treatment of dripping and soaking using Basmingro, with a dose in the previous stage. Next, the seedlings were replanted in the waters. On the 40th day, the following measurements were carried out: harvesting, weighing the 250 bands and 25 knots with samples, measuring the diameter of the thallus, and counting the number of branches. The weight and diameter of each sample were measured using a digital scale (nearest to 1 g) and a caliper (nearest to 0.01 mm). Each thallus diameter was quantified at the first branch base. The branches number and physical changes in the samples were visually determined and documented.

Figure 1. *Kappaphycus alvarezii* knots were bounded and hanged along a nylon slap rope.

Data analysis. The specific weight growth rate of seedling samples was calculated using the following formula (Husniah et al 2020):

$$SGR = \frac{\ln W_t - \ln W_0}{\ln W_t \times t} \times 100\%$$

Where: SGR - specific growth rate (%); W_t - initial weight (g); W_0 - final weight (g); t - rearing period (months).

The total weight (250 sets) of the seedlings before and after the rearing stage with Basmingro immersion treatment, measured twice per the 20 days of rearing, was compared descriptively and visually. The significance of the distinctions in their growth parameters (weight, thallus diameter, and the number of branches) from 25 bundles of initial *K. alvarezii* samples and those in harvest periods in this experimental cultivation was examined with the t-Test: Paired Two Sample for Means.

Results. In general morphology, several advantages of *K. alvarezii* with the soaking treatment using Basmingro were observed: the relatively massive diameter of the thallus, light brown in color, shiny, slippery, many more branches appearing in the main thalli, no symptoms of ice-ice disease along its body. On the other hand, *K. alvarezii* cultivated without Basmingro treatment showed a darker color, and was relatively small, indicating symptoms of ice-ice disease (Figure 2).

Figure 2. The appearance of *Kappaphycus alvarezii* seedling samples cultivated in Popalo Waters through (left) mass tissue culture using Basmingro – a sample from this study; (right) without Basmingro soaking treatment – a sample from local farmers.

The SGR of the seaweeds through mass tissue culture in Popalo Village coastal waters is presented in Figure 3. There was an increase in their SGR value.

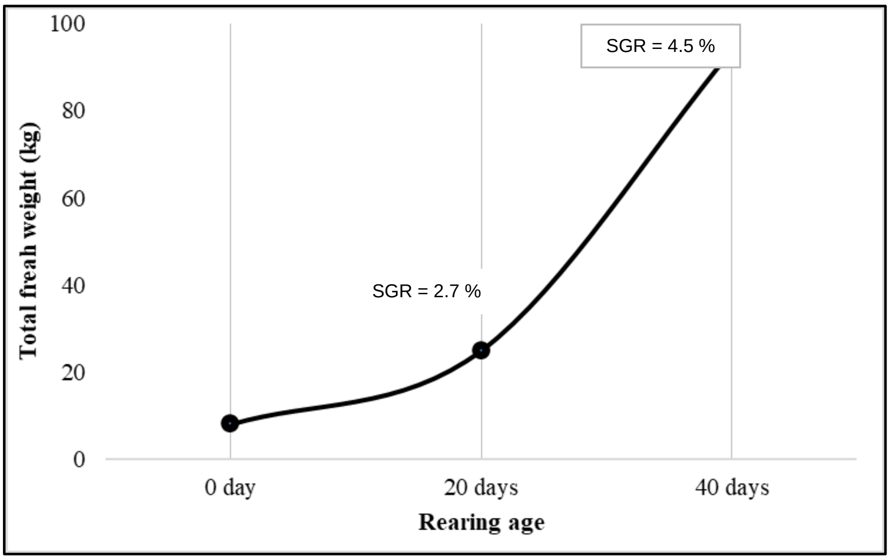

Figure 3. Specific growth rate (SGR) of fresh *Kappaphycus alvarezii* with Basmingro nutrient immersion treatment prior to the planting step.

The initial total wet weight of the *K. alvarezii* samples was 3843 g, averaging 153.72±10.49 g (n=25 knots). At 40 days of rearing, the total weight of the samples was 7785 g, averaging 311.4±77.45 g (n=25 knots). The average base branch diameter was 5.18±0.66 mm initially, and became 7.88±1.01 mm at harvest time. The average number of branches before and after 40 days of cultivation with Basmingro immersion treatment was 7.24±1.05 and 16.9±2.78, respectively. The results of the t-Test revealed that the Basmingro immersion treatment on *K. alvarezii* seedlings prior to the rearing stage lead to a positive effect on fresh weight growth (p-value=1.3×10^{-9}), diameter size (p-value=7.73×10^{-12}), and a significant increase in the number of branches (p-value=1.92×10^{-15}). The initial and harvesting data comparisons of weight, diameter, and the number of branches of each sample knots are presented in Figures 4 to 6, respectively.

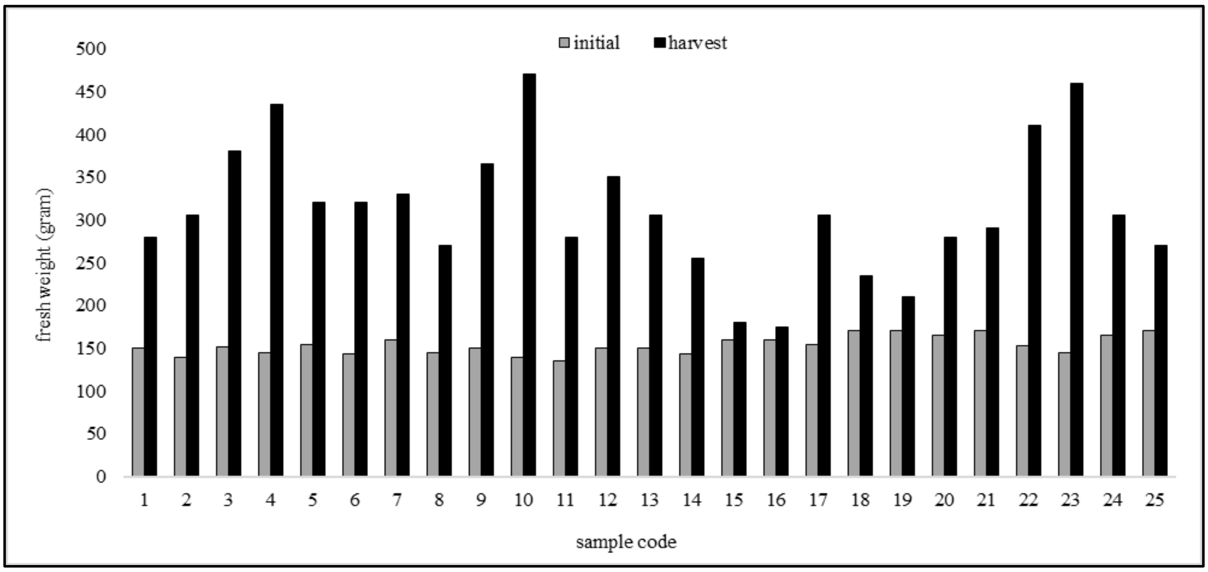

Figure 4. Fresh weight comparison of *Kappaphycus alvarezii* seedlings with mass tissue culture before and after cultivation during 40 rearing days with Basmingro immersion treatment.

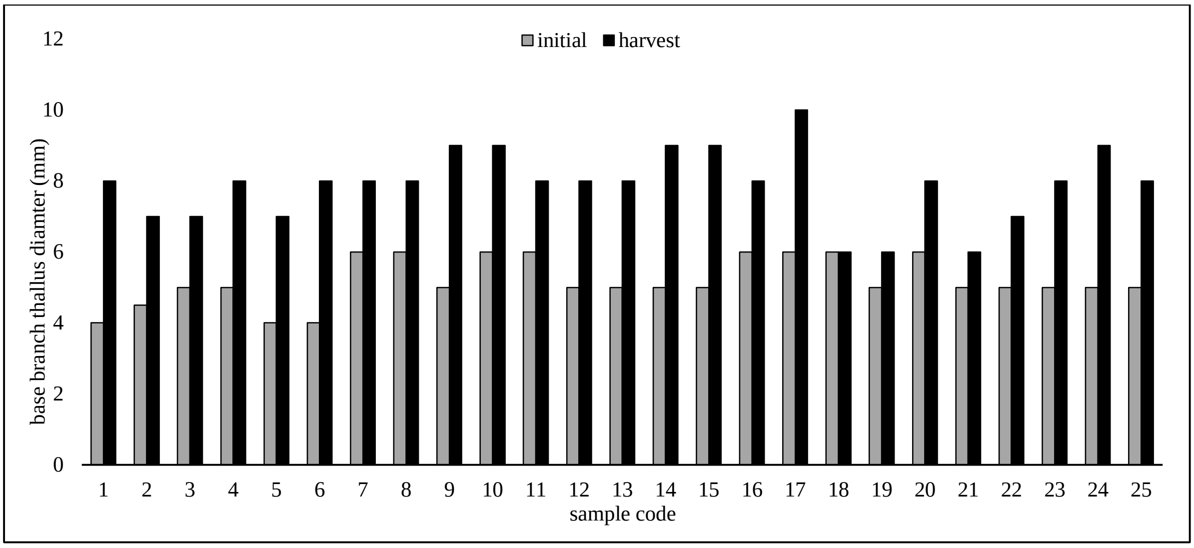

Figure 5. Comparison of stem diameters of *Kappaphycus alvarezii* seedlings with mass tissue culture before and after cultivation during 40 rearing days with Basmingro immersion treatment.

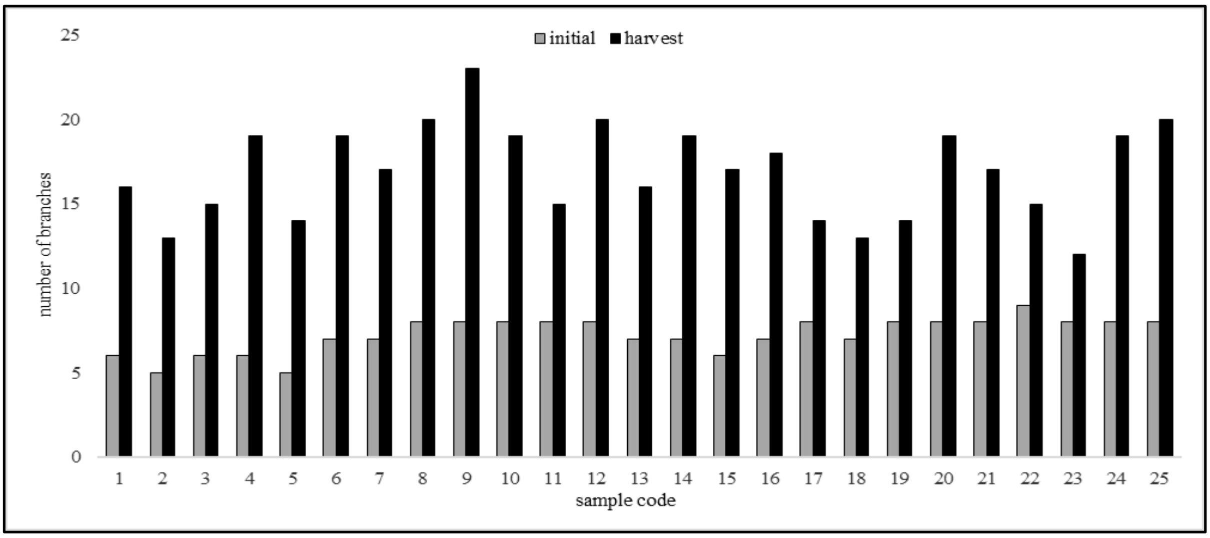

Figure 6. Comparison of the number of branches of *Kappaphycus alvarezii* seedlings with mass tissue culture before and after cultivation during 40 rearing days with Basmingro immersion treatment.

Discussion. The world demand for seaweed, including the *K. alvarezii*, is expanding every year, as it is a high economic marine commodity (Bindu & Levine 2011; Pratama et al 2021). Seaweed or algae cultivation is usually carried out in the sea, and growth depends on natural conditions when no treatments are applied. Various external factors will determine survival, including predation, water quality fluctuation and dynamics, and nutrition sufficiency. All of these aspects need to get more attention from the mariculture farmers to produce maximum yield of crops (Tuiyo 2016).

The primary habitat of *K. alvarezii* is represented by coral reef flats, requiring sunlight for photosynthesis. Therefore, it generally grows well in areas permanently submerged in water, where it can attach to the bare substrate in the form of dead corals, live corals, rocks, mollusk shells, and other complex objects (Veluchamy & Palaniswamy 2020). Naturally, the species is usually found and harvested in a community form, not solitary. *K. alvarezii* lives well in areas far from freshwater sources, 300-500 m from river mouths (Sulistiawati et al 2020). In aquaculture, it has been developed to be cultivated by tying it on a rope, so it does not require to be attached to coral substrates or other objects (Rama et al 2018), similar to the circumstances of the current experiment. The seedling rearing sites must have appropriate environmental water parameters, such as dissolved oxygen, salinity, water temperature, radiation, water depth, waves, and pH (Ateweberhan et al 2015; Nazaruddin et al 2015; Mulyani et al 2018).

The effective growth of marine alga, particularly *K. alvarezii*, is likewise strongly induced by the availability of nutrients. NO_3-N, PO_4-P, and SiO_3-Si are the required nutrients needed by the species to grow in the wild (Mandal et al 2015). The results of a study conducted by Msuya (2013) in Zanzibar, Tanzania, revealed that cultivated *K. alvarezii* with additional higher N and P nutrients had a slightly increased growth rate. In a study conducted by La Macchia Pedra et al (2017), *K. alvarezii* cultivated with shrimp effluent had a higher growth rate as well as a greater content of phenolic compounds and flavonoids. An acclimatization study by Yong et al (2015) showed that an optimum growth of *K. alvarezii* in seawater at some extent condition of density was achieved with an enrichment treatment with mixed-algae fertilizer, natural seaweed extract (NSE), also applied to the seedling stage. An experiment by Fadlillah et al (2017) showed that an hour-immersion treatment of extracted *Ulva lactuca* is an effective soaking duration to maximize growth and carrageenan content of *K. alvarezii.*

In addition to nutrition, plant growth regulators formerly referred to non-nutrient organic compounds in small amounts can promote, inhibit, and change plant physiological processes (Khan 2021). The growth regulators are chemical substances produced internally in a particular part of the plant that affect other parts of its body. They can be produced by the plant itself and also formed synthetically. Those found naturally in plants are named phytohormones or plant hormones. Phytohormones are hereinafter referred to as Plant Growth Regulators (PGRs) to distinguish them from hormones in animals. They are generally translocated to other plant parts, which provide biochemical, physiological, and morphological responses. Thus far, five main groups of PGRs are recognized, namely auxins, cytokinins, gibberellins (GAs), ethylene (ETH), and abscisic acid (ABA). Auxins, cytokinins, and gibberellins have a positive impact for plant growth. ETH can support and inhibit growth, and ABA is a growth inhibitor (Tuiyo 2016). Along with the development of the biochemistry and the chemical industry, many compounds are found to affect physiological conditions. PGR begins with the concept of hormones themselves and external nutrients needed by plants, showing their essential role in the growth, development, and other physiological processes of plants.

Basmingro is a technology categorized as a PGR in the form of a solution formulated based on the first author's staged experimental results since 2005. The technology was initiated based on the complaints of seaweed farmers due to the slow growth and susceptibility of ice-ice disease of their crops. It has a trademark with IDM number 0004387770 in 2012, a copyright hearing book number 077719 in 2015, and a patent with the registration number P28201912172. Apart from being used in the field of aquaculture (Tuiyo & Marsuci 2022), the application of the Basmingro nutrient also has been attempted in agriculture (Husain & Tuiyo 2012), although its optimal dose has not been obtained. A previous study conducted by Tuiyo (2016) with different treatment doses revealed that a dose of 0.1 mL L^{-1} is optimal to obtain the maximum growth of *K. alvarezii* in plastic bags. The optimal dose of Basmingro nutrient for to the successful growth of *K. alvarezii* seeds with a mass tissue culture method has not been published. However, the 1st author conducted several experiments with seaweed mariculture farmers in Koperasi Tunas Mekar, Koperasi Nelayan Lagundi, and UD Berkah Kotonii Popalo Gorontalo Utara Pesisir. *K. alvarezii* seedlings from mass tissue culture with Basmingro nutrient had length-SGRs of 3.5%, 3.7%, and 4.5% from each location, respectively (unpublished data).

The expression of fresh weight growth, thallus base branch diameter, and the number of branches in the present study revealed that Basmingro immersion treatment with a dose of 0.1 mL L^{-1} of seawater on *K. alvarezii* mass tissue culture in Popalo waters, North Gorontalo, significantly optimized the growth of the marine algae. Nonetheless, this study needs to be applied with treatments combined with various densities, salinities, and other variables. Moreover, the researchers must determine the specific type of bioactive compound in Basmingro that makes the product significantly affect seaweed growth rates.

Conclusions. This study has successfully revealed that the specific growth rate of weight for *K. alvarezii* increases from 2.7% in the first 20 rearing days to 4.5% in the second 20 rearing days. There is a significant (α=0.05; n=25 seedling knots) increase in wet weight, first-branch base diameter, and the number of branches of *K. alvarezii* for 40 days of nurturing through mass tissue with Basmingro solution immersion treatment.

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Conflict of Interest. The authors declare that there is no conflict of interest.

References

Asni A., Najamuddin, 2021 Analysis on carrageenan content of seaweed *Kappaphycus alvarezii* at different water condition in Bantaeng District. IOP Conference Series: Earth and Environmental Science 860:012069.

Ateweberhan M., Rougier A., Rakotomahazo C., 2015 Influence of environmental factors and farming technique on growth and health of farmed *Kappaphycus alvarezii* (cottonii) in south-west Madagascar. Journal of Applied Phycology 27:923-934.

Bindu M. S., Levine I. A., 2011 The commercial red seaweed *Kappaphycus alvarezii*—an overview on farming and environment. Journal of Applied Phycology 23:789-796.

Brown E. M., Allsopp P. J., Magee P. J., Gill C. I., Nitecki S., Strain C. R., McSorley E. M., 2014 Seaweed and human health. Nutrition Research Reviews 72(3):205-216.

Chopin T., Tacon A. G. J., 2020 Importance of seaweeds and extractive species in global aquaculture production. Reviews in Fisheries Science & Aquaculture 29(2):139-148.

Duarte C. M., Bruhn A., Krause-Jensen D., 2022 A seaweed aquaculture imperative to meet global sustainability targets. Nature Sustainability 5:185-193.

Fadlillah M., Cokrowati N., Rusdani M. M., 2017 [Effectiveness of *Ulva lactuca* extract on *Kappaphycus alvarezii* growth]. Proceedings of the 2nd International Conference on Science and Technology, Mataram-Lombok, Indonesia, pp. 398-404. [In Indonesian].

Hayashi L., Reis R. P., 2012 Cultivation of the red algae *Kappaphycus alvarezii* in Brazil and its pharmacological potential. Revista Brasileira de Farmacognosia 22(4):748-752.

Hung L. D., Trinh P. T. H., 2020 Structure and anticancer activity of a new lectin from the cultivated red alga, *Kappaphycus striatus*. Journal of Natural Medicines 75:223-231.

Husain I., Tuiyo R., 2012 [Breaking dormancy of candlenut (*Aleurites moluccana*, L. Willd) seeds soaked with basmingro organic growth regulator and its effect on seed viability]. Jurnal Agroteknotropika 1:95-100. [In Indonesian].

Husniah, Nur M., Tenriware, Tamaruddin A., Jabbar F. B. A., Tambaru R., 2020 Growth performance of seaweed *Kappaphycus alvarezii* in different planting distance using long line farming, Mandar Bay, West Sulawesi Indonesia. IOP Conference Series: Earth and Environmental Science 575:012097.

Kalasariya H. S., Yadav V. K., Yadav K. K., Tirth V., Algahtani A., Islam S., Gupta N., Jeon B. H., 2021 Seaweed-based molecules and their potential biological activities: An eco-sustainable cosmetics. Molecules 26(17):5313.

Kasim M., Mustafa A., 2017 Comparison growth of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivation in floating cage and longline in Indonesia. Aquaculture Reports 6:49-55.

Khambhaty Y., Mody K., Gandhi M. R., Thampy S., Maiti P., Brahmbhatt H., Eswaran K., Ghosh P. K., 2012 *Kappaphycus alvarezii* as a source of bioethanol. Bioresource Technology 103(1):180-185.

Khan N., 2021 Application of plant growth promoting microorganism and plant growth regulators in agricultural production and research. Agronomy 11(3):524.

Kinley R. D., Martinez-Fernandez G., Matthews M. K., de Nys R., Magnusson M., Tomkins

N. W., 2020 Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. Journal of Cleaner Production 259:120836.

La Macchia Pedra A. G., Ramlov F., Maraschin M., Hayashi L., 2017 Cultivation of the red seaweed *Kappaphycus alvarezii* with effluents from shrimp cultivation and brown seaweed extract: Effects on growth and secondary metabolism. Aquaculture 479:297-303.

Mandal S. K., Ajay G., Monisha N., Malarvizhi J., Temkar G., Mantri V. A., 2015 Differential response of varying temperature and salinity regimes on nutrient uptake of drifting fragments of *Kappaphycus alvarezii*: Implication on survival and growth. Journal of Applied Phycology 27:1571-1581.

Meinita M. D. N., Kang J. Y., Jeong G. T., Koo H. M., Park S. M., Hong Y. K., 2012 Bioethanol production from the acid hydrolysate of the carrageenophyte *Kappaphycus alvarezii* (cottonii). Journal of Applied Phycology 24:857-862.

Morais T., Inácio A., Coutinho T., Ministro M., Cotas J., Pereira L., Bahcevandziev K., 2020 Seaweed potential in the animal feed: A review. Journal of Marine Science and Engineering 8(8):559.

Msuya F. E., 2013 Effects of stocking density and additional nutrients on growth of the commercially farmed seaweeds *Eucheuma denticulatum* and *Kappaphycus alvarezii* in Zanzibar Tanzania. Tanzan. Tanzania Journal of Natural & Applied Sciences 4(1):605-612.

Muawanah U., Syafitri N., Zulham A., Yanti B. V. I., Setyawidati N. A. R., 2021 Seaweed farming and seaweed capture fishermen phenomenon in Sebatik Strait, Nunukan Regency. IOP Conference Series: Earth and Environmental Science 869:012003.

Mulyani S., Tuwo A., Syamsuddin R., Jompa J., 2018 Effect of seaweed *Kappaphycus alvarezii* aquaculture on growth and survival of coral *Acropora muricata*. AACL Bioflux 11(6):1792-1798.

Najamuddin, Hajar M. A. I., Rustam, Palo M., Asni A., 2020 Development of integrated seaweed culture and capture fisheries in Indonesia. IOP Conference Series: Earth and Environmental Science 564:012027.

Nanda S., Kumar G., Hussain S., 2021 Utilization of seaweed-based biostimulants in improving plant and soil health: current updates and future prospective. International Journal of Environmental Science and Technology 19:12839-12852.

Nazaruddin N., Sugianto S., Rizal S., 2015 The feasibility of seaweed culture in the northern coast of Simeulue Island, Aceh Province, Indonesia. AACL Bioflux 8(5):824-831.

Nielsen C. W., Rustad T., Holdt S. L., 2021 Vitamin C from seaweed: A review assessing seaweed as contributor to daily intake. Foods 10(1):198.

Palmieri N., Forleo M. B., 2020 The potential of edible seaweed within the western diet. A segmentation of Italian consumers. International Journal of Gastronomy and Food Science 20:100202.

Pratama M. F. A., Nurhayati A., Rizal A., Suryana A. A. H., 2021 Strategy for the development of seaweed cultivation in Karawang Regency, West Java Province. Asian Journal of Fisheries and Aquatic Research 11:12-22.

Raja A., Vipin C., Aiyappan A., 2013 Biological importance of marine algae- An overview. International Journal of Current Microbiology and Applied Sciences 2(5):222-227.

Rama R., Aslan L. O. M., Iba W., Nurdin A. R., Armin A., Yusnaeni Y., 2018 Seaweed cultivation of micropropagated seaweed (*Kappaphycus alvarezii*) in Bungin Permai coastal waters, Tinanggea Sub-District, South Konawe Regency, South East Sulawesi. IOP Conference Series: Earth and Environmental Science 175:012219.

Ratnawati P., Simatupang N. F., Pong-Masak P. R., Paul N. A., Zuccarello G. C., 2020 Genetic diversity analysis of cultivated *Kappaphycus* in Indonesian seaweed farms using COI gene. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 15(2):65-72.

Rudke A. R., de Andrade C. J., Ferreira S. R. S., 2020 *Kappaphycus alvarezii* macroalgae: An unexplored and valuable biomass for green biorefinery conversion. Trends in Food Science & Technology 103:214-224.

Sulistiawati D., Ya’la Z. R., Jumiyatun, Mubaraq D. Z., 2020 Water quality study in several seaweeds culture sites in the post-earthquake-tsunami Palu Central, Sulawesi

Province. Journal of Physics: Conference Series 1434:012035.

Tuiyo R., 2016 [Cultivation of marine algae (*Kappaphycus alvarezii*) using Basmingro technology in plastic bags]. UNG Press, Gorontalo, 71 p. [In Indonesian].

Tuiyo R., Marsuci R., 2022 [Increasing catfish (*Clarias* sp.) seeds through spawning techniques using basmingro technology in Bua Village]. Devotion 1:17-20. [In Indonesian].

Valderrama D., Cai J., Hishamunda N., Ridler N., Neish I. C., Hurtado A. Q., Msuya F. E., Krishnan M., Narayanakumar R., Kronen M., Robledo D., Gasca-Leyva E., Fraga J., 2015 The economics of *Kappaphycus* seaweed cultivation in developing countries: A comparative analysis of farming systems. Aquaculture Economics & Management 19(2):251-277.

Veluchamy C., Palaniswamy R., 2020 A Review on marine algae and its applications. Asian Journal of Pharmaceutical and Clinical Research 13(3):21-27.

Yong Y. S., Yong W. T. L., Thien V. Y., Ng S. E., Anton A., Yassir S., 2015 Acclimatization of micropropagated *Kappaphycus alvarezii* (Doty) Doty ex Silva (Rhodophyta, Solieriaceae) in outdoor nursery system. Journal of Applied Phycology 27:413-419.

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