Achieving High Production of Micropropagated Seaweed through Optimization of the Culture Protocol

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Sea-based seaweed farming in Bubog, Guimaras, Philippines

Kappaphycus and *Eucheuma* are the two carrageenan-producing red algae extensively cultured and farmed in tropical and subtropical waters. Seaweed production accounted for 5.4 percent of the world's aquaculture production in 2019, which was over USD 15 billion in value. With this value share, the production of seaweed farming was higher than other aquatic commodities, such as tilapia, carp, shrimps, and others (Cai *et al*., 2021). The red algae production of the Philippines started in the 1960s (Ronquillo & Gabral-Llana, 1989), and by the early 2000s, the country had become the world's top supplier of seaweed, particularly *Kappaphycus* sp., until 2007, when Indonesia outperformed it.

Problems and challenges in seaweed farming, including climate change, pests and diseases, and biosecurity issues, caused the continuous decline of the seaweed production yield (Faisan, Sollesta-Pitogo, & de la Peña, 2022). Furthermore, the deterioration of seaweed quality caused by the repetitive use of vegetative cutting methods has also become one of the most pressing concerns in seaweed farming. Unfortunately, this procedure of growing plantlets for farming is one of the most common methods many seaweed farms used in the Philippines and other countries.

Research in SEAFDEC Aquaculture Department (AQD) looks into breaking from the conventional method of farming seaweed plantlets and optimizing laboratory-based production. With this, it hopes to create a more sustainable source of propagules, better growth and survival in grow-out, and a higher carrageenan quality in cultured *Kappaphycus alvarezii*.

Break from the 'conventional method'

Traditionally, seedlings for seaweed farming are collected through a method called "vegetative cutting." This method entails taking small pieces of the healthy thallus of seaweed for planting in a separate environment that is sustainable enough to support growth (Faisan, Sollesta-Pitogo, & de la Peña, 2022). Upon reaching marketable size, it is harvested by removing the entire plant or leaving small pieces to grow into a new batch (McHugh, 2003). Conventional production of seaweed seedlings has successfully propagated in-demand eucheumatoid red seaweeds such as *Kappaphycus* sp., a species with high carrageenan content that is widely produced in the Philippines. However, this heavy reliance on the conventional and unoptimized cultivation of seedlings will eventually have some disadvantages (Jiksing *et al*., 2022).

Seaweed is the top aquaculture commodity in the Philippines and the country is the fourth top seaweed-producers in the world. Most of the production that supplies this demand is cultured by marginal seaweed farmers (Luhan & Sollesta, 2010; Tahiluddin & Terzi, 2021). For years, local farmers have had growing concerns about the deteriorating production and quality of carrageenan in cultured seaweeds as well as the shortage of seedlings. This makes the seaweed they produced unattractive to the international and export market. Seaweed health problems, including disease and pest outbreaks in seaweed farms, caused annual production losses. However, one of the main factors affecting production is the most common practice of producing seedlings—using cuttings from existing crops as plantlets for subsequent harvests. Continuous repetition of this method could erode the genetic traits of seaweeds, reducing their vigor and making growth and survival slower and weaker. In addition, seaweeds now become more susceptible to diseases and pests as this method eventually wears out the good genetic traits. This calls for a new and optimized method of producing seaweed seedlings (Hayashi *et al*., 2010; Luhan & Sollesta, 2010).

To improve the quality of plantlets, micropropagation or in vitro clonal propagation was developed. The first attempt to cultivate seaweed explants under axenic conditions was conducted by Aharon Gibor from the University of California in the 1950s (Polne-Fuller, 1988). Since then, many studies have been conducted to better laboratory-based propagule production. Dawes and Koch (1991) demonstrated the first successful branch tissue culture of *Eucheuma denticulatum*, a red seaweed that thrived in sandy to rocky coastal areas, which were usually exposed to strong water currents. Hurtado and Biter (2007) used a small section of the seaweed for tissue culture and then grow-out farming. These include studies conducted within the facilities of the SEAFDEC Aquaculture Department (AQD), where spores were used to produce young seaweeds. Seaweeds from reproductive cells were grown in a laboratory setting and were subsequently cultured in grow-out farms (Luhan & Sollesta, 2010). Modern seaweed seedling production, such as micropropagation, is a more sustainable and scalable alternative to the conventional method, which can help yield larger production and bolster the livelihood of local producers. However, these methods are far from ideal. Further studies to improve the existing systems have been one of the ongoing researches in AQD.

The benefit of 'acclimation'

Currently, the method for micropropagation of seaweeds, particularly *Kappaphycus alvarezii*, lasts for 90 days in the laboratory and is followed by direct stocking in the field nurseries without acclimation. However, survival in the sea-based nursery using the aforementioned method is only about 30–50 %. Direct planting of micropropagules in the open sea has caused stress and shock to the seaweeds because of the sudden change in environmental conditions.

A study was conducted in AQD in 2020, to improve the existing micropropagation systems by modifying the protocols. The 90-day laboratory culture of micropropagules was shortened and replaced with tank culture to produce seedstock for sea-based nursery and out-planting purposes for commercial cultivation of carrageenophytes. This tank culture period served as the acclimation phase for the micropropagules. A study by Yong *et al*., (2015) stated that the growth of acclimatized micropropagules was significantly higher compared to the non-acclimatized ones and the presence of epiphytes was not noted when these were cultured in the field. Acclimation of tissue-cultured seedlings was also recommended to achieve higher survival growth (Jiksing *et al*., 2022).

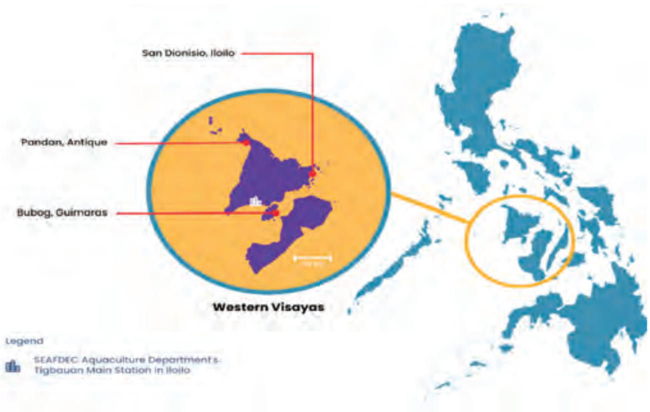

Figure 2. Collection sites of seaweed explants in Western Visayas, Philippines

The main objective of the study was to produce tank-acclimated seaweed propagules and see if the acclimation does favor better growth and survival when the seaweed plantlets are later transferred to the open sea. The seaweed explants (**Figure 1**) were collected from San Dionisio in Iloilo, Pandan in Antique, and Bubog in Guimaras (**Figure 2**). They were brought to AQD Tigbauan Main Station and stocked in rectangular concrete tanks in the seaweed hatchery (**Figure 3**). Conditioning of seaweed explants in tanks lasted for at least

Figure 1. Seaweed explants from a seaweed farm in Pandan, Antique, Philippines

Figure 3. Acclimation of seaweeds in tanks in SEAFDEC/AQD

seven days, and each tank was provided with flow-through seawater (0.5 L/min flow rate) and moderate aeration.

Once healthy explants were produced from the vegetative seaweeds, they were collected and cleaned before stocking them in culture bottles with UV-treated seawater and, later, in carboy containers for two months. After two months in the laboratory, propagules were transferred to a plastic net-covered tray and stocked in the concrete tanks. Tank-acclimation of propagules lasted for 30 days. The culture in the land-based tanks served as the acclimation area of propagules; hence, parameters such as salinity, temperature, and light intensity were closer to the natural condition.

Steady increase in micropropagule production

Improving and modifying the protocols for culturing micropropagated seaweeds led to a steady increase in production in AQD. The highest recorded yield was a total of 69,870 pieces of *K. alvarezii* propagules in 2022. This exceeded the production in the previous years, with 61,644 in 2020 and 62,751 in 2021. These are huge leaps compared to the production of 25,617 pieces in 2019 (**Figure 4**).

Moreover, the modification in stocking density and inclusion of an acclimation stage improved not only the yield but also the average survival rate, as it reached an 87 % average survival rate from 2020 to 2022. Over 100,000 micropropagated plantlets (**Figure 5**) can be produced from 5,000 explants collected from the wild for this optimized method. Moreover, the carrageenan quality of seaweeds from laboratory-produced plantlets after 45–60 days of culture in the field showed no significant difference when compared to the farmed ones in terms of gel strength, yield, and viscosity.

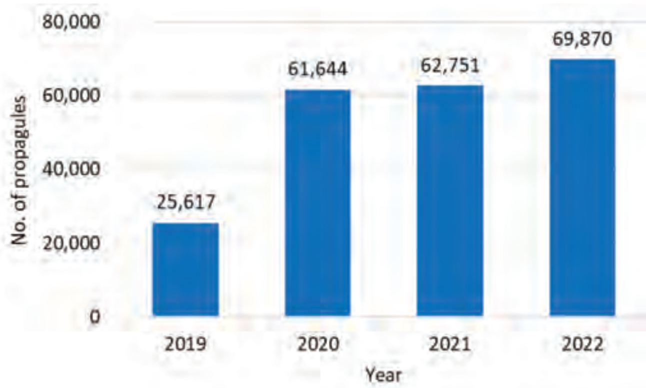

Figure 4. Increasing production of seaweed plantlets in AQD from 2019 to 2022 through modified culture protocols

Way Forward

Currently, the seaweed propagule production of AQD can only steadily supply the needs of the sea-based seaweed nursery. To increase the production of propagules in the land-based nursery, AQD is looking for an expansion of its land-based seaweed nursery operation. and aims to make healthy and high-quality seaweed plantlets available to growers in the Philippines. In addition, refining this optimized protocol in order to make it adaptable and transfer-ready to stakeholders through training courses and the production of manuals is also being targeted.

Figure 5. Seaweed micropropagules produced in AQD

References

Cai, J., Lovatelli, A., Aguilar-Manjarrez, J., Cornish, L., Dabbadie, L., Desrochers, A., Diffey, S., Garrido Gamarro, E., Geehan, J., Hurtado, A., Lucente, D., Mair, G., Miao, W., Potin, P., Przybyla, C., Reantaso, M., Roubach, R., Tauati, M. & Yuan, X. (2021). Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular No. 1229. Rome, FAO. https://doi.org/10.4060/cb5670en

Dawes C., & Koch, E. (1991). Branch, micropropagule and tissue culture of the red algae *Eucheuma denticulatum* and *Kappaphycus alvarezii* farmed in the Philippines. *J. Appl. Phycol., 3*(3), 247–257.

Faisan, Jr., J. P., Sollesta-Pitogo, H., & de la Peña, L.D. (2022). Issues and challenges in sustainable development of fisheries and aquaculture of Southeast Asian Region: Marine fishery resources: Seaweeds. In *The Southeast Asian State of Fisheries and Aquaculture 2022* (pp. 59–64). Secretariat, Southeast Asian Fisheries Development Center. http://hdl.handle.net/20.500.12066/6997

Hayashi, L., Hurtado, A. Q., Msuya, F. E., Bleicher-Lhonneur, G., Critchley, A. T. (2010). A review of *Kappaphycus* farming: prospects and constraints. In J. Seckbach, R. Einav, & A. Israel. (Eds.), *Seaweeds and their role in globally changing environments* (pp. 225–283). Cellular Origin, Life in Extreme Habitats and Astrobiology, Vol. 15. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-8569-6_15

Hurtado A., & Biter A. (2007) Plantlet regeneration of *Kappaphycus alvarezii* var. *adik-adik* by tissue culture. *J. Appl. Phycol., 19*(6), 783–786.

Jiksing, C., McMarshall, M. O., Thien, V. Y., Rodrigues, K. F., & Wilson, T. L. Y. (2022). Recent advances in seaweed seedling production: a review of eucheumatoids and other valuable seaweeds. *Algae 2022, 37*(2), 105–121. https://doi.org/10.4490/algae.2022.37.5.11

Luhan, M. R. J., & Sollesta, H. (2010). Growing the reproductive cells (carpospores) of the seaweed, *Kappaphycus striatum*, in the laboratory until outplanting in the field and maturation to tetrasporophyte. *J. Appl. Phycol.,* 22, 579–585. https://doi.org/10.1007/s10811-009-9497-7

McHugh, D. J. (2003). A guide to the seaweed industry (Fisheries Technical Paper No. 441). Food and Agriculture Organization of the United Nations.

Polne-Fuller, M. (1988). The past, present, and future, of tissue culture and biotechnology of seaweeds. In T. Stadler, J. Mollion , M-C. Verdus, Y. Karamanos, & M. H. Christiaen (Eds.), *Algal Biotechnology* (pp. 17–31). Elsevier.

Ronquillo, I. A., & M. E. Gabral-Llana. (1989). The Philippine Eucheuma seaweed industry. *Fishery Resources Journal, Philippines, 14*(1), 23–29.

Tahiluddin, A. B., & Terzi, E. (2021). An overview of fisheries and aquaculture in the Philippines. *J. Anatolian Env. and Anim. Sciences, 6*(4), 475-486. https://doi.org/10.35229/jaes.944292

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. *J Appl Phycol 27,* 413–419 (2015). https://doi.org/10.1007/s10811-014-0289-3

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