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Growth Performance and Pathological Responses of Tissue Culture and Local *Kappaphycus alvarezii* Seedlings in Marine Waters of Punti Village, Bima Bay, Indonesia

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ABSTRACT

Kappaphycus alvarezii is an economically important carrageenan-producing red alga, and tissue culture has been promoted as a means of improving seedling quality and field growth performance in Indonesian seaweed farming; however, field-based evidence comparing tissue-culture and conventional (local) seedlings under commercial cultivation conditions remains limited. This study therefore compared the growth performance and disease susceptibility of tissue-culture-derived and local *Kappaphycus alvarezii* seedlings cultivated using the longline method in the marine waters of Punti Village, Bima Bay, West Nusa Tenggara, Indonesia, over a 45-day period. Environmental conditions remained stable and within the tolerance range reported for the species, with temperature ranging from 27–30°C, salinity from 31–33 ppt, and pH from 7.5–8.1. Tissue-culture seedlings recorded a numerically higher biomass increase (70.1%) and specific growth rate (1.18% day^{-1}) than local seedlings (68.6% and 1.17% day^{-1}, respectively); however, independent-samples t-tests showed no statistically significant difference between the two seedling types ($p > 0.05$). In contrast, the seedling types differed markedly in disease response: tissue-culture seedlings exhibited pronounced susceptibility to ice-ice disease, marked by thallus structural damage and characteristic white discoloration, whereas local seedlings retained comparatively higher disease resistance. These results indicate that, under field conditions, propagation method had little influence on short-term growth but a substantial influence on disease susceptibility, suggesting that seedling resilience—rather than growth rate alone—should guide cultivation strategy in ice-ice-prone waters such as Bima Bay.

INTRODUCTION

Indonesia's marine ecosystem constitutes a globally significant natural resource, comprising a coastline of approximately 108,000 km and total marine waters spanning approximately 6.4 million km² (Coordinating Ministry for Maritime Affairs and Investment of the Republic of Indonesia, 2018). As the world's largest archipelagic country, Indonesia possesses substantial marine and fisheries resources that offer considerable potential for sustainable economic development, within which seaweed cultivation—particularly of *Kappaphycus alvarezii*—constitutes an important commodity of substantial economic and ecological value (Manurung *et al*., 2021). *K. alvarezii* is a red alga classified within the phylum Rhodophyta, class Florideophyceae, order Gigartinales, family Solieriaceae (Liao, 1996), and has attracted growing scientific and commercial interest owing to its notable biochemical properties. Indonesia is a leading global producer of seaweed, having produced 9.92 million tonnes in 2019, equivalent to 28.6% of total global cultured seaweed production (FAO, 2021, as cited in Sultana *et al*., 2023). The species is rich in bioactive compounds, including phenolic antioxidants with strong free-radical-scavenging (DPPH) activity and potential human-health benefits (Rajaram, Muralisankar, Paray, & Al-Sadoon, 2021), essential minerals, dietary fiber, and distinctive pigments such as phycoerythrin, carotenoids, and chlorophyll a (Suresh Kumar, Ganesan, & Subba Rao, 2015; Rajaram *et al*., 2021). It is also a major source of polysaccharides, particularly carrageenan—a hydrocolloid composed of potassium, sodium, magnesium, and sulfate ester derivatives (Estevez *et al.,* 2004; Rupert *et al*., 2022)—that confers anti-inflammatory, anticoagulant, antibacterial, and antiviral properties (Pradhan & Ki, 2023; Rupert *et al*., 2022), underscoring its potential for industrial and medical applications.

Nationally, the Indonesian Ministry of Marine Affairs and Fisheries estimates that potential land available for seaweed cultivation spans 12 million hectares across Indonesian waters, of which only about 0.8% has been utilized to date (Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, 2023). West Nusa Tenggara Province, particularly Bima Regency, offers considerable but largely unexploited potential for sustainable seaweed aquaculture: with a coastline of 687.43 km and marine waters covering 3,760.33 km² (Bima Regency Government, https://bimakab.go.id/halaman/33-karakteristik-wilayah), the region is well suited for marine macroalgae cultivation. A land suitability assessment by Nurlina *et al*. (2024) found that the coastal area of Bima Bay contains ±2,188.9 ha classified as highly suitable and ±2,512.69 ha classified as suitable for *K. alvarezii* cultivation, with an environmental carrying capacity supporting development across ±1,410.26 ha, equivalent to approximately 11,752 cultivation units.

Marine macroalgae cultivation offers a promising strategy for addressing food security, economic diversification, and ecosystem management, but technological constraints have historically limited its development. Recent advances in tissue culture techniques provide a promising means of overcoming these limitations, as demonstrated in several studies on seaweed propagation. Rachmawati & Abdillah (2019), for example, demonstrated the potential of tissue-culture-derived *Kappaphycus alvarezii* seedlings to improve growth performance, reporting an increase in specific growth rate of 0.67% day^{-1} (from 2.89% to 3.56%), accelerated biomass accumulation from 50–75 g to 200–225 g within a four-week cultivation cycle, and a reduction in cultivation duration from 40–45 days to 28–35 days under optimal conditions (temperature 28–30°C, salinity 30–33 ppt). Despite these promising results, tissue culture techniques remain largely confined to laboratory settings, leaving a gap between experimental findings and field-scale application; comprehensive field-based studies are therefore needed to validate and scale up these cultivation methods.

This study addresses this gap by evaluating the field growth performance of tissue-culture-derived and local *K. alvarezii* seedlings in the marine waters of Punti Village, Bima Bay. The findings are expected to provide empirical evidence to support the development of scalable seaweed cultivation strategies, with implications for both scientific understanding and practical marine resource management.

METHODS

Study Site and Experimental Design

The research was conducted from May to December 2018 in the marine waters of Bima Bay, Punti Village, Bima Regency, West Nusa Tenggara Province, Indonesia (Figure 1). A comparative experimental design was used to evaluate the growth performance of two *K. alvarezii* seedling types: tissue culture-derived and non-tissue culture (local) seedlings.

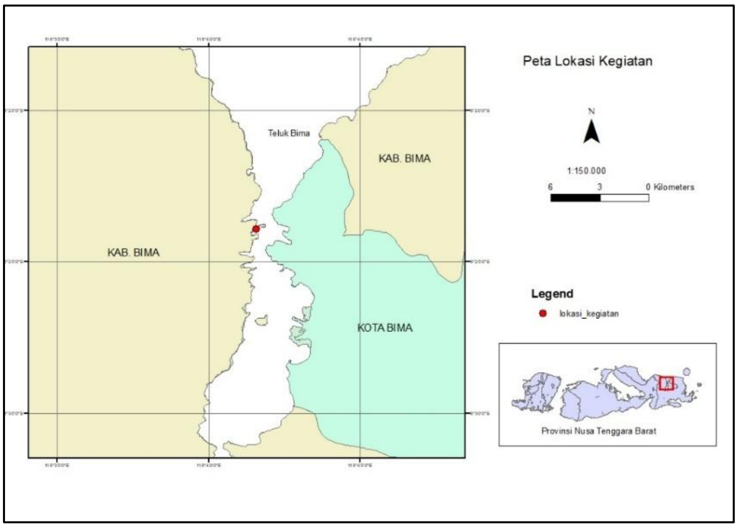

Figure 1. Research Location Map for Seaweed Cultivation Using Tissue Culture and Local Seedlings in the Waters of Punti Village, Soromandi District, Bima Regency, West Nusa Tenggara Province

Tissue culture-derived *K. alvarezii* seedlings were obtained from the Marine Aquaculture Center of Lombok, Gerupuk Installation, whereas local seedlings were sourced from community cultivation areas in Waworada Bay. The experiment followed a Completely Randomized Design (CRD) with 15 replicates per treatment, randomly allocated by lottery.

Seedling Procurement and Preparation

Tissue culture and local *K. alvarezii* seedlings were transported by land to the research site. Upon arrival, seedlings were placed in mesh bags and suspended on cultivation structures for 24 h of acclimatization. Seedlings were selected based on strict criteria, including multiple branching *thallus*, clean and bright coloration, and the absence of sliminess, breakage, foul odor, or disease (Figure 2).

Figure 2. Seedling Acclimatization After Land Transportation (A) and Seedling Selection Process (B)

Seedling Deployment

For each seedling type, 15 experimental units of 100 g biomass were prepared. *Thallus* segments were cut with a sharp knife and tied to 10-mm-diameter ropes at approximately 30-cm intervals. Each cluster was securely attached at strong *thallus* branch points to prevent detachment during cultivation. Recycled drinking-water bottles were attached to the ropes at 1.5-m intervals as floats.

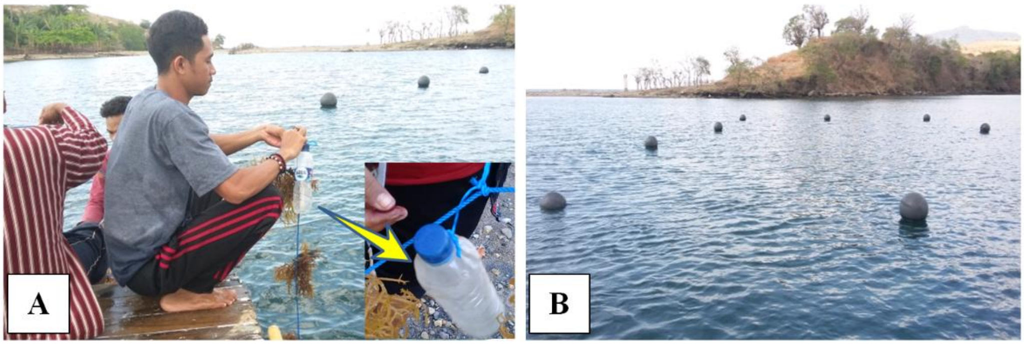

Figure 3. Field Conditions of *K. alvarezii* Cultivation Using Longline Method in Punti Bay Waters, West Nusa Tenggara, Indonesia. Notes: Flotation bottles (A) and longline system (B). University of Mataram

Seedlings were cultivated using the longline method in waters 7-10 m deep. Ropes bearing the seedlings were attached to a rectangular main longline measuring 10 m × 20 m, with 33-cm spacing between ropes (Figure 3). Seedlings were attached in shaded areas near the coastline to maintain humidity and prevent desiccation.

Maintenance and Monitoring

The cultivation period lasted 45 days. Water quality parameters (temperature, salinity, pH, and water transparency) were measured weekly, and each seedling cluster was visually inspected weekly to monitor its condition. Final biomass was recorded on day 45.

Growth Parameters Determination

Growth performance was evaluated using three parameters: absolute growth (Abs), relative growth rate (RGR), and specific growth rate (SGR), calculated following Effendie (1997) and Huisman (1976). The formulas for calculating growth parameters were as follows:

Absolute Growth (Abs) = $Wt - W0$ .... (1)

Relative Growth Rate (RGR) = $[(Wt - W0) / W0] \times 100\%$ .... (2)

Specific Growth Rate (SGR) = $\{[(Wt / W0)^{(1/t)}] - 1\} \times 100\%$ .... (3)

Where: Abs = Absolute growth (g); RGR = Relative growth rate (%); SGR = Specific growth rate (% per day); Wt = Final biomass of seedlings (g); W0 = Initial biomass of seedlings (g); t = Observation period (days)

Statistical Analysis

Growth data were analyzed using an independent-samples t-test to determine significant differences between treatments, at a significance level of $p < 0.05$. Water quality parameters were summarized using descriptive statistics.

RESULTS

Environmental Parameters

Environmental parameters are a key determinant of seaweed aquaculture productivity and sustainability. This section presents the physicochemical characteristics of the Bima Bay cultivation site and their implications for *K. alvarezii* cultivation.

Water temperature ranged from 27-30°C, typical of tropical marine waters. According to Kumar *et al*. (2020), this range represents an optimal thermal window for macroalgal growth, supporting metabolic activity and photosynthetic efficiency in *Kappaphycus alvarezii*. This is consistent with regional findings, where the highest growth rate of *K. alvarezii* in Vietnam was recorded at 25–28°C, and a higher growth rate was observed in India within a seawater temperature range of 26.0–28.0°C, collectively indicating that *Kappaphycus* cultivation performs best at 27–30°C in tropical and subtropical waters.

Salinity ranged from 31-33 ppt, indicating a stable marine environment suitable for *K. alvarezii* cultivation. This range falls within the physiological tolerance previously reported by Rachmawati & Abdillah (2019), and its narrow fluctuation reflects a stable environment favorable for seaweed biomass production and sustainable aquaculture.

The pH ranged from 7.5-8.1, indicating a slightly alkaline environment. Because pH governs the ionization of cellular metabolites and nutrient uptake, even small fluctuations can influence macroalgal metabolic processes and physiological responses (Falkowski & Raven, 2007).

Growth Performance and Pathological Observations of *Kappaphycus alvarezii*

- **Growth Performance**

Propagation method is a key factor influencing seaweed productivity and resilience. This study compared the growth performance and disease susceptibility of tissue culture and local *K. alvarezii* seedlings over a 45-day cultivation period in the waters of Punti Village, West Nusa Tenggara, Indonesia.

Tissue culture seedlings showed marginally higher growth than local seedlings, with a mean final biomass of 170.1 g/unit compared with 168.6 g/unit. This corresponded to a 70.1% biomass increase and a specific growth rate of 1.18% day^{-1} for tissue culture seedlings, versus 68.6% and 1.17% day^{-1} for local seedlings (Table 1).

Table 1. Growth Parameters of *K. alvarezii* Using Tissue Culture and Local Seedlings after 45 Days of Cultivation

Parameter	Tissue Culture Seedlings	Local Seedlings
Absolute Growth (g)	70.13 ± 14.47	68.60 ± 6.67
Relative Growth (%)	70.13 ± 14.47	68.60 ± 6.67
Specific Growth Rate (% day^{-1})	1.18 ± 0.19	1.17 ± 0.09

Note: Values are presented as mean ± standard deviation (SD); n = 15 replicates per treatment.

Statistical analysis using independent *t*-tests showed no statistically significant differences between tissue culture and local seedlings for any growth parameter, indicating comparable growth performance between the two seedling types under the same cultivation conditions.

- **Pathological Observations of *Kappaphycus alvarezii***

Ice-ice disease, a major challenge in tropical seaweed cultivation, was observed during the study. Symptoms were evident in tissue-cultured *K. alvarezii* seedlings, characterized by structural damage and characteristic white discoloration of the thallus (Figure 4; A, B, D). In contrast, local seedlings exhibited reddish-tinged thallus tips, suggesting relatively higher disease resistance under the study's environmental conditions (Figure 4; B).

The higher incidence of ice-ice disease in tissue-cultured *K. alvarezii* seedlings suggests greater sensitivity to environmental stress under field conditions. Previous studies indicate that macroalgal disease susceptibility is multifactorial: abrupt changes in salinity, water temperature, and light intensity contribute to pathogen susceptibility and stress responses in marine macroalgae (Imardjono *et al*., 1989; Hurtado & Agbayani, 2000). These interactions illustrate the complex role that environmental conditions play in seaweed health and disease progression. The differential disease response between tissue-cultured and local seedlings suggests that genetic adaptation and environmental acclimatization are important factors in seaweed disease resistance.

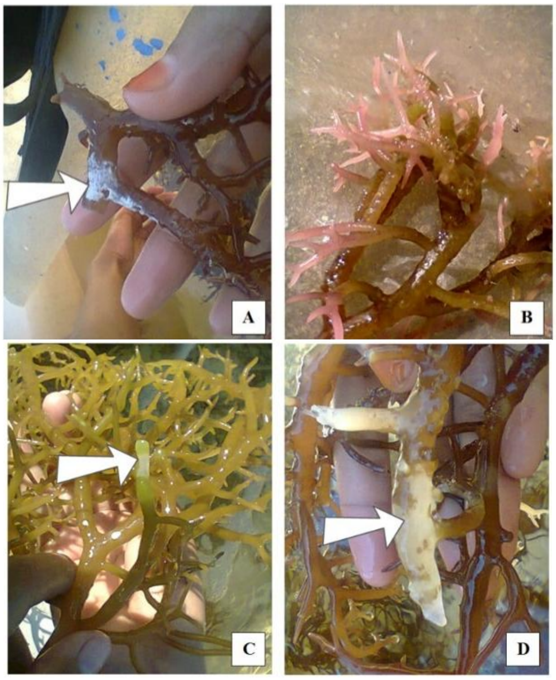

Figure 4. Symptoms of Ice-Ice Disease in Tissue Culture Seedlings (A, C, D) and Reddish Coloration at the Tip of Local Seedling Thallus (B)

DISCUSSION

Marine Environmental Parameters in Bima Bay: Implications for *Kappaphycus alvarezii* Cultivation

Environmental parameters are fundamental determinants of aquaculture productivity and ecosystem health. This study provides an integrated evaluation of the physicochemical conditions recorded at the Bima Bay cultivation site—temperature, salinity, and pH—and their relevance to *K. alvarezii* performance.

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The temperature recorded during cultivation (27–30°C) falls within the range generally considered favorable for tropical macroalgal growth, supporting photosynthetic efficiency and metabolic activity (Radiarta & Erlania, 2014). Kumar *et al*. (2020) reported that *K. alvarezii* becomes physiologically stressed once temperatures exceed approximately 33°C, with disrupted metabolic function and, at the extreme, irreversible cellular damage. Although the cultivation site remained below this threshold throughout the study, the narrow margin between the observed range and the upper thermal tolerance of the species underscores the importance of continuous temperature monitoring, particularly given the increasing frequency of marine heatwaves in tropical coastal waters.

Salinity (31–33 ppt) remained comparatively stable throughout the study, consistent with the tolerance range reported for *K. alvarezii* by Rachmawati & Abdillah (2019). Salinity governs osmoregulation and membrane transport processes in macroalgae (Hayashi *et al*., 2011), and its narrow fluctuation at Punti Village indicates a stable hydrodynamic environment—an important precondition for consistent thallus quality and carrageenan yield in tissue-culture-based cultivation systems (Yunque *et al*., 2011).

The pH range recorded (7.5–8.1) is slightly alkaline, consistent with typical open-water seawater chemistry (Menéndez *et al*., 2001; Harley *et al*., 2012). Because *K. alvarezii* relies primarily on bicarbonate ions as its photosynthetic carbon source (Wu *et al.,* 2008; Zou & Gao, 2009), pH governs the equilibrium between dissolved CO_2 and bicarbonate available for carbon fixation (Lavens & Sorgeloos, 1996; Roleda *et al*., 2012). The values recorded in this study lie within the tolerance window reported for the species, though Menéndez *et al.* (2001) note that photosynthetic rates can decline once pH rises above 8.5—a threshold not reached here, but one that remains relevant for long-term monitoring under localized ocean acidification or eutrophication-driven pH shifts (Cornwall *et al*., 2012).

Taken together, the physicochemical conditions at Punti Village fell within ranges generally supportive of *K. alvarezii* cultivation, providing a plausible environmental basis for the comparable growth performance recorded for both seedling types (Table 1).

Growth Performance of Tissue Culture and Local Seedlings

The growth performance recorded in this study—a 70.1% biomass increase and 1.18% day^{-1} specific growth rate for tissue-culture seedlings, versus 68.6% and 1.17% day^{-1} for local seedlings—did not differ statistically between seedling types ($p > 0.05$). This indicates that, under the cultivation conditions at Punti Village, propagation method had little detectable effect on growth over the 45-day trial.

These values are considerably lower than those reported in some previous studies. Rachmawati & Abdillah (2019), for example, recorded specific growth rates rising from 2.89% to 3.56% day^{-1} and biomass gains from 50–75 g to 200–225 g over a four-week cycle under controlled conditions (28–30°C, 30–33 ppt), while Satriani *et al*. (2022) and Ramadan *et al*. (2022) likewise reported higher growth rates for *K. alvarezii* cultivated with different longline spacing and genetic stock. The gap between these figures and the present findings likely reflects several factors rather than propagation method alone: differences in initial thallus quality and genetic source, greater depth and current exposure at Punti Village compared with more sheltered installations, and the absence of supplementary nutrient management in this study. Kumar *et al*. (2020) and Mandal *et al.* (2015) both note that nutrient availability and thermal regime can shift specific growth rates several-fold across sites, which is broadly consistent with the variability observed across studies here.

The absence of a significant growth difference between tissue-culture and local seedlings is itself a noteworthy field-scale finding. Tissue culture is typically promoted on the premise that laboratory-propagated seedlings carry a physiological advantage—faster initial growth, more uniform thallus morphology—that translates into superior field performance (Kasim *et al.,* 2021; Hanifah *et al*., 2025). The present results suggest that, at least under the conditions and 45-day timeframe examined here, this advantage was not realized, or was masked by site-level environmental variability. This is consistent with the broader understanding that macroalgal growth outcomes are jointly determined by genotype and environment, and that an advantage established under laboratory conditions does not automatically transfer to open-water cultivation (Lim *et al*., 2014; Kasim *et al.*, 2021; Simatupang *et al*., 2021).

From a practical standpoint, this finding is directly relevant to cultivators. Given the added cost and logistical complexity of sourcing tissue-culture seedlings from a centralized facility—in this case transported from Lombok to Bima—the marginal growth advantage recorded here may not, on its own, justify the additional investment unless disease resistance or other quality traits are also taken into account, an issue addressed directly in the following section.

Disease Susceptibility and Ice-Ice Syndrome

A more consequential difference between the two seedling types concerned disease response rather than growth. Tissue-culture seedlings showed clear signs of ice-ice disease—thallus whitening and structural degradation—whereas local seedlings largely retained a healthy, reddish-tinged thallus tip, suggesting comparatively greater resistance under the same field conditions.

Ice-ice disease is widely recognized as one of the principal constraints on *Kappaphycus* cultivation in Southeast Asia, with impacts ranging from reduced thallus quality to substantial yield loss on severely affected farms (Ward *et al.*, 2020). The condition is generally understood as a stress-mediated syndrome rather than a single-pathogen disease: abrupt shifts in temperature, salinity, or light intensity are thought to compromise thallus integrity and create conditions favorable for opportunistic bacterial colonization, which then accelerates tissue degradation (Azizi *et al*., 2018; Kumar *et al.,* 2020; Ward *et al*., 2020; Riyaz *et al*., 2021; Li *et al*., 2026).

The differential response observed here—greater susceptibility in tissue-culture seedlings, greater apparent resistance in local seedlings—is consistent with the general principle that laboratory propagation, while effective for producing clean, disease-free planting material, does not necessarily confer field-level stress tolerance. Local seedlings, by contrast, have typically undergone repeated cycles of field selection, in which individuals that survive recurring environmental stress and disease pressure are propagated onward, acting as an informal selection process for hardier stock (Paula *et al*., 1999; Parenrengi *et al*., 2020; Narvarte *et al*., 2022). If this interpretation holds, seedling resilience under field conditions may depend as much on propagation history and prior stress exposure as on genetic identity per se—a hypothesis that would benefit from direct testing through common-garden or reciprocal-transplant trials, and from comparative screening of wound-healing capacity between tissue-culture and field-conditioned seedlings.

This finding also carries direct implications for cultivation strategy. Where disease pressure is a known constraint, as observed at Punti Village, the modest growth advantage offered by tissue-culture seedlings may be offset by their greater disease susceptibility, particularly over cultivation periods extending into more stress-prone parts of the year. Combining locally acclimatized planting stock with periodic reintroduction of tissue-culture material selected for stress tolerance may therefore be a more robust strategy than reliance on either source alone.

Study Scope and Directions for Future Research

This study's environmental characterization was limited to three parameters—temperature, salinity, and pH—measured weekly. While these are the parameters most consistently linked to *K. alvarezii* growth and disease response in the literature, they do not capture the full range of factors known to influence macroalgal health, including dissolved nutrient concentrations (particularly nitrogen and phosphorus), dissolved oxygen, water clarity and light penetration, current speed, and the composition of the surrounding microbial community.

Future studies at Punti Village and comparable sites would benefit from pairing growth and disease monitoring with (i) nutrient and dissolved-oxygen profiling across the cultivation depth, (ii) microbiological sampling of both healthy and diseased thalli to characterize the bacterial communities associated with ice-ice onset, and (iii) genetic or physiological screening of local versus tissue-culture stock to test directly whether disease resistance is heritable or condition-dependent. Extending the cultivation period beyond 45 days would also help determine whether the growth parity observed here persists, narrows, or reverses over a full commercial production cycle.

CONCLUSION

Over 45 days of cultivation at Punti Village, tissue-culture and local K. alvarezii seedlings showed statistically comparable growth performance (70.1% vs. 68.6% biomass increase; 1.18% vs. 1.17% day^{-1} specific growth rate; $p > 0.05$), under environmental conditions that remained within the tolerance range reported for the species. However, the two seedling types diverged sharply in disease resistance: tissue-culture seedlings were susceptible to ice-ice disease, whereas local seedlings remained largely healthy. These findings indicate that the practical value of propagation method lies in field resilience rather than growth speed—making disease susceptibility, not growth rate, the more decisive consideration. As this conclusion is based on a single site, a single 45-day cycle, and three environmental parameters, further validation across seasons, cultivation cycles, and other sites in Bima Bay is needed before the findings are applied to routine cultivation practice. Based on this study, several recommendations can be made:

1. For cultivators at Punti Village and comparable sites in Bima Bay, prioritize locally sourced, field-acclimatized seedlings where disease pressure is a known constraint, given their greater resistance to ice-ice disease observed in this study; reserve tissue-culture seedlings for conditions or seasons where disease risk is lower, or pair them with pre-conditioning steps intended to improve field stress tolerance.
2. Extend future trials beyond the season used in this study to encompass a full commercial production cycle and multiple seasons, in order to determine whether the growth parity and the disease-susceptibility gap between the two seedling types persist, narrow, or reverse over time.
3. Broaden environmental monitoring beyond temperature, salinity, and pH to include dissolved nutrient concentrations, dissolved oxygen, water clarity, current speed, and microbial community composition, in order to identify the specific environmental triggers of ice-ice onset at this site.

4. Conduct comparative genetic and physiological screening—for example, common-garden or reciprocal-transplant trials—between tissue-culture and local seedlings to test directly whether the observed difference in disease resistance is heritable or is instead a function of prior field conditioning.
5. Local aquaculture extension services and the Bima Regency fisheries authority should factor the disease-resistance findings, and not growth rate alone, into guidance given to smallholder farmers on seedling sourcing.
6. Consider a combined cultivation strategy that relies predominantly on locally acclimatized seedlings for resilience, while periodically reintroducing tissue-culture stock to maintain genetic diversity and seedling quality, rather than depending exclusively on either source.

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