Photosynthetic Efficiency and Survival of Tissue-Cultured *Eucheuma denticulatum* Microplantlets under Varying Transport Conditions

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ABSTRACT

The cultivation of eucheumatoids (*Eucheuma and Kappaphycus*) is increasingly threatened by pests, diseases, climate change, and biosecurity concerns, leading to declining yields. Tissue culture offers a more resilient propagation approach; however, the success of outplanting depends on minimizing stress and mortality during the transfer of cultivars. This study evaluated the effects of different transport treatments on the photosystem II (PSII) photochemical efficiency and survival of tissue-cultured *Eucheuma denticulatum* microplantlets. An 8-h transport simulation followed by a 7-day laboratory culture was conducted to evaluate the effectiveness of various packaging and storage conditions. After 8 h, the maximum quantum yields (Fv/Fm) of transport treatments 1 (0.53±0.08) and 2 (0.51±0.11), in which explants were placed in plastic bags containing 250 mL of seawater stored in a styrofoam box without and with ice, respectively, did not significantly differ from their initial values (0.58±0.11 and 0.54±0.08, respectively). All explants in these treatments remained viable after 7 days. In contrast, treatments 3 (0.33±0.11) and 4 (0.44±0.09), where explants were wrapped in moistened muslin cloths and stored in a styrofoam box without and with ice, respectively, showed significantly lower Fv/Fm values compared with their initial states (0.64±0.09 and 0.63±0.11, respectively), with treatment 3 resulting in complete die-off (0.01±0.06) after 7 days. These findings suggest that transporting microplantlets in seawater-filled plastic bags, especially with ice insulation, is the most effective method for maintaining photochemical efficiency and viability during transport.

Keywords: Eucheumatoids, Live transport, Pulse amplitude modulation (PAM) fluorometry, Storage, Transport stress

INTRODUCTION

Seaweeds significantly contribute to the Philippine economy, ranking first among fishery products in 2023 with an estimated production of 1.63 million metric tons (wet weight) (Department of Agriculture Bureau of Fisheries and Aquatic Resources, DA-BFAR, 2024). The industry is dominated by eucheumatoids (*Kappaphycus* and *Eucheuma*), which are valued not only as food but more importantly as raw materials for carrageenan extraction. Although the Philippines pioneered eucheumatoid farming in the 1960s (Hurtado *et al*., 2015; Valderrama *et al*., 2013; DA-BFAR, 2022) and once led global production, output has since declined due to pest and epiphyte infestations, ice-ice syndrome, climate change, and biosecurity threats (Ward *et al*., 2020; 2022; Kambey *et al*., 2021).

To address declining productivity, tissue culture and micropropagation techniques are being adopted to produce high-quality seedstocks with desirable traits such as rapid growth and resistance to epiphytes and diseases (Reddy *et al*., 2008; Jiksing *et al*., 2022). However, the success of these efforts depends heavily on effective transport of tissue-cultured explants from laboratories to nurseries and farms.

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Current seaweed transport practices, such as using ice and seawater (Yong *et al*., 2013; Ali *et al*., 2020; Ciaramella, 2022) or wrapping thalli in seawater-moistened paper towels (Dawes and Koch, 1991; Borlongan *et al*., 2016; Hinaloc and Roleda, 2021; Gonzaga *et al*., 2025), are mostly empirical, and their physiological effects on seed stock viability remain poorly understood. For tissue-cultured microplantlets, common methods include placing explants in seawater-filled plastic bags or polyethylene terephthalate (PET) bottles stored in styrofoam boxes, with or without ice. However, the impact of these transport conditions on seedstock viability has not been thoroughly evaluated. Recent studies evaluated shipment stress in cultivated seaweed juveniles using chlorophyll fluorescence as an indicator of physiological integrity. Sato *et al*. (2022) demonstrated that *Undaria pinnatifida* sporelings maintained photosystem II (PSII) efficiency under saturated humidity for up to 72 h at low temperatures, whereas *Caulerpa chemnitzia* var. *laetevirens* exhibited irreversible declines in *PSII* efficiency at 4 °C but remained viable under room-temperature, high-humidity conditions (Terada *et al*., 2024). These findings highlight the potential of PAM-chlorophyll fluorometry for optimizing transport environments for seaweed seedstock.

This study therefore evaluates the physiological responses of *Eucheuma denticulatum* microplantlets to four transport strategies to identify the most effective approach for maintaining photosynthetic integrity and post-transport viability. The results are expected to contribute practical insights toward the development of optimized, field-adaptable transport protocols that support seedstock resilience in sea-based nurseries and grow-out farms.

MATERIALS AND METHODS

Preparation of experimental samples

Tissue-cultured *Eucheuma denticulatum* microplantlets used in this study were derived from apical segments of farmed thalli exhibiting the green color morphotype, collected from a seaweed farm in San Dionisio, Iloilo. The culture protocols were adapted from Hurtado *et al*. (2009) and Tibubos *et al*. (2017). Explants were surface-cleaned, acclimated, and cultured in UV-filtered seawater supplemented with seaweed extract and plant growth regulators. Microplantlets were maintained for approximately six months under controlled conditions in the walk-in culture room of the Seaweed Laboratory, University of the Philippines Visayas, at a temperature of 23–24 °C, salinity of 30±3 psu, incident irradiance of 110±5 µmol photons·m^{-2}·s^{-1} from LED tubes, a 13 h light:11 h dark photoperiod, and continuous moderate aeration. Healthy individuals exhibiting active growth and normal pigmentation were selected for transport trials.

A total of 300 microplantlets (approximately 2 cm in length) were used in the transport simulation experiment and distributed across four transport treatments. Photosystem II (PSII) photochemical efficiency was monitored during and after transport, and survival rates were assessed after a 7-day laboratory culture period.

Transport simulation experiment

Four transport treatments were designed to simulate live-seaweed transport under typical field conditions. In treatment 1 (T1) microplantlets were placed in clear polyethylene bags (approximately 10×30 cm) containing 250 mL UV-filtered seawater, stored in a styrofoam box without ice. Treatment 2 (T2) followed the same procedure as T1, but the styrofoam box contained ice packs to provide cooling. In treatment 3 (T3), microplantlets were wrapped in muslin cloth moistened with UV-filtered seawater and stored in a styrofoam box without ice, whereas treatment 4 (T4) was identical to T3 except that ice packs were added to maintain a lower temperature during transport.

Each treatment had three replicates, with one styrofoam box (31.5×23.5×33.5 cm) serving as a replicate and containing 25 microplantlets. For T1 and T2, a stocking density of one microplantlet per 10 mL of seawater was maintained. For T2 and T4, each styrofoam box contained four ice packs (500 mL of frozen UV-filtered seawater per pack), placed at the bottom and separated from the samples by ten layers of paper to prevent direct contact.

In total, 12 styrofoam boxes were exposed to ambient outdoor temperature and irradiance to simulate field transport conditions. PSII photochemical efficiency (maximum quantum yield, Fv/Fm) was measured at designated time points between 9:00 a.m. to 5:00 p.m. to capture treatment responses while minimizing diurnal variation.

Chronological changes in maximum quantum yield (Fv/Fm)

Fv/Fm was measured using a Junior PAM fluorometer (Heinz Walz, Germany) throughout the 8-h transport simulation. At the start of the experiment, 25 microplantlets were randomly assigned to each replicate, and Fv/Fm values of 10 randomly selected individuals per replicate (n = 30 per treatment) were recorded as the initial values. Before measurement, samples were dark-acclimated in a light-proof chamber for 10–15 min to ensure PSII reaction centers were fully relaxed. Fv/Fm was subsequently measured every 2 h during the 8-h transport period. The temperature inside and outside each styrofoam box was also recorded at 2-h intervals using a digital thermometer.

Following the transport simulation, samples were transferred to 250-mL flasks containing UV-filtered seawater and incubated under the same laboratory conditions. Fv/Fm values (n = 30 per treatment) were measured again after 16 h to assess PSII recovery.

Survival assessment

Post-transport recovery was assessed by monitoring survival over a 7-day laboratory culture period. Survival rates (%) were determined by counting viable microplantlets in each replicate. To assess physiological recovery, Fv/Fm values were measured again after 7 days. For each treatment, 10 microplantlets per replicate (n = 30 per treatment) were randomly selected for analysis.

Statistical analyses

Fv/Fm and survival data were tested for normality using the Shapiro-Wilk test, and for homogeneity of variances using Levene's test. When assumptions were met, a one-way ANOVA followed by Tukey's HSD test was applied to compare Fv/Fm across different time points: the initial measurement (0 h), post-transport (8 h), and subsequent assessments after 16 h and 7 days of laboratory culture. Survival rates were analyzed using the same procedure. When data did not meet the assumption of normality, the non-parametric Kruskal-Wallis test was used, followed by Dunn's test for pairwise comparisons. All values are presented as mean±standard deviation (SD), and all analyses were performed using R version 4.4.1 (R Core Team, 2023).

RESULTS

PSII photochemical efficiency (Fv/Fm) of Eucheuma denticulatum microplantlets across transport treatments

The maximum quantum yield (Fv/Fm) of *E. denticulatum* microplantlets varied across transport treatments over time (Figure 1, Table 1). In T1 (seawater without ice), Fv/Fm declined slightly from 0.58±0.10 to 0.53±0.08 over 8 h, further decreasing to 0.48±0.05 after 16 h, but recovering to 0.57±0.06 after seven days (Figure 1a). T2 (seawater with ice) showed a gradual decline from 0.54±0.08 to 0.51±0.11 during transport, followed by a marked recovery to 0.61±0.08 after seven days (Figure 1b). In contrast, T3 (moistened cloth without ice) exhibited the most pronounced decline, with Fv/Fm dropping from 0.64±0.09 to 0.33±0.11 over 8 h, remaining low at 0.25±0.08 after 16 h, and decreasing further to 0.01±0.06 after seven days (Figure 1c). All replicates in T3 showed depigmentation and whitening, indicative of ice-ice syndrome (Figure 2). T4 (moistened cloth with ice) also showed a significant decline from 0.63±0.11 to 0.44±0.09 during transport, with only minimal recovery to 0.49±0.08 after seven days (Figure 1d). Among all treatments, T2 demonstrated the highest post-transport recovery, whereas T3 exhibited the most severe physiological stress.

Temperature profiles inside styrofoam boxes

The transport experiment was conducted on 20 May 2024, in Miagao, Iloilo, under a heat index of 42–43 °C (Philippine Atmospheric, Geophysical

and Astronomical Services Administration, PAGASA 2024). Ambient air temperatures ranged from 34.3 °C at 9:00 a.m., peaked at 48.2–48.7 °C between 11 a.m. and 1 p.m., and declined to 33.5–37.2 °C by 3–5 p.m.

Transport treatments without ice (T1 and T3) recorded higher internal temperatures, averaging 32.6–34.5 °C throughout the day, whereas ice-treated setups (T2 and T4) maintained significantly lower temperatures ($p<0.001$), ranging from 24.6–25.3 °C (Figure 3). The highest internal temperatures were recorded between 11 a.m. and 3 p.m., with T3 reaching 35.8–39.3 °C and T1 at 34.0–35.2 °C. In contrast, T2 ranged from 26.2–30.0 °C, while T4 remained within 24.7–28.8 °C.

Survival rates after seven days of culture

Survival rates of *E. denticulatum* microplantlets differed significantly among treatments (Figure 4). T3 (moistened cloth without ice) resulted in 0% survival, with all microplantlets exhibiting thallus whitening and softening, symptoms characteristic of ice-ice syndrome. This was consistent with their near-zero Fv/Fm value (0.01±0.06). In contrast, T1 and T2 (seawater without and with ice, respectively) achieved 100% survival, with explants remaining healthy throughout the experiment. T4 (moistened cloth with ice) showed a survival rate of 49.3±41.1%, with some individuals displaying visible signs of stress. Statistically, survival in T1 and T2 was significantly higher than in T3 ($p<0.05$), while no significant differences were observed among T1 and T2, or between T3 and T4.

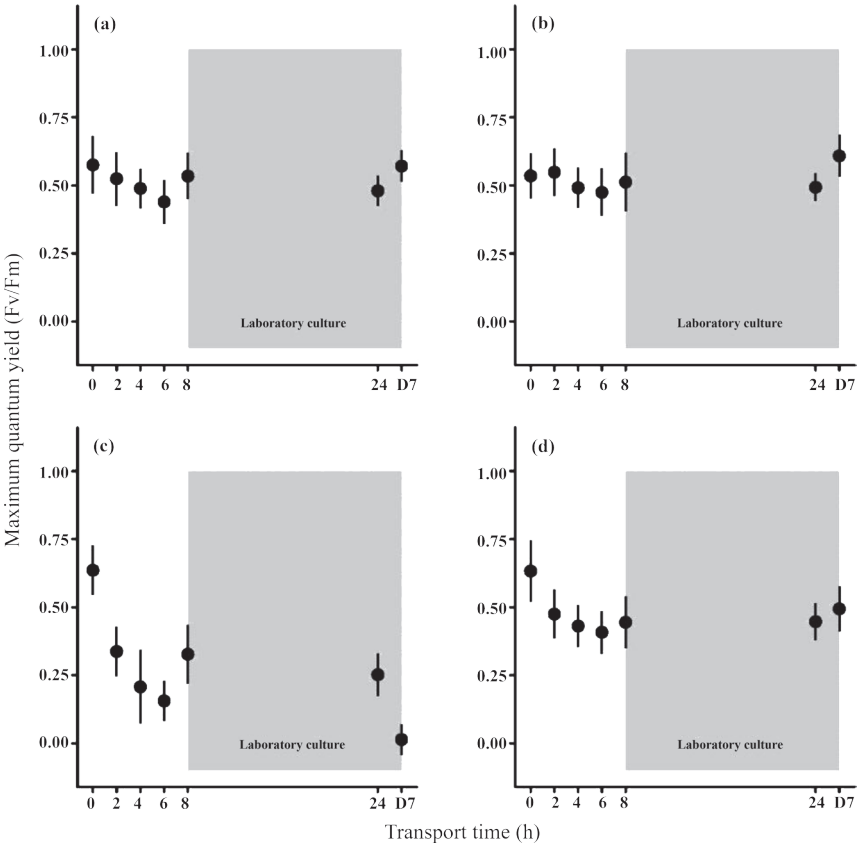

Figure 1. Chronological changes in the maximum quantum yield (Fv/Fm) of *Eucheuma denticulatum* microplantlets during the 8-h transport simulation and subsequent 7-day laboratory culture. Treatments: (a) seawater without ice, (b) seawater with ice, (c) moistened cloth without ice, and (d) moistened cloth with ice. Symbols represent mean values measured (n = 30); error bars indicate standard deviation (SD). Shaded regions denote the 7-day laboratory culture phase.

Table 1. Mean maximum quantum yields (Fv/Fm, mean±SD) over time for *Eucheuma denticulatum* microplantlets under different transport conditions. Superscript letters indicate statistical groupings based on Tukey's HSD test, with significant pairwise differences ($p<0.05$) observed across designated time points within each treatment.

Treatment	Time	Mean±SD
Seawater without ice	0 h	0.58±0.10[a]
	8 h	0.53±0.08[a]
	24 h	0.48±0.05[b]
	D7	0.57±0.06[a]
Seawater with ice	0 h	0.53±0.08[b]
	8 h	0.51±0.10[b]
	24 h	0.49±0.05[b]
	D7	0.61±0.08[a]
Moistened cloth without ice	0 h	0.64±0.09[a]
	8 h	0.33±0.11[b]
	24 h	0.25±0.08[c]
	D7	0.01±0.06[d]
Moistened cloth with ice	0 h	0.63±0.11[a]
	8 h	0.44±0.09[b]
	24 h	0.45±0.07[b]
	D7	0.49±0.08[b]

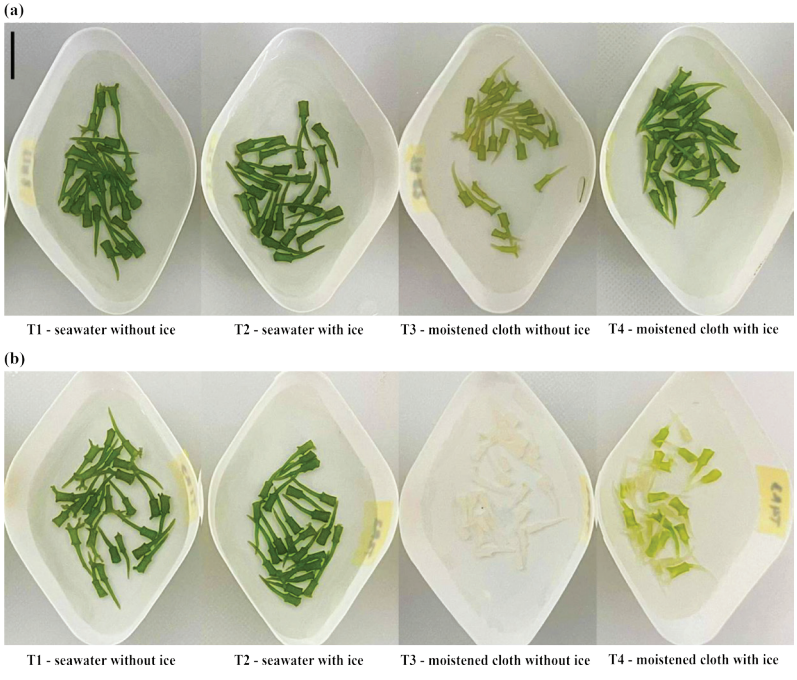

Figure 2. Representative *Eucheuma denticulatum* microplantlets subjected to different transport treatments, observed after (a) 16 h and (b) 7 days of laboratory culture, following an 8-h simulated transport experiment. Scale bar = 2.0 cm.

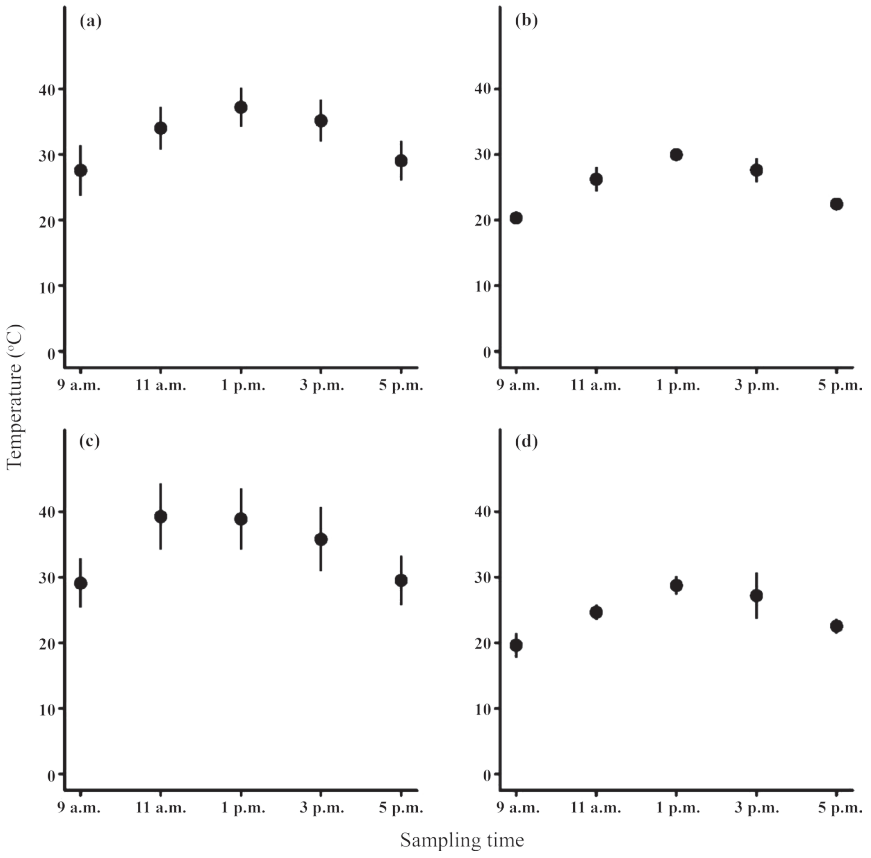

Figure 3. Temperature changes inside the styrofoam boxes across transport treatments during the 8-h simulation. Treatments: (a) seawater without ice, (b) seawater with ice, (c) moistened cloth without ice, and (d) moistened cloth with ice. Symbols represent mean values (n = 3); error bars indicate standard deviation (SD).

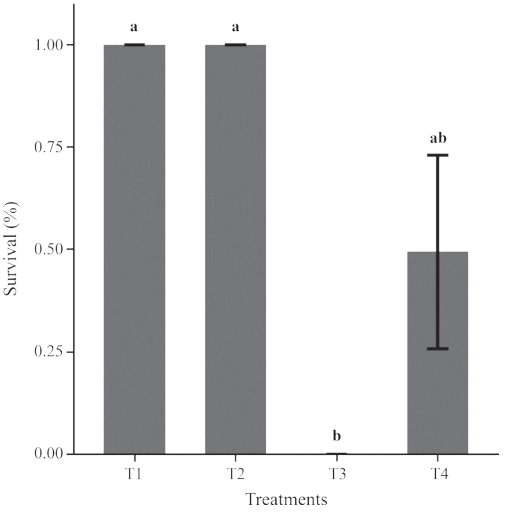

Figure 4. Survival rates (%, mean±standard deviation, SD) of *Eucheuma denticulatum* microplantlets after 7 days of laboratory culture following an 8-h transport simulation. Treatments: T1 = seawater without ice, T2 = seawater with ice, T3 = moistened cloth without ice, and T4 = moistened cloth with ice. Different letters above bars indicate statistically significant differences among treatments ($p<0.05$) and error bars represent ±SD.

DISCUSSION

Optimizing transport strategies is essential for maintaining the physiological integrity of tissue-cultured seaweeds and minimizing stress-induced mortality during transfer to sea-based nurseries. Effective handling and temperature-moisture regulation during transit are critical for preserving microplantlet viability and supporting successful acclimation under field conditions.

This study demonstrated that transport conditions significantly affected the photosynthetic performance and survival of *E. denticulatum* microplantlets. Treatments using clear plastic bags with UV-filtered seawater (T1 and T2) provided the best physiological stability, as indicated by stable Fv/Fm values and complete recovery after seven days. Although T1 (seawater without ice) exhibited a delayed Fv/Fm recovery, survival remained unaffected, suggesting that seawater provided a buffering effect against dehydration and osmotic stress. Continuous hydration and ionic support from seawater likely helped maintain cellular water potent and enzyme stability, minimizing physiological disruption during exposure to ambient heat (Eggert, 2012; Karsten, 2012). The superior performance of T2 (seawater with ice) further highlights the role of cooling in maintaining PSII function, possibly through reduced metabolic demand and slower pigment degradation.

In contrast, muslin cloth-based treatments (T3 and T4) exhibited marked declines in Fv/Fm, with T3 (moistened cloth without ice) resulting in complete mortality. The irreversible loss of PSII efficiency in these treatments likely stemmed from dehydration stress during prolonged emersion, which can damage cellular structures and impair photosynthetic machinery. Dehydration is known to induce oxidative stress and inhibit PSII activity, with the severity of these effects depending on species-specific desiccation tolerance (Flores-Molina *et al*., 2014; Shindo *et al*., 2022). T3 also experienced extreme temperatures (up to 39.3 °C), which likely exceeded the thermal tolerance of *E. denticulatum*, inhibiting enzymatic processes essential for photosynthesis (Salvucci and Crafts-Brandner, 2004; Allakhverdiev *et al*., 2008).

Although muslin cloth initially retained moisture, it dried out over the 8-h period, reducing water availability. Similar results were obtained in the brown alga *Saccharina japonica*, which maintained ΔF/Fm during short-term emersion but exhibited irreversible declines with prolonged exposure (Shindo *et al*., 2022). Cellular dehydration is known to alter membrane structures and increase intracellular electrolyte concentrations, disrupting electron transport between PSI and PSII (Gao *et al*., 2011; Hurd *et al*., 2014). The physiological decline observed in T3 and T4 supports these findings and underscores the vulnerability of *E. denticulatum* microplantlets to emersion stress. Measuring algal tissue moisture content would help quantify dehydration effects in future studies.

T4 (moistened cloth with ice) maintained lower temperatures (24.7–28.8 °C), yet Fv/Fm still declined, suggesting that dehydration, rather than heat, was the dominant stressor. The reduced survival in T4 (49.3±41.1%) further supports the conclusion that thermal regulation alone is insufficient without adequate moisture retention. The combination of dehydration and thermal stress in T3 likely triggered oxidative damage and ice-ice symptoms, resulting in complete loss of viability. Heat stress disrupts PSII by denaturing proteins, destabilizing thylakoid membranes and impairing D1 protein synthesis, leading to photoinhibition and reduced oxygen evolution (Borlongan *et al*., 2017; Kumar *et al*., 2020).

Photosynthetic impairment under stress is also linked to disrupted chlorophyll biosynthesis and pigment degradation, leading to thallus whitening (Dutta *et al*., 2009). *E. denticulatum* responds to environmental stress by producing halogenated organic compounds, notably bromoform and diiodomethane, which generate hydrogen peroxide and exacerbate oxidative stress (Mtolera *et al*., 1996). Elevated temperatures destabilize PSII, accelerate carotenoid breakdown, and reduce chlorophyll-*a* levels, further compromising photosynthetic efficiency (Eggert, 2012; Wernberg *et al*., 2016; Eismann *et al*., 2020; Zuo *et al*., 2023). Temperatures above 33 °C have been shown to reduce growth, induce pigment loss, and trigger ice-ice syndrome in *E. denticulatum* (Ganzon-

Fortes *et al*., 1993; Largo *et al*., 1995). Borlongan *et al*. (2016) reported that thermal inhibition in this species begins beyond 31 °C, marked by declining Fv/Fm and gross photosynthesis.

Temperature fluctuations recorded during the experiment aligned with observed physiological responses. T3 experienced the highest internal temperatures (35.8–39.3 °C), contributing to irreversible damage. T1 also reached elevated temperatures (34.0–35.2 °C), but survival remained unaffected, likely due to the buffering capacity of seawater, which helped maintain osmotic balance and enzymatic function. Treatments with ice (T2 and T4) maintained temperatures within the optimal range (26.2–30.0 °C), which helped preserve PSII efficiency and prevent enzymatic inhibition (Doğru, 2021).

CONCLUSIONS

Overall, this study highlights the importance of integrating thermal regulation and moisture retention into transport protocols for tissue-cultured seaweeds. Packaging microplantlets in seawater-filled plastic bags, particularly with ice insulation (T2), proved to be the most effective strategy for preserving physiological integrity, as evidenced by stable PSII photochemical efficiency and 100% survival. In contrast, the use of moistened muslin cloth without ice (T3) resulted in the poorest outcomes, primarily due to elevated temperatures and dehydration stress.

These findings emphasize the need to ensure both temperature stabilization and sustained hydration during transport to minimize stress-induced impairment. Future studies should include real-time monitoring of tissue moisture content to better correlate Fv/Fm changes with absolute water content (AWC), providing a more precise assessment of dehydration stress. Exploring alternative wrapping materials with improved water retention capacity may further enhance the viability of cloth-based transport methods. Optimizing transport strategies based on physiological response trends will improve seedstock quality and support the long-term sustainability of seaweed farming reliant on tissue-cultured planting materials.

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