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# Effects of IAA and kin on somatic embryogenesis and the effect of acadian marine plant extract powder content on micropropagule regeneration of the seaweed *Kappaphycus striatus*

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## ABSTRACT

The objective of this study was to perfect the callus culture method for propagating *Kappaphycus striatus.* Our research focuses on enhancing somatic embryo (SEs) induction from the callus and micropropagule regeneration in SEs. In order to enhance the induction rate of SEs, the 16-week-old callus was transplanted into a semi-solidified PES (0.4% agar) medium supplemented with 0.5–3.0 mg.L$^{-1}$ 3-indoleacetic acid (IAA) and 0.5–3.0 mg.L$^{-1}$ kinetin (KIN), either alone or in combination. The results showed that the transplantation of the callus into a PES medium containing IAA and KIN (0.5–3.0 mg.L$^{-1}$), both individually and in combination, significantly impacted the somatic embryogenesis of *K. striatus*. After 8 weeks of cultivation, the treatment with 1 mg.L$^{-1}$ IAA + 2 mg.L$^{-1}$ KIN exhibited the highest embryo induction rate (62.22 ± 5.09%) and the greatest number of SEs (182.00 ± 10.58 embryos/explant) compared to other treatments. Moreover, during the micropropagule regeneration stage, the commercial powdered extract of *Ascophyllum nodosum* (Acadian Marine Plant Extract Powder, AMPEP) at concentrations ranging from 5 ppm to 50 ppm was found to enhance the rate of micropropagule regeneration from SEs of *K. striatus*. The optimal regeneration of SEs of *K. striatus* into complete micropropagules occurred in an AMPEP medium at a concentration of 20 ppm. Micropropagules cultured in this medium displayed the highest survival rate (75.6 ± 5.09%), number of branches (7.67 ± 0.58 branches/micropropagule), branch length (24.7 ± 1.53; 25 ± 1.00 mm), and fresh weight (173.7 ± 8.14 mg) compared to alternative treatments.

**Keywords:** AMPEP, callus, IAA, KIN, *Kappaphycus striatus*, somatic embryos.

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## Introdution

*Kappaphycus striatus*, a member of the red seaweed phylum (Rhodophyta), is widely distributed in tropical waters and holds economic significance as a key source for extracting commercial carrageenan - a polysaccharide structure utilized in various industries [1, 2]. Our research, which has successfully enhanced the somatic embryo (SEs) induction and micropropagule regeneration in SEs of *K. striatus*, opens new possibilities for cultivating and propagating this economically and biologically important seaweed. The rich content of crude fiber, iron, omega-3 fatty acids, antioxidants [3], and bioactive compounds of interest to the pharmaceutical and biological sectors [4] in *K. striatus* underscore our findings' potential applications.

The propagation of *K. striatus* through callus culture has been investigated, involving five primary stages: axenic material preparation, callus induction and proliferation, somatic embryos (SEs) induction, micropropagule regeneration, and field cultivation of regenerated plants [5]. However, the rates of SEs induction and micropropagule regeneration from SEs in the traditional process are notably low.

Plant growth regulators, including seaweeds, play a pivotal role in governing plants' growth, differentiation, and development of plants. Control effectiveness varies based on each stage's species, concentration, and growth regulator type at each stage. Biological plant growth regulators such as auxin and cytokinin influence callus induction and embryonic differentiation processes. Auxin, for instance, stimulates callus induction and growth, with its role in embryogenesis contingent upon specific combinations and concentrations [6]. Seaweed explants require auxin in the growth medium for proper SEs development, and a medium enriched with auxin can serve as an induction setting for asexual SEs formation. The presence of auxin in the growth medium is necessary for the explant to develop into SEs when transferred to a suitable medium. Enriched medium cultures containing plant growth regulators can be considered induction environments for asexual SEs formation. Explants maintained continuously in an auxin-free medium will not form SEs [7]. In seaweed, when using α-Naphthaleneacetic acid (NAA) alone or in combination with benzylaminopurine (BAP) at a concentration of 0.1–1.0 $mg.L^{-1}$ stimulated the differentiation of asexual SEs of *K. alvarezii* [8].

Cytokinins, used for morphogenesis induction in dicotyledonous plants, exhibit varying effects on asexual embryogenesis depending on plant species, genotype, type of medium, and application timing. In the case of *K. striatus* breeding, supplementation with 1 $mg.L^{-1}$ NAA + 2 $mg.L^{-1}$ BAP has been found to stimulate callus induction. Still, further research is required to identify superior types and concentrations of growth regulators for SEs induction. However, we have yet to evaluate the SEs induction rate, and the number of SEs induced per explant is relatively low (113 embryos/explant) [5]. Therefore, further research is needed to find other types and concentrations of growth regulators better for SEs induction in *K. striatus*.

Additionally, studies have indicated that supplementing 3-indoleacetic acid (IAA) and Kinetin (KIN) enhances SEs induction in *K. alvarezii*. However, evaluation has yet to be conducted for *K. striatus* to determine the optimal concentrations of these phytochemicals for inducing SEs and assessing the rate and efficacy of SEs induction. Hence, our research focuses on the impact of individual and combined IAA and KIN to identify the suitable type and concentration for stimulating SEs induction in *K. striatus*.

Furthermore, the commercial poweredextract of *Ascophyllum nodosum* (Acadian Marine Plant Extract Powder, AMPEP) has been shown to improve survival rates and promote the growth of *K. alvarezii* micropropagules in some studies [9, 10]. Consequently, we explore the use of AMPEP during the regeneration stage of *K. striatus* micropropagules, providing novel insights into the survival rate and regeneration of complete micropropagules under *in vitro* conditions.

Therefore, our investigation delved into the impacts of both individual and combined treatments of KIN and IAA, aiming to assess their influence on the potential to induce SEs and evaluate the capacity of callus tissue to transform into SEs. Additionally, we explored the effects of AMPEP on the regeneration potential for micropropagules originating from SEs. Through these endeavors, we aimed to refine and enhance the overall process of micropropagule development in *K. striatus*.

## Materials and methods

### *Materials*

The 16-week-old callus of *Kappaphycus striatus* (F. Schmitz, 1895) Doty ex P.C. Silva, 1996 (Rhodophyta), which originates and is cultivated commercially by local farmers in Van Phong Bay, Khanh Hoa province, Vietnam (Fig. 1).

Research time: 1/2023–12/2023.

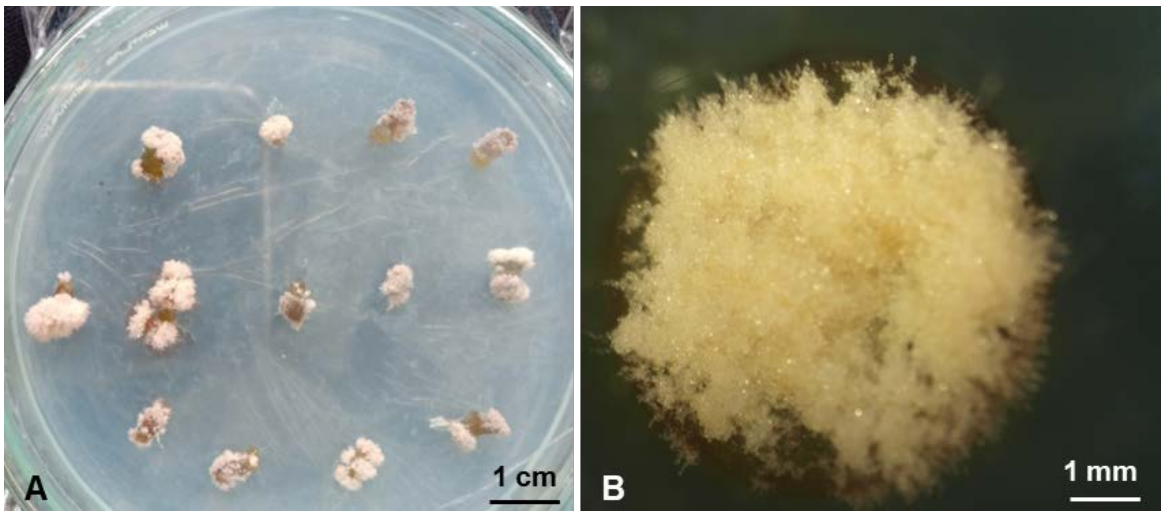


*Figure 1.* Callus of *K. striatus* as research material. A. Petri dish containing explants with the 16-week-old callus of *K. striatus*; B: The 16-week-old callus of *K. striatus* under stereo microscope

### *Methods*

*Effects of IAA and KIN on somatic embryogenesis*

*- The effects of IAA and KIN alone on somatic embryogenesis*

A 16-week-old callus of *K. striatus* (2 × 2 mm, 10 mg) was inoculated into a glass flask with 20 mL of semi-solidified PES medium (4 $g.L^{-1}$ agar), supplemented with IAA (0.5, 1.0, 1.5, 2, 2.5, 3 $mg.L^{-1}$) or KIN (0.5, 1.0, 1.5, 2.0, 2.5, 3 $mg.L^{-1}$); and control (1 $mg.L^{-1}$ NAA + 2 $mg.L^{-1}$ BAP) to determine the type and concentration of phytochemicals suitable for the ability to introduce somatic embryogenesis from *K. striatus* callus.

*- The effects of combined IAA and KIN on somatic embryogenesis*

A 16-week-old callus of *K. striatus* (2 × 2 mm, 10 mg) was inoculated into a glass flask with 20 mL of semi-solidified PES medium (4 $g.L^{-1}$agar), supplemented with combination of IAA (1.0, 1.5, 2 $mg.L^{-1}$) and KIN (1.0, 1.5, 2.0 $mg.L^{-1}$); and control (1 $mg.L^{-1}$ NAA + 2 $mg.L^{-1}$ BAP) for somatic embryogenesis induction from *K. striatus* callus (Table 1).

In somatic embryogenesis, the culture medium is a nutrient medium mixed with seawater, possessing a salinity ranging from 30‰ to 35‰ and a pH of 7.8. It is filtered by a net of 22 µm, and supplemented with 4 $g.L^{-1}$ agar (Algae culture, Himedia, India). The culture medium was sterilized by autoclaving at 121°C for 20 minutes.

Each treatment consisted of 30 glass flasks, each containing one explant. The flasks with the explant were placed under *in vitro* conditions. During the somatic embryogenesis period, explants were maintained at 24 ± 2°C

under cool white fluorescent tube lights at 35 µmol photon.$m^{-2}$.$s^{-1}$ with a 12:12-h light: dark cycle.

*Table 1.* Type and concentration of plant growth regulator

| Experience No. | Plant growth regulators | |
|---|---|---|
| | IAA (mg.$L^{-1}$) | KIN (mg.$L^{-1}$) |
| 1 | 1.0 | 1.0 |
| 2 | | 1.5 |
| 3 | | 2.0 |
| 4 | 1.5 | 1.0 |
| 5 | | 1.5 |
| 6 | | 2.0 |
| 7 | 2.0 | 1.0 |
| 8 | | 1.5 |
| 9 | | 2.0 |
| 10 | Control (1 mg.$L^{-1}$ NAA + 2 mg.$L^{-1}$ BAP) | |

After 8 weeks, the SEs induction rate was calculated by counting the number of explants with SEs. The agar containing embryogenic callus with tiny SEs was cut into blocks with a scalpel and transferred to conical flasks with 100 mL of liquid PES medium. The flasks were then placed on a portable rotary shaker at 100 rpm. After 2 weeks of culture in a liquid PES medium, the SEs separated in to individual units. The number of SEs per explant was calculated by counting the number of these single SEs.

*The effect of AMPEP content on micropropagules regeneration of the K. striatus*

The single SEs (0.5–0.6 mm) obtained from the above optimized experiment were filtered by net (100 × 100 µm) and then transferred into conical flasks with 250 mL of different liquid media with AMPEP concentrations (5–50 ppm) [11] for the regeneration of micropropagules. The SEs cultured in liquid PES medium (20 mL.$L^{-1}$) were used as a control. After 8 weeks of cultivation, the micropropagules survival rate (%), number of branches per micropropagules, micropropagule length (mm), fresh weight of micropropagules (mg), and morphology of micropropagules were recorded. Each treatment had three conical flasks containing 250 mL of medium containing 10 single SEs.

The culture medium was mixed with seawater (30–35‰) with a pH of 7.8 and was filtered by a net (22 × 22 µm). The seawater was sterilized by autoclaving at 121°C for 20 minutes. During the micropropagules regeneration period, explants were continuously aerated and maintained at 24 ± 2°C under cool white fluorescent tube lights at 55 µmol photon.$m^{-2}$.$s^{-1}$ with a 12:12 h light: dark cycle.

*Collecting and s*tatistical analysis

- *Environmental factors*

pH: determined by a pH/mV/temperature meter (EC10, Hach Company, USA); salinity (‰): measured with a handheld refractometer (ATAGO Hand Refractometer); light intensity was measured using a light intensity meter (Lutron, LM81LX, Taiwan); temperature (°C): measured with a mercury thermometer.

- *Morphological observation of somatic embryogenesis*

The morphology of SEs was observed, and the images were captured with a stereo zoom microscope (SZH10 - OLYMPUS, Japan) and under the compound light microscope ×10, ×40 (Nikon YS 100, Japan) after 8 weeks of culture. The rate of SE induction (%) was investigated after 8 weeks of culture. The number of SEs induction (No SEs/explant) and morphology of SEs were investigated after 10 weeks of culture. The cultural conditions were as mentioned above.

$$\text{The rate of SEs induction}(\%) = \frac{\text{The total number of explants with SEs}}{\text{The total number of explants}} * 100$$

Quantifying the fresh weight of *K. striatus* involves employing an analytical balance with a capacity of 200 g and the finest division of 0.0001 g. Prior to weighing, the seaweed undergoes drying with paper.

Micropropagule length is measured in the following manner: The micropropagules of *K. striatus* are carefully arranged on a flat plastic tray. A graduated ruler, with a precision of 1 mm, is then positioned alongside the

length of the micropropagules. Calculating the length of *K. striatus* micropropagules involves measuring from the attachment plate to the tip of the most extended branch of the micropropagules.

Determine the number of branches per micropropagule: Count the number of branches growing from the holdfast of micropropagules.

- *Statistical analysis*

The experiments were conducted in triplicate, with each treatment involving 30 explants. Data analysis was performed using SPSS version 16.0 (SPSS Inc., USA). In cases where a significant difference ($P \leq 0.05$) was detected for a measured parameter, means were distinguished through Duncan's multiple range test at a 5% level of significance [12].

## Results and discussion

### *Effects of IAA and Kin to induce somatic embryogenesis*

*The effect of single IAA on somatic embryogenesis*

The calluses of *K. striatus* were transplanted into a semi-solidified PES medium supplemented with a single IAA to generate SEs. As a result, after 3 weeks of culture in a semi-solidified PES medium supplemented with IAA at different concentrations, the ivory-white callus clusters gradually turned light brown. After 8 weeks of culture, the 1 $mg.L^{-1}$ IAA treatment had the highest SEs induction rate, and the 1.5 $mg.L^{-1}$ IAA treatment had the highest SEs number compared to the remaining treatments (Figs. 2, 3).

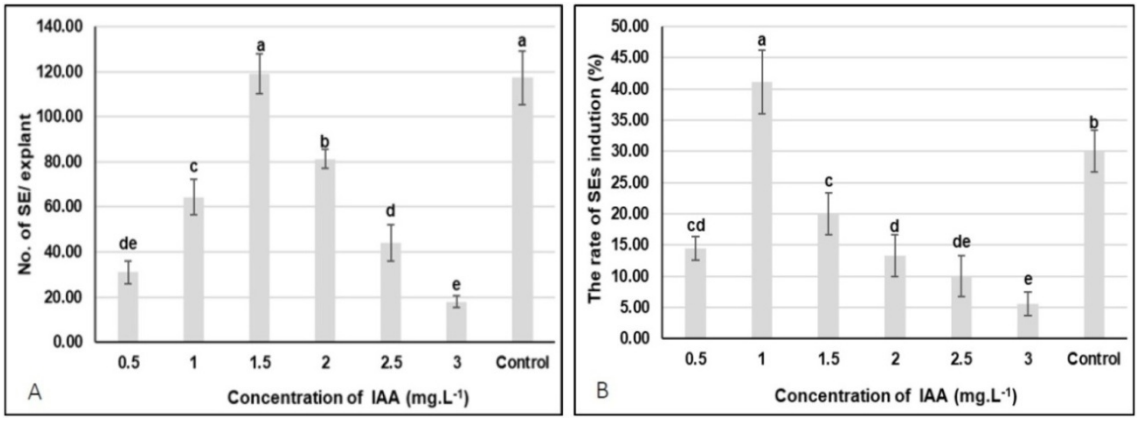


*Figure 2.* Effect of single IAA on the rate of SEs induction and the number of SEs/explants from *K. striatus* callus after 8 weeks of culture, n = 30

Supplementing a single IAA with different concentrations (0.5–3 $mg.L^{-1}$) affected the somatic embryogenesis of *K. striatus* callus. After 8 weeks of culture, the results showed explants had an induction of SEs in all treatments. Anatomizing clusters of explants, we saw densely induced tiny SEs structures. At 1.0 $mg.L^{-1}$ IAA treatment, the SEs exhibit a long spherical shape, measuring approximately 200–300 µm in length, 40–100 µm in width, and possess a light brown coloration. In contrast, at the concentration of 1.5 $mg.L^{-1}$ IAA treatment, the SEs were dense, longer (400–600 mm) but smaller (10–40) in width, and the embryos clustered, and branches appeared. Explants that do not induce SEs are opaque white to clear in color; these are dead explants. The treatment supplemented with 1.0 $mg.L^{-1}$ IAA had the highest SEs induction rate (41.11 ± 5.09%) compared to the remaining treatments. Next, the control treatment (1 $mg.L^{-1}$ NAA + 2 $mg.L^{-1}$ BAP) had an embryonic induction rate (30.00 ± 3.33%). When IAA concentration increases, the SEs induction rate tends to decrease. In the remaining treatments, the SEs induction rate (20.00 ± 3.33 – 5.56 ± 1.92%) (Figs. 2A, 3).

Besides the SEs induction rate, the number of SEs induced in each explant was also evaluated. In the treatment supplemented with 1.5 $mg.L^{-1}$, the number of SEs per explant was

(119.00 ± 8.72 SEs/explant) equal to the control treatment (1 mg.L$^{-1}$ NAA + 2 mg.L$^{-1}$ BAP) with the number of SEs (117.33 ± 11.93 SEs/explant). Next, in the treatment supplemented with 2.0 mg.L$^{-1}$, the number of SEs/explant was (81.33 ± 4.15 SEs/explant). For the treatment supplemented with 1 mg.L$^{-1}$ IAA, the number of SEs/explant was (64.33 ± 7.77 SEs/explant). In the 0.5 mg.L$^{-1}$ treatments, the number of SEs (31.00 ± 5.00) was equal to that of embryos (44.00 ± 7.94 SEs/explant). In the 3.0 mg.L$^{-1}$ treatment, the number of SEs/explant (18.00 ± 2.65 SEs/explant) was the lowest compared to the remaining treatments (Figs. 2B, 3).

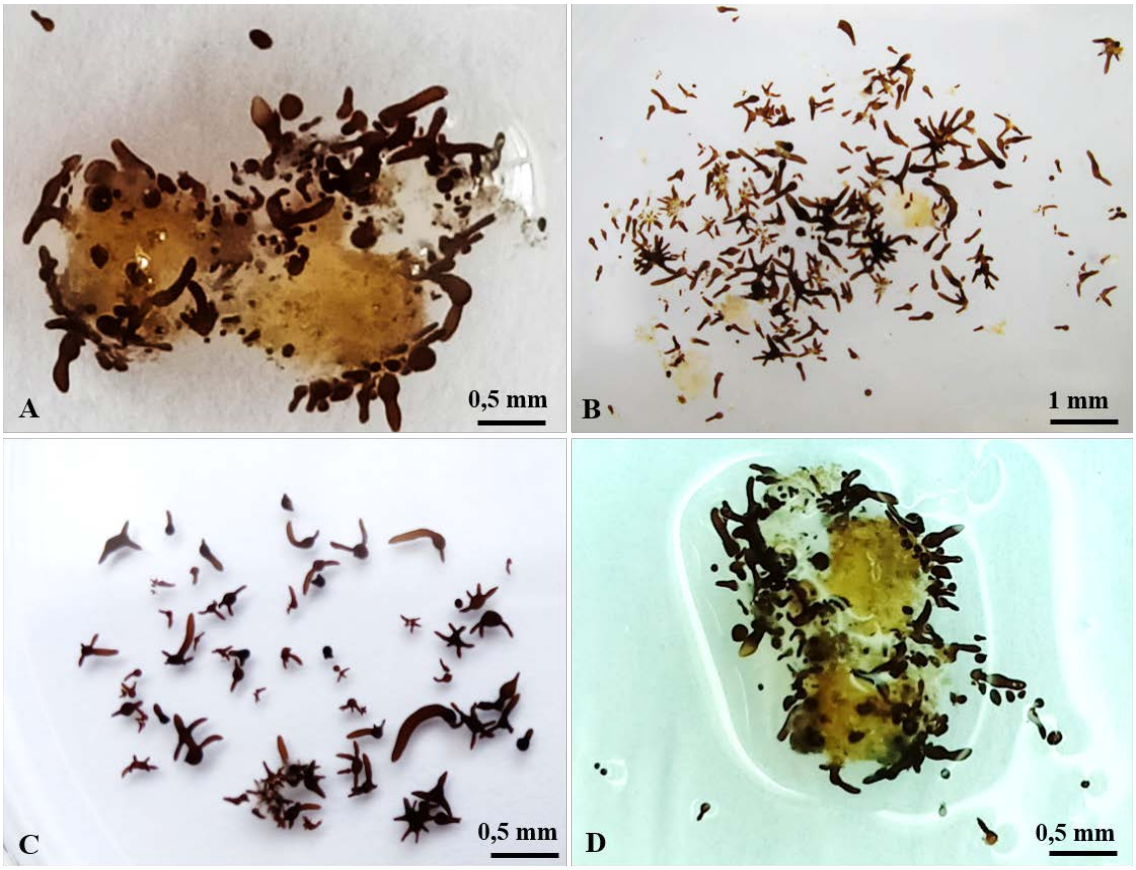


*Figure 3.* Morphology of somatic *K. striatus* in PES medium supplemented with IAA alone after 8 weeks of culture. A: 1.0 mg.L$^{-1}$ IAA; B: 1.5 mg.L$^{-1}$ IAA; C: 2.0 mg.L$^{-1}$ IAA; D: 1 mg.L$^{-1}$ IAA NAA + 2 mg.L$^{-1}$ IAA BAP

*The effect of single Kin on somatic embryogenesis*

Callus clumps were transferred into a semi-solidified PES medium, augmented with a singular concentration of KIN, to instigate the formation of SEs. Subsequently, following a 4-week cultivation period in the semi-solidified PES medium supplemented with KIN at various concentrations, the initially ivory-white callus clusters underwent a progressive transformation, turning a light brown. By the end of an 8-week cultivation period, the treatment enriched with 1.5 mg.L$^{-1}$ KIN exhibited the most noteworthy rates of SEs induction and yielded the highest number of SEs compared to the other treatments (Figs. 4, 5).

Supplementing single KIN with different concentrations (0.5–3 mg.L$^{-1}$) affected on the somatic embryogenesis of *K. stritus* callus. After 8 weeks of culture, the results showed that in all treatments, callus was induced into SEs. The SEs are spherical, about 250–500 µm long, and light brown. Explants that do not induce SEs are opaque white to clear in color; these are dead

explants. In the treatment supplemented with 1.5 mg.L$^{-1}$, the SEs induction rate (53.33 ± 3.33%) was highest compared to the remaining treatments. Next, in the 2 mg.L$^{-1}$ KIN treatment, there was a SEs induction rate (35.36 ± 1.92%). For the control treatment 1 mg.L$^{-1}$ NAA + 2 mg.L$^{-1}$ BAP), there was a SEs induction rate (30.00 ± 3.33%). In the remaining treatments, the SEs induction rate (11.11 ± 1.92 – 26.67 ± 3.33%) (Figs. 4A, 5).

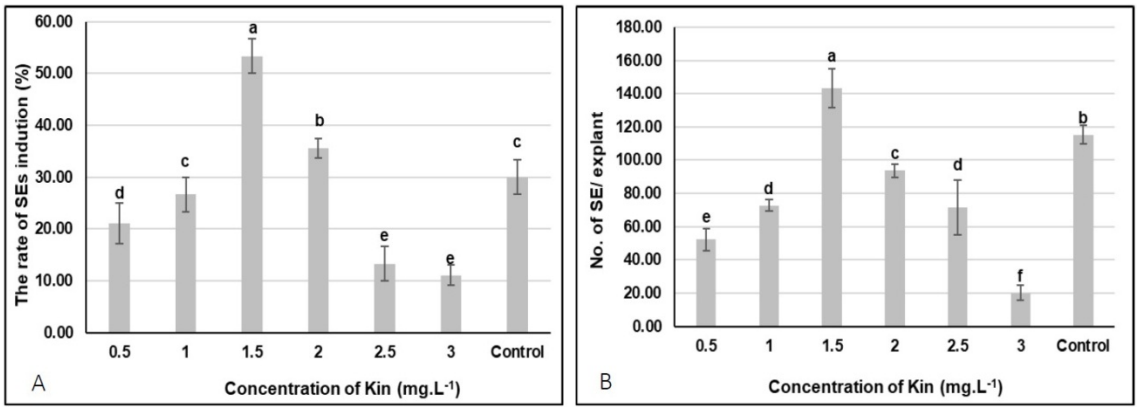


*Figure 4.* Effect of single KIN on the rate of SEs induction and the number of SEs/explants from *K. striatus* callus after 8 weeks of culture, n = 30

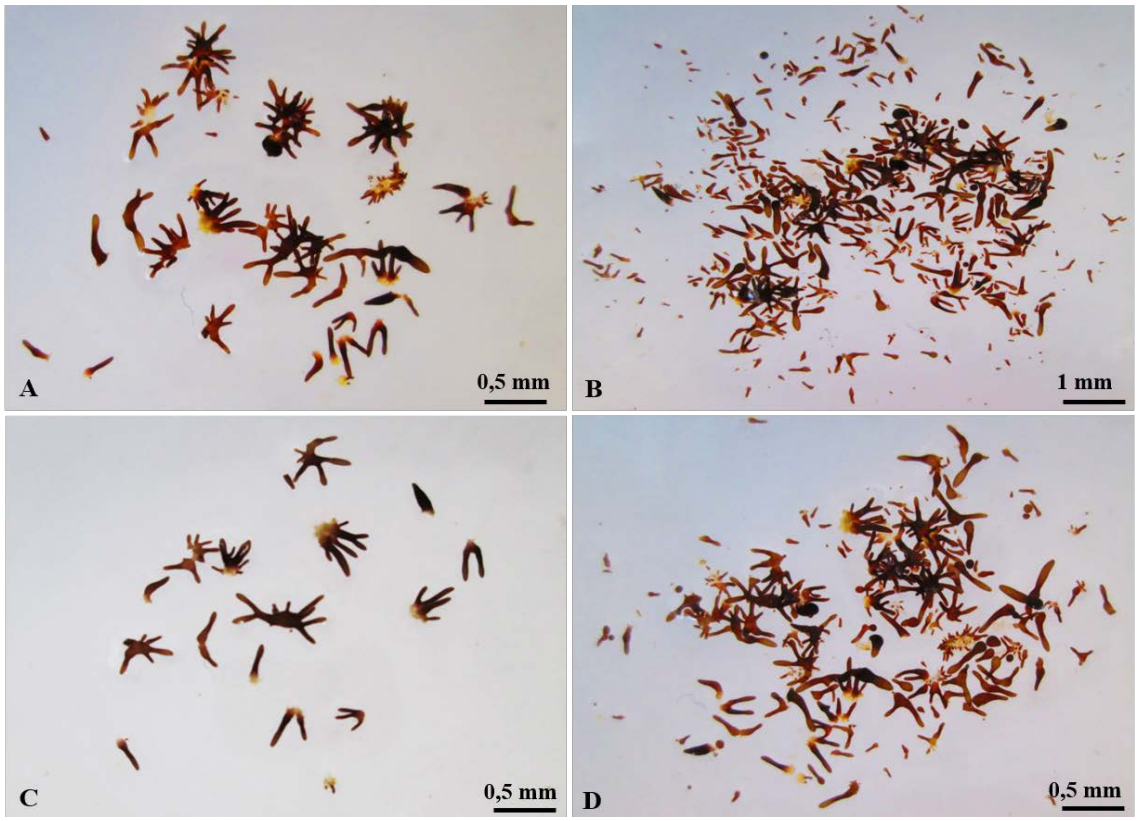


*Figure 5.* Morphology of somatic *K. striatus* in PES medium supplemented with Kin alone after 8 weeks of culture. A: 0.5 mg.L$^{-1}$ KIN; B: 1.5 mg.L$^{-1}$ KIN; C: 2.5 mg.L$^{-1}$ KIN; D: 1.0 mg.L$^{-1}$ NAA + 2.0 mg.L$^{-1}$ BAP

Besides the embryo induction rate, each explant's number of SEs induced was also evaluated. In the treatment supplemented with 1.5 mg.L$^{-1}$, the number of embryos (143.33 ± 11.85 SEs/explant) was highest compared to the remaining treatments. Next, in the 2 mg.L$^{-1}$ KIN treatment, the number of SEs/explant was (93.67 ± 4.04 SEs/explant). For the control treatment (1 mg.L$^{-1}$ NAA + 2 mg.L$^{-1}$ BAP), the number of SEs was (115.33 ± 5.51 SEs/explant). In the remaining treatments, the number of SEs/explant (20.00 ± 4.58 – 72.67 ± 3.51 SEs/explant) was lower (Figs. 4B, 5).

Based on the outcomes of the preceding two experiments, it is evident that concentrations of 1–2 mg.L$^{-1}$ of both IAA and KIN exhibit favorable effects on SEs induction. Consequently, in the forthcoming investigation, we have focused on concentrations within the range of 1–2 mg.L$^{-1}$ to explore the impact of varying ratios of IAA and KIN on the induction of SEs in *K. striatus*.

*The effects of KIN and IAA combine to induce somatic embryogenesis*

*K. striatus* callus was transplanted into a semi-solidified PES medium supplemented with KIN and IAA combined to generate SEs. As a result, after 2 weeks of culture in a semi-solidified PES medium supplemented with KIN at different concentrations, the ivory-white callus clusters gradually turned light brown. Continue to monitor the growth and development of the culture. After 8 weeks of culture, the 1 mg.L$^{-1}$ IAA + 2 mg.L$^{-1}$ KIN treatment had the highest SEs induction rate and number of SEs compared to the remaining treatments (Figs. 6, 7).

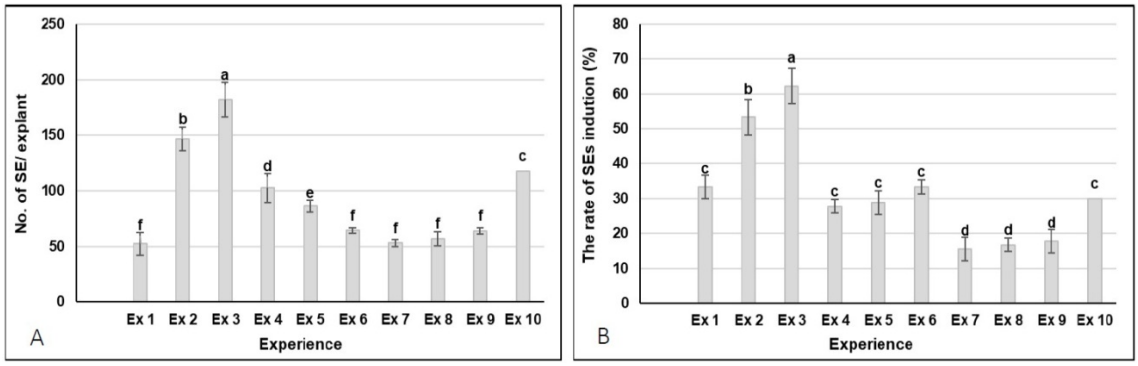


*Figure 6.* Effects of Kin and IAA combine on SEs induction from callus of *K. striatus* after 8 weeks of culture in PES medium, n = 30

Supplementing KIN and IAA (1–2 mg.L$^{-1}$) in combination affected the somatic embryogenesis of *K. striatus* callus. After 8 weeks of culture, the results showed that in all treatments, tissue explants had induction of SEs. The SEs are spherical, about 200–600 µm long, and light brown. Explants that do not induce SEs are opaque white to clear in color; these are dead explants. The treatment supplemented with 1 mg.L$^{-1}$ IAA + 2 mg.L$^{-1}$ KIN had the highest SEs induction rate (62.22 ± 5.09%) compared to the remaining treatments. Next, in the treatment of 1 mg.L$^{-1}$ IAA + 1.5 mg.L$^{-1}$ KIN, the SEs induction rate was (55.33 ± 3.33%). For the control treatment (1 mg.L$^{-1}$ NAA + 2 mg.L$^{-1}$ BAP), the SEs induction rate (30.00 ± 3.33%) was equal to the SEs induction rate (27.78 ± 5.09 – 33.33 ± 3.33). In the remaining treatments, the SEs induction rate (15.56 ± 1.92 – 17.78 ± 1.92%), was lower than other treatments (Fig. 6A).

After 10 weeks of culture, in the 1 mg.L$^{-1}$ IAA + 2 mg.L$^{-1}$ KIN treatment, the number of ESs (182.00 ± 10.58 SEs/explant) was highest compared to the other treatments. Next, in the treatment of 1 mg.L$^{-1}$ IAA + 1.5 mg.L$^{-1}$ KIN, the number of SEs was (146.67 ± 10.02 SEs/explant). In the control treatment (1 mg.L$^{-1}$ NAA + 2 mg.L$^{-1}$ BAP), the number of SEs (117.68 ± 3.96 SEs/explant) was higher than the number of SEs (52.33 ± 6.66 – 102.67 ± 15.53) when supplemented with a combination of 1.5–2.0 mg.L$^{-1}$ IAA + 1–2 mg.L$^{-1}$ KIN (Figs. 6B, 7).

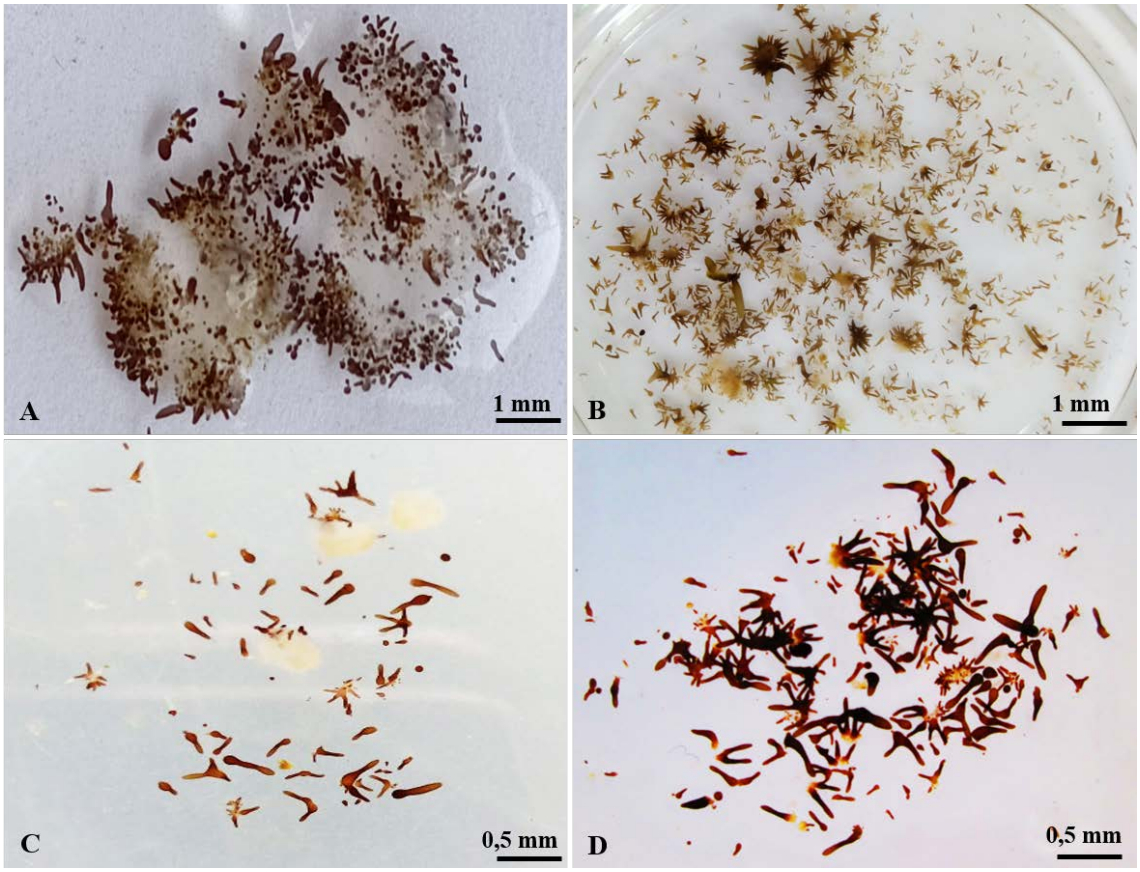


*Figure 7.* Morphology of somatic *K. striatus* in PES medium supplemented with KIN and IAA combine after 8 weeks of culture. A: 1.0 mg.L$^{-1}$ IAA + 1.5 mg.L$^{-1}$ KIN; B: 1.0 mg.L$^{-1}$ IAA + 2.0 mg.L$^{-1}$ KIN; C: 2.0 mg.L$^{-1}$ IAA + 1.0 mg.L$^{-1}$ KIN; D: 1.0 mg.L$^{-1}$ NAA + 2.0 mg.L$^{-1}$ BAP

### *The effect of AMPEP content on microprogagules regeneration of the K. striatus*

SEs were regenerated as micropropagules in an aerated seawater supplemented with different levels of AMPEP. After 8 weeks of culture, the results of AMPEP content (5–50 ppm) had an impact on the survival rate of microprogagules from SEs showing the number of branches/micropropagule, branch length, and fresh weight of micropropagules, which were different in each different treatment.

Enhancing the AMPEP medium is a routine practice undertaken following weekly seawater changes. Following the introduction of AMPEP during the initial two days, most of the culture medium exhibited clarity. However, after two days of treatments enriched with AMPEP (30–50 ppm) medium, the water showed cloudiness and foaming. In contrast, in the other treatments, the seawater remained clear for 4–6 days before gradually becoming cloudy. At the end of 8 weeks of cultivation, the survival rate of micropropagules was highest (75.6 ± 5.09%) when cultured in seawater supplemented with 20 ppm AMPEP. In the treatment supplemented with 10 ppm AMPEP and PES medium, the survival rate of micropropagules (48.9 ± 1.92%; 52.2 ± 1.92%) was equal but lower than in the supplemented medium of 20 ppm AMPEP. The survival rate is lower when adding AMPEP medium at higher concentrations (30–50 ppm). In the treatment supplemented with 30 ppm AMPEP medium, the survival rate of micropropagules was (36.7 ± 3.33%). The lowest survival rate (11.1 ± 5.09; 15.6 ± 5.09%) was in the treatment supplemented with (40–50 ppm) AMPEP medium (Table 2).

*Table 2.* The effect of different AMPEP content on micropropagules regeneration of *K. striatus*, n = 30

| Concentration of AMPEP (ppm) | Survival rate (%) | Number of branches/microprogagules | Length of branches (mm) | Fresh weight of microprogagules (mg) |
|---|---|---|---|---|
| 5 | $30.0^{c} \pm 3.33$ | $1.67^{c} \pm 0.58$ | $5.67^{e} \pm 1.53$ | $34.3^{e} \pm 4.51$ |
| 10 | $48.9^{b} \pm 1.92$ | $3.00^{c} \pm 1.00$ | $9.00^{d} \pm 1.00$ | $38.7^{e} \pm 5.51$ |
| 20 | $75.6^{a} \pm 5.09$ | $7.67^{a} \pm 0.58$ | $24.67^{a} \pm 1.53$ | $173^{a}.7 \pm 8.14$ |
| 30 | $36.7^{c} \pm 3.33$ | $5.33^{b} \pm 0.58$ | $25.00^{a} \pm 1.00$ | $158.7^{b} \pm 10.26$ |
| 40 | $15.6^{d} \pm 5.09$ | $2.33^{c} \pm 0.58$ | $15.67^{c} \pm 2.08$ | $75.3^{d} \pm 5.03$ |
| 50 | $11.1^{d} \pm 5.09$ | $2.00^{c} \pm 1.00$ | $13.33^{c} \pm 1.53$ | $82.7^{d} \pm 9.29$ |
| PES | $52.2^{b} \pm 1.92$ | $6.33^{b} \pm 0.58$ | $19.00^{b} \pm 1.00$ | $101.0^{c} \pm 7.94$ |

*Notes:* *Data are shown as mean value ± SD. Different letters in the same column indicate significant difference in Duncan's test (P ≤ 0.05).


*Figure 8.* Morphology of *K. striatus* micropropagules in an AMPEP medium after 8 weeks of cultivation. A: 5 ppm AMPEP medium; B: 20 ppm AMPEP medium; C: 30 ppm AMPEP medium; D: 40 ppm AMPEP medium; E: 50 ppm AMPEP medium; F: PES medium

Regarding the number of branches permicropropagule, at different AMPEP medium concentrations, the micropropagules regenerate different branches/micropropagules.

Notably, at 20 ppm AMPEP medium, micropropagules exhibit prolific growth, generating numerous main branches from which additional secondary branches emerge. These micropropagules showcase a luxuriant appearance characterized by pointed branch tips and dark brown coloration. The branch count per micropropagule in this setting stands at 7.67 ± 0.58, surpassing other AMPEP medium concentrations and the control. In the treatment supplemented with AMPEP medium content (30 ppm), the number of branches/micropropagules (5.33 ± 0.58

branches/micropropagules) aligns with that observed in the control treatment (PES medium) at 6.33 ± 0.58 branches/micropropagules. Meanwhile, the cultivation of SEs in AMPEP medium (5–10 ppm) yields a branch count per micropropagule ranging from 1.67 ± 0.58 to 3.00 ± 1.00 branches/micropropagules, and in the AMPEP medium content (40–50 ppm), it ranges from 2.00 ± 1.00 to 3.00 ± 0.58 branches/micropropagules (Table 2, Fig. 8).

Following 8 weeks of cultivation under varying concentrations of AMPEP medium, the branch length of micropropagules exhibited notable distinctions. Within the medium enriched with 20–30 ppm AMPEP, micropropagules displayed the lengthiest branches (24.7 ± 1.53; 25 ± 1.00 mm) compared to other treatments. In contrast, the control environment (PES) yielded microprogagules with a shorter branch length (19.00 ± 1.00 mm), surpassing those subjected to low (5–10 ppm) and high (40–50 ppm) AMPEP supplementation treatments (Table 2, Fig. 8).

Besides the number of branches and length of branches/micropropagules, the fresh weight of microprogagules is also recorded to evaluate the regeneration ability of SEs in different environments. After 8 weeks of cultivation, the fresh weight of micropropagules varied at different AMPEP concentrations. In the 30–40 ppm AMPEP treatment, the micropropagules had the highest fresh weight (157.7 ± 10.26; 173.7 ± 8.14 mg) compared to the other treatments.

## *Disccusion*

Factors affecting the induction of somatic embryogenesis are very diverse but are mainly exogenous biocompetence and other stress factors [13]. The concentration and type of phytochemicals impact the process of regeneration and SEs morphogenesis, in which phytochemicals play a prerequisite role. In higher plants, proembryonic cells are generated from callus cells with large cell spaces when stimulated by IAA, KIN, 2,4-D,… or some inducing factors and other stresses [13]. In *K. alvarezii*, when supplemented with NAA, stimulates cell division and SEs formation [8]. The *K. alvarezii* callus mass was transferred to PES medium supplemented with 1 $mg.L^{-1}$ BAP combined with 2.5 $mg.L^{-1}$ IAA and 8 $g.L^{-1}$ agar to freeze the asexual SEs induction medium after 24–35 days of culture. The cloned SEs were transferred to a liquid PES medium containing 1 $mg.L^{-1}$ BAP and 2.5 $mg.L^{-1}$ IAA. The medium was continuously shaken with a shaker for 10–15 days to create dense and mature SEs [14].

The most important plant growth regulators in the induction of somatic embryogenesis are auxins. Synthetic auxins such as IAA, 2,4-D, and NAA are commonly used to create SEs [15]. IAA is a natural auxin, has weaker effects than synthetic auxins, and is more easily broken down than 2,4-D and NAA. Auxin stimulates the formation of proembryonic cells, which are clusters of cells directed to form SEs. Once the pre-embryonic cells are formed, they can develop to the spherical SEs stage. However, if these pre-embryonic cells continue to be cultured in an auxin-containing environment, further development of the SEs is hindered by auxin. Removal or reduction of auxin in the culture medium allows preembryonic cells to develop into SEs [15]. Some plant species can form SEs and must use auxin as the only substance to regulate this growth, but other species that form SEs sometimes require the participation of cytokinin. There have been reports of the induction and formation of SEs in cultures with the sole addition of cytokinin. However, these reports are very few compared to SEs induction by auxin or auxin combined with cytokinin. IAA is a natural auxin that is sensitive to light and is easily degraded. Therefore, the use of IAA for callus formation must consider light radiation. Synthetic auxins (IBA, NAA, and 2,4-D) are more stable under light. *Citrus sinensis* embryonic callus grows and differentiates on medium containing IAA and KIN. Repeated transfer of callus tissue reduces the ability to generate SEs, and after about 2 years, the tissue lines show hormonal autotrophy. In these tissue lines, IAA in the culture medium at low concentrations (0.001 $mg.L^{-1}$) inhibited embryogenesis. IAA supplementation of 1–3 $mg.L^{-1}$ in PESI solid medium has also been reported to induce SEs yields of up to 90% in the brown seaweed

*Pelvetia siliquosa*, but at higher concentrations of 4 and 5 mg.L$^{-1}$, induction rates decreased by 50 and 5%, respectively [16]. Sulistiani et al. [17] reported that the addition of IAA (2.5–5.0 mg.L$^{-1}$) in PES medium (40–50%) produced the SEs of *K. alvarezii* at a higher rate compared to CW medium (0–20%). In this study, *K. striatus* callus induced SEs at almost all concentrations from 0.5–3.0 mg.L$^{-1}$. However, at high concentrations (2.5–3.0 mg.L$^{-1}$), IAA inhibited SEs induction, and the number of SEs induced/explant was very low.

In addition to auxin, cytokinins are used for embryogenesis induction in dicotyledonous plants. The most commonly used cytokinin in culture media is BAP, but other cytokinins such as kinetin, zeatin, and TDZ also give good results depending on the plant species. In embryonic culture, the concentration of plant growth regulators is essential for optimal explant growth. When the concentration is too low, it will not stimulate growth, whereas when it is too high, it will be toxic to the culture explants. Although the composition of the basal media can influence the response to plant growth regulators, natural phytohormones and synthetic plant growth regulators can control embryogenesis. KIN stimulates callus formation in *G. perplexa* but harms *G. tenuistipitata* [18]. The callus growth can be accelerated by the incorporation of auxin and cytokinin, such as IAA and BAP, respectively [19].

Earlier studies have demonstrated the versatile applications of AMPEP, extending beyond the micropropagation of *Kappaphycus* plantlets in the Philippines [20, 21]. Its utility extends to enhancing the growth rate of field-cultivated *Kappaphycus* in the Philippines [22] and mitigating epiphyte incidence in Brazil [23]. Additionally, AMPEP has been instrumental in improving carrageenan yield [10]. AMPEP is an effective medium for micropropagules regeneration and development in the micropropagation of *Kappaphycus* plantlets [24]. Using the AMPEP also improves survival rates and increases the heat tolerance of *K. alvarezii* micropropagules [10].

In this study, when AMPEP is added at different concentrations, micropropagules of *K. striatus* had different regeneration. The seawater environment becomes turbid quickly at high AMPEP content (40–50 ppm). The reason is that the microorganisms that thrive in AMPEP environments are organic substances that are easily decomposed. When cultured seawater contains many microorganisms, it produces many substances that are harmful to the seaweed plants, leading to the death of the micropropagules and reducing their survival rate. In the medium supplemented with a lower AMPEP content (5–10 ppm), the culture water in the tank is always stable, with slight bubbling in the last days of the water change cycle. However, the nutritional content is low; it does not have enough nutrients to help microprograms grow and develop. When micropropagules are raised in an environment with low nutrient content for a long time, the seaweed becomes stressed and leads to death, resulting in a low survival rate. While these findings hold promise for the commercial-scale development of plantlets, it is acknowledged that the existing body of AMPEP studies with *Kappaphycus* serves as a proof of concept and highlights the need for further extensive research.

## Conclusion

In summary, we conducted a study to identify the optimal embryogenic induction conditions, finding that the most favorable ratio of plant growth regulators was 1 mg.L$^{-1}$ IAA + 2 mg.L$^{-1}$ KIN. In the stage of complete micropropagule regeneration, SEs were cultured in a medium supplemented with 20 ppm AMPEP, resulting in the most successful micropropagule regeneration after 8 weeks of cultivation. Through these steps, we progressively perfected the propagation process of *K. striatus* using callus culture.

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*striatus* (F. Schmitz) Doty ex P. C. Silva, 1996 bằng phương pháp nuôi cấy mô sẹo", (No. UDSXTN.02/23–24).

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