Photosynthetic Responses of The Red Seaweed *Gracilaria fisheri* to Green and Brown Seaweed Extracts

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ABSTRACT

Seaweed extracts are widely used as biostimulants in crops for promoting growth and stress resistance. This study examined the photosynthetic responses of *Gracilaria fisheri* to extracts of green seaweeds *Chaetomorpha crassa* (Ch-SE) and *Rhizoclonium riparium* (Rhi-SE), and brown seaweeds *Padina* sp. (Pad-SE) and *Sargassum oligocystum* (Sar-SE). Thalli of *G. fisheri* were treated with varying concentrations of the extracts (0, 1, 2, 3, 4, and 5 g SE·L^{-1}) under controlled conditions: salinity of 30‰, temperature of 25–26 °C and light intensity of 200 µmol photons·m^{-2}·s^{-1}. Photosynthesis was measured using a dissolved oxygen sensor and pulse amplitude-modulated fluorometry. Results showed that light intensity rapidly increased with irradiance up to 625 µmol photons·m^{-2}·s^{-1} without photoinhibition in the SE-treated algae, unlike the control. The electron transport rate (ETR) of the SE-treated algae significantly increased compared to the control at 625 µmol photons·m^{-2}·s^{-1}. Net photosynthesis (P_{net}) of the SE-treated *G. fisheri* was two- to fivefold higher than the control. Additionally, the maximum quantum yield also increased in SE-treated algae, with the highest increase in Rhi-SE treatment, followed by Pad-SE, Ch-SE, and Sar-SE treatments. This study suggests that green and brown seaweed extracts effectively enhance photosynthesis in *G. fisheri*.

Keywords: Biostimulant, *Gracilaria*, Photosynthesis, Seaweed extract, Thai seaweed

INTRODUCTION

Seaweeds have been widely used as food and non-food materials worldwide. Despite Thailand's rich seaweed diversity, consumption of seaweeds in the country is limited. Only a few genera, such as *Gracilaria, Caulerpa*, and *Ulva*, are commonly consumed as fresh vegetables. In recent years, farming of *Caulerpa lentillifera* and *Ulva rigida* in Thailand has been promoted by the Department of Fisheries, while the red seaweed *Gracilaria fisheri* is farmed at the community level along the coastal areas in the southern part of the country, especially in the Songkhla and Pattani Provinces. Most of *Gracilaria* is naturally grown in earthen ponds. However, the production and quality of this seaweed are usually low and unstable due to environmental and climatic variations. Consequently, efforts have been recently made to enhance growth, yield, and quality of *Gracilaria* seaweed (Khreauthong *et al*., 2018; Chirapart *et al*., 2022), for example, by using the seaweed extract of *Ascophyllum nodosum* which not only improved growth and production of *G. fisheri* (Chirapart *et al*., 2022) but also inhibited epiphytic attachment.

Seaweeds are a rich source of macro- and microelements and bioactive compounds (Hurtado *et al*., 2009; Baltrusch *et al*., 2023). A number of seaweeds are used to promote the growth of plants, such as the brown seaweeds *Ascophyllum nodosum*, *Ecklonia maxima, Macrocystis pyrifera, Padina*

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Received 1 March 2024 / Accepted 1 November 2024

pavonica and *Sargassum johnstonii* (Hurtado *et al*., 2009; Kumari *et al*., 2011; Chbani *et al*., 2013; Ali *et al*., 2021; Chirapart *et al*., 2022; Hernández-Herrera *et al*., 2022), the green seaweeds *Ulva lactuca* and *Ulva flexuosa* (Reis *et al*., 2020), and the red seaweeds *Kappaphycus alvarezii* (Zodape *et al*., 2011; Trivedi *et al*., 2018a; 2018b). Currently, the extract of a wide variety of seaweeds is becoming increasingly popular; it is not only used as a biostimulant of plant growth but also to help with tolerance to biotic, abiotic, and environmental stresses (Hurtado *et al*., 2009; Khan *et al*., 2009; Chbani *et al*., 2013; Sangha *et al*., 2014; Trivedi *et al*., 2018a; Fitriyah *et al*., 2022). Seaweed extracts have been used as biostimulants to improve the germination, vegetative growth, flowering and yield of many crops (Ali *et al*., 2021; Fitriyah *et al*., 2022). Biostimulants are defined as materials other than fertilizers or plant nutrients; they can stimulate plant growth and development when applied in small quantities (Sangha *et al*., 2014). Seaweed extracts can induce changes in the physiological and biochemical processes of agricultural crops, which are related to nutrient uptake and plant growth (Jannin *et al*., 2013; Yao *et al*., 2020). The seaweed extract from *A. nodosum* has been reported to replace EDTA to chelate trace elements (Richardson *et al*., 2009). The use of seaweed extract from *Sargassum horneri* could improve yield, leaf photosynthesis, ripening time, and net returns of tomato plants (Yao *et al*., 2020). Using kelp seaweed extract in sugarcane plants reportedly results in enhanced photosynthesis and transpiration, improved water use efficiency and utilization efficiency of N-P-K nutrients, and increased plant height (Chen *et al*., 2021).

The effects of seaweed extracts on photosynthesis have been well-documented in crops (Jannin *et al*., 2013; Zermeño-Gonzalez *et al*., 2015; Di Stasio *et al*., 2017; Sosnowski *et al*., 2019; Yao *et al*., 2020; Chen *et al*., 2021) but are rarely studied in seaweeds. In particular, there have been no reports on the effects of seaweed extracts on photosynthesis in *Gracilaria* species or other Thai seaweeds. Therefore, this study was conducted to examine the effects of seaweed extracts on photosynthesis in *G. fisheri*. The aim was to investigate the photosynthetic responses of this alga to extracts from green seaweeds (*Chaetomorpha crassa* and *Rhizoclonium riparium*) and brown seaweeds (*Padina* sp. and *Sargassum oligocystum*) in laboratory conditions. The results obtained will help in understanding the effects of seaweed extracts on the photosynthetic performance of *G. fisheri* and promote growth, yield and quality in farming this seaweed.

MATERIALS AND METHODS

Seaweed collection and preparation

Samples of the brown seaweeds *Padina* sp. and *Sargassum oligocystum* were collected from the coasts of Samaesan and Nang Rong Beach in Chonburi Province, respectively. The green seaweeds *Chaetomorpha crassa* and *Rhizoclonium riparium* were obtained from sea bass fish ponds at the Samut Songkhram Fisheries Research Station, Samut Songkhram Province. The seaweed samples were thoroughly washed with freshwater to remove contaminants, then dried at room temperature. The dried seaweeds were separately ground into powder using a grinder and stored in a Ziplock bag under dry conditions in the laboratory.

For the red seaweed, thalli of *Gracilaria fisheri* were grown in an indoor tank at the Algal Bioresources Research Center, Department of Fishery Biology, Kasetsart University. Healthy thalli were selected and acclimated under controlled culture conditions in the laboratory for photosynthesis examination.

Seaweed extraction

Dried powders of brown and green seaweed were extracted according to methods modified from Sathya *et al*. (2010) and Mohanty and Adhikary (2018). Ten grams (n = 3) of each seaweed powder were soaked in distilled water at a ratio of 1:100 (w/v) for 30 min, then boiled in an autoclave at a pressure of 15 lb and a temperature of 121 °C for 20 min. After cooling, the extract was filtered through cheesecloth. The extract was considered 100% seaweed extract for each species. Then, the extracts (SEs) were further concentrated to 50 mL using a rotary evaporator and then freeze-dried for later use.

Preparation of Gracilaria fisheri for photosynthesis examination

To assess the photosynthetic response of *G. fisheri* to the seaweed extracts, healthy thalli were cut into 2 cm fragments. Five fragments per replicate were weighed and soaked in seaweed extract solutions (mixed with 20 mL·L^{-1} of the Provasoli medium) at different concentrations (0, 1, 2, 3, 4, and 5 g SE·L^{-1}) for 1 h. The experiment was conducted with six replicates per treatment, with each replicate containing five fragments (n = 30 per SE concentration). The *G. fisheri* samples were soaked daily in the extracts and grown for 7 days in 250 mL of 30‰ sterilized seawater (n = 30) at room temperature (25–26 °C) and a light intensity of 200 µmol photons·m^{-2}·s^{-1}. Following the 7-day period, photosynthetic responses were measured.

Relative electron transport rate (rETR)

Rapid light curves were generated using a pulse amplitude modulated (PAM) fluorometer (Junior–PAM, Walz/Germany) with incremental actinic illumination, increasing the photosynthetically active radiation (PAR) intensity in nine steps from 0 to 625 µmol photons·m^{-2}·s^{-1}. The rETR was calculated using the formula:

$$rETR = 0.5 \times Y \times PAR \times AF,$$

where Y represents the effective quantum yield of photosystem II (PSII), the Factor 0.5 assumes that half of the photons are absorbed by PSII, and AF is the fraction of incident light assumed to be absorbed by the sample (= 0.84) (Khreauthong *et al*., 2018).

Effect of seaweed extracts on the photosynthesis of Gracilaria fisheri

The photosynthetic rate of SE-treated samples (n = 3 per SE treatment) were measured using the light/dark bottle technique. Net photosynthetic rate (P_{net}) and dark respiration were determined by measuring the dissolved oxygen concentration (mg·L^{-1}) every 5 min for 30 min following a 30 min preincubation for acclimate (Fujimoto *et al*., 2014). Dissolved oxygen was measured using a DO meter (YSI 5000).

Effect of seaweed extracts on the maximum quantum yield (Fv/Fm)

Maximum quantum yield (Fv/Fm) at 0 µmol photons·m^{-2}·s^{-1} was measured after 7 days of culture with varying concentrations of seaweed extract (0, 1, 2, 3, 4, and 5 g SE·L^{-1}). The Fv/Fm ratio of photosystem II (PS II) was measured using a Junior-PAM (Pluse-Amplitude-Modulation) chlorophyll fluorometer after incubating the SE-treated sample in the dark for 30 min. Photosynthetic efficiency was calculated using the formula:

$$Fv/Fm = (Fm - Fo)/Fm,$$

where Fm is the maximum fluorescence level and Fo is the minimal fluorescence level.

Statistical analysis

Data were presented as the mean ± standard deviation (SD). Statistical analyses were performed using analysis of variance (ANOVA) followed by post hoc Tukey's HSD tests at a 95% confidence level.

RESULTS

The photosynthetic responses of *Gracilaria fisheri* to the extracts of the four seaweeds *Rhizoclonium riparium* (Rhi-SE), *Chaetomorpha crassa* (Ch-SE), *Padina* sp. (Pad-SE), and *Sargassum oligocystum* (Sar-SE) are shown in Figures 1–6. After seven days of culture, *G. fisheri* responded differently to the concentration of the seaweed extracts. The rapid light curves of *G. fisheri* increased until reaching an asymptote, and photoinhibition was not apparent until the PAR reached 625 µmol photons·m^{-2}·s^{-1} (Figures 1–4). The rETR of the SE-treated *G. fisheri* plants tended to be similar to that of the non-SE-treated control plants when the PAR ranged from 25–285 µmol photons·m^{-2}·s^{-1}. However, the ETR of the SE-treated algae increased when the PAR reached 625 µmol photons·m^{-2}·s^{-1}, which was significantly different from that of the non-SE-treated control (Supplementary Table S1). The non-SE control had a low rETR, ranging from 4.12±0.03–18.93±0.40 µmol e·m^{-2}·s^{-1}, while the rETR increased when the thalli of the algae were

treated with the SE of the four seaweed species, with the highest ETR of 20.70±0.22–25.48±0.51 µmol e·m^{-2}·s^{-1} when the PAR reached 625 µmol photons·m^{-2}·s^{-1}. The ETR of *G. fisheri* treated with 2 g SE·L^{-1} Rhi-SE (Figure 1) or 5 g SE·L^{-1} Sar-SE (Figure 4) was greater than that of the algae treated with Ch-SE (Figure 2) or Pad-SE (Figure 3) at the same concentration; the ETR significantly differed among the SE treatments ($p<0.05$). Notably, none of the SE-treated algae showed apparent photoinhibition when the PAR reached 625 µmol photons·m^{-2}·s^{-1}, while photoinhibition occurred in the non-SE-treated control. In addition, the relationships between the ETR and PAR of all the SE-treated *G. fisheri* were slightly curvilinear, especially in the 2 g SE·L^{-1} Rhi-SE treatment (Figure 1) and the 5 g SE·L^{-1} Sar-SE treatment (Figure 4).

The response of the photosynthetic rate of *G. fisheri* to different concentrations of seaweed extract is shown in Figure 5. This study revealed significant differences in the photosynthesis of the algae among the seaweed extract treatments (Supplementary Table S2). The net photosynthetic rate (P_{net}) of the non-SE-treated algae was 0.71±0.09 µgO_2·g^{-1}_{ww}·min^{-1} (Figure 5a). The P_{net} of the SE-treated algae markedly increased as the SE concentration increased to 1 and 2 g SE L^{-1}, respectively. The highest P_{net} of the *G. fisheri* treated with 2 g SE L^{-1} Rhi-SE and Pad-SE were 3.46±0.13 µgO_2·g^{-1}_{ww}·min^{-1} and 2.96±0.01 µgO_2·g^{-1}_{ww}·min^{-1}, respectively. The Ch-SE-treated algae showed the highest P_{net} value of 3.32±0.44 µgO_2·g^{-1}_{ww}·min^{-1} at a concentration of 4 g SE·L^{-1}. The P_{net} significantly differed ($p<0.05$) between the treatments with 1 g SE·L^{-1} and 2 g SE·L^{-1} of the four seaweed extracts (Rhi-SE, Ch-SE, Pad-SE, and Sar-SE). The P_{net} of the Rhi-SE- and Pad-SE-treated algae decreased as the concentration of the extracts increased to 3–5 g SE·L^{-1}. The P_{net} of the Ch-SE-treated algae increased until the extract concentration increased to 4 g SE·L^{-1} and decreased as the concentration increased to 5 g SE·L^{-1}. In contrast, for the Sar-SE-treated algae, P_{net} gradually increased as the treatment concentration increased from 1 to 5 g SE·L^{-1}, and P_{net} did not decrease throughout the experiment. The P_{net} of the Sar-SE-treated algae ranged from 1.47±0.07 to 2.73±0.03 µgO_2·g^{-1}_{ww}·min^{-1}. A similar response was found for gross photosynthesis. The gross photosynthetic rate (GPP) increased with increasing concentration from 0 to 2 g SE·L^{-1} for Rhi-SE and Pad-SE, from 0 to 4 g SE·L^{-1} for Ch-SE, and from 0 to 5 g SE·L^{-1} for Sar-SE (Figure 5b). The GPP of the algae treated with Rhi-SE and Pad-SE had maximum values of 5.89±0.11 µgO_2·g^{-1}_{ww}·min^{-1} and 5.41±0.04 µgO_2·g^{-1}_{ww}·min^{-1}, respectively, at a concentration of 2 g SE·L^{-1}. However, those of the algae treated with Ch-SE and Sar-SE had maximum values of 5.90±0.44 µgO_2·g^{-1}_{ww}·min^{-1} and 5.30±0.02 µgO_2·g^{-1}_{ww}·min^{-1} at concentrations of 4 g SE·L^{-1} and 5 g SE·L^{-1}, respectively. The GPP of *G. fisheri* was significantly different between the non-SE- and SE-treated algae ($p<0.05$). In this study, the dark respiration rate (R_d) was 2.19±0.18 µgO_2·g^{-1}_{ww}·min^{-1} in the non-SE-treated algae. However, the respiration rates markedly increased in the algae treated with the seaweed extracts (Figure 5c). Similar to P_{net} and GPP, the respiration rate of *G. fisheri* was significantly different between the algae treated with SE and those not treated with SE ($p<0.05$). The respiration rate was greatest (2.72±0.02 µgO_2·g^{-1}_{ww}·min^{-1}) in 1 g SE·L^{-1} Pad-SE-treated algae, followed by those treated with 1 g SE·L^{-1} Ch-SE (2.60±0.04 µgO_2·g^{-1}_{ww}·min^{-1}), 4 g SE·L^{-1} Rhi-SE (2.59±0.01 µgO_2·g^{-1}_{ww}·min^{-1}), and 4 g SE·L^{-1} Sar-SE (2.57±0.02 µgO_2·g^{-1}_{ww}·min^{-1}). The respiration rate of the algae gradually increased with increasing concentrations of Sar-SE from 1 to 5 g SE·L^{-1}, with values ranging from 2.45±0.06 to 2.57±0.02 µgO_2·g^{-1}_{ww}·min^{-1}.

In addition, this study revealed that the maximum quantum yield (Fv/Fm) of the SE-treated algae (Figure 6) was significantly greater than that of the non-SE-treated algae ($p<0.05$). The maximum quantum yield of the non-SE treatment group was 0.27 on average, but it markedly increased in the SE treatment group. The average Fv/Fm values of the algae treated with Rhi-SE, Ch-SE, Pad-SE, and Sar-SE ranged from 0.42–0.65, 0.35–0.55, 0.36–0.60, and 0.33–0.51, respectively. The Fv/Fm values were 0.65±0.01, 0.60±0.01, 0.55±0.00, and 0.51±0.00 for the algae treated with 2 g SE·L^{-1} Rhi-SE and Pad-SE, 4 g SE·L^{-1} Ch-SE, and 5 g SE·L^{-1} Sar-SE, respectively. The Fv/Fm of *G. fisheri* decreased with increasing concentrations of Rhi-SE and Pad-SE over 2 g SE·L^{-1}. However, the Fv/Fm of the algal samples treated with Ch-SE and Sar-SE increased with increasing concentrations of SE from 1–5 g SE·L^{-1}.

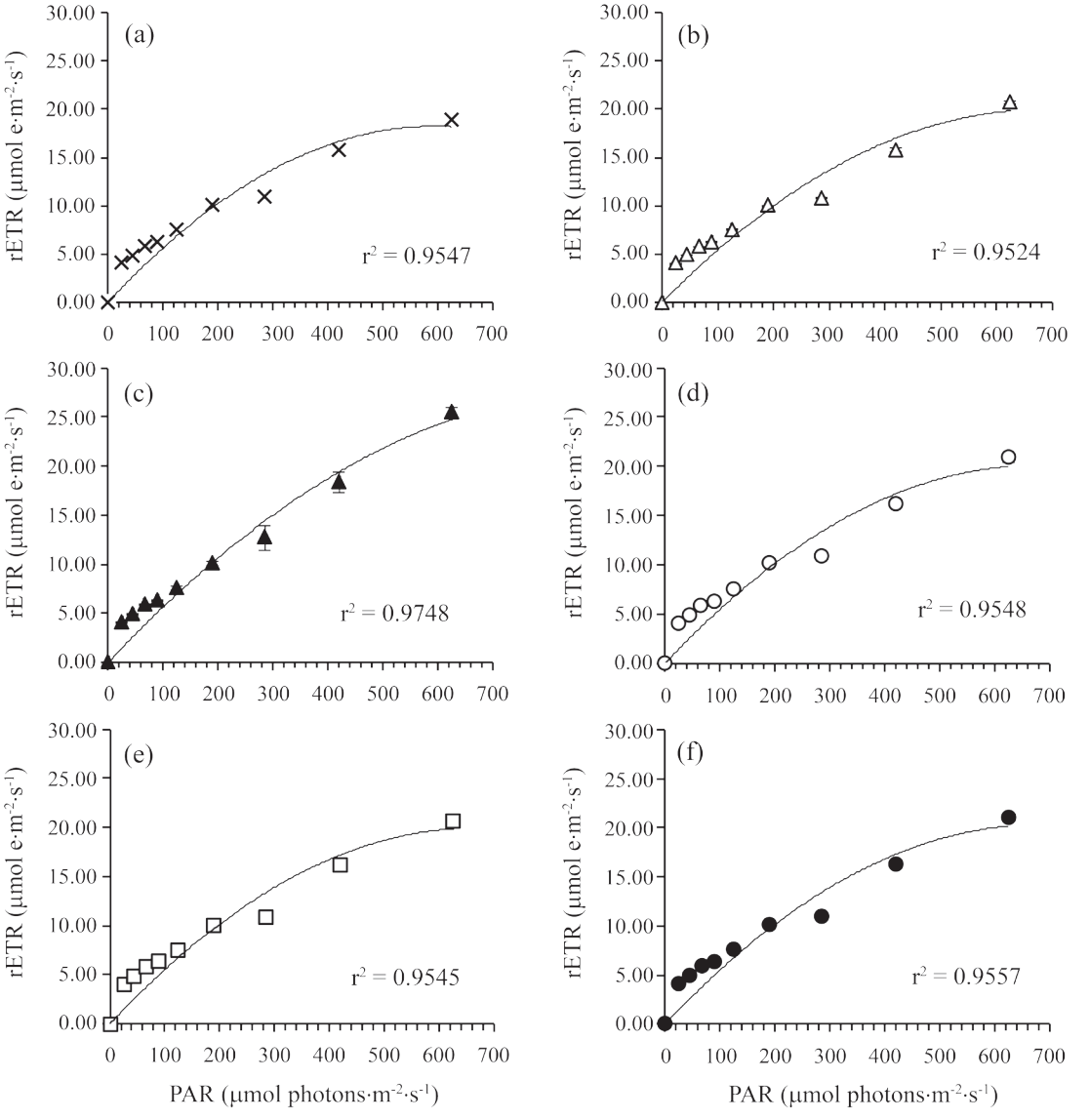

Figure 1. Influence of exposure to irradiance between 0–625 µmol photons·m^{-2}·s^{-1} on the relative electron transport rate (rETR) of Chl *a* fluorescence of *Gracilaria fisheri* treated with seaweed extract from *Rhizoclonium riparium* (Rhi-SE) at concentrations of (a) 0 g SE·L^{-1} (control), (b) 1 g SE·L^{-1}, (c) 2 g SE·L^{-1}, (d) 3 g SE·L^{-1}, (e) 4 g SE·L^{-1}, and (f) 5 g SE·L^{-1}.

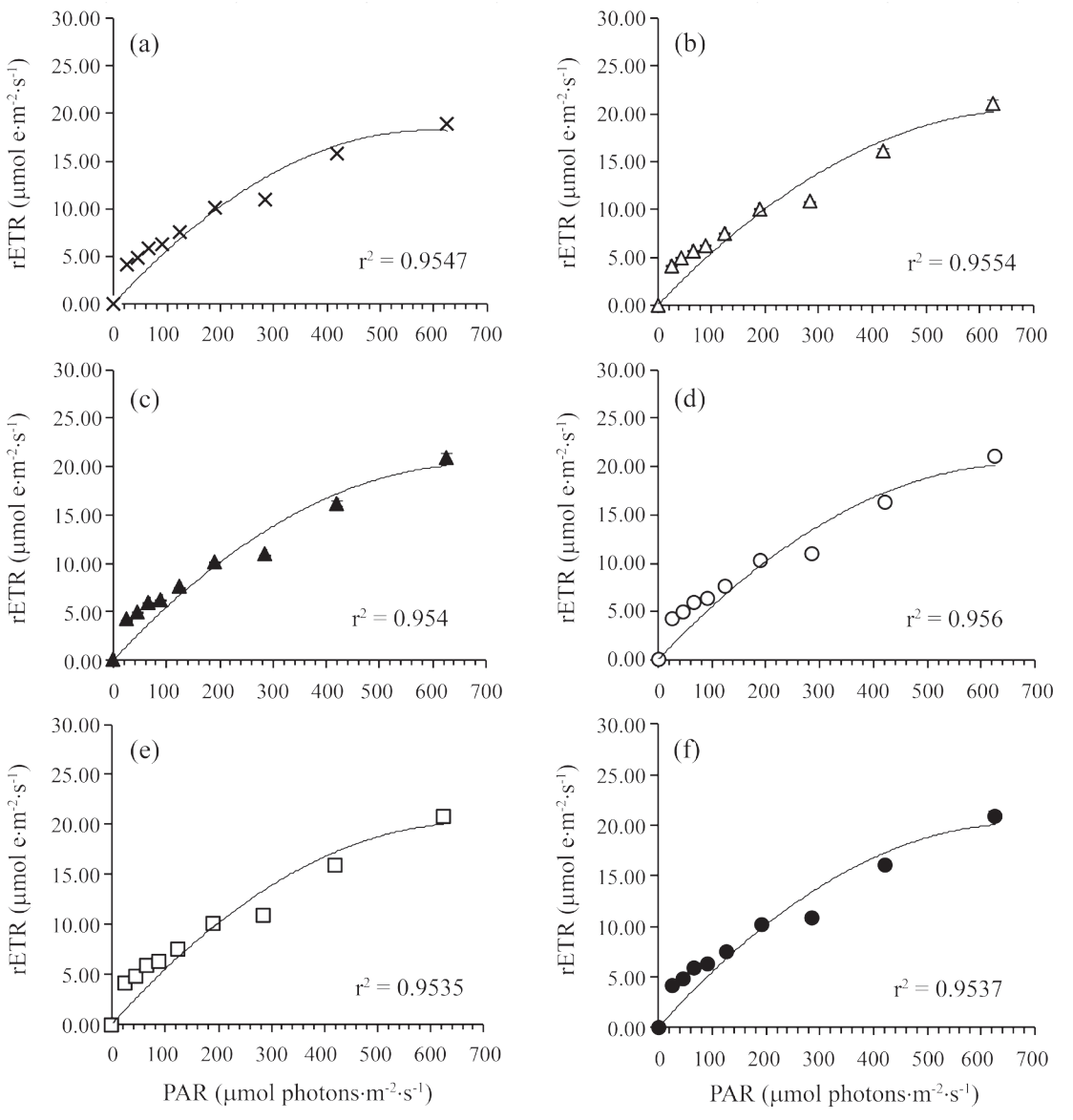

Figure 2. Influence of exposure to irradiance between 0–625 µmol photons·m^{-2}·s^{-1} on the relative electron transport rate (rETR) of Chl *a* fluorescence of *Gracilaria fisheri* treated with seaweed extract from *Chaetomorpha crassa* (Ch-SE) at concentrations of (a) 0 g SE·L^{-1} (control), (b) 1 g SE·L^{-1}, (c) 2 g SE·L^{-1}, (d) 3 g SE·L^{-1}, (e) 4 g SE·L^{-1}, and (f) 5 g SE·L^{-1}.

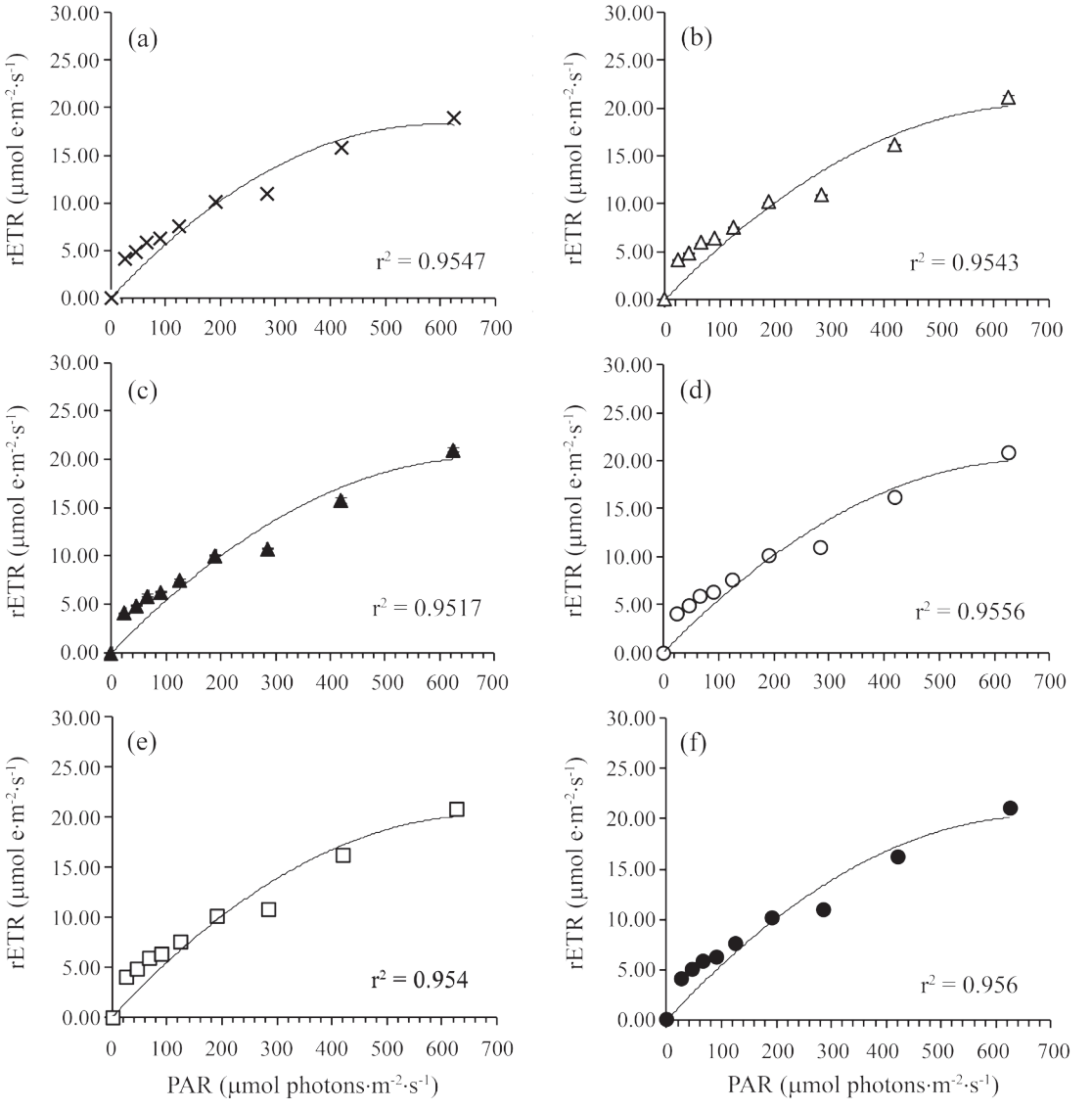

Figure 3. Influence of exposure to irradiance between 0–625 µmol photons·m^{-2}·s^{-1} on the relative electron transport rate (rETR) of Chl *a* fluorescence of *Gracilaria fisheri* treated with seaweed extract from *Padina* sp. (Pad-SE) at concentrations of (a) 0 g SE·L^{-1} (control), (b) 1 g SE·L^{-1}, (c) 2 g SE·L^{-1}, (d) 3 g SE·L^{-1}, (e) 4 g SE·L^{-1}, and (f) 5 g SE·L^{-1}.

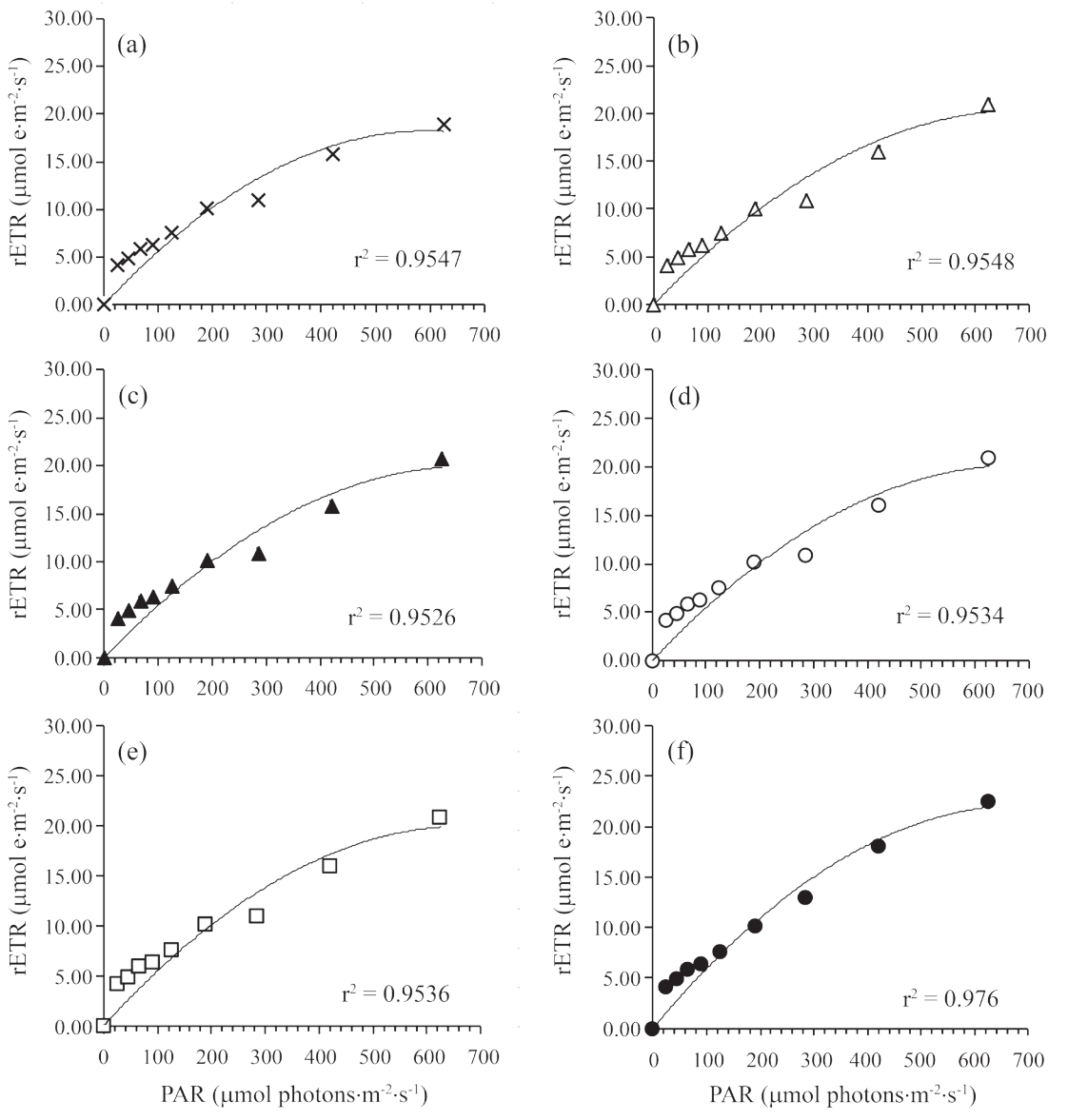

Figure 4. Influence of exposure to irradiance between 0–625 µmol photons·m^{-2}·s^{-1} on the relative electron transport rate (rETR) of Chl *a* fluorescence of *Gracilaria fisheri* treated with seaweed extract from *Sargassum oiligocystum* (Sar-SE) at concentrations of (a) 0 g SE·L^{-1} (control), (b) 1 g SE·L^{-1}, (c) 2 g SE·L^{-1}, (d) 3 g SE·L^{-1}, (e) 4 g SE·L^{-1}, and (f) 5 g SE·L^{-1}.

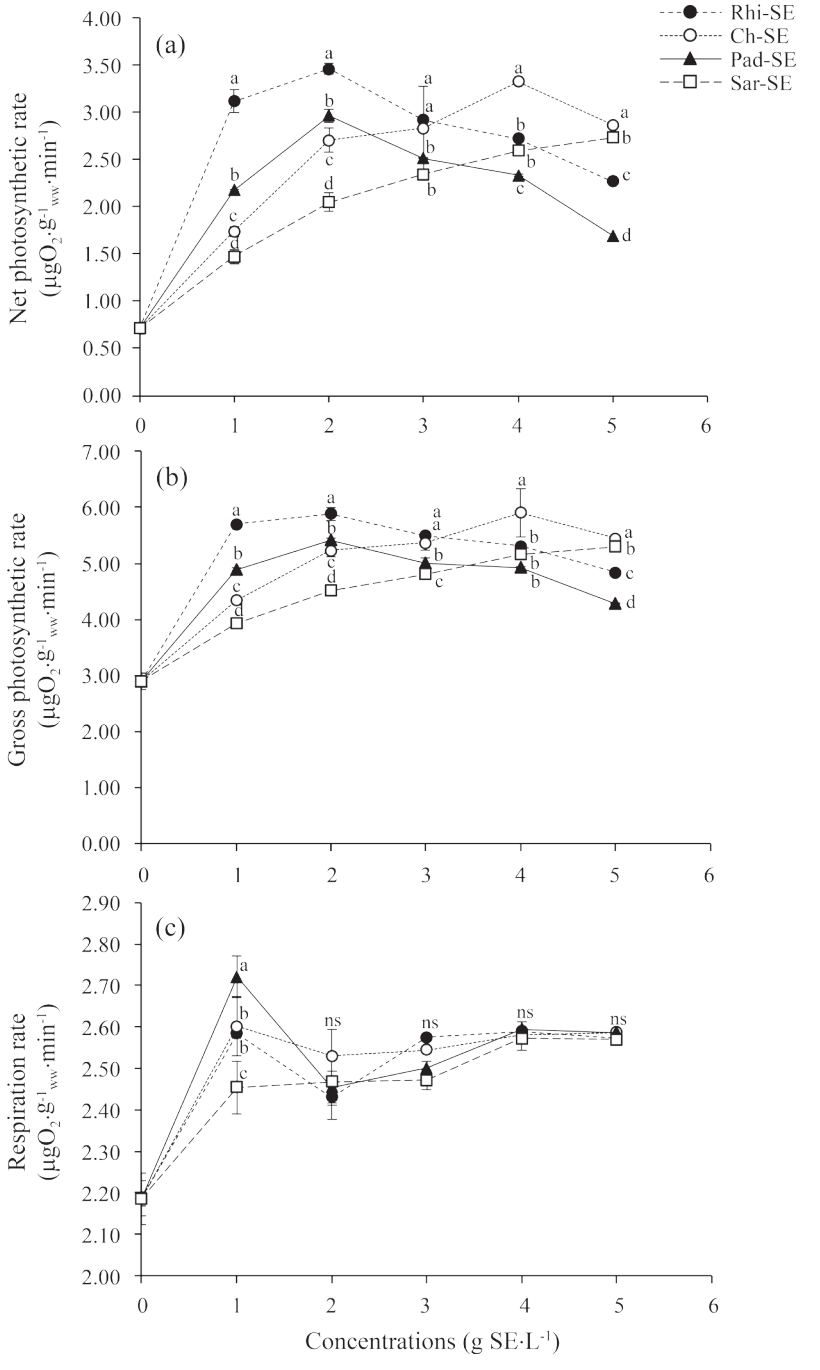

Figure 5. Response of *Gracilaria fisheri* treated with seaweed extracts from *Rhizoclonium riparium* (Rhi-SE), *Chaetomorpha crassa* (Ch-SE), *Padina* sp. (Pad-SE), and *Sargassum oiligocystum* (Sar-SE) at different concentrations of 0, 1, 2, 3, 4, and 5 g SE·L^{-1}: (a) P_{net}; (b) GPP; (c) R_d; Data are mean and error bars represent SD. Different lower case letters at each concentration denote significant differences ($p<0.05$).

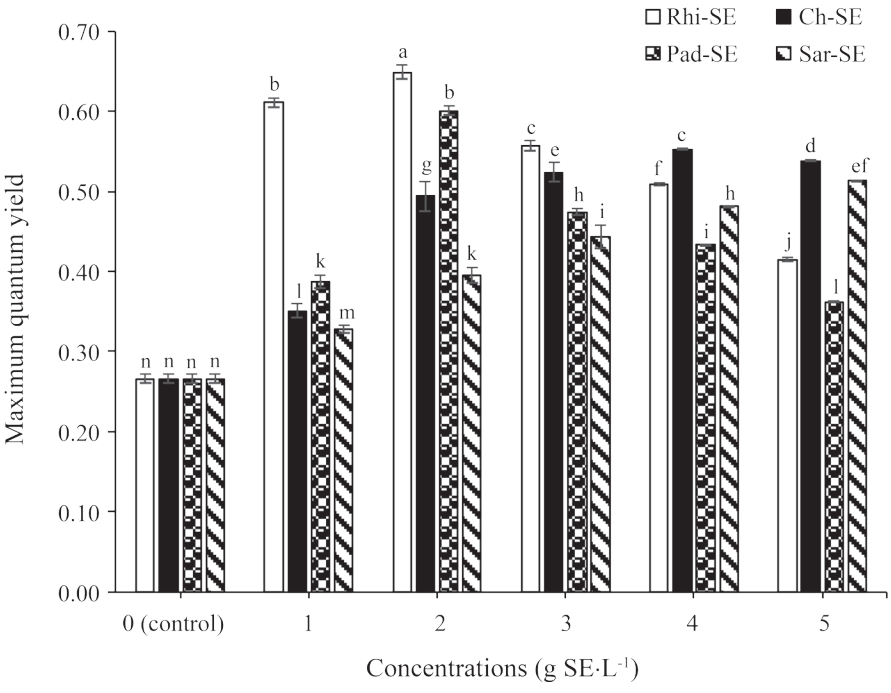

Figure 6. Maximum quantum yield (Fv/Fm) of *Gracilaria fisheri* treated with seaweed extracts from *Rhizoclonium riparium* (Rhi-SE), *Chaetomorpha crassa* (Ch-SE), *Padina* sp. (Pad-SE), and *Sargassum oiligocystum* (Sar-SE) at different concentrations (0, 1, 2, 3, 4, and 5 g SE·L^{-1}). Data are mean and error bars represent SD. Different lower case letters at each concentration denote significant differences ($p<0.05$).

DISCUSSION

Seaweed extracts are widely used as biostimulants in agricultural crops due to their growth-promoting and stress-resistant properties (Yao *et al*., 2020; Ali *et al*., 2021; Fitriyah *et al*., 2022). Several studies have shown that seaweed extracts can increase chlorophyll content and enhance photosynthetic capacity in treated plants (Spinelli *et al*., 2009; Kumari *et al*., 2011; Zermeño-Gonzalez *et al*., 2015). These extracts can induce physiological and biochemical changes associated with nutrient uptake and plant growth (Yao *et al*., 2020). In recent years, the use of *Ascophyllum nodosum* extract supplemented with 5% CO_2, has been reported to enhance the growth of *Gracilaria fisheri* (Chirapart *et al*., 2022). However, *G. fisheri* thalli have limited photosynthetic tolerance to light intensities of 100–203 µmol photon·m^{-2}·s^{-1} (Khreauthong *et al*., 2018). In this study, *G. fisheri* exhibited a curvilinear ETR at a PAR ranging from 25–285 µmol photon·m^{-2}·s^{-1}. However, when the PAR increased to 420 and 625 µmol photon·m^{-2}·s^{-1}, the ETR of the SE-treated algae increased by 9–35% compared to the untreated control. Notably, at a PAR of 625 µmol photon·m^{-2}·s^{-1}, treatment with 2 g SE·L^{-1} Rhi-SE or 5 g SE·L^{-1} Sar-SE increased the ETR by 35% and 19%, respectively. Both concentrations of the extract also improved the ETR compared to the other concentrations.

The ETR is often used to estimate the photosynthetic capacity, as demonstrated in studies on the brown seaweed *Cystoseira tamariscifolia* (Celis-Plá *et al*., 2017). In this study, the seaweed extracts Rhi-SE, Ch-SE, Pad-SE, and Sar-SE enhanced photosynthetic electron transport, thereby improving the photosynthetic capacity of *G. fisheri*. Similarly, Digruber *et al*. (2018) reported that *Ecklonia maxima* extract increased the electron transport rate of both PSI and PSII in willow plants.

Seaweed extracts have been shown to affect chlorophyll content and photosynthetic capacity in various plants (Blunden *et al*., 1996; Kumari *et al*., 2011; Zermeño-Gonzalez *et al*., 2015; Yao *et al*., 2020). For example, Blunden *et al*. (1996) reported that *A. nodosum* increased chlorophyll levels in leaves of treated plants. In this study, the P_{net} of the untreated control was lower (0.71 $\mu gO_2 \cdot g^{-1}_{ww} \cdot min^{-1}$) than previously reported (Khreauthong *et al*., 2018), where the P_{net} of *G. fisheri* peaked at 2.9 $\mu gO_2 \cdot g^{-1}_{ww} \cdot min^{-1}$ at a light saturation point of 203 $\mu mol\ photon \cdot m^{-2} \cdot s^{-1}$. However, under SE treatment, the P_{net} increased by 283% to 315% compared to the control, with maximum increases observed in Sar-SE (5 g $SE \cdot L^{-1}$), Pad-SE (2 g $SE \cdot L^{-1}$), Ch-SE (4 g $SE \cdot L^{-1}$), and Rhi-SE (2 g $SE \cdot L^{-1}$) treatments.

Lower concentrations (0, 1, and 2 g $SE \cdot L^{-1}$) of Rhi-SE and Pad-SE boosted both P_{net} and GPP of *G. fisheri*, while higher concentrations (3, 4, and 5 g $SE \cdot L^{-1}$) had the opposite effect. Similarly, Ch-SE treatments showed an increase in photosynthetic performance at concentrations up to 4 g $SE \cdot L^{-1}$, followed by a decline at 5 g $SE \cdot L^{-1}$. This pattern mirrors the findings of Kumari *et al*. (2011), who observed increased photosynthetic pigments in cucumber cotyledons with low doses of *Sargassum johnstonii* extract, followed by a decline at higher doses. Yao *et al*. (2020) similarly reported enhanced photosynthetic capacity in tomato plants treated with *S. horneri* extract, followed by a decline at higher dose. In contrast, the P_{net} and GPP of algae treated with Sar-SE consistently with higher doses reaching a peak at 5 g $SE \cdot L^{-1}$.

The increase in the net and gross photosynthetic rate, as well as respiration in *G. fisheri* is believed to be driven by the rise in chlorophyll content stimulated by low concentrations of seaweed extracts. The application of seaweed extracts has been reported to improve leaf photosynthetic capacity, largely due to the increase in photosynthetic pigments in plants (Al-Juthery *et al*., 2020; Yao *et al*., 2020; Hernández-Herrera *et al*., 2022). Spinelli *et al*. (2009) reported that using *A. nodosum* extract in apple trees increased leaf chlorophyll content by 12%, leading to a corresponding rise in photosynthesis and respiration rates. Similarly, Jannin *et al*. (2013) reported that *A. nodosum* extract increased chlorophyll content by promoting chloroplast biogenesis and reducing chlorophyll degradation in rapeseed plants, likely due to the upregulation of genes associated with photosynthesis, cell metabolism, stress response and sulfur (S) and nitrogen (N) metabolism. Di Stasio *et al*. (2017) also observed that seaweed extract improved plant nutritional status, enhanced photosynthetic rates, and increased chlorophyll content, contributing to more efficient translocation of minerals and assimilates to sinks, independent of nutrient concentrations in plants. A recent study by Hernández-Herrera *et al*. (2022) showed an increase in growth of tomato plants treated with a seaweed extract from *Padina gymnospora*, attributed to the presence of growth-promoting substances, total carbohydrates, proline and phenolic compounds in the extract.

The improvements in photosynthetic rates in *G. fisheri* under SE treatment may be attributed to the presence of growth-promoting hormones and compounds in the extracts. Growth hormones are known to involve in the regulation of photosynthesis and photoprotection under high light stress (Müller and Munné-Bosch, 2021). The plant growth hormones such as auxins, gibberellins, cytokinin, abscisic acid, and betaine have been found in both green and brown seaweed extracts (Panda *et al*., 2012; Ghaderiardakani *et al*., 2019). Betaines, in particular, mimics cytokinin activity and causes physiological changes when applied at low concentration (Panda *et al*., 2012). Betain in seaweed extract has been shown to increase chlorophyll levels (Whapham *et al*., 1993; Blunden *et al*., 1996), inhibit chlorophyll degradation, and suppress the loss of photosynthetic activity in plants (Genard *et al*., 1991; Ali *et al*., 2021). In this study, the increased photosynthetic activity in the *G. fisheri* treated with the four seaweed extracts may be due to the presence of growth-promoting compounds as well as plant growth hormones in the seaweed extracts. The four seaweed extracts used in this study also enhanced growth and induced branching of the *Gracilaria* species (data will be reported elsewhere).

Additionally, changes in pH can influence photosynthesis in freshwater algae (Dodd and Bidwell, 1971; Haraguchi and Zheng, 2022). Dodd and Bidwell (1971) observed that the flow of carbon into photosynthetic intermediates in *Acetabularia*

mediterranea was strongly influenced by pH, with maximal photosynthesis occurring at pH 7.6 to 7.7. Haraguchi and Zheng (2022) noted high dark respiration rates in *Euglena mutabilis* (Euglenophyta) at pH 7. In our study, the pH of the seaweed extracts varied slightly, ranging from 7.7 to 8.5, which may have contributed to the observed variations in photosynthesis in *G. fisheri*. However, further research is needed to fully understand the impact of seaweed extract pH on photosynthesis in this species.

The maximum quantum yield (Fv/Fm) of *G. fisheri* increased with SE treatment in this study. In the Rhi-SE and Pad-SE treatments, the Fv/Fm increased by 56% and 36%, respectively, with maximum values reaching 144% and 126%. Similarly, Ch-SE treatment resulted in increases ranging from 32% to 108%. In contrast, treatment with Sar-SE reduced Fv/Fm by 23% at 1 g SE·L^{-1} and by 93% at 5 g SE·L^{-1}. These increases in Fv/Fm were greater than the 12.4% increase in alfalfa plants treated with *Ecklonia maxima* extract (Sosnowski *et al*., 2019). Fv/Fm has been used as an indicator of photoinhibition because of its ability to balance photodamage and repair (Celis-Plá *et al*., 2017; Figueroa *et al*., 2017). A previous study showed that increasing Fv/Fm is associated with greater PSII activation, resulting from a lack of photoinhibition in nitrogen-deficient plant cells; thus, the energy required for electron transport did not decrease (Laisk *et al*., 2014). Similarly, the increase in Fv/Fm in the SE treatment in this study suggests that the seaweed extracts may help prevent photoinhibition in the cells of *G. fisheri*.

CONCLUSIONS

This study is the first to report the photosynthetic response of *Gracilaria fisheri* to Thai seaweed extracts. The extracts of four seaweed species (Chi-SE, Rhi-SE, Pad-SE and Sar-SE) effectively enhanced the photosynthetic activity of *G. fisheri*. Seaweed extracts could increase the photosynthetic electron transport of this seaweed. Treatment with Rhi-SE and Pad-SE increased the net and gross photosynthesis and respiration of *G. fisheri* at low concentrations (1–2 g SE·L^{-1}), which decreased as the concentration of the extracts increased (3–5 g SE·L^{-1}). Ch-SE increased the net and gross photosynthetic rates as the concentration increased from 1 to 4 g SE·L^{-1} but decreased when the concentration increased to 5 g SE·L^{-1}. In contrast to those in the Sar-SE treatment, the net and gross photosynthesis of the algae increased as the extract concentration increased from 1 to 5 g SE·L^{-1}. The net photosynthesis of the *G. fisheri* plants treated with the seaweed extracts increased two- to fivefold compared with that of the non-SE-treated control plants. Similarly, the maximum quantum yield of the algae treated with the four seaweed extracts increased, with the greatest increase in the Rhi-SE treatment, followed by the Pad-SE, Ch-SE, and Sar-SE treatments. Our study suggested that the application of the four seaweed extracts promoted photosynthesis in *G. fisheri* cells. However, further research is needed to elucidate the effects of these seaweed extracts on *G. fisheri* under various stress conditions, as well as the active components in the extracts that enhance the photosynthetic performance and growth of this seaweed.

ACKNOWLEDGEMENTS

This research was supported by the Graduate Scholarship of the Graduate School, Kasetsart University. Special thanks to anonymous reviewers whose remarks helped to improve this paper.

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