Light Quality Influences on Early Developmental Stage of a Tropical Seagrass, *Enhalus acoroides*: Growth and Physiological Responses

Muhammad Heemboo[1,2], Chongdee Thammakhet-Buranachai[3,4], Fonthip Makkliang[5] and
Pimchanok Buapet[1, 2 *]

ABSTRACT

The impact of light quality on the early development of the seagrass *Enhalus acoroides* remains poorly understood. To bridge this gap in knowledge, we conducted aquarium-based experiments to assess the growth and physiological responses of *E. acoroides* seeds from germination to seedling development over 15 days. We subjected the seeds to different light conditions, including darkness, white light (λ = 460−630 nm), red light (peak λ = 630 nm), and blue light (peak λ = 460 nm), all at an intensity of 100 µmol photons·m^{-2}·s^{-1}. Our results revealed unique responses in darkness and red light. Darkness stimulated shoot and root elongation, while red light resulted in the lowest shoot/root ratio. Seedlings grown in darkness displayed suppressed photosynthetic activity, as evidenced by the lowest maximum quantum yield, electron transport rates, and initial slope of the light response curve. These seedlings also had the lowest photosynthetic pigments and xanthophyll cycle-related attributes. On the other hand, red light promoted high light responses, as indicated by the lowest chlorophyll *b/a* ratio (an indicator of the size of the light-harvesting antenna) and the highest de-epoxidation state of xanthophyll (an indicator of photoprotective effort). Our findings highlight the significant impact of changes in light quality on the growth and physiological performance of *E. acoroides* during early development. Interestingly, white light and blue light resulted in similar outcomes. More research is necessary to shed light on the underlying mechanisms driving these responses and the implications for seagrass adaptation to marine environments.

Keywords: Growth, Light quality, Photosynthesis, Seagrass

INTRODUCTION

The growth of plants during the germination and early development stages is influenced by red and blue light (Chory *et al*., 1996; Spaninks *et al*., 2020). Many studies have demonstrated that red light promotes seedling germination and growth in various species (Rehman *et al*., 2020; Spaninks *et al*., 2020; Li *et al*., 2021) and that the red to far-red ratio regulates several physiological processes, including acquisition of nutrients and stress tolerance (Demotes-Mainard *et al*., 2016). Conversely, blue light plays a role in plant physiology and growth aspects, such as stomatal opening and developmental phase transitions (Inoue and Kinoshita, 2017; Spaninks *et al*., 2020). Photoreceptor-mediated blue light responses include de-etiolation, floral initiation, phototropism and diurnal rhythm (Lin, 2000).

---

[1] Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand
[2] Coastal Oceanography and Climate Change Research Center, Prince of Songkla University, Songkhla, Thailand
[3] Division of Physical Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand
[4] Center of Excellence for Trace Analysis and Biosensor, Prince of Songkla University, Songkhla, Thailand
[5] School of Languages and General Education, Walailak University, Nakhon Si Thammarat, Thailand
[*] Corresponding author. E-mail address: pimchanok.b@psu.ac.th
Received 20 April 2023 / Accepted 23 June 2023

Photoprotection is a crucial process that enables plants to avoid photodamage caused by excessive light and redox imbalance (Duan *et al*., 2020). Recent studies have shown that blue light plays a significant role in photoprotection by triggering the production of xanthophyll pigments (Samuolienė *et al*., 2021) and by increasing the abundance of the PsbS protein (Duan *et al*., 2020), which facilitates excess light energy dissipation. Additionally, blue light also induces chloroplast avoidance movement (Kagawa and Wada, 2002). However, the phenomenon of chloroplast movement has not yet been observed in larger seagrasses such as *Enhalus* and *Thalassia*, despite its importance as an essential photoprotective mechanism observed in seagrasses belonging to the genus *Halophila* (Phandee and Buapet, 2018; Buapet *et al*., 2020; Saewong *et al*., 2022). On the other hand, the contribution of red light to photoprotection remains unclear. When present in excess, both red and blue light can generate reactive oxygen species that can lead to photodamage (Shengxin *et al*., 2016; 2018; Hamdani *et al*., 2019), although some studies showed that blue light could be more harmful than red light (Ohnishi *et al*., 2005; Hamdani *et al*., 2019). Therefore, an optimal balance of light quality is critical for promoting plant growth and development while minimizing the risk of photodamage (Shengxin *et al*., 2016; 2018; Zheng and Van Labeke, 2017).

Coastal areas, estuaries, and lagoons are among the shallow marine environments where seagrasses, a group of flowering plants, grow (Short *et al*., 2007). The seagrasses play critical ecological roles as habitat and food for various marine animals, and they also help stabilize sediments and reduce erosion (Nordlund *et al*., 2016). The light spectrum in underwater environments differs from terrestrial habitats (Soong *et al*., 2013; Strydom *et al*., 2017a; 2017b; Cussioli *et al*., 2020). In shallow coastal waters, the abundance of red and blue light can vary depending on many factors, including water depth, water quality, sediment type and coastal activities (Strydom *et al*., 2017a; 2017b; Cussioli *et al*., 2020). The abundance of red light is typically higher at the water's surface, but it rapidly diminishes as the depth increases due to absorption by the water column and suspended particles (Soong *et al*., 2013). Blue light, on the other hand, can penetrate deeper into clear water, but its transmission in coastal and estuarine waters is often constrained by phytoplankton, chromophoric dissolved organic matter, and detritus, which absorb the blue light (Kirk, 2011). Therefore, existing knowledge from terrestrial models may not directly apply to seagrasses due to the distinct light spectra and environmental conditions found in underwater environments. Seagrasses have evolved to photosynthesize underwater, and their adaptation to this unique environment is reflected in the genome of *Zostera marina*, which has lost multiple genes encoding photoreceptors such as UV resistance locus 8, and cryptochromes 2 and 5 through evolutionary processes (Olsen *et al*., 2016). As a result, changes in the light spectrum may have different effects on seagrasses compared to terrestrial plants. Although seagrasses are affected by variations in light quality, how they detect and respond to these changes is not fully understood and remains a subject of ongoing research.

In this study, we evaluated the responses of *Enhalus acoroides*, a common seagrass species in tropical and subtropical coastal waters of the Indian and Pacific Oceans (Kongrueang *et al*., 2018; Artika *et al*., 2020; Liu *et al*., 2023), during seedling development under four distinct treatments: darkness, white light, red light, and blue light. We emphasized examining the plant's response during the stages of seed germination and seedling development, given their high susceptibility to environmental influences (von Arnim and Deng, 1996). This study aims to enhance our understanding of light signaling in *E. acoroides* and provide valuable insights into the species' adaptations to marine environments.

MATERIALS AND METHODS

Experimental design

A comprehensive laboratory experiment was conducted to examine the impact of light quality on the early growth stage of *Enhalus acoroides*. The research was conducted at the Division of Biological Science, Faculty of Science, Prince of Songkla University, Thailand, using an indoor aquarium system.

Seeds of *E. acoroides* were sourced from a mangrove rehabilitation center in Songkhla Province, Thailand. These seeds were placed in aquarium tanks measuring 30×20×15 cm, filled with artificial seawater (salinity = 30 psu, pH = 8.1), and allowed to acclimate in complete darkness for one night. The tanks were maintained at an ambient temperature, ranging from 28–31 °C, to ensure optimal conditions for acclimation.

Subsequently, the experiment proceeded in the same setup, spanning 15 days. During this period, the seedlings were exposed to four different light quality treatments: darkness, white light (wavelength between 460 and 630 nm), red light (with a peak wavelength at 630 nm), and blue light (with a peak wavelength at 460 nm). Irradiance was provided by light-emitting diodes (LEDs). The irradiance level was set at 100 μmol photons·m^{-2}·s^{-1}, with a photoperiod of a 12-h light and 12-h dark cycle. Each treatment tank was covered with a dark shade to prevent interference from other light sources.

Growth-related attributes

Throughout the 15-day experiment, the growth-related attributes of the *E. acoroides* seedlings were measured. Specifically, we recorded the shoot length and root length on days 0, 2, 4, 6, 9, 12, and 15. To obtain these measurements, we carefully removed the seedlings from the aquarium tanks and positioned them on a flat surface. Utilizing a ruler, we measured the length of the longest shoot and root, measuring from the base to the tip in millimeters. Each measurement was taken under the respective light conditions, except for darkness, where a subdued white light was applied.

At the conclusion of the investigation, the growth rates for both shoot and root were calculated by dividing the change in length by the number of days the experiment lasted (15 days). This allowed us to determine the elongation rate per day for shoot and root. Additionally, we calculated the shoot-to-root ratio for each treatment, which provided insights into the allocation of resources between shoot and root growth in *E. acoroides* seedlings. A higher shoot-to-root ratio would indicate a greater emphasis on shoot growth, while a lower ratio would suggest a preference for root growth. Growth measurements were collected from 12 replicates.

Photosynthetic responses

Upon concluding the experiment, we evaluated the photosynthetic responses of *E. acoroides* seedlings. Initially, we assessed the maximum quantum efficiency of Photosystem II (PSII) by calculating the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm). To ensure accurate measurements, a dark adaptation period of 20 min was provided to allow the seedlings to reach a steady state. Subsequently, Fv/Fm was determined using the Mini-PAM fluorometer (Walz, Effeltrich, Germany). This was achieved by applying a saturating pulse of light to induce maximum fluorescence and employing the formula $(Fm-F_0)/Fm$, where Fm represents the maximum fluorescence, and F_0 represents the minimum fluorescence in the dark-adapted state.

Furthermore, we evaluated the electron transport rates (ETR) in *E. acoroides* seedlings using the rapid light curve (RLC) approach described by Ralph and Gademan (2005). To accomplish this, we exposed the seedlings to a series of photosynthetic active radiation (PAR) levels, ranging from 33 to 1,472 μmol photons·m^{-2}·s^{-1}, using an optical fiber. Each PAR step lasted for 10 s. The ETR was determined by multiplying the effective quantum yield of PSII (ϕPSII) by PAR, 0.5 (considering an even distribution of absorbed photons between photosystems I and II), and the absorption factor (AF) of *E. acoroides* leaves, as reported by Kongrueang *et al.* (2018). The ETR plots were fitted against irradiance using the equation proposed by Platt *et al.* (1980), enabling us to derive the photosynthetic parameters such as asymptotic maximum levels of ETR (ETR_{max}), initial slope of the light response curve indicating photosynthetic efficiency (α), and minimum saturating irradiance (E_k). The RLC measurements were performed on six replicates.

Pigment analyses

To analyze the pigment composition in the leaves of *E. acoroides* seedlings, we employed the method outlined by Buapet *et al*. (2017). The measurement of total chlorophylls and carotenoids offers a comprehensive evaluation of the photosynthetic pigments, which collectively contribute to the plants' photosynthetic capacity.

To determine total pigment content, frozen leaves from each treatment group were homogenized using a mortar and pestle in 80% acetone. Subsequently, the supernatant was collected after centrifugation. The absorbance of the supernatant was measured at specific wavelengths (470 nm, 647 nm, and 664 nm) using a spectrophotometer (UV 1720, Yoke Instrument, China). The chlorophyll *a* and *b* content were calculated according to the method described by Porra (2002), while the carotenoid content was determined following the procedure outlined by Lichtenthaler and Wellburn (1983). The pigment contents were expressed as $\mu g \cdot g^{-1}$ plant fresh weight. These measurements were performed on six replicates.

Additionally, we examined the xanthophyll pigment content in the leaves of *E. acoroides* seedlings. Xanthophyll pigments are a specific type of carotenoid pigments that play a vital role in photoprotection by dissipating excess light energy from the photosystems' light-harvesting antenna. The levels of xanthophyll cycle pigments, namely violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z), were determined using high-performance liquid chromatography (HPLC) with a diode array detector (DAD) following the protocol described by Buapet *et al*. (2017).

To prepare the seagrass leaves for analysis, they were ground in 90% HPLC-grade acetone under low light conditions, and the resulting supernatant was collected following centrifugation. HPLC analysis was conducted using a Hewlett-Packard 1200 series system, with data acquisition and evaluation performed using Agilent Chem Station software version 1200 series. To enhance the HPLC analysis, a mixed standard solution (CaroteNature, Lupsingen, Switzerland) of V, A, and Z with a concentration of 2.0 $\mu g \cdot mL^{-1}$ and a volume of 20 µL was utilized. Optimal operating conditions for the HPLC system were determined as follows: isocratic elution with a constant flow rate of 0.60 $mL \cdot min^{-1}$, employing acetone: DI water (93:7) mobile phase, a detection wavelength of 450 nm, and a column temperature of 21 °C. The retention times for V, A, and Z were identified as 5.77, 6.29, and 6.95 min, respectively. The standards were utilized for pigment identification through co-chromatography. The xanthophyll pigment content was expressed as $\mu g \cdot g^{-1}$ plant fresh weight. The de-epoxidation state (DES) of the xanthophyll cycle was estimated as the ratio of the sum of antheraxanthin (A) and zeaxanthin (Z) to the total xanthophyll pigments (V+A+Z). These measurements were performed on six replicates.

Statistical analyses

The lengths of the leaves and roots were analyzed using repeated-measures ANOVA, with treatments as the categorical factor and days of measurements as the within-group factor. Post-hoc comparisons were conducted using the HSD (Honestly Significant Difference) test to compare the leaf and root lengths across treatments and days of measurements.

The data on elongation rates, shoot-to-root ratios, maximum quantum yield, rapid light curves parameters, and photosynthetic and xanthophyll pigment contents were analyzed using one-way ANOVA. Post-hoc comparisons were conducted using the HSD test to compare these parameters across treatments. The software used for all analyses was Statistica Academic.

RESULTS

Growth-related attributes

All treatments in the experiment exhibited 100% germination rates for *Enhalus acoroides*. The shoot and root lengths of the seedlings were significantly influenced by the treatments during the 15-day experiment (Figure 1a, 1b). Specifically, the seedlings exposed to darkness exhibited the highest shoot elongation rates (Figure 1a, 1c). There were no significant observable distinctions among the various light-quality treatments (white, red, and blue). Furthermore, the root lengths of *E. acoroides* seedlings were consistently higher in darkness compared to the other light-quality treatments, starting from day 9 and continuing until the end of the experiment, with root lengths in darkness comparable to those in red light at the end of the experiment (Figure 1b, 1c). Notably, the shoot-to-root ratio was significantly lower in the red-light treatment (1.95±0.09) compared to the other treatments, indicating a higher investment in root growth under red-light conditions (Figure 1d).

Photosynthetic responses

The rapid light curve (RLC) measurements revealed that the light use characteristics of *E. acoroides* seedlings were not affected by the treatments of light quality (Figure 2a, Table 1). However, a significant difference was observed in darkness, where seedlings exhibited the lowest ETR$_{max}$, indicating a reduced capacity for photosynthetic electron transport (Table 1).

Table 1. Mean±SD of parameters derived from the rapid light curves (RLC) determined at the end of the experiment.

Parameter	Dark	White	Red	Blue
ETRmax (μmol·m^{-2}·s^{-1})	8.09±0.54^{a}	13.13±1.90^{b}	12.43±1.29^{b}	12.33±0.86^{b}
Alpha (electrons·quanta^{-1})	0.04±0.02^{a}	0.33±0.12^{b}	0.30±0.12^{b}	0.31±0.07^{b}
Ek (μmol photons·m^{-2}·s^{-1})	204.97± 27.68^{a}	44.88±8.49^{b}	45.56±7.44^{b}	40.81±4.06^{b}

Note: Means within a row with different superscripts differ at $p<0.05$ (HSD test).

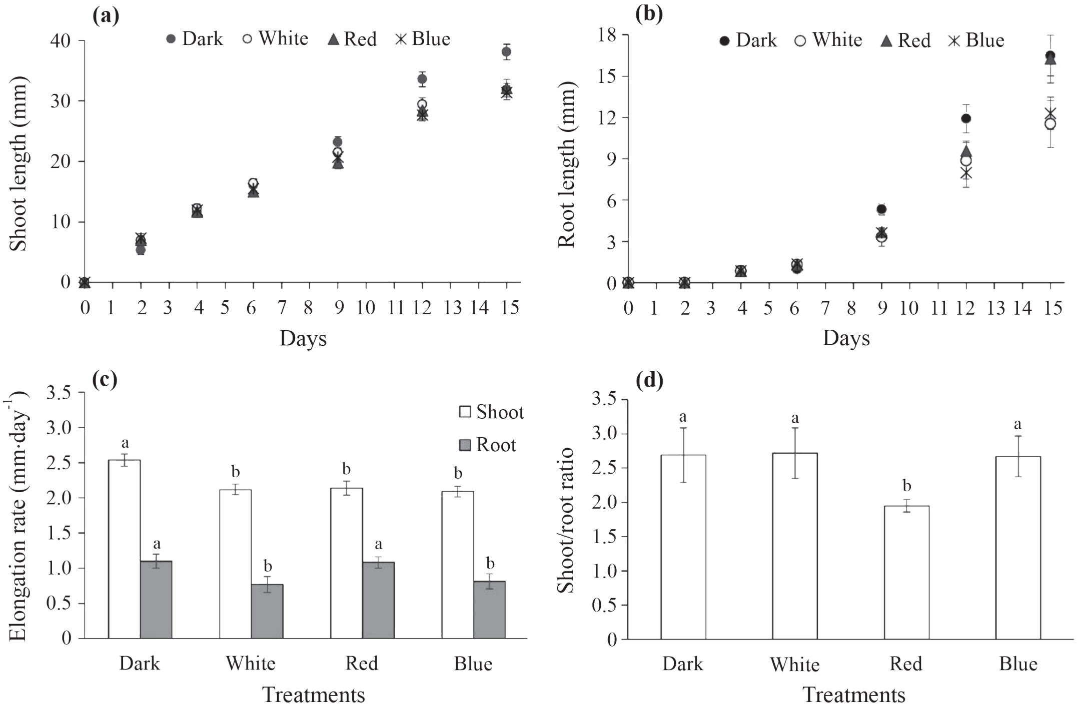

Figure 1. The effects of light quality on growth-related attributes of *Enhalus acoroides*: (a) time course of shoot length, (b) time course of root length, (c) elongation rates of shoot and root calculated at the end of the experiment, and (d) shoot/root ratio calculated at the end of the experiment. Different letters above histogram bars denote significant difference between treatments (HSD test, $p<0.05$). Error bars represent standard deviation.

Furthermore, the initial slope of the light response curve (α), representing photosynthetic efficiency, was also reduced. In contrast, the highest minimum saturating irradiance (E_k), which indicates the light level at which photosynthesis becomes saturated, was increased (Table 1). These findings suggest that darkness suppressed the photosynthetic performance of *E. acoroides* seedlings by lowering the electron transport rate and photosynthetic efficiency and increasing the light level required for saturation.

Similarly, the maximum quantum efficiency of PSII, as represented by Fv/Fm, was not influenced by the treatments of light quality (Figure 2b). The lowest Fv/Fm values (0.67±0.01) were observed in seedlings exposed to darkness (Figure 2b).

Pigment analyses

The photosynthetic pigment content was significantly influenced by treatments, as shown in Figure 3a. Seedlings exposed to red light exhibited

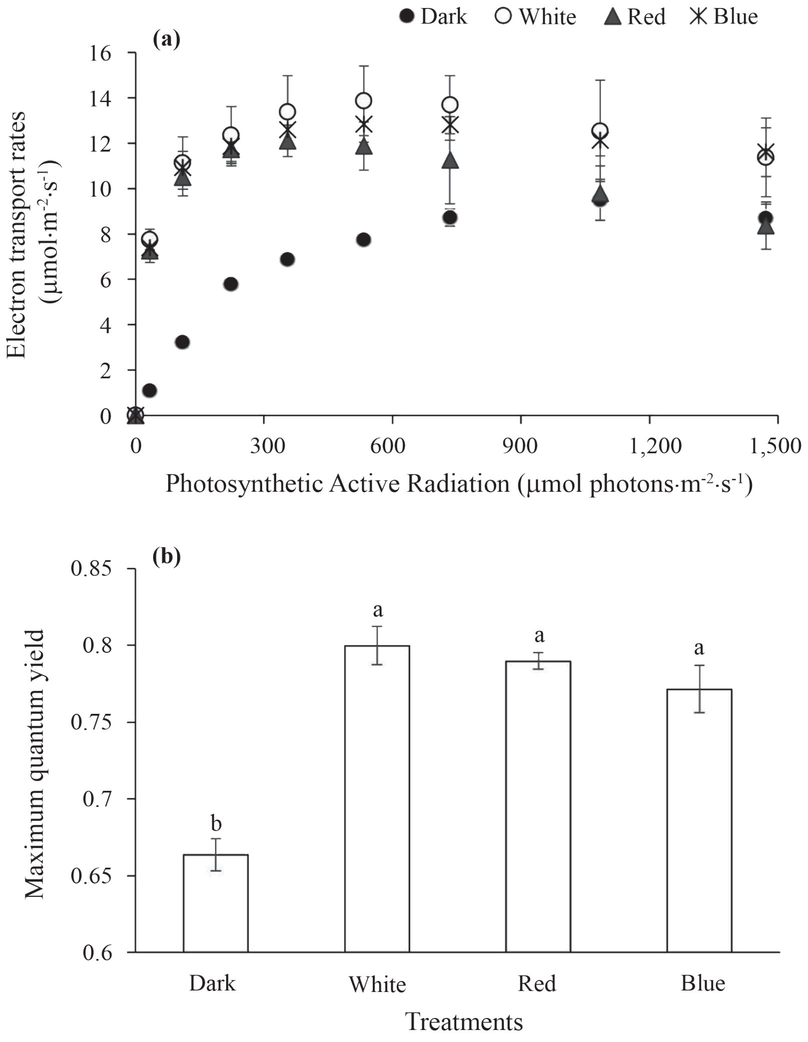

Figure 2. Effects of light quality on the photosynthetic characteristics of *Enhalus acoroides*: (a) rapid light response curves and (b) maximum quantum yield of photosystem II. Different letters above histogram bars denote significant difference between treatments (HSD test, $p<0.05$). Error bars represent standard deviation.

the highest chlorophyll *a* content, followed by white and blue light, while the lowest content was detected in darkness. Although the chlorophyll *b* content was similar among the different light treatments, it was lower in red light and lowest in darkness. The ratio of chlorophyll *b* to chlorophyll *a*, which serves as an indicator of the size of the light-harvesting antenna, was lowest in the red light treatment, and significantly different from the ratio measured in white light. Carotenoid content in *E. acoroides* significantly differed between seedlings grown in white light and darkness, with lower levels detected in darkness.

The xanthophyll pigments violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z), were identified in the leaves of *E. acoroides* seedlings, and their levels exhibited significant differences among the treatments, as depicted in Figure 3b. The xanthophyll pigments and the de-epoxidation state of the xanthophyll cycle (DES) were lowest in seagrass grown in darkness. The levels of V, A, and Z did not differ significantly among the various light qualities, but the DES was highest in the red light treatment (0.81±0.01).

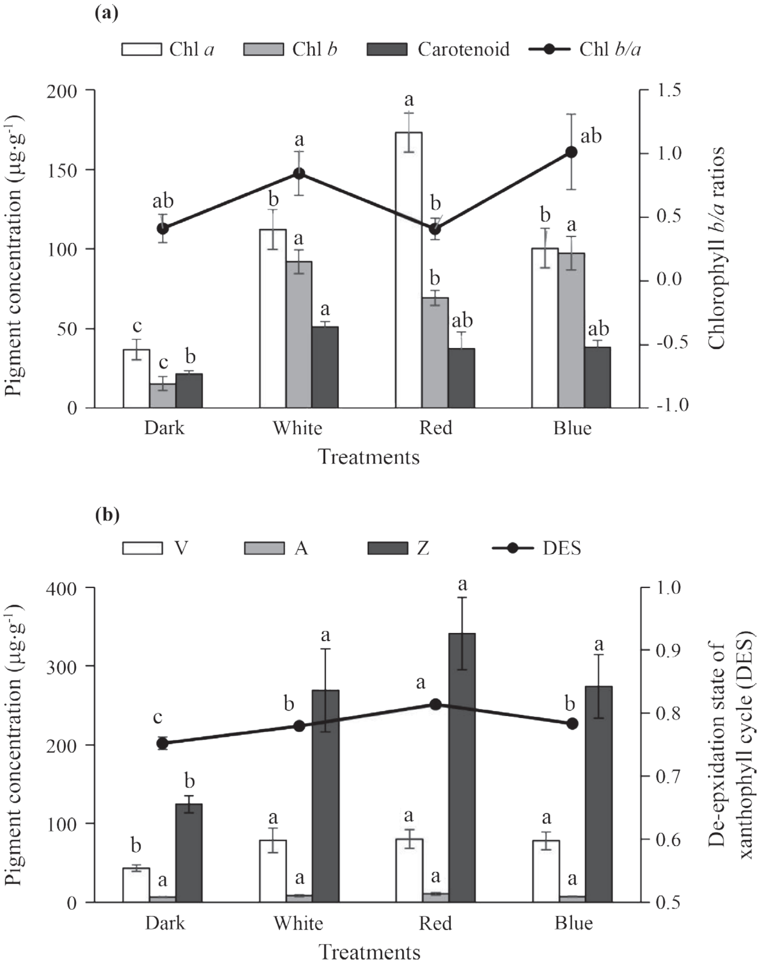

Figure 3. Effects of light quality on pigment contents of *Enhalus acoroides* determined at the end of the experiment: (a) chlorophyll *a* (Chl *a*) chlorophyll *b* (Chl *b*) carotenoid and the ratios of Chl *b/a* (b) xanthophyll pigments consisting of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) and de-epoxidation state of xanthophyll cycle. Error bars represent standard deviation. Different letters denote significant treatment differences (HSD test, $p<0.05$).

DISCUSSION

The germination rate of *Enhalus acoroides* seeds was observed to be 100% across all treatments. This finding appears to differ from the results reported by Soong *et al*. (2013), who found that *Thalassia hemprichii* seeds had a germination rate of only ~55% in white and blue light, and ≤20% in red light and darkness. In a recent study conducted by Liu *et al*. (2023), *E. acoroides* seeds had a 75% germination rate under 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white light, but this rate was reduced to 25% under burial. This suggests that the response of seed germination can vary not only among different species but also among populations. The process of seagrass seed germination is multifaceted and subject to the influence of numerous factors, such as dissolved oxygen, temperature, sediment nutrient and organic matter levels, and other related factors (Artika *et al*., 2020; Liu *et al*., 2023).

Seeds of *E. acoroides* depend on the cotyledon for germination (Kausik, 1940). A reduction in light availability is expected to hinder photosynthesis in young leaves and limit the use of stored nutrients from the cotyledon. Moreover, according to Liu *et al*. (2023), there is a possibility that *E. acoroides* seeds possess photosynthetic capabilities, similar to what has been documented in *Posidonia oceanica* (Celdran and Marín, 2013) and *Thalassia testudinum* (Celdran, 2017). As a result, germination success rate may be affected, and seedling growth rates may be impaired in darkness. Unexpectedly, leaf elongation rates in the present study were found to be highest in darkness, whereas growth rates of shoots and roots were comparable across light treatments. In the darkness, plants may allocate the limited nutrient reserves stored in their cotyledons towards the elongation of their leaves to reach the light source. This process, called etiolation, enables plants to optimize their photosynthetic efficiency and survival in light-limited environments (Jedynak *et al*., 2022). Other model plants that rely on cotyledons for germination, such as peas, have shown that while seedling development can progress at a similar rate in both dark and light conditions, it may eventually halt at an underdeveloped stage (Low, 1971). Therefore, it is likely that the elongation process would also stop if the experiment were prolonged.

The experiment results showed that seedlings grown under red light had the lowest shoot/root ratio. This finding is consistent with the trait of sun-adapted plants identified by Givnish (1988), which is characterized by a lower shoot/root ratio. However, it is important to note that different seagrass species exhibit varying shoot/root ratio trends along a depth gradient (Olesen *et al*., 2002; Terrados *et al*., 2006; Collier *et al*., 2007). Furthermore, it should be pointed out that the redox state of the root environment can significantly impact plant metabolism (Terrados *et al*., 1999). As a result, the growth response of seagrass seedlings rooted in the anaerobic sediments of their natural habitat may diverge from the outcomes observed in our experimental setup. The results indicate that plants across all light treatments exhibited similar responses in their light response curves, whereas those grown in darkness displayed distinct characteristics. Specifically, the seedlings grown in darkness showed lower maximum electron transport rates and higher saturating irradiance, consistent with the attributes of shade-adapted plants (Statton *et al*., 2018; Wutiruk *et al*., 2022). However, it is noteworthy that the photosynthetic efficiency of the seagrass grown in darkness was significantly lower, as indicated by the lower alpha value, which is likely associated with low pigment content. Indeed, the photosynthetic pigment content of the seagrass grown in darkness was lowest (Figure 3a), which is expected, as light is a crucial environmental cue for the biosynthesis of chlorophyll (Yuan *et al*., 2017) and carotenoid (Llorente *et al*., 2017).

The phenomenon of a lower maximum quantum yield (Fv/Fm) in plants that are germinated and grown in darkness has been widely documented in various studies (Garmash *et al*., 2013; Lepeduš *et al*., 2017). This decrease in Fv/Fm is most likely due to the failure of the plants to develop a fully functional photosynthetic apparatus rather than any photodamage (Garmash *et al*., 2013; Wang *et al*., 2020). However, it has been observed that exposing these plants to light can increase their Fv/Fm values, with some studies reporting values of 0.7−0.8 (Garmash *et al*., 2013; Lepeduš *et al*., 2017). This suggests that the effects of darkness on Fv/Fm may be reversible.

Of the three light treatments (white, red, and blue), the seedlings grown under red light exhibited the most distinctive pigment profile. Specifically, these seedlings had a lower chlorophyll *b/a* ratio and a higher de-epoxidation state of xanthophylls, the characteristics of high light-adapted plants. Under excess light, plants tend to have a smaller light-harvesting antenna, as this reduces the amount of excitation energy and electron transport, which can help prevent photoinhibition (Ort *et al*., 2011). The ratio of chlorophyll *b* to chlorophyll *a* is frequently employed as an indicator of the light-harvesting antenna size (Terashima and Hikosaka, 1995), and it has been shown to be responsive to changes in the light environment in seagrasses in many studies (Dattolo *et al*., 2014; Sandoval-Gil *et a*l., 2014; Procaccini *et al*., 2017). Therefore, the lower chlorophyll *b/a* ratio observed in the seedlings grown under red light indicates that these plants had a smaller light-harvesting antenna, which may have helped them better cope with high-light conditions.

The higher de-epoxidation state of xanthophylls observed in the red light treatment suggests that these seedlings had a more effective photoprotective mechanism against excess light. Specifically, zeaxanthin, a product of the xanthophyll cycle that is formed through the de-epoxidation of violaxanthin via antheraxanthin, plays a critical role in dissipating excess absorbed light energy through thermal processes (Demmig-Adams *et al*., 2012). The ratio of (A+Z)/(V+A+Z) signifies the de-epoxidation state of xanthophylls (DES), which reflects the activity of the xanthophyll cycle. The DES values observed under red light treatment were comparable to those observed during mid-day in the seagrass species *Halophila ovalis* and *Cymodocea rotundata* growing in the upper-intertidal areas (Buapet *et al*., 2017), indicating that *E. acoroides* has a high capacity and effective xanthophyll cycle even at the seedling stage. The success of the high-light adaptations was further supported by the absence of photoinhibition, as indicated by an Fv/Fm value of almost 0.8, a value typically associated with healthy plants (Ralph, 1999).

There have been inconsistent findings in prior research regarding the impact of light quality on seagrass growth and physiology. For example, Strydom *et al*. (2017b) found that *Halophila ovalis* grown under blue light had lower germination and growth but higher alpha, while red light stimulated germination. In *Posidonia australis*, blue light increased alpha and decreased chlorophyll *b/a*, while red light increased the leaf productivity of seedlings (Strydom *et al*., 2018). Soong *et al*. (2013) reported that germination rates under blue light were similar to those under white light, while red light inhibited germination in *Thalassia hemprichii*. Ma *et al*. (2021) conducted a study on *Zostera marina*, examining the expression of CHS, a crucial gene in anthocyanin biosynthesis and photoprotection, in response to different light qualities. Their findings indicated that the expression of ZosmaCHS was weaker under blue light than in red light. One possible explanation for these findings is the lack of blue light photoreceptors in the seagrass *Z. marina* due to the absence of cryptochromes 2 and 5 in its genome (Olsen *et al*., 2016). It is possible that this same explanation may also account for the comparatively weak response to blue light in *E. acoroides*.

Additionally, different seagrass species may exhibit distinct responses to light quality, potentially due to differences in their light environment. For instance, *Z. marina* and *P. australis* dominate deeper habitats where blue light is the dominant spectral component, while shallow seagrass habitats feature a higher proportion of red light (Kirk, 2011; Olsen *et al*., 2016; Strydom *et al*., 2018). Moreover, the responses of seagrass species to changes in light quality may be influenced by variations in their life traits. For instance, *Enhalus* is considered a persistent group of species and tends to exhibit higher physiological tolerance than colonizing species (Kilminster *et al*., 2015). However, the specific underlying mechanisms responsible for these varying responses to light quality among seagrass species are yet to be fully explored.

CONCLUSION

Our study reveals that red light significantly impacts the physiology of *Enhalus acoroides* during its early developmental stage by modulating the levels of photosynthetic pigments and photoprotective compounds. Notably, the seedlings exposed to blue light demonstrated similar responses to those exposed to white light. Nevertheless, various light qualities had no significant impact on plant growth rates. These results offer insight into how seagrasses regulate photosynthesis in response to light quality and its potential association with light perception mechanisms.

ACKNOWLEDGEMENTS

We acknowledge the Development and Promotion of Science and Technology Talent Project, Thailand, for providing financial support to MH and the Faculty of Science, Prince of Songkla University, for supporting PB in this research. We would also like to express our appreciation to Assistant Professor Dr. Chalongrat Daengngam for assisting us with the wavelength screening of the light source.

LITERATURE CITED

Artika, S.R., R. Ambo-Rappe, M. Teichberg, A. Moreira-Saporiti and I.G. Viana. 2020. Morphological and physiological responses of *Enhalus acoroides* seedlings under varying temperature and nutrient treatment. **Frontiers in Marine Science** 7: 325. DOI: 10.3389/fmars.2020.00325.

Buapet, P., F. Makkliang and C. Thammakhet-Buranachai. 2017. Photosynthetic activity and photoprotection in green and red leaves of the seagrasses, *Halophila ovalis* and *Cymodocea rotundata*: implications for the photoprotective role of anthocyanin. **Marine Biology** 164: 182. DOI: 10.1007/s00227-017-3215-9.

Buapet, P., L.J.Q. Low and P.A. Todd. 2020. Differing photosynthetic responses to excess irradiance in the two coexisting seagrasses, *Halophila ovalis* and *Halophila decipiens*: Chloroplast avoidance movement, chlorophyll fluorescence, and leaf optical properties. **Aquatic Botany** 166: 103268. DOI: 10.1016/j.aquabot.2020.103268.

Celdran, D. and A. Marín. 2013. Seed photosynthesis enhances *Posidonia oceanica* seedling growth. **Ecosphere** 12: 1–11. DOI:10.1890/ES13-00104.1.

Celdran, D. 2017. Photosynthetic activity detected in the seed epidermis of *Thalassia testudinum*. **Aquatic Botany** 136: 39–42. DOI: 10.1016/j.aquabot.2016.09.004.

Chory, J., M. Chatterjee, R.K. Cook, T. Elich, C. Fankhauser, J. Li, P. Nagpal, M. Neff, A. Pepper, D. Poole, J. Reed and V. Vitart. 1996. From seed germination to flowering, light controls plant development via the pigment phytochrome. **Proceedings of the National Academy of Sciences** 93(22): 12066–12071. DOI: 10.1073/pnas.93.22.12066.

Collier, C.J., P.S. Lavery, R.J. Masini and P.J. Ralph. 2007. Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. **Marine Ecology Progress Series** 337: 103–115. DOI: 10.3354/meps337103.

Cussioli, M.C., D. Seeger, D.R. Pratt, K.R. Bryan, K. Bischof, W.P. de Lange and C.A. Pilditch. 2020. Spectral differences in the underwater light regime caused by sediment types in New Zealand estuaries: implications for seagrass photosynthesis. **Geo-Marine Letters** 40: 217–225. DOI: 10.1007/s00367-020-00640-0.

Dattolo, E., M. Ruocco, C. Brunet, M. Lorenti, C. Lauritano, D. D'esposito, P. De Luca, R. Sanges, S. Mazzuca and G. Procaccini. 2014. Response of the seagrass *Posidonia oceanica* to different light environments: Insights from a combined molecular and photo-physiological study. **Marine Environmental Research** 101: 225–236. DOI: 10.1016/j.marenvres.2014.07.010.

Demmig-Adams, B., C.M. Cohu, O. Muller and W.W. Adams. 2012. Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. **Photosynthesis Research** 113: 75–88. DOI: 10.1007/s11120-012-9761-6.

Demotes-Mainard, S., T. Péron, A. Corot, J. Bertheloot, J. Le Gourrierec, S. Pelleschi-Travier, S.L. Crespel, P. Morel, L. Huché-Thélier, R. Boumaza, A. Vian, V. Guérin, N. Leduc and S. Sakr. 2016. Plant responses to red and far-red lights, applications in horticulture. **Environmental and Experimental Botany** 121: 4–21. DOI: 10.1016/j.envexpbot.2015.05.010.

Duan, L., M.Á. Ruiz-Sola, A. Couso, N. Veciana and E. Monte. 2020. Red and blue light differentially impact retrograde signalling and photoprotection in rice. **Philosophical Transactions of the Royal Society B: Biological Sciences** 375(1801): 20190402. DOI: 10.1098/rstb.2019.0402.

Garmash, E.V., O.V. Dymova, R.V. Malyshev, S.N. Plyusnina and T.K. Golovko. 2013. Developmental changes in energy dissipation in etiolated wheat seedlings during the greening process. **Photosynthetica** 51: 497–508. DOI: 10.1007/s11099-013-0044-z.

Givnish, T.J. 1988. Adaptation to sun and shade: a whole-plant perspective. **Functional Plant Biology** 15(2): 63–92. DOI: 10.1071/PP9880063.

Hamdani, S., N. Khan, S. Perveen, M. Qu, J. Jiang and X.G. Zhu. 2019. Changes in the photosynthesis properties and photoprotection capacity in rice (*Oryza sativa*) grown under red, blue, or white light. **Photosynthesis Research** 139: 107–121. DOI: 10.1007/s11120-018-0589-6.

Inoue, S.I. and T. Kinoshita. 2017. Blue light regulation of stomatal opening and the plasma membrane H1-ATPase. **Plant Physiology** 174: 531–538. DOI: 10.1104/pp.17.00166.

Jedynak, P., K.F. Trzebuniak, M. Chowaniec, P. Zgłobicki, A.K. Banaś and B. Mysliwa-Kurdziel. 2022. Dynamics of etiolation monitored by seedling morphology, carotenoid composition, antioxidant level, and photoactivity of protochlorophyllide in *Arabidopsis thaliana*. **Frontiers in Plant Science** 12: 772727. DOI: 10.3389/fpls.2021.772727.

Kagawa, T. and M. Wada. 2002. Blue light-induced chloroplast relocation. **Plant and Cell Physiology** 43(4): 367–371. DOI: 10.1093/pcp/pcf049.

Kausik, S.B. 1940. **A contribution to the embryology of *Enalus acoroides* (L. fil.), Steud.** Proceedings Indian Academy of Sciences 1940: 83–99.

Kilminster, K., K. McMahon, M. Waycott, G.A. Kendrick, P. Scanes, L. McKenzie, K.R. O'Brien, M. Lyons, A. Ferguson, P. Maxwell, T. Glasby and J. Udy. 2015. Unravelling complexity in seagrass systems for management: Australia as a microcosm. **Science of the Total Environment** 534: 97–109. DOI: 10.1016/j.scitotenv.2015.04.061.

Kirk, J.T. 2011. **Light and Photosynthesis in Aquatic Ecosystems,** 3rd ed. Cambridge University Press, New York, USA. 662 pp.

Kongrueang, P., P. Buapet and P. Roongsattham. 2018. Physiological responses of *Enhalus acoroides* to osmotic stress. **Botanica Marina** 61(3): 257–267. DOI: 10.1515/bot-2017-0108.

Lepeduš, H., M. Jakopec, J. Antunović Dunić, G. Krizmanić, S. Osmanović and V. Cesar. 2017. Temperature-dependent chlorophyll accumulation and photosystem II assembly during etioplast to chloroplast transition in sunflower cotyledons. **Acta Botanica Croatica** 76(1): 107–110. DOI: 10.1515/botcro-2016-0043.

Li, Z., Q. Chen, Y. Xin, Z. Mei, A. Gao, W. Liu, L. Yu, N.X. Chen, Z. Chen and N. Wang. 2021. Analyses of the photosynthetic characteristics, chloroplast ultrastructure, and transcriptome of apple (*Malus domestica*) grown under red and blue lights. **BMC Plant Biology** 21(1): 1–14. DOI: 10.1186/s12870-021-03262-5.

Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. **Biochemical Society Transactions** 11: 591–592.

Lin, C. 2000. Plant blue-light receptors. **Trends in Plant Science** 5(8): 337–342. DOI: 10.1016/S1360-1385(00)01687-3.

Liu, S., Z. Jiang, Y. Wu, X. Zhang and X. Huang. 2023. Combined effects of temperature and burial on seed germination and seedling growth rates of the tropical seagrass *Enhalus acoroides*. **Journal of Experimental Marine Biology and Ecology** 562: 151881. DOI: 10.1016/j.jembe.2023.151881.

Llorente, B., J.F. Martinez-Garcia, C. Stange and M. Rodriguez-Concepcion. 2017. Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. **Current Opinion in Plant Biology** 37: 49–55. DOI: 10.1016/j.pbi.2017.03.011.

Low, V.H. 1971. Effects of light and darkness on the growth of peas. **Australian Journal of Biological Sciences** 24(2): 187–196. DOI: 10.1071/BI9710187.

Ma, M., M. Zhong, Q. Zhang, W. Zhao, M. Wang and C. Luo. 2021. Phylogenetic implications and functional disparity in the Chalcone synthase gene family of common Seagrass *Zostera marina*. **Frontiers in Marine Science** 8: 760902. DOI: 10.3389/fmars.2021.760902.

Nordlund, L.M., E.W. Koch, E.B. Barbier and J.C. Creed. 2016. Seagrass ecosystem services and their variability across genera and geographical regions. **PLoS One** 11(10): e0163091. DOI: 10.1371/journal.pone.0163091.

Ohnishi, N., S.I. Allakhverdiev, S. Takahashi, S. Higashi, M. Watanabe, Y. Nishiyama and N. Murata. 2005. Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. **Biochemistry** 44(23): 8494–8499. DOI: 10.1021/bi047518q.

Olesen, B., S. Enríquez, C.M. Duarte and K. Sand-Jensen. 2002. Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. **Marine Ecology Progress Series** 236: 89–97. DOI: 10.3354/meps236089.

Olsen, J.L., P. Rouzé, B. Verhelst, Y.C. Lin, T. Bayer *et al*. 2016. The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. **Nature** 530(7590): 331–335. DOI: 10.1038/nature16548.

Ort, D.R., X. Zhu and A. Melis. 2011. Optimizing antenna size to maximize photosynthetic efficiency. **Plant Physiology** 155(1): 79–85. DOI: 10.1104/pp.110.165886.

Phandee, S. and P. Buapet. 2018. Photosynthetic and antioxidant responses of the tropical intertidal seagrasses *Halophila ovalis* and *Thalassia hemprichii* to moderate and high irradiances. **Botanica Marina** 61(3): 247–256. DOI: 10.1515/bot-2017-0084.

Platt, T., C.L. Gallegos and W.G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. **Journal of Marine Research** 38: 687–701.

Porra, R.J. 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. **Photosynthesis Research** 73: 149–156. DOI: 10.1023/A:1020470224740.

Procaccini, G., M. Ruocco, L. Marín-Guirao, E. Dattolo, C. Brunet *et al*. 2017. Depth-specific fluctuations of gene expression and protein abundance modulate the photophysiology in the seagrass *Posidonia oceanica*. **Scientific Reports** 7(1): 1–15. DOI: 10.1038/srep42890.

Ralph, P.J. 1999. Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. f. to combined environmental stress. **Aquatic Botany** 65(1–4): 83–96. DOI: 10.1016/S0304-3770(99)00033-9.

Ralph, P.J. and R. Gademann. 2005. Rapid light curves: a powerful tool to assess photosynthetic activity. **Aquatic Botany** 82(3): 222–237. DOI: 10.1016/j.aquabot.2005.02.006

Rehman, M., S. Fahad, M.H. Saleem, M. Hafeez, M. Rahman, F. Liu and G. Deng. 2020. Red light optimized physiological traits and enhanced the growth of ramie (*Boehmeria nivea* L.). **Photosynthetica** 58(4): 922–931. DOI: 10.32615/ps.2020.040.

Saewong, C., S. Soonthornkalump and P. Buapet. 2022. Combined effects of high irradiance and temperature on the photosynthetic and antioxidant responses of *Thalassia hemprichii* and *Halophila ovalis*. **Botanica Marina** 65(5): 325–335. DOI: 10.1515/bot-2022-0014.

Samuolienė, G., A. Viršilė, J. Miliauskienė, P.J. Haimi, K. Laužikė, A. Brazaitytė and P. Duchovskis. 2021. The physiological response of lettuce to red and blue light dynamics over different photoperiods. **Frontiers in Plant Science** 11: 610174. DOI: 10.3389/fpls.2020.610174.

Sandoval-Gil, J.M., J.M. Ruiz, L. Marín-Guirao, J. Bernardeau-Esteller and J.L. Sánchez-Lizaso. 2014. Ecophysiological plasticity of shallow and deep populations of the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* in response to hypersaline stress. **Marine Environmental Research** 95: 39–61. DOI: 10.1016/j.marenvres.2013.12.011.

Shengxin, C., L. Chunxia, Y. Xuyang, C. Song, J. Xuelei, L. Xiaoying, X. Zhigang and G. Rongzhan. 2016. Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the rosette stage. **Frontiers in Plant Science** 7: 1144. DOI: 10.3389/fpls.2016.01144.

Shengxin, C., C. Pu, R.Z. Guan, M. Pu and Z.G. Xu. 2018. Transcriptional and translational responses of rapeseed leaves to red and blue lights at the rosette stage. **Journal of Zhejiang University Science B: Biomedicine and Biotechnology** 19(8): 581. DOI: 10.1631/jzus.B1700408.

Short, F., T. Carruthers, W. Dennison and M. Waycott. 2007. Global seagrass distribution and diversity: a bioregional model. **Journal of Experimental Marine Biology and Ecology** 350(1–2): 3–20. DOI: 10.1016/j.jembe.2007.06.012.

Soong, K., S.T. Chiu and C.N.N. Chen. 2013. Novel seed adaptations of a monocotyledon seagrass in the wavy sea. **PLoS One** 8(9): e74143. DOI: 10.1371/journal.pone. 0074143.

Spaninks, K., J. van Lieshout, W. van Ieperen and R. Offringa. 2020. Regulation of early plant development by red and blue light: a comparative analysis between *Arabidopsis thaliana* and *Solanum lycopersicum*. **Frontiers in Plant Science** 11: 599982. DOI: 10.3389/fpls.2020.599982.

Statton, J., K. McMahon, P. Lavery and G.A. Kendrick. 2018. Determining light stress responses for a tropical multi-species seagrass assemblage. **Marine Pollution Bulletin** 128: 508–518. DOI: 10.1016/j.marpolbul.2018.01.060.

Strydom, S., K. McMahon and P.S. Lavery. 2017a. Response of the seagrass *Halophila ovalis* to altered light quality in a simulated dredge plume. **Marine Pollution Bulletin** 121(1–2): 323–330. DOI: 10.1016/j.marpolbul.2017.05.060.

Strydom, S., K. McMahon, G.A. Kendrick, J. Statton and P.S. Lavery. 2017b. Seagrass *Halophila ovalis* is affected by light quality across different life history stages. **Marine Ecology Progress Series** 572: 103–116. DOI: 10.3354/meps12105.

Strydom, S., K.M. McMahon, G.A. Kendrick, J. Statton and P.S. Lavery. 2018. Short-term responses of *Posidonia australis* to changes in light quality. **Frontiers in Plant Science** 8: 2224. DOI: 10.3389/fpls.2017.02224.

Terashima, I. and K. Hikosaka. 1995. Comparative ecophysiology of leaf and canopy photosynthesis. **Plant, Cell and Environment** 18(10): 1111–1128. DOI: 10.1111/j.1365-3040.1995.tb00623.x.

Terrados, J., C.M. Duarte, L. Kamp-Nielsen, N.S. Agawin, E. Gacia, D. Lacap, M.D. Fortes, J. Borum, M. Lubanski and T.M. Greve. 1999. Are seagrass growth and survival constrained by the reducing conditions of the sediment. **Aquatic Botany** 65: 175–197. DOI: 10.1016/S0304-3770(99)00039-X.

Terrados, J., M. Grau-Castella, D. Piñol-Santiñà and P. Riera-Fernández. 2006. Biomass and primary production of a 8–11 m depth meadow versus <3 m depth meadows of the seagrass *Cymodocea nodosa* (Ucria) Ascherson. **Aquatic Botany** 84(4): 324–332. DOI: 10.1016/j.aquabot.2005.12.004.

Von Arnim, A. and X.W. Deng. 1996. Light control of seedling development. **Annual Review of Plant Biology** 47(1): 215–243. DOI: 10.1146/annurev.arplant.47.1.215.

Wang, L., D. Leister and T. Kleine. 2020. Chloroplast development and genomes uncoupled signaling are independent of the RNA-directed DNA methylation pathway. **Scientific Reports** 10(1): 15412. DOI: 10.1038/s41598-020-71907-w.

Wutiruk, T., P. Buapet, J. Nopparat, E. Kong, S.M. Yaakub and Y.X. Ow. 2022. Acclimation to low light modifies nitrogen uptake in *Halophila ovalis* (R. Brown) JD Hooker. **Journal of Experimental Marine Biology and Ecology** 549: 151705. DOI: 10.1016/j.jembe.2022.151705.

Yuan, M., Y.Q. Zhao, Z.W. Zhang, Y.E. Chen, C.B. Ding and S. Yuan. 2017. Light regulates transcription of chlorophyll biosynthetic genes during chloroplast biogenesis. **Critical Reviews in Plant Sciences** 36(1): 35–54. DOI: 10.1080/07352689.2017.1327764.

Zheng, L. and M.C. Van Labeke. 2017. Long-term effects of red-and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. **Frontiers in Plant Science** 8: 917. DOI: 10.3389/fpls.2017.00917.