Insufficient synthesis and acylation modification of anthocyanins causes photoinactivation of the oxygen-evolving complex in Zostera marina

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Abstract

Zostera marina among seagrass suffering from global decline is a representative species in temperate regions in the Northern Hemisphere. Given our recent findings, the decline of seagrasses may be associated with the photosensitivity of the oxygen-evolving complex (OEC). Therefore, understanding the mechanism of OEC photosensitivity is key to understanding the continued decline in seagrasses. Herein, we explored the screening-based photoprotection function in *Z. marina* by examining the inactivation spectrum of OEC and the differences in photoresponse pathways following exposure to different spectrums. The OEC inactivation was spectral-dependent. High-energy light significantly reduced the PSII performance, OEC peripheral protein expression, and photosynthetic O $_2$ release capacity. The increased synthesis of carotenoids under blue light with severe OEC damage implied its weak photoprotection property in *Z. marina*. However, anthocyanins key synthetic genes were lowly expressed with inefficient accumulation under high-energy light. Furthermore, the acylation modifications of anthocyanins, especially aromatic acylation modifications were insufficient, leading to poor stability and light absorption of anthocyanins. Based on the role of blue light receptors in regulating the synthesis of anthocyanins in vascular plant, we hypothesized that the absence of blue light receptor CRY2 in *Z. marina* causes the insufficient synthesis of anthocyanins and acyl modifications, reducing the shielding against high-energy light, subsequently causing OEC photoinactivation.

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Abstract

Zostera marina among seagrass suffering from global decline is a representative species in temperate regions in the Northern Hemisphere. Given our recent findings, the decline of seagrasses may be associated with the photosensitivity of the oxygen-evolving complex (OEC). Therefore, understanding the mechanism of OEC photosensitivity is key to understanding the continued decline in seagrasses. Herein, we explored the screening-based photoprotection function in*Z. marina* by examining the inactivation spectrum of OEC and the differences in photoresponse pathways following exposure to different spectrums. The OEC inactivation was spectral-dependent. High-energy light significantly reduced the PSII performance, OEC peripheral

protein expression, and photosynthetic O_2 release capacity. The increased synthesis of carotenoids under blue light with severe OEC damage implied its weak photoprotection property in *Z. marina* . However, anthocyanins key synthetic genes were lowly expressed with inefficient accumulation under high-energy light. Furthermore, the acylation modifications of anthocyanins, especially aromatic acylation modifications were insufficient, leading to poor stability and light absorption of anthocyanins. Based on the role of blue light receptors in regulating the synthesis of anthocyanins in vascular plant, we hypothesized that the absence of blue light receptor CRY2 in *Z. marina* causes the insufficient synthesis of anthocyanins and acyl modifications, reducing the shielding against high-energy light, subsequently causing OEC photoinactivation.

Keywords : Anthocyanin; Light screening; Photoreceptor; Oxygen-evolving complex;*Zostera marina* .

Introduction

Seagrasses are a foundation species in marine ecosystems, which provide important ecosystem services by forming extensive seagrass beds (Fourqurean et al., 2012; Cullen-Unsworth et al., 2018; Costa et al., 2020). Despite their critical value, they are suffering a global decline, becoming one of the most threatened ecosystems (Orth et al., 2006). This decline has been attributed to global climate change and habitat fragmentation, eutrophication, pollution, etc., as a direct result of human activities (Burkholder et al., 2007; Duarte et al., 2018; Hall-Spencer and Harvey, 2019; Nguyen et al., 2021). At the same time, the inherent biological vulnerability of seagrasses has received little attention.

Our recent studies indicated that oxygen-evolving complex (OEC) of*Zostera marina* and *Phyllospadix iwatensis* , two representative seagrasses in temperate regions, are prone to photoinactivation under visible light (Zhao et al., 2021; Wang et al., 2022). The OEC, located on the luminal side of the thylakoid lumen of photosystem II (PSII), is the site for catalytic cleavage of water into protons and molecular oxygen in photosynthesis (Gupta, 2020). Generally, OEC photoinactivation is limited, thus, OEC stability and carbon assimilation are effectively maintained by the regulations of ascorbic acid and PSII and PSI cycle electron transport (Tan et al., 2020; Zhao et al., 2021). However, during extreme light conditions, the percentage of OEC inactivation can exceed 15%, rendering the regulation mechanisms inefficient in maintaining the photosynthetic performance, leading to irreversible damage to the photosynthetic apparatus and a potential decline in seagrass populations in the long term (Wang et al., 2022). Therefore, to further understand the intrinsic causes of seagrass population decline, understanding the mechanism of OEC inactivation is crucial.

Normally, plants trigger a series of protective mechanisms to prevent oxidative damage under stressful conditions (Takahashi and Badger, 2011). Screening-based photoprotection constitutes the first-line defense of plants against potentially harmful solar radiation (Solovchenko, 2010). The screening pigments accumulated in plants tissue structures attenuate ultraviolet (UV) or the visible part of the spectrum, reducing the excessive absorption of radiation by the photosynthetic apparatus (Shick and Dunlap, 2002; Solovchenko and Merzlyak, 2003). These screening pigments have been categorized into four groups: mycosporine-like amino acids mainly in prokaryotes, betalains, a nitrogenous compound limited to flowering plants, carotenoids (Car), and phenolic compounds commom in all plants (Solovchenko and Merzlyak, 2008). The screen function in the visible region is majorly performed by Car and anthocyanins in phenolic compounds (Hormaetxe et al., 2005; Landi et al., 2015). Carotenoids are auxiliary pigments commonly found in photosynthetic autotrophs, which transfer absorbed energy to chlorophyll a for photosynthesis and protect the plant against photodamage through the xanthophyll cycle and light screening (Frank et al., 1997; Baroli and Niyogi, 2000; Merzlyak and Solovchenko, 2002). Under unfavorable conditions, the synthesis of extrathylakoid, and extraplastidic Car is induced in microalgae to protect the photosynthetic apparatus (Boussiba, 2000; Wang et al., 2003). However, compared to microalgae, the screening function of Car in higher is relatively less studied (Merzlyak and Solovchenko, 2002; Hormaetxe et al., 2005; Merzlyak et al., 2005). Unlike Car, anthocyanins in phenolic compounds are the most studied protective pigments against stress (Chalker-Scott, 1999). Apart from their antioxidant activity, anthocyanins have a good spectral absorption property for UV and the blue-green component of visible light (Landi et al., 2015). Besides, modifications to the anthocyanins enhance their stability and light absorption capacity (Fan et al., 2008). Specifically, the resistance of plant photosynthetic tissues to photodamage is increased with the accumulation of anthocyanins (Landi et al.,

2015; Zhu et al., 2018).

Herein, to establish the mechanism of OEC photoinactivation, *Z. marina,*whose complete genome has been sequenced, was used as the research object (Olsen et al., 2016; Ma et al., 2021).*Zostera marina* is a completely submerged angiosperm in oceans, which evolved from monocotyledons land plants and returned to the marine ecosystem (Wissler et al., 2011). During its complex evolutionary process, the omission of some photoreceptors occurred. Specifically, phytochromes (PHYs) include PHYA and PHYB, lack PHYC-E, while only cryptochrome 1 (CRY1) is present in CRY, with CRY2 and CRY3 missing. In addition, UVR8 photoreceptor associated with UV is also absent (Olsen et al., 2016; Ma et al., 2021). The Mn cluster of OEC is extremely unstable and prone to photoactivated shedding (Hakala et al., 2005). However, the synthesis of screening substances regulated by photoreceptors reduces the photoactivated release of the Mn cluster through light screening (Landi et al., 2015). Therefore, the absence of photoreceptors in *Z. marina*potentially restricts the synthesis of light-shielded compounds that allow high-energy light to reach the chloroplasts easily, causing OEC photoinactivation. To verify this hypothesis, *Z. marina* was treated with different light qualities to investigate (1) the spectral dependence of OEC inactivation and (2) the differences in spectral of photoresponse pathways.

Materials and methods

Sample preparations and treatments

Zostera marina plants with intact rhizomes-systems and fresh leaves were collected from the seagrass beds in Rongcheng (37º 16'N, 122º 41'E), Weihai, Shandong province, China. Samples were pre-cultured in an aquarium at 15 °C and a 10: 14 h light: dark cycle with a minimum saturation light intensity of 70 photosynthetic photon flux density (PPFD) for 3 days. Pre-cultured samples were dark-adapted overnight prior to experimental treatment, following which leaves 2 cm above the leaf sheath were sampled for experimentation. The inactivation spectrum of OEC was determined by monitoring the prompt fluorescence kinetic on leaves exposed to light at 380, 400, 420, 450, 530, 630, 660, 725, and 400-750 nm with a light intensity of 210PPFD for 15 min. Furthermore, the PSII performance, OEC peripheral protein expression, photosynthetic O_2 release capacity, and transcriptome differences were measured on leaves exposed to white (WL, 400-750 nm), blue (BL, 420 nm) and red light (RL, 660 nm) at 210 PPFD to investigate the effects of photoreceptor absence.

Chlorophyll a fluorescence measurements

For a prompt characterization of the OEC activity, the kinetic of prompt fluorescence was monitored using a multi-function plant efficiency analyzer 2 (M-PEA-2; Hansatech, Norfolk, UK). The chlorophyll fluorescence parameters were calculated as previously described by Strasser et al. (2010). The normalized fluorescence rise kinetics of OJIP were calculated using the formula: $\Delta V\ \mathrm{t} = \Delta\ [(F_{\ \mathrm{t}} - F_{\ \mathrm{O}})/\ (F_{\ \mathrm{m}} - F_{\ \mathrm{O}})]$; the maximal quantum yield of the PSII by $F_{\ \mathrm{v}}/F_{\ \mathrm{m}} = (F_{\ \mathrm{m}} - F_{\ \mathrm{o}})/\ F_{\ \mathrm{m}}$; the degree of damage on the donor side of PSII by $W_{\ \mathrm{K}} = (F_{\ \mathrm{K}} - F_{\ \mathrm{O}})/\ (F_{\ \mathrm{J}} - F_{\ \mathrm{O}})$, and the active fraction of OEC centers by $\mathrm{OEC_{centers}} = [1 - (V_{\ \mathrm{K}}/V_{\ \mathrm{J}})]_{\mathrm{treatment}}/\ [1 - (V_{\ \mathrm{K}}/V_{\ \mathrm{J}})]_{\mathrm{control}}$. The subscript "control" and "treatment" indicated that the corresponding parameters were measured on the dark-adapted, and light-stressed treatment samples, respectively. Each measurement was conducted in triplicate.

Western blotting analysis

The peripheral protein expression was used to characterize the stability of OEC. Chloroplasts were separated from the leaf samples using the Plants Leaf Chloroplast Rude Divide Kit (GenMED Scientifics Inc, Arlington, MA, USA). The chlorophyll contents were measured as previously described by Porra et al. (1989). To compare quantitative differences, control samples with 1.25, 2.5, and 5 μg chlorophyll corresponding to 25, 50, and 100% of the control sample, respectively, were loaded on SDS-PAGE gel and separated with solubilized materials from treatment leaves containing 5 μg chlorophyll. Next, a Western blot assay with antibodies against PsbO, PsbP and PsbQ (Agrisera, Vännäa, Sweden) was performed following the protocol described by Fristedt et al. (2009). RuBisCo large subunit (RbcL) were used as equal loading controls. The chemiluminescent bands on the blots were quantified using Image Lab software (Bio-Rad) on a Gel Doc

XR+ system (Bio-Rad, Hercules, CA, USA). The sample densities were normalized to the RbcL density. Each measurement was conducted in triplicate.

Oxygen evolution rate measurements

The rate of photosynthetic O_2 evolution was determined using a liquid-phase oxygen electrode system (Chlorolab2+; Hansatech, Hercules, UK) to evaluate the overall photosynthetic performance. Leaf fragments (˜ 30 mg) were placed in the reaction chamber containing 2 mL of seawater at 15°C. The net photosynthetic rate (Pn) and the respiration rate (R) of the samples were measured within 3 min of white light irradiation (70 PPFD) and 2 min of the dark adaptation, respectively. The rate of O_2 evolution (P) was calculated as P = R + Pn and expressed as a percentage before the onset of light stress. Each measurement was conducted in triplicate.

RNA extraction, library construction and transcriptome sequencing

To establish the influence of light quality on the gene expression levels, a transcript analysis was performed. Total RNA of*Z. marina* samples exposed to BL, WL and RL for 3 h was extracted using the TRlzol Reagent (Invitrogrn). Next, the genomic DNA in the extracted RNA was digested using DNase I (TaKaRa Shuzo, Kyoto, Japan). The quality and concentration of the RNA were detected by agarose gel electrophoresis and NanoDrop ND-2000 spectrophotometer (NanoDropTechnologies), respectively. The high-quality RNA samples (OD260/OD280 [?] 1.8, OD260/OD230 [?] 2.0) were selected and used to construct the sequencing library. To prepare the RNA-seq transcriptome library, mRNA was isolated from the total RNA using oligo(dT) coupled to magnetic beads. Next, a SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA, USA) with random hexamer primers (Illumina) was used to synthesize an end-repaired, and phosphorylated double-stranded cDNA, following the manufacturer's protocol. The mRNA library was sequenced using the Illumina HiSeq xten after quantification with TBS380, generating 150bp paired-end reads. Full RNA-seq transcriptome data were submitted to the National Center for Biotechnology Information database under the accession numbers: SRR18740075, SRR18740076, SRR18740077, SRR18740078, SRR18740079, SRR18740080, SRR18740081, SRR18740082, SRR18740083, SRR18740084, SRR18740085, SRR18740086.

Bioinformatics analysis

The*Z. marina* genome v.3.1 (Ma et al., 2021) was used as the reference genome for bioinformatics analysis. Clean reads were mapped to the reference genome using Bowtie2 v2.4.1. The gene expression levels expressed as fragments per kilobase of exon model per million mapped fragments (FPKM) were calculated using Expectation-Maximization (RSEM) v1.3.1. Differentially expressed genes (DEGs) between treatments were analyzed using the DESeq2 R package. To improve the accuracy of identifying, we investigated the significant DEGs (fold change >= 2.00 and$P$ -value < 0.05). Next, the gene annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed to identify the biological pathways regulated by the significant DEGs. The biological pathways with P -value < 0.05 were considered significantly enriched. The HeatMap function in Tbtools was used for the visual analysis of the DEGs.

Totalcarotenoids andanthocyanins content analysis

The total carotenoid content was measured using a Plant Carotenoid Content Assay Kit (BC4330, Solarbio, Beijing, China), based on the spectral absorption properties of carotenoids, chlorophyll a and chlorophyll b. The total anthocyanins content was measured as previously described by Neff and Chory (1998) with some modifications. The samples were dried at -60degC for 48 h using a freeze dryer (Alpha1-2LDplus, Martin Christ, Osterode, Germany) and then ground into powder. Next, approximately 0.01g per sample was put into a 2 mL Eppendorf tube, and 1 mL of methanol acidified with 1% HCL was added. The tubes were incubated overnight in dark at 4 until the green color of the leaf tissue disappeared. Anthocyanins were separated from chlorophylls by back-extraction with 400 μL chloroform and 400 μL ddH_2O, then centrifuging the tube for 5 min at 6000 r/min and measuring the absorbance of the supernatant using a TU-1810 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China). The content of anthocyanins was calculated as OD_{530nm}-0.25OD_{657nm} to account for any interference from chlorophylls.

Finally, the total anthocyanin content was calculated as cyanidin-3-glucoside using 29600 (E 1 cm 1%) as the extinction coefficient and 449.2 as the molecular weight.

Liquid chromatography with tandem mass spectrometry

The differences in anthocyanin acylation modification were detected by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The lyophilisation of samples and analysis of extracts were all performed by MetWare Biotechnology Ltd. (Wuhan, China). The detailed protocol is presented in Methods S1.

Data analysis

All statistical analyses were performed with the SPSS 22.0 statistical package (IBM Corp, Armonk, NY, USA). All parameters were analyzed using one-way ANOVA and post hoc comparisons were performed using the tukey trend test.

Results

OEC inactivation spectrum

The standardized OJIP curves showed K points at 0.3 ms after a short period of stress at different light qualities (Fig. 1A), implying that OEC damage on the donor side of PSII was triggered at all light qualities. To further understand the photoinactivation of OEC, calculation of fluorescence at point K in a standardized manner revealed that W_{K} was gradually increased with an increase in wavelength, with the greatest increase following UV and BL exposure (Fig. 1B).

3.2 Physiological and biochemical responses to light quality

The amplitude of the standardized OJIP curve ΔV t was different across the three light qualities (Fig. 2A), implying that light quality significantly affected PSII performance. In addition, the decrease in F v/F m was light quality dependent, with the greatest decrease observed upon exposure to BL (Fig. 2B). Furthermore, a significant decrease in OEC stability was observed under BL and was characterized by a substantial reduction in OEC peripheral protection proteins, including PsbO, PsbP, and PsbQ (Fig. 2C). Overall, BL induced the inactivation of a most active OEC, evidenced by the changes in the physiological parameter, $\mathrm{OEC}_{\mathrm{centers}}$ (Fig. 2D).

3.3 Photosynthetic O_2evolution

The complete measurement process of the O_2 evolution rate was shown in Fig. 3A. The rate of O_2 evolution (overall photosynthetic performance) was gradually decreased with exposure time, with the greatest decrease occurring following BL exposure (3B). Moreover, the O_2 content change curves after 3 h of irradiation revealed that Pn and R were significantly decreased following BL exposure, implying the severe impairment of the overall leaf function (Fig. 3C).

3.4 Overview oftranscriptome analysis

A total of 129.42 GB of high-quality sequencing data with Q30 exceeding 92.53% were obtained after filtering the raw sequencing data (Table 1). Clean reads were mapped to the reference genome at a mapping rate higher than 92.13% (Table 2), implying a high level of gene expression in all treatment groups. The principal component analysis revealed that the four treatment groups were distributed in the different regions of the three-dimensional space, hence, they could be distinguished based on the light quality (Fig. 4).

3.5 Functional enrichment analysis

The KEGG analysis revealed that "Photosynthesis - antenna proteins", "MAPK signaling pathway - plant", "Glycine, serine and threonine metabolism", "Plant hormone signal transduction" and some other pathways were significantly enriched following exposure to BL, WL, and RL (Fig. 5A-C). However, "Carotenoid biosynthesis" pathway was only enriched in BL, while "Flavonoid biosynthesis" pathway was minimally enriched in BL compared to WL and RL (Fig. 5A-C). Furthermore, "Anthocyanin biosynthesis" pathway was only enriched in RL, although it was not significant (Fig. S1).

3.6 Expression of related synthetic genes and carotenoids content

Following BL exposure, nine DEGs were mapped to the "Carotenoid biosynthesis" pathway. The genes encoding Car synthesis, including*PSY* , were significantly upregulated following BL exposure, implying that BL effectively promoted Car accumulation (Fig. 6A). Indeed, Car content was significantly increased with BL exposure, consistent with the signaling pathway enrichment analysis and gene expression.

3.7 Expression of related synthetic genes and anthocyanin content

The DEGs associated with anthocyanins synthesis identified by pathway enrichment assignments were less upregulated following BL exposure than WL and RL (Fig. 7A). Specially, the genes encoding phenylalanine ammonia-lyase (PAL), a rate-limiting enzyme during polyphenol synthesis, and chalcone synthase (CHS), a key enzyme during flavonoid synthesis, were lowly expressed under BL exposure (Fig. 7A), implying a limited ability to induce anthocyanin synthesis. Furthermore, the changes in anthocyanins content during the exposure period confirmed the inefficiency of BL-induced anthocyanin synthesis. Although there was a gradual increase in anthocyanins content with exposure time across the three light qualities, it was consistently the lowest under BL exposure (Fig. 7B).

3.8 Acylated anthocyanins

A total of ten anthocyanins modified by acylation, including seven aromatic acyl-substituted, were detected in*Z. marina* following light exposure. The lowest level of aromatic acyl-substituted anthocyanins, particularly the delphinidin-3-O-(6-O-p-coumaroyl)-glucoside and pelargonidin-3-O-(6-O-p-coumaroyl)-glucoside, which may have a good light absorption ability due to the aromatic acyl-modification, was detected following BL exposure (Table 3).

Discussion

In the present work, the OEC dysfunction *in vivo* was spectral dependent, with a progressive increase in impairment with increasing light wavelength. The effects of light quality on OEC function and stability, overall photosynthetic performance, and gene expression at three typical light qualities, including composite WL and two critical lights in the visible range (BL and RL), were characterized to understand the spectral dependence of *Z. marina* . Based on chlorophyll fluorescence, protein expression, and photosynthetic O_2 evolution analyses, BL is the key light quality inducing OEC inactivation and reducing the photosynthetic rate. Transcriptome analysis further revealed that compounds with light-screening functions are involved in the *Z. marina* responses to light. Specifically, pathways associated with Car and phenolic compounds synthesis following light exposure are enriched. Considering the UV wavelengths are mostly attenuated in marine ecosystems (Olsen et al., 2016), this study focused on the light-shielding substances (Car and anthocyanins) with absorption peaks in the visible region. Interestingly, "Carotenoid biosynthesis" and "Anthocyanin biosynthesis" pathways were enriched in BL and RL, respectively, implying varying light-screening capacity in response to different light qualities.

To clarify the differences in photoprotection functions, the expression of relevant synthetic genes and the content of Car and anthocyanins were analyzed. Although the content of Car was significantly increased following BL exposure, the level of OEC inactivation in BL was still more severe than WL and RL, implying that Car were not the major photoprotector in*Z. marina* . On the other hand, anthocyanin is another substance playing an important light-shielding role in the visible region (Landi et al., 2015), which demonstrated a synthetic regulation consistent with photosynthetic properties. The key genes associated with anthocyanin synthesis as well as the anthocyanin content were upregulated and increased following light exposure, respectively. Furthermore, the degree of anthocyanin accumulation varied with light quality, implying photoreceptors regulated the process. However, unlike the significant induction of anthocyanin accumulation by exposure to BL in most plants (Kondo et al., 2014; Zoratti et al., 2014; Tao et al., 2018; Zhang et al., 2018), anthocyanin synthesis in *Z. marina* was inefficient. Plant responses to BL are mainly regulated by CRY1, and CRY2 receptors, which regulate the synthesis of secondary metabolites, such as anthocyanins (Ahmad et al., 1995), apart from mediating the hypocotyl elongation and flowering (Jenkins et

al., 2001).*Zostera marina* has a special evolutionary process, achieving the most severe habitat shift among the flowering plants, and experiencing the extensive losses of photoreceptors, including CRY2 (Olsen et al., 2016). Therefore, the inefficiency of BL-induced anthocyanin synthesis in *Z. marina* may be due to the absence of the CRY2 photoreceptor. This is consistent with the findings on Arabidopsis mutants, where the absence of the CRY2 receptor reduced anthocyanin accumulation (Li et al., 2013). At the same time, the massive synthesis of anthocyanins under RL compared to BL and WL, can be attributed to the presence of PHYA and PHYB receptors. PHYA, and PHYB are major photoreceptors under RL, which can control the expression of the genes regulating anthocyanin synthesis (Yanovsky et al., 1998). Besides, PHYC, PHYD, and PHYE receptors associated with the photoperiodic regulation of flowering in most plants were absent in*Z. marina* (Olsen et al., 2016), implying they do not have a significant effect on *Z. marina* flowering that is more temperature controlled.

More than 500 anthocyanins with specific chemical structures synthesized by plants have been identified. Their main differences are in the degree of hydroxylation of the anthocyanin chromophore and the modifications added to the backbone (Andersen and Markham, 2006; Kovinich et al., 2014). Modification in the acyl groups, especially aromatic acyl, increases the stability and light absorption ability of anthocyanins compared to non-acylated modifications (Stintzing and Carle, 2004; Luo et al., 2007; Yonekura-Sakakibara et al., 2008). Coumaric acid, caffeic acid, sinapic acid, ferulic acid, and p-hydroxybenzoic acid are the main aromatic acids involved in aromatic acyl substitution (Yonekura-Sakakibara et al., 2009). Besides, modifications on multiple aromatic acyl groups improve the anthocyanin properties (Kovinich et al., 2015), thus playing an important role in photoprotection. However, unlike the multiple acylation modifications (i.e., polyacylation) of anthocyanins in wild-type Arabidopsis (Luo et al., 2007; Yonekura-Sakakibara et al., 2012), only one acylation substitution occurs in *Z. marina* anthocyanins. Furthermore, the light shielding efficiency in BL could be worse than WL and RL, due to the lower content of aromatic acyl-substituted anthocyanins in BL, especially delphinidin-3-O-(6-O-p-coumaroyl)-glucoside and pelargonidin-3-O-(6-O-p-coumaroyl)-glucoside.

The acylation modification of anthocyanins is catalyzed by anthocyanin acyltransferases, whose activity is mainly regulated by phosphorylation and gene transcription (Fan et al., 2008). As a signal transducer, photoreceptors can induce the binding of transcription factor MYB to acyltransferase promoter, facilitating gene expression (Rinaldo et al., 2015). Our transcriptome data revealed differences in the expression of the transcription factor MYB and acyltransferases under different light qualities (Fig. S2), implying that the absence of photoreceptors in *Z. marina* may affects the acylation modification of anthocyanins through gene expression. Since anthocyanins modification is not regulated by all MYB transcription factors (Tamagnone et al., 1998; Du et al., 2009), and anthocyanin acyltransferases are anthocyanin-specific and acyl receptor-specific (Fan et al., 2008), the regulation of anthocyanin acylation in *Z. marina* requires further investigations.

Conclusion

The absence of CRY2 photoreceptor in*Z. marina* led to the insufficient synthesis of anthocyanins and reduced the level of aromatic acylated modification of anthocyanins. Thus, excessive photosynthetically effective radiation, especially the high-energy blue-green light enriched in marine ecosystems, was not effectively shielded, leading to OEC inactivation. Irreversible photoinactivation of OEC in harsh environments limits *Z. marina* growth leading to its population decline in the long run. Therefore, the absence of photoreceptors is an important intrinsic factor for seagrass degradation. The findings in this study provide a new insight for seagrasses restoration by modifying the photoreceptors through the gene-editing technology to enhance their resilience to environmental changes, thus, slowing down their population decline.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relation-

ships that could be construed as a potential conflict of interest.

Reference

Ahmad, M., Lin, C., and Cashmore, A.R. (1995). Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. *The Plant Journal* 8(5), 653-658. doi: 10.1046/j.1365-313X.1995.08050653.x.

Andersen, O.M., and Markham, K.R. (2006). *Flavonoids: chemistry, biochemistry and applications.* Boca Raton: CRC Taylor & Francis.

Baroli, I., and Niyogi, K.K. (2000). Molecular genetics of xanthophyll–dependent photoprotection in green algae and plants.*Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 355(1402), 1385-1394. doi: 10.1098/rstb.2000.0700.

Boussiba, S. (2000). Carotenogenesis in the green alga Haematococcus pluvialis: cellular physiology and stress response. *Physiologia Plantarum* 108(2), 111-117. doi: 10.1034/j.1399-3054.2000.108002111.x.

Burkholder, J.M., Tomasko, D.A., and Touchette, B.W. (2007). Seagrasses and eutrophication. *Journal of experimental marine biology and ecology* 350(1-2), 46-72. doi: 10.1016/j.jembe.2007.06.024.

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and photobiology*70(1), 1-9. doi: 10.1111/j.1751-1097.1999.tb01944.x.

Costa, A.C.P., Garcia, T.M., Paiva, B.P., Neto, A.R.X., and de Oliveira Soares, M. (2020). Seagrass and rhodolith beds are important seascapes for the development of fish eggs and larvae in tropical coastal areas.*Marine Environmental Research* 161, 105064. doi: 10.1016/j.marenvres.2020.105064.

Cullen-Unsworth, L.C., Jones, B.L., Seary, R., Newman, R., and Unsworth, R.K. (2018). Reasons for seagrass optimism: local ecological knowledge confirms presence of dugongs. *Marine Pollution Bulletin*134, 118-122. doi: 10.1016/j.marpolbul.2017.11.007.

Du, H., Zhang, L., Liu, L., Tang, X.-F., Yang, W.-J., Wu, Y.-M., et al. (2009). Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry (Moscow)*74(1), 1-11. doi: 10.1134/S0006297909010015.

Duarte, B., Martins, I., Rosa, R., Matos, A.R., Roleda, M.Y., Reusch, T.B., et al. (2018). Climate change impacts on seagrass meadows and macroalgal forests: an integrative perspective on acclimation and adaptation potential. *Frontiers in Marine Science* 5,190. doi: 10.3389/fmars.2018.00190.

Fan, D.D., Sun, M.J., Wang, L.H., and Jiao, B.H. (2008). Advance in study of acyltransferase. *Chemistry of Life* 28(6),701-703. doi: 10.3969/j.issn.1000-1336.2008.06.009.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., et al. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature geoscience* 5(7),505-509. doi: 10.1038/ngeo1477.

Frank, H.A., Chynwat, V., Desamero, R.Z., Farhoosh, R., Erickson, J., and Bautista, J. (1997). On the photophysics and photochemical properties of carotenoids and their role as light-harvesting pigments in photosynthesis. *Pure and applied chemistry* 69(10),2117-2124. doi: 10.1351/pac199769102117.

Fristedt, R., Willig, A., Granath, P., Crevecoeur, M., Rochaix, J.-D., and Vener, A.V. (2009). Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in Arabidopsis. *The Plant Cell* 21(12), 3950-3964. doi: 10.1105/tpc.109.069435.

Gupta, R. (2020). The oxygen-evolving complex: a super catalyst for life on earth, in response to abiotic stresses. *Plant Signaling & Behavior* 15(12), 1824721. doi: 10.1080/15592324.2020.1824721.

Hakala, M., Tuominen, I., Keränen, M., Tyystjärvi, T., and Tyystjärvi, E. (2005). Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1706(1-2), 68-80. doi: 10.1016/j.bbabio.2004.09.001.

Hall-Spencer, J.M., and Harvey, B.P. (2019). Ocean acidification impacts on coastal ecosystem services due to habitat degradation. *Emerging Topics in Life Sciences* 3(2), 197-206. doi: 10.1042/ETLS20180117.

Hormaetxe, K., Becerril, J.M., Fleck, I., Pinto, M., and García-Plazaola, J.I. (2005). Functional role of red (retro)-carotenoids as passive light filters in the leaves of Buxus sempervirens L.: increased protection of photosynthetic tissues? *Journal of Experimental Botany* 56(420), 2629-2636. doi: 10.1093/jxb/eri255.

Jenkins, G.I., Long, J.C., Wade, H.K., Shenton, M.R., and Bibikova, T.N. (2001). UV and blue light signalling: pathways regulating chalcone synthase gene expression in Arabidopsis. *New Phytologist*151(1), 121-131. doi: 10.1046/j.1469-8137.2001.00151.x.

Kondo, S., Tomiyama, H., Rodyoung, A., Okawa, K., Ohara, H., Sugaya, S., et al. (2014). Abscisic acid metabolism and anthocyanin synthesis in grape skin are affected by light emitting diode (LED) irradiation at night. *Journal of Plant Physiology* 171(10), 823-829. doi: 10.1016/j.jplph.2014.01.001.

Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M.S., and Grotewold, E. (2015). Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. *Plant signaling & behavior* 10(7),e1027850. doi: 10.1080/15592324.2015.1027850.

Kovinich, N., Kayanja, G., Chanoca, A., Riedl, K., Otegui, M.S., and Grotewold, E. (2014). Not all anthocyanins are born equal: distinct patterns induced by stress in Arabidopsis. *Planta*240(5), 931-940. doi: 10.1007/s00425-014-2079-1.

Landi, M., Tattini, M., and Gould, K.S. (2015). Multiple functional roles of anthocyanins in plant-environment interactions.*Environmental and Experimental Botany* 119, 4-17. doi: 10.1016/j.envexpbot.2015.05.012.

Li, Y.-Y., Mao, K., Zhao, C., Zhao, X.-Y., Zhang, R.-F., Zhang, H.-L., et al. (2013). Molecular cloning and functional analysis of a blue light receptor gene MdCRY2 from apple (Malus domestica). *Plant cell reports* 32(4), 555-566. doi: 10.1007/s00299-013-1387-4.

Luo, J., Nishiyama, Y., Fuell, C., Taguchi, G., Elliott, K., Hill, L., et al. (2007). Convergent evolution in the BAHD family of acyl transferases: identification and characterization of anthocyanin acyl transferases from Arabidopsis thaliana. *The Plant Journal*50(4), 678-695. doi: 10.1111/j.1365-313X.2007.03079.x.

Ma, X., Olsen, J.L., Reusch, T.B., Procaccini, G., Kudrna, D., Williams, M., et al. (2021). Improved chromosome-level genome assembly and annotation of the seagrass, Zostera marina (eelgrass).*F1000Research* 10(289), 289. doi: 10.12688/f1000research.38156.1.

Merzlyak, M., Solovchenko, A., and Pogosyan, S. (2005). Optical properties of rhodoxanthin accumulated in Aloe arborescens Mill. leaves under high-light stress with special reference to its photoprotective function. *Photochemical & Photobiological Sciences*4(4), 333-340. doi: 10.1039/b417802e.

Merzlyak, M.N., and Solovchenko, A.E. (2002). Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. *Plant Science* 163(4), 881-888. doi: 10.1016/S0168-9452(02)00241-8.

Neff, M.M., and Chory, J. (1998). Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. *Plant physiology* 118(1), 27-35. doi: 10.1104/pp.118.1.27.

Nguyen, H.M., Ralph, P.J., Marín-Guirao, L., Pernice, M., and Procaccini, G. (2021). Seagrasses in an era of ocean warming: a review.*Biological Reviews* 96(5), 2009-2030. doi: 10.1111/brv.12736.

Olsen, J.L., Rouze, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., 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.

Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., et al.

(2006). A global crisis for seagrass ecosystems. *Bioscience* 56(12), 987-996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Porra, R., Thompson, W., and Kriedemann, P. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 975(3), 384-394. doi: 10.1016/S0005-2728(89)80347-0.

Rinaldo, A.R., Cavallini, E., Jia, Y., Moss, S.M., McDavid, D.A., Hooper, L.C., et al. (2015). A grapevine anthocyanin acyltransferase, transcriptionally regulated by VvMYBA, can produce most acylated anthocyanins present in grape skins. *Plant physiology*169(3), 1897-1916. doi: 10.1104/pp.15.01255.

Shick, J.M., and Dunlap, W.C. (2002). Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual review of Physiology*64(1), 223-262. doi: 10.1146/annurev.physiol.64.081501.155802.

Solovchenko, A. (2010). *Photoprotection in plants: optical screening-based mechanisms.* Springer Science & Business Media.

Solovchenko, A., and Merzlyak, M. (2003). Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. *Photochemical & Photobiological Sciences*2(8), 861-866. doi: 10.1039/B302478D.

Solovchenko, A., and Merzlyak, M. (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russian Journal of Plant Physiology* 55(6), 719-737. doi: 10.1134/S1021443708060010.

Stintzing, F.C., and Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition.*Trends in food science & technology* 15(1), 19-38. doi: 10.1016/j.tifs.2003.07.004.

Strasser, R.J., Tsimilli-Michael, M., Qiang, S., and Goltsev, V. (2010). Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant Haberlea rhodopensis. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1797(6-7), 1313-1326. doi: 10.1016/j.bbabio.2010.03.008.

Takahashi, S., and Badger, M.R. (2011). Photoprotection in plants: a new light on photosystem II damage. *Trends in plant science*16(1), 53-60. doi: 10.1016/j.tplants.2010.10.001.

Tamagnone, L., Merida, A., Parr, A., Mackay, S., Culianez-Macia, F.A., Roberts, K., et al. (1998). The AmMYB308 and AmMYB330 transcription factors from Antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. *The Plant Cell*10(2), 135-154. doi: 10.1105/tpc.10.2.135.

Tan, Y., Zhang, Q.S., Zhao, W., Liu, Z., Ma, M.Y., Zhong, M.Y., et al. (2020). The highly efficient NDH-dependent photosystem I cyclic electron flow pathway in the marine angiosperm Zostera marina.*Photosynthesis research* 144(1), 49-62. doi: 10.1007/s11120-020-00732-z.

Tao, R., Bai, S., Ni, J., Yang, Q., Zhao, Y., and Teng, Y. (2018). The blue light signal transduction pathway is involved in anthocyanin accumulation in 'Red Zaosu'pear. *Planta* 248(1), 37-48. doi: 10.1007/s00425-018-2877-y.

Wang, B., Zarka, A., Trebst, A., and Boussiba, S. (2003). Astaxanthin Accumulation in Haematococcus pluvialis (Chlorophyceae) AS An active photoprotective process under high irradiance *Journal of Phycology* 39(6), 1116-1124. doi: 10.1111/j.0022-3646.2003.03-043.x.

Wang, M., Zhao, W., Ma, M., Zhang, D., Wen, Y., Zhong, M., et al. (2022). Intrinsic photosensitivity of the vulnerable seagrass Phyllospadix iwatensis: Photosystem II oxygen-evolving complex is prone to photo-inactivation. *Frontiers in Plant Science* , 399. doi: 10.3389/fpls.2022.792059.

Wissler, L., Codoner, F.M., Gu, J., Reusch, T.B., Olsen, J.L., Procaccini, G., et al. (2011). Back to the sea twice: identifying candidate plant genes for molecular evolution to marine life. *BMC evolutionary biology*

11(1), 1-13. doi: 10.1186/1471-2148-11-8.

Yanovsky, M.J., Alconada-Magliano, T.M., Mazzella, M.A., Gatz, C., Thomas, B., and Casal, J.J. (1998). Phytochrome A affects stem growth, anthocyanin synthesis, sucrose-phosphate-synthase activity and neighbour detection in sunlight-grown potato. *Planta* 205(2),235-241. doi: 10.1007/s004250050316

Yonekura-Sakakibara, K., Nakayama, T., Yamazaki, M., and Saito, K. (2008). "Modification and stabilization of anthocyanins," in*Anthocyanins* . Springer), 169-190.

Yonekura-Sakakibara, K., Nakayama, T., Yamazaki, M., and Saito, K. (2009). Modification and Stabilization of Anthocyanins. *Springer New York* , 169-190. doi: 10.1007/978-0-387-77335-3_6.

Yonekura-Sakakibara, K., Fukushima, A., Nakabayashi, R., Hanada, K., Matsuda, F., Sugawara, S., et al. (2012). Two glycosyltransferases involved in anthocyanin modification delineated by transcriptome independent component analysis in Arabidopsis thaliana. *The Plant Journal* 69(1), 154-167. doi: 10.1111/j.1365-313X.2011.04779.x.

Zhang, Y., Jiang, L., Li, Y., Chen, Q., Ye, Y., Zhang, Y., et al. (2018). Effect of red and blue light on anthocyanin accumulation and differential gene expression in strawberry (Fragariax ananassa).*Molecules* 23(4), 820. doi: 10.3390/molecules23040820.

Zhao, W., Yang, X.-Q., Zhang, Q.-S., Tan, Y., Liu, Z., Ma, M.-Y., et al. (2021). Photoinactivation of the oxygen-evolving complex regulates the photosynthetic strategy of the seagrass Zostera marina. *Journal of Photochemistry and Photobiology B: Biology* 222, 112259. doi: 10.1016/j.jphotobiol.2021.112259.

Zhu, H., Zhang, T.-J., Zheng, J., Huang, X.-D., Yu, Z.-C., Peng, C.-L., et al. (2018). Anthocyanins function as a light attenuator to compensate for insufficient photoprotection mediated by nonphotochemical quenching in young leaves of Acmena acuminatissima in winter.*Photosynthetica* 56(1), 445-454. doi: 10.1007/s11099-017-0740-1

Zoratti, L., Sarala, M., Carvalho, E., Karppinen, K., Martens, S., Giongo, L., et al. (2014). Monochromatic light increases anthocyanin content during fruit development in bilberry. *BMC plant biology*14(1), 1-10. doi: 10.1186/s12870-014-0377-1

Table 1. Summary of the raw sequencing data

Sample	Raw reds	Raw bases	Clean reads	Clean bases	Error rate (%)	Q30 (%)	GC (%)
Control 1	51696844	7806223444	51248218	7654100841	0.0254	93.7	45.89
Control 2	43195248	6522482448	42884144	6396532081	0.0247	94.34	45.91
Control 3	53426210	8067357710	52988854	7916574395	0.0256	93.52	45.55
Blue 1	47966530	7242946030	47571454	7087546538	0.0259	93.25	45.55
Blue 2	46431768	7011196968	46022620	6853807472	0.0259	93.15	45.34
Blue 3	46629886	7041112786	46209142	6869230840	0.026	93.07	45.54
White 1	47783712	7215340512	47304796	7048592380	0.0266	92.53	45.5
White 2	48806370	7369761870	48448086	7234007292	0.0253	93.81	45.84
White 3	42486188	6415414388	42179286	6296228913	0.0252	93.87	45.73
Red 1	55690780	8409307780	55279256	8264478362	0.0254	93.66	45.57
Red 2	42774264	6458913864	42383990	6336610478	0.0263	92.85	45.53
Red 3	53363724	8057922324	53060834	7781592173	0.0227	96.52	45.34

Q30 refers to the percentage of bases with sequencing quality above 99.9% in total bases.

Table 2. Summary of the transcriptome sequence alignment

Sample	Total reads	Total mapped	Multiple mapped	Uniquely mapped

Control 1	51248218	47669935 (93.02%)	3540284 (6.91%)	44129651 (86.11%)
Control 2	42884144	39508764 (92.13%)	2835227 (6.61%)	36673537 (85.52%)
Control 3	52988854	49921524 (94.21%)	3307605 (6.24%)	46613919 (87.97%)
Blue 1	47571454	44874551 (94.33%)	2337446 (4.91%)	42537105 (89.42%)
Blue 2	46022620	43045726 (93.53%)	2256231 (4.9%)	40789495 (88.63%)
Blue 3	46209142	43708260 (94.59%)	2148207 (4.65%)	41560053 (89.94%)
White 1	47304796	44443715 (93.95%)	2421034 (5.12%)	42022681 (88.83%)
White 2	48448086	45367323 (93.64%)	2510172 (5.18%)	42857151 (88.46%)
White 3	42179286	39200137 (92.94%)	2207280 (5.23%)	36992857 (87.7%)
Red 1	55279256	51774839 (93.66%)	2963989 (5.36%)	48810850 (88.3%)
Red 2	42383990	39667109 (93.59%)	2402614 (5.67%)	37264495 (87.92%)
Red 3	53060834	50230425 (94.67%)	2573786 (4.85%)	47656639 (89.82%)

Table 3. Content of acylated anthocyanins

		Anthocyanin content (μg/g)	Anthocya
	Compounds	BL	WL
aliphatic acyl-substituted	Delphinidin-3-O-(6-O-malonyl-beta-D-glucoside)	0.172±0.02	0.164±0.0
	Delphinidin-3-O-(6-O-malonyl)-glucoside-3'-glucoside	0.004±0.001	0.006±0.0
	Pelargonidin-3-O-(6-O-malonyl-beta-D-glucoside)	0.173±0.03	0.033±0.0
	Total amount	0.349	0.203
aromatic acyl-substituted	Cyanidin-3-(6-O-p-caffeoyl)-glucoside	0.417±0.02	0.400±0.0
	Cyanidin-3-O-(6-O-p-coumaroyl)-glucoside	0.015±0.01	0.017±0.0
	Delphinidin-3-O-(6-O-p-coumaroyl)-glucoside	0	0.013±0.0
	Malvidin-3-O-(6-O-p-coumaroyl)-glucoside	0.013±0.01	0.037±0.0
	Malvidin-3-O-5-O-(6-O-coumaroyl)-diglucoside	0.110±0.004	0.166±0.0
	Pelargonidin-3-O-(6-O-p-coumaroyl)-glucoside	0	0.006±0.0
	Peonidin-3-O-(6-O-p-coumaroyl)-glucoside	0.02±0.003	0.023±0.0
	Total amount	0.575	0.662

Figure 1. (A) Changes in O-J phase of normalized chlorophyll a fluorescence intensity in response to 15 min of different light qualities exposure, respectively. (B) Action spectrum of OEC photoinhibition. The inset shows the changes in W k following WL exposure. The means ± SD are calculated from three independent samples.

Figure 2. (A) Changes in normalized chlorophyll a fluorescence intensity of ΔV_{t} in response to 3 h of BL (blue circles), WL (light gray triangles) and RL (red inverted triangles) exposure, respectively. Each curve represents the average of three replicates. (B) Variations in $F_{\mathrm{v}}/F_{\mathrm{m}}$ in response to BL, WL and RL exposure, respectively. (C) Changes in three peripheral proteins of OEC are analyzed by Western blot. The significantly different value from dark (Tukey's tests, $p< 0.05$) is marked with an asterisk (*). (D) Variations in $\mathrm{OEC}_{\mathrm{centers}}$ in response to BL, WL and RL exposure, respectively. The means ± SD are calculated from three independent samples. Different letters indicate a significant difference ($p< 0.05$, one-way ANOVA).

Figure 3. (A) The typical O_2 content change curve in the reaction chamber of the Liquid-Phase Oxygen Electrode System for monitoring the O_2 evolution rate. (B) Time course of changes in the O_2 evolution rate in response to 3 h of BL, WL and RL exposure, respectively. The means ± SD are calculated from three independent samples. (C) Changes in the oxygen content change curve during the O_2 evolution rate monitoring after 3 h of BL, WL and RL exposure, respectively. The rising and falling slope of the curve under light and darkness represent the net photosynthetic rate and respiration rate, respectively.

Figure 4. Principal component analysis (PCA) of identified genes. Light blue squares represent the samples

of control, whereas red circles, green triangles, and dark blue inverted triangles indicate samples treated by BL, RL, and WL, respectively. Different spot of each color represents the biological replicates for this treatment.

Figure 5. (A-C) Enriched KEGG pathways among the DEGs in response to the BL, WL, and RL treatments, respectively. Vertical axis shows the pathway names, whereas the horizontal axis shows the enrichment factor. The size of each spot reflects the number of DEGs in this pathway, and each spot color corresponds to the different P -value ranges. Pathways with$P$ -values < 0.05 are significantly enriched.

Figure 6. (A) Expression profiles of the DEGs related to the carotenoid biosynthesis pathway. (B) Time course of changes in the carotenoid contents in response to 3 h of BL, WL and RL exposure. The means $\pm$ SD are calculated from three independent samples. Different letters indicate a significant difference ($p < 0.05$, one-way ANOVA).

Figure 7. (A) Expression profiles of the DEGs related to the anthocyanins biosynthesis. (B) Time course of changes in the anthocyanin contents in response to 3 h of BL, WL and RL exposure. The means $\pm$ SD are calculated from three independent samples. Different letters indicate a significant difference ($p < 0.05$, one-way ANOVA).

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