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Effects of light and salinity on carotenoid biosynthesis in *Ulva prolifera*

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Abstract

Ulva prolifera is a green alga that plays an important role in green tides. Carotenoid biosynthesis is a basic terpenoid metabolism that is very important for maintaining normal life activities in algae. In this study, we first reported the complete sequences of all genes in the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which is the only carotenoid synthesis pathway in *U. prolifera*. Then, we compared these genes with those of other species. Additionally, by detecting the carotenoid contents and expression levels of key genes participating in carotenoid biosynthesis in *U. prolifera* under three different light (1 000 lx, 5 000 lx and 12 000 lx) and salinity (12, 24 and 40) regimes, we found that carotenoid synthesis could be influenced by light and salinity, such that low light and high salinity could promote the synthesis of carotenoids. The results showed that the expression levels of genes involved in the MEP and the downstream pathway could affect the biosynthesis of carotenoids at the molecular level. This study contributes to a better understanding of the roles of genes participating in carotenoid biosynthesis in *U. prolifera* and the environmental regulation of these genes.

Key words: *U. prolifera*, carotenoid, MEP pathway, light, salinity

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1 Introduction

Ulva prolifera belongs to Chlorophyta, Ulvophyceae, Ulvales, Ulvaceae, *Ulva* (Gao et al., 2010) and is a bright green or pale green alga. The height of *U. prolifera* can reach 1–2 m, and the diameter can reach 2–3 mm. It has many branches, but the main branches are obvious and slender. In terms of its life history, *U. prolifera* exhibits asexual reproduction, sexual reproduction and vegetative reproduction (Lin et al., 2008; Zhang et al., 2011). In terms of its biological characteristics, *U. prolifera* can withstand various environmental stresses, such as salty, dry and high light conditions, and can survive in a wide range of temperatures (Xiao et al., 2016). The "green tide" disaster that has continuously erupted in the Yellow Sea waters of China throughout the past 10 years has brought considerable negative impacts on the ecological environment and economy of coastal areas (Zhao et al., 2018; Zhang et al., 2017). Studies of the basic metabolism of *U. prolifera*, such as lipid metabolism and terpenoid metabolism, are very important for determining its biological characteristics.

Terpenoids are the most widely distributed natural compounds in nature and play an important role in plant growth, development, light absorption, hormone synthesis, photoprotection and stress resistance (Lange et al., 2000). Terpenoids are the largest and most volatile matter released by plants (Sharma et al., 2017). Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are precursors of terpenoids. Two pathways are involved in the biosynthesis of IPP and DMAPP in organisms: the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Vranová et al., 2013; Yang and Guo, 2014). In recent years, the metabolism of terpenoids in algae has been studied intensively. It has been proven that as green algae have evolved, the MVA pathway has disappeared and only the MEP pathway exists (Lohr et al., 2012). In the synthesis of IPP and DMAPP, the MEP pathway is independent of MVA. This pathway includes eight enzymes: 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (CMS), 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate (MCS), 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) and isopentenyl pyrophosphate isomerase (IPI) (Vranová, 2012). These enzymes play an important regulatory role in the pathway. Terpenoids are secondary metabolites in living organisms. Secondary metabolites play a key role in improving the ability of organisms to survive and interact with their environment (Bennett and Wallsgrove, 1994). The production of and changes in secondary metabolites are sensitive to the environment (Kliebenstein, 2004; Zhang et al., 2014; Duan et al., 2003).

Carotenoids are terpenoid pigments derived from C40; more than 750 carotenoids have been reported in nature (Takaichi, 2011), and they have high commercial value because they are rich in nutritional value (Patias et al., 2017). Carotenoids are biosynthesized through the MEP pathway, which includes eight enzymes, and the downstream pathway, which includes key enzymes such as PSY, PDS and ZDS (Sun et al., 2008).

Microalgae, such as *Haematococcus pluvialis* and *Dunaliella*

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salina, are an alternative platform for carotenoid production (Yuan et al., 2011); however, the study of carotenoids is weak in macroalgae. In this study, we obtained the full length of all genes in MEP pathways and analyzed them through bioinformatics, which helped us to better understand the functions of these genes. Furthermore, we explored the expression levels of genes involved in the MEP pathway and the downstream pathway under different light and salinity conditions; the results lay a foundation for studying the complex molecular biology of terpenoid biosynthesis in *U. prolifera* in the future.

2 Materials and methods

2.1 *Plant materials*

Ulva prolifera samples were cultivated in the seaweed herbarium of Soochow University. The materials were cultivated in seawater consisting of 15 mg/L N and 2 mg/L P. The conditions for cultivation were 20°C, with cool-white fluorescent light provided on a 12:12 light:dark cycle. There were three light regimes: 1 000 lx was set as low light (l), 12 000 lx was set as high light (h), and 5 000 lx was set as medium light (m). In addition, there were three salinity regimes: salinity 12 was set as low salinity (L), salinity 40 was set as high salinity (H), and salinity 24 was set as medium salinity (M). These samples were cultivated at three light and salinity regimes for 5 d.

2.2 *Measurement of total carotenoids and chlorophyll a (Chl a) and chlorophyll b (Chl b)*

Samples (0.05 g) from these samples were weighed and ground into powder; and then, 5 mL 80% acetone solution was added to the powder. The samples were shaken on ice for 20 min and centrifuged at 12 000 r/min for 20 min; and then, the supernatants were collected. The absorbances (A) at wavelengths of 470 nm, 646.8 nm and 663.2 nm were obtained with a spectrophotometer (Hitachi, Japan). The contents (C, μg/mL) of total carotenoids, Chl *a* and Chl *b* were calculated with the following formulas:

$$C_{\text{total carotenoids}} = (1\,000A_{470} - 1.82C_{\text{Chl }a} - 85.02C_{\text{Chl }b})/198, \quad (1)$$

$$C_{\text{Chl }a} = 12.25A_{663.2} - 2.79A_{648.8}, \quad (2)$$

$$C_{\text{Chl }b} = 21.5A_{646.8} - 5.1A_{663.2}. \quad (3)$$

2.3 *Total RNA extraction and cDNA synthesis*

Total RNA was obtained from *U. prolifera* cultivated at different light and salinity regimes using the MiniBEST Plant RNA Extraction Kit (Takara, Japan). The RNA ratio of A_{260} to A_{280} and A_{230} to A_{280} was detected with the NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). Then, single-strand cDNA was obtained from 2 μg total RNA according to the user's manual (Takara Reverse Transcription kit, Japan).

2.4 *Cloning the full length of genes involved in the MEP pathway by RACE*

The partial sequences of genes involved in carotenoid biosynthesis of *U. prolifera* were obtained based on the transcriptome of *U. prolifera* in the NCBI Sequence Read Archive (SRP157932). Then, rapid amplification of the cDNA ends (RACE) was performed to obtain the 5′ and 3′ ends of these genes according to steps in the user's manual (SMARTer RACE5′/3′ Kit User Manual Extraction Kit (Takara, Japan)). The primers for RACE were designed using Primer Premier 5.0 and are listed in Table 1. The complete open reading frames (ORFs) of the genes were obtained using ORF Finder from the start codon to the stop codon.

2.5 *Multiple alignments of genes with different species*

The boundaries and lengths of the deduced amino acid sequences of genes were confirmed using BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences of all the species used were obtained from GenBank. The deduced amino acid sequences were analyzed using the Conserved Domain program. Multiple sequence alignment was conducted with DNA-

Table 1. The primer sequences of the genes related to terpenoids synthesis pathway in *Ulva prolifera*

Gene	Primer name	Sequence
DXS		
Core sequence	DXSF	GCTGGCGGAACTGGCCTTTACC
	DXSR	GGGCGACATCATGCACAACCTG
5′-RACE	5DXS	GCCAAGCCAGCGGCAAATGTAA
3′-RACE	3DXS	CTGTTACATTTGCCGCTGGCTTGG
	UPM	CTAATACGACTCACTATAGG-GCAAGCAGTGG TATCAACGCAGAGT
DXR		
Core sequence	DXRF	GAAGCCTACATGCGCTGCAATC
	DXRR	CAATGCGTCTCAAGCCTCCCTCA
5′-RACE	5DXR	GAAGCCTACATGCGCTGCAATC
3′-RACE	3DXR	CAATGCGTCTCAAGCCTCCCTC
HDS		
Core sequence	HDSF	GTGCTGATGCTGGGGCTGACCT
	HDSR	GAATATCTGCCACCAACGGTGTG
5′-RACE	5HDS	AGGTCAGCCCCAGCATCAGCAC
3′-RACE	3HDS	GTGCTGATGCTGGGGCTGACCT
HDR		
Core sequence	HDRF	ACGGTGGGGACCAAGGAGGAGT
	HDRR	GTCCACCGCGTCCTCGCCGCCG
5′-RACE	5HDR	CCAAAGGCAGGAAGGATGACCACA
3′-RACE	3HDR	ACACGCCCCTCCCACGCAAACT
CMS		
Core sequence	CMSF	GCGGTGCTGGGTGTTCCTGTCAA
	CMSR	CGTTCTGCAACGCTCATGTCGTC
5′-RACE	5CMS	TGGCGTCGTGACCTTGATGTTGG
3′-RACE	3CMS	CACCAACATCAAGGTCACGACGCC
CMK		
Core sequence	CMKF	ACCTGCTGAAGTGGTCGGGTGA
	CMKR	TGGCTTGATGAGGAGGAGGGGTG
5′-RACE	5CMK	TGGCTTGATGAGGAGGAGGGGTG
3′-RACE	3CMK	TACAGCGTTGCCAAGCGTACCAAA
MCS		
Core sequence	MCSF	TGCTCCCGAGTTCCAAGCGTACA
	MCSR	CCTCCCCGATTGAATCCACCTTC
5′-RACE	5MCS	TGGCATCTGTGACGCAATGGAGC
3′-RACE	3MCS	CTCCCGAGTTCCAAGCGTACAGGC
IPI		
Core sequence	IPIF	CTCCGATGGTCGCCCTGGTTCC
	IPIR	GCTTTGCGCTTAGAGCATGGTTGG
5′-RACE	5IPI	CCCCATCAACATCCTCCCACCACT
3′-RACE	3IPI	CTCCGATGGTCGCCCTGGTTCC

MAN software (DNAMAN, LynnonBiosoft, USA). The ExPASy ProtParam tool was used to perform a bioinformatic analysis of the genes.

2.6 *Gene expression analysis of carotenoid biosynthesis genes*

The expression patterns of genes involved in carotenoid biosynthesis, including those of the MEP pathway and the downstream pathway, were explored simultaneously by real-time PCR (RT-PCR). The primers for RT-PCR were designed using Primer Premier 5.0 software; 18S rRNA was used as the control for normalization; the primers are listed in Table 2; and 1 μg total RNA was used to synthesize the cDNA. The RT-PCR experiment was carried out by using the Baiyuan ASA-4800 Real Time PCR System with SYBR green fluorescence (Takara, Japan). The RT-PCR conditions were as follows: the cycling profile included a step at 95°C for 30 s, followed by 40 cycles of amplification (95°C for 5 s and 60°C for 34 s). The relative gene expression was calculated with the $2^{-\triangle\triangle CT}$ method. The experiment for each sample was repeated three times.

3 Results

3.1 *Cloning and characterization of all genes involved in carotenoid biosynthesis*

To clone partial sequences of target genes, the primers were designed to obtain core fragments. Based on the partial sequences, RACE technology was used to obtain the 3′ and 5′ regions of sequences. Sequence analyses showed that the full-length cDNA of *UpDXS* was 2 689 bp and encoding a putative amino acid protein with a molecular weight of 76.89 kDa; *UpDXR* was 2 548 bp and encoding a putative amino acid protein with a molecular weight of 46.95 kDa; *UpCMS* was 1 881 bp and encoding a putative amino acid protein with a molecular weight of 29.25 kDa; *UpCMK* was 1 131 bp and encoding a putative amino acid protein with a molecular weight of 35.46 kDa; *UpMCS* was 905 bp and encoding a putative amino acid protein with a molecular weight of 20.91 kDa; *UpHDS* was 2 509 bp and encoding a putative amino acid protein with a molecular weight of 79.52 kDa; *UpHDR* was 2 254 bp and encoding a putative amino acid protein with a molecular weight of 52.2 kDa; *UpIPI* was 1 434 bp and encoding a putative amino acid protein with a molecular weight of 27.66 kDa (Table 3). The sequences of these genes have been submitted to NCBI GenBank with accession numbers MH716005-MH716012.

Table 2. Primers of genes for qPCR

Gene	Primer name	Sequence
18SrDNA	18F1	ACCACATCCAAGGAAGGCAGCAG
	18R1	TGCGTCCCACCCGAAATCCAAC
HDS	HDSF1	CTGGGAACTTTGCAGACGGAACGA
	HDSR1	AAGACAGAATGCGGGCACTCAAGC
HDR	HDRF1	TTGCGTGGGAGGGGCGTGTTAC
	HDRR1	AGGTCAGGTTGGTGGCAACATGGG
DXS	DXSF1	GCCTGTGCGGTTTGCGATGGAT
	DXSR1	ACTTCCAGCAACACGGCATCGG
DXR	DXRF1	CGGGGAAGATGTTGATGCGCTGAA
	DXRR1	AAAACACGGCGTCCACATCCTTCC
CMS	CMSF1	GGTTCTACTCGCTGGTGGCGTTGG
	CMSR1	CTTGGCGCTCCGCTCCTGGTAA
CMK	CMKF1	TGAGTCGGTGTTCATGACGG
	CMKR1	ATACCATTCTCCCGGTTGCC
MCS	MCSF1	CTTGTTGTTCGGTGATGTGGCTCG
	MCSR1	CCGGTTTGGCTTGTTTGAAGGTCC
IPI	IPIF1	ACGGCTGCGATGTCTTTTGTAGGG
	IPIR1	GCTTTGCGCTTAGAGCATGGTTGG
GGPS	GGPSF1	AGGGGAACCTGGCGGGAATGGA
	GGPSR1	CCACCACAACGCACAGCATAACCA
PSY	PSYF1	AGACTACGCCTTGCAACACA
	PSYR1	TACAAGACTGCTGTCTGCCC
PDS	PDSF1	TACCACTGACGTGTCACAGC
	PDSR1	TGCATGCTACTTGGTGCTGA
ZDS	ZDSF1	TGGGCCTCCATGTGTTCTTC
	ZDSR1	TCTCTACTGCGCCACACTTG

Table 3. The information of the sequences

Gene	Full length/bp	5′-UTR/bp	3′-UTR/bp	Full-length ORF/bp	Molecular weight/kDa
UpDXS	2 689	196	351	2 142	76.89
UpDXR	2 548	194	1 040	1 314	46.95
UpCMS	1 881	244	827	810	29.25
UpCMK	1 131	157	20	972	35.46
UpMCS	905	166	163	576	20.91
UpHDS	2 509	82	267	2 160	79.52
UpHDR	2 254	101	734	1 419	52.2
UpIPI	1 434	265	431	738	27.66

3.2 *Comparative analysis of all the genes*

The encoded protein of *UpDXS* predicted by the NCBI Conserved Domain Search program indicated that it contained three conservative domains: domain I for DXP_synthase_N (67–352), domain II for TPP_PYR_ DXS_TK_like (393–548), and domain III for transketolase_C (573–696); the *UpDXR* protein contained three conservative domains: DXP_reductoisom (43–171), DXP_redisom_C (185–268), and DXPR_ C (301–419); the UpCMS protein contained one conservative domain: ispD (46–266); the *UpCMK* protein contained one conservative domain: ispE (35–309); the *UpMCS* protein contained one conservative domain: ispF (35–190); the *UpHDS* protein contained two conservative domains: ispG (57–344), GcpE (569–701); the *UpHDR* protein contained one conservative domain: ispH (105–450); and the *UpIPI* protein contained one conservative domain: IPP_isomerase (20–215).

The amino acid sequences of these genes and those of other proteins in GenBank were analyzed using Protein BLAST (Table 4). The results of multiple alignments of the amino acid sequences of these genes with those of other species in GenBank indicated that compared with those of other species, the amino acid sequences of genes involved in the MEP pathway were conserved.

3.3 *The contents of total carotenoids, Chl a and Chl b of different samples*

For the groups subjected to different light regimes, the contents of total carotenoids, Chl *a* and Chl *b* in the l group were (1.90±0.01) μg/mL, (7.48±0.04) μg/mL and (3.96±0.09) μg/mL, respectively. The contents of total carotenoids, Chl *a* and Chl *b* in the m group were (1.73±0.01) μg/mL, (5.33±0.06) μg/mL and (3.20±0.09) μg/mL, respectively. The contents of total carotenoids, Chl *a* and Chl *b* in the h group were (1.34±0.02) μg/mL, (4.07±0.06) μg/mL and (2.77±0.09) μg/mL, respectively. The results showed that the contents of terpenoids were highest in the samples subjected to low light, followed by the samples subjected to medium light; the samples subjected to high light had the lowest terpenoid contents (Fig. 1).

Table 4. Comparative analysis of all the genes

Genes	Species	E-values	Sequence identities	Accession numbers
UpDXS	*Raphidocelis subcapitata*	0.0	76.96%	GBF98923.1
UpDXR	*Micractinium conductrix*	0.0	75.18%	PSC72817.1
UpCMS	*Coccomyxa subellipsoidea* C-169	1×10^{-105}	67.25%	XP_005649705.1
UpCMK	*Chlorella variabilis*	2×10^{-134}	70.99%	XP_005851681.1
UpMCS	*Volvox carteri*	4×10^{-95}	72.83%	XP_002947986.1
UpHDS	*Trebouxia* sp. A1-2	0.0	69.15%	KAA6412354.1
UpHDR	*Chlorella variabilis*	0.0	66.22%	XP_005848540.1
UpIPI	*Haematococcus lacustris*	1×10^{-101}	57.20%	AAC32208.1

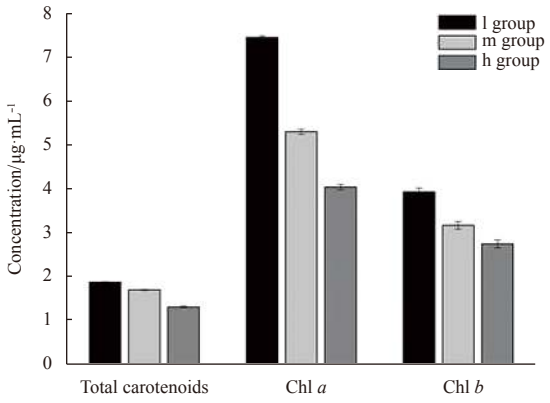

Fig. 1. The concentrations of total carotenoids, Chl *a* and Chl *b* in samples subjected to different light regimes.

For the groups subjected to different salinities, the contents of total carotenoids, Chl *a* and Chl *b* in the L group were (1.14±0.04) μg/mL, (4.20±0.05) μg/mL and (3.06±0.09) μg/mL, respectively. The contents of total carotenoids, Chl *a* and Chl *b* in the M group were (2.21±0.01) μg/mL, (7.70±0.05) μg/mL and (5.49±0.08) μg/mL, respectively. The contents of total carotenoids, Chl *a* and Chl *b* in the H group were (2.65±0.01) μg/mL, (10.23±0.06) μg/mL and (6.94±0.09) μg/mL, respectively. The results showed that the contents of terpenoids were highest in the samples subjected to high salinity, followed by the samples subjected to medium salinity; and the samples subjected to low salinity had the lowest terpenoid contents (Fig. 2).

3.4 *Expression analysis of all genes involved in the MEP pathway in different environments*

The samples were subjected to different environments, and the relative mRNA expression levels of all genes involved in the MEP pathway were detected by RT-PCR. The results indicated that the expression levels of all eight genes involved in the MEP pathway were significantly influenced by light and salinity (Figs 3 and 4). The MEP pathway and the expression of related genes in *U. prolifera* in different environments are shown in Fig. 5.

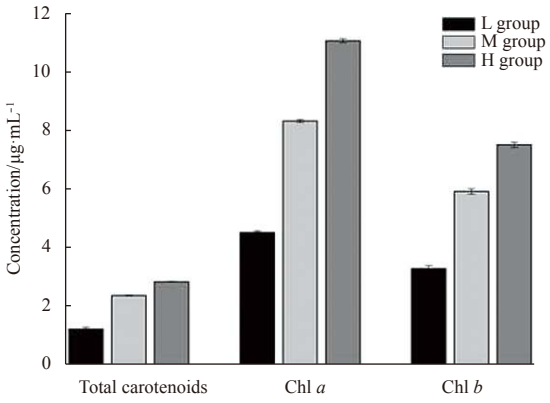

Fig. 2. The concentrations of total carotenoids, Chl *a* and Chl *b* in samples subjected to different salinities.

For the samples treated with different light regimes, the expression levels of five of the eight genes were gradually downregulated with increasing light, and seven genes had the lowest expression levels under high light. The data suggested that high light may not be beneficial to the expression of these genes.

For the samples treated with different salinities, the expression levels of all eight genes were upregulated with increasing salinity. The data suggested that high salinity may be conducive to the expression of the genes participating in the MEP pathway.

3.5 *Expression analysis of key genes involved in the downstream pathway of carotenoid biosynthesis*

The expression levels of four key genes, GGPS, PSY, PDS and ZDS, were also detected under three different light and salinity regimes (Figs 6 and 7). The carotenoid biosynthetic pathway and the expression of related genes in *U. prolifera* in different environments are shown in Fig. 8.

Under different light culture conditions, the expression levels of GGPS, PSY and PDS were downregulated with increasing light, and the results were in line with the trend of genes participating in the MEP pathway. However, the expression level of ZDS was upregulated with increasing light.

Under different salinity culture conditions, the expression levels of the genes were gradually upregulated with increasing salinity. Three of the four genes had the highest expression levels under high salinity, and the results basically conform to the trend of genes participating in the MEP pathway.

4 Discussion

Terpenoids are also called isoprenoids and are involved in almost all life activities, are metabolites required by a large class of organisms and are widely found in nature (Davies et al., 2015). Terpenoid synthesis is divided into three stages: first, the steroid precursors IPP and DMAPP are synthesized through the MEP pathway; then, the precursors IPP and DMAPP process different lengths of polyisoprene precursors; and, ultimately, the polyisoprene precursors undergo reactions to produce a series of terpenoids with different functions and activities (Lohr et al., 2012). Carotenoids are a general term for natural pigments and belong to tetraterpenoids. Carotenoids are indispensable functional pigments in the human body; however, the human body cannot synthesize these pigments, and carotenoids can only be obtained from food (Mikami and Hosokawa, 2013). Carotenoids have many functions, including enhancing immunity, preventing cancer occurrence, delaying aging, inhibiting bacterial

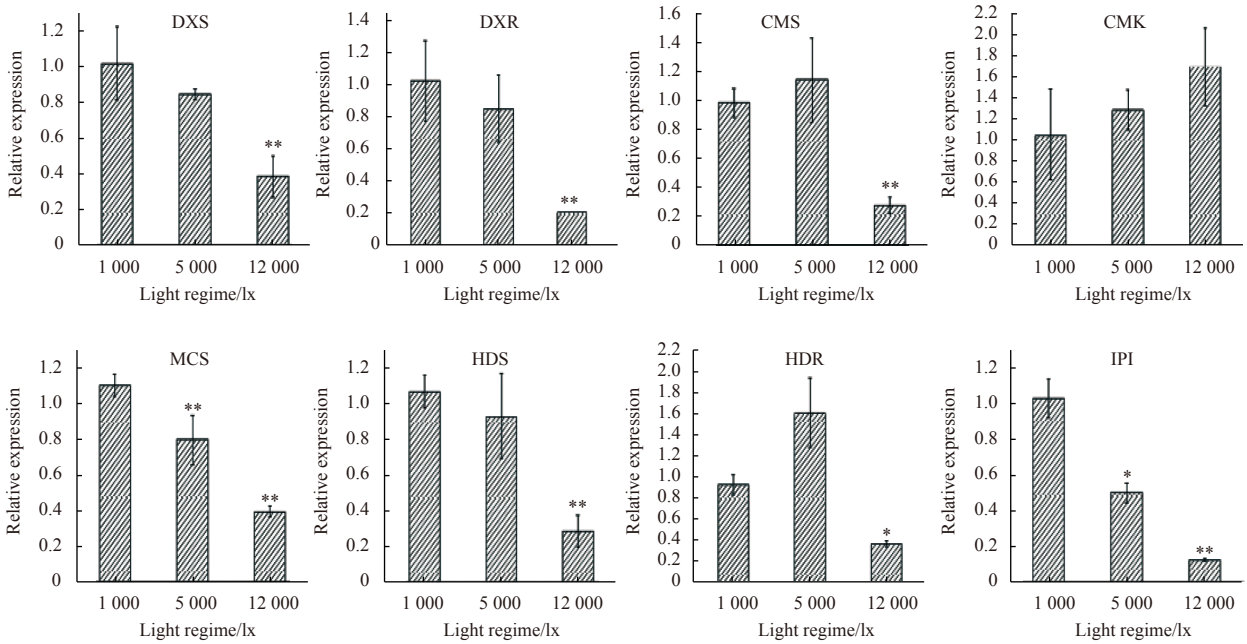

Fig. 3. The results of RT-PCR of genes in the MEP pathway of *U. prolifera* under different light regimes. The significance level was set at $P<0.05$ level and is shown with an asterisk. The highly significant level was set at $P<0.01$ level and is shown with two asterisks.

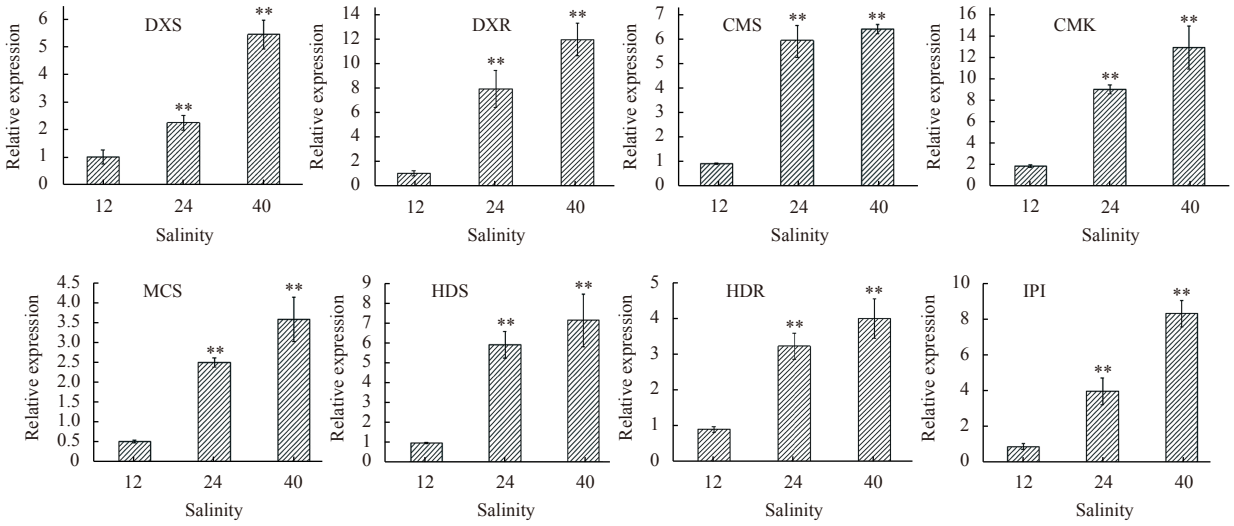

Fig. 4. The results of RT-PCR of genes in the MEP pathway of *U. prolifera* under different salinities. The significance level was set at $P<0.05$ level and is shown with an asterisk. The highly significant level was set at $P<0.01$ level and is shown with two asterisks.

growth, and resisting oxidation (Astley et al., 2004; Guedes et al., 2011; Collins, 2001; Zheng et al., 2018).

The regulation of the expression of related genes in the metabolic pathways of terpenoids plays an important role in the growth, light absorption and cell membrane synthesis of plants and is affected by various factors. The MEP pathway is an upstream pathway that regulates carotenoid biosynthesis. In the natural environment, as seawater stirs and tumbles, *U. prolifera* undergoes a transition from direct sunlight to the low light of the low layer of seawater. At the same time, the salinity changes significantly as environmental changes occur on the sea surface, such as increased CO_2 concentration in the atmosphere, which can cause heavy rainfall and further result in salinity of seawater changes. Therefore, light and salinity are important factors that affect the growth of *U. prolifera*. The expression levels of genes participating in the MEP pathway in *Arabidopsis* have been shown to significantly increase when exposed to intense light (Kliebenstein, 2004). The expression level of the GGPS gene related to carotenoid biosynthesis in *Pyropia umbilicalis* has also been shown to be affected by light, and the expression level of the gene is higher under high light (Yang et al., 2016). This study also showed that light is a factor affecting the expression levels of genes for carotenoid biosynthesis in *U. prolifera* and that the expression levels of most genes increased under lower light. The results were in accord with the conclusion in tonka beans that plants increase their light capture in a low light environment (de

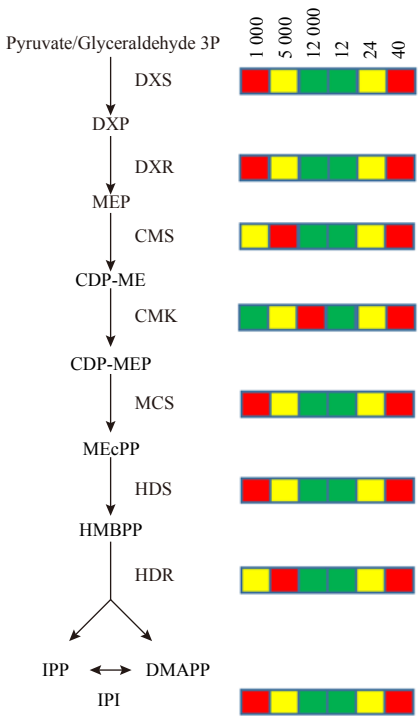

Fig. 5. The MEP pathway and the expression of related genes in *U. prolifera*. Eight enzymes participate in the steps of the MEP pathway, including DXS, DXR, CMS, CMK, MCS, HDS, HDR and IPI. 1 000: 1 000 lx, 5 000: 5 000 lx, 12 000: 12 000 lx, 12: salinity 12; 24: salinity 24, and 40: salinity 40. Red indicates high expression, yellow medium expression, and green low expression.

Carvalho Gonçalves et al., 2001). Salinity stress can induce the metabolic pathway and synthesis of some terpenoids, such as monoterpenes and carotenoids (Rivasseau et al., 2009). Aceto-acetyl-CoA thiolase is a regulatory enzyme for the synthesis of terpenoids. RT-PCR results showed that the expression level of this gene in the roots and leaves of tropical plants increased significantly under salt stress (Shi et al., 2016). In this study, the expression level of genes related to carotenoid biosynthesis in *U. prolifera* was also affected by salinity. The results showed that the expression levels of most genes were increased under higher salinity, which was consistent with the results of sunflower (Soto et al., 2011). The expression levels of these genes are influenced by two environmental factors, which indicates that environmental factors have significant effects on the expression levels of carotenoid biosynthetic genes in organisms (Wang et al., 2008). However, not all the studied genes had consistent expression trends. For example, the expression trends of CMK, HDR and ZDS under different light conditions and the expression trends of GGPS and ZDS under different salinities were different from those of other genes. We hypothesize that the biosynthesis of carotenoids is a complex process determined by the expression of multiple genes and depends on the expression trends of most genes. The specific molecular mechanism of carotenoid biosynthesis needs to be explored in future research.

Temperature, light and salinity are important environmental factors of algae (He et al., 2017). In a previous study, temperature was shown to be a critical factor affecting carotenoid biosynthesis in *U. prolifera* (He et al., 2018). To further explore the effects of the environment on carotenoid biosynthesis in *U. prolifera*, light and salinity were used as important factors for studying the expression levels of genes participating in the MEP pathway and the downstream pathway. The results indicated that, as with temperature, light and salinity can influence carotenoid synthesis in *U. prolifera*. Our results increase our understanding of the metabolism of terpenoids under different environments and lay the foundation for studying other metabolic pathways in *U. prolifera* that are influenced by the environment.

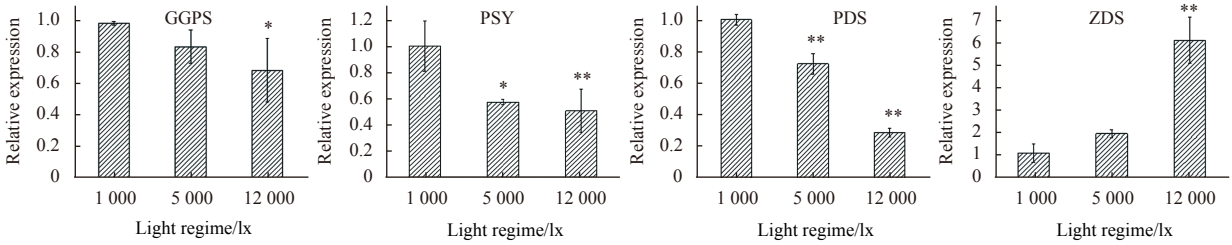

Fig. 6. The results of RT-PCR of genes in the downstream pathway of carotenoid biosynthesis in *U. prolifera* under different light regimes. The significance level was set at $P<0.05$ level and is shown with an asterisk. The highly significant level was set at $P<0.01$ level and is shown with two asterisks.

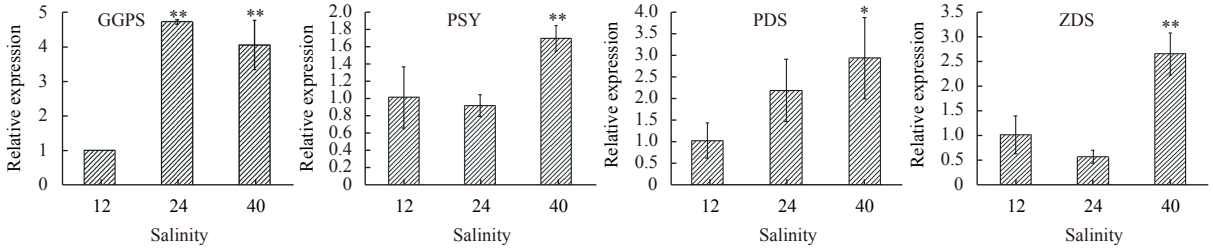

Fig. 7. The results of RT-PCR of genes in the downstream pathway of carotenoid biosynthesis in *U. prolifera* under different salinities. The significance level was set at $P<0.05$ level and is shown with an asterisk. The highly significant level was set at $P<0.01$ level and is shown with two asterisks.

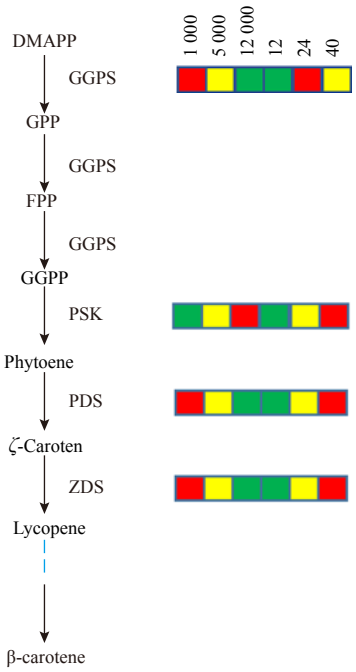

Fig. 8. Carotenoid biosynthetic pathway and the expression of related genes in *U. prolifera*. Four enzymes that participate in the steps of carotenoid biosynthesis were studied, including GGPS, PSY, PDS and ZDS. 1 000: 1 000 lx, 5 000: 5 000 lx, 12 000: 12 000 lx, 12: salinity 12, 24: salinity 24, and 40: salinity 40. Red indicates high expression, yellow medium expression, and green low expression.

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