Effect of a short-term light stress on resistance, signaling, metabolism, and cell division of *Ulva prolifera* revealed by omics

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Abstract

Ulva prolifera is the main species of green tide algae in the Yellow Sea, China, and its growth process is significantly affected by light intensity. The work used four omics to reveal the molecular mechanism of *U. prolifera* responding to high light. Four-omics conjoint analysis showed the interconversion of sugars in the algae, fatty acid synthesis, steroid synthesis, photosynthesis, pyrimidine metabolism and carbohydrate metabolism. After 12 hours of high light, the photosynthetic capacity of *U. prolifera* increased and the carbon sequestration mode changed from C3 pathway to C4 pathway. At the same time, the glucose metabolism pathway was enhanced, but the energy metabolism pathway was weakened, and the overall energy consumption showed a decreasing trend. Different resistance modes have different response mechanisms to high light stress. In addition, the growth, development and reproduction of *U. prolifera* were inhibited, indicating that *U. prolifera* may be in a dormant state after 12 hours of hight light stress, reducing energy consumption caused by unnecessary developmental physiological processes. The response mechanism of *U. prolifera* to high light stress was preliminarily obtained through the combined analysis of four kinds of omics, which provided the basis for future research.

1. Introduction

From 2007 to 2020, large-scale green tide disasters occurred in the Yellow Sea in China [1][1], which has severely affected the organisms, environment, and the lives of coastal residents in the sea area. Green tides are ecological anomaly caused by a sharp increase in green algae's biomass, and *U. prolifera* has always been a species in the outbreak of green tide algae in the Yellow Sea [3][2]. *U. prolifera* belongs to the Chlorophyta, *Ulva*les, and *Ulva* [1, 3], and has strong resistance to the complex environmental conditions in the sea. It can also resist and adapt to the increasing light intensity in the Yellow Sea from spring to summer during floating and reproduce rapidly [5][4]. *U. prolifera* reaches the Shandong Peninsula and Qingdao coastal waters in July and becomes the only surviving species.

The light intensity of algae growth is generally between 33-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [6][5], and the change of light intensity has different effects on its growth, photosynthesis, and respiration [7][6]. For *U. prolifera*, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is the lowest light intensity for algae growth, and 60-140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is the suitable light intensity (the light intensity range in the Yellow Sea area in May and June), with the algae reaching the highest daily growth rate [6][5]. With the increased light intensity after July, the growth of other early component species of green tide algae, including *Ulva linza*, *Ulva compressa*, and *Ulva flexuosa*, is inhibited at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, the instantaneous net photosynthetic performance of *U. prolifera* increases significantly at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the relative growth rate at 280 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is even higher than that under low light conditions [7, 8][11]. *U. prolifera* can even survive under 200-600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [9], showing that it has strong tolerance to high light intensity, and the mechanism among which is worth studying.

With the rapid development of high-throughput sequencing techniques, omics technology has become the mainstream for studying organisms' response to the environment. Jia *et al*. obtained many expressed sequence tags of *U. prolifera*, marking the parts that may contribute to the rapid growth of algae [13][10]. The comparative transcriptome of *U. prolifera*, *U. linza*, *U. flexuosa*, and *U. compressa* showed the difference in the construction of transcription factors and metabolic pathways of *U. prolifera*, as well as the enrichment of pyruvate kinase and nitrate transporters in these growth-related genes [15][11]. However, the current research on the effects of environmental factors on *U. prolifera* focuses on the temperature response. For example, the transcriptome is used to identify the relevant genes of *U. prolifera* involved in the carotenoid biosynthesis pathway at different temperatures [16][12]; proteomics is used to study the changes in the protein expression of *U. prolifera* at high temperatures [18][13]. The research on the response to light intensity stays at physiological ecology or focuses on individual genes, e.g., the ELIP-like genes in *U. linza* may be involved in photoprotection under high light, and low temperature and low osmotic stress. Therefore, the molecular mechanism of *U. prolifera* responding to light intensity lacks a comprehensive and systematic understanding.

The previous transcriptomics studies showed that the above four green tide algae have significant differences in response to different light intensities and provides a reference for establishing the light intensity models. On this basis, the work combined transcriptome, proteome, metabolome, and lipidome to study the environmental response of *U. prolifera* under high light intensity, thus revealing the biological mechanism of *U. prolifera*.

2. Materials And Methods

2.1 Materials

U. prolifera samples were collected from Qingdao waters (120°19'E, 36°04'N) in July 2008, and gametophytes' pure-line progeny were obtained through sterile subculturing in the laboratory. In this experiment, samples of *U. prolifera* gametophytes were subcultured in VSE medium at 20°C and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and light period/dark period = 12:12 h. After 15 days of cultivation, the algae with healthy growth and similar morphology were taken. The experiment was divided into the high light intensity treatment group (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the control group (120 $\mu mol \cdot m^{-2} \cdot s^{-1}$), with other conditions unchanged. After the two groups were cultured for 12 h, the algae were taken out immediately. After liquid-nitrogen treatment and ultra-low-temperature freeze-drying, omics tests were performed separately. The experiment set up biological replicates, where transcriptome and proteome had three replicates per group, and metabolome and lipidome had six per group. Each of the above replicates contained ten fronds.

2.2 Chlorophyll fluorescence determination

12 hours later after culture under L: D = 12 h: 12 h, the conditions of chlorophyll fluorescence were determined after 15 mins treatment in the darkness using fluorescence analyzer (PHYTO-PAM WALZ).

The parameters included maximum energy conversion efficiency, actual light energy conversion efficiency, effective energy conversion efficiency value, and coefficient of photochemical quenching. Each group had three repeats.

2.3 Photosynthetic oxygen release rate determination

12 hours later after culture under L: D = 12 h: 12 h, 0.1 g *U. prolifera* in each repeat was used to extract chlorophyll by 80% acetone. The absorbance was measured at the wavelengths of 663 nm, 645 nm and 652 nm. Each group had three repeats. [20]According to the Arnon formula [14], the chlorophyll (Chl) content was calculated as follows:

Chl a concentration (mg/L): Chl a=12.72A_{663}-2.69A_{645}

Chl b concentration (mg/L): Chl b=22.88A_{645}-4.67A_{663}

Total chlorophyll concentration (mg/L): Chl (a+b) = Chl a + Chl b =20.29A_{645}+8.05A_{663}

After the concentration of pigment is obtained, the content of various pigments per unit fresh weight in the tissue is calculated by the following formula:

$$\text{chloroplast pigment content}=\frac{\text{pigment concentration}\times\text{extraction liquid volume}\times\text{dilution ratio}}{\text{fresh weight of sample (mg / g)}}$$

2.4 Transcriptomics procedure

In the transcriptome experiment, the total RNA from *U. prolifera* samples was accurately quantified after extracting. mRNA capture and fragmentation were performed. After synthesizing the first strand, double-strand cDNA synthesis was performed. Subsequently, the library was amplified with quality testing, and the obtained cDNA library was subjected to high-throughput sequencing on Illumina Hiseq TM. Then, Fast QC quality evaluation was performed on the original sequencing data, and the quality was cut by Trimmomatic to obtain relatively accurate and valid data [22][15]. Finally, the work carried out gene annotation, RNA-seq sequencing evaluation, gene-structure analysis, expression-level analysis, expression-variation analysis, and gene-enrichment analysis [16–19].

2.5 Quantitative PCR assay

The total RNA of *U. prolifera* was extracted from each group followed by reverse-transcription into cDNA using the Fast-King cDNA first strand synthesis kit (Tiangen). Then nine differentially expressed genes (≥80%) selected from transcriptome were applied for qRT-PCR, where 18S rRNA was taken as internal reference [12, 20].The target gene primers were designed using NCBI database online tool “Primer-BLAST” (Supporting Information S1), and Tiangen's Talent fluorescence quantitative detection kit (SYBR Green)

was used for qPCR experiment, with formulate as follows: 2 × Talent qPCR PreMix 12.5 μl, positive and negative primers 0.75 μl, cDNA template 1μl, RNAase-free ddH_2O 10μl. The reaction system was placed in the FTC-2000 PCR instrument, with the program setting as follows: 3 min pre-denaturation at 95 ℃, 40 times of recycles including 95 ℃ for 30s, 60 ℃ for 30s, and 72 ℃ for 30s. All samples had four repeats, and gene differential expression was calculated by $2^{-\Delta\Delta CT}$ [28][21].

2.6 Proteomics procedure

The samples were ground by liquid nitrogen and precipitated by TCA/ acetone, then an appropriate amount of SDT lysate was added, respectively. The samples were bathed in boiling water for 15min, then treated with ultrasonic treatment and centrifuged at 12,000 g. After the supernatant was collected, the protein was quantified by BCA method[22], and the filtrate was collected by FASP enzymatic hydrolysis method[23]. The peptides were desalted by C18 Cartridge, then lyophilized and redissolved with 40 μL 0.1% formic acid solution. The peptides were quantified (OD_{280}). High performance liquid chromatography was used to separate each sample using the HPLC liquid phase system easy NLC with nanositre flow rate. After chromatographic separation, Q-Exactive mass spectrometer was used for mass spectrometry analysis. The mass charge ratio of polypeptides and fragments was collected as follows: after each full scan, 20 fragments were collected (MS2 scan). MS2 activation type was HCD, isolation window was 2 m/z, secondary mass spectral resolution was 17,500 at 200 m/z, normalized collision energy was 30 eV, and underfill was 0.1%.

2.7 Metabolomics procedure

The sample was quantitatively weighed for liquid nitrogen grinding, dissolved in methanol acetonitrile aqueous solution (v/v, 2:2:1), centrifuged at 14000 g at 4 ℃ for 20 min, and then the supernatant was taken. The supernatant was then redissolved in acetonitrile aqueous solution (acetonitrile: water =1:1, v/v) for mass spectrometry. The supernatant was taken for sample analysis after centrifugation at 14000 g at 4 ℃ for 15min.The samples were separated on an Agilent 1290 Infinity LC ultra-performance liquid chromatography (UHPLC) HILIC column. The samples were separated by UHPLC and analyzed by Triple TOF 6600 mass spectrometers (AB SCIEX, US). The obtained original data was converted into. MZML format by Proteo Wizard, and then the XCMS program was used for peak alignment, retention time correction and peak area extraction. Accurate mass number matching (< 25 PPM) and secondary spectral matching were used for metabolite structure identification, and a database built by the laboratory was retrieved. The integrity of the data extracted by XCMS was checked. The metabolites with missing values of more than 50% in the group would be removed and would not participate in the subsequent analysis. The extreme values were deleted and the total peak area was normalized for the data to ensure the parallelism of comparison between samples and metabolites. After the data were processed, it was input into the software SIMCA-P 14.1 (Umetrics, Umea, Sweden) for pattern recognition. After the data were preprocessed by pareto-scaling, multi-dimensional statistical analysis was conducted, including unsupervised principal component analysis (PCA), partial least squares discriminant (PLS-DA) and orthogonal partial least-squares discriminant (OPLS-DA) analysis. One-dimensional statistical analysis included student's *t*-test and multiple of variation analysis, and volcano maps were drawn by R software.

2.8 Lipidomics procedure

After centrifugation at low temperature and high speed, the upper organic phase was taken, and the ammonia gas was blown dry. Isopropanol solution was added for resolution during mass spectrometry analysis. The samples were centrifuged for 15 min at 14000g under 10 ℃ in the vortex, and the supernatant was taken for sample analysis. The samples were separated by Nexera UHPLC LC-30A ultra performance liquid chromatography. Electrospray ionization (ESI) positive and negative ion modes were used for detection, respectively. The samples were separated by UHPLC and analyzed by mass spectrometry with Q exactive plus mass spectrometer (Thermo Scientific, US). Peak and lipid identification (secondary identification), peak extraction, peak alignment, and quantification were performed by lipaid search software Version 4.1 (Thermo Scientific, US). In the extracted data, lipid molecules of RSD > 30% were deleted. For the data extracted by lipaid search, lipid molecules with missing value > 50% in the group were deleted, and the total peak area was normalized for the data. SimCA-P 14.1 (Umetics, Umea, Sweden) was used for pattern recognition. After the data were preprocessed by Pareto-scaling, multi-dimensional statistical analysis was conducted, including unsupervised PCA, PLS-DA and OPLS-DA analysis. One-dimensional statistical analysis included student's *t*-test and multiple of variation analysis, and R software drew volcano maps, hierarchical clustering analysis, and correlation analysis.

3. Results

3.1 Changes of photosynthetic parameters and pigment content

After 12 hs of high light intensity (400 μmol·m^{-2}·s^{-1}), the changes of photosynthetic parameters and pigment content of *U. prolifera* were shown in Figure 1. The results showed that both the maximum photochemical quantum conversion efficiency (Fv/Fm) and the maximum electron transfer efficiency (rETRmax) of PSII were significantly reduced ($p < 0.001$, Fig. 1A, B), while non-photochemical quenching (NPQ) did not change significantly ($p > 0.05$, Fig. 1c). Under the same conditions, there were no significant changes in chlorophyll a, b, and total chlorophyll content in *U. prolifera* ($p > 0.05$, Fig. 1a, b, c), meanwhile the carotenoid content was significantly decreased ($p < 0.05$, Fig. 1d). Chlorophyll a molecule harvesting light can transfer from ground state into single excited state (chlorophyll *), then back to the ground state in the following three ways: the excitation energy can be transferred to the reaction center to drive photosynthesis (Photochemical, qP), or change to chlorophyll fluorescence emission (Fluo), or as heat (NPQ). In this study, the NPQ coefficient and chlorophyll a content did not change significantly after 12 hs of high light stress, while both Fv/Fm and *r*ETRmax value decreased significantly. It showed that under the action of high light, *U. prolifera* might dissipate excess energy in the form of chlorophyll fluorescence.

3.2 Changes in photosynthetic oxygen release rate

The apparent photosynthetic rate was 73.02 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the high-light stress group and 16.10 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the control group (Fig. 2A). Under the same conditions, the respiration rate (Fig. 2B) was 90.87 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the high-light group and 13.44 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the control group. The total photosynthetic rate of *U. prolifera* (Fig. 2C) was 163.9 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the high-light group and 29.54 $O_2 \cdot g^{-1} \cdot h^{-1}$ in the control group. It seems that high light significantly improved the photosynthetic oxygenation and respiration capacity of *U. prolifera*, suggesting that *U. prolifera* needed to consume more energy to cope with high light.

3.3 Basic data of transcriptome analysis

Trimmomatic processed the raw data obtained by high-throughput sequencing to obtain the clean data. The average read length of each sample was more than 142 bp, with the total read length more than 39 Mb, the base amount more than 5.5 Gb, the GC ratio greater than 59%, and the Q30 ratio between 96.29 and 96.48%. It indicated good sequencing quality (Supporting Information S2). Trinity was used to assemble the clean data into transcripts with denovo assembly and remove redundancy. By taking the longest transcript in each transcript cluster as the unigene, 28,362 unigenes were obtained, with an average length of 1,406 bp. Wherein the longest sequence length was 26,903 bp (Supporting Information S3). After comparison, 1,579 unigene sequences were annotated in the databases of NR, KEGG, Swiss-prot, and KOG, and the numbers of annotated genes were 8,502, 1,801, 7,670, and 5,851, respectively (Supporting Information S4). Compared with the control group, there were 100 genes whose expression quantities were significantly up-regulated ($|\text{Log}_2\text{Fold Change (FC)}| > 2$, and p-value < 0.05), and 167 genes were down-regulated for *U. prolifera* under high light intensity.

3.4 Target gene verification results

The selected nine differentially expressed genes were verified by real-time fluorescence quantitative PCR, and the data were analyzed. As shown in Figure 3, under the condition of 12 h high light intensity, the expression trends of nine differentially expressed genes were similar with the transcriptomics results, indicating that transcriptome data were relatively reliable.

3.5 Basic data of proteome analysis

According to the obtained mass spectrum, the Andromeda engine integrated by Max Quant was used for identification. The filtering was completed with PSM-level FDR ≤ 1%, and filtering was performed with protein-level FDR ≤ 1%. There were 18,100 identified peptide fragments and 2,226 identified proteins. The unique peptide fragment is the protein's characteristic sequence. In this experiment, there were 309 unique peptide segments with a quantity of two (Supporting Information S5). The obtained proteins were mostly distributed between 10-50 kDa, of which 20-30 kDa had the most distribution (Supporting Information S6). Max Quant was used for the quantitative analysis of each group with Welch's t-test. It showed that the two groups contained 62 different proteins ($|FC| \geq 1.5$, and $p < 0.05$), of which 21 were up-regulated, and 41 were down-regulated.

3.6 Basic data of metabolome analysis

The chromatographic peak's response intensity and retention time in the positive and negative ion mode of the QC samples in the metabolome overlapped. SIMCA-P software was used for PCA analysis to obtain that the parallel samples of each group were closely clustered together, which showed that the experiment had good repeatability. In the positive ion mode, 3,790 ion peaks were obtained; in the negative ion mode, 3,606 ion peaks were obtained. PLS-DA measured the strength of influence and interpretation of metabolites' expression patterns on the classification of samples in each group by calculating variable importance for the projection (VIP). The PLS-DA model's evaluation parameters R_2Y=0.997 (for positive ions) and 0.971 (for negative ions) after seven interactive verification cycles. OPLS-DA was modified based on PLS-DA to filter out noises unrelated to classified information, which improved the model's analysis and effectiveness. In this model, R_2Y=1 (for positive ions) and 0.999 (for negative ions). In the above two models, R_2Y was close to one, which explained the samples' metabolic differences in the two groups. On this basis, 29 significantly different metabolites (VIP > 1 and $p < 0.05$) were identified through statistical analysis and screening. Wherein 24 were up-regulated, and five were down-regulated.

3.7 Basic data of lipidome analysis

The response intensity and retention time in the UHPLC-Obitrap MS BPC of QC samples in the lipidome showed that the experiment has good repeatability. The PLS-DA and OPLS-DA models' evaluation parameters R_2Y equals to 0.968 and 0.993, respectively, which explained the metabolic differences between samples in two groups. In this study, 558 lipid molecules were identified with 21 subclasses, mainly involving triglyceride (TG), ceramidesglycerol 1 (CerG1), diacylglycerol (DG), DGDG, diacylglycerol monoacylglycerol (DGMG), MGDG, monogalactosyl monoacylglycerol (MGMG), phosphatidylglycerol (PG), and sulfoquinovosyl diacylglycerol (SQDG) (Supporting Information S7). There were five significantly different metabolites (VIP > 1, and $p < 0.05$), among which one was up-regulated, and four were down-regulated (Supporting Information S8).

3.8 Photosynthesis of U. prolifera to high light stress

Through multi-omics joint analysis, it was found that some important genes related to the process of photosynthesis in *U. prolifera* changed significantly after 12 h of high light. Transcriptomics data showed that genes that promote chlorophyll and carotenoid synthesis were up-regulated, e.g., glutamate-1-semialdehyde 2,1-aminomutase (promote chlorophyll biosynthesis, 2.11), prolycopene isomerase (promote carotenoid biosynthesis, 2.11), photosystem II CP43 reaction center protein (promote the formation of light-trapping pigment complex,2.60).On the other hand, decreased expression of pheophytinase (chlorophyll catabolic enzymes, promote chlorophyll biosynthesis, 0.25). The genes involved in promoting the synthesis of photosystem I and electron transport Cytochrome b6-F were up-regulated, e.g., photosystem I P700 chlorophyll a apoprotein A1 (primary electron donor of photosystem I, 2.17), cytochrome b6-f complex subunit 4 (Component of the cytochrome b6-f complex, which mediates electron transfer between photosystem II (PSII) and photosystem I (PSI), cyclic electron flow around PSI, and state transitions,3.07), And at the same time, the gene expression of PSII complex was down-regulated, e.g., photosystem II protein D2 (psbD) (the D1/D2 (PsbA/PsbA) reaction center heterodimer

binds P680, the primary electron donor of PSII as well as several subsequent electron acceptors. D2 is needed for assembly of a stable PSII complex, 0.33), aldedh domain-containing protein (catalysis of an oxidation-reduction reaction in which an aldehyde or ketone group acts as a hydrogen or electron donor and reduces NAD or NADP, 0.42). Downregulation of gene expression associated with the dark reaction process in photosynthesis, e.g., carbonic anhydrase 5A (ceversible hydration of carbon dioxide,0.36). The expression of genes associated with phosphoenolpyruvate synthesis was up-regulated and metabolism was down-regulated, e.g., pyruvate, phosphate dikinase (formation of phosphoenolpyruvate, 3.63) and phosphoenolpyruvate/ phosphate translocator 1 (phosphoenolpyruvate/phosphate translocator that transports phosphoenolpyruvate (PEP) and dihydroxyacetone phosphate,0.33), phosphoenolpyruvate carboxylase 1 (through the carboxylation of phosphoenolpyruvate (PEP) it forms oxaloacetate, a four-carbon dicarboxylic acid source for the tricarboxylic acid cycle, 0.46).

Proteomics data showed that the expression of proteins related to biosynthesis of chlorophyll was up-regulated, e.g., uroporphyrinogen decarboxylase (involved in the synthesis of chlorophyll and porphyrin[24], 3.84), heat shock protein 90-5, chloroplastic (the molecular chaperones required for chloroplast biogenesis are critical for chloroplast biogenesis and maintenance,1.59), s1-domain-containing protein (participates in chloroplast biogenesis,1.74), ABC transporter C family member 1(transport of chlorophyll catabolites, 2.61), ABC transporter C family member 2(transport of chlorophyll catabolites, 1.92); And up-regulated with proteins that slow down the C3 pathway, e.g., pyridoxal 5'-phosphate synthase subunit PDX1 (catalyzes the formation of pyridoxal 5'-phosphate from ribose 5-phosphate (RBP), glyceraldehyde 3-phosphate (G3P) and ammonia, 2.04).

The combined metabolome and lipidome data showed that the expression of photosynthetic membrane involved in photosynthesis was up-regulated, e.g., DGDG (participates in the formation of photosynthetic membranes, 3.01) in lipidome. Relevant studies had shown that the biosynthesis of DGDG started from palmitic acid and became cis-9-octadecenoyl-CoA through acetylation, chain lengthening and hydrogenation [25]. The latter reacts with 3-phosphoglycerol to form lysophosphatidic acid [26], which was then deacylated to form phosphatidic acid. Phosphatidic acid as a substrate can generate both phosphatidylglycerol and diacylglycerol (DAG). DAG reacts with uridine diphosphate galactose to generate MGDG under the catalysis of MGDG synthetase, which combines with 1-phosphate-galactose and is finally formed DGDG under the catalysis of DGDG synthase [27].

[32][33][34]In summary, after 12 h of intense light stress, the expression of photosynthesis-related genes and proteins showed an overall trend of up-regulation in *Ulva* algae, indicating that short-term high light promoted the photosynthesis of *Ulva*. At the same time, the main photosynthetic carbon sequestration pathway was C3 pathway, but the accumulation of phosphoenolpyruvate suggested a shift to the C4 pathway. Combining metabolome and lipidome, it was speculated that high light induces a large amount of DGDG synthesis on the photosynthetic membrane and consumes the substrate MGDG. Those can be supplemented by MGMG. It showed that 12 h of high light stress is the turning point of *U. prolifera* tolerant to high light.

3.9 Energy metabolism of U. prolifera to high light stress

Through multi-omics joint analysis, it was found that after 12 h of intense light stress, some important genes related to the energy metabolism in *U. prolifera* body changed significantly. Transcriptomics data indicated that the expression of genes related to energy metabolism was down-regulated, such as adenylate kinase 5 (catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP, 0.28), acyl-CoA-binding domain-containing protein 5 (binds medium- and long-chain acyl-CoA esters with very high affinity, 0.46), pyruvate dehydrogenase E1 component subunit α-1 (the pyruvate dehydrogenase complex catalyzes the overall conversion of pyruvate to acetyl-CoA and CO_2, 0.46), glycerol-3-phosphate dehydrogenase [NAD (+)] 1 (involved in glycerolipid metabolism, 0.49), ADP-ribosylation factor GTPase-activating protein AGD15 (GTPase-activating protein (GAP) for ADP ribosylation factor (ARF),0.5), ELMO domain-containing protein B (binds to and increases the activity of a GTPase, an enzyme that catalyzes the hydrolysis of GTP, 0.36). The expression of genes related to redox activity was down-regulated, e.g., protein *tas* (D-threo-aldose 1-dehydrogenase activity, 0.39/0.41), cytochrome P450 4e3 (terminal oxygenase, involving in respiration,0.43), cytochrome P450 97B2 (oxidoreductase, 0.41), aldedh domain-containing protein (catalysis of an oxidation-reduction reaction in which an aldehyde or ketone group acts as a hydrogen or electron donor and reduces NAD or NADP, 0.42), amino oxidase domain-containing protein (oxidoreductase activity, 0.47).

On the other hand, transcriptome showed that the expression of genes that involve in tricarboxylic acid cycle were up-regulated, e.g., endoglucanase E-4 (catalytic activity endohydrolysis of (1, 4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans, 2.07), endoglucanase 1 (this enzyme catalyzes the endohydrolysis of 1, 4-β-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans, 2.41). And the gene expression of catalyzing starch to sucrose was also up-regulated, e.g., 4-α-glucanotransferase DPE2 (cytosolic α-glucanotransferase essential for the cytosolic metabolism of maltose, an intermediate on the pathway by which starch is converted to sucrose in leaves at night, 2.10).

Proteome showed that the expression of proteins related to energy metabolism were up-regulated, e.g., (R)-mandelonitrile lyase-like (involve in TCA cycle, 2.05), NADH: ubiquinone oxidoreductase 30 kDa subunit (electron transport involved in mitochondrial respiration, 2.31).

Metabolome showed that metabolites related to energy metabolism were up-regulated during dissimilation, e.g., L-malic acid (3.02), L-asparagine (2.39), glyceric acid (2.15), succinate (1.67), cyclohexylamine (1.16). diethanolamine (involved in phospholipid metabolism, 6.04), and ribitol (involved in riboflavin metabolism, 1.44). Meanwhile, energy metabolism-related metabolites were down-regulated, e.g., sucrose (0.84) and L-pyroglutamic acid (0.82).

In a whole, it was found that the overall trend including energy metabolism of *Ulva* after short-term high light stress was consistent and expressed as down-regulation, but glycolysis and respiration were down-regulated. These data indicated that under high light, energy metabolism of *U. prolifera* was down-regulated but energy storage was increased, which might make *U. prolifera* dormancy.

3.10 Protein biosynthesis of U. prolifera to high light stress

[35]

[36][37][38]Through multi-omics joint analysis, it was found that some important genes related to the process of protein synthesis and expression in *Ulva* changed significantly after 12 h of high light. Transcriptomics data showed that genes that promote protein synthesis were down-regulated, e.g., DOT1 domain-containing protein (histone-lysine N-methyltransferase activity,0.44), ATP-dependent DNA helicase DDM1 (plays a role in formation, organization, stability and heritability of heterochromatin,0.39), WD repeat-containing protein 5 (contributes to histone modification,0.49), RuvB-like 2 (it has single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase (5' to 3') activity suggesting a role in nuclear processes e.g., recombination and transcription,0.44), werner syndrome ATP-dependent helicase homolog (multifunctional enzyme that has both magnesium and ATP-dependent DNA-helicase activity and 3'-5' exonuclease activity towards double-stranded DNA with a 5'-overhang, 0.35); same in RNA transcription e.g., AP2-like ethylene-responsive transcription factor AIL5 (transcriptional activator, 0.33), transcriptional activator Myb (transcriptional activator,0.35), ESF1 homolog (constitute a novel regulatory system for basal transcription, 0.39), transcription initiation factor TFIID subunit 5 (TAFs are components of the transcription factor IID (TFIID) complex that is essential for mediating regulation of RNA polymerase transcription,0.39), DNA-directed RNA polymerase I subunit 1 (DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates, 0.49), RuvB-like protein 1 (proposed core component of the chromatin remodeling INO80 complex which is involved in transcriptional regulation, DNA replication and probably DNA repair,0.46), DUF2428 domain-containing protein (he posttranscriptional addition of methyl groups to specific residues in a *t*RNA molecule, 0.37), and genes that decay with RNA were up-regulated e.g., tetratricopeptide repeat protein SKI3 (involved in exosome-mediated RNA decay, 3.46);down-regulation of genes that promote protein degradation and inhibit protein synthesis, e.g., ribosome biogenesis protein BRX1 homolog (biogenesis of the 60S ribosomal subunit, 0.30), *t*RNA pseudouridine synthase A (transfer RNAs, 0.36), general transcription factor 3C polypeptide 5 (involved in RNA polymerase III-mediated transcription, 0.37), EEF1A lysine methyltransferase 1 (protein-lysine methyltransferase that selectively catalyzes the trimethylation of EEF1A at 'Lys-79', 0.41), protein SDA1 homolog (ribosomal large subunit biogenesis,0.47), 60S ribosomal export protein NMD3 (acts as an adapter for the xpo1-mediated export of the 60S ribosomal subunit,0.48), eukaryotic translation initiation factor 5 (formation of cytoplasmic translation initiation complex, 0.48), cytoplasmic *t*RNA 2-thiolation protein 2 (may act by forming a heterodimer with ctu1/atpbd3 that ligates sulfur from thiocarboxylated urm1 onto the uridine of *t*RNAs at wobble position, 0.49), protein-lysine methyltransferase METTL21D (protein-lysine N-methyltransferase that specifically trimethylates 'Lys-315' of VCP/p97, 0.50), hybrid signal transduction histidine kinase J (Acts as a receptor histidine kinase for a signal transduction pathway. This protein undergoes an ATP-dependent autophosphorylation at a conserved histidine residue in the kinase core, and a phosphoryl group is then transferred to a conserved aspartate residue in the receiver domain,0.40), Haloacid dehalogenase-like hydrolase domain-containing protein 3 (hydrolase,0.43), and nucleotide synthesis was inhibited, e.g., 5'-nucleotidase (hydrolyzes extracellular nucleotides into membrane permeable nucleosides, 0.47), pseudouridine-5'-phosphate glycosidase (Catalyzes the reversible cleavage of

pseudouridine 5'-phosphate (PsiMP) to ribose 5-phosphate and uracil. Functions biologically in the cleavage direction, as part of a pseudouridine degradation pathway,0.48), cytosolic purine 5'-nucleotidase (may have a critical role in the maintenance of a constant composition of intracellular purine/pyrimidine nucleotides in cooperation with other nucleotidases. Preferentially hydrolyzes inosine 5'-monophosphate (IMP) and other purine nucleotides, 2.51).

Proteomics data showed that the expression of proteins related to protein synthesis was down-regulated, e.g., pre-mRNA-splicing factor ATP-dependent RNA helicase DEAH3 (may be involved in pre-mRNA splicing, 0.37), eukaryotic translation initiation factor 5A-2 (the precise role of eIF-5A in protein biosynthesis is not known but it functions by promoting the formation of the first peptide bond,0.60), DNA binding helix-turn helix protein (it has the activity of transcription coactivators and participates in the transcription process,0.24), glutamate–cysteine ligase (synthesis of glutathione is involved in interpretation,0.30).photosystem I (PSI) biogenesis, 0.59), polynucleotide-3'-phosphatase ZDP (nick-sensing 3'-phosphoesterase involved in a base excision repair pathway required for active DNA demethylation, 0.46). And the expression of proteins involved in photosynthesis were up-regulated, e.g., uroporphyrinogen decarboxylase (involved in the synthesis of chlorophyll and porphyrin[24],3.83).

The combined metabolome and lipidome data showed that the expression of promoting amino acid metabolism was up-regulated, e.g., L-glutamate (1.90), L-methionine (3.06), L-glutamate (1.74).

In summary, after 12 h of intense light stress, the expression of protein synthesis related genes showed an overall trend of down-regulation in *Ulva* algae, including DNA activation, RNA transcription and protein folding and so on, meanwhile, it also inhibited nucleotide production. It showed that 12 h of high light stress is the turning point of *U. prolifera* tolerant to high light.

3.11 Signal transduction and growth of U. prolifera to high light stress

Accord to omics data, some important genes related to signal transduction and growth altered significantly after 12 h of high light stress. Transcriptomics data indicated that the expression of genes related to the second molecular were down-regulated, e.g., adenylate cyclase (plays essential roles in regulation of cellular metabolism by catalyzing the synthesis of a second messenger, 0.47), protein RRC1 (required for phytochrome B (phyB) signal transduction, 0.44). So did those in ion transport, e.g., potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2 (modulated by intracellular chloride ions and pH, acidic pH shifts the activation to more negative voltages, 0.46), sodium/calcium exchanger 3 (mediates the electrogenic exchange of Ca^{2+} against Na^{+} ions across the cell membrane, and thereby contributes to the regulation of cytoplasmic Ca^{2+} levels and Ca^{2+}-dependent cellular processes[28], 0.35), potassium voltage-gated channel subfamily H member 5 (channel properties may be modulated by cAMP and subunit assembly, 0.37), protein detoxification (enables the active transport of a solute across a membrane by a mechanism whereby two or more species are transported in opposite directions in a tightly coupled process not directly linked to a form of energy other than chemiosmotic energy, 0.48).

The genes expression of growth were down-regulated, e.g., GPCR-type G protein 2 (abscisic acid receptor. The GDP-bound form exhibits greater abscisic acid binding than the GTP-bound form. Required for seedling growth and fertility,0.49), and the genes that inhibit cell growth were up-regulated, e.g., ABC transporter G family member 31 (Together with ABCG25, export abscisic acid (ABA) from the endosperm to deliver it to the embryo via ABCG30 and ABCG40-mediated import to suppress radicle extension and subsequent embryonic growth, 2.33).

[40][39][41][42]Proteomics data showed that the expression of proteins involved in signal transduction was down-regulated, e.g., inositol monophosphatase (participates in the phosphatidylinositol signaling pathway,0.57), Calcium-dependent protein kinase 22 (may play a role in calcium as a second messenger signal transduction pathway,0.07), Transmembrane transport protein expression is up-regulated, e.g., Vesicle-fusing ATPase (involved in vesicle-mediated transport pathways,1.61),ATP-energized ABC transporter (participates in transmembrane transport mechanisms,2.63). But at the same time, the expression of related proteins mediating mitochondrial protein transport was down-regulated e.g., mitochondrial import inner membrane translocase subunit Tim9 (mitochondrial intermembrane chaperone that participates in the import and insertion of multi-pass transmembrane proteins into the mitochondrial inner membrane,0.46). The expression of ion channel-related proteins was down-regulated, e.g., UPF0187 protein At3g61320 (participates in the formation of anion channels,1.58).

Metabolomics data showed that a few metabolites involved in signal transduction were up-regulated, such as L-glutamate (1.90), adenosine (1.86), succinate (1.67), while isoleucyl-glutamate (0.71) was down-regulated.

In summary, after 12 h of high light, *Ulva* algae showed downward-regulated overall expression of signal transduction process-related genes, while the expression of intermediate metabolites were up-regulated. It was speculated that the signal transduction pathway of *U. proifera* was rapidly inhibited during 12 h irradiation, and downward-regulated trend after that time. Meanwhile, the expression of growth inhibition related genes was up-regulated, the expression of growth promotion related genes was down-regulated and, resulting in the growth and development of *U. prolifera* was inhibited.

3.12 Cell division, gametogenesis and apoptosis of U. prolifera to high light stress

According to multiple omics analysis, it was found that after 12 h of high light stress on *U. prolifera*, some important genes in the process of cell division, gametogenesis and apoptosis in *U. prolifera* had significant changes. Transcriptomics data indicated that the expression of genes involved in cell division were down-regulated, e.g., DNA mismatch repair protein MSH4 (promotes homologous recombination through facilitating chiasma formation during prophase I, 0.28), DNA replication licensing factor MCM5 (essential to undergo a single round of replication initiation and elongation per cell cycle in eukaryotic cells, 0.40), single mybhistone 3 (binds preferentially double-stranded telomeric repeats, but may also bind to the single telomeric strand, 0.40), RING finger and CHY zinc finger domain-containing protein 1 (contributes to the regulation of the cell cycle progression,0.41), chromosome transmission fidelity protein 18 homolog (involved in sister chromatid cohesion and fidelity of chromosome transmission,

0.41), origin of replication complex subunit 1A (component of the origin recognition complex that binds origins of replication, 0.42), chromosome transmission fidelity protein 18 (essential for the fidelity of chromosome transmission and also required for the DNA replication block checkpoint, 0.43), ATP-dependent DNA helicase DDX11 (cooperates also with TIMELESS factor during DNA replication to regulate proper sister chromatid cohesion and mitotic chromosome segregation, 0.43), cyclin-dependent kinase-like 4 (cyclin-dependent catalysis activity, 0.44), DNA replication licensing factor MCM6 (component of the MCM2-7 complex that may function as a DNA helicase and which is essential to undergo a single round of replication initiation and elongation per cell cycle in eukaryotic cells, 0.45), WD repeat-containing protein WRAP73 (act as regulator of spindle anchoring at the mitotic centrosome, 0.45), bloom syndrome protein homolog (participates in DNA replication and repair, 0.46), centrosomal protein of 135 kDa (involved in centriole biogenesis, 0.46), POC1 centriolar protein homolog A (may play an important role in centriole assembly and/or stability and ciliogenesis, 0.36), mitotic spindle checkpoint protein MAD2 (required for the execution of the mitotic checkpoint, 0.49), regulator of telomere elongation helicase 1 homolog (acts as an anti-recombinase to counteract toxic recombination and limit crossover during meiosis, 0.39), lys-63-specific deubiquitinaseBRCC36 (required for normal mitotic spindle assembly and microtubule attachment to kinetochores, 0.49), heat shock-like 85 kDa protein (molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction, 0.50); and some genes promoting the cell division were up-regulated, e.g., histone acetyltransferase MCC1 (histone acetyltransferase that probably regulates acetylation status of histone H3 during meiosis and may influence recombination and chromosome segregation, 2.87), protein chromatin remodeling 24 (an essential component of the spindle assembly checkpoint, chromatin remodeling factor that regulate homologous recombination and non-homologous recombination, 2.73), DNA excision repair protein ERCC-6-like (contributes to the mitotic checkpoint by recruiting MAD2 to kinetochores and monitoring tension on centromeric chromatin, 2.78), serine/threonine-protein kinase mos (suppresses the mitotic cell cycle in oocytes, forcing them to undergo meiosis II to produce haploid gametes, 2.04).

Transcriptomics data indicated that the expression of genes involved in gametogenesis were down-regulated, e.g., thioredoxin domain-containing protein 3 homolog (may be required during the final stages of sperm tail maturation, 0.30), 26S proteasome non-ATPase regulatory subunit 12 homolog A (required for gametogenesis and sporophyte development acts redundantly with RPN5B, 0.50), cilia- and flagella-associated protein 91 (may play a role in spermatogenesis, 0.40), and some genes promoting the gametogenesis were up-regulated, e.g., C-.factor (necessary for cellular aggregation, for spore differentiation, and for gene expression that is initiated after 6 hour of starvation, 2.15).

Transcriptomics data indicated that the expression of genes related to apoptosis were down-regulated, e.g., serine/threonine-protein kinase atg1 (involved in autophagy, 0.26), dnaJ homolog subfamily A member 1 (functions as co-chaperone for HSPA1B and negatively regulates the translocation of BAX from the cytosol to mitochondria in response to cellular stress, thereby protecting cells against apoptosis, 0.47), nucleotide-binding oligomerization domain-containing protein 1 (forms an intracellular sensing system along with ARHGEF2 for the detection of microbial effectors during cell invasion by pathogens,

0.37), WD repeat-containing protein 35 (may promote CASP3 activation and TNF-stimulated apoptosis[29], 0.35), and some genes promoting the gametogenesis were up-regulated, e.g.,metacaspase-1 (acts as a positive regulator of cell death, 3.14), homocysteine methyltransferase (Catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine resulting in methionine formation, methionine is precursors of plant endogenous hormones ethylene and polyamines synthesis, 2.16), and the expression of ascorbate peroxidase (may play a role in the protection of oocyte incorporated cells from rapid apoptotic degradation, 0.41) protecting cells and reducing apoptosis were down- regulated.

Proteomics data showed that the expression of proteins that promote cell division were down-regulated, e.g., SNF1-related protein kinase regulatory subunit gamma (plays redundant role with PV42a in regulating male gametogenesis and pollen tube guidance, 0.57), (R)-mandelonitrile lyase 2 (participates in the formation of propagules, 0.52). Upregulation of development-related proteins, e.g., dnaJ protein homolog 2 (plays a continuous role in plant development probably in the structural organization of compartments, 2.27).

According to omics data above, it was found that after short-term high light, the gene expression related to cell division and gametogenesis showed an overall downward trend in *U. prolifera*, at the same time, the expression of apoptosis-related genes was up-regulated, which means the reproductive development of *U. prolifera* was inhibited by high light. It was speculated that 12 h of high light was the turning point of *U. prolfera* cell division and reproduction.

3.13 Resistance of U. prolifera to high light stress

According to multiple omics analysis, it was found that after 12 h of high light stress on *U. prolifera*, some important genes in the process of resistance in *U. prolifera* had significant changes. Transcriptome showed that the expression of some genes on resistance were up-regulated. Some were involved in disease, e.g., as disease resistance protein RGA4 (that triggers a defense system which restricts the pathogen growth, 2.04), disease resistance protein TAO1 (TIR-NB-LRR receptor-like protein that contributes to disease resistance induced by the Pseudomonas syringae type III effector AvrB. Acts additively with RPM1 to generate a full disease resistance response to P.syringae expressing this type III effector, 2.08), disease resistance protein RPP5 (May have additional roles in adaptation to various stress conditions and in DNA damage tolerance, 3.10)and TMV resistance protein N (that triggers a defense system including the hypersensitive response, which restricts the pathogen growth, 2.30/3.03); some in osmotic substance synthesis, e.g., broad substrate specificity ATP-binding cassette transporter ABCG2 (part of the ATP-binding cassette family that actively extrudes a wide variety of physiological compounds, dietary toxins and xenobiotics from cells, 2.22), neurotrypsin (exocytosis, 2.30/2.35/2.36/2.91), mannitol dehydrogenase (provides the initial step by which translocated mannitol is committed to central metabolism and, by regulating mannitol pool size, is important in regulating salt tolerance at the cellular level, 2.66),thiamine thiazole synthase (involved in biosynthesis of the thiamine precursor thiazole, 2.51), protein VMS (involved in the endoplasmic reticulum associated degradation

pathway, 2.9); some in antioxidant response, e.g., hydroperoxide isomerase ALOXE3 (non-heme iron-containing lipoxygenase which is atypical in that it displays a prominent hydroperoxide isomerase activity and a reduced lipoxygenases activity, 2.36), Riboflavin biosynthesis protein PYRR (Riboflavin is involved in the antioxidant and peroxidation processes of plants, thus affecting the production of reactive oxygen species during oxidative damage and subsequent allergic reactions,2.99), some in apoptosis signal transduction, e.g., calcium-dependent protein kinase 34 (activated by calcium. Autophosphorylation may play an important role in the regulation of the kinase activity, 2.03), and although protein-tyrosine-phosphatase MKP1 (protein-tyrosine-phosphatase that acts as a negative regulator of MPK6 and MPK3 signaling by dephosphorylating and repressing MPK6 and MPK3. May be involved in salt and genotoxic stress responses, 0.41) was down-regulated, it was catalytic factor that inhibits apoptosis, and the final result is still to weaken apoptosis. And V-type proton ATPase subunit E (V-ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells, 2.51) expression can accelerate the decomposition of cells.

On the other hand, transcriptome data revealed some important genes involved in the process of stress resistance were down-regulated significantly. Some were involved in cellular defense responses, e.g., DEAD-box ATP-dependent RNA helicase 50 (probably involved in resistance to biotic and abiotic stresses, 0.22), 17.6 kDa class I heat shock protein 3 (0.27), class I heat shock protein (response to reactive oxygen species, 0.48), activator of 90 kDa heat shock protein ATPase homolog 1 (activates the ATPase activity of HSP90AA1 leading to increase in its chaperone activity, 0.50); some in DNA damage repair, e.g., deoxy ribodipyrimidine photo-lyas (photolyase involved in the repair of *UV* radiation-induced DNA damage, 0.42); some in photoprotection, e.g., carotene biosynthesis-related protein CBR (it forms photoprotective complexes within the light-harvesting antennae, 0.47) and some in cell osmotic pressure-related gene expression, e.g., molybdenum cofactor sulfurase (sulfurates the molybdenum cofactor, 0.49); some in oxidoreductase activity, e.g., Fe2OG dioxygenase domain-containing protein (oxidoreductase activity, 0.36), amino oxidase domain-containing protein (oxidoreductase activity, 0.47); and some in innate immune response, e.g., NLR family CARD domain-containing protein 3 (attenuates signaling pathways activated by Toll-like receptors and the DNA sensor sting/tmem173 in response to pathogen-associated molecular patterns, such as intracellular poly (dA : dT), but not poly (I : C), or in response to DNA virus infection, including that of herpes simplex virus 1, 2.53 / 3.16).

Proteomics data showed that the expression of proteins involved in antioxidant was down-regulated, e.g., glutathione S-transferase (involved in the redox homeostasis, especially in scavenging of ROS under oxidative stresses,0.16),ascorbate peroxidase it is one of the important antioxidant enzymes in reactive oxygen metabolism of plants,0.58), peroxidase (oxidordeuctase,0.61), alkyl hydroperoxide reductase/thiol specific antioxidant/Mal allergen (RDOX enzyme activity, antioxidant activity, 0.44). The expression of proteins involved in detoxification was down-regulated, e.g., glutamate-cysteine ligase (synthesis of glutathione is involved in interpretation,0.30). The expression of osmotic regulation related proteins was up-regulated, e.g., Delta-1-pyrroline-5-carboxylate (co-expression of rice OsP5CS1 and OsP5CS2 genes in transgenic tobacco resulted in elevated proline biosynthesis and enhanced abiotic stress tolerance,1.57). Down-regulated expression of related proteins involved in regulating protein refolding, e.g., 10 kDa

chaperonin (seems to function only as a co-chaperone, along with cpn 60, may facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix, 0.38).

According to the metabolome data, most of metabolites on anti-stress were up-regulated, including γ-L-glutamyl-L-glutamic acid (4.91), L-glutamate (1.90), D-proline (6.61), 1-aminocyclopropanecarboxylic acid (1.88), L-methionine (3.06), dimethyl sulfone (1.18), L-asparagine (2.39), (S)-2-aminobutyric acid (1.80), 2,3-dihydroxy-3-methylbutyric acid (3.73), ribitol (1.44), L-threonate (1.34), and D-lyxose (1.70). However, there were also metabolites down-regulated, e.g., galactinol (0.82), L-pyroglutamic acid (0.82), and L-glutamine (0.67).

According to omics data above, it was found that after 12 h of high light, some gene expression on stress resistance were up-regulation trend.*U. prolifera* after 12 hours of strong light, cell recognition and adhesion and other aspects of stress resistance, while the antioxidant and innate immunity and other aspects of weakened. Different stress mechanisms of Enteromorpha prolifera have different coping mechanisms to 12 h’s strong light stress.

3.14 Cell membrane synthesis and repair of U. prolifera to high light stress

Accord to omics data, some important genes related to cell membrane synthesis and repair altered significantly after 12 h of high light stress. Transcriptomics data indicated that the expression of genes related to cell membrane and cytoderm synthesis were up-regulated and lipid alienation were down-regulated, e.g., Enoyl-[acyl-carrier-protein] reductase [NADH] (catalyzes the last reduction step in the de novo synthesis cycle of fatty acids. Involved in the elongation cycle of fatty acids which are used in lipid metabolism. Required for normal plant growth, 2.23), sterol sensor 5-transmembrane protein (involved in cholesterol biosynthesis and uptake, 3.27), UDP-glucuronate 4-epimerase 1 (involved in the synthesis of the negatively charged monosaccharide that forms the backbone of pectic cell wall components, 2.19), UDP-glucose 6-dehydrogenase 5 (involved in the biosynthesis of UDP-glucuronic acid (UDP-GlcA), providing nucleotide sugars for cell-wall polymers, 2.23), callose synthase 12 (structural component of plasmodesmatal canals, 2.62), Cycloartenol-C-24-methyltransferase 1 (catalyzes the methyl transfer from S-adenosyl-methionine to the C-24 of cycloartenol to form 24-methylene cycloartenol, 0.45), cycloeucalenol cycloisomerase (converts pentacyclic cyclopropyl sterols to tetracyclic sterols, 0.45); and genes associated with tRNA synthesis are up-regulated, e.g., Cycloartenol-C-24-methyltransferase 1 (catalyzes the methyl transfer from S-adenosyl-methionine to the C-24 of cycloartenol to form 24-methylene cycloartenol, 0.45), cycloeucalenol cycloisomerase (converts pentacyclic cyclopropyl sterols to tetracyclic sterols, 0.45). tRNA 2'-phosphotransferase (Catalyzes the last step of tRNA splicing, the transfer of the splice junction 2'-phosphate from ligated tRNA to NAD to produce ADP-ribose 1"-2" cyclic phosphate, 7.56), Ribonuclease Z, mitochondrial (Probably involved in tRNA maturation, by removing a 3'-trailer from precursor tRNA, 2.04).

Proteomics data showed that the expression of proteins involved in lipid biosynthesis was up-regulated, e.g., CDP-diacylglycerol-serine O-phosphatidyl transferase 2 (lipid biosynthesis,1.97), adipocyte plasma

membrane-associated protein (Cell membrane biosynthesis, 3.31).

The combined metabolome and lipidome data showed that the expression of certain metabolites was up-regulated during the process of biosynthetic fatty acid (e.g., cis-9-Palmitoleic acid (2.28), myristic acid (2.54), palmitic acid (2.11), α-linolenic acid (1.71), linoleic acid (1.53)), and unsaturated fatty acids (carbon-fixed-3-hydroxypropionic acid (1.65) in prokaryotes, DGDG (3.01) in lipidome). There were also down-regulated expressions, e.g., 4,7,10,13,16,19-docosahexaenoic acid (0.62) in metabolomics, and MGDG (0.69) and MGMG (0.60) in lipidome.

In summary, after 12 h of intense light stress, the expression of promoting synthesis of cell membranes, cell walls and plasmodesmata related genes showed an overall trend of up-regulation in Ulva algae, indicating that short-term high light promoted the repair of cell membrane system of Ulva. Meanwhile, the expression of genes related to lipid metabolism decreased. The results showed that high light stress for 12 h might lead to cell membrane damage of *U. prolifera*, and the synthesis process of cell membrane system was increased to protect the body.

4. Discussion

4.1 High light affecting the composition of photosynthetic membranes

When the external environment changes drastically, algae have evolved multiple to avoid harm[30–32]. The algae cell membrane is a significant hydrophobic barrier separating it from the surrounding environment[33]. Therefore, maintaining or regulating the physical and biochemical properties of cell membranes is very important. Especially as the thylakoid-membrane glycerolipids for photosynthesis and photoprotection in chloroplasts, different light conditions will affect them [34, 35]. Meanwhile, unsaturated fatty acids are also important components of biofilms[36]. They can increase the fluidity of the membrane, which is important for activating the enzymes on the membrane [37, 38].

This transcriptome data showed that the transcript expressions of the sterol sensor 5-transmembrane proteins involved in sterol synthesis were up-regulated. Sterols are essential eukaryotic lipids that are required for a variety of physiological roles [39]. Under the condition of high light, the photosynthetic membrane of Enteromorpha prolifera was damaged to a certain extent, which accelerated the repair process of photosynthetic membrane to ensure the normal photosynthesis of the body [40].

Metabolome data showed that metabolite 4,7,10,13,16,19-docosahexaenoic acids involved in the synthesis of unsaturated fatty acids were down-regulated; α-linolenic acid, cis-9-palmitoleic acid, and linoleic acid were up-regulated; palmitic acid, myristic acid, and diethanolamine, involved in the biosynthesis of saturated fatty acids, were up-regulated. The biosynthesis of the two fatty acids was related, because linoleic acid was the synthetic precursor of α-linolenic acid, and the latter was the synthetic precursor of docosahexaenoic acid. The product of fatty acid metabolism was the precursor of

lipoic acid metabolism, and the expression of ferredoxin-thioredoxin reductase involved in lipoic acid metabolism was down-regulated. [54]The enzyme and thioredoxin have been recognized as the key system [41, 42] for transmitting the light-induced reduction signal to the target proteins.

Lipid group results showed 76 DGDG expressions, with OPLS-DA model VIP>1 as the standard. In the high-light experimental group, 13 DGDG were differentially expressed, and seven were up-regulated. Among them, DGDG ((16:4/18:4) +HCO_2) was up-regulated extremely significantly (p <0.01, and FC=3), and the obtained DGDG had the longest carbon chain and the lowest saturation. The work detected a total of 53 MGDG (the direct precursor of DGDG biosynthesis), where 11 differential expressions were down-regulated, including the products (MGDG (16:4/18:4) +HCOO) corresponding to the above-mentioned DGDG. Among them, the difference of MGDG ((16:0/16:4) +HCOO) was extremely significant (p=0.013, and FC=0.69), and the product was supposed to be a reaction intermediate. The work detected 20 monogalactosyl monoacylglycerols (MGMGs), of which seven were differentially expressed, and five were down-regulated. MGMG ((16:1) +HCOO, FC=0.60) and MGMG ((16:2) +HCOO, FC=0.72) had extremely significant difference (p < 0.05), with intermediate saturation. Correlation analysis showed that these two MGMGs were closely positively correlated with the expression of MGDG, and had a strong negative correlation with DGDG. Besides, 42 DAGs, 16 PGs, 6 PAs, and 6 fatty acids (including palmitic acid and cis-9-octadecenoyl-CoA) were detected in the lipid group, but no differential expression was found. Six PIs and seven PEs detected were not different, indicating that the above intermediate products did not participate in the endoplasmic reticulum reaction.

[56][57][58][32][33][34]Di-galactose diacylglycerol (DGDG) and mono-galactose diacylglycerol (MGDG) are the main membrane lipids that constitute the chloroplast photosynthetic membrane of higher plants, accounting for more than 80% of the chloroplast membrane lipids [43, 44]. Among them, DGDG is one of the most important compounds constituting photosynthetic membranes and exists in almost all biofilms[44, 45]. It accounts for more than 20% of total lipids and can replace phospholipid PC under special circumstances [46]. Moreover, DGDG plays an important role in maintaining the oligomer structure of the photosystem II light-harvesting pigment-protein complex and regulating the photosystem II and the oxygen-evolution activity of its core complex [47, 48]. In higher plants containing a large number of hexadecenoic acids, the biosynthesis of DGDG started from palmitic acid and became cis-9-octadecenoyl-CoA through acetylation, chain lengthening, and hydrogenation [25, 49]. The latter reacted with 3-phosphoglycerol to form lysophosphatidic acid [26, 50] and then deacylated to form phosphatidic acid. Phosphatidic acid as a substrate could generate phosphatidylglycerol and DAG. DAG reacted with uridine diphosphate galactose to generate MGDG under the catalysis of MGDG synthase. The latter was combined with galactose-1-phosphate and finally generated DGDG under the catalysis of DGDG synthase [27]. In summary, it was speculated that high light induced a large amount of DGDG synthesis on the photosynthetic membranes and consumed the substrate MGDG, which could be supplemented by MGMG.

4.2 Changes in photosynthetic pigments affected by high light

[64][65][66]The content of chlorophyll can be induced by light. The chlorophyll a and yield of *U. prolifera* cultured under weak light (62 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for one day were twice that under high light (324 $\mu mol \cdot m^{-2} \cdot s^{-1}$). However, within one week of culture, there was no difference in chlorophyll a yield of all samples [51]. The synthesis of chlorophyll a in the red alga *Corallina elongata* could be induced by red light pulses [52, 53], and regulated by light intensity. After five hours of light treatment, the pigment reached a steady state. When the irradiance increased, chlorophyll synthesis also increased, indicating that this steady-state was dynamic [54, 55].

For floating *U. prolifera*, the surface and lower layers of the algal mat had different photosynthetic responses[56, 57]. The surface algae mat dissipated excess energy through the quantum control of photosynthesis (energy quenching or redistribution between PSII/PSI) and reduced the photosynthetic system's damage; The lower algal mat increased Chl a and Chl b, and reduced the ratio of Chl a to Chl b to improve its ability to use light energy [56]. Therefore, *U. prolifera* has strong photosynthetic plasticity [58, 59]. Due to the waves' interference, it quickly adapted to the frequent exchange between the surface and the lower environments through the change of pigment compositions, energy quenching, and energy redistribution between PSII/PSI [69][56].

Chlorophyll synthesis and catabolism were dynamically balanced, and the change in the ratio of Chls a/b under different physiological conditions was reflected in this experiment's transcriptome data. After 12 h of high light treatment, the expressions of glutamate-1-semialdehyde aminotransferase (1.07)-related genes involved in the synthesis of photosynthetic pigment Chl b [60, 61] were up-regulated to promote the production of Chl b. The expression level of pheophorbide hydrolase (-1.98)-related genes, which was the key rate-limiting enzyme of the chlorophyll catabolism pathway [62], was down-regulated. It slowed down the conversion of Chl b to a, and reduced the ratio of Chl a/b. However, Chl a increased, thereby improving its ability to use light energy as a whole.

The joint analysis of transcription and proteome showed that the glutamate-1-semialdehyde aminotransferase involved in the synthesis of Chl b [62] was annotated in the proteome. The enzyme showed the expression in the protein group but did not change significantly. However, the key rate-limiting enzyme pheophorbide hydrolase showed significantly differential expression in the transcriptome, but no corresponding enzyme expression was found in the protein group. It was speculated that after 12 h of high light stress, *U. prolifera* had a certain time difference in the corresponding response expression system.

The contents of the photosystem's auxiliary proteins and pigments were regulated by light. Under 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the chlorophyll content in *Ulva* spp. decreased within a few minutes, while the carotenoids remained unchanged [63]. Besides, under 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the upper layer of the meadow was involved in the gene up-regulation of light adaptation (rubisco, ferredoxin, and chlorophyll-binding protein) and light protection (antioxidant enzymes, genes related to lutein cycle, and tocopherol biosynthesis), indicating the activation of more defense mechanisms [64]. In addition to common photosensitive pigments such as carotenoids and chlorophyll, seven rhodopsin types, two leuco dyes, and one

photoprotein have been found in *Chlamydomonas reinhardtii.* Wherein rhodopsin was a flavin-based photoreceptor sensitive to blue light [78][65].

This data in this study showed that the expression of photolyase (transcriptome: -1.88) was significantly down-regulated. Photolyase was a blue-light receptor that could bind to folic acid and FAD. Its significant down-regulation showed that the blue light sensitivity of *U. prolifera* weakened after 12 h of high light. Among the light-harvesting proteins, LHCX and LHCZ genes had a stronger up-regulation effect under 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than that under 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [66]. The similar proteins ElipL1, ElipL2, Cbrx and OHP in *U. linza* were also upregulated by high light within 3 hours under 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [80][67].

[81][82][83][84][85][86]*U. prolifera* has a mechanism to resist photoinhibition. When the required light energy exceeds the range that the photosynthetic system can withstand, the photosynthetic function declines and light inhibition occurs. Plants have multiple protection mechanisms in response to excessive light energy [68]. For example, *macrocystis pyrifera*, *chondrus crispus* and *Ulva lactuca* promote self-shading by increasing biomass and reduce photoinhibition [69]. The dinoflagellate uses the flavin cycle for photoprotection through heat dissipation [70]. When the light intensity exceeded 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the electron flow reached saturation, with the increased excitation pressure and NPQ [71]. When *Ulva fasciata* was exposed to 1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, protein D1 rapidly degraded, and its PES medium form was destroyed. The NPQ ability decreased to a steady-state within 110 minutes, but it quickly recovered in low light [72]. When non-photochemical quenching did not work properly, *U. fasciata* maintained NPQ by keeping a small proportion of high fast-light PSII combinations. However, there was high-thermal activity with high light because the degradation of Cyt_6f seriously hindered electrons' transmission, which led to NPQ [73].

Far-red light enhanced the circulating electron flow around photosystem I (PSI) and induced the expression of LHCSR to trigger NPQ in *U. prolifera*. Lhcb1 and CP29 were adjusted up-regulated under FRL, which meant that the photosystem II antenna size increased [87][74]. NPQ induction could be related to individual proteins, for example, *psb*S content was positively correlated. The latter played an important role in reconstructing the PSII-CLHCII super complex and the energy balance regulation of the thylakoid membrane [88][75]. Or it was regulated by zeaxanthin and triggered and controlled by the transthylakoid proton gradient (ΔpH) under high light (1,954 $\mu mol \cdot m^{-2} \cdot s^{-1}$). More importantly, it was regulated by the members of the light-harvesting complex (LHC) family. Under high light conditions, the expression of LHCSR was even higher than that of PSBS [90][77]. However, *U. prolifera*'s NPQ lacked a rapid activation mechanism under high light, and its monomeric LHC proteins only contained CP29 and CP26 instead of CP24. Meanwhile, a significant increase in the expression level of CP26 could not change the concentrations of the photoprotective proteins psbS and lhcSR, with the gradual synthesis of zeaxanthin. The atypical NPQ made *U. prolifera* more suitable for the complex sea environment [78]. The transcriptome data in this experiment also showed that under high light (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) treatment for 12 h, *U. prolifera* was involved in light capture, PSI and PSII, and the expressions of genes related to photosynthetic pigment synthesis. It was very similar to the atypical NPQ of *U. prolifera*.

4.3 High light affecting the signal transduction pathways of *U. prolifera*

Light-induced cAMP changes significantly increase stress-response proteins in *Arabidopsis*, so adenylate cyclase (ACs) may act as a light sensor in higher plants [79]. The transcript expression quantity of *cya*C, the primary subtype of adenylate cyclase in algae, is strongly affected by light, which is about 300 times stronger than that of the dark-treated control group [80]. Under subsaturated white light irradiation, the oxygen release of photosynthesis is correlated with cAMP change, showing that electron transfer can regulate the accumulation of cAMP in *G. sesquipedale* and *U. rigida*, that is, cAMP level is regulated by light intensity [81]. This transcriptome data here showed that the transcript expression of ACs was down-regulated. At the same time, metabolome data showed that the expression of related metabolites involved in signal channels was up-regulated.

Light could change calcium-dependent protein kinases. In plants, the multi-gene family of CDPKs (calcium-dependent protein kinase) encoded structurally conserved single-molecule calcium sensor/protein kinase, which played essential roles in multiple signal transduction pathways. In this experiment, after 12 h of high-light stress for *U. prolifera*, the protein content of CDPKs was significantly down-regulated, but the expressions of CDPKs-related transcripts were significantly up-regulated, indicating a large consumption of CDPKs and an increased demand. However, the transcript expressions of Calcium: Cation Antiporter, glucose 6-phosphoric acids/phosphates and phosphoenolpyruvate/phosphate anti-transporter proteins, and K^+-channel ERG-related proteins (including PAS/PAC sensor domain and K^+-channel KCNQ) were significantly down-regulated, indicating that the demands for calcium and potassium-ion transport inside and outside the membrane decreased. Besides, changes in light intensity promoted the release of cations to the outside of the cell quickly. For example, within the first two minutes, light caused the release of sodium ions in *Ulva lobata* and *Ulva expansa* to be twice that of the group in the dark, with $^{86}Rb^+$ and ^{85}Sr involved in the tracer released [95] [82]. However, in addition to HCO^{3-}, other anions such as $^{36}Cl^-$, $^{35}SO_4{}^{2-}$ and [^{14}C] acetate were not affected by light [96][83].

This data in this study showed that *U. prolifera* had significant changes in its signal transduction pathway-related proteins and metabolites within 12 h of high-light stress. Transcriptome data analysis revealed that up-regulated RAB escort proteins, up-regulated ARF and down-regulated DP-ribosylation factor-like protein 13B involved in plant ROP signal transduction pathways. The ubiquitous, conserved MPK (mitogen-activated protein kinase) cascade pathway in eukaryotic cells was widely involved in plant growth, development and plant defense responses against biotic and abiotic stresses.

4.4 Stress-resistance genes of *U. prolifera* under high-light stress

The growth and distribution of algae in water are affected by many environmental factors such as temperature, light, and chemicals in the water. When its environment changes drastically, the growth of

algae will also be inhibited. Relevant studies have shown that in order to resist adversity stress, algae stress-related proteins have gradually been discovered [84, 85].

DnaJ C chaperone protein (up-regulated), a member of the J protein family, has a molecular chaperone's characteristics and is used as a co-chaperone for HSP70 [86, 87]. It is also involved in stress resistance for abiotic stress[88]. In the proteome data, the [89]expression of *dna*J protein homologue 2 was up-regulated. Zinc finger proteins as transcription factors participated in the pathways related to plant stress resistance and signaling pathways mediated by ubiquitination [90]. In proteomics, antioxidant-related proteins' expressions were down-regulated, including ascorbate peroxidases, glutathione S transferases, and peroxidases. The expressions were up-regulated, including the metabolome-related metabolites (D-proline and dimethyl sulfone), and L-threonate which involved in ascorbic acids and alginate metabolism.

4.5 High light affecting the growth and metabolism of *U. prolifera*

Light is an essential factor in controlling the growth of algae, and light intensity affects the algae's growth and metabolism [91, 92]. When the sunshine duration exceeds 12 h per day, compared with the light intensity of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the chloroplast surface area of *Ulva* spp. under 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increases with the increasing light duration [93]. Under low light intensity, the long light period will also promote spores in *Ulva* spp. [93]. The algae's growth rate in the light period (150 $AE/m^2/s$) is significantly higher than that in the dark period. It increases by about two times within 24 hours because actin ACT expression is induced and inhibited by light and dark treatments [94].

However, in this experiment, under 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the expression transcript of SDA1 involved in regulating the actin skeleton was down-regulated, suggesting that cell growth was inhibited [95, 96]. Thus, it was speculated that the cell growth was inhibited. The enhancement of endopeptidase activity was considered the main reason for the decreased protein content during plant senescence[96], and the growth of *U. prolifera* under high light was also inhibited[97]. The possible reason was that growth-related enzymes and proteins changed, and the expression of related transcripts of serine endopeptidase was significantly up-regulated. At the same time, it is involved in the overall down-regulation of cell cycle checkpoint kinase expression of cyclin-dependent kinase CDK1 (the main engine of mitosis), impaired cell cycle regulation.

5. Conclusions

Based on four omics data analysis, high light mainly affects the mutual conversion of pentose and glucuronic acids, fatty acid biosynthesis, steroid biosynthesis, photosynthesis, pyrimidine metabolism, and carbohydrate metabolism, and other metabolic pathways, and regulates cell cycle in *U. prolifera*. DGDG and MGDG metabolites were regulated by influencing the ascorbic acid and alginate metabolism, fatty acid metabolism, and energy metabolism to control changes in the photosynthetic membranes of *U. prolifera*, thereby affecting its photosynthesis. It provides a reference for *U. prolifera* tolerating high light.

Declarations

Ethics approval and consent to participate

The main research object of this paper is algae, without any ethical problems. At the same time, all the authors of this article are willing to participate in the writing of this paper.

Consent for publication

All the authors provided their consent for publication.

Competing interests

The authors declare that they have no conflict of interest.

Availability of data and materials

All data used are present in this manuscript.

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Authors' contributions

CC and PH econceived the idea. TJ and KG designed the experiments. KG and TJ performed the experiments. KG, TJ and HZ analyzed the data. GK, YL,TJ and XL wrote and finalized the manuscript. KG and YL are co-first authors.All authors have read and approved the manuscript.

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Tables

Tables 1 to 7 are only available as a download in the Supplemental Files section.

Figures

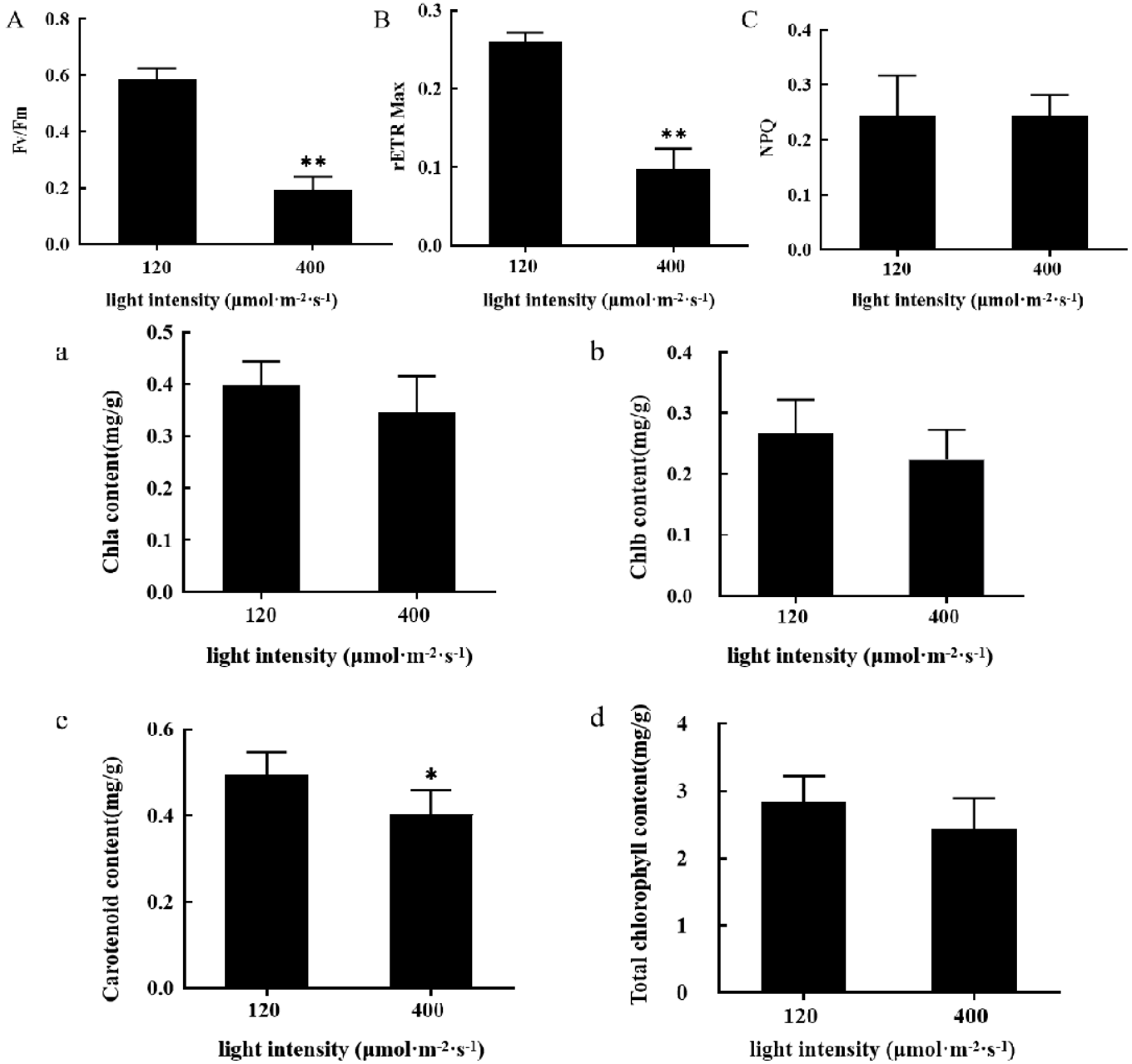

Figure 1

Results of changes in chlorophyll fluorescence parameters and photosynthetic pigment content of *Ulva* under high light intensity (400 μmol·m^{-2}·s^{-1}). A: Fv/Fm is the maximum photochemical quantum conversion efficiency change of PSII ($p < 0.001$); B: rETRmax is the maximum electron transfer efficiency ($p < 0.001$); C: NPQ is the result of non-photochemical quenching coefficient change ($p > 0.05$, Not obvious); a: the change of *Ulva* chlorophyll a content; b: the change of *Ulva* chlorophyll b content; c: the change of total chlorophyll content; d: the change of carotenoid content. The four groups of data have been significantly analyzed ($p < 0.05$).

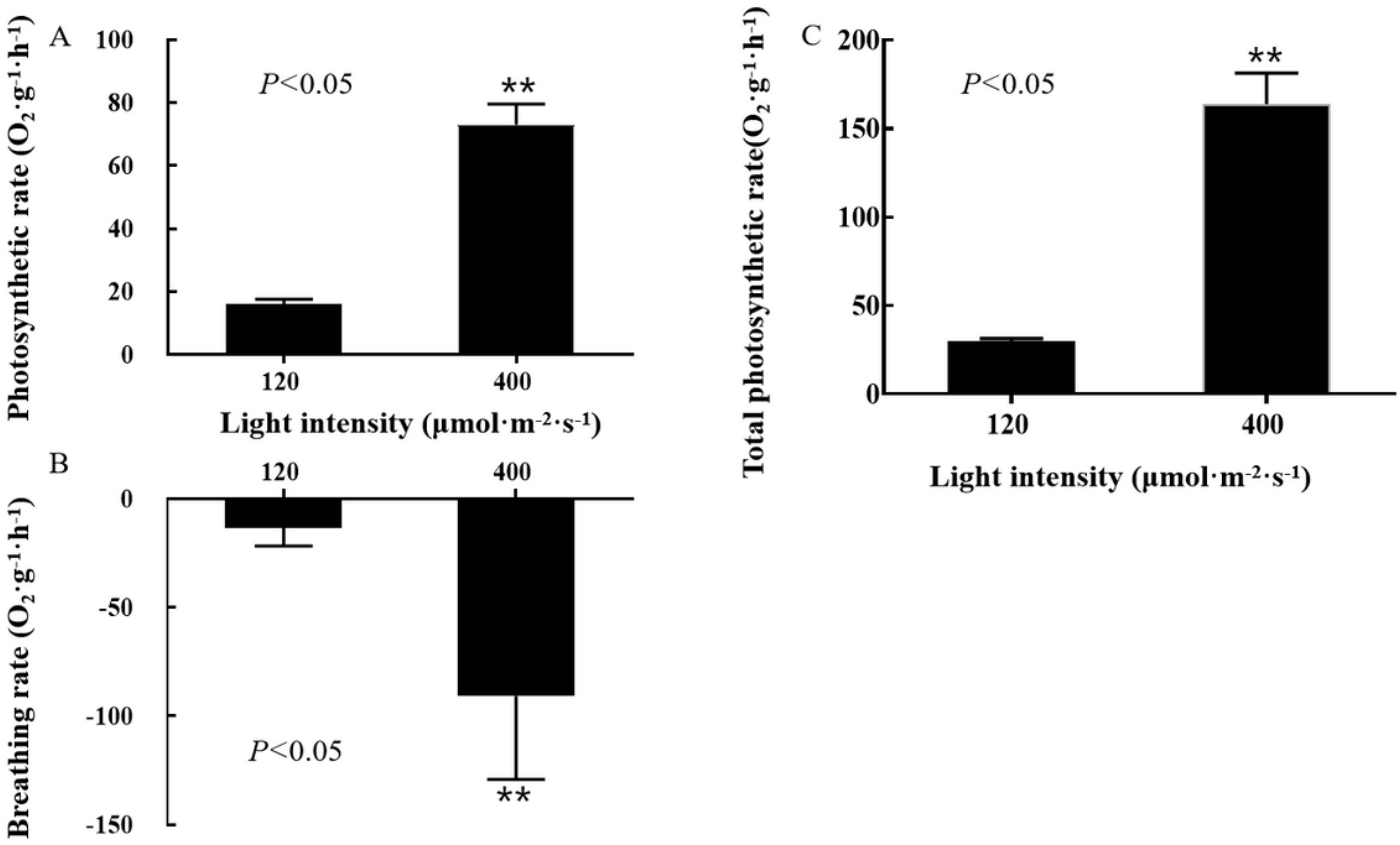

Figure 2

Results of changes in photosynthetic oxygen evolution rate of *Ulva* under high light intensity (400 μmol·m^{-2}·s^{-1})

A is the result of significant changes in photosynthetic rate ($p < 0.05$); B is the result of significant changes in respiration rate ($p < 0.05$); C is the result of significant changes in total photosynthetic rate ($p < 0.05$)

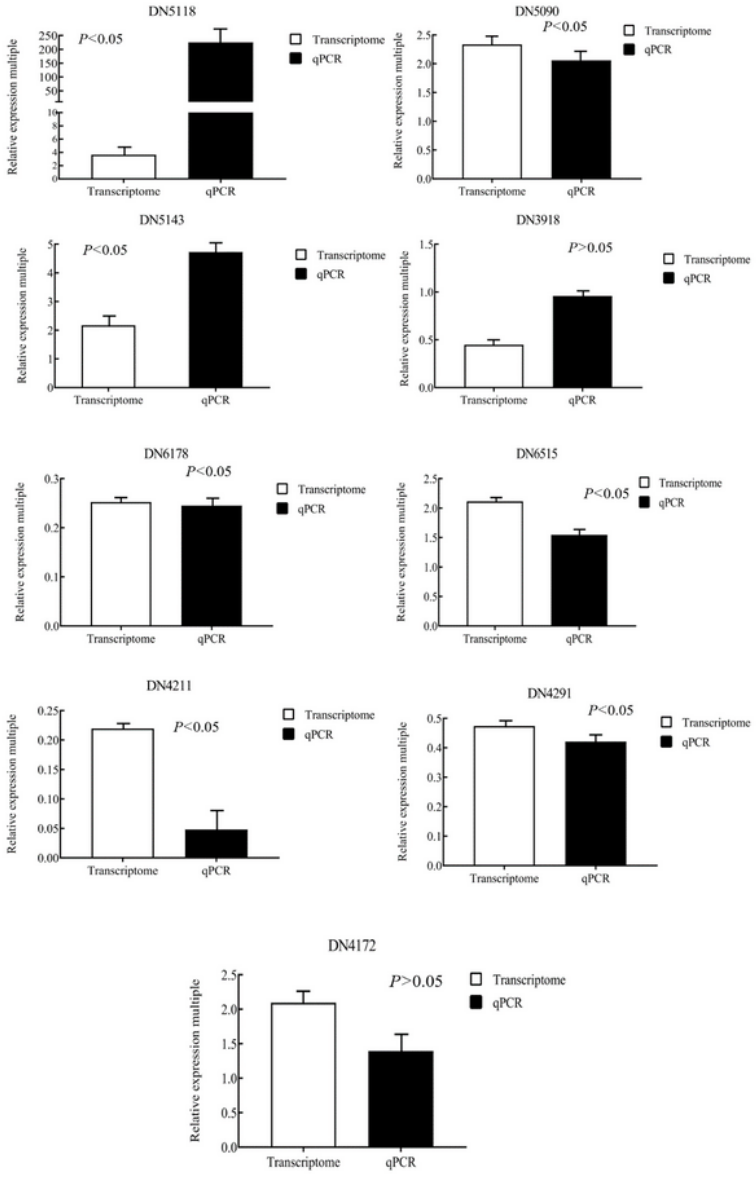

Figure 3

The nine significantly differential expressed genes in *U. prolifera* under high light intensity (400 μmol·m^{-2}·s^{-1}) were verified by qRT-PCR

Supplementary Files

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