

Physiological and transcriptomic analyses reveal the effects of phytohormones on growth and astaxanthin accumulation in *Haematococcus pluvialis*

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Research Article

Keywords: Astaxanthin, Biomass accumulation, *Haematococcus pluvialis*, Phytohormone

Posted Date: December 15th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8198834/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on March 14th, 2026.
See the published version at <https://doi.org/10.1186/s12870-026-08577-9>.

Abstract

Background

Haematococcus pluvialis is recognized as the richest natural source of astaxanthin, a high-value ketocarotenoid with potent antioxidant properties. Commercial production typically relies on a two-stage cultivation strategy, comprising a green vegetative phase for biomass accumulation and a red inductive phase for astaxanthin synthesis. However, a major bottleneck in this process is the balance between cell growth and pigment accumulation, as the stress conditions required for astaxanthin synthesis often inhibit biomass productivity. Phytohormones are critical signaling molecules that regulate growth and metabolism in photosynthetic organisms. Exploring their potential to decouple these conflicting physiological processes is crucial for optimizing industrial astaxanthin production.

Results

In this study, physiological and transcriptomic analyses were performed to evaluate the effects of five phytohormones (IAA, ABA, MeJA, ZT, and IPR) on *H. pluvialis* during its two-stage cultivation. Physiological data indicated distinct hormonal requirements for each stage: 0.01 mg L⁻¹ IAA significantly enhanced biomass in the green stage (0.37 g L⁻¹ vs. 0.29 g L⁻¹ in control), whereas 0.1 mg L⁻¹ MeJA maximized astaxanthin content in the red stage (29.22 mg g⁻¹ vs. 23.09 mg g⁻¹ in control). Transcriptomic profiling revealed that IAA promoted vegetative growth by upregulating genes involved in primary carbon metabolism (*PPDK*), cell cycle progression (*CDKB1-1*), and nutrient uptake (*ZIP9*). Conversely, MeJA treatment reprogrammed metabolism towards secondary biosynthesis by upregulating key ketolase genes (*CRTW* and *BKT3*) while downregulating competing carotenoid pathway genes (*CRTZ* and *LCYE*) and photosynthesis-related transcripts.

Conclusions

This study elucidates the distinct molecular mechanisms by which phytohormones regulate the dichotomy between growth and secondary metabolism in *H. pluvialis*. The findings suggest that the targeted application of IAA to boost biomass and MeJA to induce pigmentation offers a practical hormone guided strategy to improve two-stage astaxanthin production and provides molecular targets for further optimization.

1. Background

Astaxanthin is a high-value red ketocarotenoid characterized by a unique chemical structure of thirteen conjugated double bonds [1]. This configuration provides potent antioxidant activity, effectively scavenging reactive oxygen species and free radicals with an antioxidant capacity up to 65-fold greater than vitamin C [2, 3]. Due to its powerful bioactivity, astaxanthin has been widely applied in many

industries such as nutraceuticals, aquaculture, cosmetics, food, and animal feed [4]. The global astaxanthin market was valued at USD 1.63 billion in 2021 and is projected to reach USD 3.2 billion in 2026 [5]. Commercial astaxanthin products are classified into two main categories: synthetic and natural. Due to its lower production cost (approximately USD 1,000 per kg), synthetic astaxanthin holds the largest market share [6]. Chemically, synthetic astaxanthin exists primarily in a non-esterified form and is a racemic mixture of stereoisomers. In contrast, natural astaxanthin is highly esterified with fatty acids (95%) and consists almost exclusively of the (3S,3'S) stereoisomer [7, 8]. This specific stereochemical configuration is associated with superior antioxidant efficacy [9]. Despite its functional advantages, the global yield of natural astaxanthin remains low, and overcoming this production bottleneck is a major challenge [10].

Several microalgae are capable of synthesizing astaxanthin, with *Haematococcus pluvialis* being the richest known natural source [11]. Under environmental stress or adverse cultivation conditions, it can accumulate astaxanthin to 5% of its dry weight, a crucial self-protection mechanism against oxidative stress [11-13]. And this process is often coupled with transitions of life stages. The life stages of *H. pluvialis* can be divided into the green stage and the red stage [14]. Under favorable conditions, *H. pluvialis* predominantly exists as green vegetative cells characterized by vigorous cell growth and division [14, 15]. Upon exposure to adverse environmental conditions, green vegetative cells reduce division, thicken cell walls and accumulate large amounts of carotenoids which are primarily astaxanthin in cytoplasmic lipid droplets, resulting in transformation into the red stage [16, 17]. Consequently, the industrial production of astaxanthin from *H. pluvialis* typically employs a two-phase cultivation strategy. The green stage involves cultivation under suitable temperature, light and nutrient conditions to accumulate biomass. Once a sufficient biomass is reached, the red stage is initiated by applying stress such as high temperature, high light intensity, or nitrogen deprivation to induce astaxanthin accumulation [7, 18]. The production of astaxanthin inherently requires the application of stress conditions to induce its synthesis, which presents a delicate balance between astaxanthin accumulation and biomass growth [17, 19]. Therefore, identifying optimal stress levels or selecting appropriate inducers that maximize astaxanthin yield without compromising productivity is critical.

Phytohormones are critical endogenous signaling molecules that regulate virtually all aspects of plant growth, development, and responses to environmental stimuli [20, 21]. The roles of phytohormones have been extensively studied in higher plants, and increasing evidence indicates that these hormones also play significant roles in the physiology and metabolism of microalgae [22]. Indeed, numerous studies have demonstrated their positive effects on enhancing microalgal productivity. Absciscic acid (ABA) stimulates the activity of carbon metabolism-related enzymes, promoting lipid accumulation in *Chlorella* sp. [23]. Indole-3-acetic acid (IAA) was found to upregulate genes in the porphyrin metabolism pathway of *Phaeodactylum tricornutum*, leading to enhanced chlorophyll biosynthesis and thereby stimulated growth [24]. Zeatin (ZT) plays significant roles in the cell cycle, cell division and photosynthetic pigment synthesis of *Nannochloropsis oculata* [25]. Given the pronounced effects of phytohormones on microalgae, the application in cultivation of *H. pluvialis* offers substantial potential. Hu et al. [26] demonstrated that the combined application of salicylic acid (SA) and high-light stress increased

astaxanthin content in *H. pluvialis* by 0.33 mg L⁻¹ at 48 h. Gao et al. [27] found that carotenoid-biosynthesis genes (e.g., *psy*, *pds*, and *zds*) involved in astaxanthin production were upregulated following treatment with either SA or jasmonic acid (JA). However, previous studies have typically investigated only a limited range of hormones and have lacked comprehensive studies on the green stage.

The present study aimed to investigate the effects of five plant hormones including IAA, ABA, ZT, methyl jasmonate (MeJA) and N⁶-(Δ^2 -Isopentenyl) adenosine (IPR) on the biomass and physiological composition (chlorophyll, astaxanthin, carbohydrates and proteins) of *H. pluvialis*. Transcriptomic analysis was performed on *H. pluvialis* from treatment groups that showed significant increases in biomass and astaxanthin accumulation, which will provide a molecular level understanding of phytohormone mediated regulatory networks governing growth and astaxanthin biosynthesis. The findings of this research are expected to facilitate the optimized application of phytohormones in *H. pluvialis* cultivation for the enhanced production of high-value bioproducts.

2. Materials and methods

2.1 Microalgae strain and phytohormones

The *H. pluvialis* strain was purchased from Shanghai Guangyu Biological Technology Co., Ltd. (Shanghai, China). The strain is maintained in the Microalgae Culture Collection at the Laboratory of Applied Microalgal Biology, Ocean University of China, under the internal accession number LAMB284. Five phytohormones were obtained from MACKLIN, China: IAA ($\geq 98\%$), ABA ($\geq 98\%$), MeJA ($\geq 98\%$), ZT ($\geq 99\%$), IPR ($\geq 98.5\%$).

2.2 Algal culture and phytohormonal additions

H. pluvialis of green stage was cultivated in 300 mL Erlenmeyer flasks containing 200 mL of BG11 medium [28] under a light intensity of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a 14h/10h light-dark cycle at 22 ± 1 °C and 7.1 ± 0.1 pH. The initial inoculation density was 1×10^4 cells mL⁻¹. The phytohormones used were IAA, ABA, IPR, and ZT. Phytohormones were first prepared as concentrated stocks (1 g L⁻¹) using sterile water (with a minimal amount of ethanol when needed for solubility), stored at 4°C and used within 7 days. Before application, stocks were diluted with culture medium to the working concentrations of 0.001, 0.01, 0.1, and 1 mg L⁻¹. Controls received the same volume of sterile water (or solvent at the matching final v/v when ethanol was used). Cultivation lasted 15 days, with samples collected every 3 days for dry weight determination and samples from day 9 were taken for physiological measurement.

H. pluvialis of red stage was obtained as follows: after 15 days cultivation under the same conditions above without phytohormone supplementation, the light intensity was increased to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and four phytohormones (IAA, ABA, MeJA, and ZT) were added to medium. Cultivation was continued for another 15 days, with samples collected every 3 days to measure astaxanthin and samples from day 24 were analyzed for physiological data. All days are counted from the initial inoculation.

The choice of IPR for the green stage and MeJA for the red stage was based on our prior measurement of endogenous phytohormones in *H. pluvialis*. Endogenous IPR was abundant in the green stage and decreased in the red stage, whereas endogenous MeJA increased during the red stage [29]. For each phytohormone concentration, 3 independent biological replicates were used.

2.3 Physiological analysis

2.3.1 Measurement of dry weight

First, 20 mL of algal culture at the green stage was concentrated at 2000 g for 10 minutes to discard the supernatant. The pellets were washed twice with distilled water, then dried at 60 °C for 24 h. Finally, the dried samples were weighed to determine the dry weight.

2.3.2 Determination of total chlorophyll content

Total chlorophyll was extracted using ethanol and measured using a spectrophotometer, following the method described by Rowan [30]. Briefly, 10 mL of algal culture was centrifuged at 2000 × g for 10 min to collect the cell pellet. The pellet was resuspended in 10 mL of 95% ethanol and incubated in darkness for 24 h. Absorbance of the extract was measured at 665 and 649 nm, with chlorophyll content calculated using the following formula:

$$C_{\text{Chl a}} (\text{mg L}^{-1}) = 13.70A_{665} - 5.76A_{649}$$

$$C_{\text{Chl b}} (\text{mg L}^{-1}) = 25.8 A_{649} - 7.60A_{665}$$

$$C_{\text{Total Chl}} (\text{mg L}^{-1}) = C_{\text{Chl a}} + C_{\text{Chl b}}$$

2.3.3 Measurement of astaxanthin

The method of astaxanthin measurement referred to Zhang et al [31]. Briefly, 5 mL of culture were centrifuged at 2000 g for 10 min and the supernatant discarded. Pellets were resuspended in 3 mL dimethyl sulfoxide and heated at 80°C for 10 min. Samples were centrifuged at 2000 g for 10 min and the supernatant transferred to a clean tube. Extraction was repeated until pellets turned white. The pooled supernatant absorbance was measured at 530 nm on a UH5300 spectrophotometer (Hitachi, Japan). Astaxanthin standard (purity ≥ 98%, Solarbio, China) was used for quantification.

2.3.4 Measurement of carbohydrate

The total carbohydrate content of *H. pluvialis* was determined using the anthrone method [32]. A 2 mL of algal suspension was centrifuged at 2000 g for 10 min. The pellet was reacted with 8 mL of anthrone solution, which was prepared by mixing 0.2 g anthrone, 8 mL absolute ethanol, and 100 mL concentrated sulfuric acid (98%). The mixture was heated at 90 °C for 10 min. After cooling, the absorbance was measured at 620 nm. A standard curve was constructed by glucose standards.

2.3.5 Measurement of protein

The protein content of *H. pluvialis* was quantified using the Coomassie Brilliant Blue G-250 colorimetric assay [33]. 5 mL of algal suspension was centrifuged (2000 g, 10 min), and cell pellet was resuspended in 200 μ L of 1 M NaOH before incubation at 80 °C for 10 min. After the addition of 800 μ L of distilled water, samples were centrifuged at 3000 g for 30 min. The supernatants were collected and the process was repeated twice. All supernatants were pooled and diluted to a volume of 5 mL. 2 mL of the extract were mixed with 5 mL of Coomassie Brilliant Blue G-250 solution (1 mg mL⁻¹) and incubated at room temperature for 15 minutes. 200 μ L aliquot was added to a 96-well microplate, and absorbance was measured at 595 nm using a microplate reader (Synergy™ Mx, BioTek, USA). Bovine serum albumin was used to generate the standard curve.

2.3.6 Statistical analysis

All physiological composition data were presented as mean $\pm$ SD (n = 3). Group differences were assessed by one-way ANOVA followed by Tukey's HSD for multiple comparisons ($p < 0.05$). All statistical analyses were performed using SPSS 26.0 and Graphpad Prism (v9.5).

2.4 Transcriptome analysis

2.4.1 RNA extraction, library preparation and sequencing

Total RNA was extracted from *H. pluvialis* in the control group, the biomass optimized group and the astaxanthin optimized group using RNeasy Pure Plant Kit (TIANGEN, China). RNA integrity and quality were assessed with an Agilent 2100 Bioanalyzer. mRNA was isolated from the total RNA using oligo (dT) magnetic beads and subsequently fragmented into smaller strands. cDNA synthesis was conducted in two steps: first-strand cDNA synthesis using random hexamer primers, followed by second-strand synthesis. Library preparation involved sequential steps of end repair, A-tailing, adapter ligation, size selection, PCR amplification, and purification. The resulting libraries were pooled based on effective concentration and target sequencing depth. DNA nanoballs were generated through rolling-circle replication after 5'-phosphorylation and circularization. Sequencing was carried out on the DNBSEQ-T7 platform (MGI Tech Co., Ltd., China). All sequence data were 2 $\times$ 75 bp in length. Raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA1279618.

2.4.2 Bioinformatics Analysis

Raw sequencing reads were pre-processed to remove low-quality reads, adapter sequences, and poly-N sequences using fastp. Clean reads were then mapped to the *H. pluvialis* reference genome (NCBI RefSeq: GCA_030144725.1) using HISAT2 v2.2.1. Gene expression levels were quantified as FPKM (Fragments Per Kilobase of transcript per Million mapped reads) using StringTie v2.2.1.

Differential expression analysis was performed using DESeq2 v1.42.0 to identify genes significantly altered in the hormone treated groups compared to the control. Genes with a $|\log_2\text{FoldChange}| \geq 1$ and an adjusted pvalue < 0.05 were considered differentially expressed.

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed genes were conducted using clusterProfiler R package (v4.8.1) to identify over-represented biological processes, molecular functions, cellular components, and metabolic pathways.

2.4.3 Functional annotation

Putative gene functions were assigned by BLAST based searches. Protein sequences were queried against the Swiss-Prot database, and Gene Ontology terms were transferred from the best Swiss-Prot match where available. KEGG Orthology identifiers were assigned using the KEGG database and were used for pathway mapping.

2.5 Validation of RNA-seq data by qRT-PCR

To validate the RNA-seq results, quantitative real-time PCR (qPCR) was performed on *H. pluvialis* samples collected from the green and red stages. For the green stage, cells from the control group and the 0.01 mg L⁻¹ IAA-treated group were analyzed; for the red stage, cells from the control group and the 0.1 mg L⁻¹ MeJA-treated group were analyzed. Total RNA was extracted using the Servicebio MF168 RNA extraction kit following the manufacturer's instructions. RNA concentration and purity were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), and samples with acceptable A260/280 ratios (1.8–2.2) were used for reverse transcription. First-strand cDNA was synthesized from 1 µg of total RNA in a 20 µL reaction using the Vazyme HiScript III 1st Strand cDNA Synthesis Kit with gDNA wiper. The reverse transcription program was 37°C for 15 min followed by 85°C for 5 s.

QPCR was carried out using Vazyme Taq Pro Universal SYBR qPCR Master Mix on a BIO-RAD CFX95 Real-Time PCR System (C1000 Touch Thermal Cycler), and fluorescence data were collected with CFX Manager 3.1. Each 20 µL qPCR reaction contained 10 µL SYBR master mix, 0.4 µL of each primer (10 µM), and 2 µL of diluted cDNA (equivalent to 20 ng total RNA), with nuclease-free water added to volume. The thermal cycling conditions were 95°C for 30 s, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Following the cycling protocol, a melting curve analysis was performed from 65°C to 95°C to verify the specificity of the PCR products. Primer pairs were designed using NCBI Primer-BLAST, and primer sequences and amplicon sizes are listed in Supplementary File 1 (Table S1). Expression levels were normalized to the reference gene *gene_QJQ45_016574* and *gene_QJQ45_003696*, and relative transcript abundance was calculated using the 2^{-ΔΔCt} method. Three biological replicates were analyzed for each condition, with three technical replicates per sample. No-template controls (NTC) and no-RT controls were included in each run to ensure specificity and exclude contamination.

3. Results

3.1 Biomass and astaxanthin content under phytohormone treatments

The effects of different phytohormone treatments on biomass accumulation during the green stage of *H. pluvialis* are shown in Fig. 1A–D. All groups exhibited slow growth during the initial 3 days, then entering an exponential phase. By day 15, the biomass of the green stage control (GCon) reached 0.29 g L⁻¹. Among all treatments, 0.01 mg L⁻¹ IAA resulted in the highest biomass yield, with biomass attaining 0.37 g L⁻¹ at day 15. In addition to IAA, 0.001 and 0.01 mg L⁻¹ concentrations of ABA, ZT, and IPR also promoted growth to varying extents, whereas higher concentrations of these hormones inhibited *H. pluvialis* growth.

Astaxanthin accumulation during the red stage is presented in Fig. 1E–H. The accumulation rate peaked between days 3 and 6 and declined thereafter. On day 15, the astaxanthin content of the red stage control (RCon) was 23.09 mg g⁻¹. Treatment with 0.1 mg L⁻¹ MeJA yielded the highest astaxanthin accumulation of 29.22 mg g⁻¹. Other phytohormones also promoted astaxanthin synthesis to lesser degrees. However, phytohormone treatments at 1 mg L⁻¹ markedly inhibited astaxanthin accumulation.

3.2 Effects of phytohormones on physiological parameters

Physiological parameters under phytohormone treatments during the green stage are summarized in Table 1. Exogenous ABA, ZT, and IPR at low concentrations (0.001 and 0.01 mg L⁻¹) significantly enhanced carbohydrate content, with 0.01 mg L⁻¹ ABA producing the greatest increase. In contrast, higher phytohormone concentrations generally inhibited carbohydrate accumulation. Protein content likewise increased under low to moderate phytohormone levels but declined markedly at 1 mg L⁻¹, reaching a minimum of 27.03 ± 2.22 mg g⁻¹.

Table 1
Effects of phytohormone treatments on physiological and biochemical measurements of green stage of *H. pluvialis*

Measurements	Carbohydrate	Protein	Chlorophyll
Algal samples			
GCon	40.42 ± 1.18	38.07 ± 3.23	10.53 ± 1.05
ABA_0.001	57.46 ± 1.56 ^{**}	49.2 ± 1.39 ^{**}	11.51 ± 0.88
ABA_0.01	82.27 ± 1.77 ^{**}	43.88 ± 0.98	11.50 ± 1.56
ABA_0.1	25.39 ± 0.62 ^{**}	31.71 ± 1.25	5.36 ± 0.52 ^{**}
ABA_1	15.65 ± 0.80 ^{**}	27.03 ± 2.22 ^{**}	2.49 ± 0.23 ^{**}
IAA_0.001	36.22 ± 1.59 [*]	48.32 ± 0.9 ^{**}	11.80 ± 0.81
IAA_0.01	35.97 ± 1.41 [*]	49.11 ± 4.07 ^{**}	14.20 ± 1.31 ^{**}
IAA_0.1	32.08 ± 0.59 ^{**}	45.58 ± 2.75	11.19 ± 1.01
IAA_1	14.57 ± 0.76 ^{**}	33.81 ± 0.79	4.41 ± 0.40 ^{**}
ZT_0.001	53.03 ± 1.42 ^{**}	36.5 ± 3.32	11.48 ± 1.03
ZT_0.01	47.58 ± 3.34 ^{**}	51.13 ± 2.09 ^{**}	12.76 ± 1.23 [*]
ZT_0.1	35.73 ± 1.00 [*]	50.32 ± 1.73 ^{**}	5.23 ± 0.70 ^{**}
ZT_1	18.48 ± 0.73 ^{**}	46.51 ± 4.67 [*]	2.94 ± 0.33 ^{**}
IPR_0.001	47.47 ± 2.34 ^{**}	43.44 ± 1.95	11.12 ± 1.49
IPR_0.01	81.51 ± 0.75 ^{**}	35.66 ± 2.6	12.82 ± 1.00 [*]
IPR_0.1	39.53 ± 0.62	45.66 ± 2.73	5.63 ± 0.62 ^{**}
IPR_1	21.73 ± 0.78 ^{**}	48.67 ± 3.45 ^{**}	2.84 ± 0.30 ^{**}
Data are given as means ± SD, n = 3. Unit is mg g ⁻¹ . * <i>P</i> < 0.05, ** <i>P</i> < 0.01 compared to the control group. The numbers following phytohormone names represent treatment concentrations (mg L ⁻¹).			

Physiological responses during the red stage are presented in Table 2. Carbohydrate content was elevated in almost all groups during the red stage. The treatment group with ZT at 0.1 mg L⁻¹ achieved the highest level (159.92 ± 5.84 mg g⁻¹). Compared with the control, the treatments of 0.001 mg L⁻¹ MeJA, 0.1 mg L⁻¹ ZT, and 0.1 mg L⁻¹ IAA resulted in a significant decrease in protein content, with the lowest level observed under 0.1 mg L⁻¹ IAA (10.05 ± 0.67 mg g⁻¹). Conversely, significant increases were

observed under the treatments of 0.01 mg L⁻¹ ZT, 1 mg L⁻¹ ABA, 1 mg L⁻¹ ZT, and 1 mg L⁻¹ IAA, with 0.01 mg L⁻¹ ZT showing the highest protein content (25.46 ± 1.73 mg g⁻¹).

Table 2

Effects of phytohormone treatments on physiological and biochemical measurements of red stage of *H. pluvialis*.

Measurements	Carbohydrate	Protein	Chlorophyll
Algal samples			
RCon	95.00 ± 4.62	15.96 ± 0.48	1.64 ± 0.10
MeJA_0.001	136.97 ± 5.65**	11.04 ± 0.51**	1.35 ± 0.08
MeJA_0.01	115.15 ± 6.48*	13.32 ± 0.06	1.18 ± 0.10**
MeJA_0.1	142.40 ± 8.46**	15.96 ± 0.43	0.95 ± 0.08**
MeJA_1	89.21 ± 3.92	17.92 ± 1.18	0.37 ± 0.03**
ABA_0.001	122.73 ± 4.80**	19.02 ± 0.23	1.79 ± 0.13
ABA_0.01	120.51 ± 7.70**	18.11 ± 0.43	2.21 ± 0.14**
ABA_0.1	117.25 ± 6.23**	17.37 ± 1.17	1.20 ± 0.09**
ABA_1	95.92 ± 6.26	22.4 ± 1.68**	0.55 ± 0.04**
ZT_0.001	88.70 ± 6.01	16.13 ± 0.79	1.48 ± 0.11
ZT_0.01	121.53 ± 5.78**	25.46 ± 1.73**	2.08 ± 0.19**
ZT_0.1	159.92 ± 5.84**	11.4 ± 0.37**	1.31 ± 0.09*
ZT_1	51.94 ± 3.04**	22 ± 0.53**	0.78 ± 0.03**
IAA_0.001	108.04 ± 7.22	14.8 ± 1.2	1.78 ± 0.07
IAA_0.01	108.29 ± 6.96	17.96 ± 1.22	2.83 ± 0.16**
IAA_0.1	146.68 ± 7.30**	10.05 ± 0.67**	1.99 ± 0.10*
IAA_1	65.59 ± 4.21**	22.09 ± 0.38**	1.01 ± 0.08**
Data are given as means ± SD, n = 3. Unit is mg g ⁻¹ . *P < 0.05, **P < 0.01 compared to the control group. The numbers following phytohormone names represent treatment concentrations (mg L ⁻¹).			

3.3 Overview of transcriptome sequencing data

To deeply explore the molecular regulatory mechanisms of phytohormones on *H. pluvialis* growth and astaxanthin accumulation, we conducted transcriptome sequencing analysis on the groups with the highest biomass accumulation (0.01 mg L⁻¹ IAA), the highest astaxanthin accumulation (0.1 mg L⁻¹ MeJA), and their respective controls (GCon and RCon). Strict quality control was performed on the raw sequencing data of all samples. As shown in Table 3, all samples had sufficient sequencing data, with an average of 40-50M reads/sample. After quality filtering, 39-48M clean reads/sample were retained, meeting expectations. In terms of base quality, the Q30 percentage of all samples was above 96%, and the GC content was stable (58.73–60.54%). The mapping rate of reads aligned to the *H. pluvialis* reference genome ranged from 64.11% to 76.51%, consistent with mapping rates reported for this species using genome based alignment ($\approx$ 63.3% on average), and the high read quality together with the known highly repetitive genome indicates that such rates are expected for *H. pluvialis* [34]. In the GCon, IAA treated groups, RCon, and MeJA treated groups, 34625, 34430, 34270, and 35267 expressed genes were identified, respectively. The FPKM for each sample group is shown in Supplementary File 1: Figure S1. The results indicate that 25% of the expressed genes were in the 0-0.5 FPKM range, 35% were in the 0.5-5 FPKM range, 35% were in the 5-100 FPKM range, and 3% of genes had FPKM values exceeding 100. Functional annotation assigned 10,021 genes to GO categories, 499 to KEGG pathways, 498 to KEGG Orthology identifiers, and 6,650 to protein interaction entries.

Table 3
RNA sequencing results

Algal Samples	Raw reads	Clean reads	Error rate (%)	Q30 (%)	GC content (%)	Mapping rate (%)
GCon_1	43009230	41841168	0.02	96.66	59.42	72.31
GCon_2	42935178	41838162	0.02	96.64	59.11	76.51
GCon_3	46886006	45483570	0.02	96.62	59.26	71.84
IAA_1	46519838	45163956	0.02	96.49	58.73	65.44
IAA_2	43069706	41577938	0.02	96.76	58.97	66.52
IAA_3	47104636	45607276	0.02	96.63	58.96	68.53
RCon_1	47533786	46393270	0.02	96.65	60.54	67.61
RCon_2	46725614	45734276	0.02	96.27	60.42	68.63
RCon_3	47131428	45941918	0.02	96.51	60.41	68.32
MeJA_1	49255422	47856734	0.02	96.55	60.08	64.11
MeJA_2	47068684	45484812	0.02	96.84	60.21	64.38
MeJA_3	40264948	38984278	0.02	96.4	59.99	68.67

GO classification of 10,021 genes emphasized categories that are most informative for growth and metabolism in *H. pluvialis* (Fig. 2). At the biological process level, annotations concentrated in protein and nucleic acid metabolism and biosynthetic and macromolecule modification processes. At the cellular component level, genes were mainly associated with the membrane and intracellular organelles including the cytoplasm, nucleus and ribosome. At the molecular function level, binding and catalytic activity dominated, with nucleotide or ATP binding and hydrolase and transferase activities also prominent.

3.4 qRT-PCR validation of RNA-seq data

To experimentally validate the RNA-seq results, qRT-PCR was performed on a randomly selected subset of DEGs from both developmental stages. Six DEGs were chosen from the green stage and another six from the red stage, with the selection covering both up-regulated and down-regulated transcripts. As shown in Fig. 3, the qRT-PCR results exhibited expression trends fully consistent with those obtained from the RNA-seq analysis in both stages.

3.5 Differential expression analysis

Clustering analysis of differentially expressed genes (DEGs) showed that biological replicate samples from the same treatment group clustered together (Fig. 4), indicating good experimental reproducibility. More importantly, samples from different growth stages formed distinct clustering branches, with the GCon and IAA treated group clustering into one category, and the RCon and MeJA treated group clustering into another.

To further identify differentially expressed genes induced by phytohormones, we used $|\log_2\text{FoldChange}| \geq 1$ and $\text{padj} < 0.05$ as screening thresholds to construct volcano plots illustrating the overall distribution of DEGs between the GCon and IAA-treated groups, and the RCon and MeJA-treated groups (Fig. 5). As shown in Fig. 5A, in the comparison between the IAA-treated group and the GCon, a total of 490 significantly differentially expressed genes were identified, of which 400 genes were upregulated and 90 genes were downregulated. In the comparison between the MeJA-treated group and the RCon (Fig. 5B), a total of 3208 DEGs were identified, with 1927 genes significantly upregulated and 1281 genes downregulated. Since these genes required functional annotation, we annotated the differentially expressed genes in the Swiss-Prot database in section 3.5 to explore their potential biological functions.

3.6 Functional annotation and in-depth analysis of key DEGs

3.6.1 Key DEGs in response to IAA treatment

Transcriptome analysis revealed significant changes in *H. pluvialis* gene expression after IAA treatment. A total of 163 annotated differentially expressed genes were obtained, of which 112 were upregulated

and 51 were downregulated (Supplementary File 2, Sheet1). Key DEGs associated with growth are presented in Table 4.

Table 4
DEGs between IAA treated group and GCon group.

Protein	Gene_id	Best-hit annotation (species)	Ref symbol	log ₂ FoldChange	pvalue
Pyruvate phosphate dikinase	novel.2670	pyruvate phosphate dikinase 1 (Oryza sativa)	PPDK1	1.57	1.7E-26
Pyruvate phosphate dikinase	novel.8652	pyruvate phosphate dikinase 1 (Arabidopsis thaliana)	PPDK	1.49	6.3E-23
Pyruvate phosphate dikinase	novel.2399	pyruvate phosphate dikinase 1 (Oryza sativa)	PPDK1	1.49	3.6E-16
Pyruvate kinase	gene_QJQ45_025570	pyruvate kinase, cytosolic 2 (Oryza sativa)	PK	1.69	2.1E-4
Fructokinase	gene_QJQ45_013744	fructokinase 2 (Oryza sativa)	FRK2	1.12	1.3E-4
Zinc transporter 9	gene_QJQ45_017004	zinc transporter 9 (Oryza sativa)	ZIP9	3.54	2E-65
Zinc transporter 9	gene_QJQ45_020772	zinc transporter 9 (Oryza sativa)	ZIP9	1.43	1.5E-19
Cyclin-dependent kinase B1-1	gene_QJQ45_023291	cyclin-dependent kinase B1-1 (Oryza sativa)	CDKB1-1	1.48	2.9E-4
Cyclin B2-1	gene_QJQ45_026341	cyclin B2-1 (Oryza sativa)	CYCB2-1	1.56	4.5E-4

Three loci annotated as pyruvate phosphate dikinase (*PPDK*; *novel.2670*, *novel.8652*, *novel.2399*) were concordantly upregulated. In parallel, transcripts encoding pyruvate kinase (*PK*; *gene_QJQ45_025570*) and fructokinase (*FRK2*; *gene_QJQ45_013744*) increased, indicating coordinated enhancement of flux at the PEP/pyruvate node. Two zinc transporter 9 loci (*ZIP9*; *gene_QJQ45_017004* and *gene_QJQ45_020772*) were strongly upregulated. Transcripts for *CDKB1-1* (*gene_QJQ45_023291*) and *CYCB2-1* (*gene_QJQ45_026341*) also increased.

3.6.2 Key DEGs in response to MeJA treatment

DEGs in *H. pluvialis* after MeJA treatment were also annotated using Swiss-Prot, yielding a total of 1103 annotated differentially expressed genes. 596 were upregulated and 507 were downregulated (Supplementary File 2, Sheet2). Key DEGs associated with astaxanthin biosynthesis are presented in Table 5.

Table 5
DEGs between MeJA treated group and Rcon group.

Proteins	Gene_id	Best-hit annotation (species)	Ref symbol	log ₂ Foldchange	pvalue
β-carotene ketolase	gene_QJQ45_019472	beta-carotene ketolase (<i>Haematococcus pluvialis</i>)	CRTW	1.29	2.9E-130
β-carotene 4-ketolase 3	gene_QJQ45_024144	beta-carotene ketolase 3 (<i>Haematococcus pluvialis</i>)	BKT3	1.62	4.1E-129
β-carotene 3-hydroxylase	gene_QJQ45_001374	beta-carotene 3-hydroxylase (<i>Haematococcus pluvialis</i>)	CRTZ	-1.34	3.4E-12
β-carotene 3-hydroxylase	gene_QJQ45_030451	beta-carotene 3-hydroxylase (<i>Haematococcus pluvialis</i>)	CRTZ	-1.18	5.3E-05
Lycopene epsilon cyclase	gene_QJQ45_015099	lycopene epsilon cyclase (<i>Solanum lycopersicum</i>)	LCYE	-1.55	1.2E-72
Lycopene epsilon cyclase	gene_QJQ45_027358	lycopene epsilon cyclase (<i>Solanum lycopersicum</i>)	LCYE	-1.42	6.8E-09

Two ketolase-encoding genes were significantly upregulated: *CRTW* (*gene_QJQ45_019472*) and *BKT3* (*gene_QJQ45_024144*). Two loci annotated as β-carotene 3-hydroxylase (*CRTZ*; *gene_QJQ45_001374* and *gene_QJQ45_030451*) were downregulated, and two lycopene ε-cyclase loci (*LCYE*; *gene_QJQ45_015099* and *gene_QJQ45_027358*) likewise decreased.

3.7 Functional Enrichment Analysis

To systematically elucidate the biological effects of IAA in the green phase and MeJA in the red phase on the *H. pluvialis* transcriptome, we performed KEGG pathway and GO enrichment analyses on the identified differentially expressed genes (DEGs). The enrichment analysis results are presented as bubble plots (Fig. 6 and Fig. 7).

KEGG pathway enrichment results of IAA treated group are shown in Fig. 6A, in which multiple pathways related to carbon and energy metabolism were significantly enriched, including pyruvate metabolism, carbon metabolism, and carbon fixation in photosynthetic organisms. Additionally, biosynthesis of secondary metabolites and fatty acid metabolism were also significantly enriched.

GO functional enrichment results of IAA treated group are shown in Fig. 7A. Within biological process, the most significantly enriched term was carbohydrate metabolic process. Additional enriched biological processes included monocarboxylic acid metabolism, lipid modification, organophosphate metabolism, and organic acid metabolism. For cellular component, significantly enriched terms comprised peroxisome and microbody. In terms of molecular function, various hydrolase activities and transporter activities were enriched.

The KEGG pathway and GO enrichment analysis results for DEGs of MeJA treated *H. pluvialis* in the red phase (MeJA VS RCon) are shown in Fig. 6B and 7B, respectively. KEGG showed that metabolic pathways were the most significantly enriched pathways. Significantly enriched specific pathways included porphyrin metabolism, carbon fixation in photosynthetic organisms, carbon metabolism, and biosynthesis of secondary metabolites. Among these, biosynthesis of secondary metabolites, biosynthesis of cofactors and pentose phosphate pathway also showed enrichment.

GO result showed in terms of biological process, carbohydrate metabolic process was still significantly enriched. Photosynthesis-related GO terms such as photosynthesis, chlorophyll biosynthetic process, and their related sub-processes were all significantly enriched, and most genes showed downregulation. Simultaneously, the enrichment of tetrapyrrole biosynthetic process corresponded to porphyrin metabolism in KEGG. In terms of cellular component, photosystem, photosynthetic membrane, and thylakoid, all related to photosynthesis, were significantly enriched, and genes in these components were generally downregulated. Additionally, ATP synthase complex, plastid, and chloroplast membrane components also showed enrichment. In terms of molecular function, iron binding was significantly enriched. Notably, monooxygenase activity was also significantly enriched.

4. Discussion

4.1 Interpreting biomass and astaxanthin outcomes

As a key regulator of cell division, differentiation, and proliferation, IAA has been shown to enhance microalgal growth. For example, Salama et al. [35] reported a 1.9-fold increase in *Scenedesmus obliquus* cell density under 10^{-5} M IAA. Such biphasic dose responses to phytohormones in microalgae have been widely documented [36–38]. Similarly, Khalili et al. [39] demonstrated that 32 μ M MeJA significantly enhanced astaxanthin accumulation in *Chlorella sorokiniana*. Chen et al. [40] also reported that high concentrations of hormones such as IAA, IBA, and NAA suppressed astaxanthin production due to cellular dysfunction and increased cell mortality.

4.2 Interpreting physiological parameters

Low dose phytohormones act as metabolic signals to promote carbon fixation, whereas excessive doses impose osmotic or feedback inhibition on photosynthesis [37]. Protein content likewise increased under low to moderate hormone levels but declined markedly at 1 mg L⁻¹, reaching a minimum of 27.03 ± 2.22 mg g⁻¹, implying that optimal phytohormone dosing enhances nitrogen assimilation and protein biosynthesis, while overdose may trigger protein degradation [41]. Chlorophyll content remained stable at low phytohormone concentrations, but was significantly reduced at 1 mg L⁻¹, indicating that high hormone doses exert stress on the photosystems or inhibit chlorophyll synthesis [42]. Carbohydrate content was elevated in all groups during the red stage, which indicated that *H. pluvialis* would accumulate a large amount of carbohydrate under stress conditions [43]. Chlorophyll levels were largely suppressed in the red stage, consistent with stress-induced downregulation of chlorophyll synthesis and upregulation of astaxanthin production [44].

4.3 DEG patterns across stages

Compared to the IAA group, the expression changes in the MeJA-treated group were more drastic, with some genes having $-\log_{10}(\text{padj})$ approaching 300, indicating that MeJA induced a stronger transcriptional response in the red algal stage. These results suggest that IAA primarily induced a slight increase in gene expression during the green growth phase, while MeJA led to the activation and suppression of a large number of genes during the red phase.

4.4 Functional interpretation of IAA responsive DEGs

Genes encoding pyruvate phosphate dikinase (PPDK), such as novel.2670, novel.8652, and novel.2399, showed significant upregulation, with log₂FoldChange values ranging from 1.49 to 1.57. The enzyme PPDK catalyzes the conversion of pyruvate to phosphoenolpyruvate, a key enzyme for improving CO₂ fixation efficiency in C₄ plants [45, 46]. However, in C₃ plants such as *Oryza sativa*, PPDK primarily acts as an auxiliary enzyme in glycolysis, balancing carbon flow towards the biosynthesis of starch, proteins, fatty acids, and amino acids, especially being highly expressed in the starchy endosperm cytoplasm [47, 48]. It may also provide ATP in hypoxic regions [49]. The significant upregulation of *PPDK* may imply that IAA induces a reprogramming of carbon metabolism in *H. pluvialis* to more efficiently utilize carbon sources for the synthesis of biological macromolecules, thereby supporting rapid cell proliferation and biomass accumulation [46]. As a photoautotrophic organism, the efficiency of carbon metabolism directly affects the growth of *H. pluvialis*, thus this regulation is consistent with the known function of IAA in promoting growth and biomass increase [50].

Furthermore, genes encoding pyruvate kinase (*PK*, *gene_QJQ45_025570*) and fructokinase (*FRK*, *gene_QJQ45_013744*) were also significantly upregulated (Table 4). PK is the final enzyme in the glycolysis pathway, responsible for producing ATP and pyruvate [51]. Its gene upregulation suggested that IAA may stimulate the rate of glycolysis in *H. pluvialis*, thereby increasing ATP production efficiency to provide sufficient energy support for various cellular activities, particularly growth and biosynthesis [52, 53]. FRK converts fructose to fructose-6-phosphate, allowing it to enter the glycolysis pathway [54]. Its gene upregulation may mean that IAA promoted the ability of *H. pluvialis* to utilize different sugar

substrates, enhancing the flexibility of carbohydrate metabolism. This helped cells maintain efficient energy production and biosynthesis under different carbon source conditions, thereby supporting continuous growth [55].

In terms of ion and nutrient transport, IAA significantly affected the absorption and distribution of key elements in *H. pluvialis*. *ZIP9* genes, including *gene_QJQ45_017004* and *gene_QJQ45_020772*, both showed extremely high upregulation, with $\log_2$ FoldChange values of 3.54 and 1.43, respectively (Table 4). The ZIP9 protein belongs to the ZIP family of proteins, responsible for transporting zinc ions from the extracellular environment into the cell [56, 57]. Zinc is an important component of cell growth, proliferation, division, and apoptosis signaling, and also a structural component of many metalloenzymes and zinc finger transcription factors, and rapidly dividing cells usually require more zinc [58, 59]. The extremely high upregulation of *ZIP9* genes indicated that IAA strongly stimulated zinc absorption in *H. pluvialis* to meet its demand for zinc during rapid cell proliferation and metabolic activities [56].

The direct impact of IAA on cell division and cycle regulation in *H. pluvialis* was reflected at the gene expression level. *CDKB1-1* (*gene_QJQ45_023291*) and *CYCB2-1* (*gene_QJQ45_026341*) were both significantly upregulated, with $\log_2$ FoldChange values of 1.49 and 1.56, respectively (Table 4). CDKB is a plant-specific CDK1 ortholog that binds to cyclins to co-regulate cell division, particularly G2/M phase transition and mitosis [60, 61]. CYCB2-1 binds to CDKB1-1 to form an active complex, and both are important components of cell-cycle regulatory mechanisms [62, 63]. As an auxin, one of IAA's most significant functions is to promote cell division [64]. The significant upregulation of these genes directly provided molecular evidence that IAA promoted cell proliferation in *H. pluvialis* by accelerating cell cycle progression, which is the basis for rapid biomass accumulation [65].

4.5 Functional interpretation of MeJA responsive DEGs

Astaxanthin synthesis is a multi-step enzymatic process involving the synergistic action of several key enzymes. The gene *CRTW* (*gene_QJQ45_019472*) encoding β -carotene ketolase catalyzes a key step in the astaxanthin biosynthesis pathway (Table 5), responsible for converting β -carotene into canthaxanthin, a direct precursor to astaxanthin [66, 67]. *CRTW* transcripts were significantly upregulated. *BKT3* (*gene_QJQ45_024144*) encoding β -carotene 4-ketolase 3, was also significantly upregulated (Table 5). This is an enzyme from the *BKT* family which activates the core step of β -carotene conversion to astaxanthin, and its upregulation further confirms that MeJA treatment stimulates the synthesis of astaxanthin [68]. Genes annotated as *CRTZ* (*gene_QJQ45_001374* and *gene_QJQ45_030451*, encoding β -carotene 3-hydroxylase) were significantly downregulated (Table 5). These genes encode β -carotene hydroxylase (CHYB), a key enzyme in the astaxanthin biosynthetic pathway. CHYB hydroxylates β -carotene to β -cryptoxanthin and then to zeaxanthin, which is subsequently ketolated by β -carotene ketolase (BKT) to form adonixanthin and ultimately astaxanthin [69, 70]. *LCYE* (*gene_QJQ45_015099* and *gene_QJQ45_027358*) were significantly downregulated

(Table 5). The LCYE protein (lycopene ε -cyclase) cyclizes lycopene to delta-carotene, an enzyme in the alpha-carotene branch that competes for substrates with the β -carotene branch [71].

The expression patterns of these core synthesis genes reveal the precise regulation of the astaxanthin synthesis pathway by MeJA. The significant upregulation of the genes *CRTW* and *BKT3* promotes the conversion of β -carotene towards astaxanthin [68, 72]. Simultaneously, the downregulation of *CRTZ* and *LCYE* indicates that MeJA treatment inhibits bypass or competitive branches of the astaxanthin synthesis pathway, thereby reducing carbon flow to other carotenoids [27, 69]. This transcriptomic pattern strongly suggested that MeJA induced astaxanthin accumulation in *H. pluvialis* was not merely a general activation of carotenoid synthesis. Instead, it appears to be a fine metabolic regulatory strategy that effectively shifts carbon flow from other carotenoids to astaxanthin, maximizing target product yield by specifically upregulating key genes in the astaxanthin branch while simultaneously inhibiting competitive branches [26, 72, 73].

4.6 Interpreting enrichment results and integration

Combining the KEGG and GO enrichment results, the effect of IAA on *H. pluvialis* in the green phase primarily focuses on the remodeling of carbohydrate and energy metabolism [74]. The significant enrichment of pyruvate, carbon metabolism, and carbon fixation in photosynthetic organism pathways, along with the prominence of carbohydrate metabolic processes in GO, collectively point to IAA's strong regulation of primary metabolism [74, 75]. IAA, as a plant hormone, is known to promote cell growth and division [64]. During the highly photosynthetic green phase, enhanced carbohydrate synthesis and utilization are fundamental to supporting rapid growth [74]. Our observed differential expression of key genes encoding enzymes such as pyruvate kinase and *PPDK* further corroborates this inference [46, 51]. IAA-induced changes in these pathways and genes may optimize the allocation of photosynthetic products or promote the synthesis of specific carbon skeletons to support biomass accumulation [76, 77]. The enrichment of peroxisomes and transporter activities suggests a fine regulation of substance transport and intracellular environment by cells under IAA signaling to adapt to growth demands [78, 79].

MeJA, as a stress phytohormone, induced a transcriptomic response in red phase *H. pluvialis* that was significantly different from IAA. GO and KEGG results consistently indicated that MeJA inhibited photosynthesis and primary carbon metabolism while promoting the biosynthesis of secondary metabolites [80]. As *H. pluvialis* transitions from the green to the red phase, photosynthetic efficiency typically decreases to conserve energy for astaxanthin synthesis [81]. MeJA may act as a signaling molecule in this process, accelerating this metabolic reprogramming [72, 82].

Collectively, these findings were summarized in a mechanistic scheme (Fig. 8), explaining the principles by which IAA promotes biomass growth and MeJA boosts astaxanthin production in *H. pluvialis*.

5. Conclusion

This study systematically investigated the effects of various phytohormones on the growth and physiological parameters of *H. pluvialis* during its green and red stages. Phytohormone application significantly altered both growth and physiological indices, and 0.01 mg L⁻¹ IAA exhibited the greatest growth-promoting effect, whereas 0.1 mg L⁻¹ MeJA yielded the highest astaxanthin accumulation. Further transcriptomic profiling of these two treatments revealed that IAA promoted biomass of green stage by upregulating carbon metabolism and cell cycle genes, while MeJA induced red stage astaxanthin biosynthesis via upregulation of *CRTW* and *BKT3* and suppression of competing genes (e.g., *CRTZ*, *LCYE*). These transcriptomic insights aligned with the observed enhancements in growth and pigment accumulation. These findings provide an important theoretical basis and molecular targets for utilizing hormones to regulate *H. pluvialis* biomass and astaxanthin production.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors' contributions

Yang Sun: Writing-original draft, Investigation, Formal analysis, Data curation; **Yun Li:** Writing – original draft, Investigation, Conceptualization, Methodology. **Jing Zhang:** Investigation, Formal analysis, Data curation; **Rui Zhao:** Formal analysis, Data curation; **Jiaji Zhang:** Conceptualization, Methodology; **Mingxin Guo:** Investigation, Methodology, Data curation; **Wenjie Yan:** Investigation, Writing-review & editing, Funding acquisition; **Xu Gao:** Writing-review & editing, Project administration, Funding acquisition.

Data availability

Data will be made available on request. The transcriptomic data have been deposited in NCBI (accession number: PRJNA1279618).

Competing interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was financially supported by the Taishan Scholar Foundation of Shandong Province (No. tsqn202211067), the Fundamental Research Funds for the Central Universities (No. 202262002), the National Natural Science Foundation of China (42306135, 42176018) and the Open Project Program of

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Figures

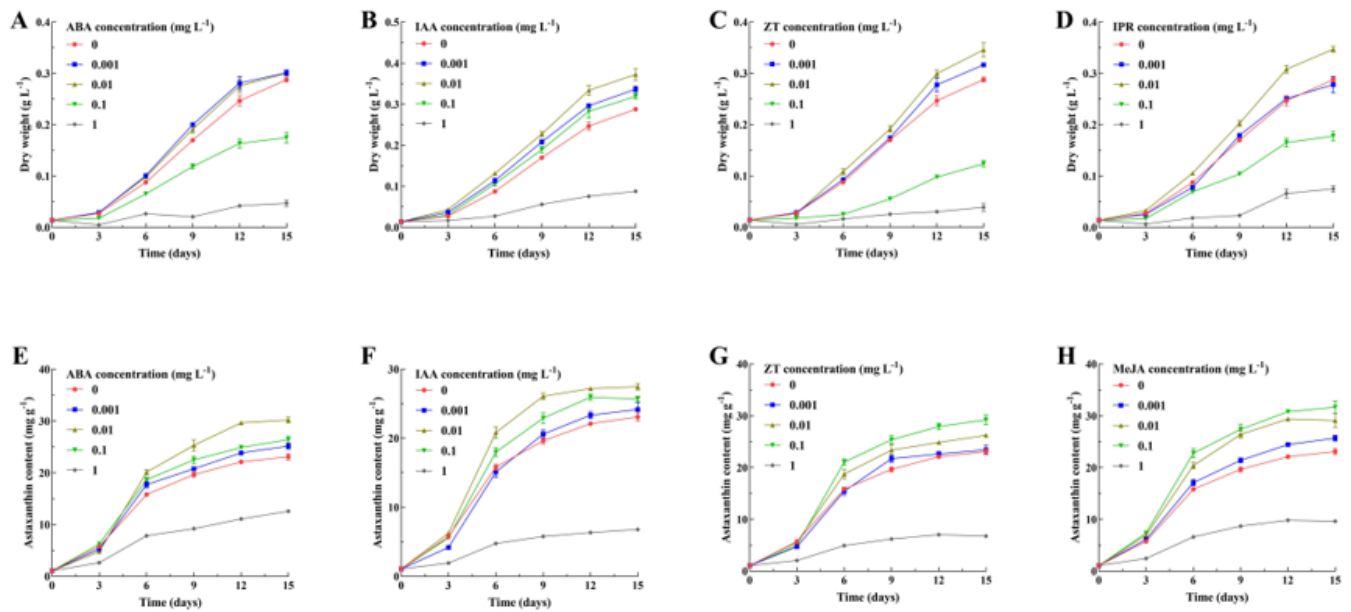


Fig. 1

Figure 1

Changes of dry weight of *H. pluvialis* in the green stage (A-D) and astaxanthin content in the red stage (E-H).

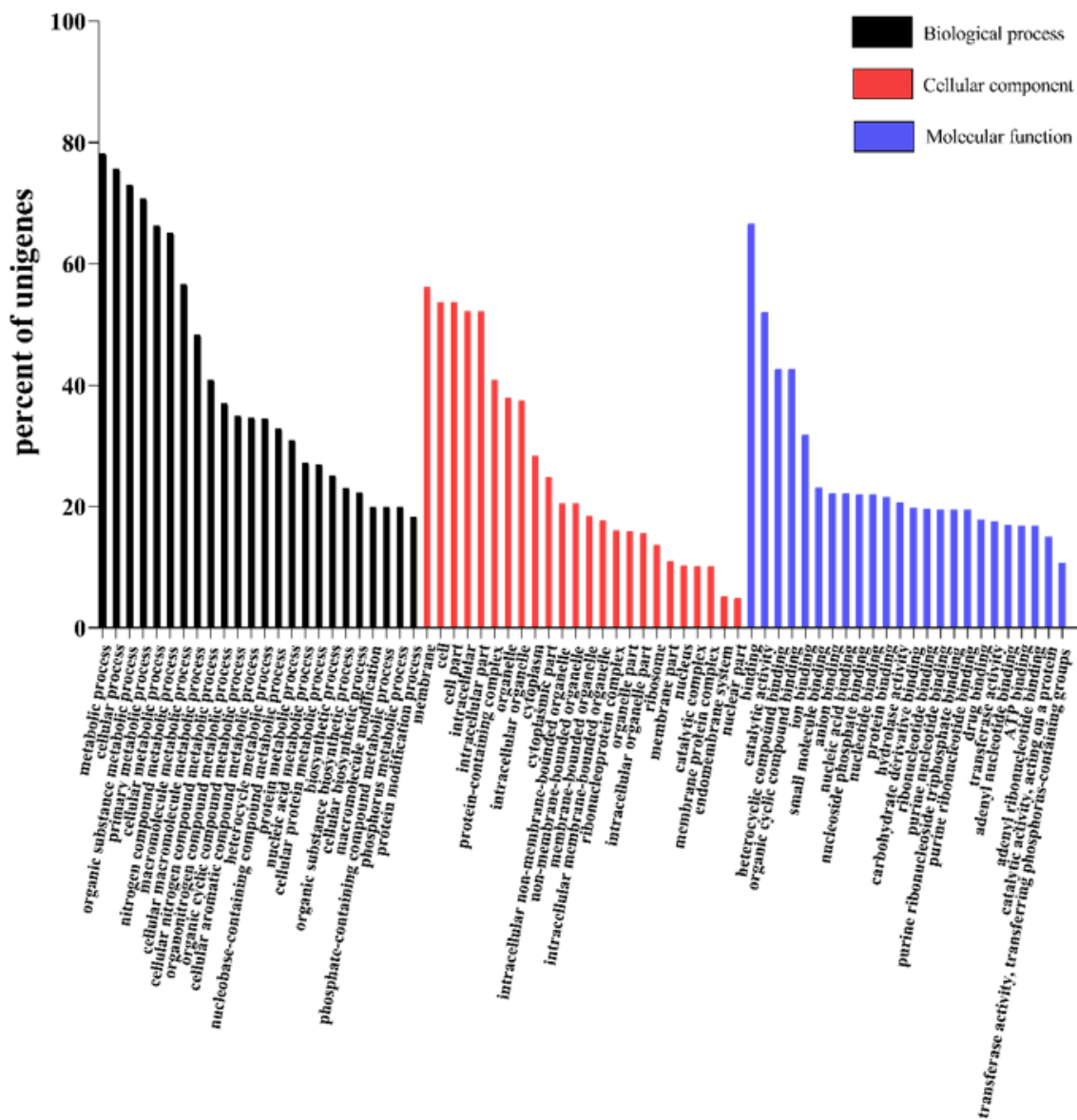


Fig. 2

Figure 2

Gene function classification of all unigenes annotated by GO. Histogram representation of Gene Ontology classification.

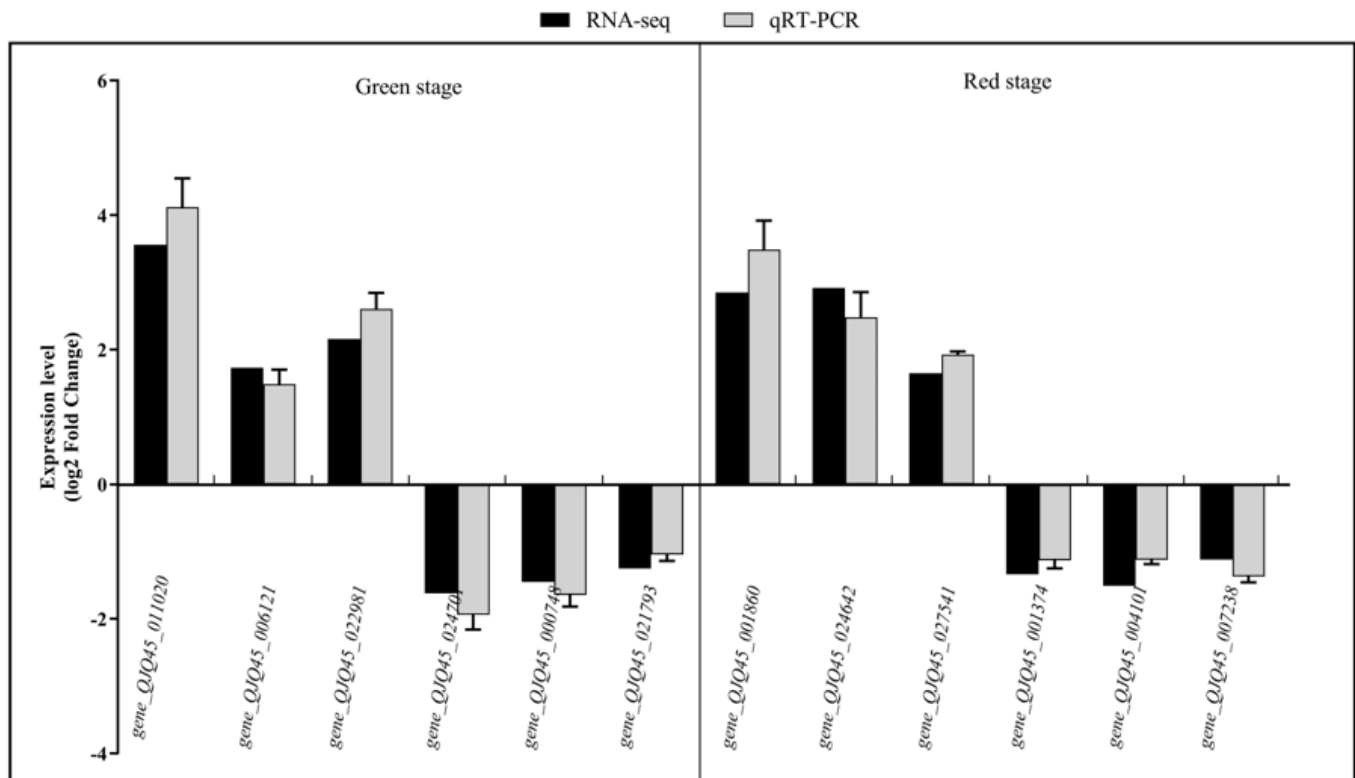


Fig. 3

Figure 3

qRT-PCR analysis of DEGs. The six DEGs on the left correspond to the green-stage validation set (control vs. 0.01 mg L⁻¹ IAA), whereas the six DEGs on the right correspond to the red-stage validation set (control vs. 0.1 mg L⁻¹ MeJA).

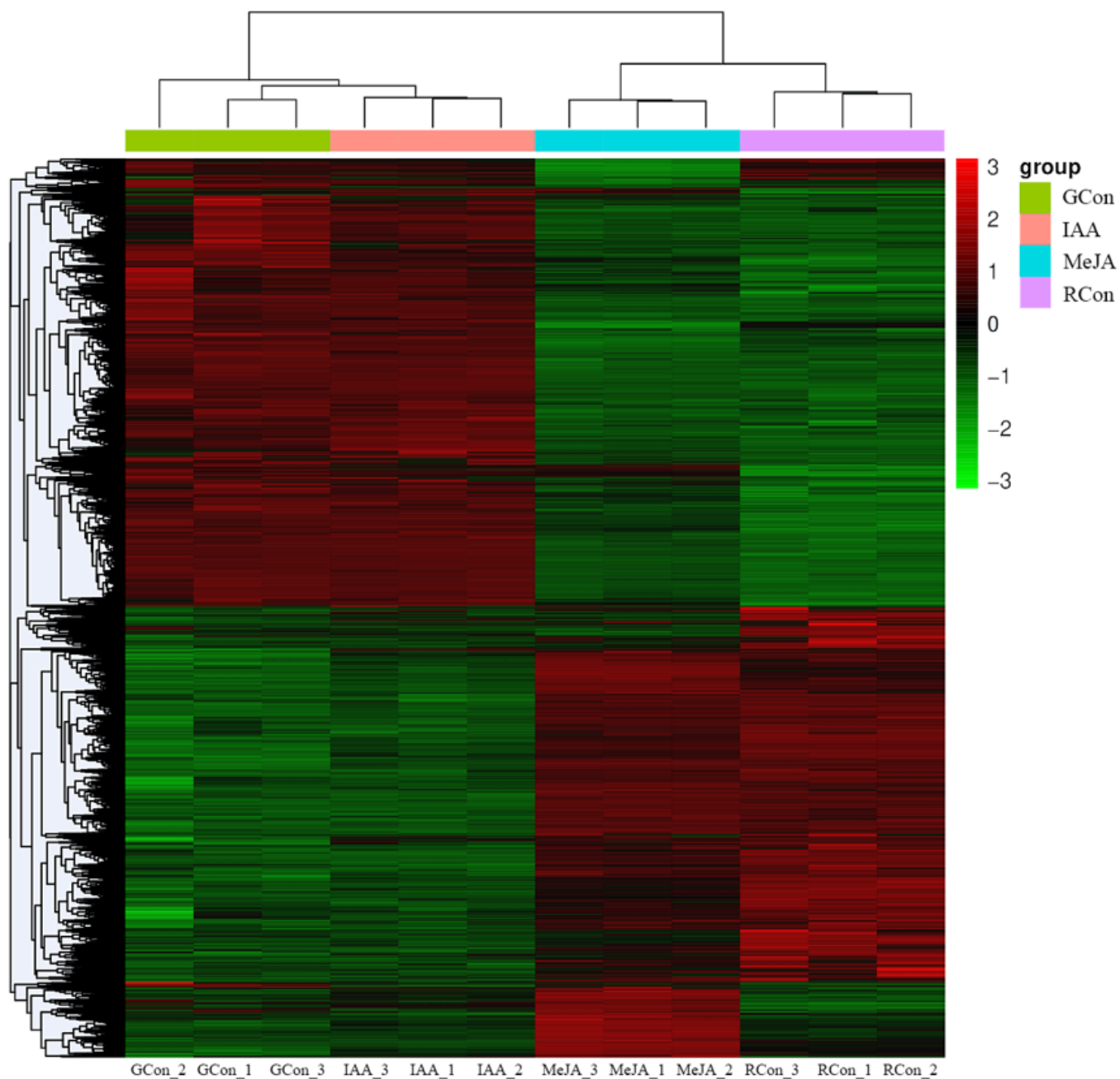


Fig. 4

Figure 4

Cluster analysis and gene expression profiling of DEGs. The X axis presents treatments, while the red and green represent up-regulation and down-regulation

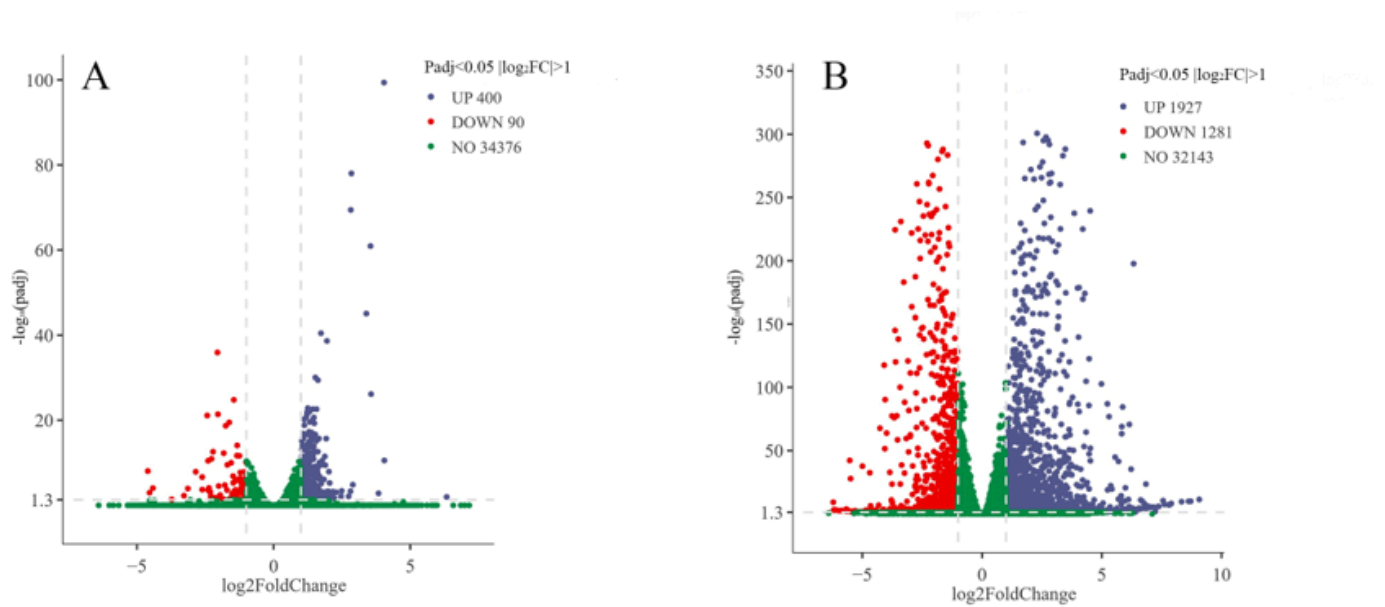


Fig. 5

Figure 5

Volcano plots of differentially expressed DEGs in response to phytohormone treatments. A: DEGs for the IAA vs GCon comparison. B: DEGs for the MeJA vs. RCon comparison. Blue dots represent upregulated genes, and red dots represent downregulated genes. Significantly upregulated genes are marked with blue dots in the figure, downregulated genes with red dots, and non-significantly differentially expressed genes with green dots.

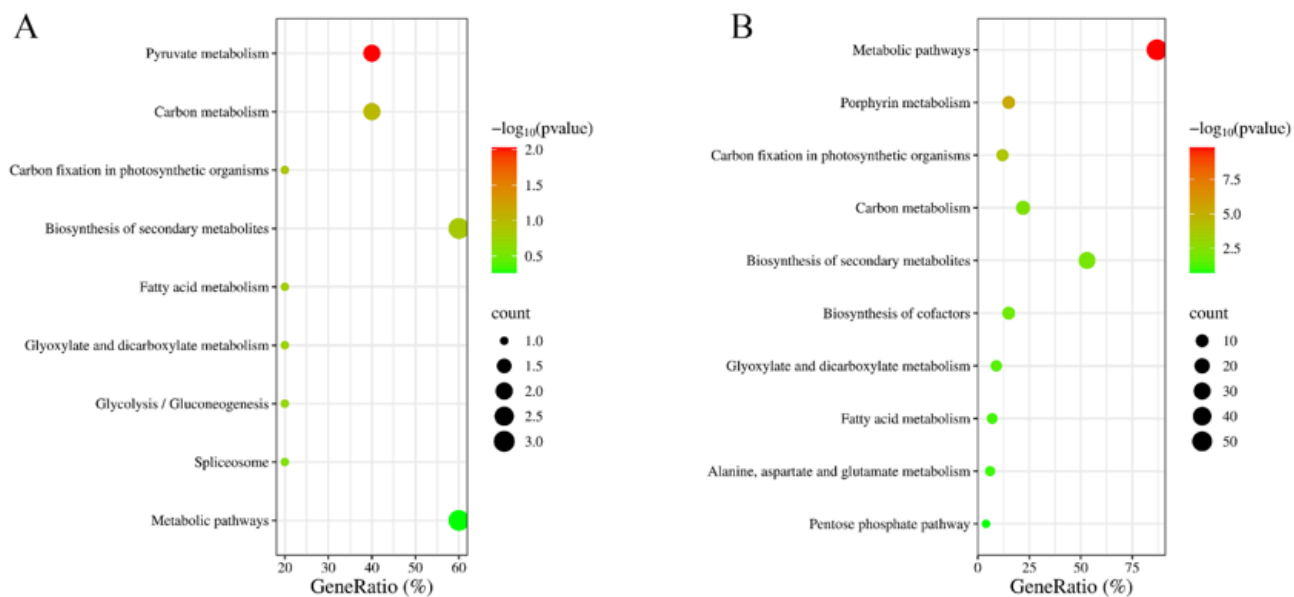


Fig. 6

Figure 6

KEGG pathway enrichment analysis of differentially expressed genes DEGs. A: IAA vs GCon comparison. B: MeJA vs RCon comparison. The size of each dot represents the number of genes enriched in the pathway (count), and the color indicates the significance of the enrichment ($-\log_{10}(\text{pvalue})$). The x-axis shows the GeneRatio, which is the percentage of enriched genes in the total number of genes in the pathway.

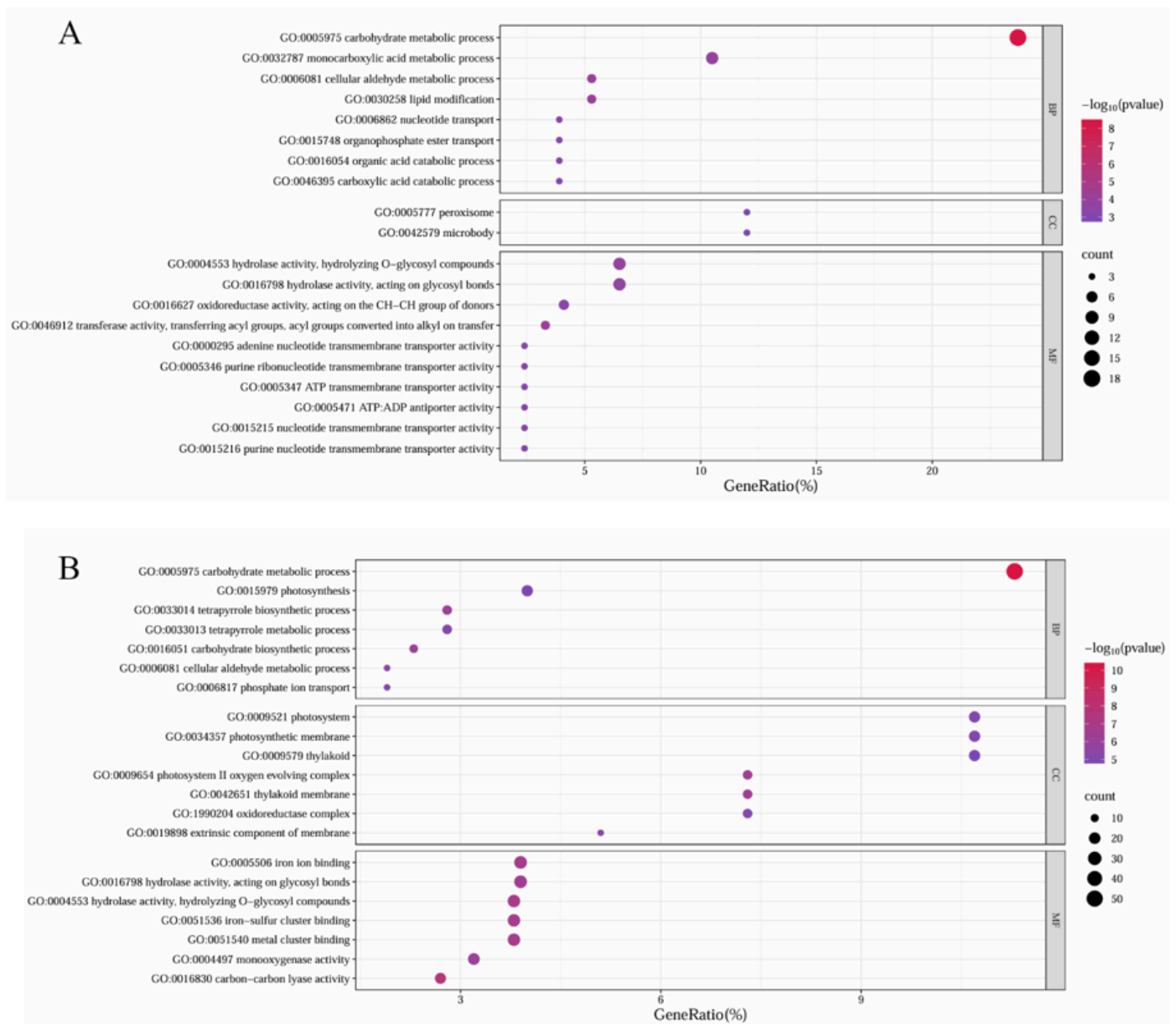


Fig. 7

Figure 7

GO pathway enrichment analysis of differentially expressed genes DEGs. A: IAA vs GCon comparison. B: MeJA vs RCon comparison. The size of each dot represents the number of genes enriched in the pathway (count), and the color indicates the significance of the enrichment ($-\log_{10}(\text{pvalue})$). The x-axis shows the GeneRatio, which is the percentage of enriched genes in the total number of genes in the pathway.

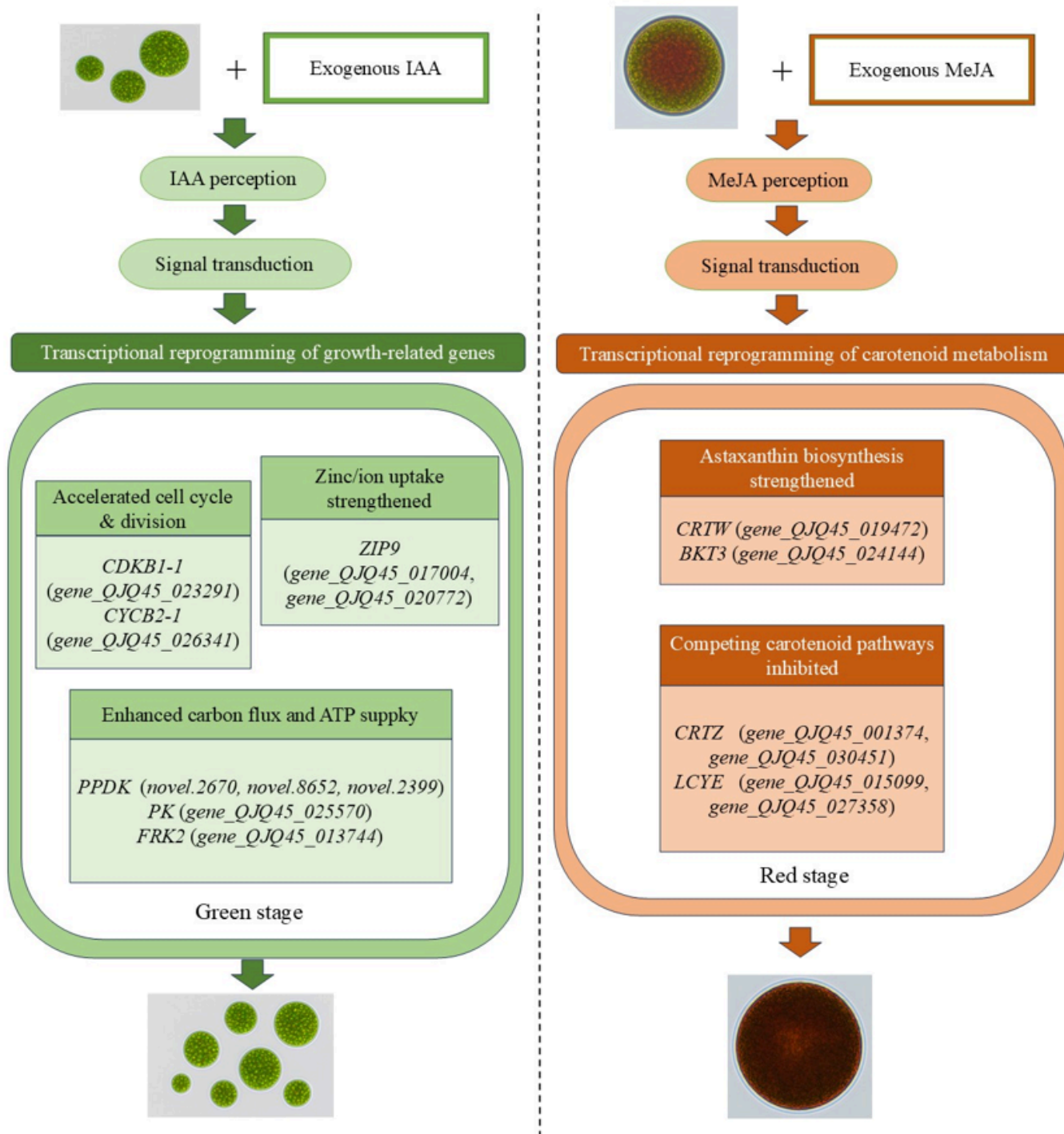


Fig. 8

Figure 8

Hypothetical models of phytohormone-regulated biomass growth and astaxanthin production in *H. pluvialis*.

Supplementary Files

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