Blue light sensor-guided opto-modulation for enhanced production of valuable metabolites in microalgae

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Abstract

Light is an essential key player in biomass and metabolite production in microalgae and the green lineage system. Light-sensing proteins (photoreceptors) can influence photosynthetic fitness by coordinating with the photosynthetic apparatus, thereby regulating biomass and various metabolic activities. However, genetic engineering is mainly employed for enhancing biomolecule production. The mechanism by which the different molecules and photoreceptors influence targeted metabolite accumulation under different light regimes is not clear. In the present study, *in-silico* and systems biology-based analysis, HPLC and label-free comparative quantitative proteomics were employed to investigate the imperative effect of different illumination conditions on metabolite production. The results indicated that blue light substantially enhances the accumulation of astaxanthin and chlorophyll via the blue light-sensor (photoreceptor). Thus, it suggests that fine-tuned illumination conditions influence molecular components in specific metabolite production. Moreover, crosstalk between photoreceptors with the protein family of carotenoid metabolic pathway, cell signalling, and identified biosynthetic gene clusters (BGCs) in green algae opens a new avenue in opto-biotechnology approaches for bioproducts production from other microalgae and the green lineage system.

Keywords: Photoreceptor, astaxanthin, Biosynthetic gene clusters, carotenoid, photosynthesis machinery.

Abbreviations: Chl a : Chlorophyll a, Chl b : Chlorophyll b, TAP : Tris-Acetate-Phosphate, PFD : Photon Flux Density, PBS: Phosphate-Buffered Saline, PFA : Paraformaldehyde. HPLC-MS/MS: High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry, MS: Mass Spectrometry

1. Introduction

Chlamydomonas reinhardtii has undergone a period of rapid development, making it a suitable platform for industrial biotechnology and the manufacturing of various high-value metabolites and bioactive peptides. They are considered a model system for genetic manipulation for overaccumulation of photosynthetic pigments such as β-carotene and lutein (Sproles et al., 2021). It shows antioxidant, antimicrobial and anti-inflammatory activity. Currently, its ability to grow at a large scale in a fermenter has recently been discovered, making it a suitable choice for the commercial production of high-value bioproducts (Masi et al., 2023). However, high biomass production and enhanced metabolite generation remain an unsolved challenge. Utilizing the photosynthetic efficiency of microalgae at its optimum might resolve these issues. Light, the most crucial parameter, affects algal physiology, altering algal biomass production and their biochemical composition (Fernandes et al., 2015; Ghedifa et al., 2021; Sforza et al., 2012; George et al., 2014; Dubey et al., 2023). The coordinated light detection, utilization, and dissipation are vital for microalgal adaptation to environments with varying light intensity (Pertroustos et al., 2016). Light, also, spatiotemporally regulates the fitness of photosynthetic machinery (Pertroustos et al., 2016). Different studies have evidenced activation of downstream components of chlorophyll and carotenoid metabolism controlled by photoreceptor activity (Im et al., 2006; Toledo-Ortiz et al., 2010). Therefore, it shows potential for improved growth and enhanced algal metabolite accumulation. In *C. reinhardtii*, exposure to blue light of low intensity significantly upregulates the genes phytoene desaturase, glutamate-1-semialdehyde aminotransferase, and light-harvesting polypeptides (LHC), which are required for chlorophyll and carotenoid biosynthesis (Im et al., 2006). Moreover, the interactive effect of light and nutrients affects photosynthesis and metabolite production (Mogany et al., 2022). Similarly, the light and temperature synergistic effect on growth kinetics and lipid accumulation was shown in *C. reinhardtii* (Li et al., 2021). Recently, a study provided a detailed understanding of how blue light perception controls starch metabolism in *C. reinhardtii* (Yuan et al., 2025). Enhanced biomass production and biohydrogen accumulation were reported to be influenced by different light quality and quantity (Lafout et al., 2025).

Hence, it is suggested that artificial modulation of algal photoreceptor(s) can alter biomass and change their biochemical makeup for improved manufacturing of valuable bioproducts. In *C. reinhardtii*, phototropin mediates regulation of photosynthesis (Petroutsos et al., 2016), starch metabolism (Yuan et al., 2025), influences expression levels of rhodopsins (Trippens et al., 2012) and can modulate the expression of the genes involved in carotenoid synthesis (Im et al., 2006). Therefore, the photosynthetic (biomass) and metabolic (lutein, β-carotene, Chla, Chlb, TAG, etc.) outputs from algae depend on the operational efficiencies of the photoreceptors involved in the algal photosynthetic and metabolic cycles. The present study examined the role of photoreceptor(s) metabolic regulation. Further, how these molecules crosstalk with each other under different illumination scenarios was assessed. Hence, the present study aims to unravel the molecular mechanism of photoreceptor-controlled algal growth systems under optimum light conditions for enhanced biomass and bioproducts accumulation.

2. Materials and methods

2.1 *In-silico* analysis for curated protein-protein networking between photosynthesis machinery, photoreceptors and different metabolic pathways

The crosstalk between enzymes of membrane lipid biosynthesis, photosynthetic pigments with photosynthesis machinery and photoreceptors in *C. reinhardtii* is shown by protein networking. The curated protein-protein network was predicted using String (version, https://string-db.org/) (Mering et al., 2003). The results were visualised using Cytoscape 3.9.1 with default layout (Shannon et al., 2003). The network analysis was performed using the top 10 betweenness and shortest. Colour range (orange red to yellowish) indicates scores ranked by the betweenness method. Curated PPI networking was created based on literature, co-expression, and proteomics data and systems biology.

2.2 *Chlamydomonas* strain and culture conditions

The wild-type (CC-125) strain of *C. reinhardtii* was procured from the Chlamydomonas Resource Center (University of Minnesota) (https://www.chlamycollection.org/). The strain was maintained on TAP (Tris-Acetate-Phosphate) at 22 ˚C. Cells were initially grown on TAP (Tris-Acetate-Phosphate) agar plates (1.5% w/v). For experiments, cells were cultivated under a 12-hour light/12-hour dark photoperiod at a photon flux density (PFD) of 80 µmol m^{-2} s^{-1} using continuous warm white light (2700 K) in an incubator shaker maintained at 22°C. For liquid culture, a primary inoculum was prepared by resuspending cells in 50 mL of TAP

medium supplemented with ampicillin (500 µg/ml) in a 250 mL Erlenmeyer flask. On the third or fourth day, when cultures reached the exponential growth phase, 1 mL of the primary culture was used to inoculate a 50 mL secondary culture in a 250 mL flask, without antibiotics. After three days, experimental cultures were established under the specified conditions (different light quality) for further analyses.

2.3.1 Growth curve analysis in *C. reinhardtii* under different illumination conditions

Experimental cultures were grown in a custom-designed multi-cultivator system comprising borosilicate glass tubes (120 mL capacity) containing 85 mL of culture medium. Cultures were exposed to monochromatic blue (450 nm), red (660 nm), and white light (2700 K) for a period of 3-4 days. Illumination was provided at a controlled photon flux density (PFD) of 120 µmol m^{-2} s^{-1}. The photon flux density was measured using an Apogee Quantum Sensor (MQ-610). To ensure proper aeration and mixing, humidified 0.2 µm filtered air was bubbled uniformly into each tube. The temperature was maintained at 22 °C using a water bath system. The absorbance was taken at 680 nm and 750 nm every 24 hours using UV/Visible Spectrophotometer (BR Biochem. Life Science Pvt Ltd). Further, a growth curve was plotted as optical density vs. the number of days for each day. Moreover, the samples were pelleted at 4000 rpm for 10 minutes at 4 °C in the mid to late exponential phase for HPLC, qRT-PCR and proteomics analysis.

2.3.2 Cell morphology under different illumination conditions

Chlamydomonas reinhardtii cells (1 mL) were harvested by centrifugation in 1.5 mL microcentrifuge tubes, and the supernatant was discarded. The cell pellet was washed twice with 1X phosphate-buffered saline (PBS). Fixation was performed by resuspending the cells in 100 µL of 2.5% paraformaldehyde (PFA) (Sigma) and incubating them on ice in the dark for 20 minutes. The cells were then transferred to room temperature for 40 minutes while maintaining them in 2.5% PFA. Following fixation, the PFA was removed, and cells were washed thrice with PBS followed by resuspension in a final volume of 50 µL PBS.

For microscopy, 2 µL of the fixed cell suspension was placed on a clean coverslip, and a glass slide was gently inverted onto it, applying slight pressure. The coverslip was sealed using nail polish along the edges. Cells were visualized using a fluorescence microscope (Zeiss Axio Observer 7 with a 63x magnification oil immersion plan- apochromatic lens with NA 1.4) at 63× magnification with oil immersion. Imaging was conducted under the GFP channel with

15% intensity and an exposure time of 40 milliseconds, as well as under bright-field illumination with 8% intensity and a 20-millisecond exposure time.

2.4 Extraction and quantification of chlorophyll and carotenoid pigments in *C. reinhardtii* exposed to different light regimes

Extraction and quantification were performed by using both a UV-Vis spectrophotometer and HPLC analysis. Chlorophyll a, b and carotenoid contents were estimated using the Arnon (1949) method. Briefly, pellets were homogenized in 80 % 1 ml of acetone and centrifuged at 10000 rpm for 10 min. The optical density was recorded at 663 nm, 645 nm and 470 nm against 80 % acetone (blank) using Carry 3500 UV-Vis Compact Peltier, (Agilent; Australia). The concentrations of chlorophyll a, b, total chlorophyll and carotenoid were calculated using the Porro (2002) equation as described below

$$\text{Chlorophyll a (mg/ml)} = 12.25\ (A663.6) - 2.55\ (A646.6)$$

$$\text{Chlorophyll b (mg/ml)} = 20.31\ (A646.6) - 4.91\ (A663.6)$$

$$\text{Total chl (mg/ml)} = 17.76\ (A646.6) + 7.34\ (A663.6)$$

$$\text{Carotenoids (mg/ml)} = (1000A470 - 3.27[\text{chl a}] - 104[\text{chl b}])/227$$

For mg/g, mg/mL amount is divided by the mass of the cells and multiplied by the total volume of the reaction.

For quantification of carotenoid pigments (lutein, astaxanthin, zeaxanthin and β-carotene), HPLC was performed. Reversed-phase HPLC method was used for the quantification using ACQUITY BEHC 18 column with ACQUITY UPLC system (Waters Corp; USA). The extracts were partitioned into diethyl ether with a flow rate of 1 mL/min, and the absorbance was recorded at 450 nm.

2.5 Comparative quantification of different photoreceptor genes in *C. reinhardtii* under different light conditions using real-time quantitative PCR

For quantification of different photoreceptor gene expression, total RNA was extracted from algal culture grown till exponential phase (O.D. 0.5-0.7 at 750 nm) using the TRIzol method (Rio et al., 2010). It is followed by cDNA synthesis using AffinityScript cDNA synthesis kit (Agilent, Santa Clara, USA) as per the manufacturer's instructions. Independent qPCR reaction

was carried out using Kappa^{TM} SYBR^{R} Fast mix as per the manufacturer's protocol with Stratagene Mx3000P machine (Stratagene, La Jolla, CA, USA). The thermal profile employed was: 10 minutes at 95 °C, 40 cycles of denaturation for 10 seconds at 95 °C, annealing for 1 minute at 60 °C and extension for 1 minute at 72 °C. The CBLP housekeeping gene was used as the internal reference for normalization of DNA quality and quantity. The relative expression of photoreceptor(s) gene was determined by the ΔΔCt approach using the threshold value (Ct). At the end of the cycles, melt curve analysis was performed, ensuring a single specific product for each gene. The qPCR experiment was conducted using two biological and two technical replicates for each sample. The primers adapted from Causo et al., (2012) are given in Table S1.

2.6 Label-free quantitative proteomics under red and blue light in the whole cell proteome of *C. reinhardtii*.

2.6.1 Sample preparation for proteomics

The sample preparation and mass spectrometric analysis were performed by Vproteomics. At first, the protein sample (25 µg) was reduced by 5 mM TCEP, followed by alkylation using 50 mM iodoacetamide. Subsequently, the protein was digested with trypsin (trypsin-to-lysate ratio 1:50) at 37 °C for 16 hours. This is followed by purification using a C18 silica cartridge. The obtained mixture was dried in a speed vacuum to concentrate it. Further, resuspension of the dried pellet was done in buffer A containing acetonitrile (2%) and formic acid (0.1%)

2.6.2 Mass spectrometric analysis of peptide mixtures

For mass spectrometric analysis, all the experiments were run on an Easy-nLC-1000 system (Thermo Fisher Scientific) coupled with an Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific). It is equipped with a nano-electrospray ion source. At first, about one µg of phosphopeptides sample was dissolved in buffer A (2% acetonitrile and 0.1% formic acid). It is then resolved using a Picofrit column (1.8-micron resin, 15 cm length). This was followed by gradient elution using a 0–38% gradient buffer B containing formic acid (0.1%) and acetonitrile (80%) at 500 nL/minute flow rate for 96 minutes, followed by 90% of buffer B for 11 minutes. At last, column equilibration was performed for 3 minutes. MS data acquisition was performed using Orbitrap Exploris 240. The conditions used were: Max IT = 60ms; AGC target = 300%; RF Lens = 70%; R = 60K, mass range = 375−1500. MS2 data was collected using the following conditions: Max IT= 60ms, R= 15K, AGC target 100%. MS/MS data

acquisition was done using a dynamic data-dependent top20 method, which chooses the most abundant precursor ions from the survey scan, employing dynamic exclusion for 30 seconds.

2.6.3 Data processing and Gene Ontology (GO) enrichment analysis

Vproteomics (New Delhi, India) performed the differential expressed protein identification. For the sample processing and RAW file generation, Proteome Discoverer (v2.5) against the Uniprot reference database was used. For dual Sequest and Amanda search, the precursor was set at 10 ppm, whereas fragment mass tolerances were set to 0.02 Da. Further, the protease was used to generate peptides. The carbamidomethyl on cysteine is considered a fixed modification, and oxidation of methionine and N-terminal acetylation are considered variable modifications for database search. The protein false discovery rate and peptide spectrum match were set to 0.01 FDR. The GO enrichment pathway analysis was done using Omicsbox tool (BioBam bioinformatics, S.L.) (Conesa et al., 2005) and g:profiler tool (Reimand et al., 2007).

2.7 Statistical analysis for data validation

Three independent biological replicates were used for each experiment. One-way ANOVA with Tukey's test, was applied for statistical analysis of data using GraphPad Prism (version 5) software. The mean values significance level at $P < 0.05$, $P < 0.01$ and $P < 0.001$ were denoted by *, ** and *** respectively.

3. Results

3.1 Curated protein-protein networking predicted a direct link between the carotenoid metabolite pathway with photoreceptors and photosynthesis machinery in *C. reinhardtii*

Photosynthesis, an important algal physiology, depends on several light-dependent components which operate effectively. It involves photosynthetic reaction centre proteins (direct involvement in photosynthesis) and photoreceptors (the light-sensing proteins). Also, the two or more photoreceptors act in a coordinated manner and show a coalescence impact on the metabolic pathways and photosynthesis efficiencies (Petroutsos et al., 2016). Therefore, the crosstalk between enzymes of membrane lipid biosynthesis, photosynthetic pigment with photosynthesis machinery and photoreceptors in *C. reinhardtii* is shown by curated protein-protein interaction using String. Protein networking showed direct regulation of pigments (Chloroplast phytoene desaturase). Besides, direct regulation of pathway intermediates of

fibrillin, plastid lipid-associated protein (PLAP) and lipid metabolism (diacylglycerol acyltransferase) by light via photoreceptor (phototropin, rhodopsin, and cryptochrome) protein was also depicted from the PPI. (Fig.1). The interactions were predicted based on known interactions, predicted interactions, text mining, proteomics, and co-expression.

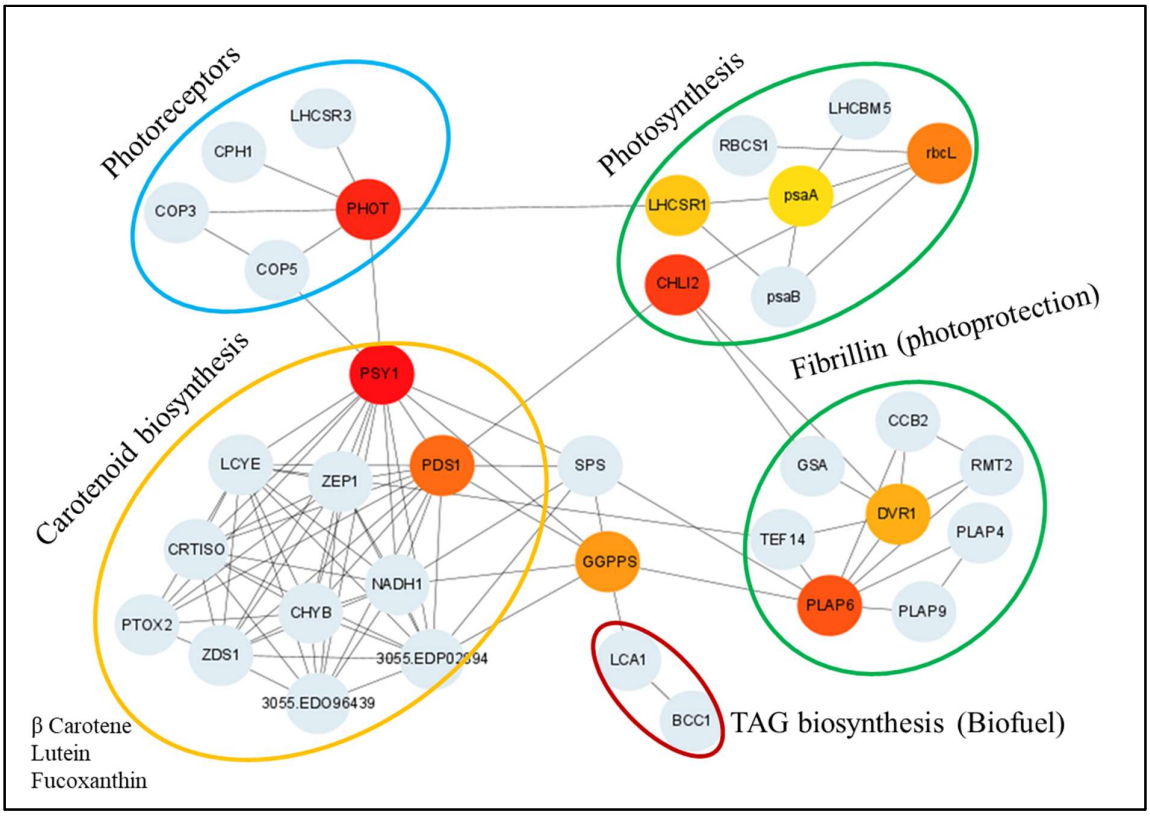


Fig. 1: The crosstalk of photoreceptors with enzymes of membrane lipid metabolism, photoprotection, and pigment biosynthesis in *Chlamydomonas reinhardtii.* String (version 11 https://string -db.org/) was used for the curated protein-protein interaction network prediction. The network was visualization and analysis was performed using Cytoscape 3.9.1 with default layout. The top 10 betweenness methods and shortest path methods were adapted for the analysis. Colour range (orange red to yellowish) indicates scores ranked by the betweenness method with their interacting partners.

3.2 Lights of different wavelengths show variation on phototropin expression and photosynthetic pigments in *C. reinhardtii*

Light imparts a significant effect on algal physiology, resulting in variation in biomass production and their biochemical composition (Fernandes et al., 2015). Based on the predicted

curated protein networking (Fig. 1), the role of different photoreceptors and their interacting partners in the light-induced production of specific metabolites and photosynthesis machinery was studied. To test the hypothesis, at first, we exposed dark-adapted synchronized cells to different light intensities for 2 hours at approximately 4000 lux. The effect of different light conditions was examined on the basis of chlorophyll amount, carotenoid content and phototropin expression. The lights of different wavelengths (blue, red, green, white, UV and dark) at 4000 lux for 2 hours exposure showed varied effects on phototropin level and photosynthetic pigments in *C. reinhardtii* (Fig. S1). Significant variation in phot expression and photosynthetic pigments was observed under different lights, in particular red and blue, as compared to white, when exposed for 2 hours at the end of the dark phase (Fig. S1A and B).

3.3 Specific growth rate remains the same under the influence of continuous white, blue and red light in *C. reinhardtii.*

Based on the above observation, we further investigated the influence of specific light regimes (red and blue light) on growth, biomass and biochemical compositions. Light quality significantly influences the growth and morphology of *Chlamydomonas reinhardtii.* Earlier studies have demonstrated that blue light delays cell division compared to red light. This might be because cultures exposed to blue light exhibited postponed DNA synthesis and cyclin-dependent kinase (CDK) activity, resulting in later cell division events (Oldenhof et al., 2004). Additionally, blue light exposure has been associated with increased cell size, suggesting that cells continue to grow before division occurs, which is what we have seen here. On the contrary, red light promotes earlier cell division and may lead to a small but large number of cells. While the specific growth rates of *Chlamydomonas reinhardtii* cultures may appear similar under red, white, and blue light conditions, notable differences emerge during the stationary phase (Fig. 2). Cultures exposed to blue light often achieve a lower optical density (OD) and exhibit a more pronounced decline compared to those grown under red light. This phenomenon could be attributed to the stress induced by high-energy blue light photons.

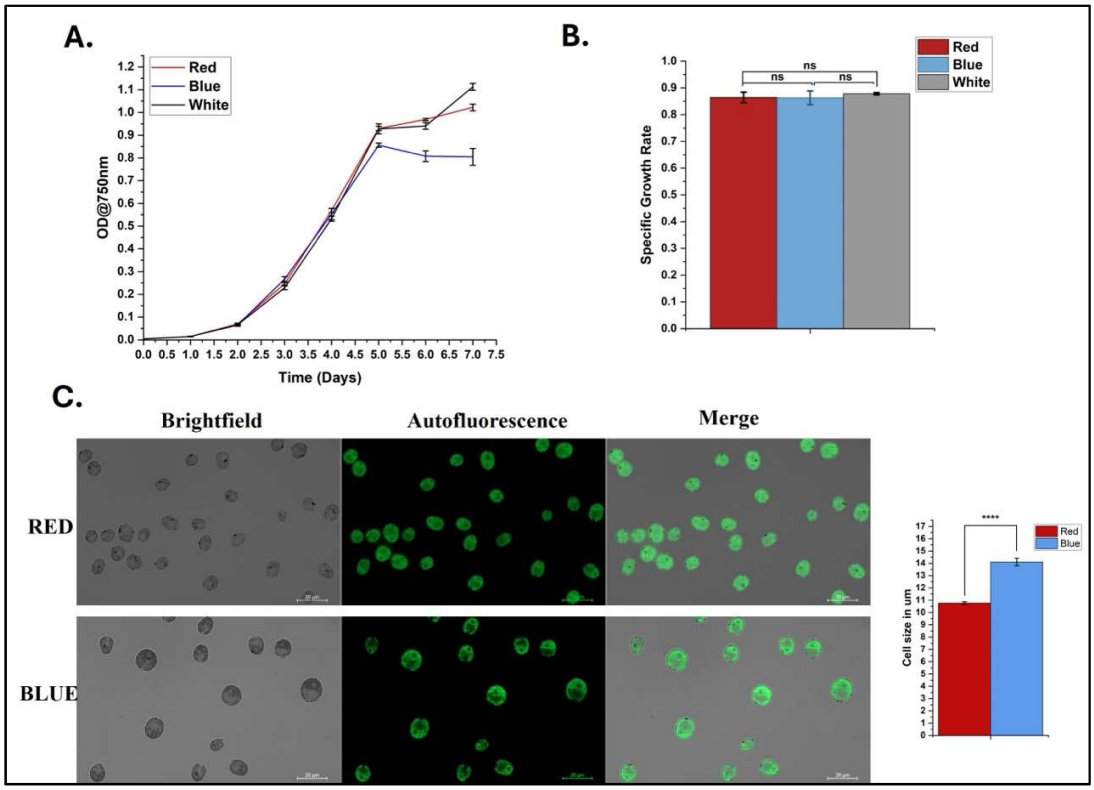


Fig 2: (A) Growth curve analysis of *C. reinhardtii* under red, white, and blue light at 120 µE. (B) Specific growth rate under the three light conditions. Data represent the mean ± SE of three biological replicates. (C) Fluorescence microscopy visualization of *C. reinhardtii* cells acclimated under red (660 nm) and blue (450 nm) light. The accompanying bar graph quantifies cell size (mean ± SE) from three independent experiments, with measurements from n = 202 cells for red light (10.762 ± 0.105 µm) and n = 84 cells for blue light (14.108 ± 0.302 µm). A t-test was performed to determine the significance.

3.4 Upregulation of chlorophyll and carotenoid content under the influence of continuous blue light in *C. reinhardtii.*

Chlorophyll and carotenoid content were assessed under different light regimes. A significant increase in chlorophyll and carotenoid content was observed in blue and red light. Carotenoid content was substantially enhanced in the presence of blue light (Fig. 3). A substantial 2-fold increase in Chl content was noted as compared to white and red light. Similarly, exposure to red light resulted in an apparent change (one-fold increase) in carotenoid content w.r.t. to white. Results indicated that different wavelengths (red, blue and white) of light significantly affected

the algal physiology in terms of chlorophyll and carotenoid content. Thus, suggesting light imparts effect on metabolite production (Fernandes et al., 2015).

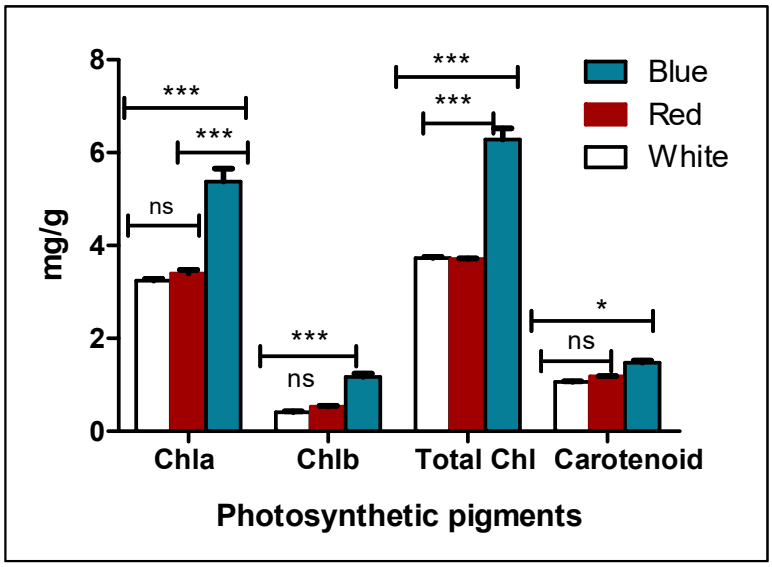


Fig. 3: Effect of different wavelengths (red, blue and white) of light on photosynthetic pigments; chlorophyll a (Chla), chlorophyll b (Chl b), total chlorophyll (total Chl) and carotenoids in *C. reinhardtii*. Values are mean ± SE (n = 4). *, **, and *** represent significant differences at $P < 0.05$, $P < 0.01$and $P < 0.001$, respectively.

Besides this, continuous exposure to blue light leads to enhanced accumulation of astaxanthin with respect to white and red light (Fig. 4). A total of one-fold increase was observed on blur light exposure. No change in other metabolites, such as zeaxanthin and lutein, was reported. Astaxanthin is an important and valuable metabolite. Due to its antioxidant properties, it is widely used as an active ingredient for anti-inflammatory and antioxidant properties in dietary supplements. Its enhanced production is mainly reported under high light stress. *C. reinhardtii* is mainly used as a genetic engineering tool for enhanced production of astaxanthin (Chen et al., 2023). Over accumulation of this is mainly reported under high light (Perozeni et al., 2025). Here, for the first time, we reported that by artificially modulating light conditions, astaxanthin production can be upregulated.

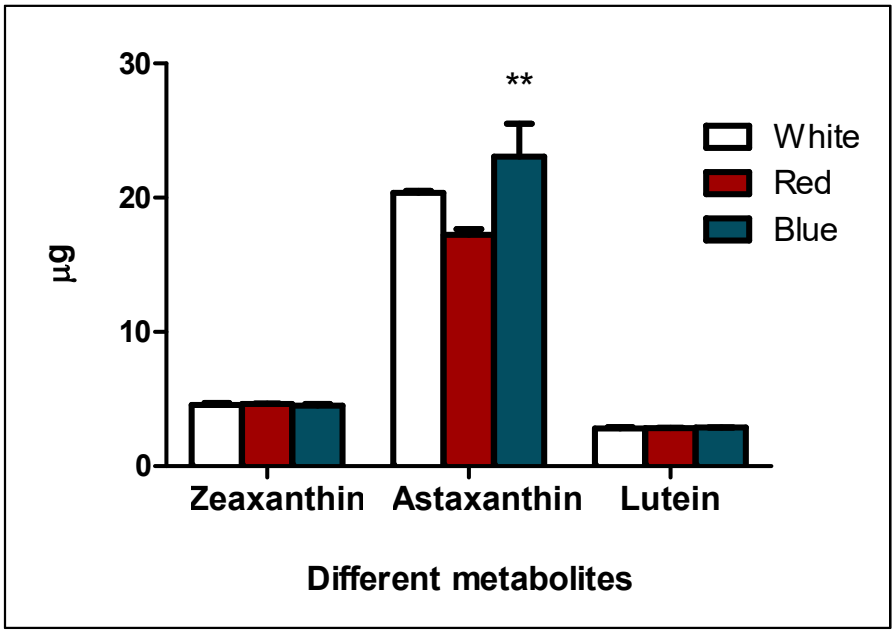
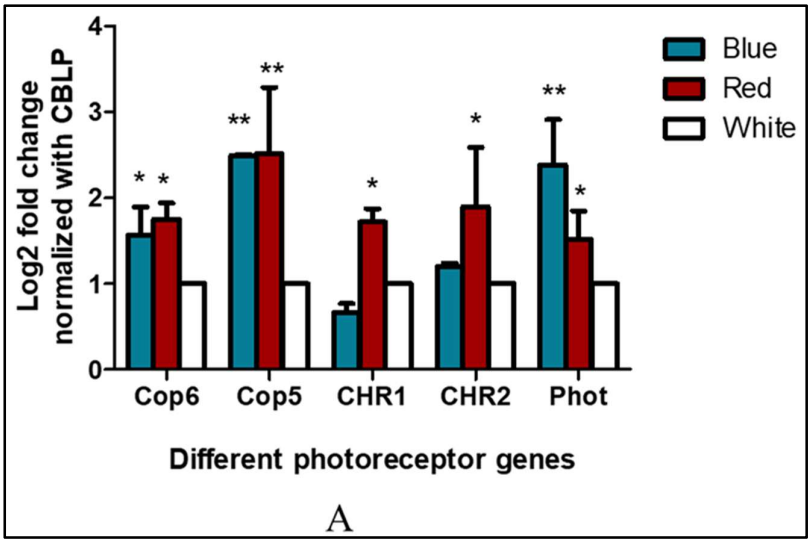


Fig. 4: Effect of different light conditions on targeted metabolite content in *C. reinhardtii*. The zeaxanthin, astaxanthin, and β-carotene were quantified using HPLC at mid-exponential phase. The standard curve for each pigment is shown in Fig S4. Values are mean ± SE (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001

3.5 Lights of blue wavelengths show variation in photoreceptor expression in *C. reinhardtii*

It is known that blue light-sensing photoreceptor phototropin is involved in maintaining the overall fitness of photosynthesis machinery and photo protection (Petroutsos et al., 2016). Blue light upregulates the gene expression of proteins important in photosynthesis, photoprotection, gametogenesis, adhesiveness in *Chlamydomonas* (Petroutsos et al., 2016). Red light is involved in regulation of gene expression of RNA-binding proteins and photosystem synthesis-associated proteins (Alizadeh and Cohen, 2010). Phototropin expression is also influenced by red light (Im et al., 2006). In the present study, phototropin expression was also upregulated under blue and red light when compared to white light (Fig. 5). Red light-sensing protein, phytochrome, is not present in *Chlamydomonas*. Therefore, upregulation of phototropin in red light is due to the involvement of phototropin interaction with some other phytochrome-like proteins, putatively red light-sensitive protein (animal-like cryptochromes, aCRY) (Beel et al., 2012). Recently, our group (Sharma et al., 2024) demonstrated phototropin interaction with

other photoreceptors such as Chlamyopsin 3, Cop3 (ChR1), Chlamyopsin 5 and 6 (Cop5 and Cop6) in C. *reinhardtii*. Here, in the present study, the expression of these photoreceptors is also influenced by different light conditions. A substantial increase in *phot, Cop5 and Cop6* gene expression were observed when exposed to red and blue light with respect to white. *CHR1* and *CHR2* expression were significantly upregulated under red light, whereas no change in their expression was observed under blue light illumination (Fig 5A). Our result suggests a crucial role of illumination conditions on photoreceptor, in turn, photo-physiological responses in algae.



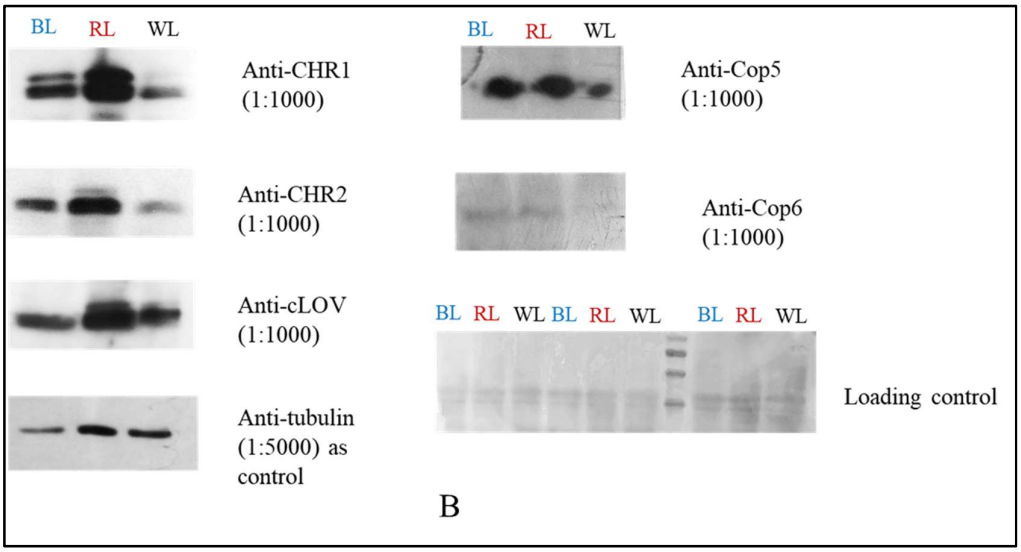


Fig. 5: Effect of different wavelengths of light on phototropin in *C. reinhardtii*. **(A)** qRT-PCR analysis of different genes like *CHR1, CHR2, Phot, Cop5* and *Cop6* in *C. reinhardtii*, after blue light and red light treatment (Control samples -sample exposed to white light). Values are mean

± SE (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001. **(B)** Immunoblot from CrTCL (*C. reinhardtii* total cell lysate) with anti-Phot (cLOV), anti-CHR1, CHR2, Cop5 and Cop6 antibodies with dilution 1:1000. Tubulin was used as control for Phot, CHR1 and CHR2, while ponceau staining was used as control for Cop5 and 6. BL- Blue light, RL- Red light, WL- white light

3.6 Label-free quantitative proteomics revealed the impact of blue light on the upregulation of metabolite content in *C. reinhardtti*.

Further, the comparative whole cell proteomics was performed to unravel the key enzymes and molecules playing a role in the upregulation of specific metabolite production under different light regimes. The data revealed a significant impact of BL and RL on the whole cell proteome of *C. reinhardtti* quantitatively. A total of 4621 proteins were identified, and their expression profiles in blue vs. red light conditions are represented by a heat map (Fig. 6, Table S2). When the BL and RL light conditions was compared, out of the total proteins identified by the label-free proteomics, 265 proteins showed significant changes in their expression (Fig. 6). The principal component analysis and correlation coefficient shows the difference and similarity between the proteome at different light conditions (Fig. S2 and S3). Among them, the key enzymes of the carotenoid metabolic (astaxanthin and zeaxanthin) pathway was found to be significantly upregulated under blue light. Further, the upregulated proteins, both in BL and RL conditions, are shown in the volcano plot.

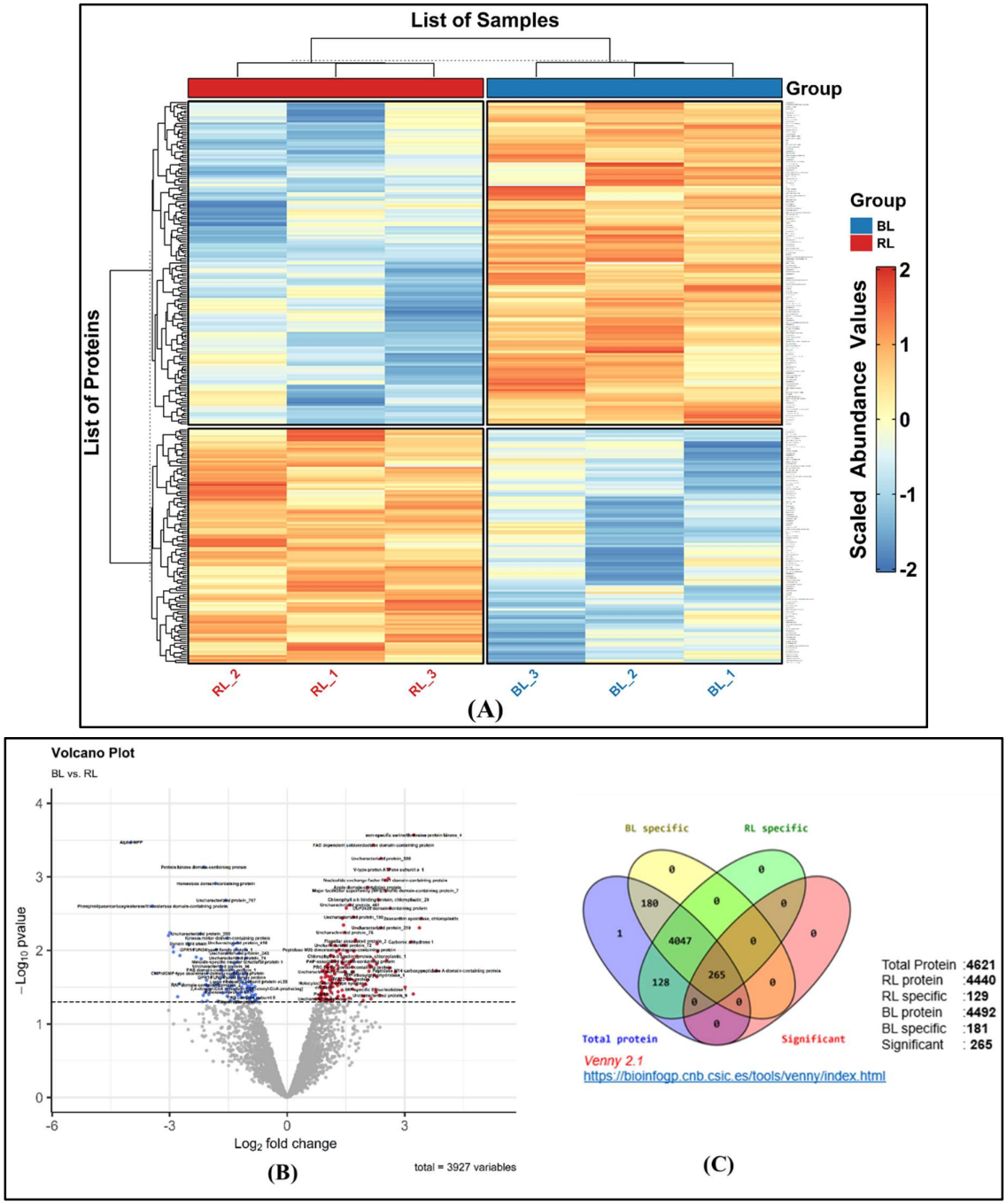
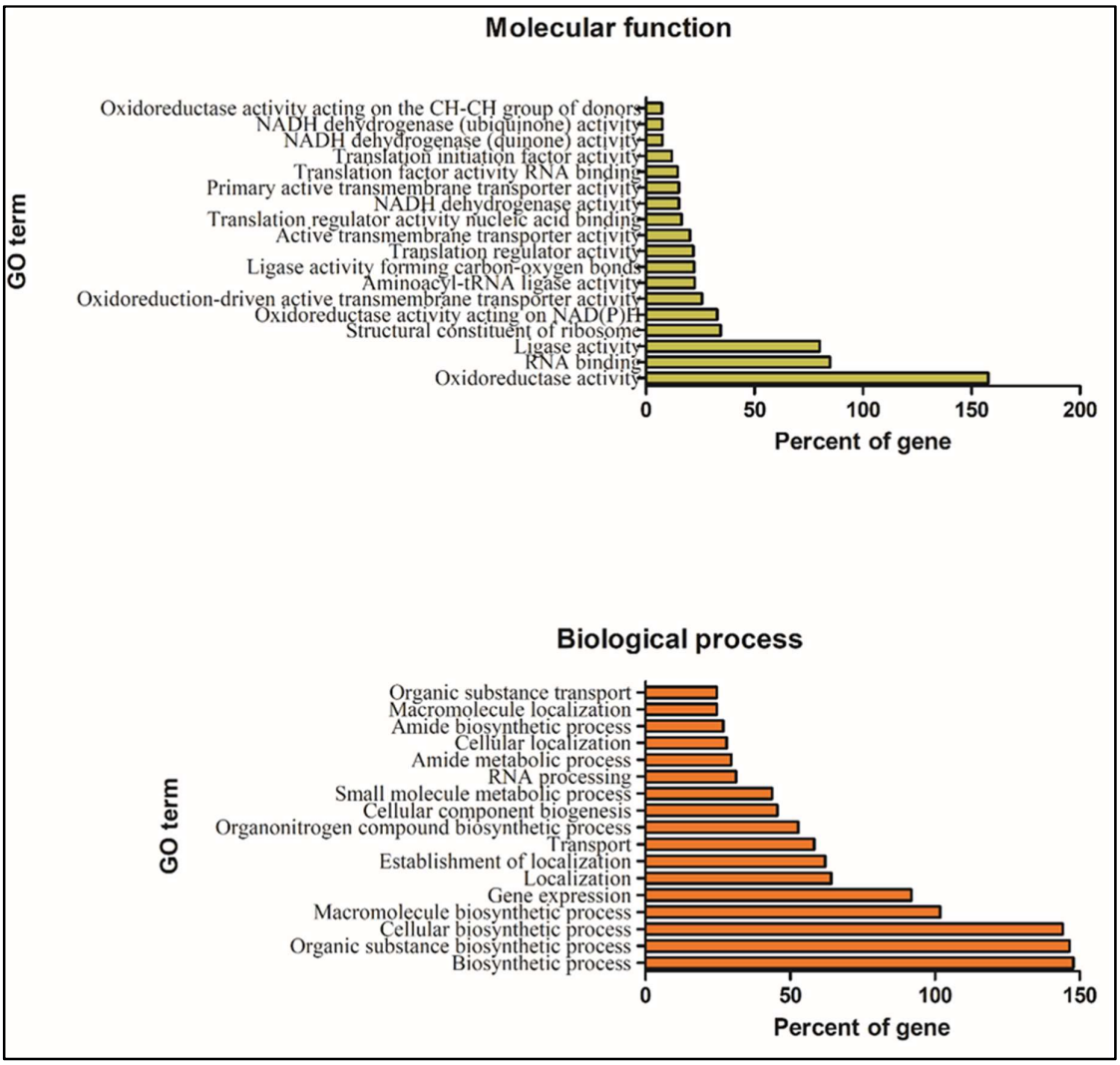


Fig. 6: Comparative proteomics of *C. reinhardtti* illuminated with blue (BL) and red light (RL). (A) The heat map for the DEPs for BL vs. RL. (B)Volcano plot showing proteins significantly downregulated (blue colour) and upregulated (red colour) for DEPs obtained for BL vs. RL. (C) Represents a Venn diagram for the distribution of protein observed under BL and RL.

3.6.1 GO enrichment analysis showed that the carotenoid metabolic pathway is regulated under blue light illumination when compared with red light

The GO enrichment analysis was performed to see the overrepresentation of different pathways. It was found that enzymes and proteins of secondary metabolic processes were greatly affected by different light regimes when compared to the differentially expressed proteins in BL vs RL (Fig. 7, Table S3). The figure 7 represents the top 20 GO terms DEP were compared between BL vs RL. The KEGG pathway analysis was done for the significantly upregulated DEP.



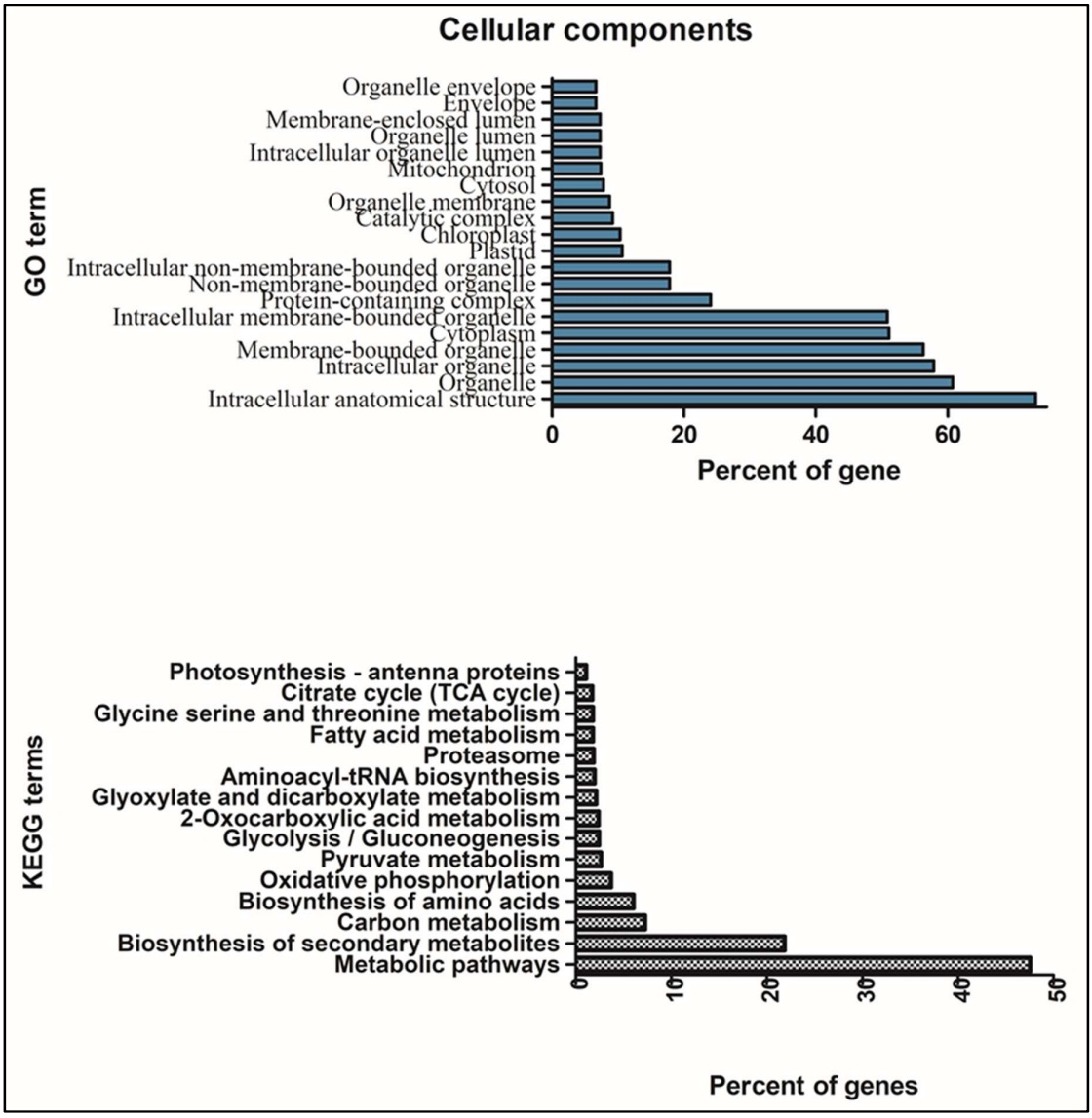


Fig. 7: The figure represents top 20 GO terms based on cellular components, molecular function, biological function and percent of genes linked to each GO term in *C. reinhardtii* in response to blue light.

Further, qRT-PCR also suggested that phytoene synthase, phytoene desaturase and zeaxanthin epoxidase revealed a higher expression only in response to blue light, whereas reduced expression was observed in red light (Fig. 8). This results corroborates with proteomics result.

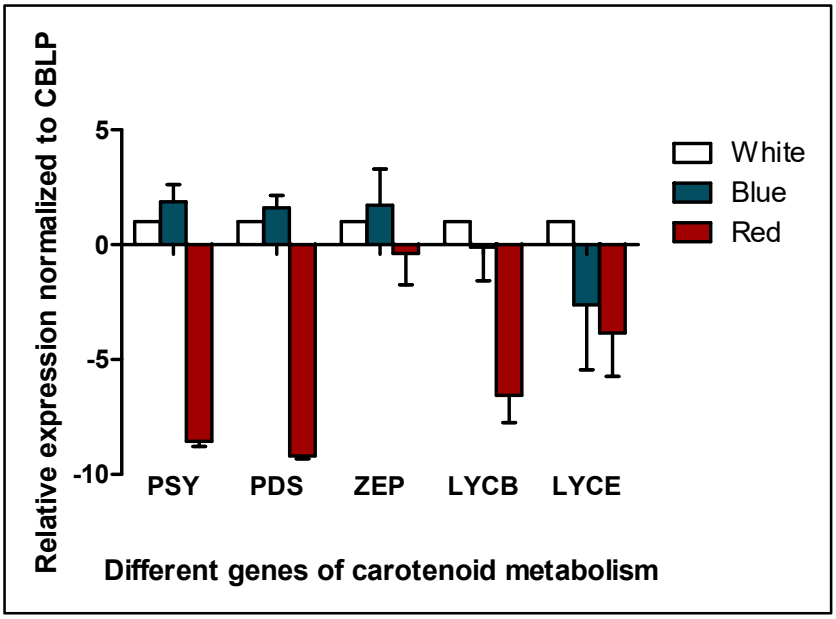


Fig. 8**:** Expression profile of different genes like *ZEP, PSY, PDS, LYCB and LYCE* in *C. reinhardtii*, after blue light and red light treatment (Control samples -sample exposed to blue light). Values are mean ± SE (n = 2, technical replicates and 2 biological replicates). PSY; Phytoene synthase, LYCB; lycopene, ZEP; zeaxanthin epoxidase, PDS; phytoene desaturase, β-cyclase and LYCE; lycopene ε-cyclase.

Further, we have selected a few DEPs identified by the label-free proteomic analysis and used for protein-protein interaction analysis. The interactome revealed that these selected proteins belonged to three different processes, i.e. photosynthesis, photorespiration and carotenoid biosynthesis (Fig. 9). All selected proteins except phototropin (Phot) and light-harvesting complex stress-related (LHCSR1) proteins showed higher expression in blue light when compared to the red-light condition (Fig. 9). This suggested that artificial modulation of algal photoreceptor(s) can regulate biomass and improve their biochemical makeup for biorefinery of high-value bioproducts. Thus, microalgae produce a plethora of valuable bioproducts and bioactive secondary metabolites, having a vital role in different industrial sectors such as medicine, cosmetics, foods, nutraceuticals, etc.

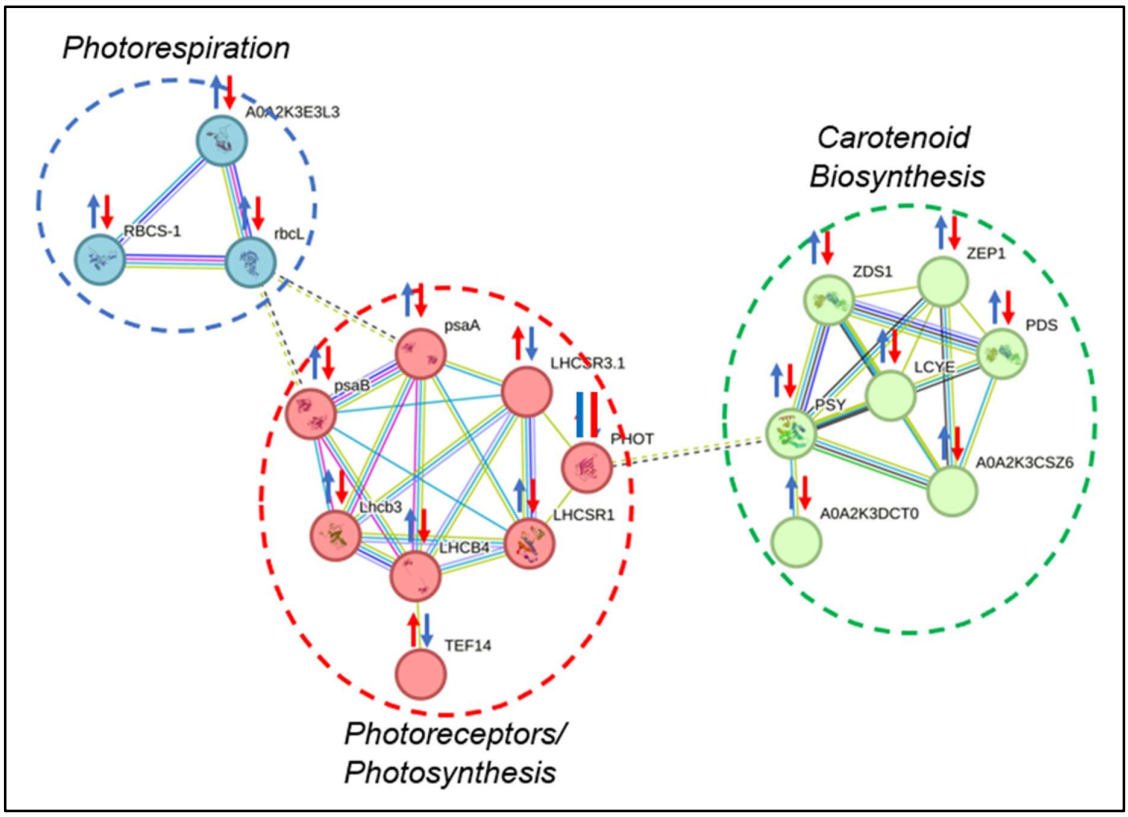


Fig. 9: Interactome of differentially expressed proteins (DEPs). Arrows shows decreased or increased expression of DEPs obtained from the proteomic analysis. Red and blue arrowhead represents expression under blue and red light.

4. Discussion

Light, being an important environmental cue, not only drives the photosynthesis, but it also spatiotemporally regulates photosynthesis machinery (Im et al., 2006; Eberhard S et al., 2006; Toledo-Ortiz et al., 2010; Fernandes et al., 2015; Pertroustos et. al., 2016; Lafout et al., 2025). Consequently, it affects biomass and biochemical constituents of algae and the green lineage system. Earlier results demonstrated that low fluence blue light in coordination with red light affects the carotenoid and chlorophyll biosynthesis (Im et al., 2006). Our result, for the first time, showed insight into the comparative molecular components when algal cells were grown under specific light quality (blue and red light). The comparative quantitative label-free proteomics data showed that enzymes and proteins involved in astaxanthin and zeaxanthin were upregulated in the presence of blue light as compared to red light (Fig. 6 and 7). On the

contrary, proteins of the light-harvesting complex, ribulose bisphosphate carboxylase/oxygenase and starch synthase were upregulated by red light. Similarly, Li et al., (2021) showed 38% increased biomass under red light illumination as compared to blue light. This data also corroborates the growth curve analysis (Fig. 2). Thus, suggested that although red light illumination positively affects the algal biomass, blue light illumination can significantly accumulate more metabolites at low biomass. Additionally, the downstream enzymes and molecules of carotenoid metabolism (phytoene synthase, phytoene desaturase, phytoene dehydrogenase, carotenoid 9,10-dioxygenase, lycopene beta-cyclase, zeta-carotene desaturase and zeaxanthin epoxidase were upregulated under blue light illumination with respect to red light (Table S2). However, most of the enzymes associated with primary metabolism remain unaffected in response to blue and red light illumination. The systems biology-based *in-silico* analysis and experimental data suggest that the biosynthetic pathway is regulated by the coordinated interactions of photoreceptors. Our study revealed interaction of photoreceptor(s) with the protein family of carotenoid and chlorophyll pathway, cell signalling and important metabolic pathways (Fig. 1 and 9).

Numerous biosynthetic pathways have been identified in bacteria, fungi, and plants that produce specialized metabolites. The enzymes and molecules pertaining to these pathways are encoded in biosynthetic gene clusters. The biosynthetic gene clusters (BGCs) are collections of interconnected genes, including operons and independently transcribed genes that collectively carry out the production of certain natural products (Medema et al., 2015). Studies of BGCs in bacteria, plants and fungi suggested that the BGCs might govern specific biomolecule production. BGCs in bacterial and fungal genomes are frequently investigated for a detailed understanding of metabolite accumulation. Numerous BGCs have been identified in plant. (Liu, et al., 2021; Bharadwaj et al., 2021; Cameron et al., 2024). Limited information is available for algal BGCs. The research for algal BGCs is mainly centralised towards the identification of BGCs (O'Neill 2020; Kwon et al., 2024). In our study, for the first time, we provided experimental evidence for BGCs-mediated biomolecule production. Based on our experimental and systems biology-based data, it is postulated that the molecular components of biosynthetic gene clusters accountable for specific metabolite production would also be influenced by different illumination conditions perceived by the native sensory photoreceptor(s). If that's the case, it would enhance the accumulation of a specific metabolite and regulation of relevant signalling pathways under carefully controlled light conditions (quality, quantity, duration, pattern and their synchronization. In the light of this discussion,

we have therefore identified the BGC present in C. reinhardtii using the plantiSMASH tool (Kautsar et al., 2017). A total of 2 BGC clusters were identified (Table S4). Among them, one cluster encodes the genes for saccharide production, and another one is putative. As we are hypothesizing that light might govern the expression of BGCs via photoreceptors, the curated protein-protein networking for each identified cluster with photoreceptor(s) shows crosstalk between proteins from the BGCs cluster and photoreceptor(s). Interestingly, PPI networking implicated that the genes/proteins of two clusters showed indirect interactions with the different photoreceptors as shown in Figure S5. Likewise, the gene cluster also showed interaction with the enzymes mediating lipids, mainly triacylglycerol metabolism, an important component of biofuel production. The important enzyme for lipid metabolism, fatty acid desaturase, was found to be upregulated under blue light illumination in DEP. Similarly, identified BGC clusters comprise genes for important steps in glycosylation (glycosyl-transferase 1). Our proteomics data also reported upregulation of glycosyl transferase family protein (Table S2). Thus, it is suggested that opto-modulation of BGCs holds potential for manufacturing important commercial bioproducts and a new biotechnological avenue for biomamufacturing of valuable products.

Conclusions and future perspective

Research towards carotenoid metabolism and metabolites related to this pathway mainly focused towards its overexpression via genetic engineering (Perozini et al., 2025). Moreover, the metabolite profiling of these valuable products was mainly conducted under high light stress, revealing the key enzymes. The present study showed the imperative effect of light on the biomanufacturing of valuable metabolites in green algae *C. reinhardtii*. Significant variation in chlorophyll and carotenoid accumulation and photoreceptor(s) gene expressions was observed under blue light illumination when compared with red light illumination. Moreover, we have offered a new aspect of opto-modulated regulated biosynthetic gene cluster-mediated metabolite production in microalgae. Additionally, this approach could also be employed for the sustainable biomanufacturing of specific metabolites simply by modulating illumination conditions. However, in-depth research is required in this area. Overall, our result suggests a crucial role of illumination conditions in the biomanufacturing of sustainable and safe metabolite production from microalgae.

Author Contributions:

A.S. and S.K. conceived the project. R.S. designed the experiments in the guidance of S.K. and A.S. and drafted manuscript with the help of A.S. H.A. and S.K. H.A. performed growth and maintenance of algal culture under different conditions, growth curve analysis and cell morphology visualization experiments for the publication. R.S. conducted immunoblotting, qRT-PCR, and HPLC, bioinformatics and data analysis. All authors reviewed and approved the final manuscript.

Author's approval:

All authors have seen and approved the manuscript. This work is original and not under consideration or published anywhere.

Conflict of interest:

The authors declare no competing interests.

Funding sources:

This research receives funding from the project grants BT/PR53866/BSA/33/107/2024 and BT/PR34567/BRB/10/1822/2019.

Acknowledgements:

A.S. and S.K. are highly thankful to the DBT project grants BT/PR53866/BSA/33/107/2024 and BT/PR34567/BRB/10/1822/2019 for providing financial assistance. RS acknowledge DBT-RA program (DBT-RA/2022/July/N/2560). H.A. acknowledge DBT (BT/PR34567/BRB/10/1822/2019) for providing Manpower. We gratefully acknowledge Dr. Shilpa Mohanty, S.K. lab member for her suggestions.

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