Photoreceptor network-modulated opto-biomanufacturing of bioactives from green lineage simply by illumination

Rajani Singh[1#], Haseeb Ul Arfin [2#], Amit Sharma[2*] and Suneel Kateriya[1*]

1. Laboratory of Optobiotechnology, School of Biotechnology, Jawaharlal Nehru University, New Delhi 110067, India
2. Photo-Biology Laboratory, Multidisciplinary Centre for Advanced Research and Studies (MCARS), Jamia Millia Islamia (JMI), Jamia Nagar, New Delhi – 110025.

# The authors contributed equally to this work.

*To whom correspondence should be addressed.

Corresponding authors' e-mail: skateriya@jnu.ac.in, asharma4@jmi.ac.in

†ORCID identifiers: R.S. 0000-0002-1520-2794; H.A. 0009-0005-8663-5502; A.S. 0000-0002-3568-1569; S.K. 0000-0001-5428-4297

Highlights

- *Chlamydomonas reinhardtii* is a green biofactory for valuable metabolite production.
- Opto-biomanufacturing of astaxanthin in *C. reinhardtii* is achieved simply by illumination.
- Biosynthetic gene clusters of metabolite production are also controlled by light.
- Photoreceptor based opto-biomanufacturing valuable bioactive is established.
- Opto-biomanufacturing opens avenues for bioactive production in various organisms.

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 photosynthesis fitness, thereby regulating biomass and various metabolic activities. However, genetic engineering is mainly employed for enhancing astaxanthin (an important therapeutic bioactive metabolite) production in *C. reinhardtii*. In the present study, for the first time, we

report opto-biomaufacturing of astaxanthin under blue light illumination in *C. reinhardtii* without any genetic modification**.** Moreover, we identified light-regulated biosynthetic gene cluster mediated metabolite production in green lineage. We employed data-driven systems biology-based analysis, array of biochemical and molecular techniques to investigate the imperative effect of different illumination conditions on metabolite production. The results indicated that blue light substantially enhances the biomanufacturing of astaxanthin and pigments via the photoreceptor. Thus, it suggests that fine-tuned illumination conditions modulate 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 lineage opens a new avenue in opto-biotechnology approaches for bioproducts generation from the green lineage system and other organisms simply by illumination.

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

Glossary:

Photosynthesis- The physio-chemical process converting light energy to chemical energy utilising carbon dioxide and water and releasing molecular oxygen as a byproduct.

Photoreceptors- Specialized cells or protein molecules that detect light and convert it into a downstream signal allowing organisms to perceive and respond to their light environment.

Label-Free Comparative Quantitative Proteomics - A technique used to determine the relative or absolute amounts of proteins in a biological sample, by comparing protein abundance between different conditions.

Biosynthetic Gene Cluster- 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.

Opto-modulation- The application of light to regulate specific physiological processes in an organism by activating or deactivating relevant cellular processes.

Opto-Biomanufacturing- The use of light or light-based tools to control biological systems for producing the desired product.

Protein-Protein Interactions Network- The computational prediction and visualization of protein interactions, to infer functional associations.

Gene Ontology- A comprehensive, hierarchically structured classification system that describes the functions of genes and proteins across all organisms, categorizing them into three main aspects: molecular function, cellular component, and biological process.

Photon Flux Density- A measure of the number of photons incident on a given surface area per unit of time to quantify the amount of light available for photosynthesis.

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 (Sforza et al. 2012; George et al. 2014; Fernandes et al. 2015; Ghedifa et al. 2021; 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. However, genetic engineering is mainly employed for astaxanthin (valuable therapeutic metabolite) production in *C. reinhardtii* (Chen et al. 2023). In the present study, we examined astaxanthin accumulation under different light regimes. Further, we uncover the underlie potential of light-regulated biosynthetic gene clusters for metabolite production in green lineage system. We provided a proof of concept for light-mediated metabolite production in green lineage without any genetic modification. 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 (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 □ 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, and zeaxanthin), 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/minute, 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).

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, and t-test were applied for statistical analysis of data using GraphPad Prism (version 5) software. The mean values significance level at $P < 0.05$, $P < 0.001$, $P < 0.01$ and $P < 0.05$ were denoted by ***, ** and * respectively.

2.8 Bio-curation of opto-modulation of biosynthetic gene clusters (BGCs) for metabolite production

For bio-curation of opto-modulation of BGCs for metabolite production, BGCs were identified across different green lineages such as *C. reinhardtii* (freshwater algae), *Klebsormidium nitens* (terrestrial algae), *Arabidopsis thaliana* (higher plant, monocot) and *Oryza sativa* (higher plant, dicot). For identification of BGCs in *C. reinhardtii* and *K. nitens*, the genome sequence of each organisms in fasta format and their annotation in GFF format were retrieved from NCBI. Further, this was uploaded in the plantiSMASH database and BGCs were identified using the default parameters and were compared with the already available BGCs in the plantiSMASH database. For BGCs annotation in *A. thaliana* and *O. sativa*, it is directly fetched from the plantiSMASH database (Kautsar et al. 2017). For Bio-curation, curated protein networking showing crosstalk between BGCs, photoreceptor(s) and carotenoid metabolism were created using String database (Mering et al. 2003).

3. Results and discussion

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.

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 bio-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* (see in supplementary files Fig. S1). Significant variation was observed in phot expression (1.5 and 1.2 times) and photosynthetic pigments (2-fold change and 3-fold change) in particular red and blue light respectively, as compared to white when exposed for 2 hours at the end of the dark phase (shown in supplementary files Fig. S1).

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). Like earlier observations (Oldenhof et al. 2006) blue light exposure is 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.

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. 3A). 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).

Besides this, continuous exposure to blue light (12 hours light/12 hours dark) for 3-4 days leads to enhanced accumulation of carotenoid metabolite with respect to red light (Fig. 3B). A total of 1.3 fold increase in lutein and zeaxanthin content was observed on blue light exposure. Several study corroborated with our results. Light quality regulates the lutein content in different algal species such as *Dunaliella salina* (Zhao et al. 2025), *Chlorella vulgaris* (Al-Nassar et al. 2024), *Haematococcus pluvialis*, *Chlamydomonas sp JSC4* (Zhao et al. 2019).

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 in different microalgae. However, studies demonstrated that *C. reinhardtii*, in native form does not make astaxanthin. Efforts have been made for accumulation of astaxanthin *in C. reinhardtii*. *C. reinhardtii* is mainly genetically engineered for enhanced production of astaxanthin (Chen et al. 2023). It is reported that astaxanthin only enhances after genetically incorporating β-carotene ketolase (BKT) and other enzymes in *C. reinhardtii* (Mei et al. 2025; Perozeni et al. 2025; Sharma et al. 2024a). Interestingly, here, for the first time, we report accumulation of astaxanthin in *C. reinhardtii* without any genetic modification simply by modulating light conditions. Continuous blue and red light illumination (12 hour light/12 hour dark photoperiodic at 120 µmol m^{-2} s^{-1}) for 3-4 days resulted in the accumulation of astaxanthin in *C. reinhardtii*. Blue light exposure causes 1.5 times enhanced accumulation when compared to red light. This is due to the fact that astaxanthin is produced at diploid zygospore stage in *C. reinhardtii* (Schwarz et al. 2025) and blue light regulates the life cycle of *C. reinhardtii* (Huang and Back 2003). It was shown that reduced phototropin in *C.*

reinhardtii leads to impairment of life cycle mainly at gametogenesis stage, mating ability and zygotes germination. Thus, it suggested that modulation of light condition results in astaxanthin accumulation in *C. reinhardtii.*

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. 4). 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. 2024b, preprint) 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 was 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 4A). Our result suggests a crucial role of illumination conditions on photoreceptor, in turn, photo-physiological responses in algae.

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 blue and red light 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. 5A). A list of differentially expressed proteins (DEPs) in *C. reinhardtii* strain CC-125 in response to blue vs. red light is given in the supplementary file (Table S1). The upregulated proteins, both in blue and red light conditions, are shown in the volcano plot (Fig. 5B). Further, when both the light conditions were compared, out of the total proteins identified by the label-free proteomics, 265 proteins showed significant changes in their expression (Fig. 5C). Among them, the key enzymes of the carotenoid metabolism such as phytoene synthase, phytoene desaturase, 15-cis-phytoene synthase, carotenoid 9,10-dioxygenase, zeaxanthin epoxidase, zeta-carotene desaturase, lycopene beta-cyclase etc., was found to be significantly upregulated under blue light. 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.5D). DEPs proteins belonging to photosynthesis, photoprotection, and carotenoid metabolisms were upregulated in blue light vs. red light. Among photoreceptors, phototropin (log 2-fold -0.5, p value 0.2) remains significantly unaffected when compared between red and blue light. Cryotochrome was upregulated while archaeal-type opsin was downregulated. The principal component analysis and correlation coefficient shows the difference and similarity between the proteome at different light conditions (see in supplementary file Fig. S2).

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. In comparison to red light, the blue light have higher impact on the DEPs, of secondary metabolic processes (Fig. 6). The list of significantly differentially expressed proteins is shown in the supplementary file (Table S2). Figure 6 is a representation of the top 20 GO terms of DEP in BL vs those in RL. The KEGG pathway analysis was done for the significantly upregulated DEP. Proteins belonging to photosynthesis, carbon metabolism, secondary metabolite production were among top 20 GO terms.

3.7 Blue light assisted opto-manufacturing of valuable metabolites

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 photosynthesis and chlorophyll metabolism were upregulated in the presence of blue light as compared to red light (Fig. 5). On the contrary, 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 (as shown in the supplementary table). However, most of the enzymes associated with primary metabolism remain unaffected in response to blue and red light illumination. Interestingly, we also found light-driven accumulation of astaxanthin. Although, the enzymes specific to astaxanthin were not found through proteomics data. This is because we have done comparative quantitative label-free proteomics between red and blue light. It would be interesting if such studies will be done comparing white light with other light conditions, at different developmental stage (Huang and Beck 2003; Schwarz et al. 2025). The systems biology-based *in-silico* analysis and experimental data in our study 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). 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.

Besides these, we studied biosynthetic gene cluster in light of opto-biomaufacturing, a new avenue in opto-biotechnology. 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 light-regulated, 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* and *Klebsormidium nitens* using the plantiSMASH tool (Kautsar et al. 2017). A total of 2 BGC clusters were identified in *C. reinhardtii*, while 7 clusters were identified in *K. nitens* (see the supplementary table S3). Among them, most of the cluster encodes the genes for saccharide production, and another one is putative or polyketides (in *K. nitens*). As we are hypothesizing that light might govern the expression of BGCs via photoreceptors, the bio-curated protein-protein networking for each identified cluster with photoreceptor(s) shows crosstalk between proteins from the BGCs cluster and photoreceptor(s) (Fig. 7). Further, we have also performed an in-depth analysis for BGCs encoding saccharides. PPI networking implicated that the genes/proteins of two clusters showed indirect interactions with the different photoreceptors in *C. reinhardtii* while direct interaction of photoreceptor in *K. nitens* with BGCs was observed as shown in supplementary figure (Fig. S4). In *C. reinhardtii*, interestingly, BGC core domain fatty acid desaturase and 3-oxoacyl-[acyl-carrier-protein] synthase (shows interaction with phytoene synthase which is

directly regulated by phototropin) were upregulated. Likewise, the gene cluster also showed interaction with the enzymes mediating lipids, mainly triacylglycerol metabolism, an important component of biofuel production. It is to note that, 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 (see supplementary table S1 and 2).

Several studies established illumination impact on flavonoid and terpenoid biosynthetic pathway in rice (Jung et al. 2013; Liu et al. 2023; Park et al. 2013; Yang et al. 2025). BGCs related to terpenoid metabolism are well identified and characterized in rice and *Arabidopsis*. Most of the BGCs in rice (*Oryza sativa* Indica) and *Arabidopsis thaliana* belongs to terpene and saccharides. Similarly, in *K. nitens* (closely related to higher plants) among 7 identified BGCs, 5 are for saccharide, one for polyketide and one for lignin. The core domain of these clusters were similar to rice and *Arabidopsis* (the data for BGCs for rice and *Arabidopsis* is available under precalculated results in plantiSMASH database). Numerous literature suggest light regulated metabolite production in higher plants. However, the mechanistic basis of light regulated biosynthetic pathway is poorly understood. Thus, it is suggested that opto-modulation of BGCs holds potential for manufacturing important commercial bioproducts and a new biotechnological avenue for biomanufacturing of valuable products.

4. Conclusions and future perspective

Research towards carotenoid metabolism and metabolites related to this pathway mainly focused towards its overexpression via genetic engineering and high light stress. 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. Here, astaxanthin biomaufacturing was observed by simply modulating light conditions in terms of quality (red and blue), intensity, and duration (continuous exposure with 12 hour light/12 hour dark phototropism for 3-4 days). Moreover, we have offered a new aspect of opto-modulated regulated biosynthetic gene cluster-mediated metabolite production in green lineage specifically in *K. nitens*, a green terrestrial alga having an evolutionary link between land and aquatic plants, *C. reinhardtii* (aquatic alga), *A. thaliana* and *O. sativa* (higher plants). This approach could also

be employed for the sustainable biomanufacturing of specific metabolites simply by modulating illumination conditions not only in microalgae but also in the green lineage system. 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, data curation and 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.

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References

Al-Nassar, H.M. and Alzurfi, S.K.L., 2024. Effect of temperature and light spectrum on the Lutein and Beta-carotenoids production in *Chlorella vulgaris* algae. International Journal of Aquatic Biology, 12(2), pp.163-170. doi.org/10.1016/j.algal.2024.103422.

Alizadeh, D. and Cohen, A., 2010. Red light and calmodulin regulate the expression of the psbA binding protein genes in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 51(2), pp.312-322. doi: 10.1093/pcp/pcq002

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24(1), p.1. doi.org/10.1104/pp.24.1.1

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A.R. and Kottke, T., 2012. A flavin binding cryptochrome photoreceptor responds to both blue and red light in *Chlamydomonas reinhardtii*. Plant Cell. 24(7), pp.2992-3008. doi: 10.1105/tpc.112.098947

Bharadwaj, R., Kumar, S.R., Sharma, A. and Sathishkumar, R., 2021. Plant metabolic gene clusters: evolution, organization, and their applications in synthetic biology. Front Plant Sci. 12, p.697318. doi.10.3389/fpls.2021.697318

Cameron, E.S., Sanchez, S., Goldman, N. et al. 2024. Diversity and specificity of molecular functions in cyanobacterial symbionts. Sci Rep 14, 18658 https://doi.org/10.1038/s41598-024 69215-8

Chen, Y.; Du, H.; Liang, H.; Hong, T.; Li, T. 2023. Enhanced Carotenoid Production in *Chlamydomonas reinhardtii* by Overexpression of Endogenous and Exogenous Beta-Carotene Ketolase (BKT) Genes. Int. J. Mol. Sci. 24, pp11382. https://doi.org/10.3390/ ijms241411382

Conesa, A., G¨otz, S., García-G´ omez, J.M., Terol, J., Tal´ on, M., Robles, M., 2005. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676. Doi.10.1093/bioinformatics/bti610

Dubey, K.K., Kumar, A., Baldia, A., Rajput, D., Kateriya, S., Singh, R., Tandon, R. and Mishra, Y.K., 2023. Biomanufacturing of glycosylated antibodies: Challenges, solutions, and future prospects. Biotechnology Adv. *69*, p.108267. DOI: 10.1016/j.biotechadv.2023.108267

Fernandes, B. D., Mota, A., Teixeira, J. A., & Vicente, A. A. 2015. Continuous cultivation of photosynthetic microorganisms: approaches, applications and future trends. Biotechnol. Adv. 33(6), 1228-1245. https://doi.org/10.1016/j.biotechadv.2015.03.004

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T. and Mishra, S., 2014. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus*–A potential strain for bio-fuel production. Bioresour Technol. 171, pp.367-374. doi.10.1016/j.biortech.2014.08.086

Ghedifa, A. B., Vega, J., Korbee, N., Mensi, F., Figueroa, F. L., & Sadok, S. 2021. Effects of light quality on the photosynthetic activity and biochemical composition of *Gracilaria gracilis* (Rhodophyta). J. Appl. Phycol. 33, 3413-3425. https://doi.org/10.1007/s10811-021-02496-y

Huang K, Beck CF. 2003. Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. Proc Natl Acad Sci U S A. May 13;100(10):6269-74. doi: 10.1073/pnas.0931459100.

Im C S, Eberhard S, Huang K, Beck C F, Grossman A R. 2006. Phototropin involvement in the expression of genes encoding chlorophyll and carotenoid biosynthesis enzymes and LHC apoproteins in *Chlamydomonas reinhardtii*. Plant J. 48(1):1-16. https://doi.org/10.1111/j.1365-313X.2006.02852.x

Jung, E.S., Lee, S., Lim, S.H., Ha, S.H., Liu, K.H. and Lee, C.H., 2013. Metabolite profiling of the short-term responses of rice leaves (Oryza sativa cv. Ilmi) cultivated under different LED lights and its correlations with antioxidant activities. Plant Sci. 210, pp.61-69. https://doi.org/10.1016/j.plantsci.2013.05.004

Kautsar, S.A., Suarez Duran, H.G., Blin, K., Osbourn, A. and Medema, M.H., 2017. plantiSMASH: automated identification, annotation and expression analysis of plant biosynthetic gene clusters. Nucleic Acids Res. 45(W1), pp.W55-W63. doi: 10.1093/nar/gkx305

Kwon, T., Hovde, B.T. 2024. Global characterization of biosynthetic gene clusters in non-model eukaryotes domain architectures. Sci Rep. 14, pp 1534 . https://doi.org/10.1038/s41598-023-50095-3.

Lafout, A.S., Mahdavi, S., Foroozandehfar, A., Tavakoli, O. and Abedi, S., 2025. Enhancing biohydrogen production and biomass accumulation in *Chlamydomonas reinhardtii* under different light spectra and intensities. International Journal of Hydrogen Energy, 126, pp.284-293. https://doi.org/10.1016/j.ijhydene.2025.03.407

Li, X., Slavens, S., Crunkleton, D.W. and Johannes, T.W., 2021. Interactive effect of light quality and temperature on *Chlamydomonas reinhardtii* growth kinetics and lipid synthesis. Algal Res. 53, p.102127. https://doi.org/10.1016/j.algal.2020.102127

Liu, Y., Singh, S.K., Pattanaik, S. et al. 2023. Light regulation of the biosynthesis of phenolics, terpenoids, and alkaloids in plants. Commun Biol .6, pp.1055. https://doi.org/10.1038/s42003-023-05435-4

Liu, Z., Cheema, J., Vigouroux, M. et al. 2021. Formation and diversification of a paradigm biosynthetic cluster in plants. Nat Commun. 11(1) pp.5354 https://doi.org/10.1038/s41467-020-19153-6

Masi, A., Leonelli, F., Scognamiglio, V., Gasperuzzo, G., Antonacci, A. and Terzidis, M.A., 2023. *Chlamydomonas reinhardtii*: a factory of nutraceutical and food supplements for human health. Molecules. 28(3), p.1185. doi: 10.3390/molecules28031185

Medema MH, Kottmann R, Yilmaz P, Cummings M, Biggins JB et al. 2015. Minimum information about a biosynthetic gene cluster. Nat. Chem. Biol. 11:625–31. doi: 10.1038/nchembio.1890.

Mei, R., Yang, H., Guo, C., Hong, Z., Hu, Z., Wu, Y., Huang, D. and Wang, C., 2025. Effects of high light exposure and heterologous expression of β-carotene ketolase on the metabolism of carotenoids in *Chlamydomonas reinhardtii*. Front Bioeng Biotechnol.13, p.1533661. doi: 10.3389/fbioe.2025.1533661.

Mering, C.V., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P. and Snel, B., 2003. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res. 31(1), pp.258-261. doi: 10.1093/nar/gkg034.

Mogany, T., Bhola, V., Ramanna, L. and Bux, F., 2022. Photosynthesis and pigment production: elucidation of the interactive effects of nutrients and light on *Chlamydomonas reinhardtii*. Bioprocess Biosyst Eng. 45(1),pp.187-201. doi: 10.1007/s00449-021-02651-2.

Oldenhof, H., Bišová, K., van den Ende, H. and Zachleder, V., 2004. Effect of red and blue light on the timing of cyclin-dependent kinase activity and the timing of cell division in *Chlamydomonas reinhardtii*. Plant Physiol Biochem. 42(4), pp.341-8. doi: 10.1016/j.plaphy.2004.02.002.

Oldenhof, H., Zachleder, V., & Van Den Ende, H. 2006. Blue- and red-light regulation of the cell cycle in Chlamydomonas reinhardtii (Chlorophyta). Eur. J. Phycol. 41(3), pp.313–320. https://doi.org/10.1080/09670260600699920),

O'Neill, E., 2020. Mining natural product biosynthesis in eukaryotic algae. Mar Drugs. 18(2), p.90. doi: 10.3390/md18020090.

Park, H.L., Lee, S.W., Jung, K.H., Hahn, T.R. and Cho, M.H., 2013. Transcriptomic analysis of UV-treated rice leaves reveals UV-induced phytoalexin biosynthetic pathways and their regulatory networks in rice. Phytochemistry, 96, pp.57-71. https://doi.org/10.1016/j.phytochem.2013.08.012

Perozeni, F., Margherita A., Matteo B., and Stefano C. 2025. "Engineered *Chlamydomonas reinhardtii* Strains for Enhanced Astaxanthin Production" Life, 15(5), pp.813. https://doi.org/10.3390/life15050813

Petroutsos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kottke, T., Mittag, M., Hegemann, P. and Finazzi, G., 2016. A blue-light photoreceptor mediates the feedback regulation of photosynthesis. Nature, 537(7621), pp.563-566. doi: 10.1038/nature19358.

Porra, R.J., 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth Res. 73, pp.149-156. doi: 10.1023/A:1020470224740.

Reimand, J., Kull, M., Peterson, H., Hansen, J. and Vilo, J., 2007. g: Profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments. Nucleic Acids Res. research, 35(suppl_2), pp.W193-W200. doi: 10.1093/nar/gkm226.

Rio, D.C., Ares, M., Hannon, G.J. and Nilsen, T.W., 2010. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc. 6, pp.pdb-prot5439. doi: 10.1101/pdb.prot5439.

Schwarz S, Bauch M, Schmitt V, Hallmann A, Lohr M. 2025. *Chlamydomonas reinhardtii*, *Volvox carteri* and related green algae accumulate ketocarotenoids not in vegetative cells but in zygospores. Plant J. 121(3):e17261. doi: 10.1111/tpj.17261.

Sforza, E., Simionato, D., Giacometti, G.M., Bertucco, A. and Morosinotto, T., 2012. Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. PloS one, 7(6), p.e38975. doi: 10.1371/journal.pone.0038975.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B. and Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13(11), pp.2498-2504. doi: 10.1101/gr.1239303.

Sharma, A., Nawkarkar, P., Kapase, V.U., Chhabra, M. and Kumar, S., 2024a. Engineering of ketocarotenoid biosynthetic pathway in *Chlamydomonas reinhardtii* through exogenous gene expression. Syst Microbiol and Biomanuf. 4(3), pp.983-995. https://doi.org/10.1007/s43393-024-00240-4

Sharma, S., Sushmita, K., Singh, R., Sanyal, S.K. and Kateriya, S., 2024b. Phototropin localization and interactions regulate photophysiological processes in *Chlamydomonas reinhardtii*. bioRxiv, pp.2024-12. **doi:** https://doi.org/10.1101/2024.12.27.630506

Sproles, A.E., Fields, F.J., Smalley, T.N., Le, C.H., Badary, A. and Mayfield, S.P., 2021. Recent advancements in the genetic engineering of microalgae. Algal Res. 53, p.102158. https://doi.org/10.1016/j.algal.2020.102158

Toledo-Ortiz, G., Huq, E. and Rodríguez-Concepción, M., 2010. Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. Proc Natl Acad Sci U S A. 107(25), pp.11626-11631. doi: 10.1073/pnas.0914428107.

Trippens, J., Greiner, A., Schellwat, J., Neukam, M., Rottmann, T., Lu, Y., Kateriya, S., Hegemann, P. and Kreimer, G., 2012. Phototropin influence on eyespot development and regulation of phototactic behavior in *Chlamydomonas reinhardtii*. Plant Cell, 24(11), pp.4687-4702. doi: 10.1105/tpc.112.103523.

Yang, J.; Zhao, Y.; Chen, Y.; Wang, Y.; Gao, M. 2025. A Comprehensive Analysis of Transcriptomics and Metabolomics Reveals Key Genes Involved in Terpenes Biosynthesis

Pathway of Litsea cubeba Under Light and Darkness Treatments. Int. J. Mol. Sci. 26, pp.2992. https://doi.org/10.3390/ijms26072992

Yuan, Y., Iannetta, A.A., Kim, M., Sadecki, P.W., Arend, M., Tsichla, A., Águila Ruiz-Sola, M., Kepesidis, G., Falconet, D., Thevenon, E. and Tardif, M., 2025. Phototropin connects blue light perception to starch metabolism in green algae. Nat Commun. 16(1), p.2545. doi: 10.1038/s41467-025-57809-3.

Zhao, X., Ma, R., Liu, X. et al. 2019. Strategies related to light quality and temperature to improve lutein production of marine microalga Chlamydomonas sp. Bioprocess Biosyst Eng 42, 435–443. https://doi.org/10.1007/s00449-018-2047-4

Zhao, T., Li, Y., Che, X. et al. 2025. Effects of monochromatic LED light quality on photosynthetic capacity and pigment content in *Dunaliella salina* under low light intensity. J Appl Phycol. https://doi.org/10.1007/s10811-025-03500-5

Figures

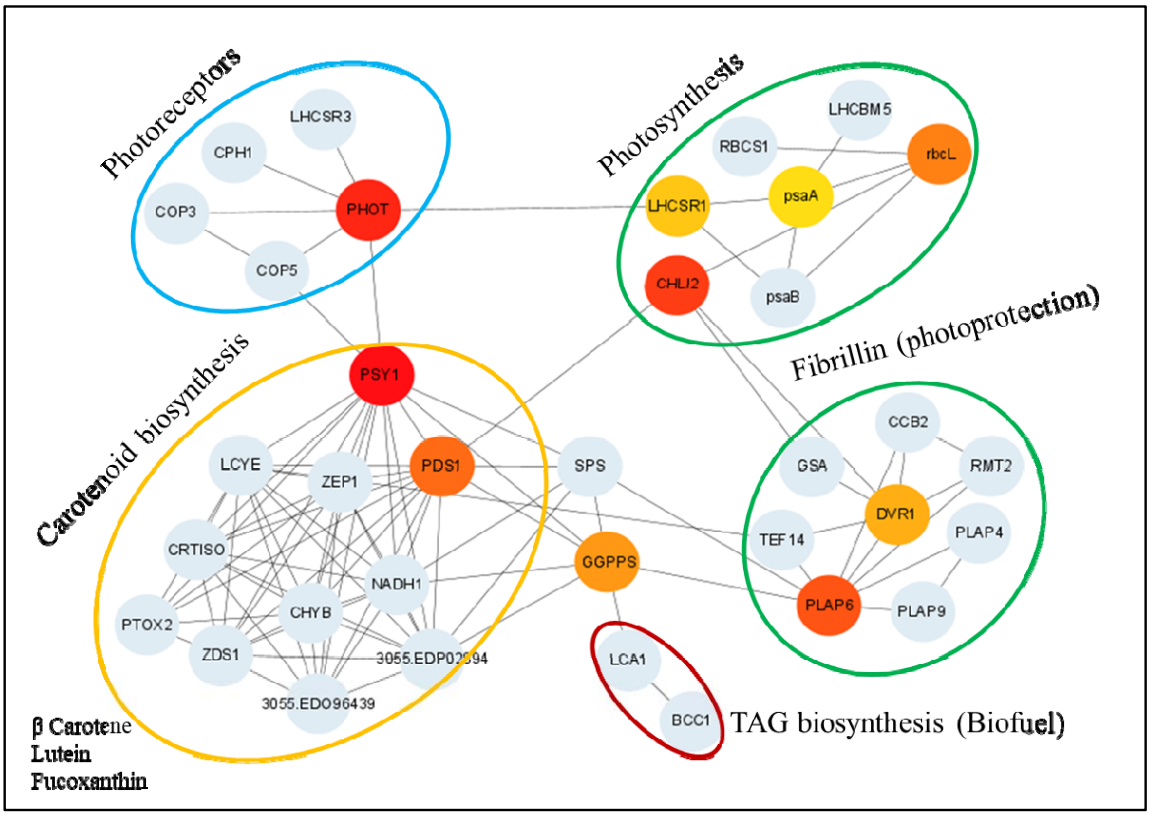


Fig. 1: The crosstalk of photoreceptors with enzymes of membrane lipid metabolism, photoprotection, and pigment biosynthesis in *Chlamydomonas reinhardtii*.

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 (red to yellowish) indicates scores ranked by the betweenness method with their interacting partners.

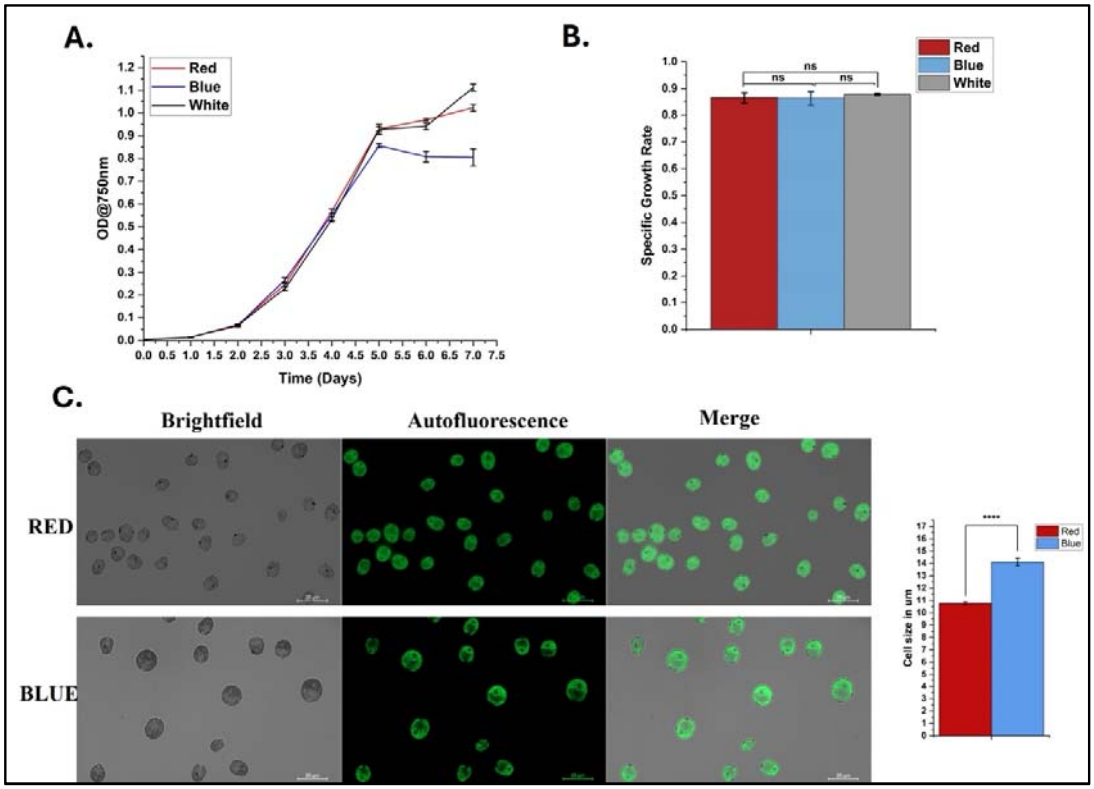


Fig. 2: Opto-modulation effect on growth rate, and morphology on *C. reinhardtii*.

(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.

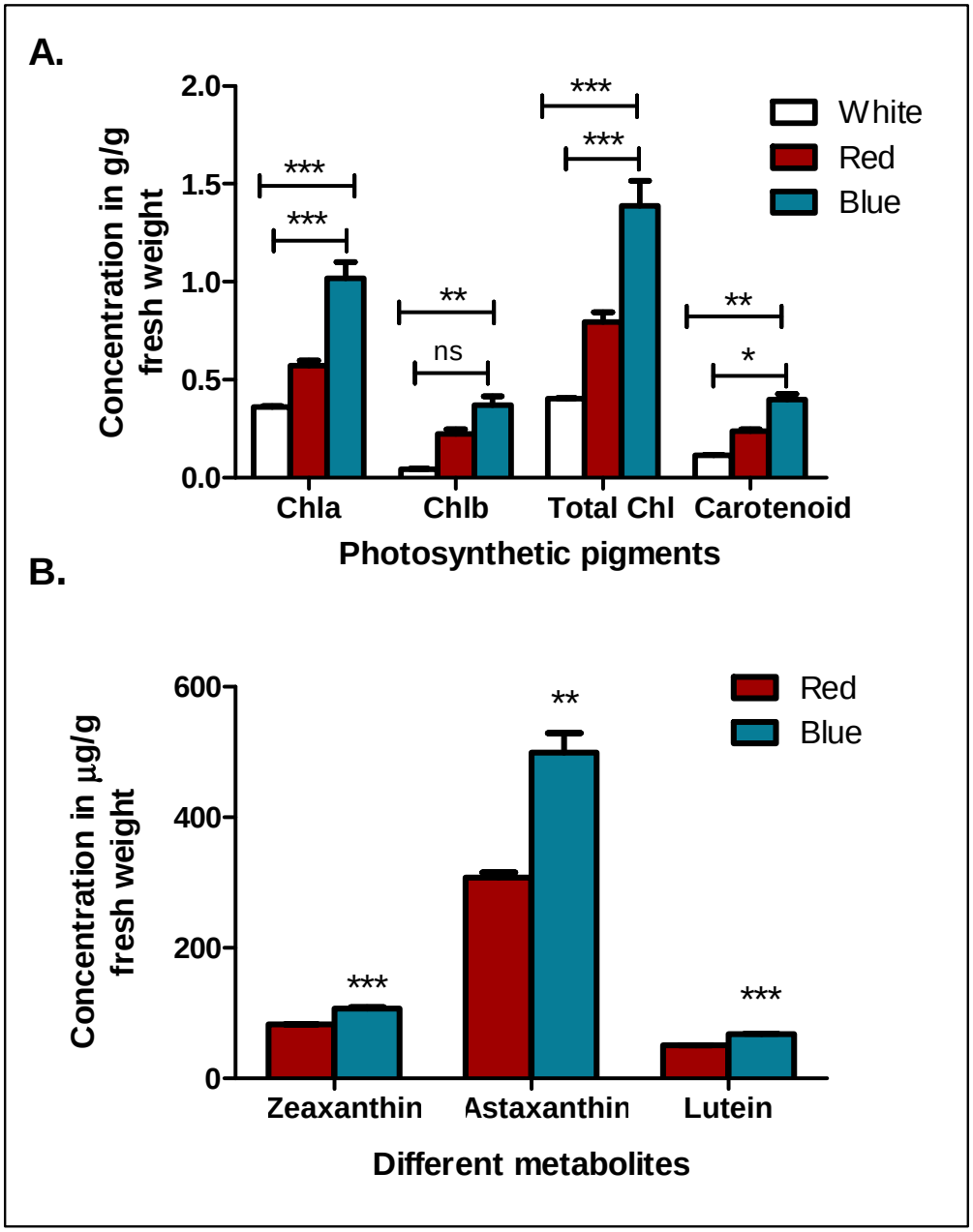
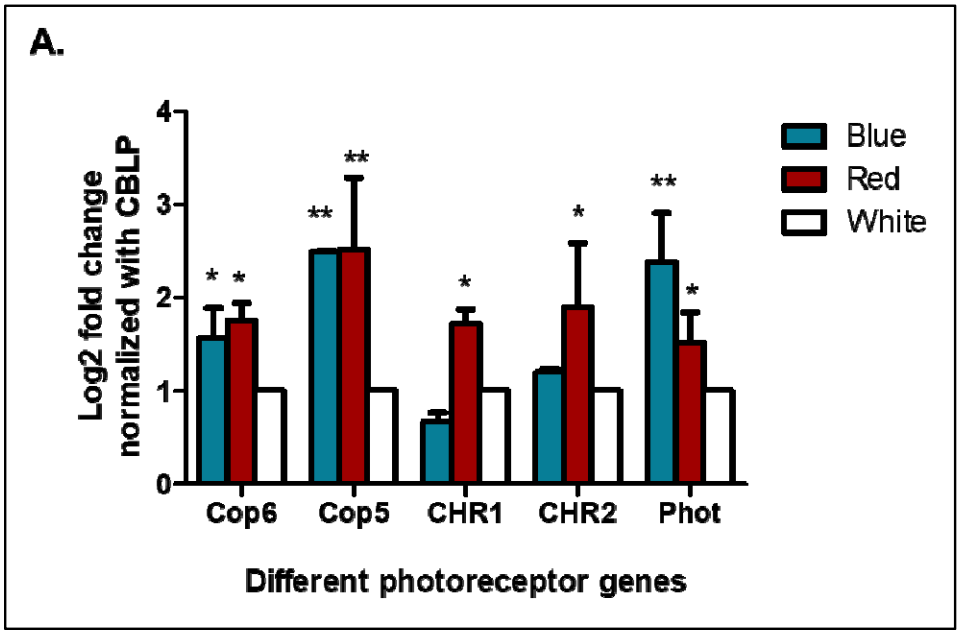


Fig. 3: Effect of opto-modulation on photosynthetic pigments and targeted metabolite content in *C. reinhardtii*.

(A): 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*. (B): 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 S3.



Values are mean ± SE (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001

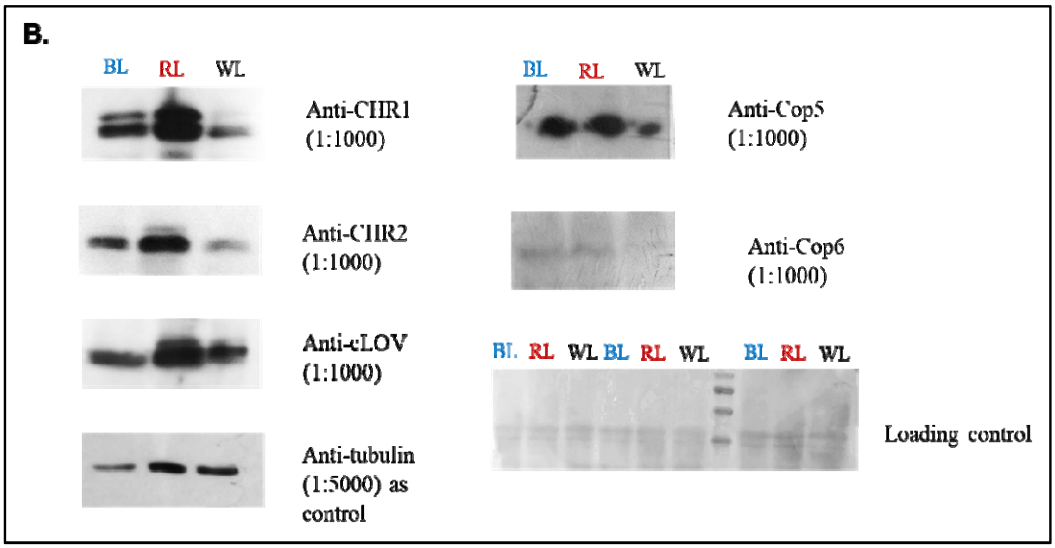


Fig. 4: Effect of different wavelengths of light on photoreceptor in *C. reinhardtii*.

(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.

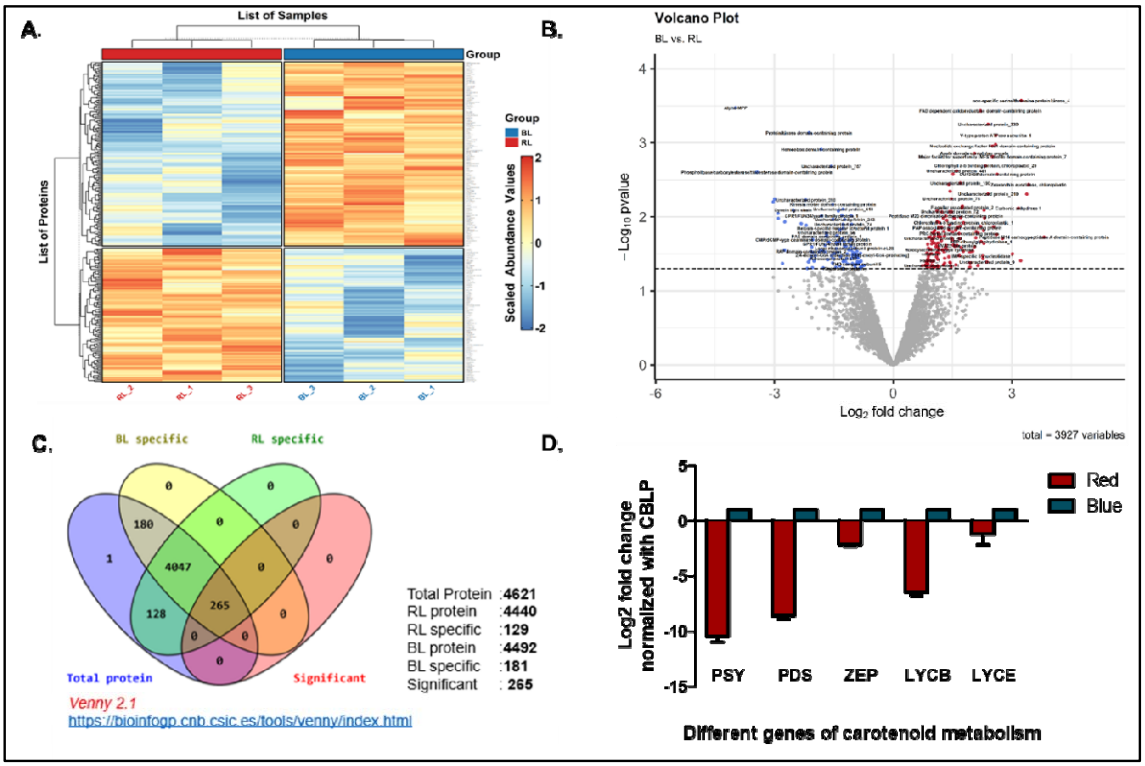


Fig. 5: Comparative proteomics of *C. reinhardtti* illuminated with blue (BL) and red light (RL). (A) The heat map for the DEPs for BL vs. RL. (B)Volcano plot showing proteins significantly downregulated (blue colour) and upregulated (red colour) for DEPs obtained for BL vs. RL. (C) Represents a Venn diagram for the distribution of protein observed under BL and RL. (D**)** Represents the 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.

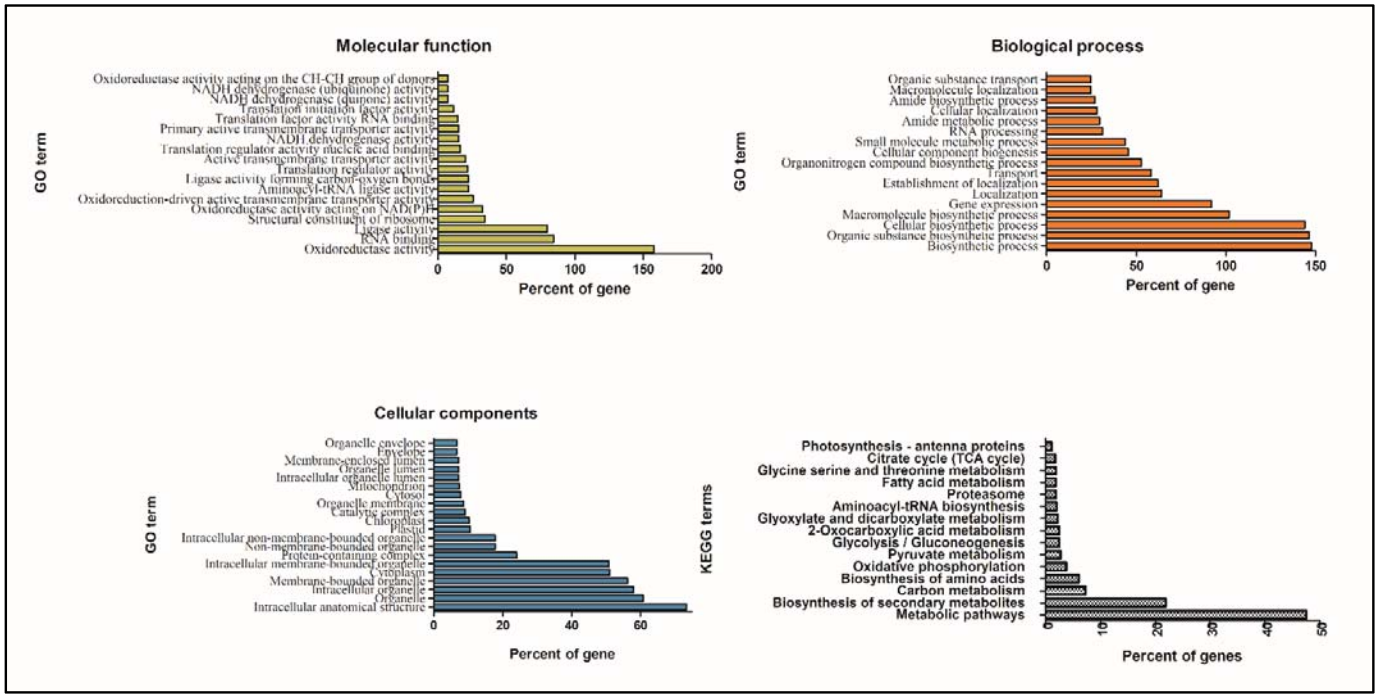


Fig. 6: The figure represents top 20 GO terms based on cellular components, molecular function, biological function and percent of genes linked to each GO term in *C. reinhardtii* in response to blue light.

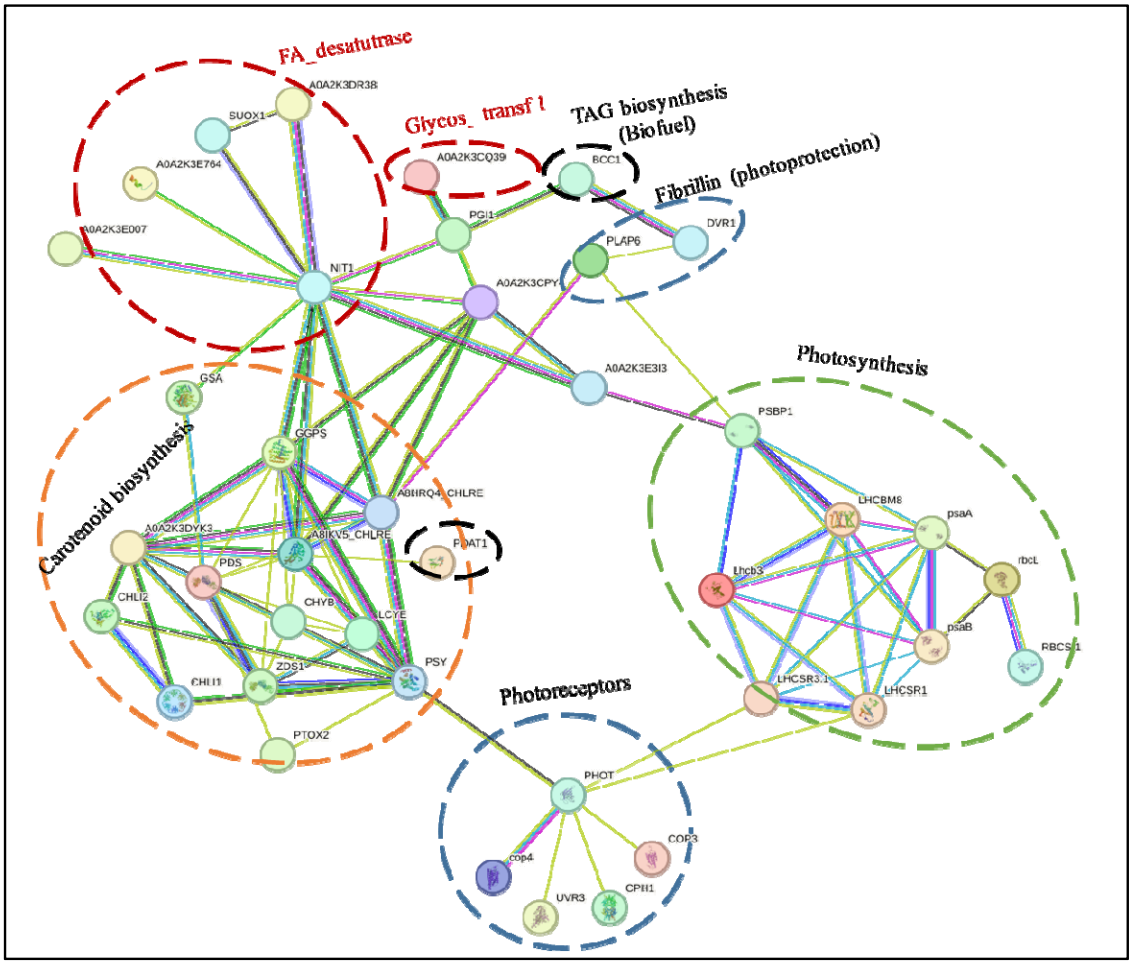


Fig. 7: Bio-curation of opto-modulation of Biosynthetic Gene Cluster for targeted metabolite production.

It shows the crosstalk of BGCs cluster and photoreceptor via predicted curated PPI networking. Here, the FA desaturase and glycosyl-transferase show secondary interaction with photoreceptor (marked in a red colour circle). Further, it also showed interaction with FA metabolism enzyme (marked in black circle). The bio-curation of PPI networking was done using String database based on text mining, co-expression, experimental data and lab proteomics data. The list of important node regulating the network is given in supplementary file (Table S4).