

Acclimation and Organization of Photosynthetic Apparatus to High Light in PGRL1 and PGR5 mutants of *Chlamydomonas reinhardtii*: A Functional and Proteomic Approach

*A thesis submitted to the University of Hyderabad for the award of
Doctor of Philosophy in Plant sciences*

By

RANAY MOHAN YADAV
(Reg. No. 16LPPH14)



Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad – 500046
India

July 2022



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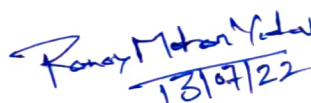
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Date: 13/11/22


Prof. S. Rajagopal

(Research Supervisor)

Prof. S. RAJAGOPAL
Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046.


13/07/22

Ranay Mohan Yadav

(Reg. No:16LPPH14)



Department of Plant Sciences

School of Life Sciences

University of Hyderabad

Hyderabad – 500046, Telangana, India

CERTIFICATE

This is to certify that the thesis entitled **“Acclimation and Organization of Photosynthetic Apparatus to High Light in PGRL1 and PGR5 mutants of *Chlamydomonas reinhardtii*: A Functional and Proteomic Approach”** submitted for the award of the degree of Doctor of Philosophy is a record of bonafide work carried out by him under my supervision per the UOH code of academic and research ethics. The contents of this report have not been submitted and will not be presented either in part or in whole for the award of any other degree or diploma in this institute or any other institute or university. The thesis fulfils the requirements and regulations of UGC and, in my opinion, meets the necessary standards for submission.

List of original Publications

This thesis is based on the following scientific research articles:

- 1- Photosynthetic Research 139, 387- 400 2019,
- 2- Photosynthetic Research 146, 247-258 2020,
- 3- Frontier in Plant Science. 25; 12:752634 2022
- 4- Plant Physiology and Biochemistry 0981-9428 2022
- 5- Plant Physiology and Biochemistry 185,144154 202

Participated and presented work in the following conferences:

- I presented the poster at the 8th International Conference on “Photosynthesis and Hydrogen Energy Research for Sustainability- 2017, organized by the Department of Plant Sciences, University of Hyderabad, Hyderabad. (Organizing secretary- Prof. S. Rajagopal)

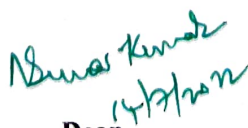
- I presented the poster at the 59th Annual conference of the association of microbiologists of India-2018, "A International Symposium on Host-Pathogen Interaction", organized from 9th- 12 December by the Department of Plant Science, University of Hyderabad, Hyderabad. (Organizing secretary- Prof. CH Venkata Ramana)
- Selected, participated and presented the work in **Japan-Asian Youth Exchange Program in Science (SAKURA Exchange program)**, Okayama and Kyoto university, japan, conducted by JSTA from August 31 to September 9 2018.
- **Best Oral presentation Awarded** in GIAN Workshop on "Chloroplast structure and function" from August 26 to September 6, 2019. (Organizing secretary- Dr Gyozo Garab)
- Participated and gave an **Oral presentation at National Conference on Frontiers in Plant Biology** Awarded held from January 31 to February 1 2020, organized by the Department of Plant Sciences, University of Hyderabad. (Organizing secretary- Dr Santosh R. Kanade and Dr Rahul Kumar)

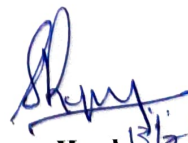
Further, the student has passed the coursework requirement for a PhD.

S. NO	Course	Name	Credits	Pass/Fail
1	PL801	Analytical Techniques	4	Pass
2	PL802	Research ethics, Data analysis and Biostatistics	3	Pass
3	PL803	Lab Work and Seminar	5	Pass


Supervisor

Prof. S. RAJAGOPAL
Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046.


Dean
School of Life Sciences
University of Hyderabad
Hyderabad-500 046.


Head 13/7/22

अध्यक्ष / HEAD
वनस्पति विज्ञान विभाग / Dept. of Plant Sciences
जैविक विज्ञान संकाय / School of Life Sciences
हैदराबाद विश्वविद्यालय / University of Hyderabad
हैदराबाद / Hyderabad-500 046, भारत / INDIA



Department of Plant Sciences

School of Life Sciences

University of Hyderabad

Hyderabad – 500046, Telangana, India

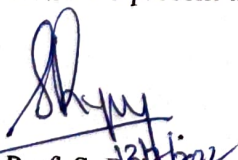
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1- Role of cyclic electron transport mutations *pgrl1* and *pgr5* in acclimation process to high light in *Chlamydomonas reinhardtii*. *Photosynthesis Research*, 146 (1), 247-258. (15%)

2- Autophagy Induced Accumulation of Lipids in *pgrl1* and *pgr5* of *C. reinhardtii* Under high Light. *Front Plant Sci.* 2022 Jan 25; 12:752634. (1%)

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Prof. S. Rajagopal

Prof. S. RAJAGOPAL
Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046.

***This thesis is dedicated to my
Beloved Parents
and Teachers***

ACKNOWLEDGEMENTS

I want to take this opportunity to extend my gratitude to my supervisor Prof. S. Rajagopal for his unrelenting encouragement and constant support, without whom this endeavour would not have been possible. Working under him gave me a true sense of freedom, broadened my scientific outlook, and inculcated a spirit of positive attitude in me. I am deeply indebted to his unfailing support in every scientific aspect like writing, communication, and presentation skills, which enabled me to complete this Ph.D program. Above all, I can't forget the care and help he showed towards me at difficult times during the entire tenure of my doctoral research and the cheerful time I had with him during the Japan trip.

I thank my doctoral committee members, Prof. A. S. Raghavendra and Dr. Irfan A. Gazhi, for their suggestions during my work. I also thank them for allowing me to use their lab facilities.

I thank the former Dean, Prof. P. Reddenna and the present Dean, Prof. S. Dayananda School of Life Sciences, former Head, Prof. Ch. Venkata Ramana, Prof. Padmaja and current Head, Prof. S. Rajagopal, Dept. of Plant Sciences, for their support in all possible ways.

I thank Prof. P. Appa Rao and Dr. Bindu Madava Reddy for allowing me to use their lab facilities.

I thank Profs. Michael Hippler (*pgr11*) and Gills Peltier (*pgr5*) provide us mutants for my research.

I thank Profs. Gyoza Garab, Yuichiro Takashi, Nathan Nelson and Antony larkum for valuable suggestions.

I sincerely thank Prof. Yuichiro Takashi and Japan-Asian Youth Exchange Program in Science (SAKURA Exchange program) for my visit to Okayama and Kyoto University, Japan, conducted by JSTA in 2018.

I am incredibly grateful to my teachers, who taught me just not science but also ethics and morals in life.

I thank Dr. Sai Kiran Madireddi and Dr. M. Suresh for their guidance and suggestion during the research.

I thank my lab members Drs. Mahesh, Dinesh, Nageswar Rao, Elsin Raju, Srilatha, Aparna, Monika, Shreya and Sangeeta. Also, thank Nisha, Divya, Kunal, Jay, Yusuf, Pavithra, Jyoti Ranjan, Jerome, Rojalin, Sailaja and Vijay for their cooperation and help during my research.

My special thanks to Mr. Ganesh, the lab assistant, for his cooperation and help during my research work.

I thank all my friends, including Shoeb, Gaurav, Saurav, Arpita, Santosh and Deepak, for their unconditional support and other research scholars of the School of Life Sciences for their cooperation and for making my stay at HCU memorable.

I thank funding agencies SERB, UGC-IFS, DBT, IoE, and CSIR for financial support. Also, thank UGC-SAP, DST-FIST, and DBT-Builder, for infrastructural support to the lab.

Words fail to express my heartfelt gratitude to my parents and all my family members, including my brother, bhabhi, Sisters, and all my niece and nephews to whom I'm forever indebted for their love, support, and endless patience; without their cooperation, I would not have come so far.

Thank you, everyone

Ranay Mohan Yadav

LIST OF ABBREVIATIONS

<u>Abbreviation</u>	<u>Description</u>
ATP	Adenosine triphosphate
BN-PAGE	Blue-native polyacrylamide gel electrophoresis
β -DM	n-dodecyl β -D-maltoside
CET	Cyclic electron transport
Chl	Chlorophyll
Cyt _b ₆ f	Cytochrome b ₆ f complex
DDT	1,4-Dithiothreitol
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DCBQ	2,4 dichloro, 2-phenyl-1,4-benzoquinone
DCPIP	Di-chloro-phenolidophenol
Fd	Ferredoxin
FNR	Ferredoxin-NADP ⁺ reductase
F _M	Maximum chlorophyll fluorescence in light
F _O	Minimum chlorophyll fluorescence in dark
F _V	Variable fluorescence (F _M - F _O)
GO	Gene Ontology
HSM	High salt medium
KD	Kilo Dalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
LHCII	Light-harvesting Complex II
LHCI	Light-Harvesting Complex I
LHCSR	Light-harvesting complex stress-related
NDH	NAD(P)H dehydrogenase
NADPH	Reduced NADP ⁺
NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NPQ	Non-photochemical quenching

PETC	Photosynthetic Electron Transport Chain
PAM	Pulse amplitude modulated
PFD	Photon flux density
PAD	Photosynthetically active radiation
PGR5	Proton gradient regulation 5
PGRL1	Proton gradient regulation like 1
PC	Photosynthetic control
PSII-LHCII	Photosystem II Light-harvesting complex II
P680	Reaction center Chlorophyll of PSII
P700	Reaction center Chlorophyll of PSI
Pm	Maximum absorbance change at P700
Pheo	Phaeophytin
PC	Plastocyanin
PSI	Photosystem I
PSII	Photosystem II
PSBS	Photosystem II Subunit S
PQ	Plastoquinone
PQH ₂	Plastoquinol
qE	Energy-dependent quenching
qI	Photoinhibitory quenching
Q _A and Q _B	plastoquinone electron acceptor
RC	Reaction centre
RT	Room Temperature

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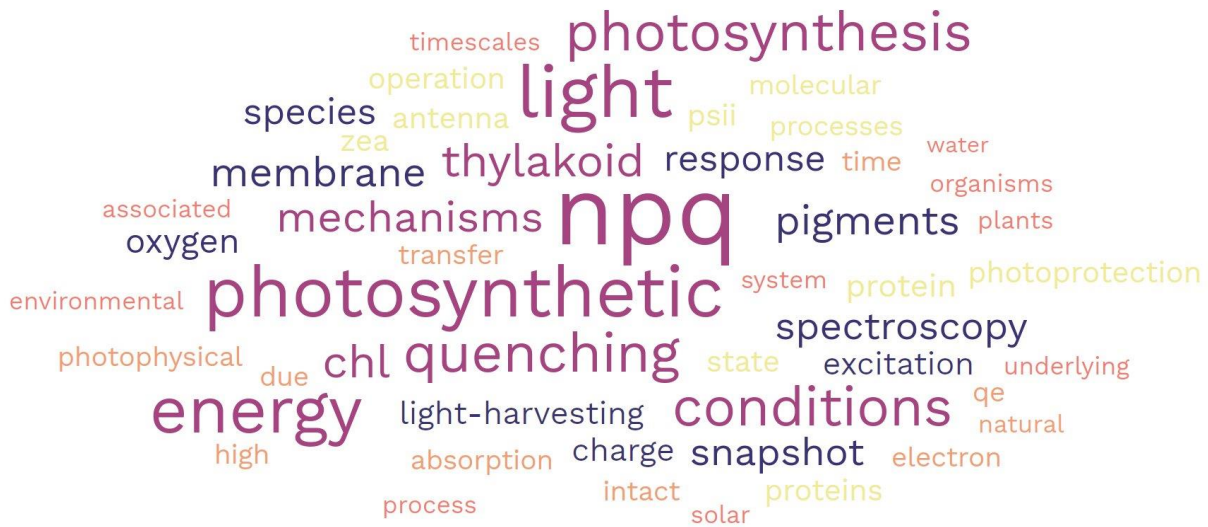
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CHAPTER - 1

Introduction



Introduction

1.1 Photosynthesis

Photosynthesis is a physiochemical process that changes light energy into the biological form of energy by using CO_2 and H_2O as a substrate to synthesize carbohydrates and release molecular oxygen within the atmosphere. Algae and plants conduct this process to capture the photons and store their energy in chemical form. Photosynthetic light reactions generate ATP and NADPH and are then utilized as a fuel for carbon fixation and other facets of metabolism. The photosynthetic pigment-protein components are entrenched in the thylakoid membranes, which distinguish as an internal compartment separating the lumen from the chloroplast stroma. The light-harvesting complexes (LHC) convey light energy to the photosystems, photosystem (PS)II and PSI and are involved in linear electron transport (LET). In the LET, PSII primarily uses light energy to generate electrons from water splitting and handovers to plastoquinone (PQ), then reduced to plastoquinol (PQH_2). The water-splitting reaction occurs at the oxygen-evolving complex (OEC) of PSII and releases molecular oxygen (O_2) and protons (H^+ ions) into the thylakoid lumen. The plastoquinol (PQH_2) is present in the thylakoid membrane and is oxidized by the cytochrome (Cyt) b_6f compound.

The Cyt b_6f complex releases protons in the lumen and energizes the reduction of plastocyanin (PC), a protein that can anchor within the lumen and ultimately reduces the ferredoxin (Fd). The reduced Fd has consumed electrons directly from PSI and gives the electron to CET or reduced to NADP^+ . The protons buildup in the lumen creates an electrochemical proton gradient (ΔpH) that activates ATP synthase to produce ATP. Thus, the LET generates both ATP and NADPH. Alternatively, in the cyclic electron transport (CET), Fd's repeatedly recycles electrons to reduce the PQ pool and releases protons in the lumen through the Cyt b_6f complex activity. In the CET, the proton gradient allows only ATP generation and no net production of reduced NADPH.

1.2 Thylakoid membrane and photosynthetic complexes

Photosynthesis occurs in chloroplasts, a specialized subcellular organelle in green algae and plants. Chloroplasts contain a double-layered membrane envelope, encompassing a widespread interior membrane arrangement known as the thylakoid. The thylakoid membrane separates two aqueous sections, the stroma and lumen, and carries the matrix for collecting photosynthetic protein complexes. Light absorption and the immediate transfer of an electron from OEC to PSII occur in the thylakoid membrane. They are mediated by other

membrane-entrenched multisubunit protein complexes like Cyt b_6f and PSI, which are interrelated to exterior LHCs (light-harvesting complexes) antenna proteins. The Cyt b_6f connects the two photosystems, while another complex, ATP synthase, functions in ATP synthesis (Figure 1.1).

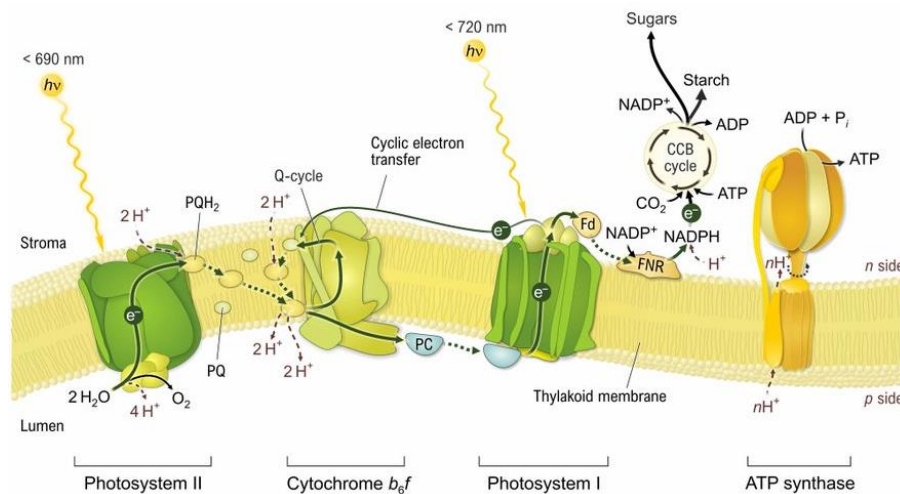


Figure 1.1 Arrangement of the photosynthetic complex on the thylakoid membrane (Adapted from Scigrafi & Agrisera).

1.3 Photosystem II and associated proteins

Photosystem II is a multiprotein membrane complex that oxidizes water molecules to generate reducing equivalents to run photosynthesis. This complex comprises almost 30 proteins dedicated to light absorption, charge separation, oxygen evolution, and photoprotection. PSII utilize light energy to split water to generate oxygen and reduces the energy that runs the photosynthetic electron transport chain (Umena et al.,

2011).

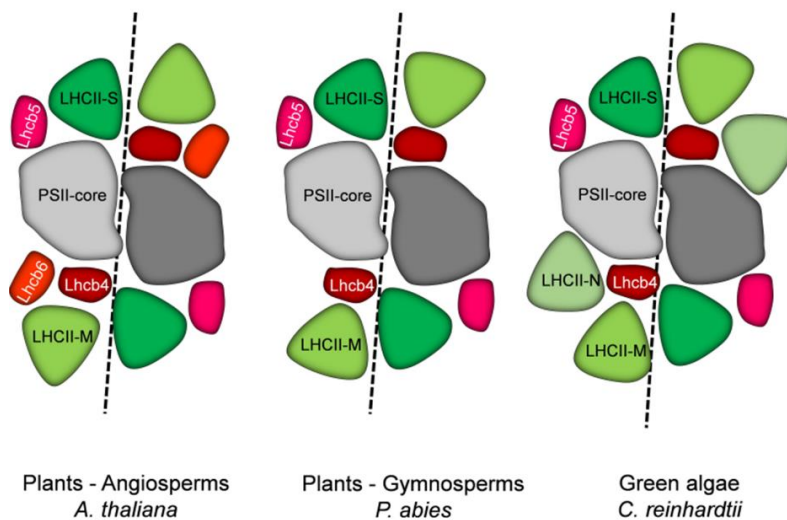


Figure 1.2 The organization of PS II supercomplexes in vascular plants and green algae.

The scheme is based on current structural data (PDB entries: 5MDX, *Arabidopsis thaliana*; 6KAF, *Chlamydomonas reinhardtii*). The putative structure of *P. abies* PSII is shown as well Kouřil *et al.*, (2016). PSII, photosystem II; Lhc, light-harvesting complex.

1.3.1 Photosystem II core complex

The PSII reaction center comprises proteins D1 and D2, containing 6 Chl *a* molecule and 2 Pheo⁻ with quinone binding sites. These proteins are bound to chlorines and quinones (Trebst and Draber, 1986), consisting of five transmembrane helices (Sayre and Wrobel-Boerner, 1994) and form the heterodimeric PSII core. The Q_A has tightly bound the binding

pocket on D2 (PsbD), while the Q_B is loosely attached to the pocket on D1 (PsbA). This reaction center contains P680, the primary electron donor donating the electron to Phaeophytin (Pheo) and quinone, i.e., Q_A via Q_B . In addition, it also includes core antenna PsbB (CP43) and PsbC (CP47), the 2 Chls linked with CP43 and CP47 help inefficient energy transfer. These proteins bind to 16 and 14 Chl *a* molecule, CP43 and CP47, respectively (Bricker and Frankel, 2002).

These proteins perform light-harvesting functions and transfer excitation energy from the peripheral LHCs to the PSII core. The chloroplast-determined fundamental proteins are PSBH, PSBI, PSBJ, PSBK, PSBL, PSBM, PSBN, PSBT, and PSBZ localized in the thylakoid membrane.

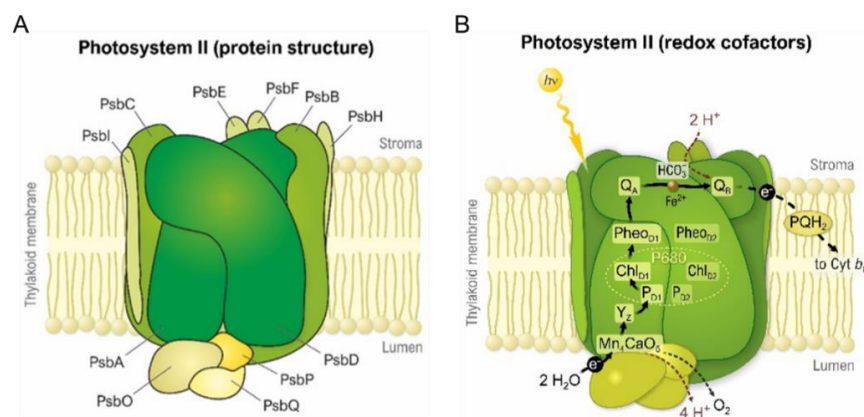


Figure 1.3 Structure of photosystem II dimer and redox co-factor (Adapted from @ Scigrafik and Agrisera)

The protein PsbH is positioned nearby Cyt b559, and the absence of this

subunit results in the loss of the PSII core. Another protein PsbI is placed on the edge of D1 and is essential for the effective gathering/constancy of PSII. The subunit of photosystem II, PsbJ is necessary for associating LHCII proteins to the PSII core. The protein PsbK (photosystem II subunit K) is tightly related to CP43 through Proline (Pro) residue of the Eukaryotic transmembrane helical (TMH) protein and helps to stabilize CP43. In the PsbK mutant, only 10 - 20% of the PSII proteins accumulate, whereas PsbL is involved in electron transfer from Yz to P680.

The Functions of PsbM and PsbN (photosystem II subunit M and N) in *C. reinhardtii* are not yet reported. The photosystem II subunit T (PsbT) is present at the interface of the dimeric core and is involved in stabilizing Q_A . PsbZ is present between PSII core and LHCII proteins. It is required for the optimum expression of CP29 and CP26 antenna protein. It plays a crucial role in plastoquinones' transition at the PSII core's Q_B place (Figure 1.3B). Most of the structural information on PSII has come from crystallographic studies of PSII particles from eukaryotic algae, cyanobacteria and higher plants (Dekker and Boekema, 2005).

The X-ray crystals from cyanobacteria and LHCII trimers have given appreciated evidence about the structural aspects of photosystem II apparatuses (Ferreira et al., 2004; Liu et al., 2004; Guskov et al.,

2009). However, due to its high complexity, it was not possible to elucidate the entire structure of the total interacting partners of PSII with X-ray crystallography; thus, with less resolution, electron microscopic single particle analysis has been used for these studies (Nield et al., 2000a; Dekker and Boekema, 2005; Tokutsu et al., 2012). Recently, a more precise resolution of the PSII complex at 1.9 Å using femtosecond X-ray pulses of the 8Å compact free-electron laser has made accurate measurements. The changes in the structure of the oxygen-evolving complex that the manganese atoms distances with the interacting partners differ from previous observations because of the reduction of manganese atoms due to irradiation by X - rays. This clear structure of OEC can give scope for developing artificial water-splitting catalysts that can be used for non-conventional fuel production (Suga et al., 2015).

1.3.2 Oxygen evolving complex (OEC)

The photosystem II (PS II) core comprises a membrane-inclusive primary reaction centre and OEC. The *C. reinhardtii* luminal extrinsic proteins, PsbO, PsbP, PsbQ, and PsbR form OEC and facilitate water oxidation. PsbO is similar to bacterial porins and functions as a water channel. The PSII subunit PsbY is found near OEC and binds to manganese (Mn). The PsbP demands chloride ions during PSII assembly, while PsbQ enhances the evolution of oxygen. Further, PsbR provides a binding site for PsbP. Cyt b559, associated with the reaction

center (RC), is necessary for PSII assembly and stability. It has α and β subunits.

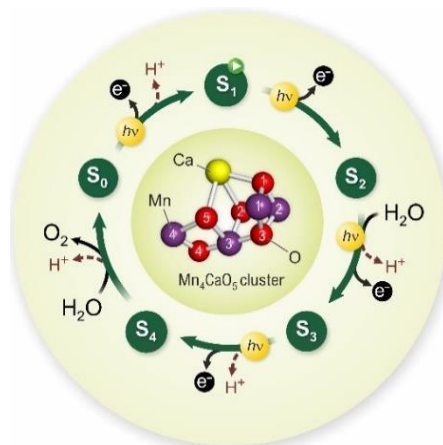


Figure 1.4 Mn_4CaO_5 Cluster (Joliot-Kok Cycle) a catalytic centre for water oxidation (Image adapted from @ Scigrafik and Agrisera).

Its residues on these subunits help coordinate heme (Minagawa and Takahashi, 2004). PSII in higher plants consists of mainly PsbO, PsbQ and PsbP proteins associated with the Mn_4CaO_5 cluster on the luminal side and some other small proteins. It is a catalytic center for water oxidation.

It is present in the pocket formed by the residues of D1 and CP43 towards the luminal side of the thylakoid membrane. It is a distorted cubane comprised of 4 Mn atoms (Mn1, Mn2, and Mn3), 5 oxygen atoms (O₁, O₂, O₃, O₅) and one Ca²⁺ ion. Another Mn is present outside the cubane (Figure 1.4). The typical bond distances of Mn - O and

Ca - O give the distorted conformation, providing flexibility for structural rearrangements and efficient water splitting. The ligand environment of the Mn cluster is formed by 6 carboxylates of C-terminal D1 and Histidine. This manganese cluster accumulates reducing power on excitation, splitting $2\text{H}_2\text{O}$ molecules into the proton (H^+) and oxygen (O_2).

1.4 PSII-LHCII complex structure and function

LHCs are peripherally arranged light-harvesting antenna systems and have an essential role in light capturing and transferring to the reaction centre. PSII-LHCII complex occurs as a dimer with a PSII core and several peripheral antennae. PSII core comprises a primary reaction center and a core antenna system with peripheral LHC trimers bordered by LHC monomers forming PSII-LHCII supercomplexes (Boekema et al., 1999; Tokutsu et al., 2012). These supercomplexes tend to create much bigger mega-compartment structures (Kouřil et al., 2012). Also, regulation of photosynthesis LHCs dissipates the absorbed excess energy as heat (thermal energy) under high light stress conditions. They can be classified into significant LHCII groups: LHCBM1-9 (M - Major) and minor LHCs LHCB4, LHCB5 and LHCB7. Based on phylogenetic relationships, major LHC is subdivided into four groups (Minagawa and Takahashi, 2004).

The category contains Type I, which includes LHCBM3, LHCBM4, LHCBM6, LHCBM8, and LHCBM9, Type II has LHCBM5,

Type III: includes LHCBM2, LHCBM7 and Type IV, which provides LHCBM1. These LHCs are described as Chl *a/b* binding antenna with three transmembrane helices. The LHC polypeptides bind to pigments like Chl *a*, Chl *b*, carotenoids, i.e., xanthophylls and leutins. The primary role of LHC is to harvest bright light energy and is also involved in photoprotective processes (Niyogi, 1999).

The essential antenna complex, LHCIIb, is organized in heterotrimers and comprises Lhcb1, Lhcb2, and Lhcb3 and binds with 60% of PSII chlorophyll molecules. Further, it was found that the Lhcb trimer is associated with a maximum of Chl *b* molecules and has a Chl *a/b* ratio of 1.45 in *C. reinhardtii*, whereas CP29 and CP26 contain less Chl pigment and have comparatively different Chl *a/b* ratio (Figure 1.5A). The structure of monomeric LHCs and LHCII trimers has not been demonstrated, but they are thought to have a topology similar to LHCII.

On the other hand, there is a difference in the pigment composition in which CP29 binds to 9 Chl (6 Chl *a* and 3 Chl *b*) (Croce et al., 2002), and CP26 binds with 8 Chl (6 Chl *a* and 2 Chl *b*) (Sandonà et al., 1998). LHCII trimer associated with 14 Chl (8 Chl *a* and 6 Chl *b*). Through analysis by the single-particle image of electron micrographs reports, the PSII core complex was enclosed by LHCII trimer binding, while the core and LHCII trimers were surrounded by a monomer antenna (Dekker and Boekema, 2005).

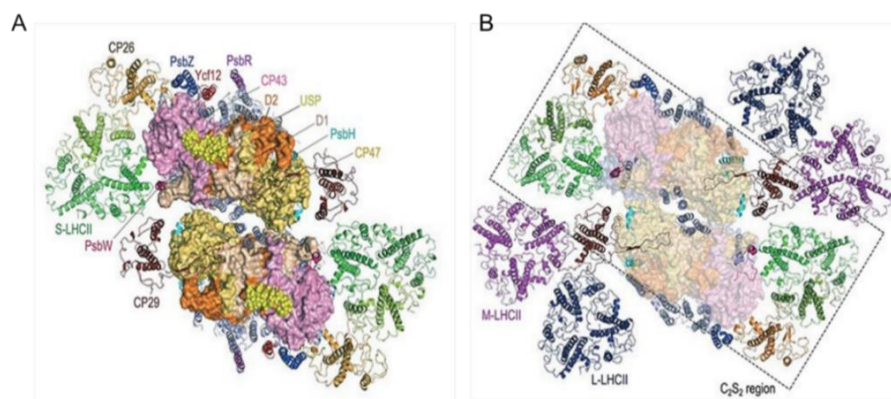


Figure 1.5 The overall architecture of PSII-LHCII supercomplexes from *C. reinhardtii*. The top view of the C_2S_2 supercomplexes from *C. reinhardtii* (PDB ID code 6KAC) D1, D2, CP43 and CP47 are shown as surface models, whereas S-LHCII, CP26, CP29 and other small subunits are presented as cartoon models (A). The top view of the $C_2S_2M_2L_2$ supercomplex from *C. reinhardtii* (PDB ID code 6KAC) stromal side M-LHCII, L-LHCII, S-LHCII, CP29, CP26 and CP24 are shown as cartoon models, while the four major PSII core subunits are presented as transparent surface models (B) (adapted from Sheng et al., 2021).

It was reported that the binding strength of LHCII trimer with PSII core (C) is grouped into three types: S-Strongly bound LHCII, M-Moderately bound LHCII, L- Loosely bound LHCII. The literature showed the $C_2S_2M_2$ kind of organization in *A. thaliana* (Caffarri et al., 2009), whereas the $C_2S_2M_2L_2$ type of PSII-LHCII supercomplex (Figure 1.5B) was observed in green algae *C. reinhardtii* (Tokutsu et al., 2012). In *C. reinhardtii*, six trimers are attached to the PSII core only through CP29 and CP26, as CP24 is absent in green algae (Minagawa and Takahashi, 2004). Due to the absence of CP24 protein, the L-LHCII trimer (L-

Loosely bound) directly binds to the PSII core, which could directly transfer excitation energy to the PSII core (Tokutsu et al., 2012).

1.5 Photosystem I and associated proteins

PSI is light-motivated plastocyanin-dependent ferredoxin oxidoreductase. It transfers electrons from the luminal side plastocyanin to the stromal side ferredoxin by using light energy to cross the membrane barrier. PS I work as a part of LET and CET. The PSI of eukaryotes comprises 14 polypeptides: PSAA, PSAB, PSAC, PSAD, PSAE, PSAF, PSAG, PSAH, PSAL, PSAJ, PSAK, PSAL, PSAN, and PSAO (Figure 1.6). PsaM is present only in cyanobacteria (Germano et al., 2002). The PsaA, PsaB and PsaC form the core of PSI as they carry all the cofactors for charge separation. It consists of a P700 reaction center. The reaction center of PSI consists of a FeS cluster that acts as a terminal electron acceptor (redox co-factor) and allows the reduction of the low potential electron acceptor like ferredoxin. The PSI core complex occurs as a trimer in cyanobacteria (Boekema et al., 1994), whereas it is a monomer in higher plants and algae (Boekema et al., 2001) (Figure 1.6).

The trimeric form of PSI has a higher cross-sectional area that favours high quantum efficiency under low light conditions, while the monomeric form is more suitable for state transitions. The reaction centre comprises PsaA and PsaB heterodimer; this is again associated with seven polypeptides PsaF, G, H, I, J, K and L polypeptides on the

periphery (Figure 1.7A). The subunits like PsaG, PsaH and PsaN are present only in eukaryotes and are involved in binding to LHCs (Amunts et al., 2007).

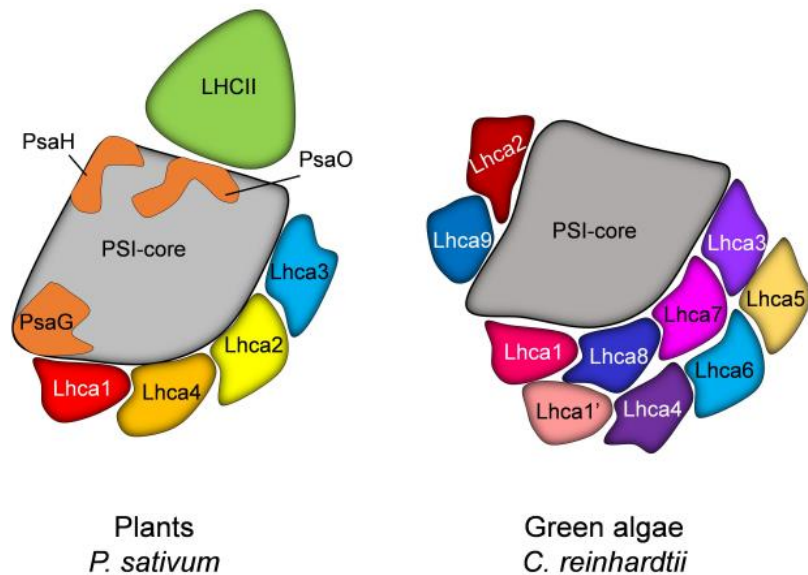


Figure 1.6. Organization of the PS I antenna system in vascular plants and green algae. The scheme is based on current structural data available (PDB entries: 5L8R, *Pisum sativum*; 6JO6, *Chlamydomonas reinhardtii*; 6L4U, *Chaetoceros gracilis*). PSI, photosystem I; Lhc, light-harvesting complex; PsaG, PsaH, PsaO subunit of PSI (Arshad et al., 2022)

The reaction centre of PSI displays striking resemblances through green sulphur bacteria consisting of iron-sulphur [4Fe-4S] clusters as the terminal acceptors, PSAA and PSAB form the RC of PSI that mediates electron transfer reactions in *C. reinhardtii*. The PSAA and PSAB bind the iron-sulphur cluster F_X . The PSAC fixes the iron-

sulphur cluster F_A and F_B (Figure 1.7B).

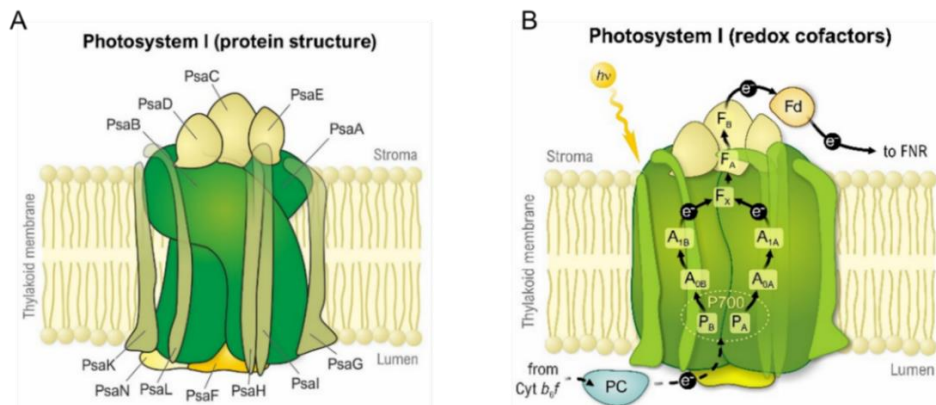


Figure 1.7 The structure of photosystem I and its redox cofactors (Image adapted from @ Scigrafik and Agrisera).

The PSI subunits PSAD, PSAE, and PSAC form the dockage place for Fd towards the stromal region. Subunit PSAF and PSAN participate in the transport of electrons from PC to the luminal side of the P700. The PSAJ binds to PSAF and stabilizes it due to its hydrophobic nature. Another subunit, PSAG and PSAK, is found close to PSAA and PSAB and plays a role in interacting with the outer antenna. The PSI subunit PSAH, PSAI, PSAL, and PSAL are involved in state transitions and bind to LHCI. PSAI and PSAL are engaged in the trimerization of PSI monomers in cyanobacteria (Yang et al., 2015).

1.6 Structure and function of PSI-LHCI complex

LHCs monitor the time scales of energy transfer reactions in photosynthesis. They harvest energy from light sources. RCs could have

evolved without LHCs, but they have to become an integral part of photosystems. The structure of the *C. reinhardtii* supercomplex PSI-LHCI-LHCII is observed laterally as the thylakoid membrane towards the stromal side (Figure 1.8A) and the plane of the membrane. The cartoon model shows protein subunits and is coloured differently (Figure 1.8B, Huang et al., 2021). The presence of LHCs clears all these hurdles by increasing the cross-sectional area for photon capturing and continuously supplying photons to RC (Cogdell et al., 2008).

C. reinhardtii genome has 10 types of LHCI polypeptides. LHCA 1-10 forms two crescent rings on one side of the PSI core. Thus, they are bound to the core as two layers towards the PsaJ PsaF and PsaG components of PSI (Figure 1.8A). The possible explanation for this one-sided vacancy is that it provides room for shuttling LHCII that migrate to PSI during state transitions. The current structure reveals the detailed arrangement of pigments and other cofactors, especially within LHCI and various interactions between the PSI core and LHCI (Qin et al., 2015; Mazor et al., 2017). As revealed by single-particle analysis, the PSI of *C. reinhardtii* contains ten LHCI proteins arranged in a double half-ring shape.

The *C. reinhardtii* PSI-LHCI complex is less stable than higher plants. The external belt contains LHC consisting of LHCA2, LLCA4, LHCA5, LHCA6, and LHCA9, while the inner belt has LHCA1,

LHCA3, LHCA7 and LHCA8. The LHCII occupies the site near LHCA1 in the crystal structure of higher plants, as revealed by the superposition of electron micrograph images and the crystal structure.

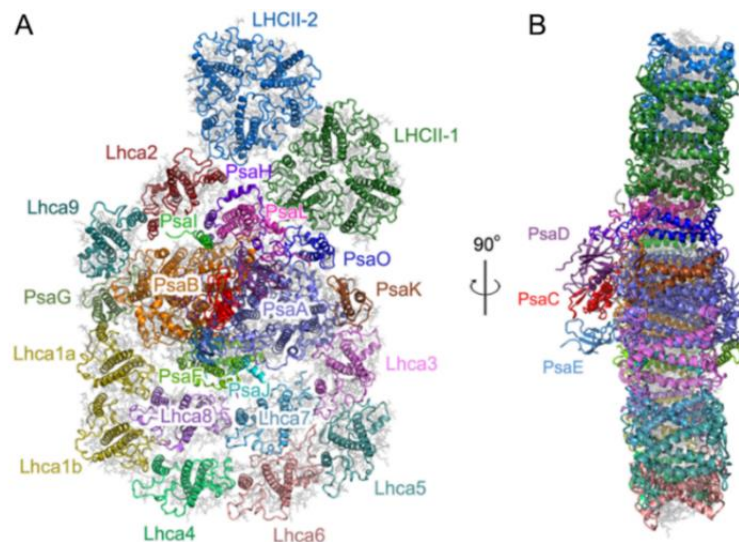


Figure 1.8 Architecture of the PSI-LHCI-LHCII supercomplex of *C. reinhardtii*. The overall view of the C_2S_2 supercomplexes from *C. reinhardtii* (PDB ID code 6KAC) D1, D2, CP43 and CP47 are shown as surface models, whereas S-LHCII, CP26, CP29 and other small subunits are presented as cartoon models (A). The top view of the $C_2S_2M_2L_2$ supercomplex from *C. reinhardtii* (PDB ID code 6KAC) stromal side M-LHCII, L-LHCII, S-LHCII, CP29, CP26 and CP24 are shown as cartoon models, while the four major PSII core subunits are presented as transparent surface models (Sheng et al., 2021) (B).

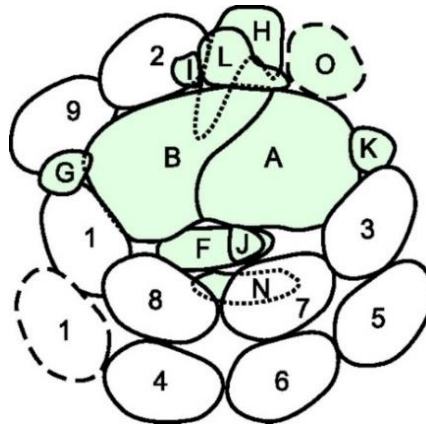


Figure 1.9 A model of the 10 LHCI subunit configuration in PSI-LHCI in *C. reinhardtii* was suggested by Ozawa *et al.*, 2018.

The PSAL, PSAK, LHCA2 and LHCA9 are loosely destined to the supercomplex (Drop *et al.*, 2011), as suggested by their loss from supercomplex even at low concentrations of mild detergent. The subunit PsaF, PsaG and PsaK participate in stabilizing the PSI-LHCI complex. PsaH is oriented away from LHCs in the PSI- LHCI supercomplex (Figure 1.9).

1.7 Cytochrome b_6f (Cyt b_6f) Complex

The Cyt b_6f catalyzing the transfer of electrons from plastoquinol to plastocyanin is a plastoquinol-plastocyanin reductase enzyme originating in the thylakoid membrane of plants and algae (Figure 1.10). It is involved in LET and CET. It also provides a Qo site for binding plastoquinone, activating the LHCII phosphorylation for state transition. It has an essential role in the regulation of photosynthesis.

During photosynthesis, the Cyt b_6f complex handovers electrons from PSII to PSI and pumps protons into the thylakoid lumen to generate pmf later used to synthesize ATP by enzyme ATP synthase. The knowledge of the structural organization of the Cyt b_6f complex comes from the crystal studies of the thermophilic bacterium *M. luminous* (Kurusu et al., 2003) and *C. reinhardtii* (Stroebel et al., 2003).

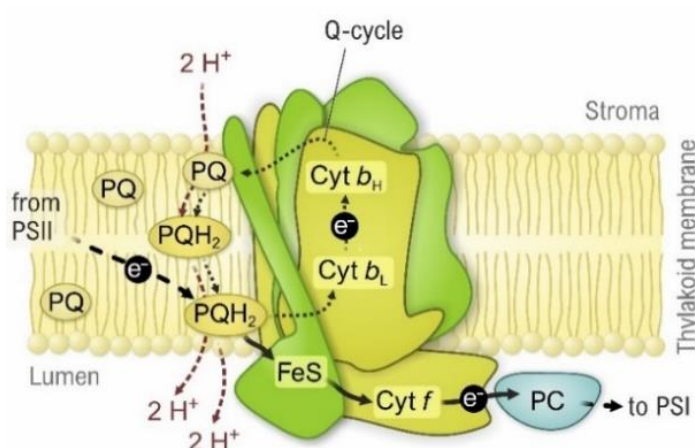


Figure 1.10 Structure of Cytochrome b_6f Complex
(Image adapted from @ Scigrafik and Agrisera).

The Cy b_6f complex is present as a dimer in the thylakoid membrane. Each monomer of the Cyt b_6f complex comprises four large subunits: Cyt b_6 , subunit IV, Cyt f , the Rieske iron-sulfur protein (Figure 1.10), and small subunits PetG, PetL, PetM and PetN. These subunits bind to the redox-active prosthetic groups. Each monomer is composed of 13 transmembrane helices. Nine of them form four large subunits, and the

remaining four are located away from the dimer. One Chl *a* molecule, one β -carotene and seven prosthetic groups are attached to each monomer. The other five prosthetic groups, two *b*-hemes, one [Fe₂S₂] cluster, and two *c*-hemes, showed redox properties (Stroebel et al., 2003)

1.8 ATP synthase

The ATPase synthase complex is also called F₁F₀-ATP synthase. It exists in the chloroplast of green plants, prokaryotes and mitochondria. This complex is divided into three specific parts: (i) A spherical-shaped and hydrophilic headpiece (F₁), (ii) A minor F₀ membrane-destined fraction and (iii) A region of the stalk for linking F₁ and F₀.

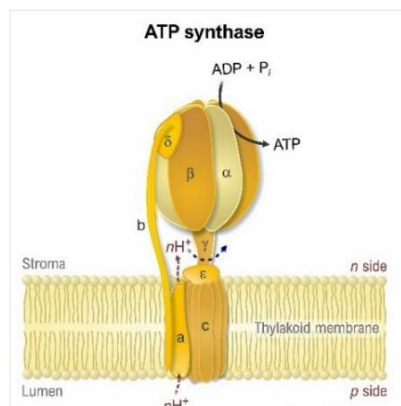


Figure 1.11 Structure of ATP Synthase
(Image adapted from @ Scigrafik and Agrisera).

The centre stack is in the stalk region and connected to the peripheral stator (Figure 1.11). This gradient rotates the CF₀ complex of ATP

synthase with 14 proton binding sites and produces 3 ATP molecules per rotation. But LET could turn CF₀ by 0.86 rounds producing only 2.6 ATP molecules.

1.9 Photosynthetic protein complexes and their function

Light is seized by the LHCs pigments protein, which mainly transports light energy by the chlorophylls to the reaction centers. The LHCs antenna organization did not do any chemistry, but the LHCs absorb light and are excited, photons moving from one pigment to another through a biophysical method. The Chl RC participated in converting light energy into the biologically available form of energy. The RC of the PSII core comprises an exclusive Chl *a* dimer P680, promoted to an excited state (P680*), by direct absorption of photon or energy transfer from the LHCs antenna. The excited P680* quickly transfer the electron Pheo, a first electron acceptor of ETC and then Pheo instantly moves to plastoquinones (Figure 1.3B). Further electron reaches Q_B, a mobile plastoquinone after receiving two electrons from plastoquinone Q_A and two protons from the stroma are completely reduced into plastoquinol.

The plastoquinol (PQH₂) is free from the Q_B pocket present in the PSII core protein D1 and concurrently ejects the protons into the lumen by twice oxidation of Cyt b₆f. Further, the electrons are transported from Cyt b₆f to PC, which ultimately reduces the P700⁺, a chlorophyll pair of PSI RC, similarly to PSII RC, excites and transfers an electron to the primary electron acceptor of PSI. The electron from

excited P700* is transferred through 3 iron-sulphur (Fe-S) clusters to ferredoxin (Fd) and further reduced to NADP⁺ to NADPH by the activity of the ferredoxin- NADPH-oxidoreductase (FNR), an enzyme present towards the stromal side of the membrane.

The primary electron donor (P680⁺) of PSII is a highly oxidizing molecule re-reduced by electrons derived from water. The photolysis of H₂O takes place in the OEC and is catalyzed by the Mn₄CaO₅ cluster that releases the total 4 electrons from H₂O; simultaneously, it releases 1 oxygen molecule and protons into the thylakoid lumen. At the same time, the Q₀ site of the Cyt b₆f complex transferred a few H⁺ ions into the thylakoid lumen, and the lumen is acidified and generates *pmf* together with the proton generated through H₂O oxidation. These proton motive forces create an electrical component ($\Delta\Psi$) and energy component (ΔpH) across the thylakoid membrane. The fraction of *pmf* is utilized for ATP synthesis by the ATPase enzyme.

1.10 Photosynthetic electron transport system

The photosynthetic electron transport system consists of multiprotein mega complexes situated in the thylakoid membrane of the chloroplast. Thylakoid membrane consists of the appressed region called grana and a non-appressed region called stroma, where these complexes are distributed according to membrane specific manner. The photosynthetic electron transport chain (PETC) consists of two photosystems (PS II and I) and a series of electron carriers that work together to produce NADPH and ATP, called the linear electron transport

(LET).

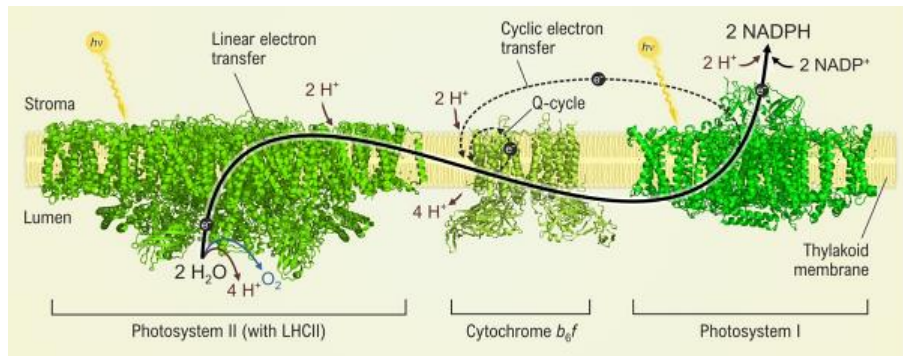


Figure 1.12 Schematic representation of photosynthetic electron transport chain components and flow of electrons from water to the final acceptor NADP^+ (Image adopted from @Scigrafik and Agrisera).

Adding to LET, another electron transport method is present in the thylakoid membrane. Thylakoid membrane contains mainly four complexes: PSII, PSI, Cyt b_6f , and ATP synthase complex, along with mobile carrier plastocyanin and partially mobile carrier ferredoxin (Figure 1.12). The energy generated through the LET is used for CO_2 fixation into carbohydrates, photorespiration, and numerous additional metabolic progressions such as nitrogen and sulfur metabolism.

1.11 Linear electron transport

The primary purpose of photosynthetic electron transport is to produce reducing power for the Calvin - cycle in the form of ATP and NADPH.

The Z-scheme of electron transport can better explain this process.

The PSII and PSI contain reaction center Chl a called P680 and P700,

respectively (named based on their absorption maximum). Excitation of these reaction centers depends on the absorption of incident light directly or through adjacent antennas (containing Chl and carotenoids) by the resonance energy transfer from the adjoining antenna to the RC. This rapid reaction takes a few femtoseconds (10^{-15} seconds). On excitation, $P680^*$ loses one electron to Pheo; it forms oxidized $P680$ ($P680^+$) and reduced (Pheo $^-$), whereas $P700^*$ oxidizes to create $P700^+$ and gives an electron to A_0 , generating reduced A_0^- . The pheo $^-$ donates the electron to PSII bound protein Q_A to Q_B . The Q_B receives two electrons and two protons from stroma to form PQH_2 .

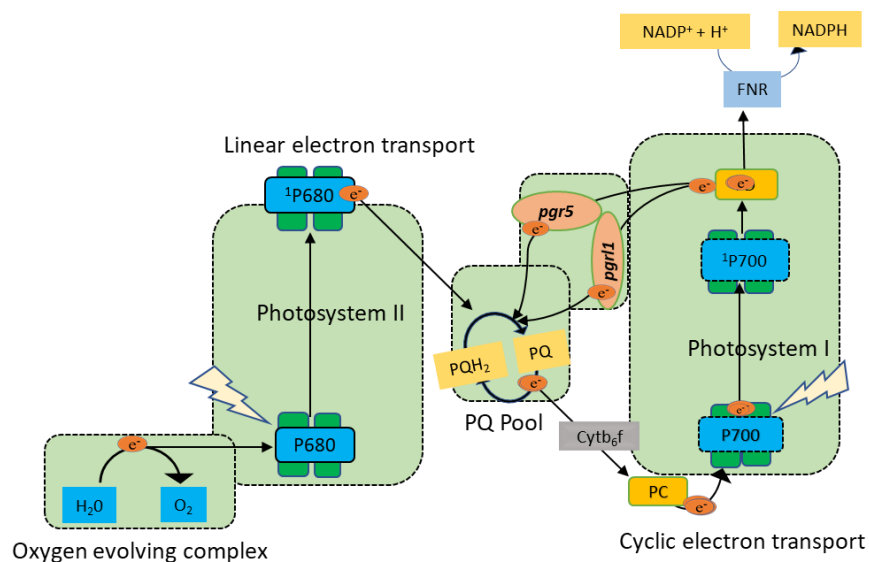


Figure 1.13 Linear and Cyclic electron transport pathways in photosynthesis.

This reduced PQH_2 detaches from PSII and travels through the

hydrophobic core of the thylakoid membrane to the Cyt b_6f complex donating the electron to FeS cluster Rieske protein. This electron is transferred to copper-containing mobile carrier protein plastocyanin (PC) that carries a single electron to oxidized $P700^+$ to recover lost electrons by excitation (Figure 1.13).

In PSI, the reduced A_0 (A_0^-) transfers the electron to final acceptor $NADP^+$ via various intermediate redox carriers in order of A_1 , a phylloquinone to $F_X - F_A - F_B$, and the immobile FeS protein to mobile ferredoxin. The transfer of an electron from ferredoxin to $NADP^+$ to form NADPH is catalyzed by ferredoxin-NADP reductase (FNR). The missing electron from $P680^+$ is recovered by splitting water molecules via tyrosine (Y_Z) and tetra manganese clusters in the PSII reaction center. A total of eight photons of light are required to transfer four electrons from two molecules of water to two molecules of $NADP^+$, giving rise to two molecules of NADPH and a single O_2 molecule. Generation of a ΔpH across thylakoid membrane occurs at several sites. Photolysis of water molecule gives 2 protons ($2H^+$), Q_B , after acquiring electrons from Q_A , binds to 2 H^+ from the stroma and is released into the lumen when transferring an electron to the Cyt b_6f complex. In addition, the Q-cycle Cyt b_6f complex adds more H^+ to the lumen to develop a ΔpH . This transmembrane ΔpH generates ATP from ADP and Pi by ATP synthase

1.12 Cyclic electron transport

When LET does not meet ATP demand for carbon fixation, the electron transport switches to cyclic electron transport. The electrons are cycled around PSI in cyclic electron transfer (CET) and only generate ATP. The CET pathways are much more investigated in plants than in *C. reinhardtii*; based on the literature, it has three types: First, proton gradient regulation 5 (PGR5), Second, PGR-LIKE1 (PGRL1), and third, NDH (NAD(P)H dehydrogenase) mediated CET as reported earlier (Munekage et al., 2002; DalCorso et al., 2008).

The CET pathways transfer the electron directly from ferredoxin to the PQ pool and then back to PSI through the Cyt b_6f complex (Figure 1.13, Yamori and Shikanai, 2016). During the CET, Cyt b_6f generate *pmf* through protons impelled from the stroma to the lumen, utilized in ATP production. The CET fine-tune the ATP/NADPH balance to meet the cell's metabolic requirement. The excess CET acidifies the lumen and is downregulated by the Cyt b_6f activity. The high *pmf* induces a non-photochemical quenching (NPQ) component called energy-dependent quenching (qE), preventing the over-reduction of the photosynthetic electron transfer chain and preventing photodamage by dissipation of excess light energy as heat (Suorsa et al., 2012). The higher plants and algae contain PGRL1 and PGR5 protein in the thylakoid membrane and are involved in CET. The absence of these proteins might be the

perturbation of *pmf*.

A *pgr5* mutant of *Chlamydomonas* revealed diminished ΔpH and limited CET capacity across the thylakoid membrane (Johnson et al., 2014). Another CET alternative pathway proposed in *C. reinhardtii* is NDA2, a type of NADPH-oxidoreductase (Jans et al., 2008). The NDA2 is a ferredoxin-dependent oxidoreductase in *Chlamydomonas* and a PGR5-PGRL1 complex in algae (Tolleter et al., 2011; Johnson et al., 2014). The PGRL1 physically interacts with PGR5 and links with PSI (DalCorso et al., 2008). In *C. reinhardtii*, PGRL1 form PSI LHCI, PSII LHCII supercomplex.

The switch between linear and cyclic electron transport, the Cyt b_6f , and Fd-NADPH oxidoreductase (FNR) balance energy between the photosystems (Iwai et al., 2010). A small protein complex consisting of the PGR5 and PGRL1 works as PGR5/PGRL1-dependent CET (DalCorso et al., 2008). The PGR5 system is the master regulator of photosynthetic energy transduction, enabling plants to cope with changes in light conditions (Munekage et al., 2002; Suorsa et al., 2012; Tikkanen et al., 2015).

The CET handovers the electrons PQ pool via Cyt b_6f to PC and ultimately reduces to PSI acceptors and donor side. In this process, translocation of protons occurs into the lumen by Q-cycle of Cyt b_6f complex, thus increasing ΔpH required for ATP production. This

process produces additional ATP necessary for carbon fixation. The CET pump proton (H^+ ions) into lumen to boost the *pmf* across the thylakoid membranes (Armbruster et al., 2017). The *pmf* subjected to redox regulation of the PETC has two components one is membrane potential ($\Delta\Psi$), and the other is energy component (ΔpH) accountable for the ATP synthesis by the chloroplast ATPase (Yamori and Shikanai 2016).

1.13 High light photoprotection in *C. reinhardtii*

Photosynthetic organisms need light for photosynthesis. Too much light damages the photosynthetic machinery and decreases and rate of photosynthesis efficiency.

It can adversely affect the photosynthetic organisms' growth and viability and further adapt to diverse photoprotective mechanisms to ease excess light's detrimental effects. *C. reinhardtii* has evolved through multiple tools to avoid and minimize the potential restorative damage to cope with extra light. Photosynthetic organisms are exposed to extensive stress factors like high salinity, drought, temperature fluctuations and high light (Aro et al., 1993) etc., leading to decreased photosynthetic efficiency or photoinhibition. PSII activity is more susceptible to environmental cues than other complexes.

The efficiency of PSII decreases very rapidly due to environmental stress. The reactive oxygen species (ROS) is one of the significant causes that inhibit PSII repair, thus supporting damage to its

proteins and cofactors mainly attributed to the PSII photoinhibition (Murata et al., 2007). Due to environmental stress, plants have evolved diverse mechanisms to cope with photodamage.

Along with the photosynthetic process, the downstream of the PSII is affected by other abiotic stress factors, i.e., light. For example, there can be a reduced amount of acceptor pool downstream of PSII or PSI. The carbon fixation process can have a bottleneck for energy usage, thus leading to an imbalance between the photosynthetic apparatus's energy harvesting and utilization capacities. This imbalance can lead to the overexcitation of chlorophyll molecules, possibly forming triplet chlorophyll that can directly generate ROS.

The excitation state of chlorophyll singlet state $^1\text{Chl}^*$ is short-lived. It returns to its ground state in four ways: first, by fluorescence, second in photochemistry, third by elimination of extra energy as heat and fourth, by transferring its energy to additional pigment (Figure 1.14).

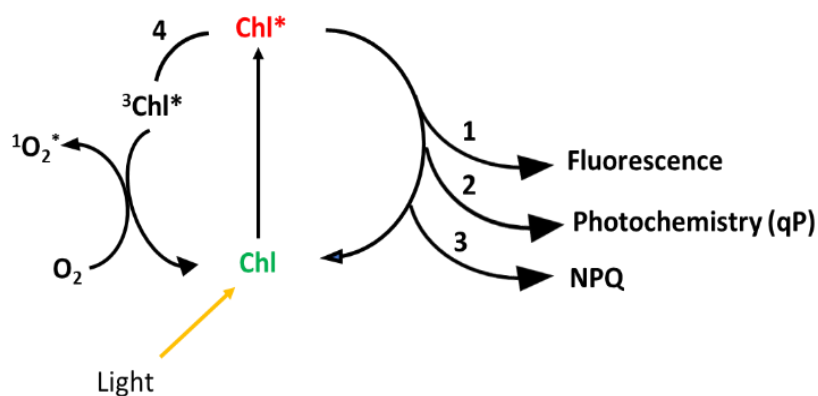


Figure 1.14 Fate of excited chlorophyll and its energy transfer mechanism.

Under abiotic stress conditions, when absorption of light energy is not utilized and under saturating light conditions, the excitation energy is long-lived, leading to triplet state $^3\text{Chl}^*$ crossing the energy barrier. This $^3\text{Chl}^*$ can directly donate the electron to ground state O_2 to form excited superoxide $^1\text{O}_2$. The superoxide is predominantly generated at PSII and LHCs, whereas H_2O_2 , O_2^- and OH^- are developed at PSI due to electron transfer from ferredoxin and NADPH (Asada, 2006). Photosynthetic organisms should check ROS production to minimize the damage to the photosynthetic machinery. They have developed many mechanisms to avoid and scavenge the ROS produced by the photosynthesis process.

The *C. reinhardtii*, a model organism for studying photosynthesis, regulates the process differently, from avoiding light absorption to complete changes in gene expression profile for photo acclimatization. The photoprotection mechanisms comprise short-term adjustment that triggers in seconds of light exposure and long-term acclimatization that can last from a few hours to days.

The short-term responses for the photoprotective mechanisms in *C. reinhardtii* include negative phototaxis, alteration of electron transport, rearrangement of LHCs and the thermal dissipation of excess light energy. In contrast, the long-term responses are transcriptional

changes, protein degradation and PSII repair, antenna size changes, PSI and PSII stoichiometry and antioxidant accumulation.

1.14 Effect of high light on photosynthesis

Photosynthetic efficiency is increased with increasing light intensities at a certain level. If light intensities continuously increase for a certain period, the photosynthetic efficiency is saturated at the maximum photosynthetic rate ($P_{\max}$). Suppose the light captivation continues to expand beyond the organism's carbon assimilation capacity, resulting in reduced photosynthetic activity and photodamage (Figure 1.15). Under high light stress, plant and algae dissipate the excess light energy exceeding their ability.

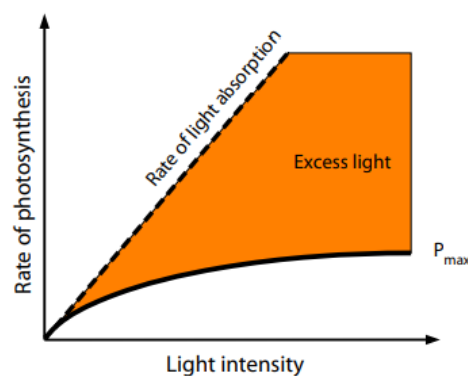


Figure 1.15 Rates of photosynthesis and light absorption.

The additional light energy makes cell damage and indirectly affects the efficiency of the photosystem. Photosynthetic efficiency is proportional to the amount of light absorbed in limiting light conditions,

but in high light, when the system reaches maximum photosynthetic efficiency, the remaining excess light is still absorbed. This leads to incontrollable over-excitation of photosynthetic apparatus that can produce ROS and their byproducts that cause photooxidative injury to the cell components (Niyogi, 1999).

The direct absorption of light at the OEC leads to the destruction Mn cluster and PSII damage (Takahashi et al., 2010). Nonfunctional OEC changes the integrity of the PSII RC and excess chlorophyll and carotenoids play a crucial role in dissipating extra light energy. PSII RC damage compensates by the PSII repair mechanism mainly involves D1 degradation and synthesis to prevent PSII damage (Rochaix, 2014; Takahashi and Badger, 2011). The PSII damage and repair mechanism determines the degree of photoinhibition and photosynthesis.

1.15 Strategies used by *Chlamydomonas* to survive increasing light stress

1.15.1 Phototaxis- movement against light

The light-induced phenomenon of *C. reinhardtii* is phototaxis occurring due to photoreceptors, mainly channel rhodopsins channelrhodopsin-1 (ChR1) and channelrhodopsin-2 (ChR2). They are located in the eyespot apparatus, where they can sense photon flux quality and quantity (Govorunova et al., 2004). When exposed to high light, channel

rhodopsins cause depolarization of membrane that trigger action potential to change the swimming direction away from light, known as negative phototaxis (Hegemann and Bruck, 1989). The redox mechanism of cytoplasm regulates the direction of movement by phototaxis. To some extent, cell homeostasis reduces oxidative damage by changing the cell cytoplasm and photosynthetic activity (Wakabayashi et al., 2011).

1.15.2 Acclimation, aggregation and programmed cell death (PCD)

Photosynthetic organisms deal with variable light energy throughout the day by continuously changing into the photosynthetic machinery. *Chlamydomonas* cells can grow normally (green cells) under optimum light.

However, several acclimatization mechanisms might be activated to resist the cells better adapt or counterattack on a slightly larger scale under moderate light conditions (as a light green cell).

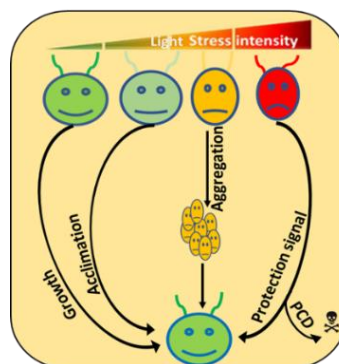


Figure 1.16 Effect of high light on *C. reinhardtii* (Carpentier et al., 2019).

Under high light stress, cells can form palmelloids or aggregates (orange cells), multicellular structures that can be detached when cells recover or away from the high light stress. In severe high light (as a red cell), it can go either PCD or release of protection signal to return to the normal state (Figure 1.16).

1.15.3 Rearrangement of the minor antenna from the Core complex

Light-harvesting's antenna size and magnitudes are changed by the effect of high light quantity and growth conditions (Smith et al., 1990). A perfect assembly among the antenna size and light intensities must be poised to minimize damage by rearranging the LHCII antenna from the core (Figure 1.17). The synthesis and degradation of LHCs depend on the environmental light condition; it increases with an increase in light intensity. It is already suggested that numerous mechanisms and pigment molecules associated with the peripherally LHCs provide a competitive benefit to the photosynthetic organisms living in conditions where the light intensity limits and effectively protects them from preventing growth (Kirk, 1994).

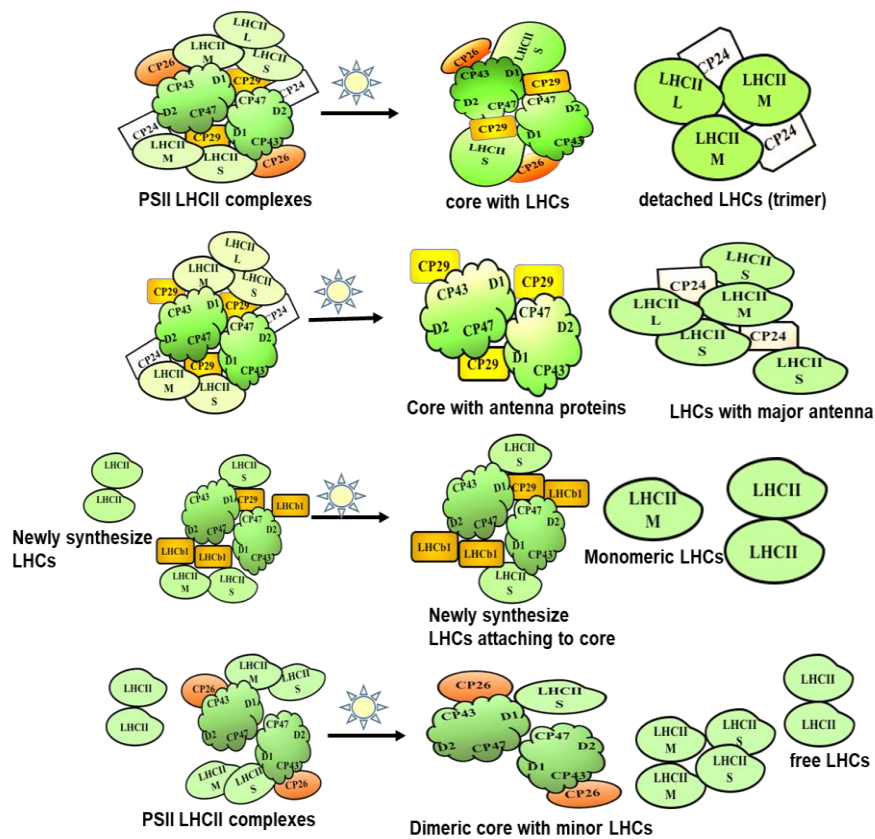


Figure 1.17 Rearrangement of trimeric and monomeric LHCs from the core under high light.

1.15.4 - Mechanisms of PSI photoinhibition and photoprotection

The donor and acceptor side regulations balance the PSI photoprotection and photoinhibition.

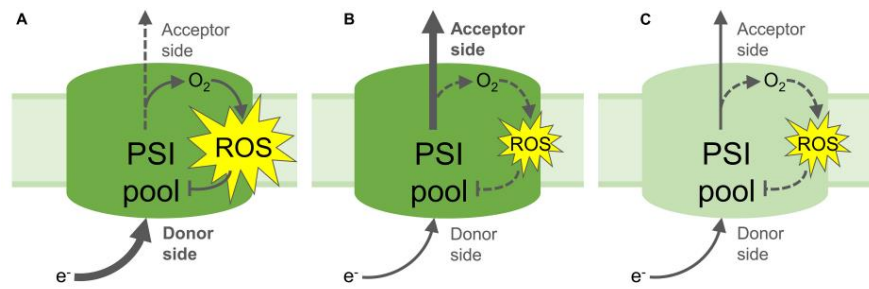


Figure 1.18 Mechanism of PSI acceptor and donor side regulation for photoprotection (Lima-Melo et al., 2021).

This mechanism is enabled when the electron flow increases toward the PSI donor side, minimizing ROS and preventing PSI inactivation (Figure 1.18A). Few photoprotective pieces of machinery like NPQ, photosynthetic control (PC), and D1 damage minimize the electron transport towards the PSI donor side, limiting ROS production and preventing PSI photoinhibition (Figure 1.18B). The CET increase the movement of electrons by Cyt b_6f towards the PSI, minimizing ROS productions and PSI damage.

PSI photoinhibition down-regulates the electron's transport towards the acceptor side by the fractional deactivation of the PSI pool, down-regulating ROS production (Figure 1.18C). Yellow stars represent ROS production; electron transport signifies by arrows, the high rate of electron transport indicates by the heavy arrow, and dashed arrows characterize minimum rates of electron transport. A pale green PSI pool (Figure 1.18C) represents a limited PSI pool inactivation by ROS-induced FeS cluster and protein damage.

1.16 Acclimation mechanism preventing *Chlamydomonas* from excess light through non-photochemical quenching

The excess energy is dissipated during high light utilization as heat, called NPQ. The decreased PSII fluorescence characterizes NPQ through the dissipation of extra light energy. This includes short-term and long-term adaptations under rapid fluctuations or longer illumination.

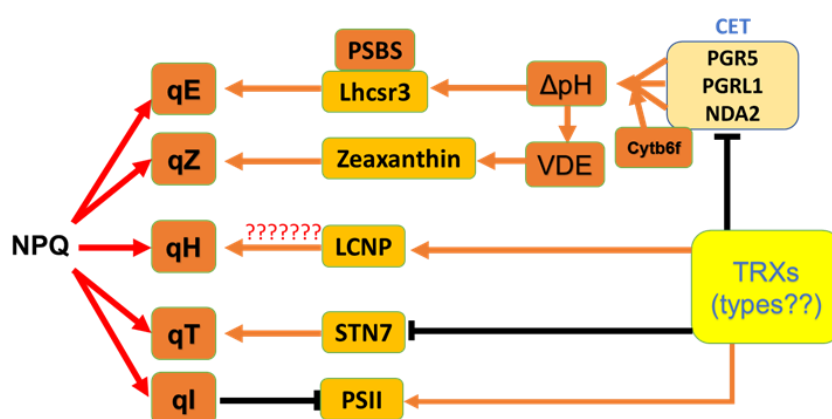


Figure 1.19 Component of NPQ and its co-regulators
(González et al., 2021)

There are four types of NPQ mechanisms based on the time and exposed light intensities, i.e., Energy-dependent quenching (qE), Zeaxanthin-dependent quenching (qZ), State transition quenching (qT) and photoinhibitory quenching (qI) (Figure 1.19).

1.17 Energy quenching (qE): quickly adjustable component of NPQ

The qE is the maximum quickly adjustable quenching. qE can be induced within seconds of exposure to HL and relax within a few seconds to a few minutes. This mechanism requires the buildup of high *pmf* inside the lumen, leading to the activation of the photoinducible LHCSR (Light-harvesting complex stress-related) and PSBS (Photosystem II subunit S) proteins. The excess illumination causes an imbalance between the absorption and the assimilation process. The ATP production is hampered due to the unavailability of precursors ADP and pi.

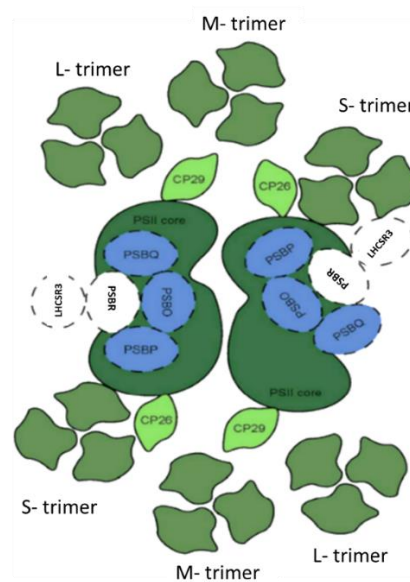


Figure 1.20 Interaction between PSBR and LHCSR3 within PSII-LHCII supercomplexes under high-light conditions.

This leads to the thylakoid lumen acidification, a signal for induction of qE. It is induced by feedback de-excitation of excited chlorophylls that

can protonate proteins associated with PSII. LHCSR is the family of proteins expressed in *C. reinhardtii* under excess illumination. Acidification of lumen triggers the mechanism to communicate LHCSR3 (Light-harvesting complex stress-responsive 3) by Ca^{2+} sensing receptor protein. There are three types – LHCSR3.1, LHCSR3.2 sensitive to light changes, and LHCSR1 expressed under limiting CO_2 . These proteins contain xanthophyll and lutein molecules that can dissipate excess energy. The LHCSR3 is bound to the PSII-LHCII complex from the PSII-LHCII-LHCSR3 complex to form a dissipative energy complex, and the requirement of PsbR is suggested for its binding to the PSII-LHCII complex. In *Chlamydomonas*, LHCSR3 may interact with PSII as well as PSI.

A recent study indicates that PSBR contributes to making a recognition site for LHCSR3 within the PSII-LHCII supercomplex (Figure 1.20). But contemporary evidence has confirmed that LHCSR3 is not directly bound to the PSII core but not to the LHC trimer. The LHCSR and PSBS proteins have protonated domains inducing a conformational change at acidic pH leading to dissipation of extra excitable energy as heat (Figure 1.21; Liguori et al., 2013; Ballottari et al., 2016). It has been suggested that plants and diatoms contain PSBS and LHCSR protein, respectively, whereas mosses and *Chlamydomonas* accumulate both PSBS and LHCSR family.

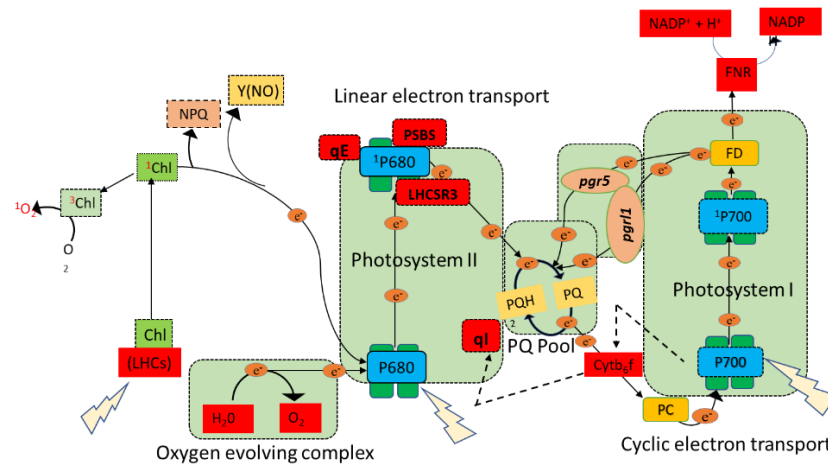


Figure 1.21 High light-induced LHCSR3 and PSBS proteins change the amount of qE and qI quenching.

There is currently no evidence that PSBS besides LHCSR3 participates in establishing qE in *C. reinhardtii*, although expression of PSBS in the algae has been reported under nitrogen deficiency (Miller et al., 2010). At the same time, acidic pH activates the zeaxanthin cycle in which the violaxanthin de-epoxidase enzyme converts violaxanthin into Zeaxanthin, inducing high light photoprotection.

1.18 Zeaxanthin quenching (qZ)

The zeaxanthin quenching begins from a few min to an hour of environmental changes. It is mainly designated in plants and is independent of ΔpH (Nilkens et al., 2010). The zeaxanthin cycle starts a thylakoid enzyme violaxanthin de-epoxidase (VDE), converting violaxanthin to antheraxanthin (epoxide) and antheraxanthin to

zeaxanthin (no epoxides) and vice versa. The accumulation conversion of pigment depends on the type and severity of stress. Zeaxanthin is formed under high light stress. The zeaxanthin cannot synthesize by *C. reinhardtii npq1* mutant under high light due to the knockout of the VDE gene (Niyogi et al., 1997a). But under the low light condition, the minimal amount of zeaxanthin is epoxidized, completing the xanthophyll cycle by reverting and restoring the violaxanthin.

1.19 Photoinhibitory quenching (qI) and repair of photodamage

High light causes photooxidative damage to PSII reaction center D1 protein. The damaged D1 is efficiently removed by thylakoid FtsH protease and replaced by a new D1 protein. The severity of stress and the chloroplast state control the D1 degradation and repair. High light reduces the PSII repair process (Murata et al., 2007), leading to the photoinhibitory quenching by non-functional PSII reaction centers (Murata et al., 2012). These mechanism to overcome pressure at PSII by transferring the electron towards the PSI, preventing D1 damage. The qI quenching results from photoinhibition of PSII activity (Figure 1.21). It prevents PSII damage by managing the electron flow toward the PSI acceptor side and exceeds the electron pooling through CET. It is irreversibly damaged when the capacity of the electron acceptors is exceeded under high light. From this, we can say that qI is also a type of photoprotective mechanism that prevents photosynthetic machinery

(Tikkanen et al., 2014) under high light, but the molecular role of qI is still unknown.

1.20 *C. reinhardtii*: a model organism for photosynthesis research

The genus *Chlamydomonas* (Greek: *chlamus*: cloak or mantle; *monas*: solitaer) is the name given to the unicellular flagellates named by Ehrenberg. The *C. reinhardtii* species was described by Danreard in 1888 and named after the Ukrainian botanist Ludwig Reinhard who isolated this species in 1876. By 1960 it became a widely used strain in laboratory work.

Scientific classification:

Kingdom: Plantae
Phylum: Chlorophyta
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Chlamydomonadaceae
Genus: *Chlamydomonas*
Species: *C. Reinhardtii*

In 1945, this species was redefined based on the wild-type strain isolated in Massachusetts. The redefined features are two equivalent flagella emerging from the anterior side of the cell body, containing a cell wall, chloroplast and chromatophore with single pyrenoids. For subsequent reasons, *C. reinhardtii* has appeared as a

powerful model for photosynthesis study.

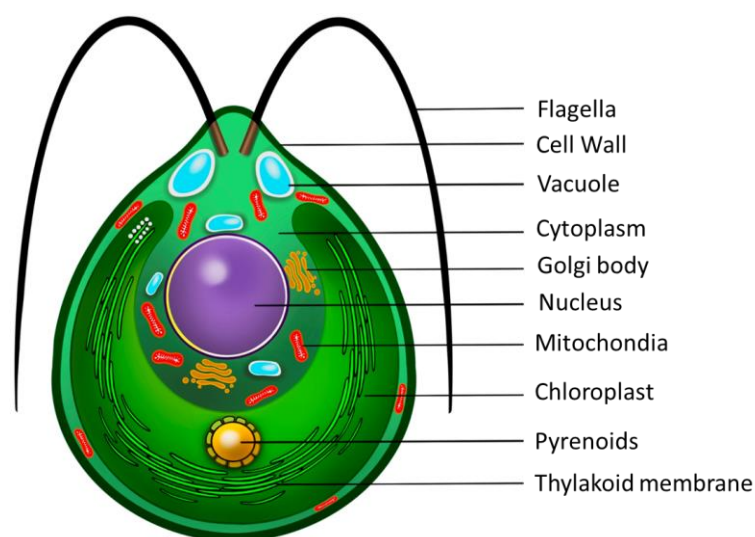


Figure 1.22 *C. reinhardtii* cell architecture contains pair of flagella, cell wall, central nucleus, the cup-shaped chloroplast, thylakoid membrane, cytoplasm, starch-containing pyrenoid, mitochondria, Golgi vesicle, vacuoles (kind gift from Mr. Jyothi Ranjan Rath, my colleague PhD student).

Primarily, *C. reinhardtii* can grow mixotrophically, so the photosynthetic function can be dispensable when grown in acetate. This property provides a gap for isolating and maintaining deficient mutants in photosynthesis. This helps to study light-sensitive mutants and their photosynthetic apparatus assembly. Secondly, in *C. reinhardtii*, the establishment of the gene is vital in the nuclear, chloroplast and mitochondrial genome. The effective transformation protocols for the chloroplast and nuclear genome have been established, allowing one to

isolate nuclear genes by the genomic release of nuclear photosynthetic mutants with suitable cosmid libraries. Powerful spectroscopic methods have been developed to study photosynthetic electron flow in this organism. Combining biochemical, biophysical and genetic approaches make it possible to study many characteristics of photosynthesis.

1.21 Aim of research

The growth of the world population has been increasing; however, agricultural production is not balanced with the increase in the world's population. In contrast, a progressive global decline in cereal yield cannot match the rate at which the world population is growing. On the other hand, the fast decline in the finite oil reserves has raised considerable interest in converting biomass into biofuel as alternate energy. The food, fibre and energy problems can be solved by increasing agricultural productivity.

Thus, the only possible way is to increase plant productivity in the limited land resource available. This can be done only by algae and plants, that is, photosynthesis research. The general information about the structure and function of photosynthetic machinery has been significantly collected; until now, the active assembly and its regulation of the energy alteration mechanism still need widespread knowledge and research. Since almost all chemical energy, most notably food and fuel, is provided by photosynthesis. So we need to improve our

understanding of the photosynthetic organism and process and its regulation mechanism, which is required to develop the emerging techniques and tools that goal to improve photosynthetic efficiency and effort toward the invention of green, clean and renewable energy sources.

1.22 The objective of the study

The dynamic nature of photosynthetic apparatus, under different abiotic stress conditions, mainly under different light regimes, has been studied extensively. Yet, there is a lack of information about the actual role of *pgrl1* and *pgr5* mutants deficient in CET, one of the energy-generating pathways. Further research also needs to be done to study the absence of these mutants and how these mutants affect photosynthetic complexes. The regulation of proton motive force leads to changes in membrane potential, and dynamic changes in photoprotective proteins were addressed to gain information about the functional relationship between CET and the utilization of this energy in a dark reaction.

Keeping the above questions in mind, the objectives of the thesis has been framed as follows:

- 1- Role of cyclic electron transport mutations *pgrl1* and *pgr5* in acclimation process to high light from *Chlamydomonas reinhardtii*.
- 2- Interplay between LHCSR3 and PSBS proteins plays a photoprotective role in *pgr5* of *Chlamydomonas reinhardtii* under high light.
- 3- High light-induced changes in whole-cell proteomic profile and supercomplex organization in *pgrl1* and *pgr5*

CHAPTER – 2

Materials and Methods



Materials and methods

2.1 Materials

2.1.1 Chlamydomonas Strain

I have used *C. reinhardtii* wild-type strains 137c, mutants *pgrl1* (as a gift from Prof. Gilles Peltier, CEA - CNRS - Aix Marseille Université, France), *pgr5* (from Prof. Michael Hippler, University of Munster) and *npq4* were obtained from the Chlamydomonas Resource Center University of Minnesota.

2.2 Methods

2.2.1 Growth condition and light treatment

The *C. reinhardtii* cells were grown in a TAP (Tris-acetate phosphate) medium using a white bright LED light at 120 rpm in an Algaetron growth chamber (AG 230-ECO, Czech Republic). For experimental analysis, I have taken the cell at mid-log phase ($\sim 3.6 \times 10^6$ cells ml⁻¹) of WT (137c) and mutants (*pgrl1*, *pgr5*), inoculated for suspension culture. Further, the suspension culture flask was kept in Algaetron chambers in growth light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) as control, moderate high light (250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions till it reaches to early logarithmic phase ($\sim 3.6 \times 10^6$ cells ml⁻¹). The chlorophyll content was analysed using 80% acetone (Porra et al., 2002). I have used 3 or 4 biological replicates for all the experiments. The *pgrl1* and *pgr5* mutants create, to some extent, different from wild-type backgrounds 137AH and T222+, respectively.

The strains 137AH and T222+ belong to the 137c lineage. I have grown separately, analyzed growth, and compared numerous photosynthetic parameters of the cells growing in all three light conditions that did not significantly differ among the two WT lines. Therefore, I have carefully chosen 137c, the ancestor of *pgr5*, as a reference strain for study.

2.2.2 Cell density and cell number

Cell number and mobility were monitored under the microscope as an indicator of cell health. For routine determination of *C. reinhardtii*, a cell number calibration curve was created relating the optical density at 730 nm recorded on a UV-visible spectrophotometer. The cells were fixed from 1mL cells in 0.025% iodine and measured with a hemocytometer for cell number and cell count.

2.2.3 Spot test analysis

Chlamydomonas cells were collected at the exponential growth phase and transferred into a fresh medium. The 10 μl cells dilution from 10^{-3} to 10^{-1} cell ml^{-1} were spotted at each row on TAP medium plates, allowed to dry and grown in $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (optimum growth light) and $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (high light) for 6 - 7 days in Algaetron growth chamber (AG 230 – ECO, Czech Republic). LED panels supplied homogeneous light, and a controlled temperature of 25°C was maintained at the level of the plate.

2.2.4 Chlorophyll and carotenoid determination

The chlorophyll pigment was extracted with 80% acetone. The cells were centrifuged for 5 min at room temperature (rpm of 10,000), and the absorbance of the solution was measured by a UV-visible spectrophotometer (Perkin Elmer) at 480, 645, and 663 nm. The concentrations of Chl *a*, Chl *b*, and carotenoids (Car) were determined using the equations of Lichtenthaler (1987) and Porra et al., (1989) as described somewhere else (Zivcak et al., 2014). Chl/carotenoid ratios were calculated with the chl pigment extract's absorption spectrum with the individual carotenoid pigments' spectra.

2.2.5 Fast Chl *a* fluorescence measurement

The OJIP fluorescence transients were registered by a Plant Efficiency Analyzer (Hansatech Instr. Ltd, Kings Lynn, Norfolk, UK) for one second with excitation light wavelength at 650 nm in liquid cell cultures. A light intensity of 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was applied to generate the maximum quantum yield of PSII (F_v / F_m) photochemistry, calculated as $F_v / F_m = (F_m - F_o) / F_m$, where F_v is the variable fluorescence, F_o is the initial fluorescence level registered at 50 μs after the onset of illumination, and F_m is the maximum fluorescence. Minimum fluorescence (F_o) (i.e., measured in the dark-adapted state) was determined after the culture had been subjected to at least 20 min of darkness in the dark chamber and immediately before the measurement of F_m . Fluorescence was detected by a PIN-photodiode after passing

through a long-pass filter (50% transmission at 720 nm).

The electron transport efficiency between Q_A and Q_B quinones of PSII was determined by the parameter $1 - V_J = 1 - (F_J - F_O) / (F_m - F_O)$, where F_J represents the fluorescence level at 2 ms after the onset of illumination (Strasser et al. 2000; Kodru et al. 2015).

2.2.6 Chlorophyll fluorescence and P700 absorbance change

Chlorophyll *a* fluorescence and P700 absorption changes were measured using a DUAL PAM-100 fluorometers (Walz, Germany) using moderate and high light cells. The cells were transferred into a six-well plate and incubated for 20 minutes, stirring at 120 rpm in the dark. I recorded a light response curve to perform quantum yield measurements by changing the actinic light intensity stepwise PAR ranging from 3 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. While recording light response curves, samples were exposed to light intensity for < 4 min, followed by a saturating pulse (SP) of 4000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was superimposed in the incidence of AL to get light-adapted chl fluorescence, F_m' . The photochemical efficiency of PSII, $F_v / F_m = (F_m - F_o) / F_m'$ was calculated.

The light-response curve-based quenching parameters q_N and q_L were recorded according to established protocols (Walters and Horton, 1991). The amount of open PSII reaction centre (RC) calculated as $q_L = q_P \times (F_o' / F')$ shows the oxidized PQ pool (Baker, 2008), and q_P is defined as photochemical quenching, $q_P = 1 - (F_s - F_o) / (F_m - F_o)$.

The F_o' was calculated as $F_o / (F_v / F_M + F_o / F_M \times F_o)$ (Oxborough and Baker, 1997). The regulated and non-regulated energy dissipation was measured at every 1 min interval with continuous high actinic light (AL) for 10 min and followed by 5 min relaxation by switching off AL using a saturating pulse (SP) of 8000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ photon flux density. The rapidly (for 10 min light treatment) induced constituent of NPQ was allocated to the energy-quenching (qE), and then the slowly relaxing (for 5 min of dark relaxation) part was specified to photoinhibitory quenching (qI) was calculated as follows: $qE = F_M / F_M'' - F_M / F_M'$ and $qI = F_M / F_M - 1$ (Krause and Jahns, 2003). where F_M' is the maximum fluorescence yield, F_M'' is the quenched level of maximum fluorescence reached under illumination for different times with actinic light, and F_M is the maximum fluorescence after 25 min of dark exposed samples afterwards illumination with actinic light.

The fraction of non-regulatory quantum yield of energy dissipation is calculated as $Y(\text{NO}) = F_s / F_m$. F_m' (Kramer et al., 2004). The redox changes of P700 were analyzed by monitoring the absorbance changes at 830 nm with reference to 875 nm. The P700 absorbance constraints were followed and calculated by Klughammer and Schreiber, 1994; The P_M displays the maximum photo oxidizable P700; P_M' , the maximum photo-oxidized P700 by SP under AL; P , the P700 photo-oxidized under AL. The quantum yield of active PSI is denoted as $(Y(I))$, i.e., $Y(I) = (P_M' - P_M) / P_M$; the donor-side limitation of PSI signifies the quantum yield of non-photochemical energy

dissipation of PSI as $Y(ND)$, $Y(ND) = P / P_M$; and the acceptor-side limitation of PSI as $Y(NA)$, $Y(NA) = (P_M - P_{M'}) / P_M$. The sum of all these quantum yields is; $Y(I) + Y(ND) + Y(NA) = 1$. The value can vary between 0 to 1, where 0 indicates a P700 fully oxidized state of PSI measurement, and 1 is P700 fully reduced state.

2.2.7 ECS measurements

The electrochromic pigment shift (ECS, P515), as well as *pmf* partitioning analyses, were performed on a DUAL-PAM-100 (Walz, Germany), prepared through a custom-built emitter-detector P515/535 module (Cruz et al., 2001). *Chlamydomonas* cells were incubated with continuous stirring in the dark for 20 min for ECS measurement. The signals of ECS were normalized contrary to the 515 nm absorbance change brought by a single turnover flash (ECSstf), measured by dark-adapted cells before 10 min of AL treatment followed by keeping the same cells for 5 min in the dark, followed by high ECSstf. The *pmf* difference is measured by the rise and fall of the ECS signal by switching on and off the AL using three different wavelengths, 53, 256, and 553 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for each sample measurement. This AL intensity closely mimics the light in which the *Chlamydomonas* strains were grown at 50, 250, and 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The components of the proton motive force (*pmf*), proton gradient (ΔpH) and membrane potential ($\Delta\psi$) can be obtained by analysing dark-light-dark changes in the P515 signal. The ΔpH and $\Delta\psi$ components were calculated by turning off the actinic light after the 30s, using dark-light-dark

relaxation kinetic. All the experiments were performed on an equal chlorophyll basis.

2.2.8 Chloramphenicol-based photoinhibitory experiment

TAP mediums are used for photoinhibition experiments to grow cells at optimum growth and high light conditions. I have collected 50 ml of cells after 48 h growth and diluted the cell cultures in fresh TAP medium to an equal chlorophyll basis, allowed them to stabilize for an hour at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and then transferred them to very high light at $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 2 h with 0.1 mg/mL chloramphenicol (Kuroda et al., 2014). The 50 ml cell culture was collected by centrifugation, and the cell pellet was resuspended in 100 μl of 20% glycerol. The remaining cell pellet was frozen in liquid nitrogen and stored at -20°C to analyze the proteins.

2.2.9 Photosynthetic O₂ evolution and respiratory O₂ consumption measurement

The light-saturated oxygen evolution and consumption rate (under $\sim 600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were measured by injecting the 2 ml of each sample in an electrode chamber, a Clark-type oxygen electrode (Hansatech Instruments Ltd, Norfolk, United Kingdom) at 24°C . Artificial electron acceptor 0.5 mM DCBQ (2,5-dichloro-1,4-benzoquinone) and 100 mM NH_4Cl and 2ml each injected into the electrode reaction.

To keep the cell mixture in the dark for two minutes, switch the light for O₂ evolution rates measurement for consecutive 3 min continuous light illumination (~500 - 600 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Another 3-min incubation followed the light until the straight bend line had not appeared. Respiratory oxygen consumption of 100 mM Sodium azide, 50 mM Methyl viologen, 10 mM DCPIC, 50 mM DCMU and 500 mM Ascorbate is detected in the dark; the cell is kept in the dark until the chamber oxygen is not entirely consumed. The oxygen electrode was calibrated with air-saturated water (100 % oxygen) by adding sodium dithionite to achieve 0% oxygen. The final chlorophyll concentration for each replicate, 20 $\mu\text{g ml}^{-1}$, was used. For better activity, cells were mixed with buffer 5mM Tris-HCL (pH 7.5), 10 mM MgCl₂, 5 mM CaCl₂, 30 mM KCl, 0.25 mM Sorbitol, (Nakalai, Japan). Experiments were the average of 3 biological replicates.

2.2.10 Circular dichroism measurement

The Circular-dichroism (CD) spectra of control and high light treated cells isolated thylakoid were measured using a J-810 spectropolarimeter (Jasco Inc., Easton, MD, USA). The spectrum's wavelength range (400-800 nm) was recorded using a quartz cuvette 1 cm path length and at 3 bandwidths. The sample thylakoid was dissolved into a thylakoid suspension buffer 20 mM Tris (pH 7.5), 0.3 M sorbitol, 10 mM MgCl₂, 10 mM NaF and 5 mM CaCl₂ with the concentration of 20 $\mu\text{g/ml}$ (Subramanyam et al., 2006). The three scans were repeated with a 100

nm/min continuous scan speed for each replicate. Three independent experiments were taken for the measurement.

2.2.11 Blue native PAGE (BN-PAGE) analysis of high light treated thylakoid samples

The thylakoid membranes were isolated from cells grown in high light dissolved in thylakoid resuspension buffer containing 0.2 M Sorbitol, 5 mM Tris-HCl (pH 7.5), 5 mM CaCl₂ (Subramanyam et al. 2010). BN-PAGE was performed with a 4% stacking and 4-12.5%-gradient resolving gel polymerised from a 32:1 Bis-acrylamide: acrylamide mixture (Järvi and Suorsa, 2011). Thylakoid was isolated from the control and the light-treated sample containing 1 mM PMSF, 1 mM ACA and 1 mM benzamidinium hydrochloride as a protease inhibitor. A total of 10 µg of Chl was loaded per lane, with a final Chl concentration of 0.5 mg ml⁻¹ and a final detergent concentration of 1% β - DM. Native protein markers from GE Healthcare (UK).

2.2.12 Gel electrophoresis and immunoblotting

SDS-PAGE (Sodium dodecyl sulphate-Poly acrylamide gel electrophoresis) separated the proteins from cells or thylakoids. Cells were harvested by centrifugation, and the pellet was resuspended in 20% glycerol. Cells were solubilized into a 2X sample buffer containing 1 M Tris HCl (pH 6.8), 10% 1 mM DTT, 100% glycerol, and 10% SDS slowly mixed with pipetting.

The sample was heated at 100 °C for 1 min and then centrifuged at 8,600 x g for 10 min to collect the supernatant and loaded the sample on an equal Chl basis. The protein separated and was transferred into a nitrocellulose membrane using a semi-dry trans-blot system (Bio-Rad, USA). The transferred blots were blocked with TBST containing 4 % skimmed milk (w/v) for 2 h at RT. All antibodies were diluted in 50 mM Tris, 150 mM NaCl, 0.1% (v/v) Tween 20 mixed in 5% (w/v) non-fat dry skimmed milk. All the antibodies were diluted in 50 mM Tris, 150 mM NaCl, 0.1% (v/v) Tween-20 in 4 % (w/v) skimmed milk.

The nitrocellulose membrane was incubated with primary antibodies (photosystem II and I and associated LHCs) raised in rabbit and the antibody dilutions were as follows: PsbA_1:5000; PsbB_1:5000; CP47_1:2000; CP43_1:10,000; PsbO_1:5000; LhcSR3_1:1000; Ferredoxin_1:1000; Cyt b6_1:5000; AtpC_1:10,000; CP29_1:10,000; CP26_1:10,000; Cyt f_1:10,000; PsbS_1:5,000; Lhcb1_1:10,000; Lhcb2_1:5000; Lhcb3; Lhcb2; Lhcbm5 (1:5000). For PSI-LHCI complex: PsaA_1:1000 and PsaC (1:1000); PsaD_1:5,000, PsaH_1:10,000; PsaG_1:10,000; and PsaF (1:10,000) (all antibodies were purchased from Agrisera, Sweden). The LHCI antibodies following dilutions: Lhca1 (1:5000); Lhca2 (1:5000) Lhca3 (1:5000); Lhca5 (1:300); Lhca6 (1:300); Lhca7 (1:250); and, Lhca8 (1:3500) (few antibodies were raised in our laboratory) were used. Subsequently,

Secondary antibody (HRP-conjugated anti-rabbit, 1:20,000 dilution; Agrisera) treatment was performed for 1h RT at 15 rpm. Chemiluminescence signal detection was performed using ECL Western Blotting solution (Bio-Rad) and the ChemiDoc Imaging System (BioRad).

2.2.13 Protein extraction and quantification of *C. reinhardtii* cells

C. reinhardtii control and 500 light treated cells were grown and harvested at 3000 rpm for 5 min. Cell pellets were finely mixed in 10% glycerol, dipped in liquid nitrogen, and stored at -80 °C for further analysis. The cell pellet was taken into a 15 ml falcon tube (Genaxy, India) and suspended in 4 ml of extraction buffer containing 0.5 M Tris-HCl (pH 7.5), 0.7 M sucrose, 0.1 M KCl, 50 mM EDTA, 2% β -mercaptoethanol, and 1 mM PMSF. After thorough mixing, an equal volume of Tris-saturated phenol (pH 7.5) was added to the extracted suspension. The whole suspension was mixed for 30 min at 4°C in a rotor spin cyclomixer. Tris-saturated phenol was prepared by mixing an equal volume of Tris-HCl (pH 7.5) and phenol with continuous stirring for 3-4 h. The lower phenolic layer was separated and added an equal volume of Tris-HCl (pH 7.5) with constant stirring for 2-3 h. The lower phenolic layer was collected and stored in an amber glass bottle at 4°C. The sample mixture was centrifuged at 5000 x g for 30 min at 4°C. The upper phenolic phase was collected carefully, and an equal volume of extraction buffer was added.

This step was repeated repeatedly, and the phenolic stage was re-extracted. Four volumes of ice-cold 0.1 M ammonium bicarbonate in methanol were added to the final collected phenolic phase and incubated overnight at -20°C for protein precipitation. The next day, the samples were centrifuged at 10000 x g for 30 min at 4°C. The pellet was washed thrice with ice-cold methanol, twice with acetone, and air-dried for a few minutes. The final pellet was dissolved in 200 µl of the rehydration solution containing 8 M urea, 2 M thiourea, 30 mM DTT, 4% CHAPS, 0.8% IPG buffer of pH range 4-7 (GE, health care). The protein concentration was determined using Bradford reagent (Bio-Rad) with BSA as standard (standard curve of 0 -100 mg ml⁻¹ concentration).

2.2.14 nLC-MS/MS analysis

100 µg of the final pellet was treated with 100 mM DTT for 1 h at 95°C followed by 250 mM iodoacetamide (IDA) for 45 min at room temperature in the dark. The sample suspension was incubated with trypsin at 37°C for overnight digestion. The trypsin digested peptides were extracted in 0.1% formic acid solution at 37°C for 45 min. The solution mixture was centrifuged at 10000 x g, and the supernatant was collected in the fresh tube for vacuum drying. The final sample was solubilized in 20 µl of 0.1% formic acid. For separation of peptides, 10 µl of injection volume was loaded on the C18 UPLC column, and peptides separated Waters Synapt G2 Q-TOF for MS and MS/MS analysis.

For LC-MS analysis, 10 μ l of the sample was injected into the ACQUITY UPLC system (Waters, UK) equipped with ACQUITY UPLC BEH C18 column (Waters, UK) (150 mm X 2.1 mm X 1.7 μ m), A SYNAPT G2 QTOF (Waters, UK) and an electrospray ionization (ESI) source.

The sample analysis was run on the positive mode by applying 3500 V capillary voltage and 30 L cone gas flow per hour. The source and desolvation gas flow was maintained at 1.8 and 800 L per h, and the temperatures of source and desolvation were 150°C and 350°C, respectively. The protein range was used from 50 Da to 150 Da. The trap and the transfer collision energy were continuously maintained at 6 V. The ramp collision energy was set at 20 V and increased up to 45 V. The total acquisition time was 60 min, and the solution flow rate was 300 nl/ min. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). A linear 60 min gradient consisted: of solvent A 98% and solvent B 2% for 1min, solvent A and B 50% for 29 min, solvent A 20% and solvent B 80% for 15 min, followed by 15 min solvent A 98% and solvent B 2%. A Wash solution was used at the end of each program to reduce carry-over between samples.

2.2.15 Protein identification

The raw data acquired from the above analysis was processed using PLGS software 3.0.2 (Waters; identification and expression algorithm),

within which data processing and database search was performed. The sample being *C. reinhardtii* proteins for two sample sequences in FASTA format were downloaded from swiss-prot and used to search peptides present in samples. On each run, the sample was processed using the following search parameters in the software: peptide tolerance 50 ppm, fragment tolerance 100 ppm, min number of fragments matches for peptides, the minimum number of fragments matches for proteins five and carbamidomethylation of cysteine and oxidation of methionine were selected as fixed and variable modifications respectively. Uniprot (*C. reinhardtii*, reviewed protein) was used as the database against which the search was done.

2.2.16 Gene Ontology, KEGG pathways and bioinformatic analysis

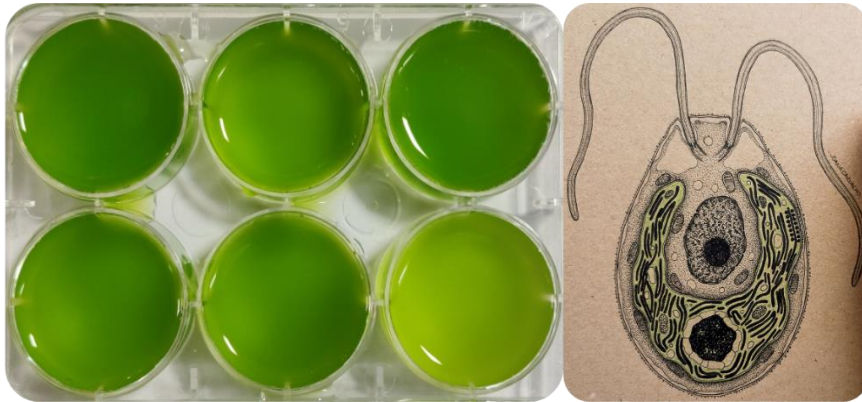
This study's identified protein species were annotated based on their molecular function, biological process, and cellular component with Gene Ontology (GO) annotation using Uniprot. Hierarchical cluster analysis was performed using the R statistical package based on correlation values. Network analysis was performed by using Cytoscape bioinformatics software version (3.7.2).

2.2.17 Statistical analyses

All statistical analyses were performed on three biological replicates ($n = 3$). Hierarchical cluster matrices and protein-protein networks were constructed using the R statistical package based on correlation values and $P < 0.05$.

CHAPTER – 3

*Role of cyclic electron transport mutations
pgrl1 and pgr5 in the acclimation process to
high light from C. reinhardtii*



Objective 1: Role of cyclic electron transport mutations *pgrl1* and *pgr5* in the acclimation process to high light from *C. reinhardtii*

3.1 Introduction

Photosynthesis harnesses solar energy and converts the biologically available form into chemical energy. Photosynthesis is a highly regulated process involving different electron transfer pathways for generating ATP and NADPH. The significant portion of electron flux required for generating ATP and NADPH is provided by linear electron flow (LEF). This process involves the transfer of electrons from water, the primary electron donor, via photosystem (PS) II, several redox carriers and PSI to the terminal electron acceptor NADP^+ . These electron transfer reactions are coupled with proton translocation from stroma to thylakoid lumen that generates proton motive force for the generation of ATP by ATP synthase. The Calvin-Benson cycle and other downstream metabolic reactions utilise the ATP and NADPH produced in this process. The stoichiometric proportions of ATP to NADPH required for the carbon fixation are slightly imbalanced towards NADPH in LEF. Therefore, the additional demand for ATP is compensated by the cyclic electron flow (CEF) (Alric, 2010).

CEF involves the NADPH and ferredoxin (FD) reoxidation through plastoquinone (PQ), cytochrome (Cyt) b_6f complex, and PSI. This process generates the transmembrane proton gradient utilized in ATP synthesis (Kramer et al. 2004a).

In vascular plants and green algae, the CEF occurs through two pathways, an antimycin-sensitive pathway that involves proton gradient regulator like 1 (PGRL1) and proton gradient regulation 5 (PGR5) proteins and antimycin-insensitive NADPH dehydrogenase (NDH) pathway. PGRL1 and PGR5 were first discovered in *A. thaliana* and are considered essential for CEF and photoprotection (Munekage et al., 2002; DalCorso et al., 2008).

PGRL1 is found to be elusive ferredoxin – plastoquinone reductase that accepts electrons in PGR5 dependent manner (Hertle et al., 2013). In *C. reinhardtii*, the deficiency of PGR5 resulted in diminished growth, less proton motive force across the thylakoid membrane and reduced CEF around PSI, indicating the importance of these proteins in photosynthetic efficiency (Munekage et al. 2004; Tolleter et al. 2011). Photoprotection is one of the essential phenomena in photosynthesis, and a set of mechanisms that confer photoprotection under high light is known as non-photochemical quenching mechanisms (NPQ) (Erickson et al., 2015). The induction of NPQ is dependent on the thylakoid lumen acidification, and the latter is modulated by the CEF (Johnson et al., 2014). In *C. reinhardtii*, the PGRL1 and PGR5 are co-regulated and co-expressed in response to several environmental stimuli (Petroutsos et al., 2009; Peltier et al., 2010). Although the involvement of PGR5 in cyclic electron flow is under debate, the increased photosensitivity and reduced luminal pH in PGR5 mutant have shown its importance in photosynthesis (Johnson et al., 2014).

The green algae, *C. reinhardtii*, can grow photo-autotrophically using CO₂ and photoheterotrophically with acetate. An increased acetate concentration can decrease the photosynthetic carbon fixation without changes in respiration and PSII efficiency (Heifetz et al., 2000). Acetate enters the glyoxylate pathway at the expense of ATP and is metabolized into triose, whereas CO₂ is reduced to triose in photosynthesis. It was shifting cells to the low concentration of CO₂ that resulted in the activation of NPQ and ATP, requiring a carbon concentrating mechanism (CCM). Thus, to meet the demand for ATP, LEF of photosynthesis turns to CEF (Miyachi et al., 2003). Besides carbon limitation, exposure to high light can lead to the over-reduced intersystem electron pool due to the unavailability of the NADP⁺ at PSI, leading to the generation of reactive oxygen species (ROS) (Ort and Baker, 2002). Therefore, CEF is the possible mechanism to recycle the electrons in the intersystem electron pool and provide enough proton motive force for ATP synthesis to be utilized for CCM and repair mechanisms. Therefore, different substrate conditions influence the photosynthesis process and its underlying mechanisms. The suggested role of CEF under varying substrate/light conditions needs to be tested. In the present work, I compared the growth, chlorophyll content and photosynthetic parameters of mutants that are impaired in one of the pathways of cyclic electron transport. I used varying light intensities and substrate combinations to dissect the photosynthetic response of the WT, *pgrl1* and *pgr5* mutants.

I have observed a precise expression of NPQ in these mutants (*pgr11* and *pgr5*) impaired in CEF. The results were obtained from the mutants carrying a defect, i.e., reduced cell growth and density, fluorescence, electron transport rate, PSII yield and pigment ratio (Chl *a/b*). These mutants have reduced capability to develop a strong NPQ indicating that the role of CEF is crucial for photoprotection even under heterotrophic growth conditions.

3.2 Results and discussion

Abiotic stresses limit plant productivity; understanding the physiological processes that underlie stress injury and plant protection mechanisms from the stresses are of immense importance to agriculture and the plant's survival. The dynamic fluctuation of light intensity from low to high has recently become a subject of experimental research. However, the molecular mechanisms of photosynthetic responses to high light remain clarified. Our group reported recently that the WT has grown in TAP medium in high light 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ the growth has been faster; however, the cell growth under 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was slower (Nama et al., 2019). The acclimation process in autotrophic growth has not been addressed sufficiently under such conditions. Since *pgr11* and *pgr5* play a significant role in cyclic electron transport (DalCorso et al., 2008; Peltier; Munekage et al., 2002), the role of these genes/proteins in the acclimation process in high light has not been studied adequately.

Here, I have examined the comparative and physiological function of mutants *pgrl1* and *pgr5* in photoautotrophic and photoheterotrophic conditions. I measured growth patterns, cell density, pigment composition, chlorophyll fluorescence, and NPQ - a photoprotective mechanism under high light in heterotrophic and autotrophic mediums. These results clearly show that both *pgrl1* and *pgr5* CEF defective mutants suffered under high light, with reduced growth and efficiency, pigment composition and electron transport rate as the primary target of photodamage.

3.3 Compressive stress affects growth, cell morphology and cell density

To evaluate the effect of high light on growth phenotypes of the WT, 137c and mutants *pgrl1* and *pgr5*, the cell suspensions were spotted on TAP and HSM - agar plates and allowed to grow up to 96 h in low light ($50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) (Figure 3.1A and 3.1B). Phototrophic growth of *pgr5* is impaired in high light but elevated growth in WT 137c and both control and high light intensities. I observed the impaired growth of *pgrl1* and *pgr5* in high light than in low light. However, the *pgr5* strain could not grow photo-autotrophically under $500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Figure 3.1D). On the other hand, the wild-type 137c show better and faster growth in high light and the low light cell culture on agar TAP and HSM plate (Figure 3.1A and 3.1B).

For the growth and cell density measurement, wild-type 137c and mutants, *pgrl1* & *pgr5*, were grown on photoheterotrophic and photoautotrophic medium at three light intensities, 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (low light) and 250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, and 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (high light).

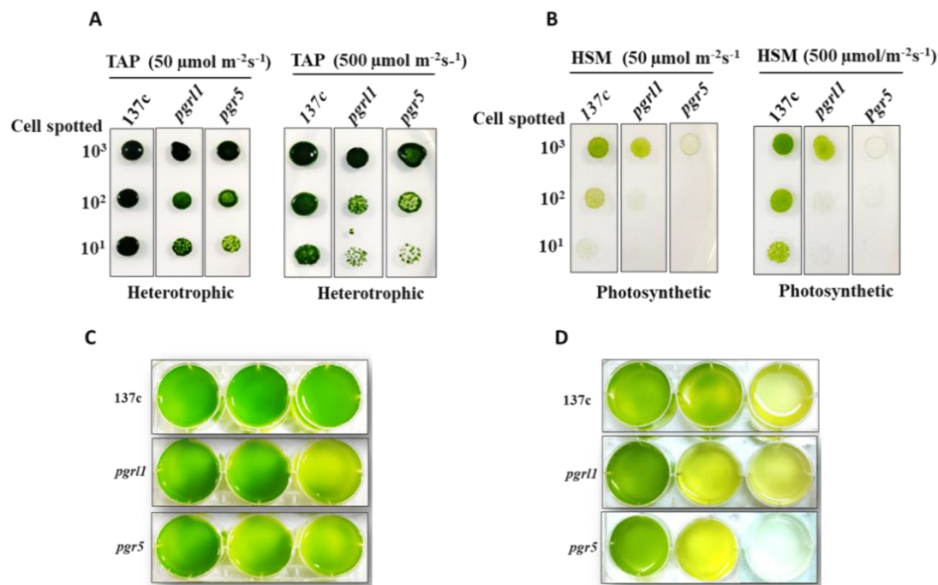


Figure 3.1 Comparisons of growth in TAP and HSM. The wild-type (137c) and *pgrl1* and *pgr5* strains at control (50) and high light (250, 500) intensities (as indicated in $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) on TAP and high salt medium (HSM) in agar as spot test (A and B) and cell suspension culture (C and D).

Cells grown at high light intensities (250 and 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) showed comparatively better and faster growth when compared to high light grown mutants (*pgrl1* and *pgr5*) cells in both TAP and HS medium (Figure 3.2A and 3.2C). For different light conditions, the cell count was evaluated by hemocytometer at each 24h time interval, resulting in higher values of cell densities for high light (250 and 500

$\mu\text{mol photons m}^{-2}\text{s}^{-1}$) in wild type 137c, although the lower values were substantially observed in low light ($50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) in *pgrl1* and *pgr5* (Figure 3.2B and 3.2D). This shows that the growth of HSM cells is indeed limited by the unavailability of inorganic carbon (Fischer et al., 2006). The availability of acetate as an organic carbon and energy source for better growth in TAP cultures compensated for growth on an even limited growth rate in HSM cultures. Similar reports have been observed with an increase in the light intensity, the *C. reinhardtii* growth and biomass increased (Nama et al., 2019).

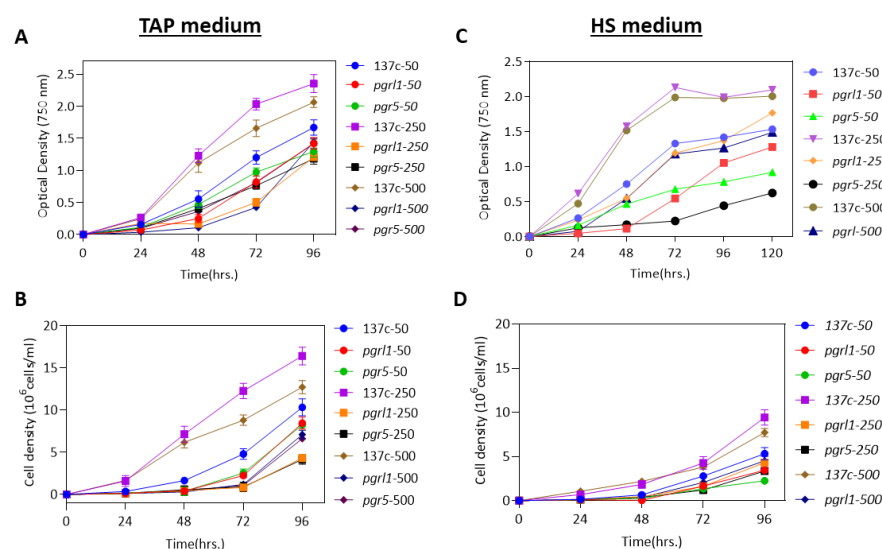


Figure 3.2 Cell density and cell number of *C. reinhardtii* under different growth conditions. Size-dependent distribution of cells and the correlation of optical densities (A and C) and cell numbers (B and D) are shown for cultures grown in TAP and HSM medium in $50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ as control, and high light (250 and $500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) was analyzed throughout for every 24 h in three independent experiments (average $\pm$ SE). Here *pgrl1* and *pgr5* with their background strain as WT (137c).

These results suggest that heterotrophic growth is not affected by the varying light conditions in wild type, whereas the growth of *pgrl1* and *pgr5* are negatively affected. Therefore, although the photosynthetic process is not required in the heterotrophic growth conditions, high light can limit the growth in mutants impaired in CEF, suggesting its role in photoprotection even under heterotrophic growth. Under photoautotrophic conditions, the growth of *pgrl1* and *pgr5* is severely damaged under high light, indicating the obligate role of these proteins.

3.4 Evaluation of photosynthetic pigments content under high light stress

The study of variation in pigment composition that can indicate the organism's photosynthetic properties at the given condition significantly affected the growth when the cells were grown in the high light in both HSM and acetate supplemented media. Under heterotrophic growth, total Chl of wild-type 137c and mutant *pgrl1* increased with light intensity, where *pgr5* showed a slight increase at 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and decreased at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ than control (Figure 3.3A).

Conversely, under autotrophic growth, total Chl in WT increased marginally with light intensity, whereas *pgrl1* and *pgr5* decreased with light intensity (Figure 3.3B). The increase in the total Chl content of *pgrl1* at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ is not apparent, and the same is reflected in its growth, i.e., on a cell basis (Table 3.1, Figure 3.1C). The

analysis of Chl *a/b* content and their ratio positively correlates with the reaction center to LHC ratio (Terashima and Hikosaka 1995).

On the other hand, in mutants, the reduction in chlorophyll content was associated with an expected increase in the Chl *a/b* ratio (Terao et al., 1985). Here, under heterotrophic growth conditions, 137c and *pgrl1* have shown a marginal decrease between 10-15% in Chl *a/b* ratio, whereas *pgr5* has demonstrated a 45% decrease (Figure 3.3C).

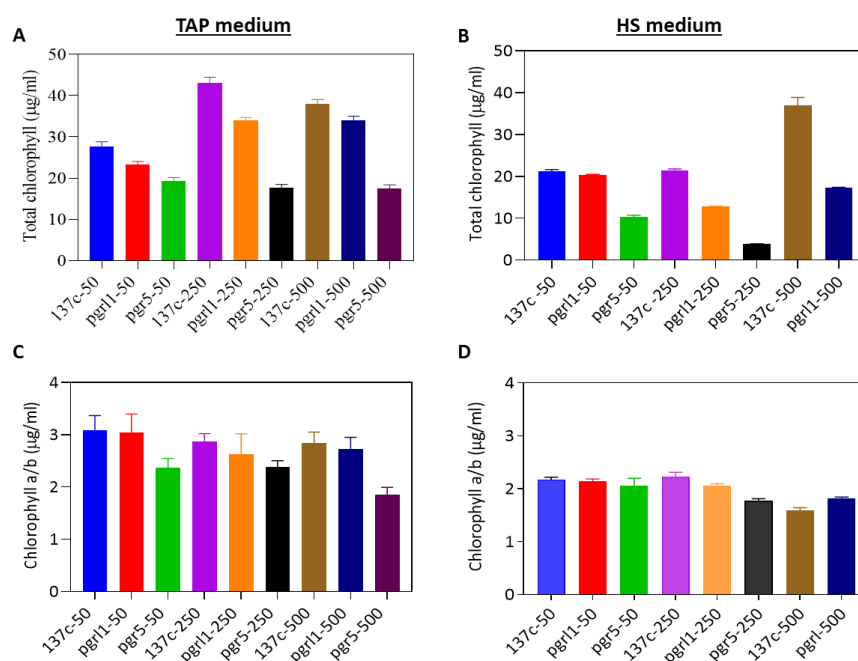


Figure 3.3 Photosynthetic pigment composition. The total chlorophyll content (A and B) and Chl *a/b* ratio (C and D) of fully-grown culture in TAP and HSM medium exposed to control and high light in WT (137c) and *pgrl1* & *pgr5* mutants. Bars show mean values $\pm$ standard error.

In autotrophic growth conditions, the Chl *a/b* ratio in 137c and *pgrl1* decreases to 28% and 15%, respectively, whereas *pgr5* failed to grow at high light (Figure 3.3D). The mid-log phase at the cells showed a

marginal change in Chl *a/b* ratio in high light conditions (Nama et al., 2019).

In the latter growth phase, I observed decreased trends in the Chl *a* and *b*, which is the leading cause of the decrease in chl ratio, especially in cells grown in high light in the HSM (Figures 3.4B and 3.4D). The cells grown in acetate Chl/ carotenoid ratio have increased with light intensity in *137c* and *pgrl1*, whereas it decreased in *pgr5* (Figure 3.5C). In autotrophically grown cells, the mutants displayed a reduced Chl/Car ratio with increasing light intensity (Figure 3.5D and Table 3.2).

Table 3.1 Chlorophyll /Optical density of the wild type (137c) and mutants (*pgr5*, *pgrl1*) in TAP.

Strain	50 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	250 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	500 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)
<i>137c</i>	10.60	13.99	17.10
<i>Pgrl1</i>	10.45	22.55	18.04
<i>pgr5</i>	12.75	15.21	10.03

Chlorophyll/Optical density of the wild type (137c) and mutants (*pgr5*, *pgrl1*) in HSM.

Strain	50 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	250 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	500 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)
<i>137c</i>	9.87	9.27	10.08
<i>Pgrl1</i>	14.24	7.23	8.46
<i>Pgr5</i>	10.32	5.70	–

Table 3.2 Total Carotenoid/Optical density of the wild type (137c) and mutants (*pgr5*, *pgrl1*) in TAP.

Strain	50 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	250 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	500 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)
<i>137c</i>	3.55	2.64	2.35
<i>Pgrl1</i>	3.58	4.24	5.62
<i>pgr5</i>	2.84	3.18	2.99

Total Carotenoid/Optical density of the wild type (137c) and mutants (*pgr5*, *pgrl1*) in HSM.

Strain	50 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	250 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)	500 ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$)
<i>137c</i>	6.79	2.40	8.70
<i>Pgrl1</i>	9.52	10.05	6.90
<i>Pgr5</i>	4.39	10.28	–

On the contrary, in mutants, the reduction in chlorophyll content was associated with an expected increase in the Chl *a/b* ratio (Terao et al., 1985). This indicates that the variation in carbon substrate can affect carotenoid biosynthesis, an essential mechanism for photoprotection. Interestingly, in autotrophic growth, the Chl/carotenoid ratio was influenced by the individual value of the total chlorophyll content, and the decrease was primarily observed in *pgr5* mutants (Figure 3.5D, Figure 3.3D). Change in Chl or carotenoid could be due to the generation of $^1\text{O}_2$ species, which may be significantly damaged by ROS, agreeing to the previous report (Triantaphylides et al., 2008).

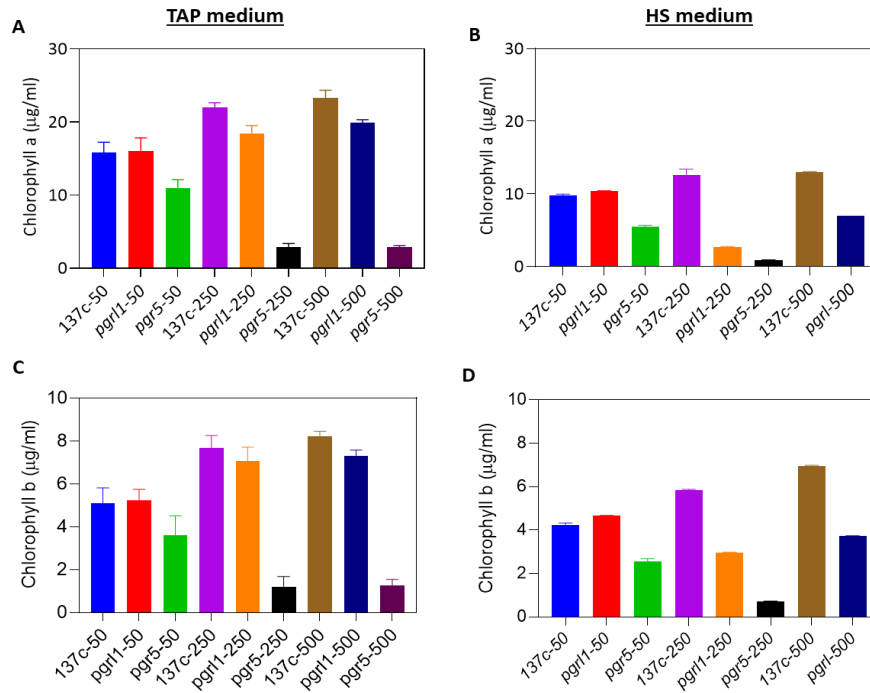


Figure 3.4 Photosynthetic pigment Chl a and b of WT (137c) and *pgr1*, *pgr5* mutant, the fully-grown culture of *C. reinhardtii* in TAP and HSM medium exposed to control/high light. Bars show mean values $\pm$ standard.

Interestingly, the increase of the individual value of carotenoid in wild type (137c) in both low and high light but the decrease in mutants (*pgr1* and *pgr5*) unevenly in TAP and HSM is determined probably by high light-induced oxidative damage (Figure 3.5A and 3.5B). Unlike $O_2^{\cdot-}$ and H_2O_2 is not detoxified through enzymatic reactions; it is quenched chemically or physically by various antioxidants (Triantaphylides and Havaux, 2009), such as carotenoids. Chemical quenching involves direct oxidation of the quencher.

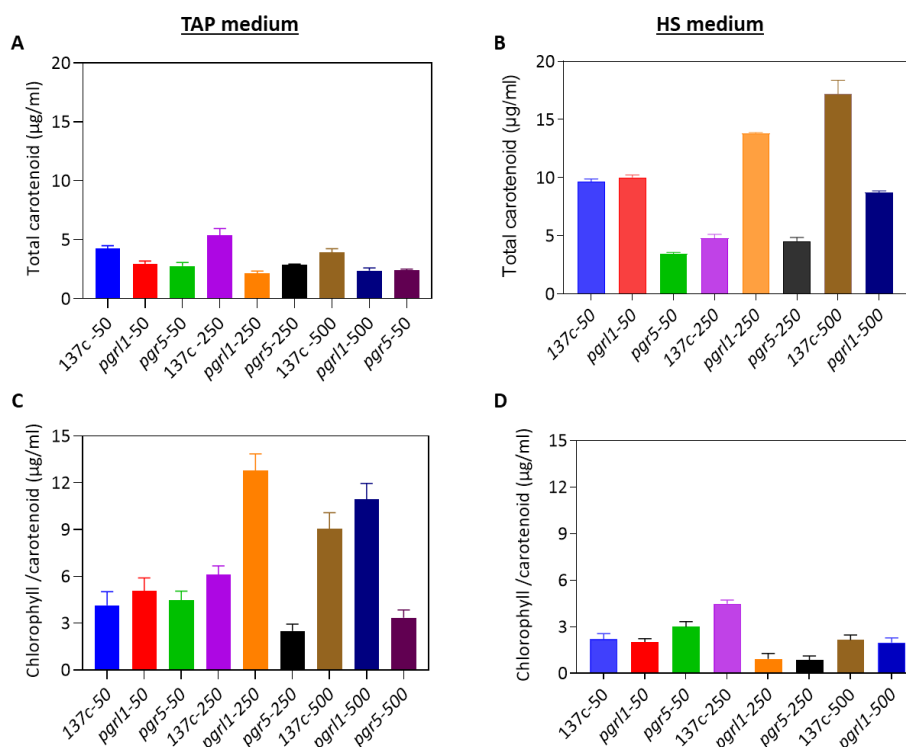


Figure 3.5 Total carotenoid (A and B) and chlorophyll/carotenoid ratio (C and D) of WT (137c) and pgr1, pgr5 mutant grown in TAP and HSM under 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ as control and HL (250, 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) intensities. Bars show mean values $\pm$ standard error.

The oxidation of α -tocopherol generates 8-hydro-peroxy-tocopherone, which requires a reduction to regenerate the quencher. Physical quenching occurs by transferring energy without oxidation, which is the primary mechanism of carotenoids (carotenes and xanthophylls).

The β -carotene is presumed to quench $^1\text{O}_2$ in the reaction center of PSII, which is the leading site of $^1\text{O}_2$ formation. However, in LHCs, xanthophylls quench 3Chl^* directly, thereby preventing $^1\text{O}_2$ generation (reviewed by Krieger- Liskay, 2005; Triantaphylides and Havaux, 2009). These two classes of lipophilic antioxidants, tocopherols, and

carotenoids, have been well studied and established for their antioxidant and photoprotective roles. Thus, the protection of cells and adaption/acclimation of the cells in WT and mutants could determine the ROS production, which is uneven in low/high light projected by an excess generation of carotenoids (Table 2).

However, these mutants (*pgr11* and *prg5*) are sensitive as they produce more ROS and less carotenoids. Also, it is reported that violaxanthin (Vio) and neoxanthin (Neo) were reduced while lutein (Lu) and β -carotene (β -C) showed different values in high light grown cells (Nama et al., 2019). These pigments are ultimately converted to zeaxanthin to protect the cells from high light stress from the NPQ mechanism (Jahns et al., 2009).

3.5 Chl *a* fluorescence induction and photosynthetic activity

Simultaneous chlorophyll fluorescence measurements were performed to analyze the status and performance of PSII photochemistry (Figure 3.6A). The Chl *a* fluorescence induction kinetics articulate the photochemical activity of PSII of oxygen-evolving organisms in a dark-adapted state inflexion (Govindjee et al., 2017). The induction curve shows characteristic feature that varies over time, i.e., (Papageorgiou, 1975) two transient phases which have been represented as a fast wave (up to hundreds of milliseconds) known as OJIP and a slow wave (seconds to tens of minutes) named as PSMT (Strasser and Srivastava, 1995; Kodru et al., 2015).

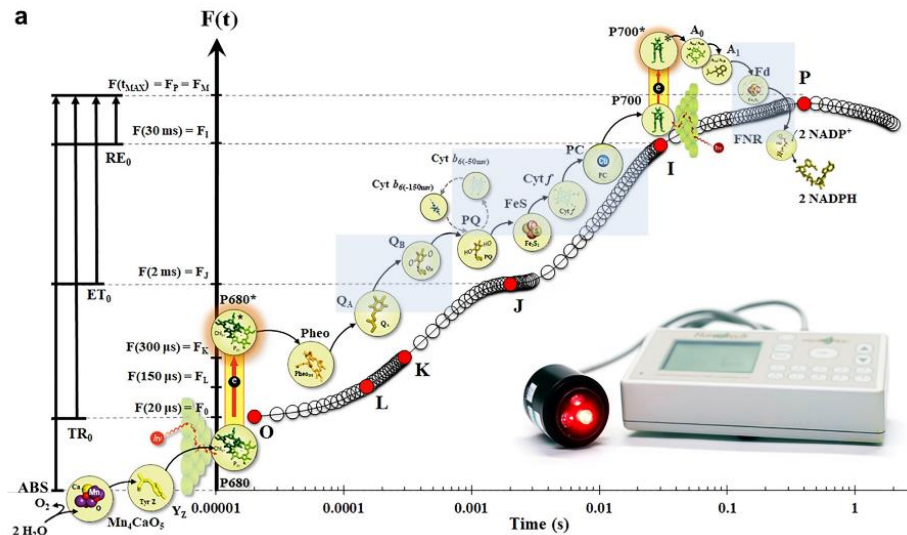


Figure 3.6 Possible relationship between chl *a* fluorescence transient curve and photosynthetic electron transport. O, J, I, and P are the starting points of the different phases, where O is for the origin (minimal), P for the peak (maximal), and J and I are for the intermediate stage.

The OJIP transients were measured, which are represented as follows: O, minimal fluorescence (F_0); O – J phase that reflects the reduction of Q to Q^- ; J – I and I – P phases involved in reducing the PQ pool and the electron acceptor side of PSI; at P, maximum fluorescence level (F_m), diminished electron transfer between the PSII reaction center and NADP (Kodru et al., 2015). As demonstrated in Figure 3.6, *pgr5* and *pgrl1* had a significantly more reduced PQ in darkness when compared to WT (137c).

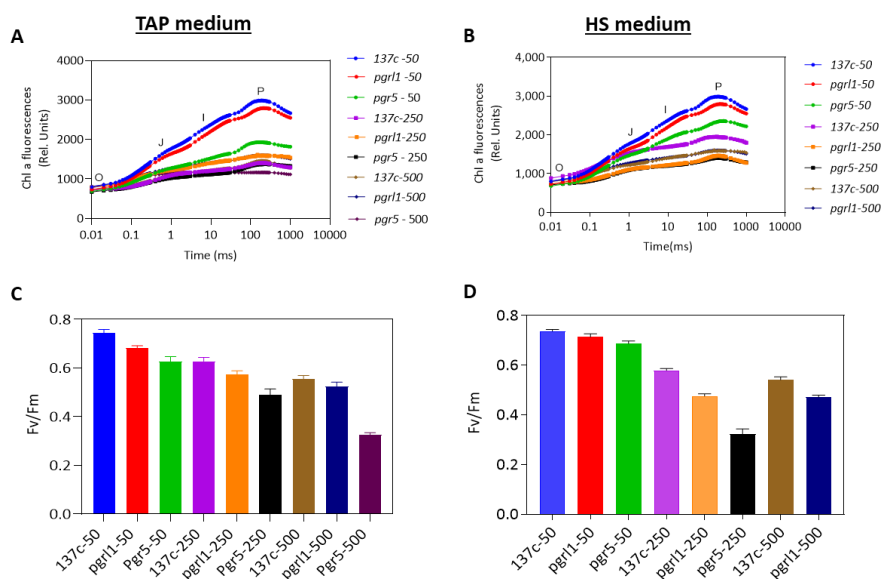


Figure 3.7 Chl a fluorescence transient (OJIP) and Maximum photosynthetic efficiency of PSII. The WT (137c) and mutants (pgrl1 and pgr5) cells dark incubated at 20 min room temperature followed by cultures, were excited by an array of three light-emitting diodes peaking at 650 nm at a photon flux density of $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ obtained as OJIP curve (A, and B) and Maximum photosynthetic efficiency of PSII denoted as FV/FM in both TAP and HS medium (C and D). The fluorescence intensity at J increases in pgrl1 and pgr5 with the increase in light intensity in three independent experiments.

Fluorescence results show the OJIP transient curves for all the high light-treated TAP and HSM cell samples. An increase of O - J phase in pgrl1 ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), J - I phase in 137c ($250, 500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), J - I phase in pgr5 ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), O - J - I phase in pgr5 ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and initial fluorescence (F_0) as represented in Figure 3.7A and 3.7B. The change of phases in each point is probably due to the sudden employment of a flash of high light

to the culture and the detachment of the core antenna from the reaction center (Stirbet et al., 2014).

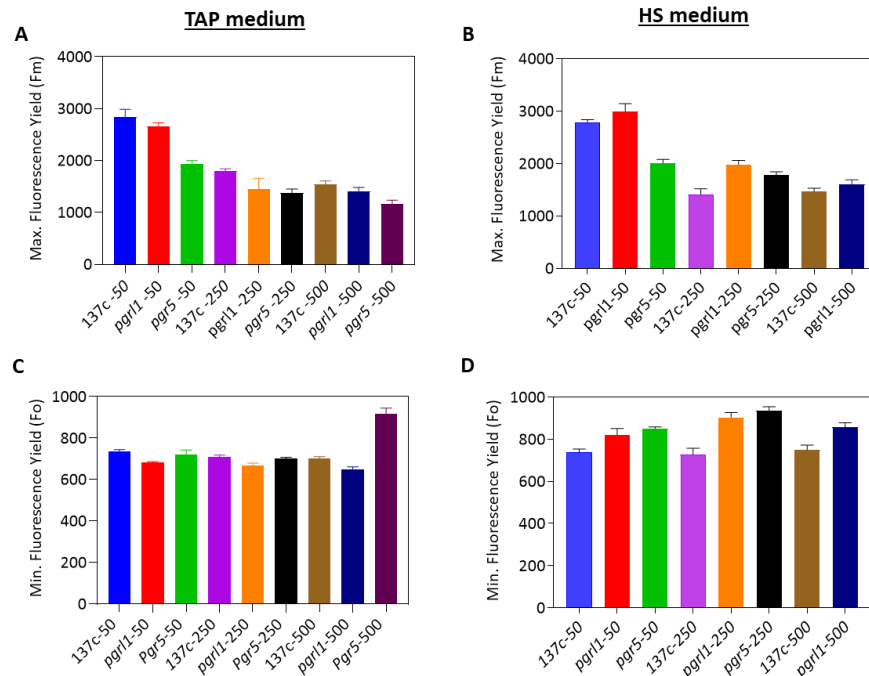


Figure 3.8 Maximum chlorophyll fluorescence (F_m; A and B) and Minimum chlorophyll fluorescence (F_o; C and D) during control (50 μmol photons m⁻² s⁻¹) high light (250,500 μmol photons m⁻² s⁻¹) grew cultures of WT (137c) and *pgr1* & *pgr5* mutants of *C. reinhardtii* in both HSM and TAP medium. Bars shows mean values ± standard error.

The difference in F_v/F_m at the J phase of the transient (F_J, 3 ms after the illumination) between dark-adapted and pre-illuminated culture indicates the PQ's redox state pool in darkness (Stirbet et al., 2014; Tóth et al., 2007). The CEF mutant had a more oxidized PQ pool in darkness than WT, while there was a significant difference between *pgr1*, *pgr5*, and WT, suggesting that the *pgr1*/ *pgr5* complex is the main CEF pathway contributes to the dark reduction of the PQ pool. The

differences in the dark redox state of PQ were also supported by a high dark-adapted apparent F_o value, a steeper initial slope of the transient, and a decreased area above the O - J and O - J - P phase of the OJIP transient in both *pgr5* and *pgrl1* when compared to WT (Tóth et al., 2007).

The increase of minimal fluorescence (F_o , Figure 3.8C and 3.8D) in high light in wild type and especially in mutant HS medium determines low photochemical yield under high light stress conditions. On the other side, the maximal fluorescence (F_m , Figure 3.8A and 3.8B) had much lower values in mutants, which may indicate a low PSII excitation cross-section and low fluorescence value; thus, this high light treated samples likely had a high to low fluorescence in darkness. All changes and differences (F_v/F_m) observed in WT (137c) grown in high light (250 & 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) were reduced to 20 to 30 % in both HSM and TAP, but this decline goes up to 35 - 50% in mutants *pgrl1* and *pgr5* (Figure 3.7C and 3.7D).

Even less F_v/F_m values in high light treated mutant *pgrl1*, and *pgr5* (500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) in TAP and *pgr5* (250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) in HSM show that even at low photochemical yield, the cells acclimatized to stress through various adaptive and photoprotective mechanisms. Changes in the photosynthetic efficiency are observed in the cells grown in varying growth conditions. Compared with WT and

pgrl1, the *pgr5* is extremely sensitive to the increase in light intensity and is more pronounced in autotrophic growth conditions.

3.6 Light induction curves of the cells grown under different growth conditions

The light induction curve parameters were measured under high light facilitated to assess the regulation of electron transport and photoprotective responses of WT (137) and mutants (*pgrl1* and *pgr5*) in HSM and TAP medium (Figure 3.9). Light-response curves have been widely used to estimate the photosynthetic parameters that provide information on the photosynthetic activity, the photo-acclimation state of photosynthetic organisms, and their driven processes. This curve mainly includes detailed information on electron transport capacity and limitation of the two photosystems by simultaneous fluorescence measurement that reflects the interplay of the light reactions of PSII and PSI connected via the electron transport chain.

Here, I compared the photosynthetic parameters for high light-grown cells (250 and 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ growth as control. In high light conditions, there is a marginal change observed in ETR(II) in 137c and *pgrl1* in 250 and 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ but reduced ETR (II) has been observed in both high light-grown cells in mutant *pgr5* and *pgrl1* of TAP and HSM medium (Figure 3.9A and 3.9B). These results, reflected in lower values of Y(II) in *pgrl1* and *pgr5* reduced to 30 to 40% in high light (250, 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) in both TAP and HSM, known to be a fraction of energy

that is photochemically used in PSII (Figure 3.9C and 3.9D) and the excess of light energy was dissipated. Most of the energy was converted into heat to pH-dependent regulation of energy conversion, which expressed a high NPQ.

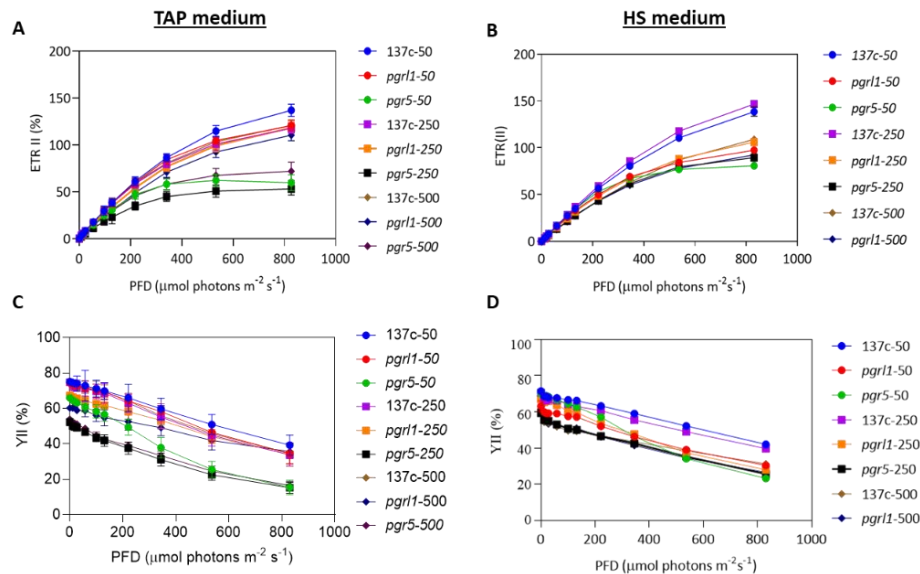


Figure 3.9 Results of simultaneous measurements of chlorophyll fluorescence and PSII photochemistry by Dual-PAM 100 system (Walz, Germany) performed in WT (137c) and mutants (pgrl1 and pgr5) both grown in Algae-Tron-230 under control and HL in both TAP and HSM. Electron transport rate (ETR; A and B) and Efficient quantum yield of PSII photochemistry (YII; C and D) were analyzed in three independent experiments. Error bars show mean values $\pm$ standard error among replicates ($n = 3$).

In wild type (137c), high NPQ was observed in the TAP medium, while low NPQ was observed in pgrl1 and pgr5 in high light-grown TAP and HSM cells; this could be due to the low capacity of energy quenching in high light (Figure 3.10A and 3.10B). NPQ is a process in which excess excitation energy reaching PSII is used for photochemistry and dissipated as heat non-radiatively (Derks et al., 2015). As I have

observed no significant differences in the activity of linear electron transport in comparison to other genotypes, I focused on cyclic electron transport around PSI, which plays a role in the buildup of the trans thylakoid proton gradient and is essential to keep NPQ high in extreme light conditions (Johnson, 2011; Shikanai, 2014).

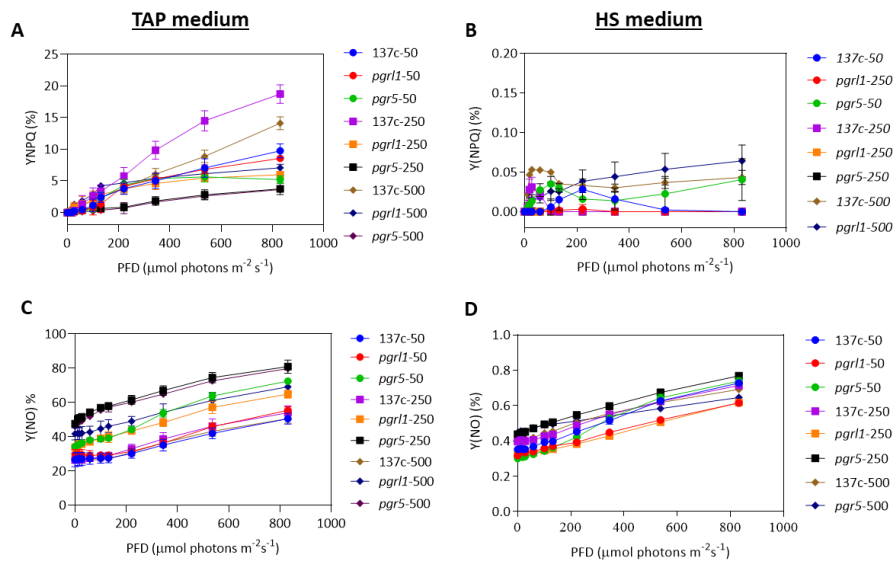


Figure 3.10 Quantum yield of non-photochemical quenching (NPQ; A and B) and quantum yield of non-regulated energy loss (NO; C and D) of control and high light grown cultures of WT (137c) and mutants (*pgrl1* and *pgr5*) *Chlamydomonas reinhardtii*. Error bars show mean values $\pm$ standard error among replicates (n = 3).

The fraction of non-regulated energy loss (NO) was increased within the expected range of 20-25% in the mutants, except for WT control in TAP and HSM. However, *pgrl1* and *pgr5* mutants grown at 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, in which higher values of Y(NO) indicate some insufficiency in the regulation of electron transport (Figures 3.10C and 3.10D). Generally, the high values of Y(NO) reflect the inability of a

plant to protect itself against damage by excess illumination (Klughammer and Schreiber, 2008; Zhao et al., 2017).

Our group also reported recently that high light (1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) induced NPQ and a low level of Y(NO) in WT in *C. reinhardtii* (Nama et al., 2019) in acetate medium. Moreover, the CEF mutant exhibits increased photosensitivity compared with the wild type. Therefore, the results suggest that besides NPQ, NO also plays a photoprotective role during high light acclimation despite a low growth rate. This low NPQ rate may be due to less influx of protons from the Q cycle in the case of *pgrl1* and *pgr5*.

3.7 NPQ kinetics measurement of cells grown under different light conditions

I have presented and discussed results on the kinetics of NPQ induction and its relaxation, as obtained by using a PAM instrument (Walz) for the different *Chlamydomonas* strains grown and tested under TAP medium, using both low light (50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and high light (250 and 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

Dark-induced NPQ revealed a clear induction of high NPQ under both high light intensities in wild type (137c) and was severely compromised in the *pgrl1* and *pgr5* mutant (compare the blue, green and red curves in Figure 3.11). The NPQ in the *pgr5* mutant was reduced approximately by 30-35 % of the total wild-type control (Figure 3.11 (A-C)). A similar finding was reported for the *pgr5* mutant in

Arabidopsis (Munekage et al., 2002) as well as the *Atpgrl1* (DalCorso et al., 2008; Hertle et al., 2013) and *Crpgrl1* (Tollete et al., 2011) mutants. The fluorescence quenching elicited by high light quickly reversed in the dark in both the wild-type and the *pgr5* mutant, which is typically observed for the energy-dependent or pH-dependent qE component of NPQ.

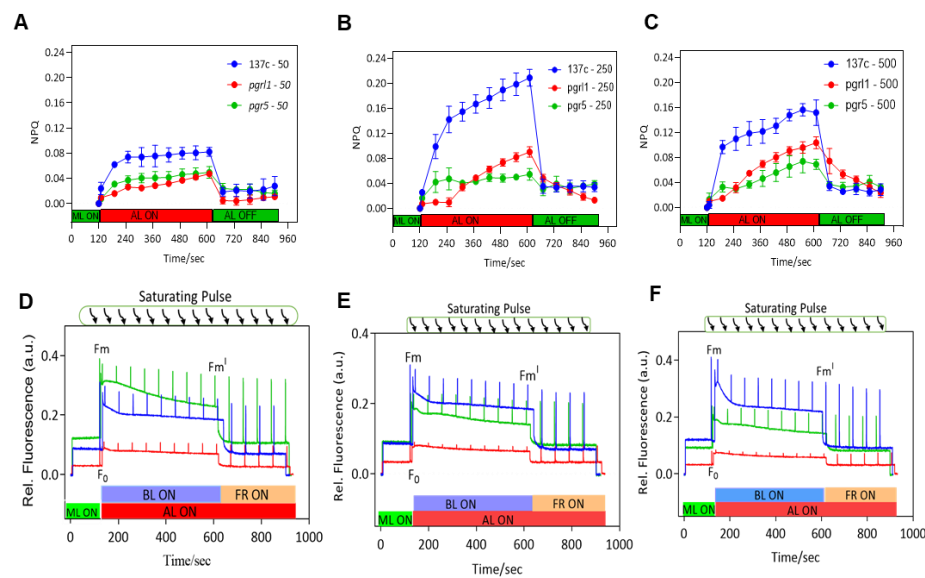


Figure 3.11 Chlorophyll a fluorescence data was obtained with Pulse Amplitude Modulation (PAM) instrument (Walz) in saturated pulse mode for four *Chlamydomonas* wild type and mutants (*pgrl1* and *pgr5*) strains grown in TAP medium under High light. Time course of NPQ induction (A-C) and chlorophyll fluorescence kinetics (D-F) in wild-type and mutants were dark-adapted for 40 min inducing state II conditions (complete oxidation). Subsequently, cells were treated with high AL for 10 min (Red bar indicating saturating pulse per minute) and recovered for 5 min, as shown on top of the blue bar on the x-axis. The dark adaptation and the measurements were done at room temperature. Bars show mean values $\pm$ standard error ($n = 3$ biological replicates).

After comparing, the 137c (250 and 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) showed a faster induction of NPQ but slower rate relaxation, indicating a complete reduction of the PQ pool and it's time for rearrangement of the antenna from state II to state I. I have then compared NPQ values of all the strains to determine whether the mutants improved NPQ quenching capacity or not. As expected, only the WT showed a high NPQ value (5.0 ~ 10.0) induced by high light, which is necessary for qE quenching (Figures 3.11 B and 3.11 C). I have obtained two significant findings; first, all three light (50, 250 and 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) WT showed a much higher NPQ value than the mutant cells (NPQ = 2.0 - 5.0). Second, WT showed a rapid increase in the level of NPQ from the dark-adapted state, which probably correlates with the state of de-epoxidation of the xanthophyll cycle pool in the thylakoid membranes. In higher plants, NPQ drops faster when actinic light is switched off.

In *C. reinhardtii*, switching the actinic light off caused a fast decrease in NPQ and a subsequent parameter increase during the dark incubation period. The maximum of 'dark NPQ' was reached after 10 min of darkness, and then a slow NPQ relaxation occurred in *pgrl1* and *pgr5* mutant (Figures 3.11B and 3.11C). The NPQ is calculated as $(F_m - F_m') / F_m'$, the increase in NPQ reflects the decrease in F_m' , such a decrease occurring in *C. reinhardtii* during incubation in darkness following actinic light exposure was described (Allorent et al., 2013). I confirmed the increase in the de-epoxidation state by high-performance NPQ levels observed in the WT relaxed quickly upon a dark transition to reduce the proton gradient across the thylakoid membranes; the most

significant part of the NPQ value observed here was from qE quenching.

As most of the fluorescence measured by PAM devices comes from PS II (Kalaji et al., 2017), the decrease in PS II excitation results in the reduction of F_m' measured (Figure 3.11 (D-F)). For the chlorophyll fluorescence kinetics measurement, when *C. reinhardtii* cells were grown under high light conditions, wild-type and mutant cells exhibited the differential fluorescence kinetic behaviour, which is shown in Figure 3.11 (D-F), that the dark-adapted minimum fluorescence yield (F_0) and the maximum fluorescence yield in the dark-adapted state (F_m) as measured by a click on the F_0 and F_m after 2 min before switch on the saturating light pulse (Figure 3.10, Red arrow) were the different set of fluorescence value in the wild-type (Figure 3.10 D-F, Blue colour) and the *pgrl1* and *pgr5* mutant (Figure 3.11 D-F, Green and Red colour).

However, chlorophyll fluorescence kinetics were still different, i.e., very high in *pgr5* than the wild-type control ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), but in high light (250 and $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), the lack of the chlorophyll fluorescence kinetics in both *pgrl1* and *pgr5* because of the lack photoprotection at PSI level, i.e., mutant of cyclic electron flow. The chlorophyll fluorescence kinetics of *pgr5* grown in high light in the acetate-containing medium were similar to the kinetics reported for *pgrl1* (Tolleter et al., 2011) that exactly matched with high light-grown (250 and $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) *pgrl1* and *pgr5* showing a lack of a transient rise in fluorescence after 2 min of illumination compared to the

wild type grown in high light.

My data shows that the maximum fluorescence values (F_m) in samples grown under low light were significantly higher than those grown under high light. This agrees with the fluorescence results presented in Figure 3.7 obtained with a Handy Pea instrument (Hansatech). Johnson et al., (2014) reported that the PGR5/PGRL1-Fd CEF does not symbolize a valve in electron transport as it only recycles electrons without being a sink: in acceptor side-limited conditions, which show an increase in Chl fluorescence observed when PGR5/PGRL1-Fd CEF is active. Interestingly, *pgr11* and *pgr5* behave differently in high light, but *pgr5* is more sensitive to high light. As mentioned above, the high light has more tendency to produce more ROS; hence, the *pgr5* produces more ROS and is damaged more specifically in photoautotrophic conditions.

3.8 Conclusion

Light is an essential component in regulating the growth and development of land plants and algae. *Chlamydomonas reinhardtii* is acclimatized to high light intensities when the cells are grown for longer durations and exhibit more growth and biomass.

This acclimation is mainly due to the dissipation of excess light through NPQ, which is one of the photoprotective mechanisms. Along with the NPQ mechanisms that provide sinks for extra excitation energy, multiple electron transport pathways potentially serve as alternative routes for electrons (or 'safety valves for photosynthesis) in excess light

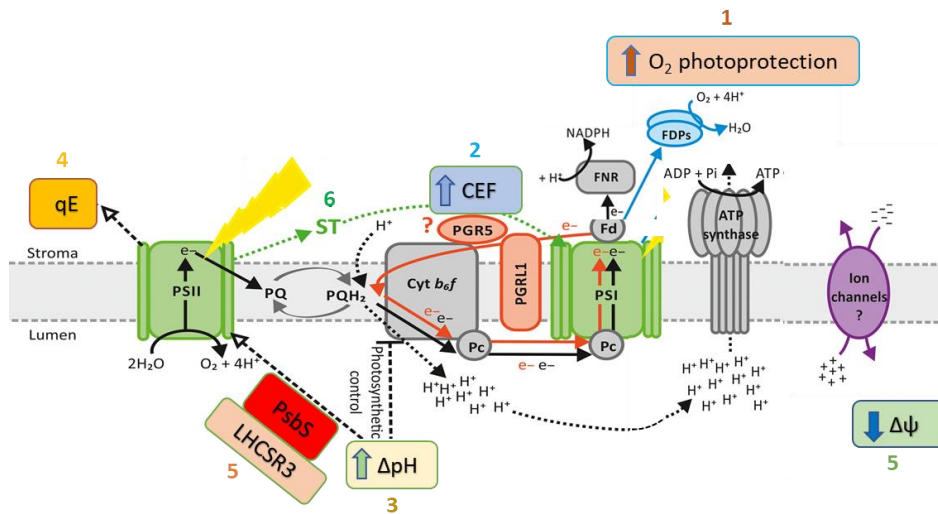
conditions.

The CEF through PGR5 and PGRL1 is crucial for lumen acidification (indirectly related to NPQ induction, i.e., photoprotection) and supplies a high amount of ATP required for the progression of the Calvin-Benson Cycle. Pigment analysis has shown that high value of total chlorophyll, Chl *a/b* and carotenoid in WT and low value compared in CEF mutants of *pgr11* and *pgr5* under excess light conditions. Also, an increased growth rate and increased NPQ can act as a sink for excess, reducing equivalents from the chloroplast. CEF and mitochondrial respiration may serve as a valve for electrons in the *pgr11* and *pgr5* mutants (Dang et al., 2014).

It is clear that the variation in the supply of carbon substrate impacts the growth and photosynthetic parameters. Under high light, the *pgr5* mutant failed to grow in autotrophic conditions, whereas the presence of acetate in the medium has rescued growth to some extent, but under high light heterotrophic conditions, it is interesting to observe that *pgr5* growth is reduced, which indicates the importance of this gene under high light. Thus, *pgr11* and *pgr5* are essential; however, *pgr5* is more vital for photoprotection.

CHAPTER – 4

Interplay between LHCSR3 and PSBS proteins plays a photoprotective role in pgr5 of C. reinhardtii under high light



Objective 2: Interplay between LHCSR3 and PSBS proteins plays a photoprotective role in *pgr5* of *C. reinhardtii* under high light

4.1 Introduction

Photosynthesis is the basis for most life on earth. Photosynthetic organisms have developed multiple photoprotective mechanisms and alternative electron transport pathways to adjust photosynthesis in high light. The two photosystems (PSII and PSI) are interconnected via a series of electron carrier systems to reduce the NADP^+ . The cytochrome (Cyt) b_6f complex functionally interconnects the two photosystems (Cramer and Hasan, 2016), accepting electrons from plastoquinol (PQH_2) and donating electrons to plastocyanin (PC) called LET. CET operates around the PSI and preferentially contributes to ATP synthesis (Allen, 2003). The CET in higher plants operated in three ways: (1) PGR5 dependent CET, (2) PGRL1 dependent CET, and (3) NADH dehydrogenase (NDH)-dependent CET pathways (Munekage et al., 2004). Proton gradient regulation 5 (PGR5) is involved in controlling photosynthetic electron transfer, but the CET route's detailed mechanism is still elusive. The *C. reinhardtii*, *pgr5*, and *pgrl1* also match *Arabidopsis pgr5* and *pgrl1*, signifying the role of these proteins in cyclic electron transport (Dang et al., 2014; Johnson et al., 2014). Interestingly, *Chlamydomonas* lack chloroplast NDH-complex (Maul et al., 2002), but a type-II NDH (NDA2) has been determined to perform the reduction of the PQ pool (Desplats et al., 2009).

The CET enhances photosynthetic control, a pH-dependent down-regulation of the PQ pool by Cyt b_6f complex. The CET mutants attributed impaired thylakoid lumen acidification and lack of photosynthetic control to enhanced stromal redox pressure (Alric et al., 2014). Subsequently, the donor and acceptor side limitations, the two regulative mechanisms prevent PSI photoinhibition and significantly contribute to photoprotection. Thus, the *pgr5* takes the electron from the PSI donor side to increase luminal acidification via Cyt b_6f , called donor-side regulation, Y(ND) (Suorsa et al., 2012). The downstream of PSI bears an electron sink which avoids the over-reduction of P700 by generating ROS called acceptor-side regulation, Y(NA) (Takagi et al., 2016).

The PGRL1 and PGR5 proteins are actively involved in the CET pathway, a backflow of electrons from Fd to the PQ pool with the help of the Cyt b_6f complex, generating a transmembrane proton gradient via pumping H^+ s into the thylakoid lumen (Hertle et al., 2013). Additionally, LET pumps extra protons into the thylakoid lumen coupled with CET to generate a proton motive force (*pmf*). Light-induced charge separation and coupled H^+ transfer into the thylakoid lumen create a *pmf* composed of transmembrane electric potential gradient ($\Delta\psi$) and H^+ concentration gradient (ΔpH). The *pmf* partitioning into ΔpH and $\Delta\psi$ can vary with the light environment, proposed as fine-tuning for photosynthesis acclimation (Avenson et al., 2005).

The mechanism by which *pmf* is partitioned and fine-tuned to achieve rapid photosynthetic acclimation is poorly understood. A critical factor is the ionic composition of stroma and thylakoid membrane counterions (Cl^- influx, Mg^{2+} , and K^+ efflux) electrically balance H^+ uptake into the thylakoid lumen (Hind et al., 1974). Hence, these ion fluxes would rapidly adjust the *pmf* partitioning by efficiently regulating the contribution of the transient electrical potential difference component ($\Delta\psi$). The *pmf* redistribution from ΔpH to $\Delta\psi$ downregulates the NPQ but leaves ATPase activity unaffected (Davis et al., 2017; Armbruster et al., 2017). NPQ is a mechanism acclimating to excess light that dissipates extra light energy into heat (Goss and Lepetit, 2015).

The CET-generated *pmf* has a photoprotective role as ΔpH -dependent energy (qE), quenching which signifies photosynthetic control (Kramer et al., 1999). Three aspects are necessary for qE: (1) De-epoxidation of xanthophylls cycle, (2) A low thylakoid lumen pH, and (3) the expression of PSBS/LHCSR3 proteins. The NPQ induction and relaxation kinetics are often constituted of qE and photoinhibitory quenching (qI) (from seconds to minutes), a regulatory mechanism that protects against photoinhibition (Müller et al., 2001). In fluctuating light conditions, the *pgr5* cannot activate qE quenching and is exceptionally prone to PSI photodamage (Mosebach et al., 2017; Jokel et al., 2018). The LHCSR3 and PSBS, NPQ-dependent proteins, characterize photosynthesis and photoprotection (Niyogi and Truong, 2013).

During land colonisation, PSBS is extant in plants and LHCSR in eukaryotic algae (Gerotto and Morosinotto, 2013). The moss *Physcomitrella patens* hold PSBS and LHCSR protein and together respond to extreme light conditions (Gerotto et al., 2011). The expression of LHCSR3 requires a chloroplast calcium sensor (CAS) pathway (Ca^{2+} signalling) for active photosynthesis (Petroutsos et al., 2011 & Maruyama et al., 2014). Still, its accumulation did not reinstate in light-sensitive phot mutant (Petroutsos et al., 2016). The earlier work showed that PSBS is transiently accumulating in *C. reinhardtii* in the HS medium (in the absence of acetate), but its accumulation and degradation are transiently regulated by CO_2 availability (Correa-Galvis et al., 2016b).

These proteins' expression and accumulation have not yet been reported in the TAP medium (acetate as carbon source). In this study, I have used three strains of *Chlamydomonas*, a WT, *pgr5*, and *pgrl1*, to understand the role of *pmf* in regulating the expression of LHCSR3 and PSBS under high light in an acetate medium. Here, I reevaluated the role of *pgr5* under high light and concluded that *pgr5* has a dysfunctional LHCSR3 protein. Further, I added the P515 measurement to access the role of *pmf* and trans-thylakoid pH gradient formation in varying actinic light intensities. I have also shown the role of each *pmf* component ΔpH and $\Delta\psi$ in *pgrl1* and *pgr5* in photoacclimation and photoprotection. Further, it demonstrated the regulative role of the donor and acceptor side of PSI in photoprotection.

4.2 Results

This study independently performed a relative analysis of the photoprotective mechanism from WT, *pgrl1*, and *pgr5* mutants of *C. reinhardtii* to assess the impact of these alternative electron transport pathways under high light. The functional interplay of these four proteins, PGR5, PGRL1, LHCSR3, and PSBS, has not yet been studied extensively, but its mechanistic role is decisive in photoprotection.

4.2.1 Photochemical properties changes under high light

I have grown the 137c (hereafter, the 137c will call it WT) and *pgrl1*, *pgr5*, and *npq4* mutants in different light conditions. The spot test result shows that WT grows typically, while mutants *pgrl1*, *pgr5*, and *npq4* are slightly reduced in optimal growth light in higher dilution (lower concentration; Figure 4.1A). However, the growth of WT was increased while mutants (*pgrl1* and *pgr5*) showed diminished growth under high light (Figure 4.1B). In the high light, there was a noticeable difference in growth among WT, *pgrl1*, and *pgr5* (Figure 4.2A). In high light, *npq4* mutant showed more biomass but reduced photosynthetic activity. I have analyzed the maximum P700 oxidation (Pm) level and maximum yield of PSII (F_v/F_m) from *C. reinhardtii* grown in three different light conditions. Here, I have normalized WT as 100% in optimum growth light for comparing the F_v/F_m and Pm (Actual values of F_v/F_m and Pm are shown in a separate table as Table 4.1).

In WT, the P_m is reduced by 19 and 25%, while F_v/F_m decreased only by 4 and 17% in moderate and high light, respectively (Figure 4.1C and 4.1D). However, the high-light grown *pgrl1* and *pgr5* cultures showed much lower P_m than optimal growth light conditions (Figure 4.1D). In constant moderate high light, the F_v/F_m was reduced by 16% in *pgrl1* and 24-25% in *pgr5* (Figure 4.1C), but at the same light condition, the P_m was reduced by ~52% equally in both *pgrl1* and *pgr5*, respectively indicating the importance of *pgrl1/pgr5* CET in high light (Figure 4.1D).

Spot test and photosystems photochemistry

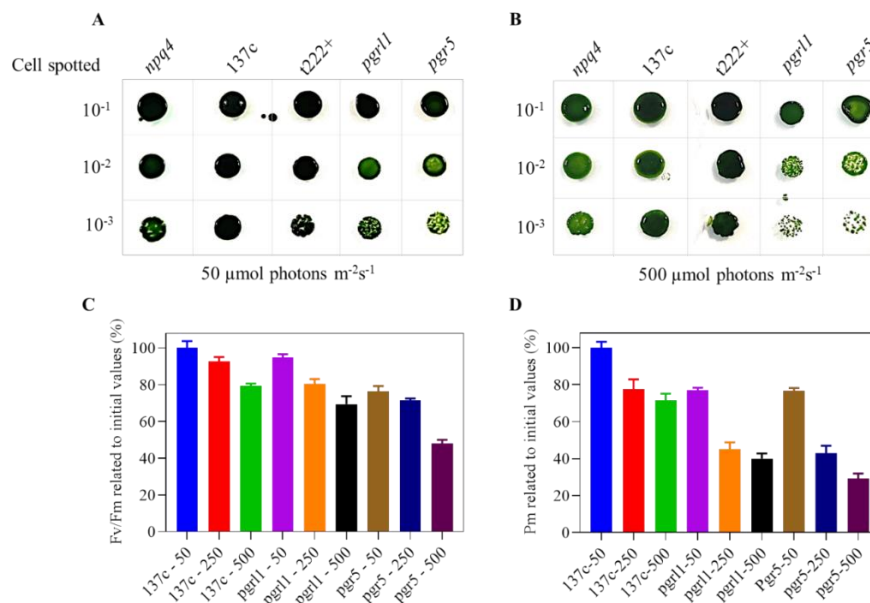


Figure 4.1 Spot test and PSII and PSI photochemistry. Effect of photoinhibition on CET mutant *pgrl1* and *pgr5*. Spot tests for growth of WT (137c, t222+) and mutants *pgrl1*, *pgr5*, *npq4* of *C. reinhardtii*. The cells were spotted with different dilutions (10^{-1} , 10^{-2} and 10^{-3}) grown for seven days in TAP medium at A, Growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and B, High light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. C, Light response curves showing the maximal quantum efficiency of

PSII measured as F_v/F_m , and D , the maximum level of the P700 signal of PSI (P_m , represents oxidation of P700), were measured at growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and treatment with constant moderate ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. After dark incubation for 20 min, all the measurements were taken, and error bars indicate the mean $\pm$ SD ($n = 4$). This experiment was repeated independently with similar results.

Culture growth and PSII (F_v/F_m) and PSI (P_m) photosynthetic activity (Real value)

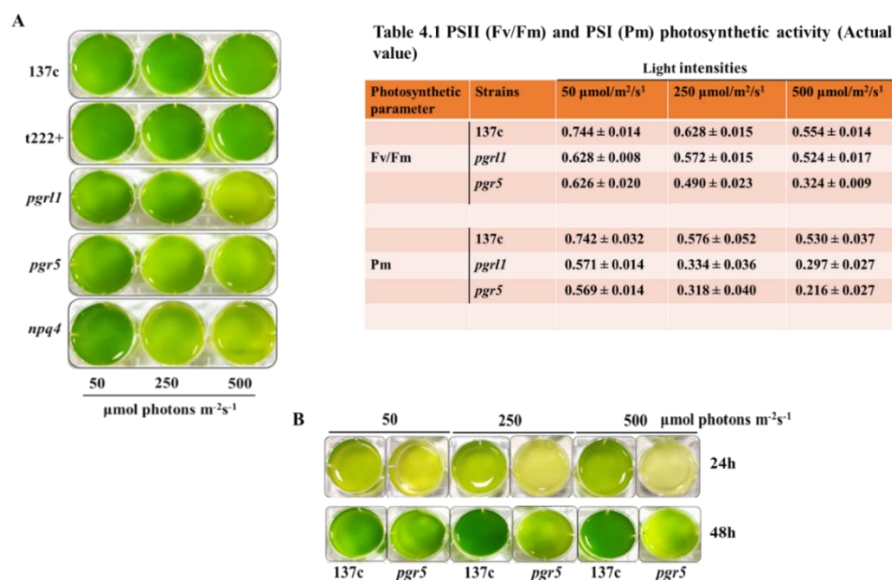


Figure 4.2 Phenotypic Growth and photosynthetic parameter in TAP medium. A, Heterotrophic cell suspension cultures growth of wt (137c, t222+) and mutants pgrl1, pgr5, npq4 strains of *C. reinhardtii*. B, represents PSII (F_v/F_m) and PSI (P_m) photochemistry (actual values) and C, represents 24 h and 48 h cell culture growth at constant growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions.

On the other hand, the F_v/F_m decreased by 27%; however, the P_m reduced by 56% in pgrl1 under high light. In high-light grown pgr5

cells, Fv/Fm was reduced by 48%, and Pm decreased significantly, by 67%, indicating that the PGR5-dependent CET is more susceptible than the *pgrl1*-dependent CET in high light.

4.2.2 High light induces donor side limitation in *Pgrl1* and *pgr5* mutant

I have studied the PSI and PSII photochemistry by measuring chlorophyll fluorescence and P700 absorption changes in experimental light conditions (Figure 4.3). The P700 absorbance change provides four different parameters, which are: -1) PSI Y(I) yield represents P700, 2) ETR(I) represents electron transfer within the PSI, CET, or transfer electrons Fd to NADPH⁺, 3). In contrast, acceptor-side limitation Y(NA) signifies the reduced fraction of P700, which could not share the electrons to Fd, and 4) Y(ND), indicates donor-side limitation, i.e., the extreme buildup of electrons at the Fd side.

The ETR I was less affected in the mutant than WT under optimal growth light conditions (Figure 4.3A). The ETR (I) was reduced by 77 and 82% in *pgrl1* and 87 and 95 % in *pgr5* in moderate and high light though increasing the AL from 0 to > 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.3A). However, in WT, the ETR (I) decreased by 45% in high light but a notable increase by 43% at moderate-high light than in optimal growth light conditions.

Though I correlate, PSI yield with electron transport rate, the *pgrl1* and *pgr5* defects strictly reduced the electron carriers of ETC (Electron transport chain). In WT, immediately after the onset of actinic

illumination, the $Y(I)$ increased by 13% in moderate light, whereas it decreased by 19% in high light compared to optimum growth light.

Energy partitioning in PSI

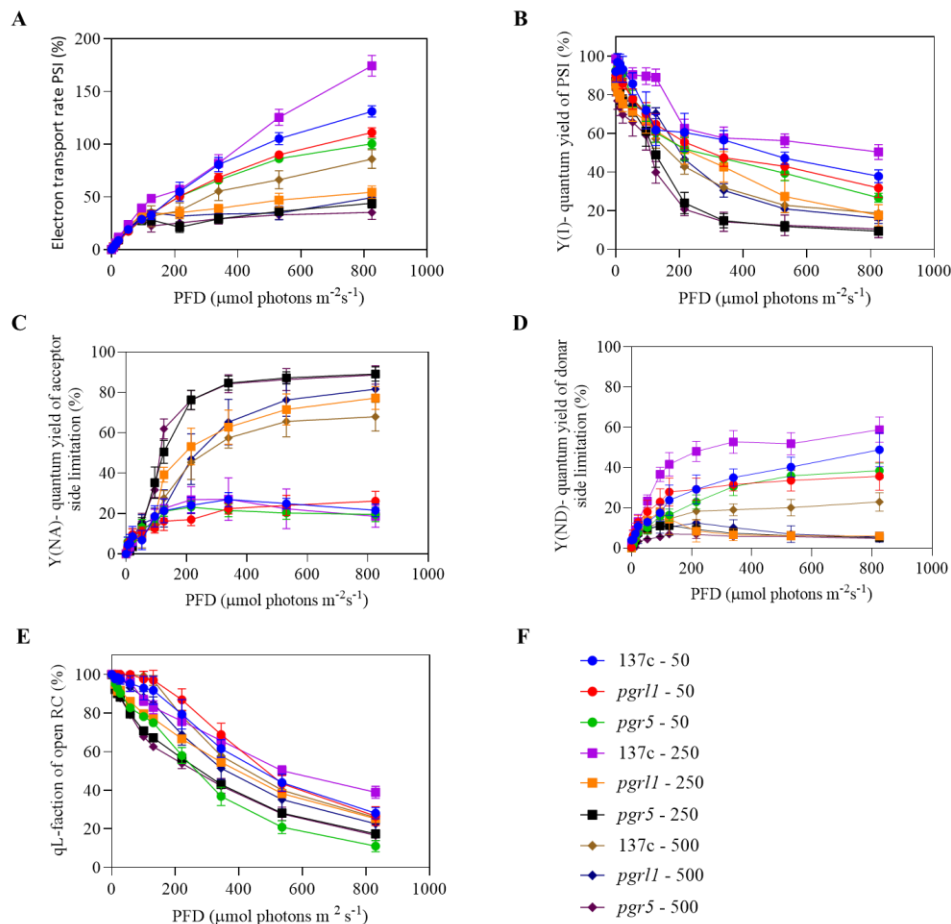


Figure 4.3 Light-dependent changes of PSI photochemistry in *C. reinhardtii*. The light intensity dependence of PSI and PSII photosynthetic parameters was monitored in the wild-type (wt) 137c, pgrl1 and pgr5 mutant cells were exposed to constant growth light, moderate, and high light. A, The ETRI are the effective electron transport rate of PSI. B, $Y(I)$ is the quantum yield of PSI. C, $Y(NA)$ is the PSI acceptor-side limitation. D, $Y(ND)$ is the PSI donor-side limitation. E, qL represents oxidation of the PQ pool was detected simultaneously, and F, represents the abbreviation of all the strain's growth parameters.

The Y(I) levels were diminished approximately by 27% in *pgr5* and 20% in *pgrl1* in moderate and high light, respectively, than WT optimum growth light at the end of AL illumination from 0 to > 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.3B).

Interestingly, the Y(ND) increased by 10% in moderate light-grown cells of WT illuminated during AL from 0 to 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

On the contrary, Y(ND) was reduced severely by 42 - 43% in *pgrl1* and *pgr5* in moderate and high light than WT control light (Figure 4.3D).

The decrease of Y(ND) in mutants might be the low operation of photosynthetic control via the Cyt b_6f and the reduction of proton motive force in the thylakoid lumen. However, it is difficult to differentiate the electron between the donor and the acceptor's side. In contrast to Y(ND), the oxidized P700, Y(NA)) is always higher in *pgrl1* and *pgr5*, showing the accumulation of electrons or the absence of electron sink towards the PSI donor side. I observed slightly lower Y(NA) levels ~18 - 20%, similar to WT at moderate and growth light conditions.

The high light grew WT showed higher Y(NA) levels, increased by 46%, and this increase was even higher, i.e., ~80% in *pgrl1* and ~89% in *pgr5*, when actinic light illumination from 0 to 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.3C). The oxidation state of the PQ pool represents by qL (a fraction of the open reaction centre). At the onset of initial PAR light intensity (from 0 to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the fraction of the open reaction centre (oxidized state) was higher in WT, *pgrl1* than *pgr5* in optimal growth conditions (Figure 4.3E). In high

light conditions, the qL was decreased by approximately 4 - 5% in WT and *pgrl1*. Whereas in moderate and high light, qL was reduced by ~10% in *pgr5* compared to WT growth light at the end of the exposure of PAR light from 0 to > 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Figure 4.3E).

4.2.3 P700 oxidation kinetics has severely decreased in *pgrl1* and *pgr5* high light

The P700 kinetics showed maximum oxidation of P700 in response to the illumination of rSP (repetitive short-pulse). In excess light, the P700 always tries to keep all the electron carriers in an oxidized form to adjust the light utilization and disperse the extra excitation energy from PSI (Shimakawa et al., 2018). The LET or CET electrons reduced the photo-oxidized P700 (P700^+) via the Cyt b_6f complex and PC (Jensen et al., 2007). The oxidation and reduction value varies between 0 (all the RCs in an open state) and 1 (RCs in the closed state), which is practically impossible, as shown in Figure 4.4, where some fraction is already oxidized before AL illumination. This fraction is narrowed down in high light-grown cells, either WT or *pgrl1* and *pgr5*. Even in WT, the P700 oxidation was highly decreased in high light.

A minor decrease at moderate light than the WT growth light conditions, indicating the corresponding relation between the electron source and sink, promotes oxidation of P700, thus avoiding PSI photoinhibition (Figure 4.4G). The PGR5 and PGRL1 are proposed to be significant constituents of PSI-CET and are essential for the oxidation of P700. The P700 oxidation was decreased significantly in *pgr5* and *pgrl1* in high light compared to WT in growth light conditions

(Figure 4.4). The *pgr5* and *pgr11* are reduced severely under high light and cannot keep P700 oxidized for a more extended period, delicately controlled by several types of molecular machinery on PSI donor and acceptor sides.

Kinetics of oxidized P700 induced by an rSP

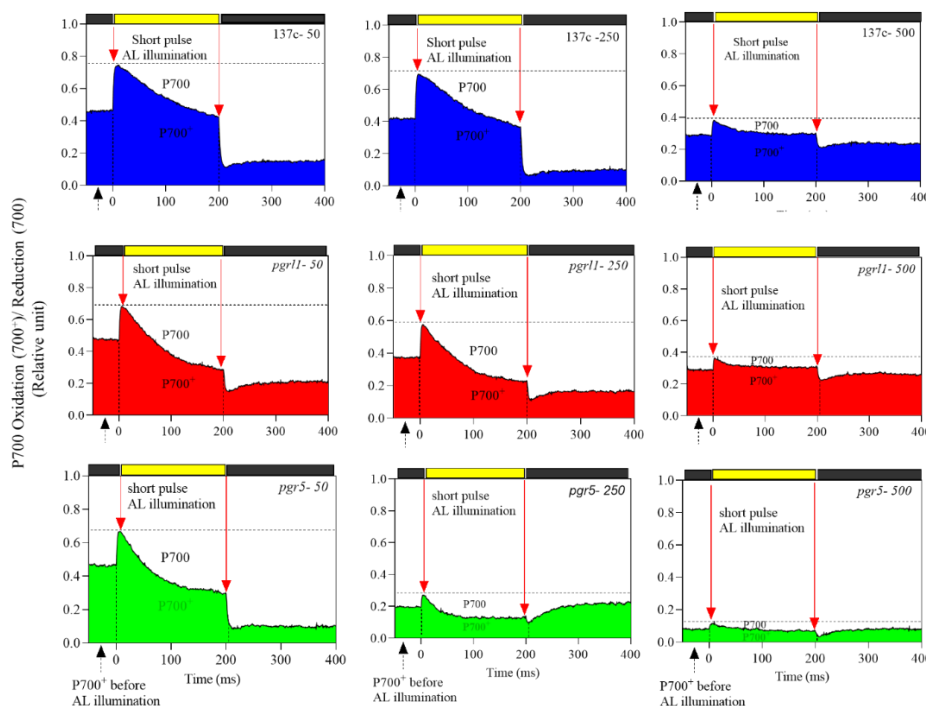


Figure 4.4 represents the functional kinetics of P700 oxidation induced by a short rSP. The P700 already oxidized before SP illumination, i.e., $P700^+$ before AL, is shown below as a black arrow upward. rSP illumination further oxidizes P700 to its maximum and lower than the oxidation obtained in the dark (before AL), showing P700 reduction (white area) accumulates under the steady-state AL illumination. However, oxidized $P700^+$ produced during SP illumination is not entirely reduced (coloured shaded area during rSP) because P700 in PSI experiences turnover with the rate-determining step of $P700^+$ reduction in the P700 photooxidation reduction cycle. The Y-axis shows the oxidation ratio of P700 from 0 to 1, and the X-axis shows the time for SP illumination: (Red arrow: indicates SP illumination starts and end; Black downward arrow: indicates P700 reduction after SP illumination) by rSP (red arrow from the yellow bar below the tracing) to generate total photo oxidizable P700 ($P700^+$) and

reduced P700 after the end of the rSP (black bar). Experiments were performed at least three times. Short-pulse was illuminated every 10 s, and average data over three short-pulse illuminations are shown. All measurements were taken after 20 min of dark adaptation with continuous stirring. Error bars indicate the mean $\pm$ SD ($n = 4$).

These results indicate the electron accumulation imbalance on PSI's acceptor side due to the absence of electron sink in *pgr5* and *pgrl1* under high light. I can see that in mutant *pgrl1* and *pgr5*, PSI acceptor side Y(NA) is hampered due to the absence of electron sink towards the PSI donor side, indicating PSI photoinhibition (Figure 4.3C).

Light response-based quenching parameter and Non-regulated energy dissipation

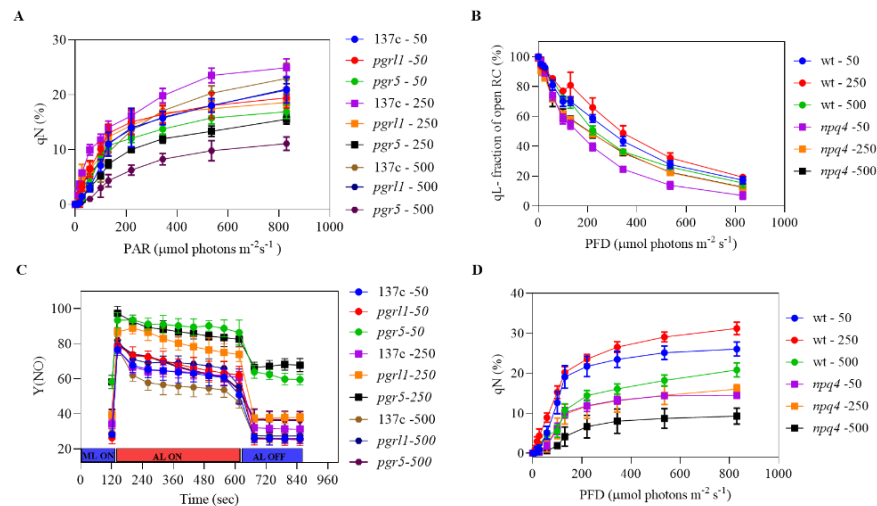


Figure 4.5 NPQ induction kinetics were measured at room temperature. A, The Pulse-amplitude fluorometric-based regulated time-course analysis of NPQ at room temperature of *npq4* and *pgr5* mutants on 20 min dark-adapted samples. B, representative blot showing a lack of LHCSR3 expression in *npq4* and *pgr5* and PsaF served as the loading control. C, represents non-regulated energy dissipation Y(NO) and D,

light-response curve based non-photochemical quenching (qN), calculated in 137c, pgrl1 and pgr5 at growth light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate (250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Values represent means $\pm$ SD ($n = 3$).

The PSI inactivation in *Synechococcus* mutant (sp. PCC7002) has been detected between 200 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ that generated ROS is 10 times smaller than direct sunlight (Shimakawa et al., 2016b). The PSI acceptor side is the leading site for ROS, which is the basis of PSI oxidative damage (Asada, 2000; Shimakawa et al., 2018). Further, I measured the qL in *npq4* mutant and showed that it decreased severely in high light than the WT at the early start of AL from 0 to 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.5B).

In *npq4* mutant, qL decreased $\sim 5 - 6\%$ in moderate and high light than WT at optimal growth light, increasing the AL from 0 to $>800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.5B). It indicates that nearly all the reaction centres are closed at the end of the exposure of AL $> 800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ because there is no NPQ in the *npq4* mutant. Previously reported that qL was partially reduced in *npq4* mutant and led to a reduction of the Q_A redox state in *A. thaliana* (Li et al., 2002), in rice (Yamori et al., 2015) and *M. polymorpha* (Ueda et al., 2012).

4.2.4 High light-induced LHCSR3 and PSBS protein regulate the qE component of NPQ

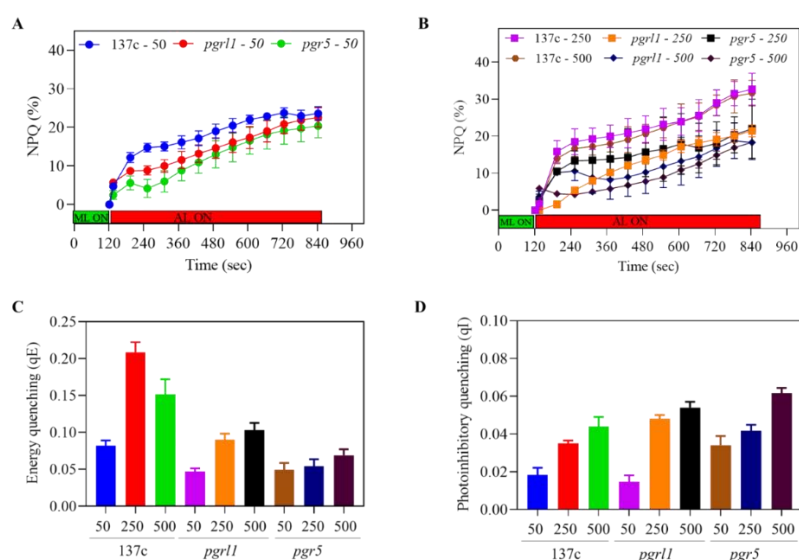
To characterise the potential defects in *pgrl1* and *pgr5* in the regulation of photosynthesis, samples were dark incubated for 25 min and exposed to high AL to observe a striking perturbation of NPQ kinetics. At optimal growth light, the NPQ showed a minor decrease in *pgrl1* and *pgr5* than the WT (Figure 4.6A).

As expected in WT, there is a rapid increase of NPQ within 30-45 s exposure to AL and a maximum of 30 - 34% in moderate and high-light conditions (Figure 4.6B). Conversely, in *pgrl1* and *pgr5*, the NPQ was augmented initially but failed to reach a similar level as the WT grown in high light conditions (Figure 4.6B).

Additionally, I measured the light-response based 'non-photochemical quenching' qN parameter, which indicates that the thermal dissipation of excitation energy was increased maximum in the WT, almost by 25% and 23% at moderate and high light, respectively (Figure 4.5A). However, it reached only by 20% in *pgrl1* and ~15% in *pgr5* moderate and high light, as observed similar to NPQ measurement (Figure 4.5A and 4.6B). On the other hand, *npq4* mutant, qN increased by 30% in moderate high light and 22 - 24% in WT at optimum growth light. But in high light, qN was reduced by 20% in WT and 10% in *npq4* mutant when increasing the PAR light from 0 to > 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.5D).

The persistence of qE and qI components of NPQ was made possible by their induction and relaxation kinetics by switching on/off AL illumination. The initiation and loss of photoinhibition-associated quenching is a composite process that happens minute-to-hourly. A decrease of NPQ components in *pgr5* and *npq4* displays the importance of Δ pH-dependent photosynthesis in high light. I observed the highest qE (non-photochemical induction of energy quenching) in WT grown in moderate high light, and diminished qE was exhibited in *pgrl1* and *pgr5* mutants (Figure 4.6C). The *pgr5* fails to develop qE and is highly prone to photodamage in moderate light (Johnson et al., 2014; Mosebach et al., 2017) and fluctuating light (Jokel et al., 2018).

NPQ induction kinetics and its components Energy-dependent quenching (qE) and Photoinhibitory quenching (qI)



Light-harvesting stress-related protein (LHCSR3) and time- based PSBS analysis

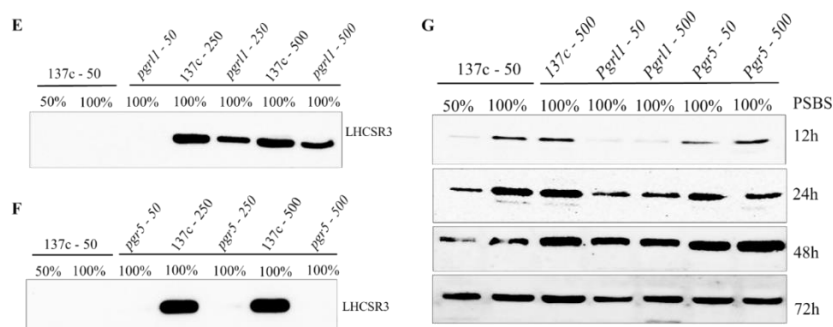


Figure 4.6 Photochemical performance of PSII measured by chlorophyll fluorescence in *C. reinhardtii*. NPQ induction was estimated at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ of actinic light illumination for 15 min at continuous A, growth light and B, moderate and high light. Blue bars represent dark along with ML, and the red bar represents actinic light illumination. The NPQ induction/relaxation kinetics with continuous actinic light for 10 min gives C, Energy-dependent quenching (qE), and in the absence of actinic light for 5 min, relaxation offers D, Photoinhibitory quenching (qI) were calculated. High light-induced stress protein LHCSR3 in E, pgr11 and F, pgr5 mutant under growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions and G, represents the time-based expression of PSBS protein from 12 h to 72 h interval in 137c, pgr11 and pgr5 under growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The values shown here are the mean of three biological replicates (mean $\pm$ SD).

Another NPQ relaxing component, qI (photoinhibitory quenching), was lower in WT and *pgr11* but increased in *pgr5* in optimal growth light. However, the *pgr11* and *pgr5* possess a higher photoinhibitory quenching (qI) in high light conditions (Figure 4.6D) accompanied by PSII photodamage (Krause and Weis, 1991). Any

precise mechanisms do not explain the Y(NO) contribution. Nevertheless, the Y(NO) persisted higher in *pgrl1* and *pgr5* but decreased in WT under high light (Figure 4.5C), indicating the inability of PSII to utilize the incident radiation for photosynthesis (Kramer et al., 2004). Further, I immunoblotted the photoinducible protein LHCSR3 to correlate with NPQ data in high light conditions.

The NPQ data shows that LHCSR3 protein accumulated in the WT and *pgrl1* mutant in high light (Figure 4.6E). In contrast, I was surprised that the high light grown *pgr5* mutant lacked complete LHCSR3 protein in the TAP medium (Figure 4.6F) but accumulated in the HS medium in moderate light ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, Figure 4.10D) conditions. Additionally, I verified another photoactive protein, PSBS, primarily present in higher plants and mosses at different time points (from 12 h to 72 h) in optimal growth and high light conditions. Then, I saw that the accumulation of PSBS protein was continuously increased in *pgr5* mutant, similar to WT, from 12 h to 72 h in optimal growth and high light conditions (Figure 4.6G).

However, in growth and high light conditions, the PSBS protein accumulation was much higher in *pgr5* than in *pgrl1* (Figure 4.6G). The earlier reported PSBS for NPQ induction in plants (Li et al., 2000). However, its role in photoprotection in *C. reinhardtii* is limited in the HS medium (Correa-Galvis et al., 2016b). Recent reports show that in *C. reinhardtii*, the PSBS was expressed in the early stage of short-term

high light (Allorent et al., 2013; Tibiletti et al., 2016) and under UV-B treatment (Allorent et al., 2016).

4.2.5 Induction and relaxation kinetics component of NPQ and quenching parameter

Further, I confirmed the absence of LHCSR3 in *pgr5* blotted with *npq4* mutant, which lacks LHCSR3 expression (Figure 4.7B). The *npq4* mutant showed similar NPQ (reduced NPQ) as *pgr5* in growth and high light condition (Figure 4.7A). So, I can say that *pgr5* and *npq4* mutant showed a clear relationship between the LHCSR3 expression and NPQ induction. At the same time, the *npq4* was faulty in qE and showed a lower NPQ than the WT in high light (Figure 4.7C). In contrast, the qE was about 11% and 25% in *npq4* in moderate and high light, respectively (Figure 4.7C).

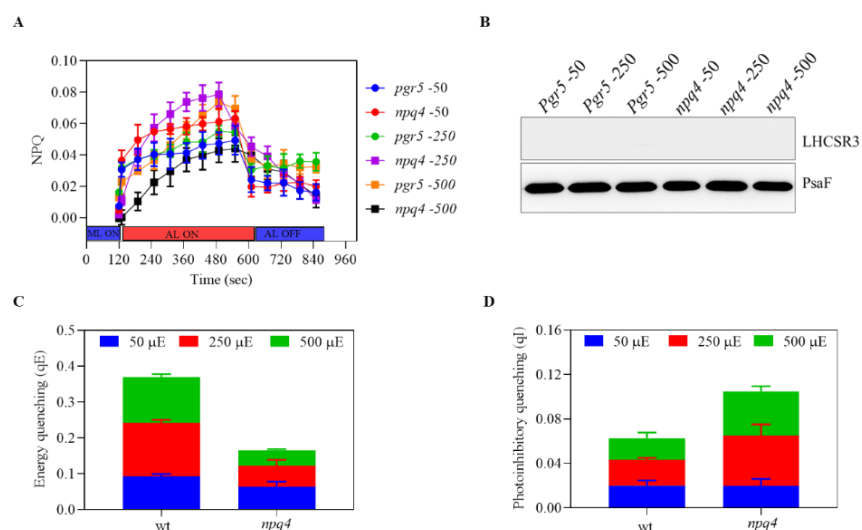


Figure 4.7 Non-photochemical quenching; mechanisms are divided into qE and qI according to their induction/relaxation kinetics. A, Energy quenching (qE); B, Photoinhibitory quenching (qI); C, light response curve-

based qN , a non-photochemical quenching component and D , qL represents oxidation of the PQ pool was calculated in wt and *npq4* mutant in growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. Error bars indicate the mean $\pm$ SD ($n = 4$).

The LHCSR3 deficient *npq4* was the utmost prone to photodamage, and the qI was described for approximately 25% and 50% in moderate and high light, respectively, than growth light (Figure 4.7D). The reduction of the qE component in *npq4* throughout the growth may indicate that some other factors, either photoinhibition or electron transport carrier, are restrictive in high light.

4.2.6 Importance of *pmf* for ΔpH -dependent photosynthesis, which controls optimal PSI activity in *pgrl1* and *pgr5*

A readily measurable proxy of ΔpH across the membranes is called NPQ. Though, NPQ relies on the luminal pH and PSBS (Tian et al., 2019) or LHCSR3 proteins. I measured the dark-adapted electrochromic pigment shift (ECS, P515) to authenticate the NPQ as a representation of ΔpH . ECS represents the dark-light-dark analysis, the difference in absorbance at 550 - 515 nm signal (ECS signal) corresponding to the total *pmf* formed under illumination (Bailleul et al., 2010). I have measured the P515 ECS signal from different AL to mimic the respective light intensities grown for our experiments, i.e., AL $53 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, AL $256 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and AL $553 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The *pgrl1* and *pgr5* are defective in PSI CET, primarily

caused by the reduced qE, the component of NPQ (Figure 4.6C), and secondarily less *pmf* was observed (Figure 4.8A).

In WT, the magnitude of *pmf* was large in AL of 53 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (0.205), reduced by approximately half (0.109) in AL of 256 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and highly reduced (0.052) in AL of 553 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In *pgr11*, the *pmf* was 0.143 in AL 53 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 0.073 in AL 256 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and further decreased by 0.042 in AL 553 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.8A). In *C. reinhardtii*, the qE component of NPQ depends on the content of LHCSR3 protein, whereas its expression and function depend on thylakoid lumen acidification.

Slow dark-light-dark induction transients of P515 (550 – 515 nm difference signal) measurement for *pmf* partitioning into ΔpH and $\Delta\psi$ components

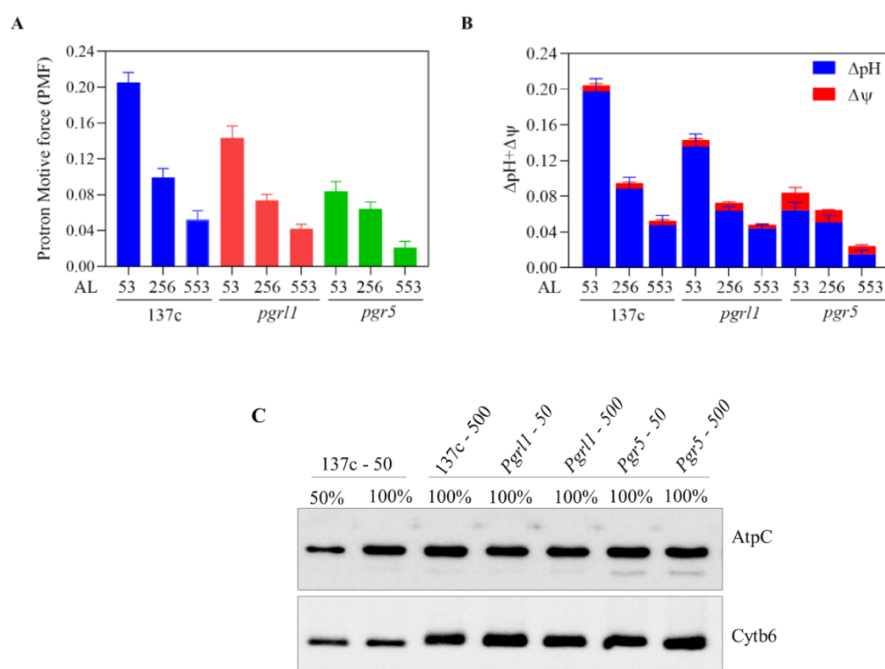


Figure 4.8 P515 Measurement for total *pmf* and its components (ΔpH and $\Delta \psi$) generation in CET deficient mutant *pgrl1* and *pgr5* in varying AL intensity. A, represents total proton motive force (*pmf*), B represents *pmf* components ΔpH and $\Delta \psi$ together were calculated by *C. reinhardtii* cells were dark-adapted for 20 min before the recording of rapid P515 induction/ relaxation kinetics for 10 min of continuous actinic light followed by switching off the actinic light and after the 30s to calculate both the components of *pmf*. ECS signals were analyzed at different AL 53, 256 and 553 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, mimicking all growth light conditions. C, Immunoblot analysis changes in AtpC and Cyt b6 protein content in wt, *pgrl1* and *pgr5* under constant growth light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

However, *pgr5* lacks LHCSR3 accumulation in high light (Figure 4.6F) but induced significant *pmf* (0.083) in AL 53 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

In contrast, it decreased by 0.064 and 0.021 in high AL 256 and 553 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively, in *pgr5* mutant (Figure 4.8A). Usually, ΔpH increases by an increase in high light illuminations for a certain period; afterwards, it diminishes via downregulation of Cyt b₆f activity via photosynthetic control (Y(ND)), leading to the reduction of the PQ pool. The ΔpH (ΔpH_{ECS}) and $\Delta \psi$ ($\Delta \psi_{\text{ECS}}$) components can be extracted from total *pmf* (Cruz et al., 2001). The total *pmf* drives protons through ATP synthase; therefore, its activity is essential in regulating thylakoid luminal pH (Armbruster et al., 2017). The relative amplitudes of ΔpH and $\Delta \psi$ formed in the WT, *pgrl1*, and *pgr5* are different at the onset of different AL intensities and similarly detected in the higher plants (Shikanai et al., 2017).

The part of the ΔpH was formed in WT and *pgrl1* and *pgr5* in optimal growth light, mainly depending on linear electron transport than CET. The WT, *pgrl1* and *pgr5* shows the maximum ΔpH in WT (137c) (0.1973 ± 0.02738), *pgrl1* (0.1352 ± 0.0277) and *pgr5* (0.0600 ± 0.0091) at $53 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ intensity (Figure 4.8B). A long induction of AL intensity ($53 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) makes higher ΔpH in both WT and mutants, which could be significantly contributed by the LET than CET. However, the ΔpH getting decreased (0.0882 ± 0.0171) in 137c, (0.0637 ± 0.0046) in *pgrl1*, and finally decreased (0.0504 ± 0.0080) in *pgr5* at increasing AL at $253 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Further, the ΔpH was decreased in 137c (0.0473 ± 0.0160), *pgrl1* (0.0439 ± 0.0042) and highly saturated in *pgr5* (0.0148 ± 0.0108) from high AL $553 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.8B) due to the long exposure of high AL could induce both photosystems photodamage.

On the other hand, transmembrane potential difference ($\Delta\psi$) was increased more in *pgr5* than *pgrl1*, and a significant decrease was observed in WT due to unequal charge separation through the thylakoid membrane from high AL (Figure 4.8B). In AL of $53 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the *pgr5* is showing maximum $\Delta\psi$ (0.0198 ± 0.0060), a relatively low (0.0140 ± 0.006) at AL of $256 \mu\text{mol m}^{-2} \text{s}^{-1}$ and reduced further (0.0065 ± 0.0014) from $553 \mu\text{mol photos m}^{-2} \text{s}^{-1}$. Similarly, this reduction was more in *pgrl1* from AL $553 \mu\text{mol m}^{-2} \text{s}^{-1}$ (0.004 ± 0.0010) and equal in both 53 and $256 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ is (0.0080 ± 0.0013 , 0.0088 ± 0.0012) respectively (Figure 4.8B). In WT (137c), the

membrane potential decreased with increasing AL intensity, and this decrease was significant (0.0072 ± 0.0018) in WT at $53 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and (0.0062 ± 0.0015) at $256 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and finally reduced to (0.0048 ± 0.0015) in $553 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 4.8B).

Interestingly, the $\Delta\psi$ value is still higher in *pgr5* than in WT and *pgrl1* in all AL intensities. I detected the probable *pmf*-reliant on adjustments of photosynthetic alteration, in which a more significant fraction of *pmf* stored as $\Delta\psi$ strongly affect the photodamage of PSII. Increased ΔpH , a component of *pmf*, can induce energy-dependent quenching qE by protonating the PSII subunit of PSBS (Takizawa et al., 2007). Few studies on higher plants indicate the relationship between down-regulation of ATP synthase activity and high *pmf* (Kohzuma et al., 2009; Davis et al., 2016). Our results showed a similar protein content of the Atpc subunit of ATP synthase complex in WT, mutant *pgrl1*, and *pgr5* (Figure 4.8C). The similar expression of AtpC in *pgrl1* and *pgr5* might be directly linked to the H^+ generated into the thylakoid lumen utilized by the ATP synthase complex for energy generation rather than *pmf* in high light. In WT, a minor increase of AtpC protein content was found in high light conditions (Figure 4.8C).

On the other hand, the Cyt b_6 regulates Q-cycle; its protein content is less abundant in optimum growth light whereas relatively high in WT under high light. However, the protein accumulation of Cyt b_6 majorly increased in *pgrl1* and *pgr5* compared to WT in optimum growth and high light (Figure 4.8C). The Cyt *b6f* complex restricts

electron transport rate via photosynthetic control when the thylakoid lumen acidifies under fluctuating light (Allahverdiyeva et al., 2015).

4.2.7 Effect of rapid P515 relaxation kinetics induced by ECS_{STF} on high light grown WT and CET mutants

P515 absorbance changes inclined by a saturating single turnover flash normalized by ECS signal were recorded in WT, *pgrl1* and *pgr5* samples before and after AL illumination with rapid ECS_{STF}. ECS_{STF} before AL illumination. A slow rise and decay phase after dark adaptation reflect the high membrane integrity, while a fast decline after illumination shows high ATPase activity and this kinetics is functionally characterized by intact photosynthetic apparatus.

P515 measures membrane potential across the thylakoid membrane due to the Chl *b* pigment and magnitude of the carotenoid electrochromic shift (Junge et al., 1968; Velthuys et al., 1978).

Normalized ECSstf analysis

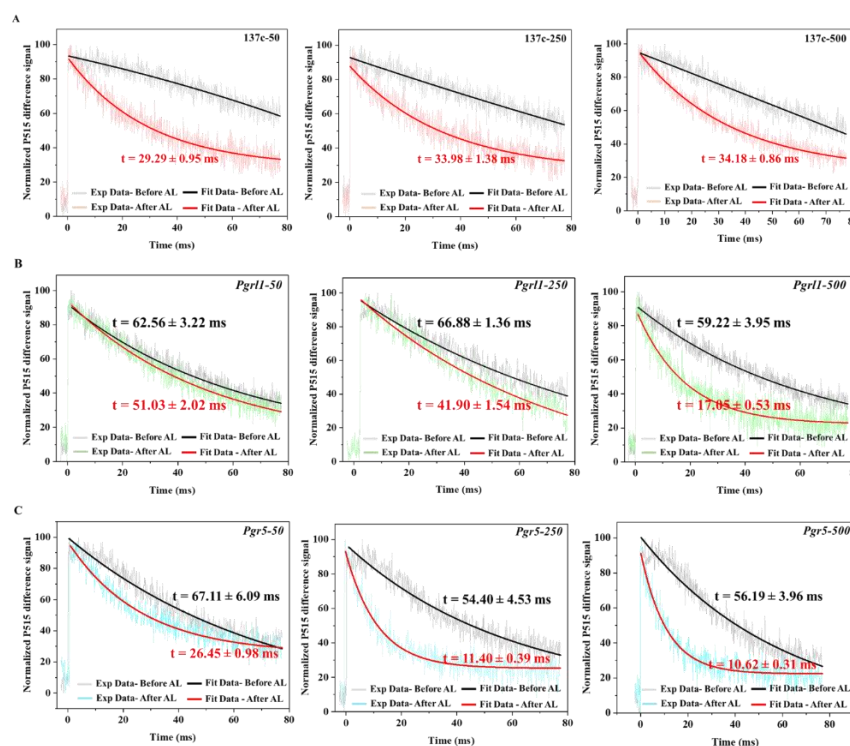


Figure 4.9 The normalized P515 absorption kinetics for studying functional ATP synthase activity induced by a first rapid single turnover flash (ECSstf), measured by dark-adapted cells before AL and then 10 min of AL treatment followed by keeping the same cells for 5 min in the dark followed by second rapid ECSstf (After AL) of the *C. reinhardtii* A, 137c, B, pgr11 and C, pgr5 grown under different light conditions. A single turnover saturation flash ($10,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was applied for all the measurements. All the measurements were performed in at least three biological replicates ($n = 3$); average values and standard deviations are indicated.

ECS_{STF} displayed no significant change in the P515 signal in WT before AL but showed a rapid decline after AL illumination in moderate and high light (Figure 4.9A). The rapid rise in signal within a fraction of a second activates primary charge separation in PSII and PSI reaction

centers and the reversible ATPase in the thylakoid membrane, increasing the H⁺ conduction across the membrane.

However, the rapid signal rise was followed by a milder reduction up to 40% in WT cells before AL illumination but a rapid decayed of 60% after AL in moderate ($t_{137c} = 33.98 \pm 1.38$ ms) and high light ($t_{137c} = 34.18 \pm 0.86$ ms, Figure 4.9A) due to the regulation of Q-cycle by Cyt b₆f via photosynthetic control (Velthuys et al., 1978). Moreover, in *pgrl1* and *pgr5*, the rapid decay up to 75-80% signal in moderate ($t_{pgrl1} = 41.90 \pm 1.54$ ms, $t_{pgr5} = 11.40 \pm 0.39$ ms) and high light ($t_{pgrl1} = 17.05 \pm 0.53$ ms, $t_{pgr5} = 10.62 \pm 0.31$ ms) grown samples (Figure 4.9B and 4.9C). The rapid decline of the P515 signal in *pgrl1* and *pgr5* reflects the relaxation induced by H⁺ outflow via the ATPase.

4.2.8 Differential expression of D1 and LHCSR3 prevent photoinhibition in *pgrl1* and *pgr5* under high light

Increased light sensitivity and loss of quantum efficiency of photosystems were detected in *pgrl1* and *pgr5* in high light (Figures 4.1C and 4.1D). The PSII reaction center protein D1 is more susceptible to photo-oxidizing conditions and appears more common in PSII photoinhibition (Adir et al., 2003).

Differential expression of D1 and LHCSR3 and PSI associated Proteins

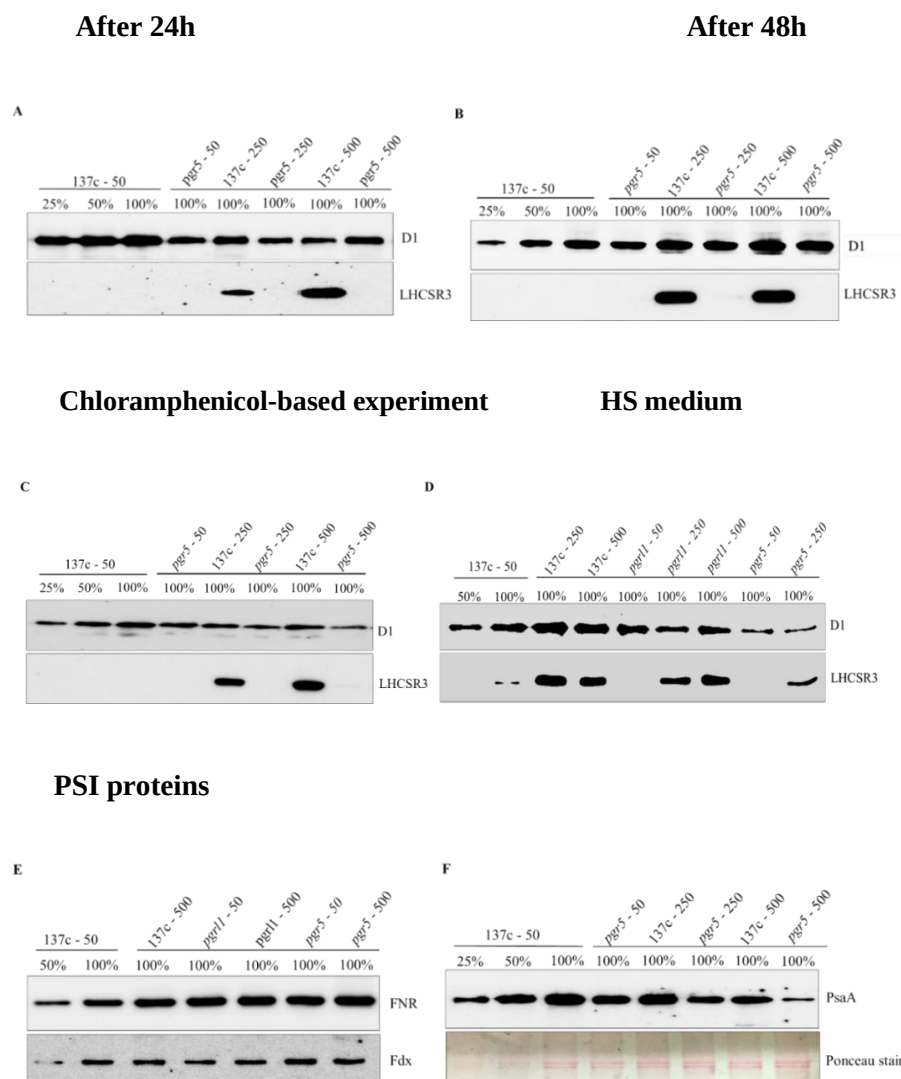


Figure 4.10 Immunoblot analysis of selected proteins from *Chlamydomonas reinhardtii* wt, *pgr1* and *pgr5* mutant. Immunoblot analysis for D1 and LHCSR3 protein content A, after 24 h; B, after 48 h; C, in chloramphenicol (CAM) 100 $\mu\text{g/ml}$ in TAP medium; and D represents D1 and LHCSR3 contents in cells grown in HS medium of wt (137c) and mutant (*pgr1*, *pgr5*) at growth light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate (250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. The PSI proteins essential for CET function E, Fdx (Ferridoxin), FNR and F, and PsaA in wt and mutant (*pgr1*, *pgr5*) at growth (50 μmol

photons m⁻² s⁻¹) and high light (500 μmol photons m⁻² s⁻¹) conditions.

LHCSR3, a potent quencher of excitation energy, is expected to protect PSII reaction center protein under high light stress. As our results suggested, LHCSR3 protein accumulated in WT and *pgrl1* but not in *pgr5* in moderate and high light (Figure 4.6E and 4.6F). Further, I have measured their protein expression to understand the relationship between D1 and LHCSR3 in the *pgr5* mutant. The results show that D1 protein content is minimum in WT and *pgr5* after 24 h (Figure 4.10A), but after 48 h growth, its protein content was increased in high light conditions (Figure 4.10B).

On the other hand, the protein content of LHCSR3 was decreased in the WT after 24 h (Figure 4.10A), but its protein accumulation was increased after 48 h in moderate and high light (Figure 4.10B). However, I can infer that LHCSR3 and D1 protein content are regulated throughout the high light-grown cells to prevent (or counterbalance) PSII photoinhibition. Further, I observed the effect of high light on PSII core protein D1 degradation and its synthesis in the presence of chloramphenicol (CAP, chloroplast protein synthesis inhibitor). For this, samples were treated to high light (600 μmol photons m⁻² s⁻¹) for 2 h (Figure 4.10C).

In the absence of translation inhibitors (CAP), D1 protein content was increased after 48 h in WT and *pgr5* in moderate and high

light (Figure 4.10B). But moderate and high light grown WT and *pgr5* (lacking LHCSR3) cells showed decreased D1 content when treated with chloramphenicol (Figure 4.10C). It is difficult to establish a clear relationship between D1 and LHCSR3, but LHCSR3 is expected to protect PSII reaction center D1 under high light stress (Kuroda et al., 2014). The result shows that the D1 (Figure 4.10B) and PSBS (Figure 4.6G) protein content increased in high light in the *pgr5*. However, this clearly shows that in the absence of LHCSR3 in *pgr5* mutant, the PSBS protein might be helping to recover D1 or to protect PSII under high light (Figure 4.6G).

Additionally, I grew the cells under a photoautotrophic (high salt minimal medium - HS medium) medium and examined the D1 and LHCSR3 protein content in optimum growth and high light conditions (Figure 4.10D). The D1 protein content was decreased in *pgr11* in high light. However, the *pgr5* cannot grow in high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in the HS medium (Figure 4.10D). The *pgr5* could grow only till moderate high light ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and shows a minor decrease in LHCSR3 protein content compared to WT and *pgr11* under high light conditions (Figure 4.10D). Furthermore, I observed the reductions of PSI core protein PsaA in WT, but this decrease was even more severe in *pgr5* mutant under high light conditions (Figure 4.10E). The protein content of another PSI subunit, Fdx (Ferredoxin), which plays an integral role in CET, was not significantly changed in WT and *pgr11* and *pgr5* strains throughout the growth, indicating that Fdx is a

highly regulated protein under high light stress because Fdx comprises multiple electrons sink at PSI donor side (Figure 4.10E).

PSII and PSI core proteins in TAP and HS growth medium

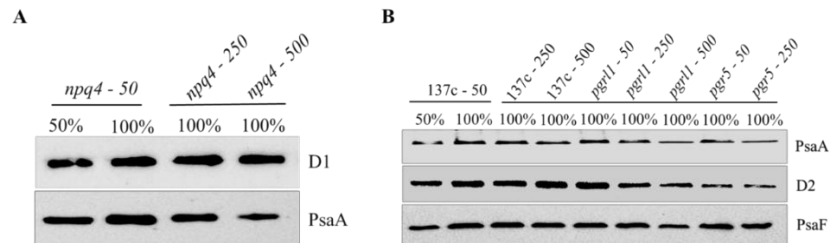


Figure 4.11 Immunoblot analysis of core proteins PSI and PSII of *Chlamydomonas reinhardtii* in Tris-acetate and HS medium. D1 and PsaA expression in *npq4* mutant A, and PsaA, D2 and PsaF in HS medium B, in 137c, *pgr11* and *pgr5* at growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) high light ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in *Chlamydomonas reinhardtii*. The western blots shown here are representative of at least three biological replicates.

Note- The *pgr5* cannot grow high light in $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the HS medium.

However, the other essential PSI protein, FNR, is also extensively involved in CET; its protein content increases in WT and mutants under high light conditions (Figure 4.10E). I have also checked the D1 and PsaA protein accumulation in *npq4* mutant (lacking LHCSR3 protein), which shows no significant difference in D1 content under all growth conditions (Figure 4.11A). I have observed a considerable decrease in PsaA protein content in moderate and high light, indicating that long-term exposure to high light causes PSI photodamage (Figure 4.11A).

These results suggest that the absence of LHCSR3 in *npq4* prevents the synthesis and assembly of PSI and PSII RC protein complexes in high light. Further, I checked the PSI protein PsaA, which showed a more severe decrease in mutants (*pgrl1* and *pgr5*) than WT in the HS medium (Figure 4.11B). The PSI protein PsaF detected the same protein level in WT and mutant strains grown under high light conditions (Figure 4.11B). Therefore, a PSII core protein, D2, had similar protein accumulation in WT under high light than growth light conditions. But I noticed a severe reduction of D2 accumulation in *pgrl1* and *pgr5* compared to WT in high light (Figure 4.11B).

LET, and CET generates *pmf* that regulates the expression of PSBS and LHCSR3 in *C. reinhardtii*

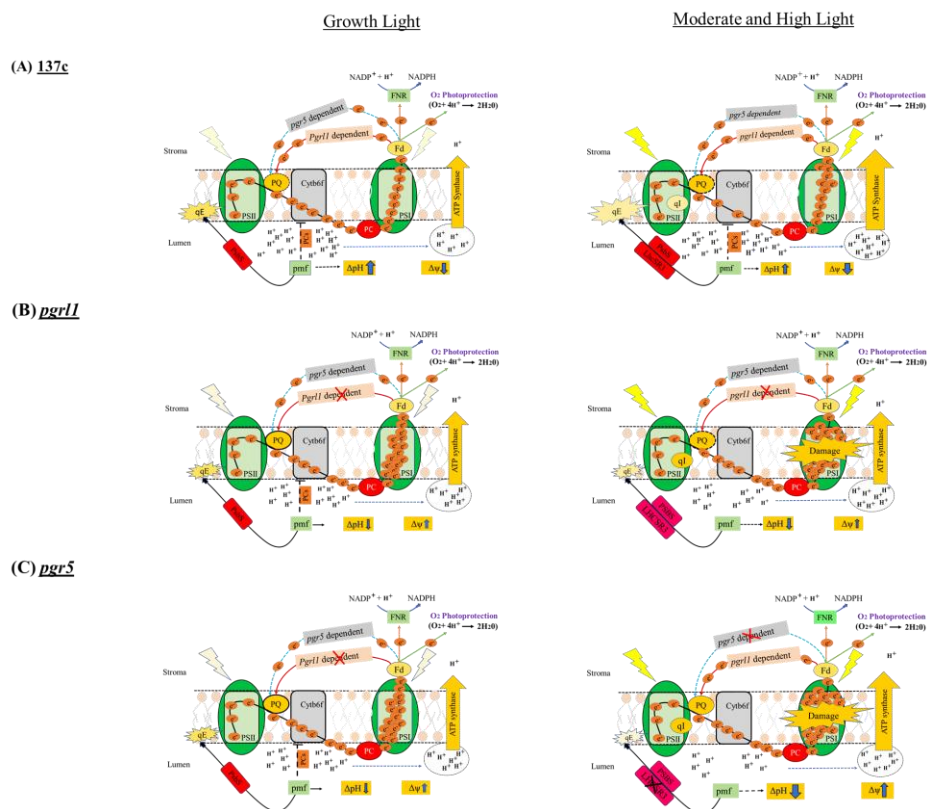


Figure 4.12 Graphical representation of LET and CET for pmf generation regulates the expression of PSBS and LHCSR3 in *C. reinhardtii*. The black line with electrons from PSII to PSI marks LET. The CET around PSI, via the PGRL1-Fd dependent CET (Red colour line) and PGR5-Fd dependent CET (Blue colour line), is specified from Fd to the PQ pool as an electron donor. The Black dash arrows determined from PMF to Cyt b_6f are photosynthetic control, mediated by lumen acidification and donor side limitation Y(ND), leading to the photoinhibitory quenching (qI) at the PSII level. The LET and CET contribute to the generation of pmf (green box) and its components, ΔpH and $\Delta \psi$ (Yellow boxes). Here I have taken $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ as growth light and 250 and 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ together as a high light. Here I have used the wt 137c to compare all the datasets generated in *pgrl1* and *pgr5* mutant at growth and high light conditions. This model was entirely based on our findings suggesting that CET protein *pgrl1* and *pgr5* is a solid and quick electron gateway that works instantly at the onset of high light. Here, Fd's role is crucial to deciding the fate of the electron; either it will go towards the CET or reduce NADP^+ to NADPH or for O_2 as a terminal acceptor. Several photoprotective mechanisms are brought into motion at different time scales when *C. reinhardtii* cells grow in growth and high light intensity. The LET and CET are enough to create total pmf regulated by the ion channels in the thylakoid membrane. Here, the cells inducing qE, a non-photochemical quenching component because of pmf generated by lumen acidification, regulate the differential expression of PSBS and LHCSR3 in growth and high light conditions. The pmf is necessary to induce photoprotective light-harvesting complex stress-related protein 3 (LHCSR3) absent in *pgr5* mutant and PSBS, which dissipates excess energy as qE, a component of non-photochemical quenching (NPQ) and qI, is generated downregulation of the photosynthetic control via Cyt b_6f activity. The ΔpH and $\Delta \psi$ are generated in wt 137c at growth light compared with *pgrl1* and *pgr5* growing in growth light, and the same wt 137c is produced in high light individually compared with high-light grown *pgrl1* and *pgr5*. The thick arrow is denoted upward, and the thin arrow is downward, indicating the amount of ΔpH and $\Delta \psi$ is increased or decreased in the individual strain under particular grown light conditions.

4.3 Discussion

4.3.1 High-light induced maximum PSI photoinhibition in *pgr5* and *pgrl1*

The cells lacking *pgr5* are challenging to survive under high light, while *pgrl1* is grown at a lower rate in high light (Figure 4.1B). The *C. reinhardtii pgr5* has phenotypic features almost matching *pgrl1* (Johnson et al., 2014). The growth of the WT was unaffected in high light, showing higher biomass (Figures 4.1A and 4.1B) and reduced photosynthetic activity (Figures 4.1C and 4.1D). The *pgr5* mutant marked a severe Pm reduction but marginally restored Fv/F_M activity, indicating that PSII is relatively more intact than PSI in high light conditions (Figure 4.1C and 4.1D). I can relate this to our protein data where PsaA is reduced more in *pgr5*, but it shows a high turnover of D1 in high light conditions (Figure 4.10F and 4.10B). This outcome indicates that reduced photosynthetic activity of the photosystems in high light could be pathway sensitivity, i.e., absence of *pgrl1* and *pgr5* mediated cyclic electron transport.

4.3.2 Donor and acceptor side regulation balance the PSI photoinhibition in high light

I examined the two primary essential CET functions in PSI photoprotection linked to luminal acidification: (1) Induce non-photochemical quenching (NPQ) and photosynthetic control, Y(ND); (2) Balancing the stromal-side redox state, overcoming the acceptor-side

limitation, $Y(NA)$, by rebalancing ATP and NADPH ratios for carbon fixation. The PSI yield was reduced more in *pgrl1* and *pgr5*, shifting PSI photochemistry from the donor side to the acceptor side, preventing the PSI photodamage from high light (Figures 4.3B, 4.3C and 4.3D). Conversely, high light subsequently makes a higher $Y(NA)$ in *pgr5* and *pgrl1* than in the WT (Figure 4.3C). It is expected that in high light, the *pgrl1* and *pgr5* cells cannot stabilise the electron transport through PSI, subsequent compromised growth (Figure 4.1A, 4.1B and Figure 4.2A).

The electron transport pressure is less at the donor side $Y(ND)$, but the maximum at the acceptor side $Y(NA)$, specifically in *pgr5*, could not induce pivotal photosynthetic control under high light (Figure 4.3C and 4.3D). The CET and photosynthetic control must be stable to maximize the LET; indeed, in WT, this mechanism showed higher photosynthetic control $Y(ND)$ than in the mutants (Figure 4.3D). The lesser $Y(NA)$ would subordinate to higher $Y(ND)$, and it is academically tricky to measure each mechanism's involvement in PSI photodamage independently. A lack of PSI donor side $Y(ND)$ has formed a profound limitation in PSI photoinhibition in *A. thaliana* under excess light (Munekage et al., 2002).

Therefore, these mutants could not perform efficient photosynthetic electron transport and showed severe photodamage of PSI during moderate and high light. The qL was also higher in WT in moderate high light and was lower in *pgrl1* and *pgr5* due to the weak operation of the photosynthetic control or donor-side limitation $Y(ND)$ when illumination light from 0 to $> 800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure

4.3D and 4.3E). The *pgrl1* and *pgr5* mediated CET are crucial to keeping P700 in an oxidized state. The high light predominantly decreased the P700 redox kinetics in *pgrl1* and *pgr5* (Figure 4.4). These results indicate that, unlike WT, *pgrl1* and *pgr5* cannot photo-accumulate P700⁺ due to the absence of PGR1 and PGR5 dependent CET, which most likely generates the extra electron sink for moving electrons from PSII to PSI.

4.3.3 Increased non-regulated energy loss Y(NO) in *pgr5* and *pgrl1* prevent photodamage

The Y (NO) constitutive energy loss due to non-regulated energy dissipation remained higher in *pgrl1* and *pgr5* than in WT moderate and high light (Figure 4.5C). The high Y(NO) could eventually prevent *pgrl1* and *pgr5* against photodamage caused by a higher fraction of closed PS II reaction centres. However, in the case of *pgrl1* and *pgr5*, the data strongly suggests that the high Y(NO) levels reflect the same as the Δ pH-independent “sustained” energy quenching (Brooks et al., 2013). The *pgrl1* and *pgr5* relaxed for an extended period than the WT in high light, and this could be considered a form of “sustained quenching (qH)”, as suggested earlier in *Arabidopsis thaliana* in cold and high light (Malnoë et al., 2018). Our data also demonstrate that the maximum NPQ induction in WT and moderately in *pgrl1* is the combined effect of LHCSR3 and PSBS protein under high light conditions (Figure 4.6A, 4.6B, 4.6E and 4.6G).

The lumen's acidification activates qE, dissipating excess energy (Niyogi and Truong, 2013). In higher plants, PSBS are the main qE effector proteins facilitating energy dissipation in high light (Peers et al., 2009), HS medium with regulated CO₂ in *C. reinhardtii* (Correa-Galvis et al., 2016) and nutrient starvation conditions (Finazzi and Minagawa, 2015; Devadasu et al., 2021). However, in high light, WT exhibits more significant qE than *pgrl1* and *pgr5* (Figure 4.6C).

The absence of LHCSR3 proteins in *pgr5* is insufficient for inducing the NPQ component of qE so it might be solely PSBS protein involved in NPQ generation and photoprotection under high light conditions. This shows the importance of these proteins in regulating energy loss (NPQ), which keeps the reaction centers in an oxidized state to prevent PSII photoinhibition. On the contrary, *pgrl1* and *pgr5* showed higher photoinhibitory quenching, qI than the WT, indicating the maximum photodamage has occurred in high light conditions (Figure 4.6D), where its qE is lower (Figure 4.6C). Nevertheless, the high-value Y(NO) and photoinhibitory quenching (qI) specify the PSII damage or cannot cope with the high electron pressure from PSII to PSI under high light conditions.

4.3.4 High light-induced photodamage decreases the ΔpH but increases the $\Delta\psi$ component of pmf in *pgrl1* and *pgr5*.

During optimum growth light, I observed a considerable accumulation of pmf and ΔpH in the *pgrl1* and *pgr5* but decreased in moderate and high light conditions (Figures 4.8A and 4.8B).

On the other hand, the *pgrl1* and *pgr5* mutants showed that excess *pmf* is stored as $\Delta\psi$ than WT in optimal growth and high light condition (Figure 4.8B). This indicates that H^+ transports out of the lumen in exchange with counter ions or K^+ by K^+/H^+ antiporter in the thylakoid membrane (Horton et al., 1996). Therefore, less acidification of its lumen may increase the possibility of electrical potential difference components ($\Delta\psi$) across thylakoid membranes, leading to the inactivation of photosynthesis's light reactions (Figure 4.8B). The high amplitude of $\Delta\psi$ and slower relaxation of NPQ component (part as photoinhibitory quenching) are the leading cause of PSII photoinhibition as previously reported in *A. thaliana* in fluctuating light conditions (Davis et al., 2016).

PSI donor-side Y(ND) over-reduction regulates the Cyt b_6f , as a photosynthetic control is minimal in *pgrl1* and *pgr5* in high light. The increased expression of Cyt b_6 in *pgrl1* and *pgr5* in high light (Figure 4.8C) increases the tendency of Cyt b_6f to transfer electrons through the PQ pool to acidify thylakoid lumen for maximum ATP synthesis. Based on this concept, I conclude that the mutual activity of the Cyt b_6f and photosynthetic control, Y(ND), regulation of ΔpH and $\Delta\psi$ required to balance the rate of electron transport and photoprotection in high light. The immunoblot analysis of WT, *pgrl1* and *pgr5* grown cells showed no downregulation of ATP synthase content in high light (Figure 4.8C).

Our data is in line with the recent report showing a low level of *pmf*, leading to a minor reduction in ATP synthesis under fluctuating light in the *pgr5* mutant of *A. thaliana* (Sato et al., 2019). The *pgr5*

function might be directly related to H^+ regulation through ATP synthase (mainly for energy generation and growth) or indirectly affects the lumen acidification, possibly via the Cyt b_6f function in the absence of one of the routes of CET. The rapid relaxation of the P515 signal in *pgrl1* and *pgr5* cells might indicate faster ATP synthesis-related H^+ outflow from the thylakoid lumen to the stroma to maintain the H^+ -ATP synthase activity (Figure 4.9B and Figure 4.9C). From these results, I can say that the rapid decline of the P515 signal is highly dependent on the physiological status of the sample and the lack of lumen acidification by which ATP synthase requires H^+ to grow in high light.

4.3.5 A regulative role of PSBS and LHCSR3 aid photoprotection in high light

The LHCSR3 and PSBS are essential proteins in *C. reinhardtii* for photoprotection. The LHCSR3 is expressed in both WT and *pgrl1*, but *pgr5* lacks complete expression of LHCSR3 in high light conditions. However, PSBS another high light expressing protein expressed in WT, *pgrl1* and *pgr5* mutant, but its accumulation varies with time under high light (Figure 4.6G). The cells lacking functional *pgr5* (no LHCSR3 protein content, Figure 4.6F) are challenging to survive under high light in a TAP medium (after 24 h, Figure 4.2 and after 48 h, Figure 4.2B), while *pgrl1* grow at a lower rate under such conditions (Figure 4.2A) and has lesser LHCSR3 protein content compared to WT (Figure 4.6E).

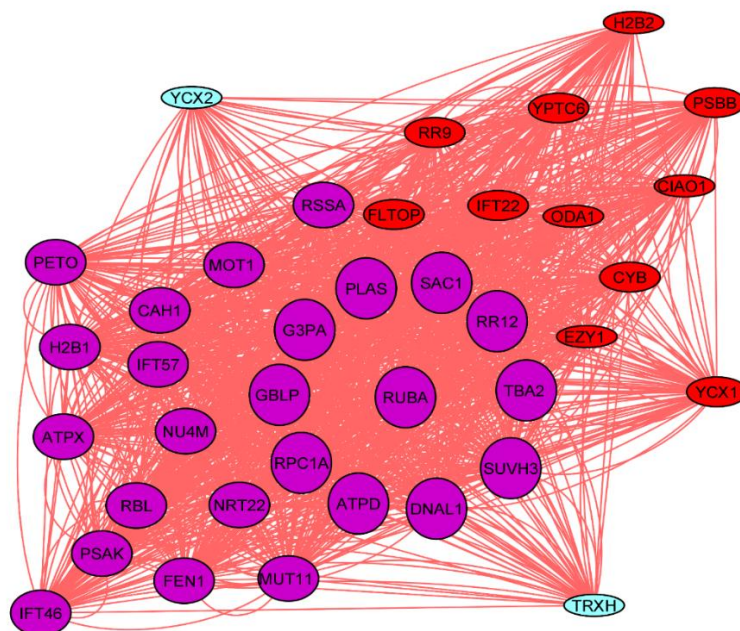
Our data suggest that cells lacking LHCSR3 protein in *pgr5* might be the deactivation of the negative controllers of LHCSR3 gene expression (Franco et al., 2021) or LHCSR3 phosphorylation diminished under prolonged high light conditions (Scholz et al., 2019). The lack of LHCSR3 expression in *pgr5* mutant grown with acetate under HL can be explained because the functional redundancy modulated by carbon availability (acetate medium) or photochemistry can be avoidable under heterotrophic nutrition conditions. As photosynthesis under high light can result in ROS generation and an alternative carbon source (acetate) is available, the organism resorts to complete heterotrophism under CET compromised conditions. The LHCSR3 and PSBS accumulate in *pgrl1* (Figure 4.6E and 4.6G), but higher PSBS protein accumulation was observed in *pgr5* similar to WT (Figure 4.6G), signifying that PSBS could play a decisive role in high light photoprotection. The high protein content of PSBS, especially in *pgr5*, mediates efficient electron transport downstream of PSII to prevent high light-induced photodamage in high light conditions. This suggests that *pgrl1* is a stable CET and stabilizes photosynthesis better than *pgr5* in high light in *C. reinhardtii*. Some reports show that in the absence of PGR5, the PGRL1 CET is steady in fluctuating light conditions (Dal Corso et al., 2008; Hertle et al., 2013; Johnson et al., 2014). However, our data suggest that PSBS is predominantly involved in high light photoprotection in CET mutants *pgr5* and *pgrl1*, and LHCSR3 plays an additive role in high light photoprotection under high light conditions.

4.4 Conclusion

I conclude that LHCSR3 and PSBS can promote photoprotection from high light in *C. reinhardtii* despite the absence of either PGRL1 or PGR5 CET in high light conditions. The PSBS already has (as in higher plants) an essential function in endorsing qE activation, but the lack of specific pH regulation is restricted to LHCSR3 activation in the *pgr5* mutant of *C. reinhardtii*. The transient Y(NA) increase in *pgrl1* and *pgr5* indicates that CET aids as a safety regulator on the acceptor side, avoiding PSI photoinhibition. Further, the reduced ΔpH (*pmf*) in *pgrl1* and *pgr5* increased $\Delta\psi$ in *pgr5*, balancing the photochemistry and photo-acclimation in optimal growth and high light conditions. These results indicate that *pgrl1* and *pgr5* are vital in the photoprotection of PSI, mainly arising from active CET. Our results display the handy relationship and the functional interplay between the four proteins PGR5, PGRL1, PSBS, and LHCSR3 in the photoprotection mechanism of both PSII and PSI. The occurrence of PGR5 and LHCSR3 (in *Pgrl1* mutant) and the absence of PGRL1 and LHCSR3 (in *pgr5* mutant), or the presence of all four proteins in the WT, is not sufficient to release the high electron pressure in *C. reinhardtii* under high light conditions (Figure 4.12). Our results provide convincing evidence that these four proteins, PGRL1 LHCSR3, PSBS, and PGR5, work together (LHCSR3 and PSBS in *pgrl1* mutant) or independently (PSBS in *pgr5* mutant) during high light photoprotection.

CHAPTER -5

*High light-induced changes in whole-cell
proteomic profile and supercomplex
organisation in pgrl1 and pgr5 mutants of C.
reinhardtii*



Objective 3: High light-induced changes in whole-cell proteomic profile and supercomplex organisation in *pgrl1* and *pgr5* mutants

5.1 Introduction

Light is a predominant need for photosynthesis. The change in the quantity of light can influence photosynthetic efficiency. The green plants and algae have been adapted to various photoprotective mechanisms to adjust the abiotic stress condition. Optimum photosynthesis requires a sensitive balance between photoreception and its utilization. This balance involves exposure to optimum light intensity and a constant supply of nutrients. Plants that grow under natural conditions do not always receive the optimum light and nutrients, leading to photosynthetic imbalance. The major challenge for photosynthetic organisms is efficiently acclimated to the highly dynamic light conditions.

High light is particularly problematic because excess light energy can damage the protein complexes involved in photosynthetic electron transport. Continuous high light affects the charge separation and electron transport which imbalance the reducing powers of NADPH and ATP required for carbon fixation. Absorption of light energy and its conversion into biologically available chemical form is carried out by the coordinated function of specialized thylakoid membrane protein complexes like photosystems (PS) I and II along with their light-harvesting (LHC) antenna LHC I and II, Cyt b_6f complex and ATP-

synthase complex. These protein complexes are well-organized as supercomplexes in the appressed sections of the thylakoid membrane (Wietrzynski et al., 2020; Shen et al., 2019). In *C. reinhardtii*, these supercomplexes consist of PSII-LHCII, PSI-LHCI, LHCs trimer, monomers, and other small subunits like CP26 and CP29 (Croce and Amerongen., 2020). The antenna proteins associate with RCs, allowing overexcitation of energy from the membrane until it drives stable photochemistry (Bennett et al., 2018).

PSII has been modified to minimise ROS generation due to RC complex strategy and redox tuning (Brinkert et al., 2016; Rutherford et al., 2012) and supporting mechanism dissipating excess energy (Peers et al., 2009; Roach et al., 2020). Photosynthetic organisms have evolved with different agencies to dissipate extra energy absorbed by photosynthetic machinery. These mechanisms are collectively called non-photochemical quenching (NPQ). The energy quenching, qE, is mediated by the LHCSR3 antenna after the protonation of its lumen residues (Peers et al., 2009; Tian et al., 2019). qE is the rapid and most studied part of NPQ (Niyogi, 2000). In green algae, *C. reinhardtii*, the induction of qE requires gene products of LHCSR e.i., LHCSR1, LHCSR3.1/3.2, and PSBS (Peers et al., 2009; Correa-Galvis et al., 2016). PSBS protein does not form complex with pigments (Bonente et al., 2008). More likely, its function depends on the protonation of the luminal-exposed glutamine residues, which can further activate the zeaxanthin and lutein-dependent quenching process in LHCs through

chlorophyll-carotenoid charge transfer (Bonente et al., 2008; Niyogi et al., 2001; Ahn et al., 2008). PSI is particularly resistant to photoinhibition under oxidative stress conditions due to the high efficiency of protective mechanisms that regulate the flow of electrons to the PSI donor side, including NPQ, lumen pH-dependent regulation of Cyt b_6f activity, and even PSII photoinhibition (Tikkanen and Aro, 2014). Electron consumption at the PSI acceptor side through the Calvin-Benson cycle, photorespiration, and cyclic and pseudo-cyclic electron flow are also protective factors that prevent PSI over-reduction (Yamori, 2016). The current studies are based on “omics” tools encompassing metabolomics, transcriptomics, and proteomics. Proteomics must be an essential match to biochemical and biophysical data to improve the biological perspective on plant biology. A dynamic approach to discovering how an organism reacts to a specific abiotic stress condition is identifying how it changes the protein expression.

In a modern protein era, proteome analysis quantifies the expression of the most protein in an organism in peptide expression levels. This technique can determine the relative levels of peptides in abiotic stress and normal physiological condition. The difference or similarity in expression levels under different light conditions can be revealed by differential expression in the peptide levels of specific sets of proteins. The impact or influence of high light on a particular biological process can be reported by changes in the proteome levels of

a specific protein that has a functional role in the performance of photosynthesis. Detailed knowledge about all pathways and biological processes is required to determine the reasons for acclimation and biomass production of microalgae under high light conditions.

Hence, a new detailed proteome analysis platform is necessary to elucidate these advantages and identify potential targets applicable in higher plants to improve crop production. Proteomics is a promising approach that could deliver insights and knowledge of the protein, which helps improve and utilize conventional breeding and genetic engineering to make new traits or recombinant plants with better-quality agronomical characters (Gao et al., 2019). Notably, several efforts have been accomplished to achieve gene expression in alfalfa in salt conditions (Jin et al., 2010; Postnikova et al., 2013).

Proteomics has numerous benefits in correlation with genomics and metabolomics technologies, like a wide range of enzymes reroutes the metabolic pathways and extra sensitivity for differential gene expression. The gene sequence analysis changes were measured during the biomass production in *C. reinhardtii* utilizing proteomics, declaring new awareness for fine-tuning and redesigning the internal metabolism. High throughput techniques have rapidly been discovered and helped us better understand plants' transcriptome and proteome changes during light and dark or high light (Vogel et al., 2014; Liang et al., 2016; Crisp et al., 2017; Huang et al., 2019).

The present study characterized bio-physiological, bio-chemical study integrated with nLCMS/MS-based proteomic analysis to enumerate the universal database reserve and determine how *C. reinhardtii* assigns resources in response to high light. The efficient approaches include changes in the characteristics of bio-physiological and biochemical parameters and the change of whole-cell proteome in WT, *pgrl1* and *pgr5* mutants. Our results will deeply reflect the importance of high light photoprotection mechanisms and give the idea to develop new approaches to attain maximal biomass and productivity. To address these questions in high light conditions, we have grown WT, *pgrl1*, and *pgr5* of *C. reinhardtii* cells in a TAP medium. No reports have been documented in the proteomic responses of microalgae *C. reinhardtii* in high light intensities, mimicking natural conditions.

The present study examined the effect of high light at different intensities on green microalga *C. reinhardtii* through proteomic analysis. More specifically, we investigated the role of PETC (Photosynthesis electron transport chain) proteins and metabolic pathways employing proteomic profiling, which participates in the acclimation process of *C. reinhardtii* under high light stress. More specifically, the chloroplast proteins are involved in the dark reaction and other cytosol and mitochondria metabolic proteins. Further, I have correlated our proteomic studies with the physiological and biochemical studies of these mutants grown in high light.

5.2 Results

5.2.1 High light changes oxygen evolution/consumption in *pgrl1* and *pgr5* mutants

Factually, oxygen evolution, spectrometry, and biochemical measurements were used to quantify PSII damage under abiotic stress (Tyystjärvi, 2013; Chow and Aro, 2005). The cell culture was grown in growth and high light. Each WT, *pgrl1* and *pgr5* cell was used along with artificial electron acceptor 2-phenyl-1, 4-benzoquinone (PBQ) and ammonium chloride (NH₄Cl) for oxygen uptake and 100 mM Sodium azide, 50 mM Methyl viologen, 10 mM DCPIC, 50 mM DCMU and 500 mM Ascorbate for oxygen consumption, detected in the dark.

The Results demonstrate that the WT cells are acclimating better when grown under high light, and their oxygen-evolving capacity is increased by around 47% (194 $\mu\text{mol/ mg Chl/ h}$) in moderate light and 36% (180.33 $\mu\text{mol/ mg Chl/ h}$) in high light conditions (Figure 5.1A). A significant decrease in oxygen evolution in *pgr5* has been observed in moderate and high light 51.58 % and 66.35 %, respectively, than WT growth light conditions.

On the other hand, in the *pgrl1*, a minor decrease is observed by 8.4 % in high light conditions. This result of the *pgr5* relates to the changes observed in the Chl fluorescence study. The chl fluorescence has mainly decreased in *pgrl1* and *pgr5* mutants in moderate and high light than in WT growth light conditions. When cells were dark-

adapted, the WT and *pgrl1* strain had an almost similar O₂ consumption observed in high light (Figure 5.1B).

Measurement of oxygen evolution/consumption

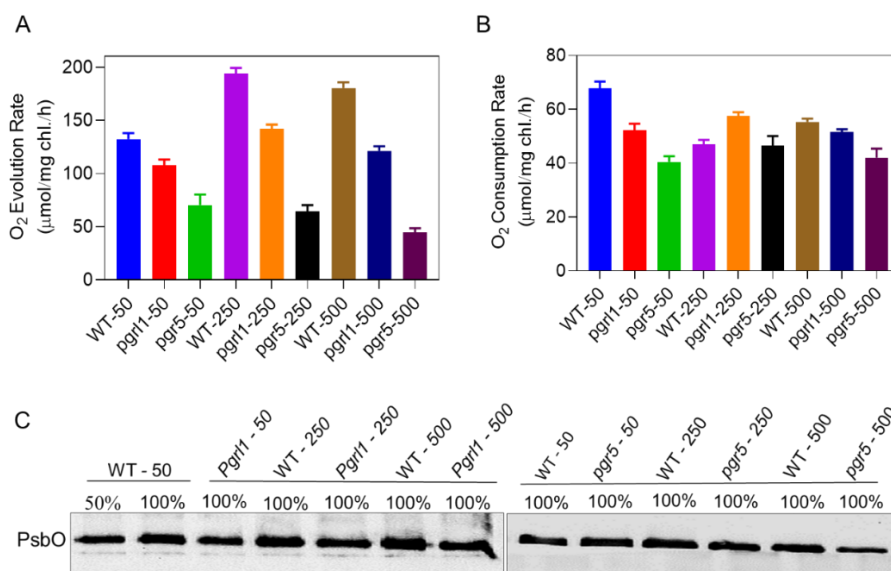


Figure 5.1. Measurement of oxygen evolution/consumption was performed using 1mM NH₄Cl and 1mM 2,4 dichloro, 2-phenyl-1,4-benzoquinone (DCBQ). The oxygen-evolving activity of cells was measured by taking an equal concentration of chlorophyll 10 μg/ml, supplemented with the artificial electron acceptor DCBQ, phenyl-p-benzoquinone (PBQ), and ammonium chloride for PSII activity (A). Comparison of oxygen consumption rates of the wild type (WT), knockdown strains, *pgr5* and *pgrl1*, Cells were grown with growth and high light in TAP medium (B). Oxygen consumption measurements were carried out with cells incubated in a TAP medium containing NaN₃, DCMU, Ascorbate and DCPIP at a final concentration of 1 mM. Values mean 3 SD of three replicates. Immunoblot analysis of PsbO subunit in WT, *pgrl1* and *pgr5* (C). Oxygen evolution recordings were measured at 25 °C under saturating light (500) using a Clark-type oxygen microelectrode (Hansateck Instrument Ltd, Norfolk, England) stuck inside the oxygen electrode chamber. The electrode was calibrated in a medium flushed with air (O₂ saturated) and in the presence of sodium hydrosulfite (anoxia).

There is a significant decrease in dark-induced O₂ consumption in moderate and high light, corresponding to nearly 15 % and 24 % in *pgrl1* and 32 % and 38 % in *pgr5* than WT growth light conditions. Further, we correlate this result with biochemical changes; the oxygen-evolving complex subunit of PsbO slightly decreased in *pgrl1* and *pgr5* in moderate and high light than WT, as shown in the O₂ uptake experiment (Figure 5.1C). These results propose that WT cells adjust to high light stress by adaptative mechanism but indicating that *pgrl1* and *pgr5* high light impact drastically damaged PSII agrees with the fluorescence result.

5.2.2 Structural changes in thylakoid membrane under high light

Thylakoid membrane structure and composition were analyzed using CD spectrometry and BN-PAGE. Thylakoid membrane is a dynamic structure; it can undergo numerous structural changes to acclimate under different environmental conditions (Nagy et al., 2014). CD determine the structural change of supercomplexes macro-organization and pigment–pigment interactions.

The WT and mutants CD spectra are presented in Figures 5.2 (A-B). The CD spectra of the thylakoid membrane are divided into the Qy region (600 to 700 nm or towards the red region) and the Soret region (400 to 550 nm or blue region). The CD spectra of the thylakoid membrane revealed the features called *psi*-type CD bands. The magnitude of the *psi*-type band is proportional to the size of the ordered array at a given set of conditions.

The visible region of the CD spectrum of *C. reinhardtii* cells consists of two intense *psi*-type bands at around (-) 676 and (+) 690 nm (Nagy et al., 2014). The positive band at about 690nm (Chl *a*) and the negative band at about 674nm (Chl *b*) originated from Chl molecules associated with macrodomains of photosynthetic proteins.

Protein pigment interaction changes in the thylakoid membrane under high light

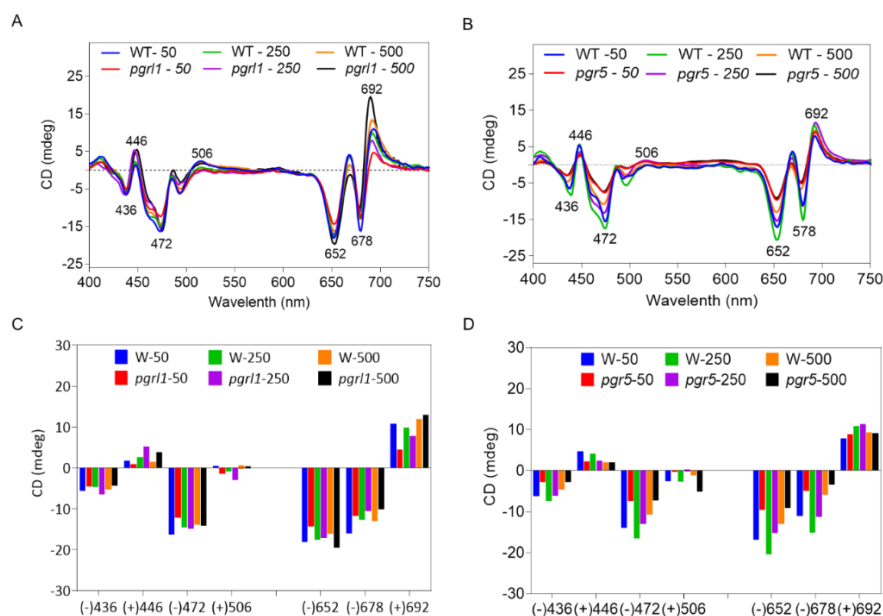


Figure 5.2 CD spectra of wild-type and mutants (A and B). Spectra were recorded at room temperature, baseline-corrected and normalized to equal chlorophyll of the samples (10 $\mu\text{g/ml}$). The total amplitude of the *psi*-type signal (i.e., the difference between the positive and negative amplitudes is shown as a bar (C and D) diagram) of WT, *pgr1* and *pgr5* cells grown in growth and high light intensity, which received the same parameter and three accumulations with the scan speed of 100 from three independent experiments, on different batches.

These *psi*-type bands are given rise by the presence of large well-ordered arrangements (size ranging from 200 to >400 nm) of PSII-LHCII complexes in the appressed region of the thylakoid membrane. In WT, there are marginal changes in the red *psi*-type around (+) 692 nm signal amplitude, especially in moderate than high light, whereas I detected a significant increase in the *psi*-type signal from *pgrl1* and *pgr5* grown under high light than WT growth light (Figure 5.2A). On the other hand, another *psi* type band around (-) 678 is significantly decreased in *pgrl1* and *pgr5* in moderate and high light than WT control. This indicates that *pgrl1* and *pgr5* are crucial for maintaining the thylakoid dynamics and structure arrangement under high light. The blue region *psi*-type band at around (+) 506nm is attributed to carotenoids found in a long-range ordered structure and at about (-) 650 nm negative band, characteristic of LHCII trimers (Garab and Amerongen, 2009).

The positive band at 506 nm in the Soret region originates from the β -carotene of the core complex (Tóth et al., 2016) and exhibits no or weak signal but decreases drastically in especially *pgr5*, which might be due to a decrease in protein content of the core complex in cells subjected to high light (Figure 5.2B). The CD spectra of LHCII trimers are similar under normal and high light but slightly increased in moderate high light in WT. At the same time, *pgrl1* trimers are increased, but in the *pgr5*, peak intensity is drastically reduced

compared to WT control, suggesting that the LHCII trimers were significantly affected in CET mutants under high light.

The spectra have a more complex structure in the blue region (400 to 450 nm) because of an abundance of Chl *a* (+) 436 nm) and Chl *b* (+) 446 nm) and the dominant peak at (-) 472 nm for LHCII. There is no such difference in peak intensity (-) 436 nm WT and *pgrl1* in moderate high light but decreased in *pgrl1* and *pgr5* high light. On the other hand, the peak intensity of (+) 446 nm increased in *pgrl1* moderate and high light but reduced significantly in *pgr5* high light than in WT control (Figure 5.2A and 5.2B). The LHCII band shows (-) 472 nm similar rise in peak WT and *pgrl1* moderate and high light but decreased dramatically in *pgr5* high light condition as shown in Figure 5.2C and 5.2D).

It is clear that though there is acclimation, the LHCs to reaction center assembly changed, describing that the LHCs are detached from the reaction center. The data agree with blue native gels that disturbed supercomplexes and mega complexes. However, these disturbed mega and supercomplex were restored as the monomer, and trimer content is reduced significantly in *pgr5* but slightly increased in *pgrl1*, shown from blue native gels. This is one of the reasons for the dramatic change in the CD signal in the red region.

5.2.3 Protein pigment interaction and changes in macro-organization of supercomplexes

Thylakoid membranes comprise several multiprotein membrane complexes that run photosynthetic light reactions. Their composition, distribution, and stoichiometry may vary according to the changes in environmental conditions. Thylakoids are composed of three structures: grana, stroma, and grana margins. The appressed regions of thylakoids, grana are enriched with PSII-LHCII supercomplexes, non-appressed parts, and stroma with PSI-LHCI and ATPase complexes (Garab and Amerongen, 2009). Grana margins have been suggested to have both photosystems' PS (I and II) form mega complexes (Järvi et al., 2011). To study the arrangement and changing aspects of thylakoid membrane protein-pigment complexes under high light, WT and *pgr1* and *pgr5* were grown in a TAP medium, and thylakoids were isolated. Before BN Native-PAGE, proteins are separated from the thylakoid membrane using mild non-ionic detergent, β -dodecyl maltoside (β -DM) (1% working concentration).

Structural composition changes in macro-organization of supercomplexes under high light stress.

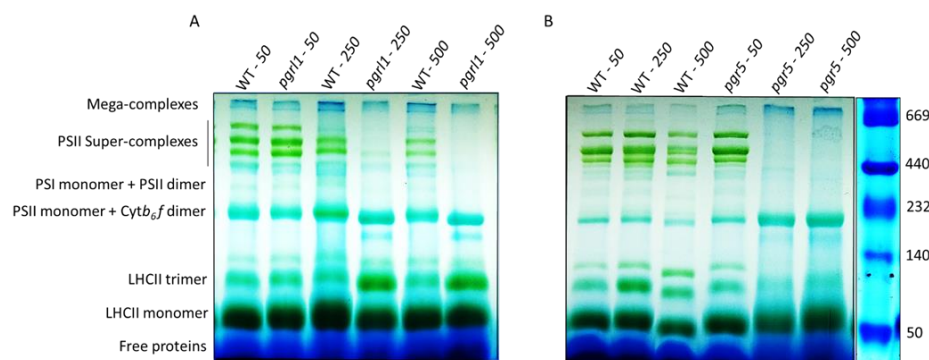


Figure 5.3 *Pigment-protein complexes and supercomplexes of representative BN-PAGE gel. Thylakoid membranes from WT, pgr11 and pgr5 samples were solubilized with 2% β -DM and separated using native PAGE. Samples containing equal amounts of chlorophyll (10 μ g) were loaded in each lane. (B) Representative BN-PAGE gel with Native marker.*

We selected to use β -DM for its ability to solubilize most of the thylakoid membrane, but it comes at the expense of losing gentle hydrophobic connections in protein multiplexes (Wittig et al., 2006). Figure 5.3 (A-B) shows typical patterns of thylakoid membrane protein complexes separated in BN-PAGE. Four bands of PSII-LHCII supercomplexes between 669 - 440 KDa, PSII and PSI monomers and Cyt b_6f complex devoid of LHCs, near 440 - 230 KDa and lower three bands consist of different hierarchical combinations LHC trimer and monomer have been resolved from the thylakoids under high light. The localization of the Cyt b_6f complex is under debate, but many studies reported its occurrence near to PSII dimer (Anderson, 1982; Albertsson et al., 1991).

The largest PSII supercomplexes isolated from *C. reinhardtii* are named as strongly bound trimer (S-trimer), moderately bound trimer (M-trimer), and loosely bound trimer (L-trimer) depending on their binding strength with the PSII core complex as $C_2S_2M_2L_2$. Here, in this BN-PAGE, the four high molecular weight bands as supercomplex (~ 669 KDa) consist of different combinations of PSII core with S and M trimers and the largest supercomplex observed is $C_2S_2M_2$, as

interactions with L-trimers are lost during solubilization with (β -DM) (Wittig et al., 2006).

The WT under moderate and high light stress shows a marginal decrease of PSII-LHCII supercomplex than WT growth light (Figure 5.3A). However, *pgrl* intensity of PSII-LHCII supercomplexes decreases, which is even more severely declined in *pgr5* in moderate and high light (Figure 5.3A and 5.3B). The reduction of PSII LHCII supercomplexes reflects in the form of LHCII trimer and monomer. This depends on the light sensitivity of the strain grown in different light conditions and the concentration of the solubilizing agent. In WT and *pgr5*, the fraction of LHCs is stored as a monomer than *pgrl1*; trimer is restored more under moderate and high light conditions (Figure 5.3A). The loosely bound LHCII complexes in mega complexes and supercomplexes (top bands) were dissociated and further accumulated as LHCII trimers. In WT and mutants, these changes are less noticeable under high light stress.

5.3.4 Western blot analysis of proteins from *C. reinhardtii* WT and *pgrl1*, *pgr5* mutants: Accumulation of photosynthetic and light-harvesting proteins

Immunoblot analysis was performed on WT, *pgrl1* and *pgr5* grown in growth (50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), moderate (250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and high light (250 and 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) regimes. Samples were loaded on an equal chlorophyll basis. The proteins from the gels

were transferred to nitrocellulose membranes and immunoblotted against specific antibodies.

The PSII (D1, D2, CP43 & CP47) and PSI (PsaA, PsaD, PsaG, PsaH & PsaF) core proteins along with both photosystems (PSII and PSI) LHCs (light-harvesting complex) proteins were analysed. The changes in the stromal and luminal subunits (PsaG, PsaH and PsaD) can distract the assembly of PSI. The proteins representing PSII core PsbA (D1) and PsbD (D2) accumulated more in *pgrl1* and *pgr5* than WT under high light stress. However, D1 and D2 did not differ significantly in *pgr5* grown under moderate light than the WT control condition (Figure 5.4A and 5.4B). The PSII major antenna protein CP43 is decreased noticeably in *pgr5* while slightly decreased in *pgrl1* mutant than WT control under moderate and high light.

Immunoblot analysis of proteins from *C. reinhardtii* WT and *pgrl1*, *pgr5* mutants

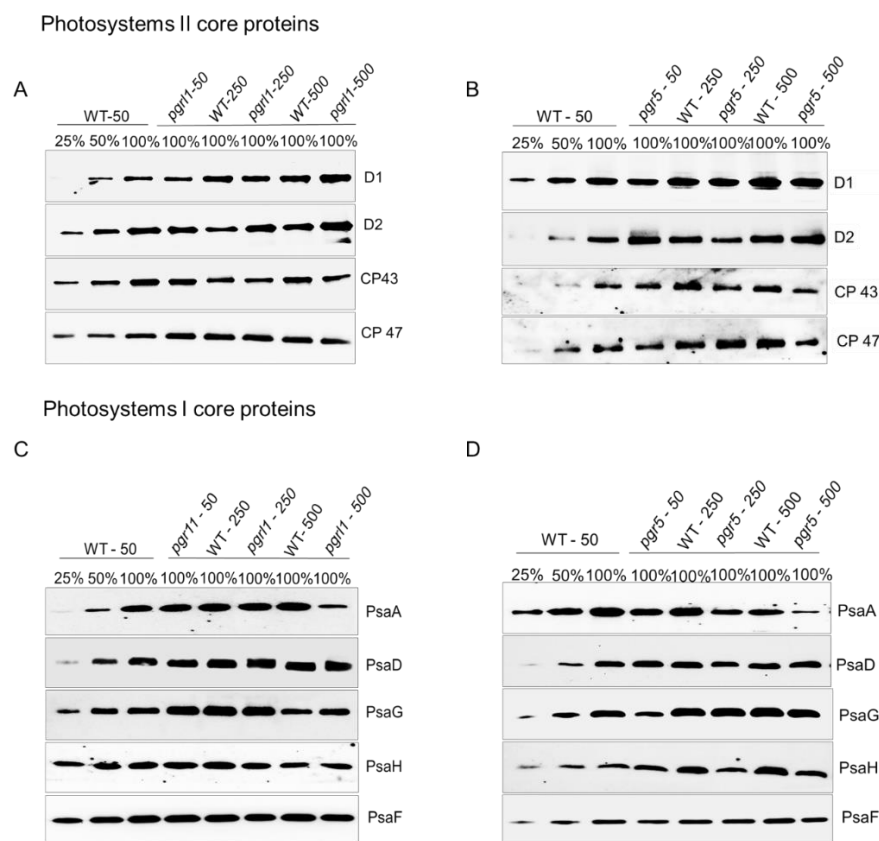


Figure 5.4. Immunoblot analysis of core (PSII and PSI) proteins (A-D) selected from *C. reinhardtii* wild-type (wt) and *pgrl1*, *pgr5* mutant. The WT, *pgrl1* and *pgr5* mutant lines were grown under constant growth light ($50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and moderate ($250 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) conditions. The western blots shown here are representative of at least three biological replicates.

However, another antenna protein, CP47, significantly reduced in high light in *pgr5* but increased dramatically in *pgrl1* and *pgr5* in moderate light than WT Control. The protein content of PsbA (D1) slightly increased in *pgrl1* and *pgr5* in high light than WT control (Figure 5.4A). In high light stress conditions, CP 43 and CP47 showed a reduced

amount in *pgr5* due to changes in chlorophyll and carotenoid molecules around the PSII protein, which coordinates the cofactors to transfer electrons to PSI (Figure 5.4B). The PGR1 and PGR5 proteins are part of the PSI complexes and are extensively involved in CET for energy generation. The absence of this protein affects the culture growth and other photosynthetic parameters related to both PSI and PSII in high light.

Additionally, I checked the outcome of high light on *pgr1* and *pgr5* strains and how the absence of these proteins affects other PSI proteins under high light studied. The PsaA subunit of the PSI showed a more than 50% decrease under moderate light while over 75% decrease under high light in *pgr5* (Figure 5.4 D). Although PsaA protein content was unaffected in *pgr1* under moderate light but significantly decreased in high light than WT control. At that time, there was a marginal decrease of PsaD and PsaG protein content in *pgr1* and WT under high light, but these proteins are unaffected under moderate light than WT control (Figure 5.4C).

The stromal subunits PsaC and PsaD act as a binding site for ferredoxin, which leads to an efficient electron transport chain (Fischer et al., 1998). Contracting the *pgr1*, PsaD is marginally decreased in moderate light but increased slightly in *pgr5* high light. At the same time, PsaG is accumulated in WT and *pgr5* under moderate and high light than WT control. On the other hand, another subunit PsaD is noticeably accumulated in WT and *pgr5* under moderate and high light

than WT control. Together mutants confirmed that the protein content of PsaF did not change from WT under moderate and high light conditions showing the arbitrary presence in the PSI complexes leaves unaffected (Figure 5.4C and 5.4D). Parallel results have been described earlier in *A. thaliana pgr5* and *pgrl1* (DalCorso et al., 2008). The immunoblot analyses of the LHCII Chl *a/b*-binding proteins were relatively consistent between WT, and *pgrl1* mutant accepted the *pgr5* under high light.

The Lhcb1 protein increased marginally in *pgrl1* and *pgr5* in high light, while the mutation unaffected the accumulation of Lhcb2 in *pgrl1* but slightly reduced in *pgr5* in high light than WT control (Figure 5.5A and 5.5B). The minor light-harvesting complexes Lhcb4 (CP29) and Lhcb5 (CP26) have been proposed to play a crucial role in the zeaxanthin-dependent (qZ) high light-induced regulation of NPQ in *C. reinhardtii*. The Lhcb4 and Lhcb5 proteins are almost unaffected in WT but increased in *pgrl1* under moderate and high light than in WT control (Figure 5.5A).

However, in *pgr5*, the Lhcb4 protein is decreased slightly while significantly in moderate and high light than WT control, indicating that *pgr5* was affected more than *pgrl1* under high light (Figure 5.5B). Similarly, we can see that high light-induced photoprotective protein LHCSR3 is accumulated more in WT and *pgrl1* but absent in *pgr5* under moderate and high light.

Further, we examined the protein content of the light-harvesting complex of PSI that is arranged in two layers towards the stromal side of the PSI core and is essential for efficient energy transfer and protection of PSI under photodamage. We suggest four PSI light-harvesting proteins, Lhca1, Lhca8, Lhca7, and Lhca3, are situated in the internal layer. Another four PSI light-harvesting subunits, Lhca1, Lhca4, Lhca6 and Lhca5, are positioned in the outside layer at the PSAF side of the core. Two light-harvesting subunits, Lhca2 and Lhca9 associated with PSAB (Ozawa et al., 2018). Interestingly, the light-harvesting complex of PSI the Lhca1, Lhca4, Lhca5 and Lhca7 are relatively stable in *pgrl1* high light, while in high light, extensively affected the Lhca2, Lhca3, Lhca6 and Lhca9 in *pgrl1* and *pgr5* (Figure 5.5C and 5.5D).

Unusually, the association of Lhca1, Lhca7, Lhca8, and Lhca3 is more steady than Lhca4, Lhca5, and Lhca6 light-harvesting proteins are positioned in the innermost layer (Drop et al., 2011). The Lhca1 is almost unaffected in *pgrl1* and *pgr5* in moderate and high light than WT control (Figure 5.5C and 5.5D).

The Lhca2 and Lhca9 present near the PsaG subunit increased in *pgrl1* and *pgr5*, except for the Lhca9 in *pgrl1* decreased under moderate and high light than WT control. The site of Lhca3 closeness to PSAB is reliable on previously reported results (Moseley et al., 2002).

Western blotting analysis of all the LHCs of photosystem II and I subunit

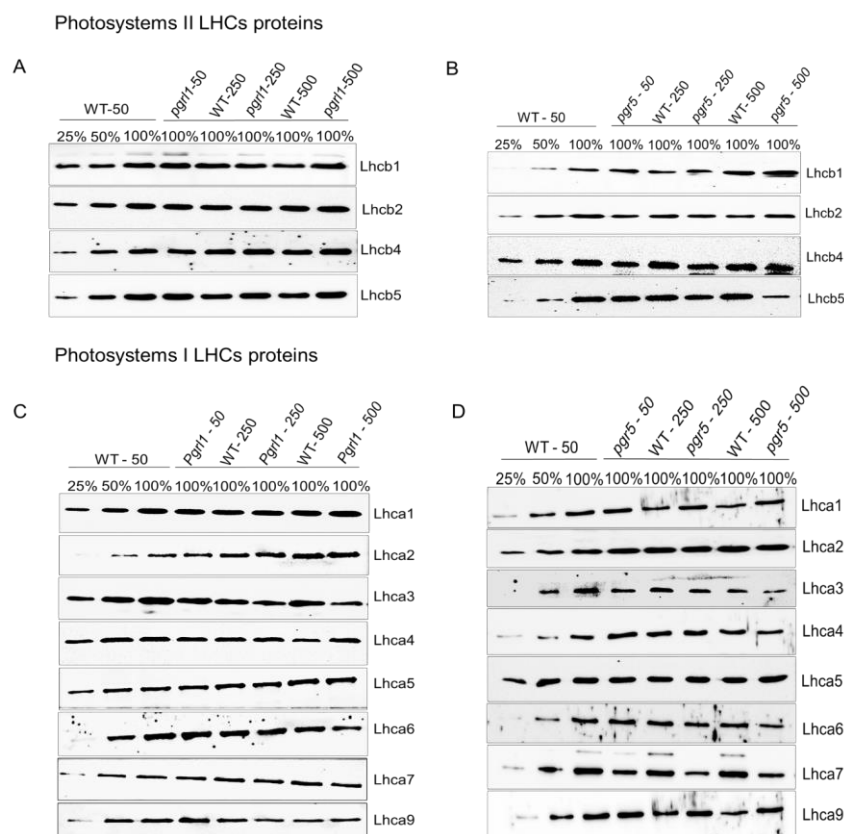


Figure 5.5 Immunoblot analysis of selected Light-harvesting complex protein of PSII and PSI (A-D) from *C. reinhardtii* wild-type (WT) and *pgrl1*, *pgr5* mutant were grown under constant growth light ($50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and moderate ($250 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and high light ($500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) conditions. The western blots shown here are representative of at least three biological replicates.

The Lhca3 are the present outer side of the edge in the inner ring, and Lhca6 at the outer ring is reduced significantly in *pgrl1* and *pgr5* under moderate and high light than WT control. Another 2 LHCs, Lhca4 and Lhca5, are likely located in the outer ring of PSI polypeptide and

unaffected under moderate and high light in *pgrl1* and *pgr5* than WT control (Figure 5.5C and 5.5D). The accumulation of the inner subunit of Lhca7 is unchanged in *pgrl1*, whereas it decreased significantly in *pgr5* under moderate and high light than WT control.

5.2.5 High light changes protein coverage up, down-regulated, and differentially expressed proteins (DEPs) between treated versus control.

The current study combined the nLC-MS/MS technique and targeted proteome to unknot the essential proteins accountable for resistance to high light *C. reinhardtii*. We speculated whether the CET mutants might be related to variations in PETC proteins and downstream metabolism in *C. reinhardtii*. Total-cell proteomes were taken from cultures grown under optimal growth and high light conditions to address the following question (Figure 5.6A). A Venn diagram of each protein was established, and we got almost proteins 295 in WT, 306 in *pgrl1* and 299 in *pgr5* under growth light while 306 in WT, 306 in *pgrl1* 286 in *pgr5* in high light. We have placed almost 249 DEPs in WT_50 and 284 DEPs in high light stress from the entire proteome. Of the total, 284 proteins showed common expression in growth light (WT_50) and high light treated W_500 (Figure 5.6B).

17 DEPs in WT_50 while 52 DEPs in WT_500 show unique expression in low and high light conditions. Further, I have annotated and enriched the *C. reinhardtii* proteome with the sample identified peptide after

nLC-MS/MS analysis categorised based on molecular weight and protein sequence coverage (Figure 5.7A and 5.7B).

Experimental setup for protein extraction, enrichment and database search

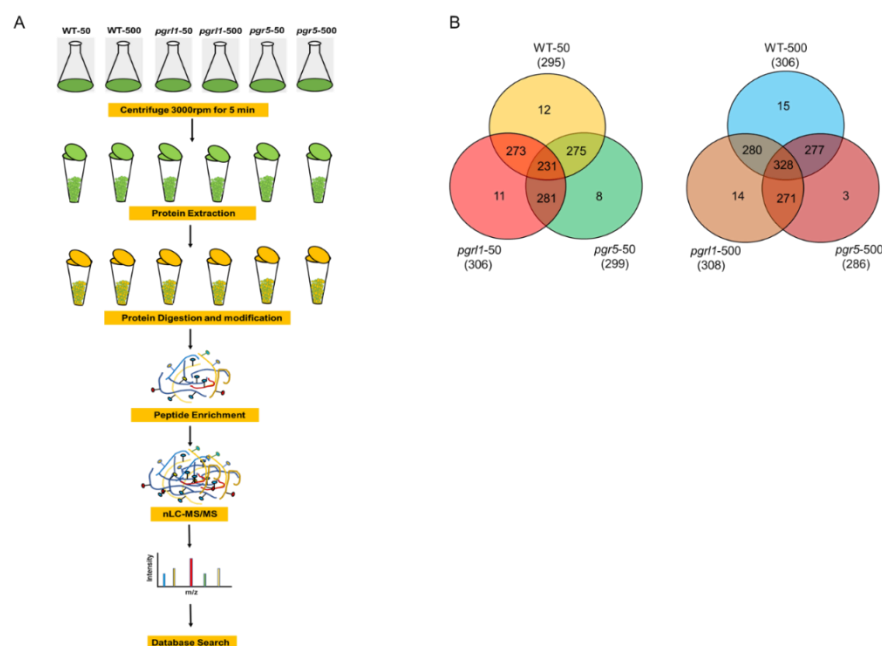


Figure 5.6 Overview of the experimental design for analyzing the proteins in WT and mutants in growth and high light condition. (A) The proteins were extracted with methanol: chloroform: water extraction solution, derivatized and measured with GC-MS. Proteins were isolated, digested, and desalted using a combined method. Subsequently, peptides were enriched and analysed by LC-MS/MS database software. (B) A Venn diagram of each protein was shown, which share common, and exclusively expressed proteins WT, pgr11 and pgr5 in growth and high light condition.

We got 6654 peptides in WT control light condition (denoted as WT_50, here 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ is light intensity in growth light), and 14822 peptides in high light treated (WT_500, here 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ is light intensity at high light) condition.

C. reinhardtii Whole-cell proteome analysis at the high light condition

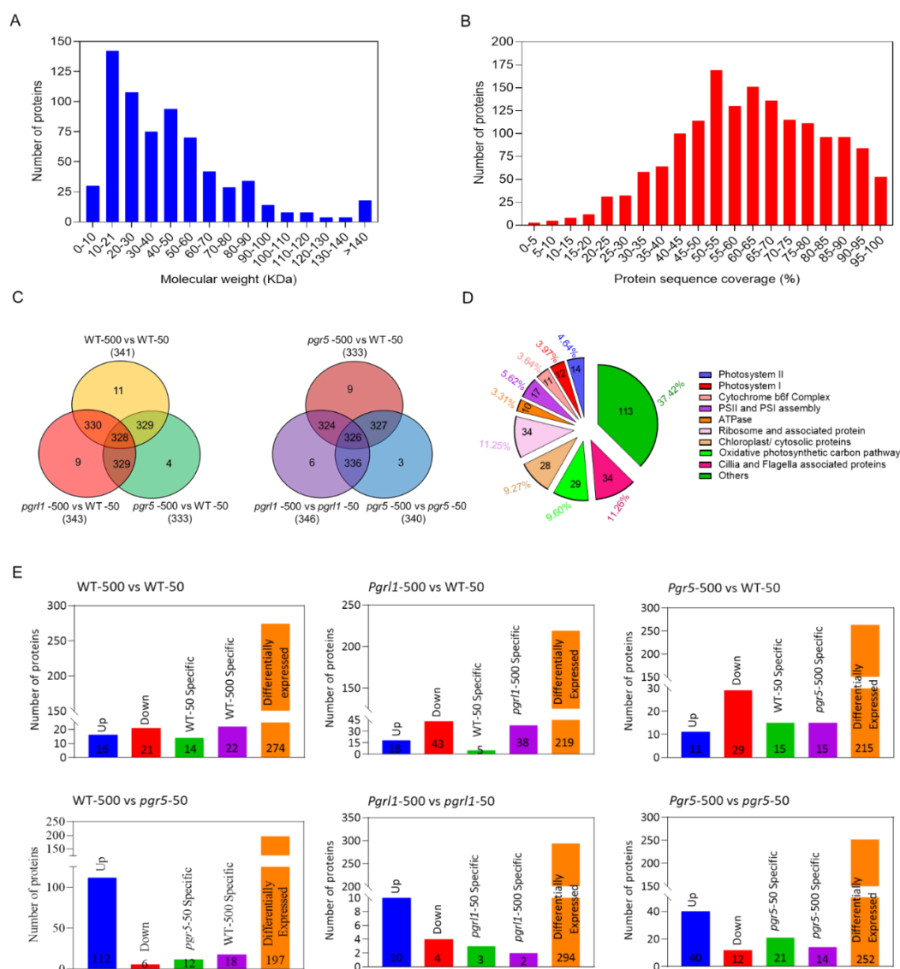


Figure 5.7 Protein coverage, up, down and differentially expressed proteins (DEPs) between treatments versus control. (A) Total numbers of proteins against different molecular weights. (B) The number of proteins against additional protein sequence coverages. (C) Venn diagrams showing the significantly different protein levels light treatment versus control. (D)

The total number of proteins shared unique expression levels represented as a percentage. (E) The comparative proteome of the total number of upregulated and downregulated proteins.

The mutant *pgrl1* showed 23133 peptides at growth light (*pgrl1_50*) and 20515 at high light (*pgrl1_500*) conditions. On the other hand, mutant *pgr5* showed 12733 peptides at growth light (*pgr5_50*) but only 4362 peptides at the high light (*pgr5_500*) conditions. Further, we enriched all peptides into proteins, which share common, and exclusively expressed proteins in treated versus control, as shown in Figure 5.7C and Table 5.1 to Table 5.6.

Overall, 302 proteins from several functional groups were significantly affected together in treated and control, out of which 14 (4.64%) were associated with PSII, 12 (3.97%) were involved in PSI, 11 (3.64%) were in Cyt b_6f complex, 10 (3.31%) were engaged in ATPase assembly, and 17 (5.62%) were involved in photosystems (PSI and PSII) assembly. Proteins associated with photosynthesis 64 (22%), 29 (9.60%) proteins related to mitochondria, 28 (9.27%) proteins related to chloroplast/cytosol, 34 (11.25 %) proteins related to translational machinery, 34 (11.26 %) associated with Cilia/Flageller assembly, followed by 113 (37.42%) proteins associated with primary and secondary metabolism (Figure 5.7D).

These up and down-regulated proteins are primarily engaged in photosynthesis, metabolic pathway, glycolysis, protein synthesis, cilia and flagella-associated protein, and protein involved in cytoskeleton assembly. We have compared *Chlamydomonas* proteome treated versus control. In WT_500 vs WT_50, we identified 274 as DEPs; 16 proteins are up-regulated, 21 are downregulated, and 14 and 22 are exclusively

expressed in WT_50 and WT_500, respectively (Figure 5.7E). In *pgrl1_500* vs WT_50, we identified 219 as DEPs; 18 proteins are up-regulated, 43 are downregulated, and 5 and 38 are exclusively expressed in WT_50 and *pgrl1_500*, respectively. In *pgr5_500* vs W_50, we have identified 215 as DEPs; 40 proteins are up-regulated, 12 are downregulated, and 21 and 14 are exclusively expressed in WT_50 and *pgr5_500*, respectively. In WT_500 vs *pgr5_50*, *pgrl1_500* vs *pgrl1_50* and *pgr5_500* vs *pgr5_50*, the proteins involved in up, down, DEPs, treated and control (Figure 5.7E).

5.2.6 Changes in photosynthetic and high light-inducible proteins of *C. reinhardtii* under high light

DEPs contributing to photosynthesis were defined under high light. The water oxidizing complex, PSBO_P12853 (Oxygen-evolving enhancer protein 1_chloroplastic) and PSBP_P11471 (Oxygen-evolving enhancer protein 2_ chloroplastic) is up-regulating ($\geq 2FC$) in WT_500 vs *pgr5_50*, *pgrl1_500* vs WT_50 and *pgr5_500* and PsbQ_P12852 (Oxygen-evolving enhancer protein 3_ chloroplastic) were differentially regulated in all the variants (Figures 5.8A). Only water oxidizing protein PSBO_P12853 is downregulated ($\leq 0.5FC$) in *pgr5* (*pgr5_500* vs WT_50) high light conditions.

The RuBisCO subunit RUBA_Q42694 (RuBisCO large subunit-binding protein subunit alpha_chloroplastic) and RUBB_Q42693 (RuBisCO large subunit-binding protein subunit beta-1), a chaperone protein involved in RuBisCO assembly, were deferentially regulated in

WT (WT_500 vs WT_50), but RUBA_Q42694 is downregulated in *pgrl1* (*pgrl1*_500 vs WT_50) and RUBB_Q42693 in *pgr5* (*pgr5*-500 vs W-50) mutant under high light. Another subunit of RuBisCO assembly, RUBC_Q42695 (RuBisCO large subunit-binding protein subunit beta-2), binds RuBisCO small and large subunits and is occupied in the enzyme assembly is differentially regulated in WT and *pgrl1* but downregulated in *pgr5* (*pgr5*_500 vs W_50) high light conditions (Figure 5.8B).

The PSII reaction center proteins PSBA_P07753 (D protein) and PSBD_P06007 (D2 protein) are upregulated more than 2- fold change expression in WT high light than the *pgr5* growth light condition (WT_500 vs *pgr5*_50) and differentially regulated ($\leq 2FC$ and $\geq 0.5FC$) in *pgrl1* and *pgr5* under high light than WT growth light (*pgrl1*_500 vs WT_50 and *pgr5*_500 vs WT_50) or *pgrl1* and *pgr5* (*pgrl1*_500 vs *pgrl1*_50 and *pgr5*_500 vs *pgr5*_50) growth light conditions (Figure 5.8C).

On the other hand, the major antenna proteins PSBB_P37255 (PS II CP47 reaction center protein) and PSBC_P10898 (PS II CP43 reaction center protein) are up-regulated in both WT and *pgr5* high light than the *pgr5* growth light condition (WT_500 vs *pgr5*_50 and *pgr5*_500 vs *pgr5*_50 (Figure 5.8C) and differentially regulated in all other variants except the *pgrl1*_500 vs *pgrl1*_50 is downregulated.

Three PSI RC proteins, PSAB_P09144 (PSI P700 chlorophyll a apoprotein A2), PSAE_P12352 (PSI reaction center subunit III

chloroplastic) and PSAB_P12356 (PSI reaction center subunit III_chloroplastic) is increased more than 2- fold change expression in WT (WT_500 vs WT_50, WT_500 vs *pgr5_50*) under high light.

The other PSI PSBB_P09144 is the only protein up-regulated in *pgr5* high light than the growth light (*pgr5_500* vs *pgr5_50*) conditions. The two PSI core proteins downregulated $< 0.5\text{FC}$ expression in *pgr5* are PSAA_P12154 (PSI P700 chlorophyll a apoprotein A1) and PSAG_P14224 (PS I reaction center subunit V_chloroplastic), as it decreases in protein blot under high light conditions (*pgr5_500* vs *pgr5_50*).

Other PSI proteins like PSAC_Q00914 (PSI iron-sulfur center), PSAD_Q39615 (PSI reaction center subunit II_chloroplastic) and PSAH_P13352 (PSI reaction center subunit VI_chloroplastic) are differentially regulated ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in all the variants in WT, *pgr11*, and *pgr5* treated vs Control light condition (Figure 5.8D). The possible role of PSAH_P13352 could be provided with the docking site of the LHC I antenna protein to the core complex.

High light changes photosynthetic electron transport and rubisco subunit in *C. reinhardtii*

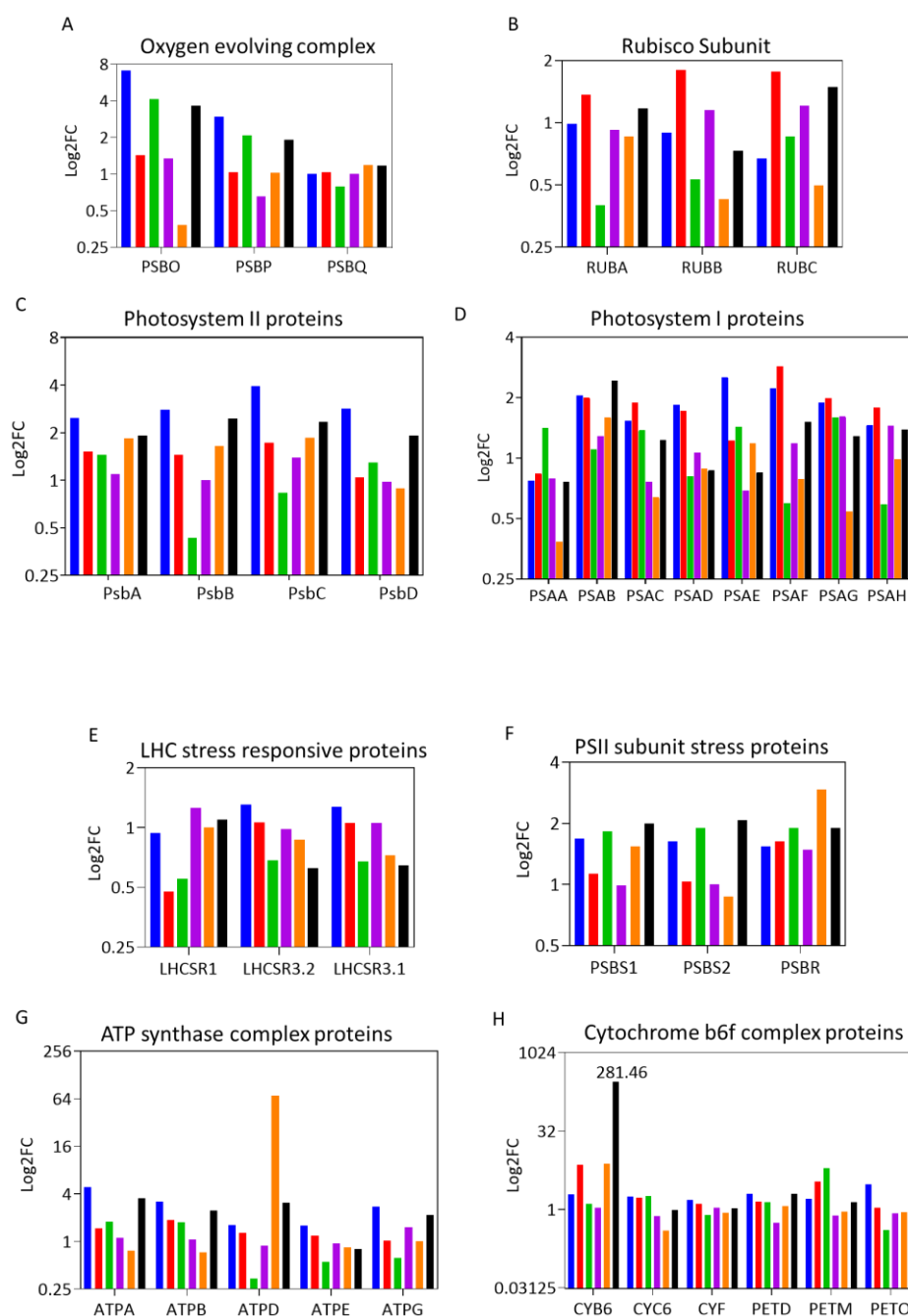


Figure 5.8 Proteins participating in photosynthetic machinery (PETC: Photosynthetic electron transport chain) involved in OEC (A), RubisCO subunit (B), PSII (C) and PSI (D) proteins are significantly affected under high light stress and are shown as log₂FC are shown (n=3). Proteins expressed in high light stress belonging to NPQ generation (E and F), ATP synthesis

(G) and Cyt *b₆f* complex (H) are mainly involved under high light and are significantly affected and shown as proteins with *p* value < 0.05 (*n*=3).

The stress-related proteins LHCSR3 and PSBS were differently expressed ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in *pgrl1* and *pgr5* in high light (Figure 5.8E and Figure 5.8F). The LHC stress-related protein 1 (LHCSR1_P93664) is mainly involved in zeaxanthin-dependent quenching and increased $>2\text{FC}$ expression in *pgrl1* and *pgr5* (*pgrl1*_500 vs *pgrl1*_50 , *pgr5*_500 vs *pgr5*_50 and *pgr5*_500 vs WT_50) mutant than WT (WT_500 vs WT_50) in high light conditions. The LHC stress-related protein 3.1 (LHCSR3.1_P0DO19) and LHCSR3.2_P0DO19, induced by lumen acidification, were decreased ($\log_2\text{FC}$ expression) in *pgrl1* and *pgr5* mutants than WT high light.

The LHCSR3.2_P0DO19 has increased $\log_2\text{FC}$ expression in WT high light (WT_500 vs WT_50, W_500 vs *pgr5*_50) than the WT and *pgr5* control light condition (Figure 5.8E). This data agrees with the previous chapter, where the LHCSR3 expression is not seen in *pgr5* and less in *pgrl1* under high light. However, *pgrl1* has decreased $\log_2\text{FC}$ expression of LHCSR3.2_P0DO19, whereas its expression was lacking in *pgr5* under high light conditions.

This finding aligns with NPQ data, showing that *pgr5* cannot generate NPQ, and *pgrl1* has a limited NPQ than WT (See Objective 1 Figure 3.11B, and 3.11C). Another high light expressing protein PSBS presence into two isoforms, one is PSBS1 _A8HPM2 (PSII protein PSBS1) which $\log_2\text{FC}$ expression is higher in *pgr5* (*pgr5*_500 vs

pgr5_50 and *pgr5_500* vs WT_50) and *pgrl1* (*pgrl1_500* vs WT_50) than WT (WT_500 vs *pgr5_50*) high light condition. On the other side, PSBS2_A8HPM5 (PSII protein PSBS2), the log2FC expression increased majorly into *pgr5* (*pgr5_500* vs *pgr5_50*) than *pgrl1*(*pgrl1_500* vs WT_50) and WT (WT_500 vs *pgr5_50*) under high light.

It shows that the LHCSR3.1_P0DO19 and LHCSR3.2_P0DO19 are majorly expressed proteins in WT, while PSBS2_A8HPM5 and PSBS1_A8HPM2 are significantly expressed in *pgr5* and *pgrl1* mutants, is the major effector protein under high light photoprotection in *C. reinhardtii* (Figure 5.8F). The proteomic data agrees with the expression levels of PSBS protein (Objective 2, Figure 4.6G). The PSBR_A0A2K3DMP5 (PSII protein PSBR chloroplastic) protein is essential for the steady binding of LHCSR3 to PSII-LHCII and is crucial for efficient quenching and maintaining the stability of the PSII-LHCII-LHCSR3 supercomplex under high light.

The ATP synthase subunit ATPA_P26526 (ATP synthase subunit alpha_chloroplastic), ATPB_P06541 (ATP synthase subunit beta_chloroplastic) (ATP synthase subunit beta_chloroplastic), and ATBG_P12113 (ATP synthase gamma chain_chloroplastic) are essential for proton uptake from the lumen to balance the proton conductivity on the thylakoid membrane are up-regulated in WT (WT_500 vs *pgr5_50*) and *pgr5* (*pgr5_500* vs *pgr5_50*) and

differentially regulated in *pgrl1* (WT_500 vs *pgrl1_50*) under high light stress (Figure 5.8G).

The ATPD_Q42687 (ATP synthase delta chain_chloroplastic) and ATPE_P07891 (ATP synthase epsilon chain_ chloroplastic) are upregulated in WT (WT_500 vs *pgr5_50*, WT_500 vs WT_50), but ATPD_Q42687 is increased exceptionally 64 times log2FC expression in *pgr5* (*pgr5_500* vs WT_50), showing the importance of CET for energy generation (Figure 5.8G). But these proteins ATPD_Q42687 and ATPE_P07891 are downregulated in *pgrl1*(*pgrl1_500* vs W_50) than WT, indicating the significance of CET under high light stress. The *pgrl1* and *pgr5* mutants established a substantial reduction in FC expression in CYF_P23577 (Cyt f) and CYC_P15451 (Cyt C) of the Cyt b₆f complex than WT high light.

Under both conditions, *pgrl1_500* vs *pgrl1_50* and *pgr5_500* vs WT_50 were more downregulated in CYC_P15451 (~ one-third of log2FC expression of the WT). The log2FC expression of CYF_P23577 ($p < 0.05$) did not significantly change in *pgrl1* and *pgr5*. Another essential Cyt b₆f complex protein is CYB6_Q00471 (Cyt b₆) which log2FC expression is increased 281 times in *pgr5* (*pgr5_500* vs *pgr5_50*) than WT high light (Figure 5.8H, WT_500 vs WT_50 and WT_500 vs *pgr5_50*).

The PETD_P23230 (Cyt b₆f complex subunit 4), another type of Cyt b₆f complex protein, was similarly expressed in WT, *pgrl1* and *pgr5* in all high light conditions, except for a slight downregulation of

PETD_P23230 was observed in *pgrl1* (*pgrl1*_500 vs *pgrl1*_50) mutant under the HL condition (Figure 5.8H). The PETM_Q42496 (Cyt b₆f complex subunit 7_ chloroplastic) and PETO_Q9LLC6 (Cyt b₆f complex subunit 5_ chloroplastic) have differentially expressed ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in *pgrl1* and *pgr5*, except for PETM_Q42496, Cyt b₆f subunit encoded by nucleus apparently effective in signaling pathway (Schneider et al., 2001) whose expression is upregulated ($\geq 2\text{FC}$) in *pgrl1* (*pgrl1*_500 vs WT_50), and WT (WT_500 vs *pgr5*_50 and WT_500 vs W_50) under high light. Similar results have been described earlier in *A. thaliana pgr5* and *pgrl1* (DalCorso et al., 2008).

5.2.7 High light changes the metabolism of chloroplast, cytosol and mitochondria

C. reinhardtii is a mixotroph that can act as an autotroph or heterotroph, depending on medium conditions. The control light-grown cultures have a robust carbon fixation potential, but downstream metabolites were changed when grown in high light, where the growth was affected. The glycolytic enzyme ALFC_Q42690 (Fructose-bisphosphate aldolase 1_chloroplastic) are essential enzyme in the energy pathways of algae; it was differentially regulated ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in WT, *pgrl1* and *pgr5* under high light (Figure 5.9). The enzyme G3PA_P50362 (Glyceraldehyde-3-phosphate dehydrogenase A_ chloroplastic) involved in the glucose metabolic process was up-regulated in *pgr5* (*pgr5*_500 vs *pgr5*_50), ($\geq 2\text{FC}$) while down-regulated in *pgrl1* (*pgrl1*_500 vs WT_50) in high light.

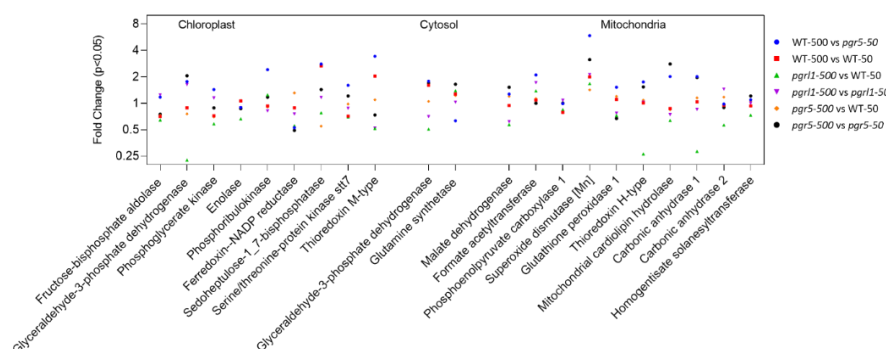


Figure 5.9 Proteins participating in chloroplast, cytosol and mitochondria are significantly affected under high light are shown. Proteins with fold change and p value, $p < 0.05$, are shown ($n=3$).

Similarly, it is differentially regulated in WT (WT_500 vs pgr5_50 and WT_500 vs WT_50) under high light. Simultaneously, protein KPPR_P19824 (Phosphoribulokinase_chloroplastic) is engaged in the Calvin cycle, part of carbohydrate biosynthesis, and was only up-regulated in WT (WT_500 vs pgr5_50) and differentially regulated in pgrl1 and pgr5 (pgrl1_500 vs WT_50) and pgr5 (pgr5_500 vs WT_50) under high light.

The enzyme FENR_P53991 (Ferredoxin- NADP reductase_chloroplastic) is vital for adjusting the cyclic and non-cyclic electron transport to meet the requirement of ATP, and reducing power was differentially regulated in WT (WT_500 vs WT_50) and pgrl1 (pgrl1_500 vs WT_50). However, it was downregulated in pgr5 (pgr5_500 vs pgr5_50) under high light. The TRXM_ P23400 (Thioredoxin M-type_chloroplastic) are vital regulatory players receiving information about the redox state of the PETC and participate in reversible oxidation of the active center dithiol to a disulfide was

highly up-regulated more than three-fold-change ($>3 \log_2\text{FC}$) in WT (WT_500 vs *pgr5_50* and WT_500 vs WT_50) and was differentially regulated in *pgrl1* (*pgrl1_500* vs W_50) and *pgr5* (*pgr5_500* vs WT_50) under high light.

The M type of TRX is known to activate NADP-malate dehydrogenase to hold the CET indirectly. The enzyme ENO_P31683 (Enolase) was differentially regulated in WT (WT_500 vs WT_50), *pgrl1* (*pgrl1_500* vs WT_50) and *pgr5* (*pgr5_500* vs WT_50) under high light. The key enzyme STT7_Q84V18 (Serine/threonine-protein kinase *stt7_ chloroplastic*) is required for state transition by phosphorylating LHC II external antenna differentially regulated ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in WT, *pgrl1* and *pgr5* in high light (Figure 5.9).

The S17P_P46284 (Sedoheptulose-1,7-bisphosphatase) chloroplastic protein is highly upregulated (more than $>2\text{FC}$) in WT (WT_500 vs *pgr5_50* and WT_500 vs WT_50) and was differentially regulated in *pgrl1* (*pgrl1_500* vs W_50) and *pgr5* (*pgr5_500* vs WT_50) in high light. Comparing the whole cell proteome of *C. reinhardtii*, high light grown cultures suggested metabolic enzyme was strongly affected in *pgrl1* and *pgr5*. The tricarboxylic acid (TCA) cycle MDHM_Q42686 (Malate dehydrogenase_ mitochondrial) was differentially regulated in WT (WT_500 vs WT_50), *pgrl1* (*pgrl1_500* vs WT_50) and *pgr5* (*pgr5_500* vs WT_50) under high light (Figure 5.9). The MDHM_Q42686, a mitochondria enzyme, synthesizes extra reducing power for chloroplast by converting oxaloacetate to malate by

malate shuttle shunt.

This way, an additional NADPH pool is synthesized and regenerated (Scheibe, 2004). The mitochondrial protein GPX1_P83564 (Glutathione peroxidase 1) was up-regulated in WT (W_500 vs *pgr5_50*) and may constitute a glutathione peroxidase-like protective system against oxidative stresses and synthesizing PUFA through arachidonic acid metabolic pathways was differentially regulated in *pgrl1* and *pgr5* in high light. The protein PAPP1_P81831 (phosphoenolpyruvate carboxylase 1) is involved in the carboxylation of phosphoenolpyruvate (PEP) forms oxaloacetate was differentially regulated ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in WT, *pgrl1* and *pgr5* high light. This enzyme is activated by glutamine and dihydroxyacetone phosphate and inhibited by glutamate and malate. Superoxide dismutase (Mn), mitochondrial (SODA), destroys superoxide anion radicals within the cells.

It is toxic to biological systems and was highly up-regulated ($> 5\text{FC}$) in WT (WT_500 vs *pgr5_50*) and was more than two-fold change ($>2\text{FC}$) up-regulated in *pgrl1* (*pgrl1_500* vs W_50) and more than the three-fold difference ($> 3\text{FC}$) up-regulated in *pgr5* (*pgr5_500* vs WT_50) under high light stress; though, its expression was flexible and statistically significant ($p < 0.05$). Whereas TRXH_P80028 (thioredoxin H-type) protein contributes to several redox responses through the reversible oxidation of the dithiol into disulfide by taking the electron from the ferredoxin, with the activity of enzyme FTR (ferredoxin: thioredoxin reductase) were differentially regulated ($\leq 2\text{FC}$ and $\geq$

0.5FC) in WT, *pgrl1* and *pgr5* and its H form is known to activate several cytosolic enzymes (Figure 5.9).

The CAH1_P20507 and CAH2_P24258 (Carbonic anhydrase 1 and 2) are present in the mitochondria and responsible for the reversible carbonate dehydratase activity for the formation of CO₂ were up-regulated in WT (WT_500 vs *pgr5*_50) and significantly downregulated ($< 0.2 \log_2\text{FC}$) in *pgrl1* (*pgrl1*_500 vs WT_50) under high light stress. We also observed that the protein mitochondrial cardiolipin hydrolase (PLD6_A8IW99) was up-regulated in more than 2FC expression in WT (WT_500 vs *pgr5*_50) and *pgr5* (*pgr5*_500 vs *pgr5*_50), proposed to be involved in the lipid catabolic process and act as a cardiolipin hydrolase to generate phosphatidic acid at the mitochondrial surface. The metabolic protein Homogentisate solanesyltransferase (HSTC_A1JHN0) is involved in PQ synthesis but also participates in carotenoids and abscisic acid (ABA) biosynthesis pathways were differentially regulated ($\leq 2\text{FC}$ and $\geq 0.5\text{FC}$) in WT, *pgrl1* and *pgr5* grown high light.

5.2.8 Heat map representing the comparative expression of the differentially abundant proteins (DAPs) between treated and control

To elucidate the heatmap analysis, we employed the expression value of WT, *pgrl1* and *pgr5* samples and normalized it into a matrix, Spearman correlation coefficient (r) on selected DEPs from data points.

Hierarchical cluster analysis of proteins presents significantly altered abundances. Most of the proteins were up-regulated in WT, and few were in the *pgrl1*, but the majority of proteins in down-regulated in *pgr5* mutant in high light conditions. The heatmap study categorized the identified DEPs into four distinctive categories: photosynthetic electron transport protein, Calvin cycle and metabolism protein, light-harvesting pigment, photoprotection protein, and protein involved in translational machinery. The photosynthetic electron transport protein was divided into 42 sub-categories into three major clusters: PS II and PS I protein subunits, Cyt b_6f complex, ATP synthase, RuBisCO subunits, and electron carrier proteins (Figure 5.10A).

During the light reaction of the photosynthesis, we identified 10 DEPs from Cluster 1 (PSBB_P37255, PSBD_P06007, PSAA_P12154, PSAB_P09144, CYB6_Q00471, CYB_P23662 (Cytochrome b), ATPF_P12356, CCSA_P48269 (Cyt c biogenesis protein CcsA), RBL_P00877 (Ribulose biphosphate carboxylase large chain), PLAS_P18068 (Plastocyanin chloroplastic), in which most of the proteins are in *pgrl1*, but few of them (PSBB_P37255, ATPF_P12356, CYB6_Q00471) in WT up-regulated than *pgr5* in high light. Cluster 2 includes 18 proteins (PSBA_P07753, PSBC_P10898, PSAH_P13352, PETD_P23230, UCR1A, PHOT_Q8LPD9 (Phototropin), CYC_P15451, PETO_Q9LLC6, ATPE_P07891, RUBC_Q42695, PSAD_Q39615, ATPG_P12113, ATPD_Q42687, PETM_Q42496, RUBB_Q42693, RBS1_P00873 (Ribulose biphosphate carboxylase small chain 1_ chloroplastic), RBS2_P08475 (Ribulose biphosphate carboxylase small

chain 2_{chloroplastic}),) in 13 are upregulated in WT high light (Figure 5.10A). However, 13 proteins that are up-regulated in *pgr5* were associated with PSII chlorophyll-binding protein D1, PSI protein, ATP synthase subunit, Rubisco subunit, Cyt c subunit, and phototropin protein in growth light conditions.

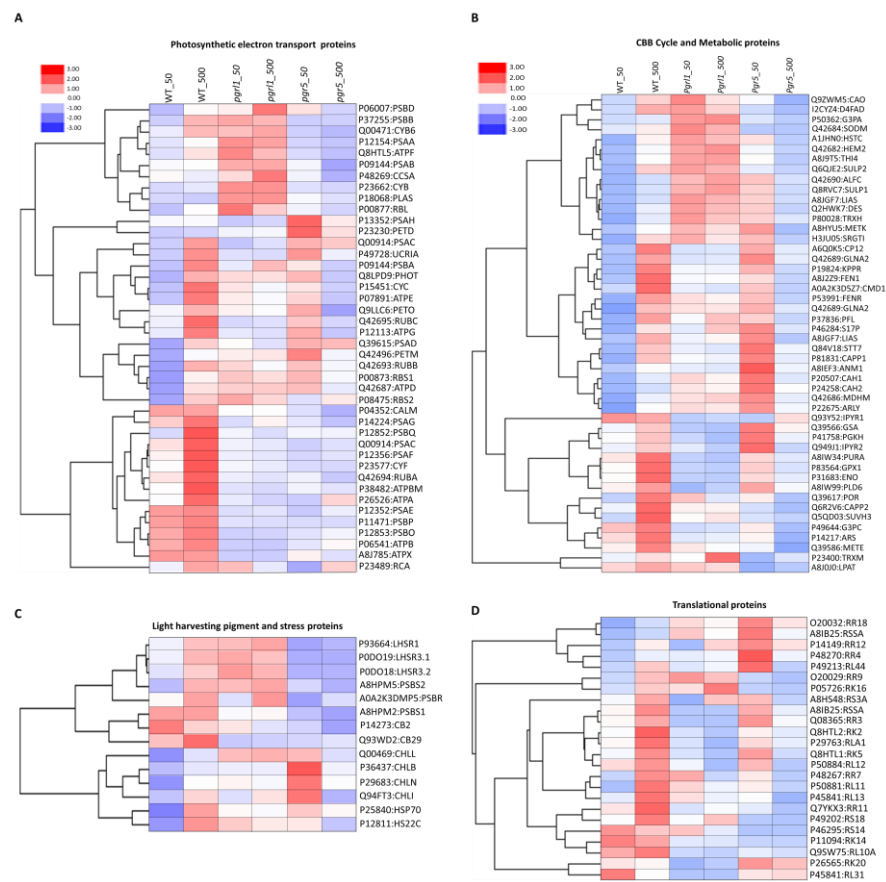


Figure 5.10 Global protein expression levels in *C. reinhardtii* in response to high light stress. Hierarchical clustering heatmap representing the relative expression of the DAPs between light treatment and control. Hierarchical clustering based on the abundant levels of protein identified was performed using Gene Cluster 3.0 software with the similarity metric and linkage method. The resulting clusters were visualized using Java Tree View software.

Cluster 3 includes [CALM_P04352 (Calmodulin), PSAG_P14224, PSBQ_P12852, PSAF_P12356, PSAC_Q00914, CYF_P23577, RUBA_Q42694, PSAE_P12352, PSBO_P12853, PSBP_P11471, ATPBM_P38482, ATPX_A8J785 (ATP synthase subunit b' chloroplastic), ATPA_P26526, ATPB_P06541 and RCA_P23489 (Ribulose biphosphate carboxylase/oxygenase activase_chloroplastic) 15 proteins exclusively involved in oxygen-evolving proteins, Cyt f protein and ATP synthase subunit upregulated in WT high light conditions.

A total of 250 DEPs were divided into 49 sub-categories of the Calvin cycle and metabolism protein and categorized into three significant clusters (Figure 5.10B). Cluster 1 includes (CAO_Q9ZWM5 (Chlorophyllide a oxygenase_chloroplastic), D4FAD_I2CYZ4 (Acyl-lipid (7-3)-desaturase_chloroplastic), G3PA_P50362, SODM_Q42684 (Superoxide dismutase (Mn)_mitochondrial), HSTC_A1JHN0 (Homogentisate solanesyltransferase_chloroplastic), HEM2_Q42682 (Delta-aminolaevulinic acid dehydratase_chloroplastic), THI4_A8J9T5 (thiamine thiazole synthase_chloroplastic), SULP1_Q8RVC7, SULP2_Q6QJE2 (Sulfate permease 1 and 2_chloroplastic), ALFC_Q42690, LISC_A8I2V9 (Lipoyl synthase_chloroplastic), DES_Q2HWK7(Acyl-lipid omega-13 desaturase), TRXH_P80028 ()), SRGT1_H3JU05 (Peptidyl serine alpha-galactosyltransferase), METK_A8HYU5 (S-adenosylmethionine synthase), 15 DEPs with the maximum number observed in the dark reaction are up-regulated in *pgrl1* in growth and high light conditions (Figure 5.10B).

Cluster 2 includes (CP12_ A6Q0K5 (Calvin cycle protein CP12_chloroplastic), GLNA2_Q42689 (Glutamine synthetase_chloroplastic), KPPR_P19824 (Phosphoribulokinase_chloroplastic), FEN1_A8J2Z9 (Flapendonuclease1), CMD1_A0A2K3D5Z7 (5-methylcytosine-modifying enzyme), FENR_P53991, GLNA1_Q42688 (Glutamine synthetase cytosolic isozyme), PFL_P37836 (Formate acetyltransferase), S17P_P46284, LIAS_A8JGF7 (Lipoyl synthase_mitochondrial), STT7_Q84V18 (Serine/threonine-protein kinase stt7_chloroplastic), CAPP1_P81831 (Phosphoenolpyruvate carboxylase 1), ANM1_A8IEF3 (Protein arginine N-methyltransferase 1). It also contains CAH1_(P20507), CAH2_(P24258), MDHM_Q42686 (Malate dehydrogenase_mitochondrial), ARLY_P22675 (Argininosuccinate lyase), 17 DEPs in which seven (7) are up-regulated in WT high light, and 15 are up-regulated in *pgr5* growth light than *pgr1* are involved at carbon fixation, protein lipoylation, modifiable cyclic and non-cyclic electron transport, supplying additional ATP and reducing power and in CO₂ uptake.

Cluster 3 includes (TRXM_P23400 (Thioredoxin M-type_chloroplastic), G3PC_P49644 (Glyceraldehyde-3-phosphate dehydrogenase_cytosolic), GPX1_P83564 (Glutathione peroxidase 1_mitochondrial), GSA_Q39566 (Glutamate-1-semialdehyde 2_1-aminomutase_chloroplastic), IPYR1_Q93Y52 (Soluble inorganic pyrophosphatase 1_chloroplastic), PURA_A8IW34 (Adenyl succinate synthetase_chloroplastic), POR_Q39617 (Protochlorophyllide reductase_chloroplastic), PGKH_P41758 (Phosphoglycerate

kinase_chloroplastic), CAPP2_Q6R2V6 (Phosphoenolpyruvate carboxylase 2), ARS_P14217 (Arylsulfatase), ENO_P31683 (Enolase), IPYR2_Q949J1 (Soluble inorganic pyrophosphatase 2), METE_Q39586 (5-methyl tetra hydropteroyl triglutamate-homocysteine methyltransferase), SUVH3_Q5QD03 (histone-lysine N-methyltransferase_H3 lysine-9 specific SUVH3), PLD6_A8IW99 (Mitochondrial cardiolipin hydrolase), LPAT_A8J0J0 (1-acyl-sn-glycerol-3-phosphate acyltransferase), 16 DEPs in which commonly up-regulating in WT high light.

Five proteins are exclusively up-regulated in *pgr5* growth light and are involved in the glycolytic process (G3PC_P49644, PGKH_P41758, GSA_Q39566, ENO_P31683), purine nucleotide biosynthesis (PURA_A8IW34), light-independent chlorophyll biosynthesis (POR_Q39617) and lipid biosynthesis (LPAT_A8J0J0) (Figure 5.10B). The Light-harvesting pigment and photoprotection protein was subdivided into 14 sub-categories into two major clusters.

Cluster 1 includes (LHR3.1_P0DO18, LHSR1_P93664, LHSR3.2_P0DO19, PSBS1_A8HPM2, PSBS2_A8HPM5, PSBR_A0A2K3DMP5, CB29_Q93WD2, CB2_P14273,) 5 photoprotective (LHCSR and PSBS) and two Chl a/b binding proteins (CB29 and CB2) up-regulated in WT and *pgr11* but downregulated in *pgr5* high light (Figure 5.10C). We have found that Cluster 2 includes (CHLB_P37824 (Light-independent protochlorophyllide reductase subunit B), CHLL_Q00469 (Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein), CHLN_P29683 (Light-independent

protochlorophyllide reductase subunit N), CHLI_Q94FT3 (Magnesium-chelatase subunit ChlI_chloroplastic), HSP70_P25840 (Heat shock 70 kDa protein), HS22C_P12811 (Heat shock 22 kDa protein_chloroplastic), and H2A_P50567 (Histone protein), 7 DEPs associated with chlorophyll pigment-binding protein up-regulated in WT high light and whereas two heat shock protein up-regulated in *pgr5* growth light conditions.

The protein involved in translational machinery was further subdivided into 24 sub-categories into 2 clusters. Among them, minor clusters containing five (RR18_O20032, RR4_P48270, RR12_P14149 (30S ribosomal protein S7_chloroplastic), RSSA_A8IB25 (40S ribosomal protein SA), RL44_P49213 (60S ribosomal protein L44)) DEPs were up-regulated in *pgr5* growth light. In contrast, significant clusters including nineteen (RR2A_O47027, RR3_Q08365, RR7_P48267, RR9_O20029, RR11_P14149 (30S ribosomal proteins_chloroplastic) RS3A_A8HS48, RS14_P46295, RS18_P49202 (40S ribosomal protein), RK2_Q8HTL2, RK5_Q8HTL1, RK14_P11094, RK16_P05726, RK20_P26565 (50S ribosomal proteins_chloroplastic), RLA1_P29763, RL10A_Q9SW75, RL11_P50881, RL12_P50884, RL13_O48513, RL31_P45841 (60S ribosomal protein L13) DEPs in which 17 were up-regulated in WT and remaining 2 (RK20_P26565, and RL31_P45841) are up-regulated in *pgr5* high light condition (Figure 5.10D).

5.2.9 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

The GO enrichment analysis includes a biological process (BP), cellular component (CC), and molecular function (MF) of up, down-regulated, and DEPs whose p value is <0.05 under high light stress (Figure 5.11). No proteins significantly changed in function type between WT and *pgr5*, confirming minimal protein turnover under high light treatment.

Gene ontology analysis in WT₅₀₀ vs WT₅₀ high light proteomics reveals a special enrichment for PSII. I detected considerable changes in proteins growing in high-light WT samples. Out of 350 DEPs, 18 were involved in 18 BP, 15 proteins in 14 CC, and 23 were engaged in 35 MF categories. We have also separated some core proteins from the groups WT₅₀₀ vs WT₅₀, mainly involved in ETR, CET, and water photolysis. KEGG pathway showed that 16 DEPs are primarily interested in photosynthesis, oxidative phosphorylation and metabolic pathway, i.e., glycolysis and TCA cycle (Figure 5.11A).

Gene Ontology (GO) enrichment analysis of the global proteomic dataset of lightly treated *pgrl1*₅₀₀ vs WT₅₀ cultures revealed that *pgrl1* was enriched over WT in which 39 proteins were involved in 83 biological functions, 32 types of proteins in 88 MF, and 45 types of proteins in 58 CC, including protein, unfolding, PSII repairing and oxidative responses related to ROS. KEGG pathway showed that 15 DEPs are mainly involved in 3 major categories, i.e., photosynthesis, oxidative phosphorylation and metabolic pathway (Figure 5.11B).

GO and KEGG Pathway analysis

(A) WT-500 vs WT-50

	GO Term	Protein Counts	
BP	Photosynthesis [GO:0015979]	8	Enrichment Fold Change
	Photosynthesis, light reaction [GO:0019684]	4	
	Photosynthesis, dark reaction [GO:0019685]	2	
	Chlorophyll biosynthetic process [GO:0015995]	4	
	Photosynthetic electron transport chain [GO:0009767]	6	
MF	Chlorophyll binding [GO:0016168]	2	
	Tetrapyrrole binding [GO:0046906]	3	
	H ⁺ transmembrane transporter activity [GO:0008509]	2	
	Cation binding [GO:0043169]	2	
	4 iron, 4 sulfur cluster binding [GO:0051539]	3	
CC	Chloroplast [GO:0009507]	15	
	Chloroplast thylakoid membrane [GO:0009535]	7	
	Photosystem [GO:0009521]	5	
	Cell and cell part [GO:0005623]	23	
	Membrane protein complex [GO:0098796]	5	
KEGG	Photosynthesis [Cre00195]	5	
	Porphyrin and chlorophyll metabolism [Cre00860]	4	
	Metabolic pathways [Cre01100]	9	
	ABC transporters [Cre02010]	2	

(B) *pgrl1*-500 vs WT-50

	GO Term	Protein Counts	
BP	Photosynthesis [GO:0015979]	13	Enrichment Fold Change
	Photosynthesis, light reaction [GO:0019684]	5	
	Photosynthesis, dark reaction [GO:0019685]	2	
	Chlorophyll biosynthetic process [GO:0015995]	3	
	Photosynthetic electron transport chain [GO:0009767]	4	
MF	Chlorophyll binding [GO:0016168]	3	
	Tetrapyrrole binding [GO:0046906]	4	
	H ⁺ transmembrane transporter activity [GO:0008509]	4	
	Cation binding [GO:0043169]	9	
	4 iron, 4 sulfur cluster binding [GO:0051539]	3	
CC	Chloroplast [GO:0009507]	25	
	Chloroplast thylakoid membrane [GO:0009535]	15	
	Photosystem [GO:0009521]	7	
	Cell and cell part [GO:0005623]	45	
	Membrane protein complex [GO:0098796]	11	
KEGG	Photosynthesis [Cre00195]	11	
	Porphyrin and chlorophyll metabolism [Cre00860]	14	
	Metabolic pathways [Cre01100]	4	

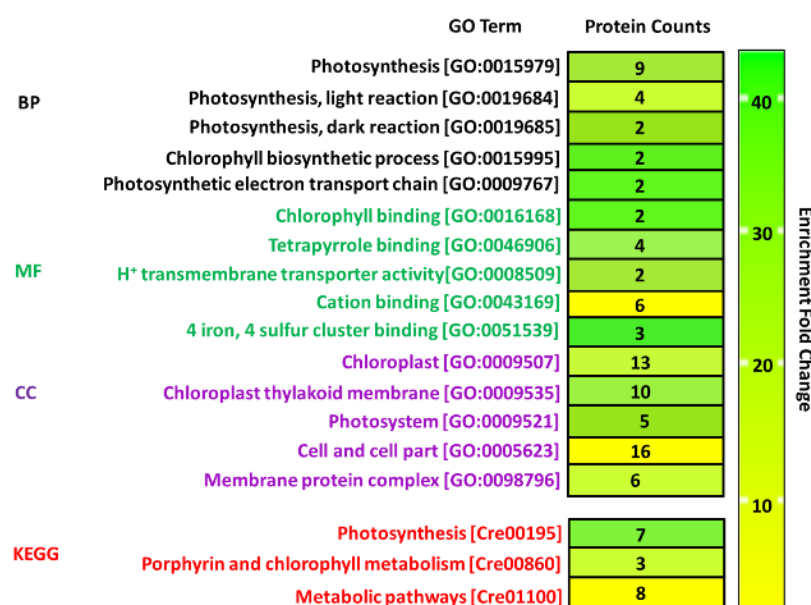
(C) *pgr5*-500 vs WT-50

Figure 5.11 Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis on light stress-induced up-/down-regulation of expressed proteins are divided into three categories of Go Term, including GO function and ID, Protein count and Enrichment fold change. Panels (A, B and C) represent the up-/down-regulation of proteins induced by WT-500 vs W-50, *pgrl1*-500 vs *pgrl1*-50 and *pgr5*-500 vs *pgr5*-50 under light treatment. Panels (A, B and C) represent the up-/down-regulation of proteins induced by high light. Abbreviations: BP1-5 (biological process). MF1-5 (molecular function). CC1-5 (cellular components). The top 3 biological pathways were presented as KEGG pathways as follows for up, down and differentially regulated proteins: (1) BP1 (biological process), Photosynthesis; BP2, Photosynthesis, light reaction; BP3, Photosynthesis, dark reaction; BP4, chlorophyll biosynthetic process; BP5, photosynthetic electron transport chain ; (2) MF1 (molecular function), chlorophyll-binding; MF2, tetrapyrrole binding; MF3, H⁺ ion transmembrane transporter activity; MF4, cation binding; MF5, 4 iron, 4 sulfur cluster binding; (3) CC1-5 (cellular components) - chloroplast, chloroplast thylakoid membrane, Photosystems, Cell and Cell part, Membrane protein complex. However, the KEGG pathway represents the top three pathways in differentially abundant proteins involved in Photosynthesis,

Porphyrin and chlorophyll metabolism, and Metabolic pathways.

Quantitative proteomics was performed on *pgr5_500* vs WT_50 under high light conditions, through which 294 proteins were quantified in all functional categories. In which 15 proteins were involved in 15 different biological functions, 11 proteins in 12 MF, and 16 types of proteins involved in 31 CC categories. In contrast, *pgr5* lacked GO terms related to carbohydrate metabolism, TCA cycle and PSII electron transport (Figure 5.11C).

KEGG pathway also showed that 9 DEPs are mainly engaged in 3 major categories, i.e., photosynthesis, oxidative phosphorylation and metabolic pathway. These proteins show differential protein expression in high light and play an essential role in photoprotection. In contrast, *pgr5* proteins remarkably decrease in high light, including assembly and stabilization of PSII, OEC complex assembly and oxygen evolution, representing a crucial for the regulation of photosynthetic-related protein has not been reported yet in algae so far. Notably, few photosynthesis-related proteins were not detected in WT high light.

5.2.10 Principal component analysis (PCA) analysis

High Light treatment and control samples are depicted in Red and green colours. The first principal component (PC1) explained 45.2% of the variation, with the 24.1% second PC2 in the WT sample being associated with the light treatments (Figure 5.12A). The first principal component (PC1) explained that 63.5% of the variation in the data was

associated with 18.5% second PC2 in the *pgr11* sample being related to high light treatments (Figure 5.12B).

The third PC1 37.8% and the PC2 24.6% principal components separated the *pgr5* light-treated samples (Figure 5.12C). Principal component analysis (PCA) revealed variation in the proteome profile between the control and high light treatments based on the first principal components (PC1) irrespective of 3 different samples (n=3 for each sample of control and light treated); they were separated based on the second principal components (PC2).

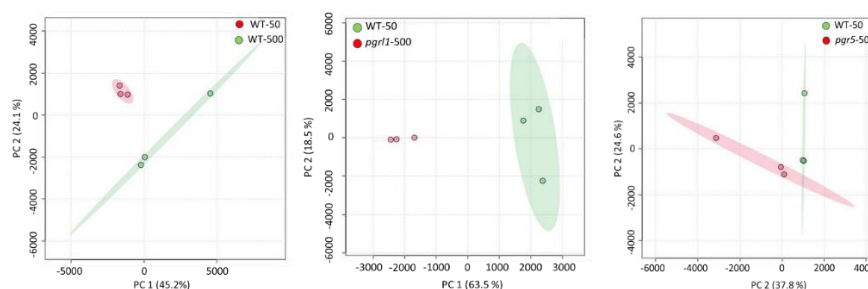


Figure 5.12 Principal component analysis (PCA) of three biological samples, i.e., WT and *pgr11* and *pgr5* mutants grown at control $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and light treated with $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Three different technical replicates were used for each biological sample.

Among these three samples (9 variants, each sample in triplicate) showed noteworthy changes between treated and control ($p < 0.05$) (Figure 5.12) and were engaged in PETP and carbohydrate biosynthesis.

5.2.11 Volcano plot and protein network analysis of DEPs regulated protein under high light stress

Volcano plots represent the proteins with substantial alterations made by high light in WT, *pgrl1* and *pgr5*. Following light treatment, 274 DEPs significantly changed in WT_500 vs WT_50, with 34 up-regulated and 19 down-regulated proteins (Figure 5.13A), while 219 DEPs significantly changed in the *pgrl1* treated versus control (*pgrl1*_500 vs WT_50), with 18 up-regulated and 124 down-regulated (Figure 5.13C). Comparing the *pgr5*_500 vs WT_50, a total of 215 DEPs are significantly identified, of which 18 are up-regulated and 25 are down-regulated after light treatment (Figure 5.13E).

Based on the volcano plot, we have taken the DEPs and elucidated the protein-protein interaction network of selected fold values of ≤ 0.86 (Down-regulated - low abundant protein) and ≥ 1.2 (Up-regulated-high abundant protein) in treated vs control in WT_50 vs WT_50, *pgrl1*_500 vs WT_50 and *pgr5*_500 vs WT_50. The nodes were shown in circles with two colours (Red- up-regulated protein, Green- downregulated) to simplify further the correlation network, based on the degree of interactions.

For the WT_50 vs WT_50 DEPs correlation network, every data point was recognised as a node, and a total of 28 nodes (each node represents a single protein) formed 76 edges (neighboring interactions); on average, each node shared 2-3 edges with adjacent nodes (Figure 5.13B). The red nodes belong to proteins associated with PETC PSII (PSBA_P07753, PSBB_P37255, PSBC_P10898, PSBD_P06007), OEC

(PSBO_P12853), PSI (PsaA_P12154), ATP synthase (ATPF_P12356, ATPBM_P38482) high light-induced protein (LHSR3.2_P0DO19), and protein involved in inter flagellar assembly (IFT57_Q2XQY7, IFT81_Q68RJ5, IFT74_Q6RCE1 (Intraflagellar transport protein) are up-regulated.

The green nodes belonging to heat shock protein HSP70_P25840, PSI PSAH_P13352, L-serine amino-acid biosynthesis PSP1_A8JEM3 (O-phosphoserine phosphohydrolase) and protein involved in inter flagellar assembly (IFT27_A8HN58 (Intraflagellar transport protein 27) are down-regulated (Figure 5.13B). To simplify the *pgrl1_500* vs WT_50 correlation network, the 52 nodes were categorized in circles by two colours (red and green) based on the degree of interactions. Green nodes comprise a dense network containing 172 interaction edges with neighboring nodes. Interestingly, the green nodes belong to proteins related to PETC (PSBB_P37255, PSBC_P10898, PSBQ_P12852, PSAF_P12356, PSAK_P14225), plastocyanin (PLAS_P18068), ATP synthase subunits (ATPD_Q42687, ATPF_P12356), Cyt b_6f subunit for CET (PETO_Q9LLC6, PETM_Q42496) inter flagellar assembly (IFT81_Q68RJ5, IFT57_Q2XQY7, IFT74_Q6RCE1), inorganic carbon uptake (CEMA_Q37050), light-independent chlorophyll biosynthesis (CHLI_Q94FT3), CCM proteins (CAH1_P20507, CAH2_P24258), flagellar radial spoke protein or flagellar bending (RSP2_Q6UBQ3, RSP4_Q01656, RSP5_Q27YU7) and PsbB mRNA maturation factor (MBB1_Q9FNS4_chloroplastic) are down-regulated.

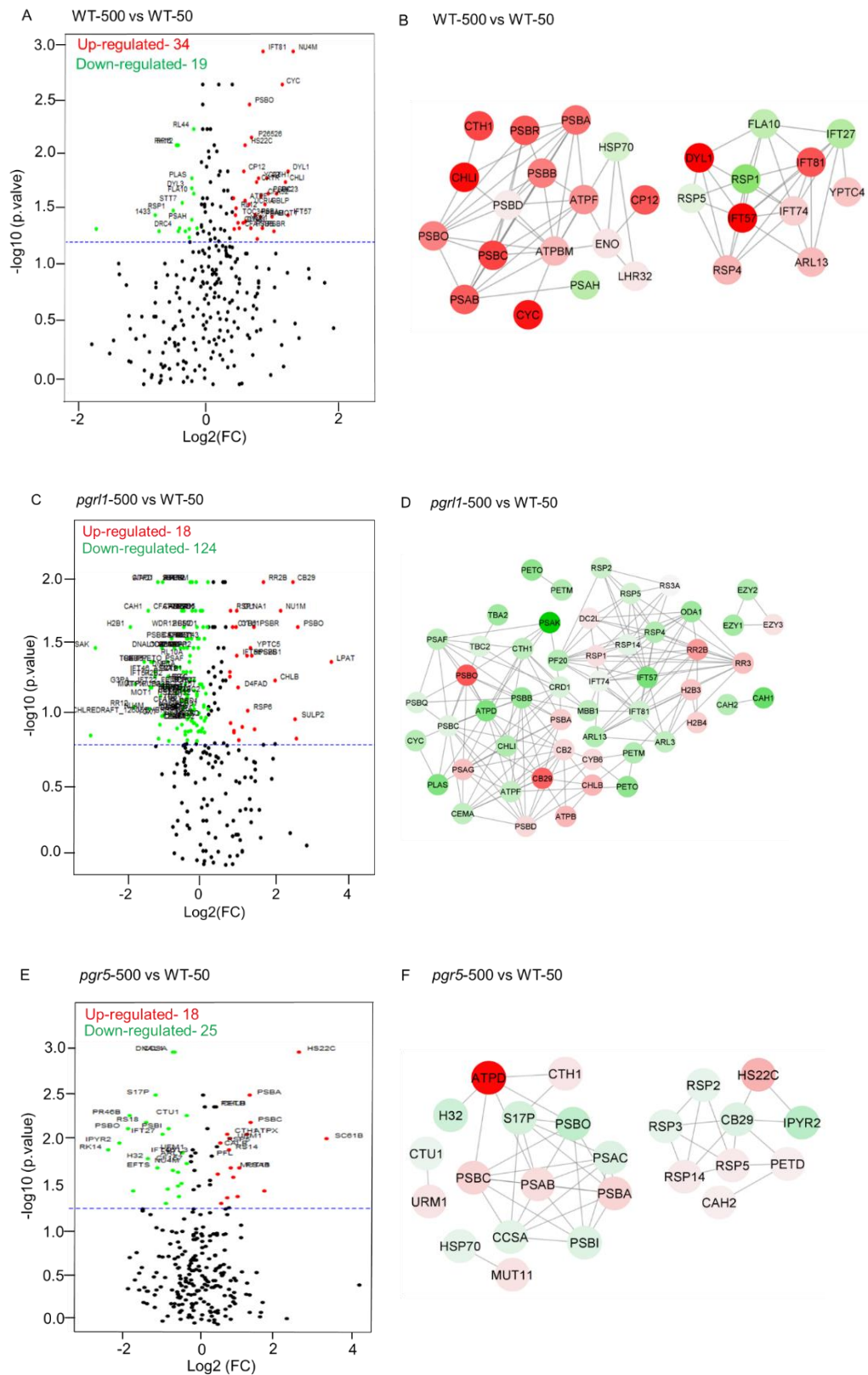


Figure 5.13 Volcano plot and correlation networks analysis of DEPs in WT, *pgr1* and *pgr5* mutants under high light stress. Volcano plots (Figure A, C and E) and protein-protein interaction networks (Figure B, D and F) represent the proteins with significant differences induced by WT-500 vs WT-50, *pgr1*-500 vs WT-50 and *pgr5*-500 vs WT-50. Proteins with significant differences and large fold changes are shown in red scatters as upregulated and green as down-regulated. X and Y-axis indicate a considerable difference in terms of both the two-sided Student's *t*-test (*p* value 0.05) and the fold cutoff (>1.2 and <0.86).

For the DEPs correlation network, every data point was reported as a node (each node represents a single protein) and each forming edges (neighboring interactions) with adjacent nodes (Figures 5.13B, 5.13D and 5.13E). Further, red nodes formed a dense network containing an average of ~15 interaction edges with neighboring nodes, which correspond to protein species related to PSII (PSBA_P07753, PSBD_P06007), PSI (PSAG_P14224), chlorophyll-binding/ light-harvesting (CB29_Q93WD2, CB2_P14273), light-independent chlorophyll biosynthesis (CHLB_P37824), translational protein (RR2B_Q8HUH1 (putative 30S ribosomal S2-like protein), RR3_Q08365), and histone protein for DNA binding or nucleosome remodeling (H2B3_P54346 (Histone H2B.3), H2B4_P54347 (Histone H2B.4), are up-regulated (Figure 5.13D). Similarly, in *pgr5*_500 vs WT_50, 24 nodes formed a solid interaction network with 47 edges. To simplify the correlation matrix, each node was represented with a specific colour (Red- Up-regulated and Green – down-regulated) based on the degree of interaction.

Further, green nodes formed a network containing an average of 2 interaction edges with neighboring nodes, which correspond to proteins related to PSII (PSBI_Q8DJZ6, PSBO_P12853), PSI (PSAC_Q00914), heat shock protein (HSP70_P25840, H32_Q6LCW8 (Histone H3 type 2), chlorophyll-binding/ light-harvesting (CB29_Q93WD2), flagellar bending/ calmodulin-binding (RSP2_Q6UBQ3, RSP3_P12759 (Flagellar radial spoke protein 2 and 3), and metabolic reaction (IPYR2_Q949J1, S17P_P46284) are down-regulated.

Further, red nodes formed a weak network with neighboring nodes, which correspond to proteins related to PSII (PSBA_P07753, PSBC_P10898), PSI (PSAB_P09144), ATP Synthesis (ATPD_Q42687), flagellar bending/ calmodulin-binding RSP4_Q01656 (Flagellar radial spoke protein 4), RSP14_A8HNV0 (Radial spoke protein 14), heat shock protein (HS22C_P12811 (Heat shock 22 kDa protein_chloroplastic), and Cyt b₆f subunit for CET (PETD_P23230) are up-regulated (Figure 5.13F).

5.2.12 Correlation network of up-regulated and down-regulated proteins

In this study, each node represented individual proteins with different colours based on the degree of interaction. The correlation network shows the 12 upregulated proteins (maximum interaction 2-16), 16 down-regulated proteins and most of the highly interconnected

(maximum interaction 2-20) proteins in WT_50 vs WT_500 (Figure 5.14A).

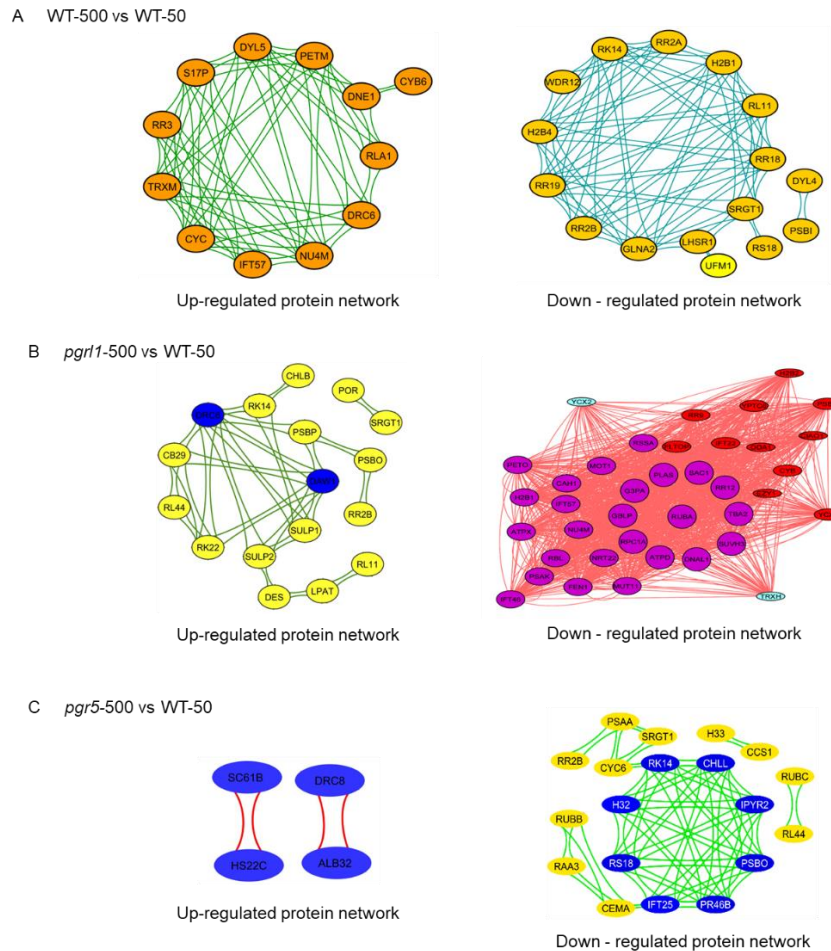


Figure 5.14 A, B, and C is the Correlation network of up-regulated (≥ 2.0 FC) and downregulated (≤ 0.5 FC) proteins treated vs control in WT (WT_500 vs WT_50), *pgr11* (*pgr11*_500 vs *pgr11*_50) and *pgr5* (*pgr5*_500 vs *pgr5*_50) based on the Pearson correlation coefficient with probability threshold $P < 0.05$.

A correlation network of *pgr11*_500 vs WT_50 contains 17 up-regulated the protein in blue colour showing maximum interaction 13-14 with yellow colour proteins (total interaction 48) and 38 down-regulated proteins and most of the highly interconnected (maximum interaction

78), and the interaction order was purple colour node (72 - 74 interaction) > Red colour node (60 - 68 interaction) > yellow colour node (42 - 48 interaction), respectively (Figure 5.14B). On the other hand, the correlation network of *pgr5_500* vs WT_50 contain 4 up-regulated proteins, in which blue colour shows the minor interaction (total interaction 4) and 19 down-regulated protein, and the interaction order was blue colour node (10 -15 interaction) > yellow colour node (2 -10 interaction), respectively (Figure 5.14C).

5.3 Discussion

Light stress has developed a thoughtful risk to *Chlamydomonas* growth and biomass development. Though, the regulating mechanisms and adaptation in *Chlamydomonas* have not been identified adequately in high light. This study comprehensively analysed crucial PETC and metabolic protein directly implicated in high light stress responses. I have addressed the entire proteome profile and its relation to the cell's function in photosynthesis efficiency under high light from WT, *pgr11*, and *pgr5* mutants of *C. reinhardtii*.

5.3.1 CET mutation reduced the capacity of oxygen uptake under high light stress

A high oxygen evolution has been observed for cells grown in high light WT compared to growth in the light, indicating the impact of high light was not drastic on PSII damage.

The light-induced O₂ uptake in the *pgrl1* and *pgr5* mutants was significantly reduced than in WT, demonstrating that the mutants' lack of CEF affected acclimation to high light (Figure 5.1A). This result correlates with Chl fluorescence, which is similarly reduced in the *pgrl1* and *pgr5* (Figure 3.7A and 3.7B). Therefore, O₂ evolved either by chlororespiration, Mehler reaction, or the malate shunt, stimulating the recurrence of PSII photochemistry (Schreiber and Vidaver, 1974). This result suggests that cells adjust to high light stress, indicating the adaptive mechanism. We correlate this result with biochemical changes in the oxygen-evolving complex subunit PsbO decreased in *pgrl1* and *pgr5* mutants (Figure 5.1C). Even proteome data also showed that all the subunits of oxygen-evolving complex are down-regulated in *pgrl1* and *pgr5* than WT in high light conditions (Figure 5.8A). This result suggests that WT is readily acclimatized to the high light stress by an adaptive mechanism. However, the *pgrl1* and *pgr5* mutant's prone to damage both the photosystems. Remarkably, after adding the mitochondrial inhibitors, the net O₂ evolution was abolished, and the respiratory-dependent non O₂ consuming pathway started downstream of photosynthesis in *C. reinhardtii*.

5.3.2 The pigment-protein composition and membrane stoichiometry were affected under high light in *pgrl1* and *pgr5*

In WT cells, the LHCSR3 and PSBS are defined under high light; therefore, the thylakoid membrane architecture and composition of PSII-LHCII supercomplexes are not much affected (Figure 5.3A).

The weaker *psi*-type CD signal in *pgrl1* and *pgr5* cells grown under high light indicates significant rearrangement in the macromolecular arrays of the PSII-LHCII complex (Figures 5.2A and 5.2B). The positive band at 506 nm in the Soret region originates from the β -carotene of the core complex (Tóth et al., 2016) and exhibits no or weak signal that might be due to a decrease in protein content core complex in cells subjected to high light stress (Figure 5.2C and 5.2D).

The same result was observed from the composition of thylakoid membrane protein complexes in BN-PAGE analysis (Figure 5.3). In *C. reinhardtii*, PSII-LHCII comprises two reaction center cores and six LHCII trimers (Järvi et al., 2011). In *pgrl1* and *pgr5*, most of the LHCs of supercomplex are associated with higher-order LHCs (LHC II trimers) or LHCII monomers under high light (Figure 5.3A and 5.3B). From BN-PAGE, due to solubilization with β -DM, loosely bound trimers dissociated from PSII-LHCII supercomplexes, leaving different core combinations with trimers and monomer of *pgrl1* and *pgr5* in high light (Figure 5.3).

The abundance of supercomplexes was not altered significantly in the *pgrl1*, but a dramatic change was observed in *pgr5* since it lacks LHCSR3 expression (Objective 2, Figure 4.6F). The BN-PAGE result complements the CD results, where higher-order structures in the thylakoid membrane are affected under high light conditions. These observations conclude that the expression of LHCSR3 in high light could photo-protect the photosystems to prevent the over-excitation energy transfer from LHCII to RC.

5.3.3 The protein profile analysis from immunoblotting provides insight into the *pgrl1* and *pgr5* under high light

The D1 and D2 proteins are the reaction centre-binding proteins of PSII that carry out essential redox components for charge separation. The protein level changes in the cells grown in high light showed a marginal increase of D1 and D2 in WT (Figure 5.4A and 5.4B). In high light-grown cells, the D1 and D2 proteins accumulated more in *pgrl1* and *pgr5* mutants; the continuous repair mechanisms change its abundance.

PSII core protein synthesis and its photodamage repair depend on many factors that could affect the process of their acclimation resulting in minimal change in its content. The photodamage of PSII repair was inhibited by ROS production (Murata et al., 2007), and the translational initiation of PsbA transcripts activated through redox signal associated with electron transport in PSI and reduction of the plastoquinone pool (Trebitsh and Danon, 2001).

The closely associated with these core proteins are core antennae chlorophyll-binding proteins such as CP43 and CP47. Its association with the PSII supercomplex, transfer's excitation energy from light-harvesting complexes to the chlorophyll-binding protein CP43 is selectively inhibited compared to CP47, preventing excess excitation energy from the overloading reaction center can changing core antenna proteins' content (Kim et al., 2017).

The PsaA is significantly decreased in *pgr5* than *pgr11* shows reduced protein content due to changes in chlorophyll and carotenoid molecules around the protein, which coordinates the cofactors to transfer electrons to ferredoxin (Figure 5.4C and 5.4D). The reduction of stromal subunits PsaD can distract the assembly of PSI. The associated stromal subunits PsaD and PsaE act as a binding site for ferredoxin, which leads to an efficient electron transport chain (Fischer et al., 1998). Further, PsaH is a docking site for LHCII during state transitions (Lunde et al., 2000). These results elucidate no change in protein expression, and also, the energy balance through PSII to PSI is not hampered under high light conditions as expected.

Further, we observed the sensitivity of PsaG to high light conditions indicating that the assembly of PSI core to LHCI may be disturbed (Ozawa et al., 2010). The LHCII chlorophyll a/b-binding proteins were relatively consistent between WT and the *pgr11* except for *pgr5* under high light. In the LHCII trimer, Lhcb1 and Lhcb3, peripherally arranged, depicted a drastic change in high light, while Lhcb2 near the PSII core showed no difference in protein content. This result indicates that high light mainly affects LHCS arranged peripherally in the supercomplexes. The LHCS monomeric protein, Lhcb5, reveals the decreased content while there was no change in Lhcbm5, indicating that light stress mainly affects outermost arranged light-harvesting antennas. Light-harvesting complex proteins Lhcb3 and Lhcb5 were more tightly associated with PSII core than Lhcb1 and Lhcb2 (Iwai et al., 2008).

This might be the reason for the inhibition of outer antennae proteins. An isoform of Lhcb4 connects trimeric LHCII with the PSII core; it stabilizes the PSII-LHCII dimer and binds to the monomeric core with the help of LHCII, suggesting its primary role in regulating the excitation energy flow and non-photochemical quenching process (Caffarri et al., 2009). Interestingly, the Lhcb4 has no significant change; thus, Lhcb4 might increase the rate of non-photochemical quenching that is generally more in high light conditions (Figure 5.5A and 5.5B).

Further, we examined the content of light-harvesting complex proteins of PSI that are arranged in two layers towards the stromal side of the PSI core. The outer half-ring of the supercomplex includes Lhca4, Lhca5, and Lhca6, whereas the inner half ring of the supercomplex composes of Lhca1, Lhca3, Lhca7, and Lhca8 with Lhca3 located near to PsaK, as previously suggested (Drop et al., 2011; Yadavalli et al., 2011a).

In assumption, eight LHCI subunits bind to PSAF in two layers from PsaG to the PsaK subunit. The innermost layer contains Lhca 1, 4, 6 and 5, and the outside layer comprises Lhca 3, 4, 6, and 7 decreased severely in *pgr5* might be the distracting assembly of PSI -LHCI under high light conditions (Figure 5.5C and 5.5D). Earlier, our group reported that under strong light conditions ($1000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), most of the inner LHCI proteins were affected due to ROS production in WT (Nama et al., 2019).

Interestingly, under moderate or (500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) light, we can say that the light-harvesting complexes of PSI are stable, but their composition might be affected due to a significant decrease in their binding site (PsaG and PsaH) under high light conditions (Figure 5.5C and 5.5D). However, the stoichiometry of the PSI and PSII LHCs are maintained, indicating that *C. reinhardtii* acclimates rapidly by changing the antenna proteins under high light conditions.

5.3.4 Our proteome supports the role of CET in the maintenance of photosynthetic and photoprotection

Here we have discussed the comparative analysis of western blot and proteome data of PETC protein. The phosphorylation and repair cycle of PSII prevent D1 degradation (Koivuniemi et al., 1995). Our data show significant down-regulation of PSBA_P07753 (D1) and PSBD_P06007 (D2) in *pgrl1* and *pgr5* than in WT high light, indicating that D1 damages more under high light conditions.

The primary antenna proteins PSBB_P37255 (CP43) and PSBC_P10898 (CP47) are down-regulated, especially in *pgr5* than *pgrl1*, as we can see in our western blot data where the protein content was decreased in *pgr5* more than the *pgrl1* (Figure 5.8C). These outcomes designate that damaging of the catalytic subunits D1 and D2 leads to the downregulation of the reaction centres associated with antenna protein PSBC_P10898 (CP43) and PSBB_P37255 (CP47) in the mutant under high light conditions (Figure 5.8C).

This might be due to light stress primarily phosphorylated to D1, D2, and CP43 in threonine residues at N-terminal (Elich et al., 1992; Booi-James et al., 2002) by the serine and threonine kinase (Vainonen et al., 2005; Rochaix et al., 2012). The data shows that the downregulation of D1 in mutants is a consequence of the other core components acting coordinately upstream to controlling PSII photochemistry and possibly facilitating the transition between linear and CEF under high light conditions. The PSI reaction center proteins, PSAA_P12154, PSAB_P09144, PSAC_Q00914, PSAD_Q39615, PSAE_P12352, PSAF_P12356, PSAG_P14224, and PSAH_P13352, are downregulated in *pgrl1*, and *pgr5* than WT, reflecting the importance of this protein in maintenance of the PSI assembly and CET under high light (Figure 5.8D). The LHCSR3 protein regulated NPQ (Peers et al., 2009) and substantial change in *pgrl1* and *pgr5* compared to WT, signifying that CET and high light changed NPQ at the post-translational level.

The NPQ is activated in the PSII antenna and disperses extra energy as heat (Ruban, 2016). Similarly, The *Chlamydomonas* exhibited a substantial downregulation on LHCSR3.1_P0DO19 and LHCSR3.2_P0DO19 in *pgrl1* and *pgr5* due to the absence of CET under high light conditions. Another report showed that the LHCSR3 expression was supplementary for NPQ initiation in *Chlamydomonas* (Bonente et al., 2011). LHCSR1_P93664 was up-regulated in *pgrl1* and

pgr5 and might be zeaxanthin cycle compensated without CET under high light (Figure 5.8E).

This finding aligns with NPQ data, showing that *pgr5* cannot induce NPQ, but *pgr11* has a lower NPQ than WT (Objective 2 Figure 4.6B) under high light. The PSBS1_A8HPM2 and PSBS2_A8HPM5 were up-regulated, especially in *pgr5*; limited *pmf* and LHCSR3 expression might increase the abundance of another photoprotective protein in *Chlamydomonas* under high light stress (Figure 5.8F). The PSBR_A0A2K3DMP5 is highly up-regulated in *pgr5* mutant, which offers that PSBR is essential for the steady binding of LHCSR3 to PSII–LHCII supercomplexes and required for effective quenching under high light (Xue et al., 2015). These results agree with objective 2 Figure 4.6G, where the *pgr5* induced PSBS protein under high light stress.

The ATP synthase and Cyt *b*₆*f* are two primary components of the PETC regulating the thylakoid lumen acidification or *pmf* under high light conditions. The ATP synthase subunit ATPA_P26526, ATPB_P06541, ATPE_P07891 and ATPG_P12113 are up-regulated in WT than *pgr11* and *pgr5* under high light. Although *pgr5* displays higher upregulation ATPD levels than WT, suggesting that most of the H⁺ are utilized for energy generation in the absence of CET in high light (Figure 5.8G). The Cyt *b*₆*f* complex highly regulates the thylakoid lumen acidification and CET pathway. The Cyt *b*₆*f* complex subunit CYF_P23577, CYC6_P08197 (Cyt C6_chloroplastic), PETD_P23230, PETO_Q9LLC6 and PETM_Q42496 are up-regulated in WT than *pgr11*

and *pgr5* under high light (Figure 5.8H).

Another subunit of Cyt *b*₆*f* complex CYB6_Q00471 is extensively involved in the regulation of lumen acidification and is highly up-regulated in *pgr5* mutant showing the importance of CET under high light stress.

5.3.5 Comparing whole-cell proteomes metabolite revealed significant changes in mutants under high light

It is known that photosynthesis is affected by high light. The change in photosynthesis would affect the metabolism of the cell. Also, the absence of the CET pathway alters the whole metabolic flux, leading to reroutes of the metabolic pathway and compositions under high light conditions to acclimate to the high light. The glycolytic enzyme Fructose-1,6-bisphosphate aldolases (FBAs) convert fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The Fructose bis-phosphate aldolase (ALFC_Q42690) was differentially expressed in WT, *pgrl1* and *pgr5* in high light involved in gluconeogenesis and starch biosynthesis (Figure 5.9). The regulation of G6PA and PRK activity has been investigated in *Chlamydomonas* (Huppe et al., 1994).

The G6PDH is the central governing enzyme of the pentose phosphate pathway, whereas PRK is a Calvin cycle enzyme involved

in carbon assimilation. The activity of G6PDH was inhibited in the light and activated in the dark. On the contrary, PRK was light-activated and interfered during the night. In addition, G3PA_P50362, engaged in glycerol synthesis, was the most up-regulated in *pgr5* under high light. The G3PA_P50362 is also related to the conversion of DHAP to sn-glycerol-3-phosphate, and finally, glycerol kinase (GK) or G3P phosphatase (GPP) produce glycerol (Driver et al., 2017). The G3P is a predecessor for TAG biosynthesis and increases lipid synthesis under salinity stress (Herrera-Valencia et al., 2012). The Ferredoxin-NADP reductase (FENR_P53991), a chloroplastic protein involved in ATP and NADPH synthesis, was downregulated in *pgr5* under high light.

These results suggested that, as in higher plants, reduction by the Fd-TRX system regulates G6PDH and PRK activity to optimize carbon reduction and oxidation in the chloroplast following the supply of electrons and response to the metabolic demands of ATP. The protein ENO_P31683 synthesized pyruvate from the D-glyceraldehyde 3-phosphate pathway for glycolysis, and carbohydrate degradation was differentially regulated in WT, *pgr11*, and *pgr5* in high light. Thioredoxin M-type (TRXM_P23400, chloroplastic) are vital regulatory players receiving information about the redox state and participating in reversible oxidation was highly up-regulated in WT. The M type of TRX is known to activate NADP-malate dehydrogenase to hold the CET indirectly (Figure 5.9).

The TCA cycle protein malate dehydrogenase

(MDHM_Q42686) was differentially regulated in WT, *pgrl1* and *pgr5* under high light (Figure 5.9). The MDHM converts oxaloacetate to malate, generating extra reducing power (Scheibe, 2004).

Another mitochondrial enzyme, Glutathione peroxidase 1 (GPX1_P83564), was up-regulated in WT and may constitute a glutathione peroxidase-like protective system against oxidative stresses synthesizing PUFA through arachidonic acid metabolic pathways was differentially regulated in *pgrl1* and *pgr5* in high light. Phosphoenolpyruvate carboxylase 1 (Ppc1), the carboxylation of phosphoenolpyruvate (PEP) forms oxaloacetate was differentially regulated ($>0.5 \log_2FC < 2.0$) in WT, *pgrl1* and *pgr5* in high light. It is activated by glutamine and dihydroxyacetone phosphate and inhibited by glutamate and malate. The enzymes of the CBB cycle are highly up-regulated in WT, including the complex involved in RuBisCO assembly (RUBA_Q42694 and RuBB_Q42693 proteins). The Phosphoribokinase converts ribulose-5-phosphate to RuBP (ribulose-1,5-bisphosphate), the leading CO₂ accepting enzyme differentially regulated in *pgrl1* and *pgr5* under high light. Rubisco uses RuBP as a substrate, creating 2 molecules of 3-phosphoglycerate.

High expression fructose bisphosphate enhances fixing CO₂ fixation and RuBP renewal (Lefebvre et al., 2005) through refining photosynthesis in stress (Driever et al., 2017). The reduced amount of P3GA_P50362 (Glyceraldehyde-3-phosphate dehydrogenase A_

chloroplastic), synthesized by RuBisCO, activates high carbon sinks to fixed CO₂ in high light-grown cultures. At last, the overexpression of these necessary CBB enzymes maintained the strong protein translation abilities, i.e., the 24 ribosomal proteins in which 17 were up-regulated in WT and seven were up-regulated in *pgr5* under high light.

The heat map data shows that 250 DEPs were divided into 49 Calvin cycle and metabolism protein sub-categories. The Carbonic anhydrase 1 (CAH1_P20507) and 2 (CAH2_P24258) proteins play a crucial role in CCM responsible for the reversible carbonate dehydratase activity for the formation of CO₂ were up-regulated in WT, and remarkably downregulated *pgr11* and *pgr5* under high light stress (Figure 5.9). The enhanced CBB protein in WT supports healthy photosynthetic growth. When photosynthesis is compromised, the starch converts into sugars and other metabolites to deliver carbon and energy under abiotic stress. Our recent laboratory reports show that *pgr11* and *pgr5* accumulated significant lipids to acclimate to high light (Chouhan et al., 2022). The over-reduction of the PETC generates additional NADPH and NADH by mitochondria, which balances the equilibrium by cellular redox homeostasis under salt stress (Wingler, 2002).

5.3.6 Heat map, Volcano plot, and Gene Ontology analysis revealed the CET involved enrichment of photosynthesis proteins but significantly lacks Light-harvesting complex

The Gene Ontology analysis (Figure 5.11) and volcano plot data (Figure 5.13) revealed an unexpected enrichment of photosynthetic targets not described in previous studies in *Chlamydomonas* or *Arabidopsis* under high light. The upregulated and downregulated protein in the volcano plot has been taken for GO analysis.

Similar to our heatmap data, proteins involved in GO mainly engage in chlorophyll biosynthetic, photosynthetic electron transport chain, chlorophyll-binding, heat shock protein, light-harvesting, translational protein, and protein involved in inter flagellar assembly (Figure 5.11).

I have identified only two light-harvesting pigment chlorophyll a/b binding proteins CB29_Q93WD2 (Chlorophyll a-b binding protein CP29) CB2_P14273 (Chlorophyll a-b binding protein of LHCII type I chloroplastic) up-regulated in Wt and *pgr11* but downregulated in *pgr5* in high light that shows agreement with our protein data (Figure 5.10C). The remaining PSII-LHCII and PSI-LHCI proteins are not identified during the analysis and might be lost during protein extraction or are underneath the nLC /MS/MS detection limit. These discrepancies between databases, i.e., the loss of proteins between releases or truncated sequences, highlight to need for new gene prediction algorithms and implementation strategies for proteomics data analysis.

5.4 Conclusion

Targeted proteome analysis was conducted to compare the photosystem-related and central metabolic protein abundance in WT, *pgrl1*, and *pgr5*. The physiological and biochemical data show that the *pgr5* is additionally prone to high light and compromised photosynthesis. The analysis showed that the CET- dependent regulation of significant metabolic protein abundance at the WT level is more than in mutants.

This present study reveals an nLC/MS/MS-based proteomic method to distinguish the possible molecular mechanism of CET on photosynthesis and metabolomic profile in response to high light. Our results specify a robust relationship between CET and Cyt b_6f that impacts lumen acidification and NPQ. I have shown that mutants share mutual targets of CET that control photosynthetic machinery and compensation mechanisms, including regulation of *pmf* by Cyt b_6f and ATP synthase activity that indirectly affect the photoprotective protein in high light.

We have also identified increased expression of PSBS protein in *pgr5* mutant than WT act independently, controlling NPQ in high light. In total, ~330 proteins were identified from the Chlamydomonas database and divided into 240 DAPs, 20 down-regulated and approximately 35 up-regulated. In the GO and KEGG pathway studies, I recognized that high light highly affected the photosynthesis, oxidative phosphorylation, and metabolic pathway, i.e., glycolysis and TCA cycle protein, to prevent abolishing carbon assimilation; few of them play a

significant role in photoprotection. The limitation of metabolite appears to be accessible via the TCA cycle affecting the restriction of CET, and the generation of additional NADPH by LET directly supplies the energy to carbon metabolism to eliminate the ROS and neutralize the deleterious inhibitory effects. I have started untying the impact of these vital CET mutants in high light, though some questions still remain.

Future efforts would address the role of these CET proteins *pgrl1* and *pgr5* in affecting the photosynthetic electron transport protein and inducing the down-regulation of cellular metabolism in chloroplast independently or in coordination with mitochondria over different targets to maintain cellular homeostasis.

Appendix documents

Table 5.1 List of proteins expressed in WT_500 vs WT_50 (treated vs control) of *C. reinhardtii*

S.No.	Abbreviation	ProteinDescription	Fold change	p-value	GO Description and Pathway
		Down-regulated protein (FC ≤ 0.5)			
1	GLNA2	Glutamine synthetase, chloroplastic	0.04460096	0.800	Glutamine biosynthetic process
2	RR19	30S ribosomal protein S19, chloroplastic	0.170332992	0.018	Translation
3	RR2A	30S ribosomal protein S2, chloroplastic	0.195929575	0.289	Translation
4	SRGT1	Peptidyl serine alpha-galactosyltransferase	0.278037308	0.435	Transferring glycosyl groups
5	RR2B	Putative 30S ribosomal S2-like protein	0.292292572	0.040	Translation
6	RS14	40S ribosomal protein S14	0.336216482	0.521	Translation
7	H2B4	Histone H2B.4	0.349937766	0.401	Protein heterodimerization
8	H2B1	Histone H2B.1	0.367879441	0.081	Protein heterodimerization
9	RS18	40S ribosomal protein S18	0.371576687	0.101	Translation
10	RK14	50S ribosomal protein L14, chloroplastic	0.398519034	0.127	Translation
11	RL11	60S ribosomal protein L11	0.410655759	0.612	Translation
12	DYL4	Dynein 14 kDa light chain	0.410655759	0.241	Outer dynein arm assembly
13	RR18	30S ribosomal protein S18, chloroplastic	0.414782914	0.313	Translation
14	UFM1	Ubiquitin-fold modifier 1	0.418951547	0.620	Protein ufmylation
15	PSAA	PSI P700 chlorophyll a apoprotein A1	0.440431658	0.386	Photosynthesis
16	WDR12	Ribosome biogenesis protein	0.467666431	0.244	LSU-rRNA from tricistronic rRNA
17	LHSR1	LHC stress-related protein 1, chloroplastic	0.477113911	0.989	Non-photochemical quenching
18	PSBI	Photosystem II reaction center protein I	0.481908981	0.361	Photosynthesis
19	CCS1	Cytochrome c biogenesis, chloroplastic	0.481908981	0.746	Cytochrome complex assembly
		Differential regulated protein (≤ 2.0 FC ≥ 0.5)			
20	IF172	Intraflagellar transport protein 172	0.506616989	0.505	Multicellular organism development
21	CHLB	Light-independent protochlorophyllide	0.516851321	0.864	Light-independent chl biosynthetic
22	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	0.522045789	0.812	Protein urmylation
23	CEMA	Chloroplast envelope membrane protein	0.532591804	0.242	Proton transmembrane transporter
24	THI4	Thiamine thiazole synthase, chloroplastic	0.537944435	0.183	Thiamine biosynthetic process
25	1433	14-3-3-like protein	0.537944435	0.030	Flagellar arrangement and assembly
26	IFT56	Intraflagellar transport protein 56	0.543350861	0.121	Intraciliary transport
27	CHLL	Light-independent protochlorophyllide	0.543350861	0.244	Photosynthesis, Dark reaction
28	DRC2	Dynein regulatory complex subunit 2	0.543350861	0.133	Axonemal dynein complex assembly
29	CFA20	Cilia- and flagella-associated protein 20	0.559898376	0.042	Axoneme, Cilium assembly
30	POR	Protochlorophyllide reductase, chloroplastic	0.565525443	0.956	Chlorophyll biosynthetic process
31	EZY2	Ezy-1 protein	0.571209062	0.915	Uncharacterized protein
32	IDLC	28 kDa inner dynein arm light chain	0.571209062	0.655	Cell motility
33	DRC11	Dynein regulatory complex protein 11	0.571209062	0.833	Ciliary/flagellar motility
34	YCF78	membrane protein ycf78	0.58274824	0.694	Cell growth
35	RAA3	Trans-splicing factor Raa3, chloroplastic	0.58274824	0.519	mRNA processing, RNA splicing
36	SULT2	Proton/sulfate cotransporter 2	0.588604987	0.271	Sulfate transmembrane transporter
37	PSAG	Photosystem I RC subunit V, chloroplastic	0.594520559	0.081	Photosynthesis
38	CCSA	Cytochrome c biogenesis protein CcsA	0.594520559	0.675	cytochrome complex assembly
39	CHLB	Light-independent protochlorophyllide	0.600495585	0.417	Light-independent chl biosynthetic
40	IFT43	Intraflagellar transport protein 43	0.612626388	0.103	Cell projection organization
41	EFTU	Elongation factor Tu, chloroplastic	0.625002269	0.388	Translational elongation
42	DYL2	Dynein 11 kDa light chain	0.625002269	0.722	Microtubule-based process
43	RTCB	RNA-splicing ligase RtcB homolog	0.625002269	0.179	tRNA splicing
44	RPC2B	DNA-directed RNA polymerase subunit beta	0.63128364	0.882	transcription, DNA-templated
45	RSP1	Flagellar radial spoke protein 1	0.65050909	0.027	Flagellar bending

46	SC61B	Protein transport protein Sec61 beta	0.65050909	0.315	Intracellular protein transport
47	GSA	Glutamate semialdehyde aminomutase	0.657046828	0.419	Chlorophyll biosynthetic process
48	RR4	30S ribosomal protein S4, chloroplastic	0.663650253	0.300	Translation
49	NRT21	Nitrate transporter 2.1	0.663650253	0.446	Nitrate assimilation
50	POC16	Centriole proteome protein 16	0.670320042	0.148	Flagellum assembly
51	RK16	50S ribosomal protein L16, chloroplastic	0.670320042	0.007	Mitochondrial translation
52	RUBC	RuBisCO large subunit- beta-2	0.670320042	0.103	RuBisCO assembly
53	PCDP1	Cilia- and flagella-associated protein 221	0.677056884	0.134	Cilium assembly and movement
54	SULP1	Sulfate permease 1, chloroplastic	0.677056884	0.654	Uptake of sulfate
55	DRC7	Dynein regulatory complex subunit 7	0.683861412	0.489	Regulation of flagellar motility
56	POB15	Proteome of basal body protein 15	0.683861412	0.042	Motility and sensing stimuli
57	RCA	RuBP carboxylase/oxygenase activase,	0.683861412	0.430	Activation of RuBisCO
58	RR12	30S ribosomal protein S12, chloroplastic	0.683861412	0.007	Translation
59	DAAF3	Dynein assembly factor 3, axonema	0.690734327	0.217	Dynein complex assembly
60	DYI2	Dynein, 78 kDa intermediate chain	0.690734327	0.166	Motor activity, Arm assembly
61	DRC4	Dynein regulatory complex subunit 4	0.690734327	0.039	Dynein complex assembly
62	ALFC	Fructose-bisphosphate aldolase 1	0.704688094	0.262	Glycolytic process
63	STT7	Serine/threonine-protein kinase	0.71177032	0.023	State transition
64	CF251	Cilia- and flagella-associated protein 251	0.71177032	0.078	Cilium movement
65	PGKH	Phosphoglycerate kinase, chloroplastic	0.718923724	0.946	Glycolytic process
66	SULP2	Sulfate permease 2, chloroplastic	0.718923724	0.289	Sulfate transport
67	PSAD	PSI reaction center subunit II, chloroplastic	0.718923724	0.612	Ferredoxin-docking protein
68	CB2	Chl a-b binding protein LHCI type I	0.718923724	0.720	Light-harvesting as a light receptor
69	MOC1	Holliday junction resolvase MO	0.726149042	0.388	DNA recombination
70	RR11	30S ribosomal protein S11, chloroplastic	0.726149042	0.111	Translation
71	RSSA	40S ribosomal protein SA	0.733446954	0.042	Translation
72	DES	Acyl-lipid omega-13 desaturase	0.740818212	0.635	Linoleic acid metabolic process
73	EZY1	Ezy-1 protein	0.748263574	0.857	Uncharacterized protein
74	FEN1	Flap endonuclease 1	0.763379486	0.183	Base-excision repair
75	IFT27	Intraflagellar transport protein 27	0.763379486	0.040	GTPase activity
76	DNAL1	Dynein light chain 1, axonemal	0.763379486	0.047	Motor flagellar motility
77	CMD1	5-methylcytosine-modifying enzyme 1	0.771051593	0.052	5-methylcytosine catabolic
78	PLAS	Plastocyanin, chloroplastic	0.786627865	0.014	Electron transfer P700 and cyt b6f
79	PSAH	PSI reaction center subunit VI, chloroplastic	0.786627865	0.033	Docking of LHC I to PSI core
80	CAPP1	Phosphoenolpyruvate carboxylase 1	0.786627865	0.923	TCA cycle
81	DYL3	Dynein 18 kDa light chain	0.786627865	0.017	Motor activity
82	PF20	Flagellar WD repeat-containing protein Pf20	0.786627865	0.116	Microtubule bridges in flagella
83	BOP1	Ribosome biogenesis protein BOP1	0.794533599	0.245	Maturation of 5.8S rRNA
84	RSP3	Flagellar radial spoke protein 3	0.794533599	0.064	Regulation of cilium-cell motility
85	RPOB1	DNA-directed RNA polymerase subunit beta	0.794533599	0.110	Transcription, DNA-templated
86	RR9	30S ribosomal protein S9, chloroplastic	0.802518799	0.419	Translation
87	FLA10	Kinesin-like protein FLA10	0.802518799	0.019	Intraciliary anterograde transport
88	RL44	60S ribosomal protein L44	0.802518799	0.005	Translation
89	DRC5	Dynein regulatory complex subunit 5	0.810584251	0.743	Ciliary/flagellar motility
90	MUT11	Protein Mut11	0.810584251	0.093	'Lys-4' histone H3 methylation
91	PHOT	Phototropin	0.818730751	0.487	Blue light signalling, NPQ induction
92	ADT	ADP,ATP carrier protein	0.826959136	0.039	Catalyzes exchange of ADP and ATP
93	H2B3	Histone H2B.3	0.835270205	0.044	DNA repair, Chromosomal stability
94	CAPP2	Phosphoenolpyruvate carboxylase 2	0.835270205	0.209	Carbon fixation, TCA cycle
95	RSP2	Flagellar radial spoke protein 2	0.843664815	0.064	Flagellar bending
96	IPYR2	Soluble inorganic pyrophosphatase 2	0.852143792	0.680	Phosphate metabolic process
97	PSAF	PSI reaction center subunit III, chloroplastic	0.860707971	0.131	Electron transfer from PC to P700
98	CFA99	Cilia- and flagella-associated protein 99	0.869358235	0.038	Ciliary/flagellar motility
99	PLD6	Mitochondrial cardiolipin hydrolase	0.869358235	0.037	Lipid catabolic process
100	KTU	Protein kintoun	0.869358235	0.010	Dynein complex assembly
101	DRC10	Dynein regulatory complex protein 10	0.869358235	0.087	Maintains microtubule sliding
102	CFA73	Cilia- and flagella-associated protein 73	0.869358235	0.024	Regulation of microtubule activity
103	CFA43_	Cilia- and flagella-associated protein 43	0.878095435	0.005	Flagellar organization and function
104	H2B2	Histone H2B.2	0.878095435	0.003	Nucleosome remodelling
105	KLP1	Kinesin-like protein KLP1	0.878095435	0.095	Microtubule-based movement
106	HSP70	Heat shock 70 kDa protein	0.878095435	0.002	Protein maturation, degradation
107	RL10A	60S ribosomal protein L10a	0.878095435	0.062	Translation
108	LIAS	Lipoyl synthase, mitochondrial	0.878095435	0.021	Lipoylation peptidyl-N6-lipoyl-L-lysine.
109	YCF3	Photosystem I assembly protein Ycf3	0.878095435	0.119	Chaperone- PSI subunits assembly
110	FENR	Ferredoxin--NADP reductase, chloroplastic	0.886920439	0.290	Regulating the cyclic and non-CEF

111	PSAC	Photosystem I iron-sulfur center	0.886920439	0.378	Binding of Psd and Psae to PSI
112	G3PA	G3P dehydrogenase A, chloroplastic	0.886920439	0.042	Glucose metabolic process
113	BSD2	BUNDLE SHEATH DEFECTIVE 2, chloroplastic	0.886920439	0.236	Chaperone-mediated protein folding
114	HAP2	Hapless 2	0.886920439	0.123	Protein insertion into the membrane
115	RUBB	RuBisCO large subunit-binding beta-1	0.895834136	0.024	Assembly of the enzyme oligomer
116	DRC1	Dynein regulatory complex protein 1	0.895834136	0.066	Dynein complex assembly
117	LISC	Lipoyl synthase, chloroplastic	0.895834136	0.169	Protein lipoylation
118	RR7	30S ribosomal protein S7, chloroplastic	0.895834136	0.878	Translation
119	RL13	60S ribosomal protein L13	0.895834136	0.728	Translation
120	ASNA1	ATPase ARSA1	0.895834136	0.025	ATPase activity
121	ALB31	ALBINO3-like protein 1, chloroplastic	0.904837417	0.316	Assembly and activity of LHC I and II
122	CFA74	Cilia- and flagella-associated protein 74	0.904837417	0.028	Cilium movement, Cell motility
123	ARS	Arylsulfatase	0.913931182	0.157	Phenol metabolic process
124	EFTS	Elongation factor Ts, mitochondrial	0.913931182	0.011	Translation elongation factor activity
125	CFA53	Cilia- and flagella-associated protein 53	0.913931182	0.273	Cilium assembly and movement
126	RSP5	Flagellar radial spoke protein 5	0.923116348	0.021	Regulation of flagellar bending
127	CFA91	Cilia- and flagella-associated protein 91	0.923116348	0.005	Cilium movement
128	RBL	RuBP carboxylase large chain	0.923116348	0.121	Photorespiration
129	KPPR	Phosphoribulokinase, chloroplastic	0.93239382	0.185	Reductive pentose-phosphate cycle
130	HSTC	Homocysteine methyltransferase	0.93239382	0.087	Methionine biosynthetic process
131	HSTC	Homogentisate solanesyltransferase	0.93239382	0.008	Carotenoid and PQ biosynthetic
132	TBA2	Tubulin alpha-2 chain	0.941764535	0.044	Alters the microtubule cytoskeleton
133	MDHM	Malate dehydrogenase, mitochondrial	0.941764535	0.070	Carbohydrate metabolic process
134	CCD39	Coiled-coil domain-containing protein 39	0.941764535	0.009	Cilium movement
135	METK	S-adenosylmethionine synthase	0.941764535	0.415	S-adenosylmethionine biosynthetic
136	CHLN	Light-independent protochlorophyllide	0.941764535	0.059	Light-independent chl biosynthetic
137	TBA1	Tubulin alpha-1 chain	0.951229424	0.034	Microtubule-based process
138	CF157	Cilia- and flagella-associated protein 157	0.951229424	0.050	Cilium movement, Cell motility
139	RK20	50S ribosomal protein L20, chloroplastic	0.951229424	0.247	Translation
140	NRT22	Nitrate transporter 2.2	0.951229424	0.005	Nitrate assimilation, Nitrate transport
141	SUVH3	Histone-lysine N-methyltransferase	0.951229424	0.015	Methyltransferase repressed chromatin
142	CAH2	Carbonic anhydrase 2	0.951229424	0.136	CCM, Carbonate dehydratase activity
143	CLPP	ATP-dependent protease proteolytic	0.96078944	0.575	chymotrypsin-control protein misfolding
144	RR14	30S ribosomal protein S14, chloroplastic	0.970445534	0.102	Translation
145	IFT46	Intraflagellar transport protein 46	0.970445534	0.024	Cilium-dependent cell motility
146	RK2	50S ribosomal protein L2, chloroplastic	0.970445534	0.093	Mitochondrial translation
147	ATPI	ATP synthase subunit a, chloroplastic	0.980198674	0.986	ATP synthesis coupled proton transport
148	ZYS3	Zygote-specific protein 3	0.980198674	0.508	Cell development
149	DAAF1	Leucine-rich repeat-containing ODA7	0.990049834	0.394	Cell motility and localization
150	RUBA	RuBisCO large subunit- alpha, chloroplastic	0.990049834	0.165	Assembly of the enzyme oligomer
151	NAC2	PsbD mRNA maturation factor, chloroplastic	0.990049834	0.098	mRNA processing- 5' UTR of psbD
152	MBB1	PsbB mRNA maturation factor, chloroplastic	0.990049834	0.040	mRNA processing via 5'-UTR of psbB
153	ACT	Actin	0.990049834	0.007	Cytoplasmic streaming
154	TBC2	Tbc2 translation factor, chloroplastic	1	0.126	PsbC mRNA for translation initiation
155	YCX3	14.4 kDa protein in 16S rRNA region	1	0.149	Determine phylogeny
156	CAO	Chlorophyllide a oxygenase, chloroplastic	1	0.048	Chlorophyll b biosynthetic process
157	IFT22	Intraflagellar transport protein 22	1.010050167	0.430	Cellular availability of IFT particles
158	RSP14	Radial spoke protein 14	1.010050167	0.092	Cell motility, Endocytosis
159	TRXH	Thioredoxin H-type	1.010050167	0.056	Cell redox homeostasis
160	RAA2	Trans-splicing factor Raa2, chloroplastic	1.010050167	0.102	RNA splicing, mRNA processing
161	ATPG	ATP synthase gamma chain, chloroplastic	1.02020134	0.008	ATP synthesis coupled proton transport
162	CFA58	Cilia- and flagella-associated protein 58	1.02020134	0.120	Cilium-dependent cell motility
163	YPTC5	GTP-binding protein YPTC5	1.02020134	0.121	GTPase activity, protein transport
164	PSBS2	Photosystem II protein PSBS2	1.030454533	0.225	Nonphotochemical quenching
165	PSBD	Photosystem II D2 protein	1.040810773	0.013	Photosynthetic Assembly of PSII
166	DAW1	Dynein assembly WDR repeat domains 1	1.040810773	0.062	Intraciliary transport and assembly
167	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	1.040810773	0.051	Photosynthesis
168	CFA65	Cilia- and flagella-associated protein 65	1.040810773	0.002	Cell projection organization
169	DC2L	Cytoplasmic dynein 2 intermediate chain 1	1.040810773	0.373	Regulates flagellar dynein activity
170	CAH1	Carbonic anhydrase 1	1.040810773	0.111	Carbonate dehydratase activity
171	PSBP	Oxygen-evolving enhancer 2, chloroplastic	1.040810773	0.161	Photosynthesis oxygen evolution
172	SLT2	Sodium/sulfate cotransporter 2	1.051271097	0.254	Potassium ion transport
173	PURA	Adenylosuccinate synthetase, chloroplastic	1.051271097	0.323	"de novo" AMP biosynthetic process
174	LHR32	LHC stress-related protein 3.1, chloroplastic	1.051271097	0.012	Nonphotochemical quenching
175	CCD40	Coiled-coil domain-containing protein 40	1.051271097	0.541	Assembly of the dynein complex

176	ENO	Enolase	1.061836545	0.040	Glycolytic process
177	LHR32	LHC stress-related protein 3.2, chloroplastic	1.061836545	0.025	Nonphotochemical quenching
178	DRC9	Dynein regulatory complex protein 9	1.061836545	0.122	Regulator of ciliary/flagellar motility
179	PETO	Cytochrome b6-f complex subunit	1.061836545	0.080	LET between PSII and I, CET around PSI
180	DRC3	Dynein regulatory complex subunit 3	1.083287066	0.591	Cilium-dependent cell motility
181	PFL	Formate acetyltransferase	1.083287066	0.056	Convert pyruvate and coA
182	PESC	Pescadillo homolog	1.094174288	0.399	Maturation of 5.8S rRNA
183	FLTOP	Protein Flattop homolog	1.094174288	0.018	Regulator-cilium basal body docking
184	YCX2	Uncharacterized protein in 16S rRNA region	1.10517092	0.041	Phylogenetic relationship
185	GPX1	Glutathione peroxidase 1, mitochondrial	1.10517092	0.170	Arachidonic acid metabolic process
186	YCX6	12.3 kDa petA-petD intergenic region	1.11627807	0.062	Chloroplast based function
187	CFA44	Cilia- and flagella-associated protein 44	1.11627807	0.058	Cilium-dependent cell motility
188	IFT74	Intraflagellar transport protein 74	1.127496849	0.029	Cilium assembly
189	TBG	Tubulin gamma chain	1.127496849	0.066	Cytoplasmic microtubule organization
190	PSBS1	Photosystem II protein PSBS1	1.127496849	0.259	Nonphotochemical quenching
191	RPOB2	DNA-directed RNA polymerase beta C	1.127496849	0.069	Transcription, DNA-templated
192	CRD1	Mg-protoporphyrin IX monomethyl ester	1.138828378	0.702	Photosynthesis, Chl biosynthetic
193	CFA61	Cilia- and flagella-associated protein 61	1.138828378	0.012	Cilium movement and organization
194	CYB	Cytochrome b	1.138828378	0.220	Respiratory electron transport chain
195	CALM	Calmodulin	1.138828378	0.018	Calcium-mediated signalling
196	CALR	Calreticulin	1.138828378	0.331	Protein folding
197	RPC1A	DNA-directed RNA polymerase subunit beta	1.1502738	0.069	Transcription by RNA polymerase II
198	RL31	60S ribosomal protein L31	1.16183425	0.057	Translation
199	YPTC4	GTP-binding protein YPTC4	1.16183425	0.048	Protein transport and vesicular traffic
200	RPOA	DNA-directed RNA polymerase alpha	1.16183425	0.182	Transcription, DNA-templated
201	ARLY	Argininosuccinate lyase	1.173510867	0.071	Arginine biosynthetic via ornithine
202	HEM2	Aminolevulinic acid dehydratase	1.185304853	0.089	Chlorophyll biosynthetic process
203	IPYR1	Soluble inorganic pyrophosphatase 1	1.185304853	0.498	Phosphate metabolic process
204	ATPE	ATP synthase epsilon chain, chloroplastic	1.185304853	0.367	ATP synthesis coupled proton transport
205	RS3A	40S ribosomal protein S3a	1.197217372	0.058	Translation
206	ARL13	ADP-ribosylation factor-like protein 13B	1.197217372	0.021	Control ciliary axoneme structure
207	NU5M	NADH-ubiquinone oxidoreductase chain 5	1.197217372	0.002	NADH dehydrogenase activity
208	TEKT	Tektin	1.197217372	0.028	Assembly of flagella and cilia
209	SLT3	Probable sodium/sulfate cotransporter 3	1.197217372	0.175	Potassium ion and Sulfate transport
210	ATPBM	ATP synthase subunit beta, mitochondrial	1.209249595	0.021	ATP synthesis coupled proton transport
211	PSAE	PSI reaction center subunit IV, chloroplastic	1.221402762	0.679	Photosynthesis
212	RSP4	Flagellar radial spoke protein 4	1.221402762	0.040	Cell motility, cilium assembly
213	CFA70	Cilia- and flagella-associated protein 70	1.221402762	0.271	Axoneme- regulates ciliary motility
214	SFAS	SF-assemblin	1.233678052	0.079	Striated microtubule-fibres assembly
215	TOC34I	Translocase of chloroplast 34, chloroplastic	1.233678052	0.030	Transport cytoplasm to chloroplast
216	RL12	60S ribosomal protein L12	1.246076729	0.026	Translation
217	RK5	50S ribosomal protein L5, chloroplastic	1.246076729	0.106	Translation
218	H4	Histone H4	1.258600015	0.147	Nucleosome remodeling
219	ASNA2	ATPase ARSA2	1.258600015	0.074	Protein insertion into the ER membrane
220	PR46B	Coiled-coil domain-containing protein 103	1.271249144	0.312	Cilia motility
221	GLNA1	Glutamine synthetase cytosolic isozyme	1.271249144	0.035	Glutamine biosynthetic process
222	CF300	Cilia- and flagella-associated protein 300	1.271249144	0.205	Structure organization and motility
223	ANM1	Protein arginine N-methyltransferase 1	1.284025417	0.124	Asymmetric dimethylation of a flagellum
224	ATPD	ATP synthase delta chain, chloroplastic	1.284025417	0.112	ATP synthesis coupled proton transport
225	CFA77	Cilia- and flagella-associated protein	1.284025417	0.039	Cell motility
226	CYF	Cytochrome f	1.284025417	0.993	CEF around PSI
227	LPAT	Acyl-sn-glycerol-phosphate acyltransferase	1.323129814	0.973	CDP-diacylglycerol biosynthetic process
228	DYI3	Dynein, 70 kDa intermediate chain	1.336427477	0.035	cell motility, dynein arm assembly
229	CP12	Calvin cycle protein CP12, chloroplastic	1.349858824	0.012	Assembly of complex of PRK/GAPDH
230	CFA45	Cilia- and flagella-associated protein 45	1.363425117	0.210	Cell motility
231	HS22C	Heat shock 22 kDa protein, chloroplastic	1.363425117	0.007	Protein maturation and degradation
232	ATPF	ATP synthase subunit b, chloroplastic	1.363425117	0.022	ATP synthesis coupled proton transport
233	YCX1	12.2 kDa protein in 16S rRNA region	1.377127754	0.034	Conserved phylogeny
234	RK22	50S ribosomal protein L22, chloroplastic	1.377127754	0.066	Translation
235	SAC1	Putative sulfur deprivation regulator	1.390968147	0.066	Potassium ion transport
236	ODA1	Outer dynein arm protein 1	1.390968147	0.150	Cell motility
237	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	1.404947596	0.357	Fatty acid biosynthetic
238	UCRIA	Cyt b6-f complex Fe-S subunit, chloroplastic	1.41906754	0.024	CET around PSI
239	PSBO	Oxygen-evolving enhancer 1, chloroplastic	1.433329435	0.003	Primary site of water splitting
240	GLE	Autolysin	1.433329435	0.378	Cell wall organization

241	PSBB	Photosystem II CP47 RC protein	1.447734622	0.039	Photosynthesis, PET in PSII
242	P26526	ATP synthase subunit alpha, chloroplastic	1.462284582	0.006	ATP synthesis coupled proton transport
243	ALB3.2	ALBINO3-like protein 2, chloroplastic	1.476980773	0.538	Assembly of LHC I and II
244	H32	Histone H3 type 2	1.476980773	0.251	Chromosomal stability.
245	H32	Histone H3 type 3	1.491824707	0.196	Nucleosome remodelling
246	PSBA	Photosystem II protein D1	1.521961536	0.030	Photosynthesis, LET in PSII
247	CP100	Cilia- and flagella-associated protein 100	1.521961536	0.162	Cell motility, Cillium movement
248	H31	Histone H3 type 1	1.537257535	0.088	DNA binding, Chromosome stability
249	YPTC6	Ras-related protein YPTC6	1.537257535	0.209	GTPase activity
250	CATR	Caltractin	1.537257535	0.015	Cell cycle, Cell division
251	SLT1	Sodium/sulfate cotransporter 1	1.537257535	0.330	Potassium ion transport
252	RL401	Ubiquitin-60S ribosomal protein L40	1.552707215	0.049	Translation
253	CIAO1	Cytosolic Fe-S protein assembly CIAO1	1.552707215	0.152	Required iron-sulfur cluster assembly
254	RBS2	RuBisCO small chain 2, chloroplastic	1.568312167	0.122	Photosynthesis, Photorespiration
255	YPTC1	GTP-binding protein YPTC1	1.568312167	0.118	Protein transport, vesicular traffic
256	ARF1	ADP-ribosylation factor 1	1.568312167	0.133	Vesicle-mediated transport
257	YCX5	Uncharacterized trnR-chlB intergenic region	1.568312167	0.014	Tag-protein stability
258	PSAB	PSI P700 chlorophyll a apoprotein A2	1.599994191	0.031	Primery electron donor of PSI
259	G3PC	G3P dehydrogenase, cytosolic	1.599994191	0.319	Glycolysis, carbohydrate metabolism
260	CFA52	Cilia- and flagella-associated protein 52	1.616074385	0.020	Cell motility
261	PSBR	Photosystem II protein PSBR, chloroplastic	1.632316236	0.039	Energy-dependent quenching
262	IFT81	Intraflagellar transport protein 81	1.648721271	0.001	Transport of tubulin within the cilium
263	GBLP	Guanine nucleotide-binding protein- beta	1.682027618	0.024	Transcription initiation and termination
264	CTH1	Mg-protoporphyrin monomethyl ester	1.716006899	0.014	Chlorophyll biosynthesis
265	PSBC	PS II CP43 reaction center protein	1.733253039	0.019	PET in photosystem II
266	RBS1	RuBisCO small chain 1, chloroplastic	1.750672504	0.091	Photosynthesis, Photorespiration
267	RTL	Reverse transcriptase-like protein	1.786038401	0.816	cDNA synthethesis
268	MOT1	Molybdate transporter 1	1.803988368	0.031	Molybdate ion transporter activity
269	CB29	Chlorophyll a-b binding protein CP29	1.840431425	0.042	LHC Facilitates the State 1 to State 2
270	DRC8	Dynein regulatory complex protein 8	1.858928051	0.269	Regulates microtubule sliding
271	DIP13	13 kDa deflagellation-inducible protein	1.858928051	0.134	Protein stability
272	ATPB	ATP synthase subunit beta, chloroplastic	1.87761057	0.066	ATP synthesis coupled proton transport
273	RK23	50S ribosomal protein L23, chloroplastic	1.896480852	0.019	Translation
274	SODM	Superoxide dismutase [Mn], mitochondrial	1.993715528	0.440	Destroys superoxide anion radicals
		Up-regulated proteins (FC ≥ 2.0)			
275	CYC	Cytochrome c	2.013752683	0.002	Carrier protein of mitochondrial ETC
276	TRXM	Thioredoxin M-type, chloroplastic	2.033991215	0.690	Cell redox homeostasis
277	CHLI	Mg-chelatase subunit ChII, chloroplastic	2.075080647	0.015	Insertion of Mg into protoporphyrin
278	RLA1	60S acidic ribosomal protein P1	2.117000017	0.098	Translational elongation
279	IFT57	Intraflagellar transport protein 57	2.1382762	0.030	Maintenance and formation of cilia
280	DYL1	Dynein 8 kDa light chain, flagellar outer arm	2.1382762	0.012	Microtubule-based process
281	NU4M	NADH-ubiquinone oxidoreductase chain 4	2.247907992	0.001	Electrons NADH to the respiratory chain
282	DNE1	DNA endonuclease I-Crel	2.293318702	0.281	Endonuclease activity, Intron homing
283	DRC6	Dynein regulatory complex subunit 6	2.386910865	0.381	Regulator of ciliary/flagellar motility
284	RR3	30S ribosomal protein S3, chloroplastic	2.637944535	0.091	Translational
285	S17P	Sedoheptulose-bisphosphatase	2.664456293	0.110	Starch and Sucrose biosynthetic process
286	DYL5	Dynein 16 kD light chain, flagellar outer arm	3.12676832	0.122	Cell redox homeostasis
287	PETM	Cyt b6f complex subunit 7, chloroplastic	3.421229602	0.310	CEF around PSI and state transitions.
288	CYB6	Cytochrome b6	7.099327336	0.441	CEF around PSI and state transitions.
		Control specific protein			
289	FER	Ferredoxin, chloroplastic	Control_W50		Electron transfer for metabolic reactions
290	PSBE	Cytochrome b559 subunit alpha	Control_W50		PETC, Charge separation
291	URM1	Ubiquitin-related modifier 1 homolog	Control_W50		Endonuclease activity, Intron homing
292	IFT25	Intraflagellar transport protein 25	Control_W50		Intraciliary transport
293	CYC6	Cytochrome c6, chloroplastic	Control_W50		Electron carrier cyt b6f and PSI
294	PETD	Cytochrome b6-f complex subunit 4	Control_W50		Transferring electrons within CET
295	UFM1	Ubiquitin-fold modifier 1	Treated_W50		Protein ufmylation
		Treated specific protein			
296	PSAK	PSI RC subunit psaK, chloroplastic	Treated_W500		Photosynthesis

297	PSAJ	PSI RC subunit IX	Treated_W500		Organization of the PsaE and PsaF
298	RR8	30S ribosomal protein S8, chloroplastic	Treated_W500		Translation
299	RK36	50S ribosomal protein L36, chloroplastic	Treated_W500		Translation
300	MOC2A	Molybdopterin synthase sulfur subunit	Treated_W500		Mo-molybdopterin cofactor biosynthetic
301	PSBJ	Photosystem II reaction center protein J	Treated_W500		Photosynthesis, Charge separation
302	H2A	Histone H2A	Treated_W500		Chromosomal stability
303	ARL3	ADP-ribosylation factor-like protein 3	Treated_W500		Cytokinesis and cilia signalling
304	PSBT	Photosystem II reaction center protein T	Treated_W500		Photosynthesis, PSII Dimerization
305	GP1	Vegetative cell wall protein gp1	Treated_W500		Major component of outer cell wall
306	NU2M	NADH-ubiquinone oxidoreductase chain 2	Treated_W500		NADH dehydrogenase activity
307	NU1M	NADH-ubiquinone oxidoreductase chain 1	Treated_W500		NADH dehydrogenase activity
308	PSBL	PSII reaction center protein L	Treated_W500		PSII assembly and dimerization.
	PSBW	PSII RC W protein, chloroplastic	Treated_W500		Stabilizes dimeric photosystem II
309	TBB	Tubulin beta-1/beta-2 chain	Treated_W500		Microtubule-based process
310	YCF4	Photosystem I assembly protein Ycf4	Treated_W500		Assembly of the PSI complex

Table 5.2 List of proteins expressed in *pgrl1_500* vs WT_50 (treated vs control) of *C. reinhardtii*.

S.No.	Abbreviation	Description	Fold change	P-value	GO description
		Down-regulated protein (FC ≤ 0.5)			
1	FLTOP	Protein Flattop homolog	0.101266466	0.042	Regulator of cilium basal body docking
2	PSAK	PSI RC subunit psaK, chloroplastic	0.109700644	0.005	Photosynthesis
3	H2B1	Histone H2B.1	0.205975089	0.003	DNA binding
4	RR12	30S ribosomal protein S12, chloroplastic	0.218711891	0.021	Translation
5	G3PA	G3P-dehydrogenase A, chloroplastic	0.225372653	0.012	Glucose metabolic process
6	TRXH	Thioredoxin H-type	0.26447725	0.007	Cell redox homeostasis
7	CAH1	Carbonic anhydrase 1	0.283654029	0.002	Carbonate dehydratase activity
8	NU4M	NADH-ubiquinone oxidoreductase chain 4	0.292292572	0.022	ATP synthesis coupled transport
9	ATPX	ATP synthase subunit b', chloroplastic	0.295230158	0.013	ATP synthesis coupled proton transport
10	GBLP	Guanine nucleotide-binding-subunit beta	0.298197268	0.007	Transcription initiation and termination
11	MUT11	Protein Mut11	0.304221247	0.013	Involved 'Lys-4' histone H3 methylation
12	IFT57	40S ribosomal protein S18	0.310366955	0.007	Translation
13	IFT57	Intraflagellar transport protein 57	0.323033258	0.010	Maintenance and formation of cilia
14	IFT46	Intraflagellar transport protein 46	0.323033258	0.009	Cilium-dependent cell motility
15	SAC1	Sulfur deprivation response regulator	0.326279793	0.054	Potassium ion transport
16	ATPD	ATP synthase delta chain, chloroplastic	0.339595511	0.001	ATP synthesis coupled proton transport
17	RR9	30S ribosomal protein S9, chloroplastic	0.343008499	0.045	Translation
18	PLAS	Plastocyanin, chloroplastic	0.349937766	0.032	Electron transfer from P700 and cyt b ₆ f
19	MOT1	Molybdate transporter 1	0.353454695	0.016	Mo- transmembrane transporter activity
20	YCX1	12.2 kDa protein in 16S rRNA region	0.364218983	0.026	Conserved phylogeny
21	DNAL1	Dynein light chain 1, axonemal	0.364218983	0.005	Motor activity for flagellar motility
22	RBL	RuBP carboxylase large chain	0.364218983	0.107	Carboxylation of RuBP in PPP
23	IFT22	Intraflagellar transport protein 22	0.367879441	0.012	Cellular availability of IFT particles
24	CIAO1	Cytosolic Fe-S protein assembly protein	0.367879441	0.001	Required for Fe-S cluster assembly
25	CYB	Cytochrome b	0.379083027	0.025	Respiratory electron transport chain
26	RUBA	RuBisCO large subunit-alpha, chloroplastic	0.398519034	0.013	Assembly of the RuBisCO enzyme
27	PETO	Cyt b ₆ f complex subunit petO, chloroplastic	0.402524213	0.007	LET between PSII and I, CETaround PSI
28	H2B2	Histone H2B.2	0.406569669	0.010	Nucleosome remodelling
29	RSSA	RuBP carboxylase large chain	0.410655759	0.048	Protein refolding
30	RR4	40S ribosomal protein SA	0.427414922	0.025	Translation
31	PSBB	Photosystem II CP47 RC protein	0.431710535	0.004	PET in photosystem II
32	FEN1	Flap endonuclease 1	0.444858065	0.388	DNA replication, Base-excision repair
33	TBA2	Tubulin alpha-2 chain	0.453844786	0.144	Alters the microtubule cytoskeleton
34	YPTC6	Ras-related protein YPTC6	0.453844786	0.726	GTPase activity
35	NRT22	Nitrate transporter 2.2	0.453844786	0.103	nitrate assimilation, nitrate transport
36	ODA1	Outer dynein arm protein 1	0.453844786	0.005	Cilium movement involved cell motility
37	DNE1	DNA endonuclease I-Crel	0.472366553	0.008	Endonuclease activity, Intron homing
38	YCX2	Uncharacterized 16S rRNA protein	0.472366553	0.463	Phylogenetic relationship
39	RR7	30S ribosomal protein S7, chloroplastic	0.472366553	0.015	Translation
40	EZY1	Ezy-1 protein	0.477113911	0.035	Uncharacterized protein
41	SUVH3	Histone-lysine N-methyltransferase	0.477113911	0.084	Repressed chromatin function
42	RPC1A	DNA-directed RNA polymerase subunit beta	0.481908981	0.329	Transcription by RNA polymerase II
43	RCA	RuBisCO/oxygenase activase, chloroplastic	0.50157607	0.008	Activation of RuBisCO
		Differential regulated protein (≤ 2.0 FC ≥ 0.5)			
44	G3PC	G3P-dehydrogenase, cytosolic	0.506616989	0.013	Carbohydrate metabolism
45	CALM	Calmodulin	0.506616989	0.005	Calcium-mediated signalling
46	RL403	Ubiquitin-60S ribosomal protein L40	0.516851321	0.032	Translation
47	TRXM	Thioredoxin M-type, chloroplastic	0.516851321	0.217	Cell redox homeostasis
48	THI4	Thiamine thiazole synthase, chloroplastic	0.516851321	0.014	Thiamine biosynthetic process
49	CFA77	Cilia- and flagella-associated protein	0.522045789	0.002	Cell motility
50	TBB	Tubulin beta-1/beta-2 chain	0.522045789	0.018	Microtubule-based process
51	ANM1	Protein arginine N-methyltransferase 1	0.527292432	0.839	Asymmetric dimethylation of a flagellum
52	RUBB	RuBisCO large protein subunit beta-1	0.532591804	0.022	Assembly of the enzyme oligomer

53	CCD39	Coiled-coil domain-containing protein 39	0.532591804	0.053	Inner dynein arm assembly
54	EZY2	Ezy-1 protein	0.532591804	0.009	Uncharacterized protein
55	NAC2	PsbD mRNA maturation factor, chloroplastic	0.532591804	0.021	mRNA processing- 5' UTR of psbD mRNA
56	PESC	Pescadillo homolog	0.537944435	0.080	Maturation of 5.8S rRNA
57	WDR12	Ribosome biogenesis protein	0.537944435	0.003	LSU-rRNA from tricistronic rRNA
58	ZYS3	Zygote-specific protein 3	0.537944435	0.005	Cell development
59	CHLI	Mg-chelatase subunit ChII, chloroplastic	0.537944435	0.002	Insertion of Mg into protoporphyrin ring
60	DYI3	Dynein, 70 kDa intermediate chain	0.543350861	0.073	Cell motility, Outer dynein arm assembly
61	UCRIA	Cyt b6-f complex Fe-S subunit, chloroplastic	0.543350861	0.052	Mediates LET and CET around PSI
62	TBA1	Tubulin alpha-1 chain	0.548811623	0.108	Microtubule-based process
63	CFA58	Cilia- and flagella-associated protein 58	0.548811623	0.087	Cilium-dependent cell motility
64	RSP4	Flagellar radial spoke protein 4	0.548811623	0.025	Cell motility, cilium assembly
65	SC61B	Protein transport protein Sec61 subunit beta	0.548811623	0.041	Intracellular protein transport
66	PETM	Cyt b6f complex subunit 7, chloroplastic	0.548811623	0.015	CEF around PSI and state transitions.
67	CFA70	Cilia- and flagella-associated protein 70	0.548811623	0.019	Axoneme- regulates ciliary motility
68	ATPE	ATP synthase epsilon chain, chloroplastic	0.548811623	0.099	ATP synthesis coupled proton transport
69	BOP1	Ribosome biogenesis protein BOP1 homolog	0.554327299	0.014	Maturation of 5.8S rRNA
70	LHSR1	LHC stress-related protein 1, chloroplastic	0.554327299	0.056	Non-photochemical quenching
71	ARL13	ADP-ribosylation factor-like protein 13B	0.554327299	0.038	Control ciliary axoneme structure
72	FENR	Ferredoxin--NADP reductase, chloroplastic	0.554327299	0.012	Regulating the cyclic and non-CEF
73	DAAF1	Leucine-rich containing protein ODA7	0.565525443	0.062	Cell motility and localization to organelle
74	ACT	Actin	0.565525443	0.051	Cytoplasmic streaming
75	CAH2	Carbonic anhydrase 2	0.565525443	0.004	CCM, Carbonate dehydratase activity
76	ASNA1	ATPase ARSA1	0.565525443	0.009	ATPase activity
77	RL401	Ubiquitin-60S ribosomal protein L40	0.571209062	0.005	Translation
78	CFA99	Cilia- and flagella-associated protein 99	0.571209062	0.005	Ciliary/flagellar motility
79	DRC8	Dynein regulatory complex protein 8	0.571209062	0.266	Regulates microtubule sliding
80	MDHM	Malate dehydrogenase, mitochondrial	0.571209062	0.047	Carbohydrate metabolic process
81	KTU	Protein kintoun	0.571209062	0.017	Axonemal dynein complex assembly
82	HS22C	Heat shock 22 kDa protein, chloroplastic	0.571209062	0.016	Protein maturation and degradation
83	PF20	Flagellar WD repeat-containing protein Pf20	0.571209062	0.028	Inter-microtubule bridges in flagella
84	TOC34I	Translocase of chloroplast 34, chloroplastic	0.571209062	0.373	Transport- Cytoplasm to Chloroplast
85	CYC	Cytochrome c	0.576949804	0.004	Final mitochondrial ETC protein carrier
86	YPTC4	GTP-binding protein YPTC4	0.576949804	0.038	Protein transport and vesicular traffic
87	CF251	Cilia- and flagella-associated protein 251	0.576949804	0.108	Cilium movement
88	NRT21	Nitrate transporter 2.1	0.576949804	0.000	Nitrate assimilation
89	PGKH	Phosphoglycerate kinase, chloroplastic	0.58274824	0.014	Glycolytic process, Reductive PPP
90	PSAH	Photosystem I RC subunit VI, chloroplastic	0.588604987	0.063	Docking for LHC I antenna
91	PSAF	PSI RC subunit III, chloroplastic	0.594520559	0.007	Electron transfer from PC to P700
92	RTCB	RNA-splicing ligase RtcB homolog	0.594520559	0.400	tRNA splicing
93	CATR	Caltractin	0.594520559	0.026	Cell cycle, Cell division
94	RR4	30S ribosomal protein S4, chloroplastic	0.600495585	0.011	Translation
95	CP12	Calvin cycle protein CP12, chloroplastic	0.600495585	0.000	Assembly of complex of PRK/GAPDH.
96	RL10A	60S ribosomal protein L10a	0.600495585	0.006	Translation
97	RPOA	DNA-directed RNA polymerase subunit alpha	0.600495585	0.001	Transcription, DNA-templated
98	HAP2	Hapless 2	0.600495585	0.001	Protein insertion into the membrane
99	CTH1	MG-protoporphyrin IX monomethyl ester	0.60653066	0.009	Photosynthesis, chlorophyll biosynthesis
100	DYL2	Dynein 11 kDa light chain	0.612626388	0.037	Microtubule-based process
101	ATPG	ATP synthase gamma chain, chloroplastic	0.618783398	0.060	ATP synthesis coupled proton transport
102	SFAS	SF-assemblin	0.618783398	0.004	Striated microtubule-fibres assembly
103	CFA61	Cilia- and flagella-associated protein 61	0.618783398	0.029	Cilium movement, Cilium organization
104	CEMA	Chloroplast envelope membrane protein	0.618783398	0.029	Transmembrane transporter activity
105	DAAF3	Dynein assembly factor 3, axonema	0.625002269	0.023	Axonemal dynein complex assembly
106	DRC1	Dynein regulatory complex protein 1	0.625002269	0.029	Dynein complex assembly
107	ARL3	ADP-ribosylation factor-like protein 3	0.625002269	0.002	Cytokinesis and cilia signalling
108	SULT2	Proton/sulfate cotransporter 2	0.625002269	0.001	Sulfate transmembrane transporter
109	PLD6	Mitochondrial cardiolipin hydrolase	0.637628159	0.020	Lipid catabolic process
110	RLA1	60S acidic ribosomal protein P1	0.637628159	0.005	Translational elongation
111	ATPF	ATP synthase subunit b, chloroplastic	0.637628159	0.031	ATP synthesis coupled proton transport
112	ALFC	Fructose-bisphosphate aldolase 1	0.644036423	0.022	Glycolytic process
113	HSTC	Homocysteine methyltransferase	0.644036423	0.011	Methionine biosynthetic process
114	CHLL	Light-independent protochlorophyllide	0.644036423	0.000	Photosynthesis, Dark reaction
115	CFA73	Cilia- and flagella-associated protein 73	0.65050909	0.031	Regulation of microtubule motor activity
116	RPOB1	DNA-directed RNA polymerase beta N	0.65050909	0.014	Transcription, DNA-templated
117	ENO	Enolase	0.663650253	0.002	Glycolytic process

118	CFA20	Cilia- and flagella-associated protein 20	0.663650253	0.132	Axoneme, Cilium assembly
119	CFA44	Cilia- and flagella-associated protein 44	0.670320042	0.051	Cilium-dependent cell motility
120	ATPBM	ATP synthase subunit beta, mitochondrial	0.670320042	0.001	ATP synthesis coupled proton transport
121	LHR32	LHC stress-related protein 3.1, chloroplastic	0.677056884	0.015	Nonphotochemical quenching
122	RSP2	Flagellar radial spoke protein 2	0.677056884	0.048	Flagellar bending
123	RBS2	RuBisCO small chain 2, chloroplastic	0.683861412	0.003	Photorespiration, Carbon fixation
124	LHR32	LHC stress-related protein 3.2, chloroplastic	0.683861412	0.016	Nonphotochemical quenching
125	RPOB2	DNA-directed RNA polymerase beta C	0.683861412	0.033	Transcription, DNA-templated
126	CALR	Calreticulin	0.683861412	0.860	Protein folding
127	RBS1	RuBisCO small chain 1, chloroplastic	0.690734327	0.004	Photosynthesis, Photorespiration
128	ALFC	Serine/threonine-kinase, chloroplastic	0.697676316	0.038	State transition
129	CAPP2	Phosphoenolpyruvate carboxylase 2	0.697676316	0.005	Carbon fixation, Tricarboxylic acid cycle
130	PURA	Adenylosuccinate synthetase, chloroplastic	0.704688094	0.024	"de novo" AMP biosynthetic process
131	RR11	30S ribosomal protein S11, chloroplastic	0.704688094	0.004	Translation
132	IFT81	Intraflagellar transport protein 81	0.71177032	0.012	Transport of tubulin within the cilium
133	GLE	Autolysin	0.718923724	0.037	Cell wall organization
134	METK	S-adenosylmethionine synthase	0.726149042	0.002	S-adenosylmethionine biosynthesis
135	GPX1	Glutathione peroxidase 1, mitochondrial	0.726149042	0.596	Arachidonic acid metabolic process
136	RSP5	Flagellar radial spoke protein 5	0.733446954	0.002	Regulation of flagellar bending
137	CFA45	Cilia- and flagella-associated protein 45	0.733446954	0.038	Cell motility
138	FLA10	Kinesin-like protein FLA10	0.733446954	0.072	Intraciliary anterograde transport
139	HSTC	Homogentisate solanesyltransferase	0.733446954	0.027	Carotenoid and PQ biosynthetic process
140	DRC7	Dynein regulatory complex subunit 7	0.740818212	0.012	Regulation of flagellar motility
141	RTL	Reverse transcriptase-like protein	0.740818212	0.243	cDNA synthetesis
142	CFA91	Cilia- and flagella-associated protein 91	0.748263574	0.002	Cilium movement
143	PCDP1	Cilia- and flagella-associated protein 221	0.748263574	0.035	Cilium assembly and movement
144	CF157	Cilia- and flagella-associated protein 157	0.755783741	0.013	Cilium movement, Cell motility
145	RK23	50S ribosomal protein L23, chloroplastic	0.755783741	0.330	Translation, Large subunit assembly
146	CMD1	5-methylcytosine-modifying enzyme 1	0.763379486	0.003	5-methylcytosine catabolic process
147	DYL4	Dynein 14 kDa light chain	0.763379486	0.688	Outer dynein arm assembly
148	TBG	Tubulin gamma chain	0.763379486	0.063	Cytoplasmic microtubule organization
149	ALB31	ALBINO3-like protein 1, chloroplastic	0.771051593	0.021	Assembly and activity of LHC I and II
150	CP100	Cilia- and flagella-associated protein 100	0.771051593	0.951	Cell motility, Cilium movement
151	CRD1	Mg-protoporphyrin IX monomethyl ester	0.771051593	0.020	Photosynthesis, Chl biosynthetic process
152	RAA3	Trans-splicing factor Raa3, chloroplastic	0.771051593	0.197	mRNA processing, RNA splicing
153	POB15	Proteome of basal body protein 15	0.778800783	0.039	Motility and sensing stimuli
154	CFA52	Cilia- and flagella-associated protein 52	0.778800783	0.029	Cell motility
155	KLP1	Kinesin-like protein KLP1	0.778800783	0.121	Microtubule-based movement
156	IFT43	Intraflagellar transport protein 43	0.778800783	0.004	Cell projection organization
157	HSP70	Heat shock 70 kDa protein	0.778800783	0.887	Protein maturation and degradation
158	CYF	Cytochrome f	0.778800783	0.062	CEF around PSI
159	S17P	Sedoheptulose bisphosphatase, chloroplastic	0.778800783	0.039	Starch and Sucrose biosynthetic process
160	DYI2	Dynein, 78 kDa intermediate chain	0.786627865	0.053	Motor activity, Arm assembly
161	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	0.794533599	0.027	Photosynthesis
162	RR2A	30S ribosomal protein S2, chloroplastic	0.794533599	0.695	Translation
163	TBC2	Tbc2 translation factor, chloroplastic	0.802518799	0.015	PsbC mRNA for translation initiation
164	PSAD	PS I reaction center subunit II, chloroplastic	0.810584251	0.065	Photosynthesis, Ferredoxin-docking site
165	YCF3	Photosystem I assembly protein Ycf3	0.810584251	0.333	Chaperone-like assembly of the PSI
166	ARLY	Argininosuccinate lyase	0.818730751	0.003	Arginine biosynthetic via ornithine
167	IDLC	28 kDa inner dynein arm light chain	0.818730751	0.226	Cilium involved in cell motility
168	CF300	Cilia- and flagella-associated protein 300	0.826959136	0.022	Structure organization and motility
169	CFA53	Cilia- and flagella-associated protein 53	0.826959136	0.001	Cilium assembly and movement
170	POC16	Centriole proteome protein 16	0.835270205	0.082	Flagellum assembly and maintenance
171	PSBC	Photosystem II CP43 reaction center protein	0.835270205	0.009	PET in photosystem II
172	SLT3	Probable sodium/sulfate cotransporter 3	0.835270205	0.025	Sulfate transport
173	RSP14	Radial spoke protein 14	0.843664815	0.003	Cell motility, Endocytosis
174	EFTU	Elongation factor Tu, chloroplastic	0.843664815	0.056	Translational elongation
175	CAPP1	Phosphoenolpyruvate carboxylase 1	0.843664815	0.021	Carboxylation of PEP into oxaloacetate
176	1433	14-3-3-like protein	0.843664815	0.451	Flagellar arrangement and assembly
177	RUBC	RuBisCO large subunit- beta-2	0.860707971	0.067	RuBisCO assembly, Protein refolding,
178	DRC4	Dynein regulatory complex subunit 4	0.869358235	0.012	Axonemal dynein complex assembly
179	CCD40	Coiled-coil domain-containing protein 40	0.878095435	0.023	Assembly of dynein regulatory complex
180	CAO	Chlorophyllide a oxygenase, chloroplastic	0.878095435	0.811	Chlorophyll b biosynthetic process
181	DRC10	Dynein regulatory complex protein 10	0.886920439	0.200	Maintains and regulates microtubule
182	FER	Ferredoxin, chloroplastic	0.904837417	0.003	Fe-S protein for metabolic reactions

183	IFT25	Intraflagellar transport protein 25	0.904837417	0.090	Intraciliary transport
184	ALB3.2	ALBINO3-like protein 2, chloroplastic	0.913931182	0.008	Assembly and activity of LHC I and II.
185	IFT74	Intraflagellar transport protein 74	0.913931182	0.001	Cilium assembly
186	CHLB	Light-independent protochlorophyllide	0.913931182	0.913	Light-independent chl biosynthetic
187	YCX3	14.4 kDa protein in 16S rRNA region	0.923116348	0.015	Determine phylogeny
188	CFA65	Cilia- and flagella-associated protein 65	0.93239382	0.019	Cell projection organization
189	YPTC1	GTP-binding protein YPTC1	0.93239382	0.003	Protein transport, vesicular traffic
190	CFA43_	Cilia- and flagella-associated protein 43	0.941764535	0.050	Flagellum axoneme organization
191	IPYR2	Soluble inorganic pyrophosphatase 2	0.941764535	0.010	Phosphate-containing metabolic process
192	TEKT	Tektin	0.951229424	0.080	Assembly of flagella and cilia
193	BSD2	BUNDLE SHEATH DEFECTIVE 2, chloroplastic	0.951229424	0.503	Chaperone-mediated protein folding
194	IF172	Intraflagellar transport protein 172	0.96078944	0.070	Multicellular organism development
195	LISC	Lipoyl synthase, chloroplastic	0.990049834	0.072	Protein lipoylation
196	ARF1	ADP-ribosylation factor 1	0.990049834	0.005	Vesicle-mediated transport
197	DRC11	Dynein regulatory complex protein 11	0.990049834	0.711	Regulator of ciliary/flagellar motility
198	CHLB	Light-independent protochlorophyllide	0.990049834	0.022	Light-independent chl biosynthesis
199	SLT1	Sodium/sulfate cotransporter 1	1	0.709	Potassium ion transport
200	CLPP	ATP-dependent Clp protease proteolytic	1.02020134	0.271	Chymotrypsin control misfolded proteins
201	RS3A	40S ribosomal protein S3a	1.02020134	0.110	Translation
202	DYL3	Dynein 18 kDa light chain	1.030454533	0.013	Motor activity, Calcium ion binding
203	DRC3	Dynein regulatory complex subunit 3	1.040810773	0.009	Cilium-dependent cell motility
204	ASNA2	ATPase ARSA2	1.061836545	0.081	Tail-anchored protein insertion into ER
205	PHOT	Phototropin	1.061836545	0.007	Blue light signalling, NPQ induction
206	RSP3	Flagellar radial spoke protein 3	1.083287066	0.011	Cilium-dependent cell motility
207	RK2	50S ribosomal protein L2, chloroplastic	1.083287066	0.001	Mitochondrial translation
208	RK5	50S ribosomal protein L5, chloroplastic	1.094174288	0.005	Translation
209	PSAB	PSI P700 chlorophyll a apoprotein A2	1.10517092	0.050	Primary electron donor of PSI
210	FLA10	Light-independent protochlorophyllide	1.127496849	0.001	Light-independent chl biosynthetic
211	CCS1	Cytochrome c biogenesis, chloroplastic	1.138828378	0.011	Cytochrome complex assembly
212	RL12	60S ribosomal protein L12	1.138828378	0.003	Translation
213	DRC9	Dynein regulatory complex protein 9	1.138828378	0.970	Regulator of ciliary/flagellar motility
214	GSA	Glutamate semialdehyde aminomutase	1.138828378	0.171	Chlorophyll biosynthetic process
215	DC2L	Cytoplasmic dynein 2 intermediate chain 1	1.173510867	0.374	Regulates flagellar dynein activity
216	ADT	ADP,ATP carrier protein	1.173510867	0.053	Catalyzes the exchange of ADP and ATP
217	EZY3	Ezy-1 protein	1.197217372	0.064	Uncharacterized protein
218	GLNA2	Glutamine synthetase, chloroplastic	1.221402762	0.009	Glutamine biosynthetic process
219	IPYR1	Soluble inorganic pyrophosphatase 1,	1.221402762	0.790	Phosphate-containing metabolism
220	DRC2	Dynein regulatory complex subunit 2	1.221402762	0.010	Axonemal dynein complex assembly
221	RSP1	Flagellar radial spoke protein 1	1.246076729	0.002	Flagellar bending
222	KPPR	Phosphoribulokinase, chloroplastic	1.246076729	0.031	Reductive pentose-phosphate cycle
223	CYB6	Cytochrome b6	1.284025417	0.003	CEF around PSI and state transitions.
224	YCF4	Photosystem I assembly protein Ycf4	1.284025417	0.479	Assembly of the photosystem I complex
225	PSBD	Photosystem II D2 protein	1.296930074	0.957	Assembly of PSII complex
226	PR46B	Coiled-coil domain-containing protein 103	1.309964465	0.334	Cilia motility
227	ARS	Arylsulfatase	1.323129814	0.039	Phenol-containing metabolic process
228	CB2	Chl a-b binding, LHCII type I, chloroplastic	1.323129814	0.037	Light-harvesting as a light receptor
230	PSAC	Photosystem I iron-sulfur center	1.377127754	0.126	Binding of PsuD and PsuE to PSI
231	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	1.377127754	0.003	Protein urmylation
232	IFT56	Intraflagellar transport protein 56	1.390968147	0.006	Intraciliary transport
233	GLNA1	Glutamine synthetase cytosolic isozyme	1.390968147	0.002	Glutamine biosynthetic process
234	H2B4	Histone H2B.4	1.404947596	0.781	Protein heterodimerization activity
235	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	1.41906754	0.013	Unsaturated and fatty acid biosynthetic
236	PSAA	PSI P700 chlorophyll a apoprotein A1	1.41906754	0.464	Photosynthesis
237	PSAE	PSI reaction center subunit IV, chloroplastic	1.433329435	0.047	Photosynthesis
238	PSBA	Photosystem II protein D1	1.447734622	0.060	Photosynthetic electron transport in PSII
239	RAA2	Trans-splicing factor Raa2, chloroplastic	1.462284582	0.133	RNA splicing, mRNA processing
240	UFM1	Ubiquitin-fold modifier 1	1.476980773	0.527	Protein ufmylation
241	RL13	60S ribosomal protein L13	1.476980773	0.258	Translation
242	SLT2	Sodium/sulfate cotransporter 2	1.537257535	0.034	Potassium ion transport
243	H2B3	Histone H2B.3	1.537257535	0.704	DNA binding
244	NUSM	NADH-ubiquinone oxidoreductase chain 5	1.537257535	0.689	ATP synthesis
245	PSAG	Photosystem I RC subunit V, chloroplastic	1.599994191	0.116	Photosynthesis
246	DRC5	Dynein regulatory complex subunit 5	1.632316236	0.249	Ciliary/flagellar motility
247	RR3	30S ribosomal protein S3, chloroplastic	1.648721271	0.073	Translational
248	RPC2B	DNA-directed RNA polymerase subunit beta	1.665291179	0.006	Transcription, DNA-templated

249	RSP6	Flagellar radial spoke protein 6	1.69893226	0.023	Cilium assembly
250	ATPB	ATP synthase subunit beta, chloroplastic	1.750672504	0.195	ATP synthesis coupled proton transport
251	YPTC5	GTP-binding protein YPTC5	1.768267039	0.005	GTPase activity, protein transport
252	ATPA	ATP synthase subunit alpha, chloroplastic	1.786038401	0.347	ATP synthesis coupled proton transport
253	MOC1	Holliday junction resolvase, chloroplastic	1.803988368	0.115	DNA recombination
254	CYC6	Cytochrome c6, chloroplastic	1.803988368	0.256	Electron carrier between cyt b6f and PSI
255	PSBS1	Photosystem II protein PSBS1	1.822118844	0.006	Nonphotochemical quenching
256	PSBR	Photosystem II protein PSBR, chloroplastic	1.896480852	0.003	Energy-dependent quenching-NPQ
257	PSBS2	Photosystem II protein PSBS2	1.896480852	0.080	Nonphotochemical quenching
258	YCF78	membrane protein ycf78	1.915540783	0.036	Cell growth
259	HEM2	Aminolevulinic acid dehydratase	1.934792385	0.096	Chlorophyll biosynthetic process
260	GP1	Vegetative cell wall protein gp1	1.934792385	0.127	component of the outer cell wall W6
261	EFTS	Elongation factor Ts, mitochondrial	1.934792385	0.081	Translation elongation factor activity
		Up-regulated proteins (FC ≥ 2.0)			
262	PSBP	Oxygen-evolving enhancer 2, chloroplastic	2.075080647	0.255	Photosynthesis, Oxygen evolution
263	DES	Acyl-lipid omega-13 desaturase	2.1382762	0.501	Linoleic acid metabolic process
264	SRGT1	Peptidyl serine alpha-galactosyltransferase	2.247907992	0.052	Transferring glycosyl groups
265	RR2B	Putative 30S ribosomal S2-like protein	2.247907992	0.001	Transferase activity
266	DAW1	Dynein assembly WDR repeat domains 1	2.611696417	0.120	Intraciliary transport and assembly
267	RL11	60S ribosomal protein L11	2.664456293	0.073	Translation
268	POR	Protochlorophyllide reductase, chloroplastic	2.773194711	0.051	Chlorophyll biosynthetic process
269	CHLB	Light-independent protochlorophyllide	2.773194711	0.011	Light-independent chl biosynthesis
270	NU1M	NADH-ubiquinone oxidoreductase chain 1	3.034358438	0.002	NADH dehydrogenase activity
271	RL31	60S ribosomal protein L31	3.632786417	0.450	Translation
272	CB29	Chlorophyll a-b binding protein CP29	3.78104355	0.001	LHC Facilitates State 1 to 2 transition
273	SULP2	Sulfate permease 2, chloroplastic	3.935350714	0.028	Sulfate transport
274	RK14	50S ribosomal protein L14, chloroplastic	4.05519987	0.045	Translation
275	PSBO	Oxygen-evolving enhancer1, chloroplastic	4.137120263	0.003	Primary site of water splitting
276	SULP1	Sulfate permease 1, chloroplastic	4.85495602	0.605	Uptake and assimilation of sulfate
277	LPAT	Acyl-sn-glycerol-3-phosphate acyltransferase	7.53832479	0.007	CDP-diacylglycerol biosynthesis
278	RK22	50S ribosomal protein L22	38.09184072	0.027	Translation
279	RL44	60S ribosomal protein L44	41.26439529	0.607	Translation
280	DRC6	Dynein regulatory complex subunit 6	278.6621495	0.172	Regulator of ciliary/flagellar motility
		Treated specific protein			
281	RK16	50S ribosomal protein L16, chloroplastic	Treated_P500		Mitochondrial translation
282	RK16	50S ribosomal protein L16, chloroplastic	Treated_P500		Mitochondrial translation
283	atpH	ATP synthase subunit c, chloroplastic	Treated_P500		ATP synthesis coupled proton transport
284	PSAJ	Photosystem I reaction center subunit IX	Treated_P500		Organization of the PsaE and PsaF subunit
285	RR19	30S ribosomal protein S19, chloroplastic	Treated_P500		Translation
286	RR8	30S ribosomal protein S8, chloroplastic	Treated_P500		Translation
287	SODM	Superoxide dismutase [Mn], mitochondrial	Treated_P500		Destroys superoxide anion radicals
288	H31	Histone H3 type 1	Treated_P500		DNA binding, Chromosome stability
289	ND6	NADH-ubiquinone oxidoreductase chain 6	Treated_P500		NADH dehydrogenase activity
290	MOC2A	Molybdopterin synthase sulfur carrier	Treated_P500		Mo-molybdopterin cofactor biosynthesis
291	PSBJ	Photosystem II reaction center protein J	Treated_P500		Photosynthesis, Charge separation
292	H2A	Histone H2A	Treated_P500		Chromosomal stability
293	H4	Histone H4	Treated_P500		Nucleosome remodelling
294	H32	Histone H3 type 3	Treated_P500		Post-translational modifications
295	PSBT	Photosystem II reaction center protein T	Treated_P500		Photosynthesis, Dimerization of PSII
296	RR14	30S ribosomal protein S14, chloroplastic	Treated_P500		Translation
297	CCSA	Cytochrome c biogenesis protein CcsA	Treated_P500		cytochrome complex assembly
298	PSBE	Cytochrome b559 subunit alpha	Treated_P500		PETC, Charge separation
299	URM1	Ubiquitin-related modifier 1 homolog	Treated_P500		Endonuclease activity, Intron homing
300	IFT27	Intraflagellar transport protein 27	Treated_P500		GTPase activity, Form IFT complex B
301	RK20	50S ribosomal protein L20, chloroplastic	Treated_P500		Translation
302	NU2M	NADH-ubiquinone oxidoreductase chain 2	Treated_P500		NADH dehydrogenase activity
303	COX1	Cytochrome c oxidase subunit 1	Treated_P500		Oxidative phosphorylation
304	PSBK	Photosystem II reaction center protein K	Treated_P500		Photosynthesis
305	PSBN	Protein PsbN	Treated_P500		Role in photosystem I and II biogenesis
306	DYL1	Dynein 8 kDa light chain	Treated_P500		Microtubule-based process

307	H32	Histone H3 type 2	Treated_P500		Chromosomal stability
308	PSBL	Photosystem II reaction center protein L	Treated_P500		PSII assembly and dimerization.
309	RS27	40S ribosomal protein S27	Treated_P500		Microtubule-based process
310	YCX6	12.3 kDa petA-petD intergenic region	Treated_P500		chloroplasts based function
311	PSBW	PSII reaction center W protein, chloroplastic	Treated_P500		Stabilizes dimeric photosystem II
312	LIAS	Lipoyl synthase, mitochondrial	Treated_P500		Lipoylation peptidyl-N6-lipoyl-L-lysine.
313	PETD	Cytochrome b6-f complex subunit 4	Treated_P500		Transferring electrons within the CET
314	PFL	Formate acetyltransferase	Treated_P500		Form pyruvate and coA
315	DIP13	13 kDa deflagellation-inducible protein	Treated_P500		Protein stability
316	YCX5	Uncharacterized trnR-chlB intergenic region	Treated_P500		Tag-protein stability
317	RS14	40S ribosomal protein S14	Treated_P500		Translation
318	RR18	30S ribosomal protein S18, chloroplastic	Treated_P500		Translation
		Control specific protein			
319	ATPI	ATP synthase subunit a, chloroplastic	<u>UOH_W_50</u>		ATP synthesis coupled proton transport

Table 5.3 List of proteins expressed in *pgr5_500* vs WT_50 (treated vs control) of *C. reinhardtii*.

S.No.	Abbreviation	Description	Fold Change	P-value	GO Description
		Down-regulated protein (FC ≤ 0.5)			
1	CHLL	Light-independent protochlorophyllide	0.013568556	0.010	Photosynthesis, Dark reaction
2	RK14	50S ribosomal protein L14, chloroplastic	0.298197268	0.012	Translation
3	IPYR2	Soluble inorganic pyrophosphatase 2	0.343008499	0.010	Phosphate-containing metabolic process
4	PSBO	Oxygen-evolving enhancer 1, chloroplastic	0.382892894	0.007	Primary site of water splitting
5	PSAA	PSI P700 chlorophyll a apoprotein A1	0.382892894	0.399	Photosynthesis
6	PR46B	Coiled-coil domain-containing protein	0.390627836	0.005	Cilia motility
7	CYC6	Cytochrome c6, chloroplastic	0.390627836	0.184	Electron carrier between cyt b6-f and PSI
8	IFT25	Intraflagellar transport protein 25	0.410655759	0.034	Intraciliary transport
9	RAA3	Trans-splicing factor Raa3, chloroplastic	0.418951547	0.324	mRNA processing, RNA splicing
10	RUBB	RuBisCO large subunit-protein beta-1	0.427414922	0.162	Assembly of the enzyme oligomer
11	RL44	60S ribosomal protein L44	0.440431658	0.273	Translation, Response to cycloheximide
12	CB2	Chl a-b binding protein, LHClI type I	0.440431658	0.415	Photosynthesis, LHC as light receptor
13	RR2B	Putative 30S ribosomal S2-like protein	0.458406024	0.234	Translation
14	RL11	60S ribosomal protein L11	0.463013077	0.053	Translation
15	CEMA	Chloroplast envelope membrane protein	0.463013077	0.056	Proton transporter activity
16	ATPI	ATP synthase subunit a, chloroplastic	0.467666431	0.567	ATP synthesis coupled proton transport
17	CCS1	Cytochrome c biogenesis protein	0.472366553	0.392	Cytochrome complex assembly
18	RS18	40S ribosomal protein S18	0.481908981	0.006	Translation
19	SRGT1	Peptidyl serine-galactosyltransferase	0.486752242	0.426	Transferase activity
20	H33	Histone H3 type 3	0.491644208	0.586	Nucleosome remodelling
21	RUBC	RuBisCO large subunit- beta-2	0.49658531	0.431	RuBisCO assembly, Protein refolding,
22	H32	Histone H3 type 2	0.49658531	0.015	Chromosomal stability.
		Differential regulated protein (≤ 2.0 FC ≥0.5)			
23	CRD1	Mg-protoporphyrin IX monomethyl ester	0.511708569	0.062	Photosynthesis, Chl biosynthetic process
24	RR2A	30S ribosomal protein S2, chloroplastic	0.532591804	0.296	Translation
25	PSAG	Photosystem I RC subunit V, chloroplastic	0.543350861	0.647	Photosynthesis
26	IDLC	28 kDa inner dynein arm light chain	0.543350861	0.474	Cilium movement involved in cell motility
27	S17P	Sedoheptulose-1,7-bisphosphatase	0.548811623	0.003	Starch and Sucrose biosynthetic process
28	RL401	Ubiquitin-60S ribosomal protein L40	0.554327299	0.267	Translation
29	RR12	30S ribosomal protein S12, chloroplastic	0.554327299	0.086	Translation
30	METK	S-adenosylmethionine synthase	0.554327299	0.413	S-adenosylmethionine biosynthesis
31	EFTS	Elongation factor Ts, mitochondrial	0.559898376	0.019	Translation elongation factor activity
32	IPYR1	Soluble inorganic pyrophosphatase 1	0.571209062	0.176	Phosphate-containing metabolic process
33	CF300	Cilia- and flagella-associated protein 300	0.58274824	0.000	Axonemal organization and motility
34	DRC11	Dynein regulatory complex protein 11	0.58274824	0.384	Regulator of ciliary/flagellar motility
35	DNE1	DNA endonuclease I-CreI	0.594520559	0.915	Endonuclease activity, Intron homing
36	IFT27	Intraflagellar transport protein 27	0.600495585	0.008	GTPase activity, Form IFT complex B
37	ZYS3	Zygote-specific protein 3	0.600495585	0.181	Cell development
38	SULT2	Proton/sulfate cotransporter 2	0.600495585	0.092	Sulfate transporter activity
39	RL403	Ubiquitin-60S ribosomal protein L40	0.618783398	0.304	Translation
40	CB29	Chlorophyll a-b binding protein CP29	0.625002269	0.046	LHC Facilitates the State 1 to 2 transition
41	PSAC	Photosystem I iron-sulfur center	0.637628159	0.032	Required for binding of PsuD, PsuE to PSI
42	THI4	Thiamine thiazole synthase, chloroplastic	0.637628159	0.800	Thiamine biosynthetic process
43	PSBI	Photosystem II reaction center protein I	0.644036423	0.007	Stability and assembly of PSII
44	NRT21	Nitrate transporter 2.1	0.644036423	0.177	Nitrate assimilation
45	RL10A	60S ribosomal protein L10a	0.657046828	0.129	Translation
46	DES	Acyl-lipid omega-13 desaturase	0.670320042	0.588	Linoleic acid metabolic process
47	RR14	30S ribosomal protein S14, chloroplastic	0.677056884	0.094	Translation
48	IFT43	Intraflagellar transport protein 43	0.677056884	0.412	Cell projection organization
49	CMD1	5-methylcytosine-modifying enzyme 1	0.683861412	0.020	5-methylcytosine catabolic process
50	DNAL1	Dynein light chain 1, axonemal	0.683861412	0.001	Motor activity for flagellar motility
51	ADT	ADP,ATP carrier protein	0.690734327	0.635	Catalyzes the exchange of ADP and ATP

52	PGKH	Phosphoglycerate kinase, chloroplastic	0.697676316	0.587	Glycolytic process, Reductive PPP
53	CCSA	Cytochrome c biogenesis protein CcsA	0.697676316	0.001	Cytochrome complex assembly
54	ACT	Actin	0.697676316	0.188	Cytoplasmic streaming
55	YCF3	Photosystem I assembly protein Ycf3	0.704688094	0.218	Chaperone-like assembly of PSI subunits
56	DAAF3	Dynein assembly factor 3, axonemal	0.71177032	0.098	Axonemal dynein complex assembly
57	KLP1	Kinesin-like protein KLP1	0.718923724	0.434	Microtubule-based movement
58	ALFC	Fructose-bisphosphate aldolase 1	0.726149042	0.181	Glycolytic process
59	LHR31	LHC stress-related protein 3.1	0.726149042	0.240	Nonphotochemical quenching
60	DYI2	Dynein, 78 kDa intermediate chain	0.726149042	0.021	Motor activity, Arm assembly
61	ATPB	ATP synthase subunit beta, chloroplastic	0.726149042	0.181	ATP synthesis coupled proton transport
62	YCX3	14.4 kDa protein in 16S rRNA	0.733446954	0.504	Determine phylogeny
63	RSP3	Flagellar radial spoke protein 3	0.733446954	0.030	Protein localization to organelle
64	CFA61	Cilia- and flagella-associated protein 61	0.733446954	0.123	Cilium movement, Cell organization
65	DRC2	Dynein regulatory complex subunit 2	0.733446954	0.461	Axonemal dynein complex assembly
66	EFTU	Elongation factor Tu, chloroplastic	0.740818212	0.236	Translational elongation
67	IFT74	Intraflagellar transport protein 74	0.740818212	0.603	Cilium assembly
68	HSP70	Heat shock 70 kDa protein	0.740818212	0.039	Protein maturation and degradation
69	TBA2	Tubulin alpha-2 chain	0.748263574	0.348	Alters the microtubule cytoskeleton
70	TBA1	Tubulin alpha-1 chain	0.748263574	0.290	Microtubule-based process
71	DAAF1	Leucine-rich repeat-containing protein	0.748263574	0.727	Cell motility and localization to organelle
72	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	0.748263574	0.141	Unsaturated and fatty acid biosynthetic
73	DRC4	Dynein regulatory complex subunit 4	0.748263574	0.508	Axonemal dynein complex assembly
74	FEN1	Flap endonuclease 1	0.755783741	0.314	DNA replication, Base-excision repair
75	GSA	Glutamate semialdehyde aminomutase	0.755783741	0.869	Chlorophyll biosynthetic process
76	G3PA	G3P-dehydrogenase A, chloroplastic	0.755783741	0.713	Glucose metabolic process
77	RL13	60S ribosomal protein L13	0.755783741	0.495	Translation
78	RSP2	Flagellar radial spoke protein 2	0.763379486	0.028	Flagellar bending
79	DRC9	Dynein regulatory complex protein 9	0.763379486	0.373	Regulator of ciliary/flagellar motility
80	PHOT	Phototropin	0.763379486	0.696	Blue light signalling, NPQ induction
81	CCD39	Coiled-coil domain-containing protein 39	0.771051593	0.592	Cilium movement
82	PCDP1	Cilia- and flagella-associated protein 221	0.771051593	0.170	Cilium assembly and movement
83	IFT46	Intraflagellar transport protein 46	0.771051593	0.013	Cilium-dependent cell motility
84	CFA20	Cilia- and flagella-associated protein 20	0.771051593	0.094	Axoneme, Cilium assembly
85	RCA	RuBisCO/oxygenase activase, chloroplastic	0.778800783	0.420	Activation of RuBisCO
86	PSAF	PSI RC subunit III, chloroplastic	0.786627865	0.069	Electron transfer PC to P700
87	1433	14-3-3-like protein	0.794533599	0.155	Flagellar arrangement and assembly
88		Dynein regulatory complex subunit 7	0.802518799	0.325	Regulation of flagellar motility
89	YCF78	membrane protein ycf78	0.802518799	0.538	Cell growth
90	MOC1	Holliday junction resolvase, chloroplastic	0.802518799	0.340	DNA recombination
91	RR9	30S ribosomal protein S9, chloroplastic	0.810584251	0.308	Translation
92	RPOB1	DNA-directed RNA polymerase beta	0.810584251	0.385	Transcription, DNA-templated
93	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	0.818730751	0.005	Protein urmylation
94	NU4M	NADH-ubiquinone oxidoreductase chain 4	0.818730751	0.017	Electrons NADH to the respiratory chain
95	PLAS	Plastocyanin, chloroplastic	0.835270205	0.827	Electron transfer P700 and cyt b6f
96	RK20	50S ribosomal protein L20, chloroplastic	0.835270205	0.461	Translation
97	POC16	Centriole proteome protein 16	0.843664815	0.350	Flagellum assembly and maintenance
98	IFT22	Intraflagellar transport protein 22	0.843664815	0.776	Cellular availability of IFT particles
99	PLD6	Mitochondrial cardiolipin hydrolase	0.843664815	0.224	Lipid catabolic process
100	RR3	30S ribosomal protein S3, chloroplastic	0.843664815	0.281	Translation
101	ATPE	ATP synthase epsilon chain, chloroplastic	0.843664815	0.243	ATP synthesis coupled proton transport
102	RUBA	RuBisCO large subunit-alpha, chloroplastic	0.860707971	0.199	Assembly of the enzyme oligomer
103	CF157	Cilia- and flagella-associated protein 157	0.860707971	0.016	Cilium movement, Cell motility
104	UCRIA	Cyt b6-f complex Fe-S subunit	0.860707971	0.320	Mediates LET and CET around PSI
105	ARLY	Argininosuccinate lyase	0.860707971	0.014	Arginine biosynthetic via ornithine
106	RAA2	Trans-splicing factor Raa2, chloroplastic	0.860707971	0.469	RNA splicing, mRNA processing
107	CYF	Cytochrome f	0.860707971	0.211	CEF around PSI
108	RSP1	Flagellar radial spoke protein 1	0.869358235	0.831	Flagellar bending
109	DRC3	Dynein regulatory complex subunit 3	0.869358235	0.357	Cilium-dependent cell motility
110	CFA43	Cilia- and flagella-associated protein 43	0.869358235	0.588	Flagellum organization and function
111	LHR32	LHC stress-related protein 3.2	0.869358235	0.192	Nonphotochemical quenching
112	PSBS2	Photosystem II protein PSBS2	0.869358235	0.649	Nonphotochemical quenching
113	PETO	Cyt b6f complex subunit, chloroplastic	0.869358235	0.256	LET between PSII and I, CET around PSI
114	UFM1	Ubiquitin-fold modifier 1	0.878095435	0.012	Protein ufmylation
115	POR	Protochlorophyllide reductase	0.878095435	0.602	Chlorophyll biosynthetic process
116	TOC34I	Translocase of chloroplast 34, chloroplastic	0.878095435	0.058	Protein from the cytoplasm to chloroplast

117	PSBD	Photosystem II D2 protein	0.886920439	0.053	Photosynthetic, Assembly of PSII complex
118	IF172	Intraflagellar transport protein 172	0.886920439	0.369	Multicellular organism development
119	DRC10	Dynein regulatory complex protein 10	0.886920439	0.102	Regulates sliding in axonemes
120	PSAD	PSI RC subunit II, chloroplastic	0.886920439	0.183	Ferredoxin-docking protein
121	SUVH3	Histone-lysine N-methyltransferase	0.886920439	0.698	Repressed chromatin functions
122	RPOA	DNA-directed RNA polymerase alpha	0.886920439	0.255	Transcription, DNA-templated
123	HAP2	Hapless 2	0.886920439	0.194	Protein insertion into the membrane
124	BOP1	Ribosome biogenesis protein homolog	0.895834136	0.116	Maturation of 5.8S rRNA
125	CLPP	ATP-dependent Clp protease proteolytic	0.895834136	0.106	chymotrypsin-control misfolded proteins
126	DRC1	Dynein regulatory complex protein 1	0.895834136	0.033	Dynein complex assembly
127	KPPR	Phosphoribulokinase, chloroplastic	0.904837417	0.020	Reductive pentose-phosphate cycle
128	RPC1A	DNA-directed RNA polymerase beta	0.904837417	0.958	Transcription by RNA polymerase II
129	ASNA1	ATPase ARSA1	0.904837417	0.461	ATPase activity
130	YCX2	Protein in 16S rRNA region	0.913931182	0.174	Phylogenetic relationship
131	DYL3	Dynein 18 kDa light chain	0.913931182	0.013	Motor activity, Calcium ion binding
132	CAPP2	Phosphoenolpyruvate carboxylase 2	0.913931182	0.116	Carbon fixation, Tricarboxylic acid cycle
133	POB15	Proteome of basal body protein 15	0.923116348	0.089	Motility and sensing stimuli
134	CFA58	Cilia- and flagella-associated protein 58	0.923116348	0.248	Cilium-dependent cell motility
135	DRC5	Dynein regulatory complex subunit 5	0.923116348	0.196	Ciliary/flagellar motility
136	ARL13	ADP-ribosylation factor-like protein 13B	0.923116348	0.582	Control ciliary axoneme structure
137	CAO	Chlorophyllide a oxygenase, chloroplastic	0.923116348	0.097	Chlorophyll b biosynthetic process
138	CFA52	Cilia- and flagella-associated protein 52	0.93239382	0.112	Cell motility
139	CFA65	Cilia- and flagella-associated protein 65	0.93239382	0.423	Cell projection organization
140	PF20	Flagellar WD repeat-containing Pf20	0.93239382	0.425	Inter-microtubule bridges in flagella
141	PURA	Adenylosuccinate synthetase, chloroplastic	0.951229424	0.030	"de novo" AMP biosynthetic process
142	LPAT	Acyl-sn-glycerol-phosphate acyltransferase	0.951229424	0.228	CDP-diacylglycerol biosynthetic process
143	FLA10	Kinesin-like protein FLA10	0.951229424	0.422	Intraciliary anterograde transport
144	YPTC4	GTP-binding protein YPTC4	0.96078944	0.254	Protein transport and vesicular traffic
145	TBC2	Tbc2 translation factor, chloroplastic	0.970445534	0.062	PsbC mRNA for translation initiation
146	METE	Homocysteine methyltransferase	0.970445534	0.518	Methionine biosynthetic process
147	ARS	Arylsulfatase	0.980198674	0.100	Phenol-containing metabolic process
148	STT7	Serine/threonine- kinase, chloroplastic	0.980198674	0.864	State transition
149	PSAH	Photosystem I RC subunit VI, chloroplastic	0.990049834	0.287	Photosynthesis, Docking for LHC I
150	ARF1	ADP-ribosylation factor 1	0.990049834	0.105	Vesicle-mediated transport
151	CFA99	Cilia- and flagella-associated protein 99	1	0.777	Ciliary/flagellar motility
152	LHSR1	LHC stress-related protein 1, chloroplastic	1	0.163	Non-photochemical quenching
153	RLA1	60S acidic ribosomal protein P1	1	0.141	Translational elongation
154	CAPP1	Phosphoenolpyruvate carboxylase 1	1	0.122	Carboxylation of PEP into oxaloacetate
155	CFA73	Cilia- and flagella-associated protein 73	1	0.389	Regulation of microtubule motor activity
156	ATPG	ATP synthase gamma chain, chloroplastic	1.010050167	0.003	ATP synthesis coupled proton transport
157	RR11	30S ribosomal protein S11, chloroplastic	1.010050167	0.085	Translation
158	HEM2	Aminolevulinic dehydratase, chloroplastic	1.02020134	0.561	Chlorophyll biosynthetic process
159	CHLN	Light-independent protochlorophyllide	1.030454533	0.360	Light-independent chl biosynthetic
160	RPC2B	DNA-directed RNA polymerase beta	1.030454533	0.304	Transcription, DNA-templated
161	PSBP	Oxygen-evolving enhancer 2, chloroplastic	1.030454533	0.004	Photosynthesis, Oxygen evolution
162	RR7	30S ribosomal protein S7, chloroplastic	1.040810773	0.319	Translation
163	SAC1	Putative sulfur deprivation regulator	1.040810773	0.414	Potassium ion transport
164	CFA44	Cilia- and flagella-associated protein 44	1.040810773	0.138	Cilium-dependent cell motility
165	G3PC	G3P dehydrogenase, cytosolic	1.051271097	0.234	Carbohydrate metabolism
166	ENO	Enolase (Fragment)	1.061836545	0.334	Glycolytic process
167	CHLB	Light-independent protochlorophyllide	1.061836545	0.372	Light-independent chl biosynthetic
168	HSTC	Homogentisate solanesyltransferase	1.061836545	0.007	Carotenoid, Plastoquinone biosynthetic
169	ASNA2	ATPase ARSA2	1.072508182	0.596	Tail-anchored insertion ER membrane
170	DRC6	Dynein regulatory complex subunit 6	1.072508182	0.339	Regulator of ciliary/flagellar motility
171	RBS2	RuBP carboxylase small chain 2	1.083287066	0.894	Photosynthesis, Carbon fixation
172	TRXH	Thioredoxin H-type	1.083287066	0.188	Cell redox homeostasis
173	TRXM	Thioredoxin M-type, chloroplastic	1.094174288	0.026	Cell redox homeostasis
174	TBG	Tubulin gamma chain	1.094174288	0.119	Cytoplasmic microtubule organization
175	BSD2	BUNDLE SHEATH DEFECTIVE 2	1.094174288	0.711	Chaperone-mediated protein folding
176	RBL	RuBisCO carboxylase large chain	1.094174288	0.029	Carboxylation of RuBP in PPP
177	SLT3	Probable sodium/sulfate cotransporter 3	1.10517092	0.106	Sulfate transport
178	DIP13	13 kDa deflagellation-inducible protein	1.10517092	0.059	Protein stability
179	RK2	50S ribosomal protein L2, chloroplastic	1.11627807	0.727	Mitochondrial translation
180	PESC	Pescadillo homolog	1.127496849	0.809	Maturation of 5.8S rRNA
181	NAC2	PsbD mRNA maturation factor	1.127496849	0.648	mRNA processing- 5' UTR of psbD mRNA

182	CCD40	Coiled-coil domain-containing protein 40	1.127496849	0.389	Assembly of dynein regulatory complex
183	PFL	Formate acetyltransferase	1.127496849	0.014	Form pyruvate and coA
184	SFAS	SF-assemblin	1.1502738	0.100	Striated microtubule-associated assembly
185	CAH1	Carbonic anhydrase 1	1.1502738	0.330	Carbonate dehydratase activity
186	CIAO1	Probable cytosolic Fe -S protein assembly	1.1502738	0.392	Required iron-sulfur cluster assembly
187	SULP2	Sulfate permease 2, chloroplastic	1.16183425	0.944	Sulfate transport
188	CFA70	Cilia- and flagella-associated protein 70	1.16183425	0.887	Axoneme- regulates ciliary motility
189	PETD	Cytochrome b6-f complex subunit 4	1.16183425	0.004	Transferring electrons within the CET
190	GLNA2	Glutamine synthetase, chloroplastic	1.173510867	0.171	Glutamine biosynthetic process
191	CFA74	Cilia- and flagella-associated protein 74	1.173510867	0.327	Cilium movement, Cell motility
192	CFA74	Dynein 14 kDa light chain	1.173510867	0.174	Outer dynein arm assembly
193	CFA77	Cilia- and flagella-associated protein 77	1.173510867	0.722	Cell motility
194	CAH2	Carbonic anhydrase 2	1.173510867	0.253	CCM, Carbonate dehydratase activity
195	CHLB	Light-independent protochlorophyllide	1.173510867	0.379	Light-independent chl biosynthetic
196	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	1.185304853	0.369	Photosynthesis
197	PSAE	PSI RC subunit IV, chloroplastic	1.185304853	0.241	Photosynthesis
198		Light-independent protochlorophyllide	1.185304853	0.004	Light-independent chlorophyll synthesis
199	MDHM	Malate dehydrogenase, mitochondrial	1.197217372	0.099	Carbohydrate metabolic process
200	GPX1	Glutathione peroxidase 1, mitochondrial	1.197217372	0.104	Arachidonic acid metabolic process
201	CFA53	Cilia- and flagella-associated protein 53	1.197217372	0.727	Cilium assembly and movement
202	CALR	Calreticulin	1.197217372	0.011	Protein folding
203	ANM1	Protein arginine N-methyltransferase 1	1.209249595	0.740	Asymmetric dimethylation of a flagellum
204	SLT1	Sodium/sulfate cotransporter 1	1.209249595	0.989	Potassium ion transport
205	GLNA1	Glutamine synthetase cytosolic isozyme	1.221402762	0.370	Glutamine biosynthetic process
206	YPTC1	GTP-binding protein YPTC1	1.221402762	0.698	Protein transport, vesicular traffic
207	ATPBM	ATP synthase subunit beta, mitochondrial	1.221402762	0.022	ATP synthesis coupled proton transport
208	RL12	60S ribosomal protein L12	1.233678052	0.237	Translation
209	LIAS	Lipoyl synthase, mitochondrial	1.246076729	0.230	Lipoylation peptidyl-N6-lipoyl-L-lysine.
210	RSP5	Flagellar radial spoke protein 5	1.258600015	0.010	Regulation of flagellar bending
211	EZY1	Ezy-1 protein	1.258600015	0.384	Uncharacterized protein
212	GLE	Autolysin	1.258600015	0.225	Cell wall organization
213	RSP14	Radial spoke protein 14	1.271249144	0.046	Cell motility, Endocytosis
214	RSP4	Flagellar radial spoke protein 4	1.271249144	0.272	Cell motility, cilium assembly
215	SLT2	Sodium/sulfate cotransporter 2	1.284025417	0.288	Potassium ion transport
216	CHLI	Magnesium-chelatase subunit	1.296930074	0.280	Insertion of Mg into protoporphyrin ring
217	IFT81	Intraflagellar transport protein 81	1.309964465	0.268	Transport of tubulin within the cilium
218	H4	Histone H4	1.309964465	0.218	Nucleosome remodelling
219	FENR	Ferredoxin--NADP reductase, chloroplastic	1.309964465	0.102	Regulating the cyclic and non-CEF
220	IFT56	Intraflagellar transport protein 56	1.323129814	0.479	Intraciliary transport
221	DC2L	Cytoplasmic dynein 2 intermediate chain 1	1.323129814	0.152	Regulates flagellar dynein activity
222	DAW1	Dynein assembly WDR repeat domains 1	1.336427477	0.053	Intraciliary transport and assembly
223	CFA91	Cilia- and flagella-associated protein 91	1.336427477	0.681	Cilium movement
224	RBS1	RuBisCO small chain 1, chloroplastic	1.336427477	0.100	Photosynthesis, Photorespiration
225	MBB1	PsbB mRNA maturation factor	1.349858824	0.802	mRNA processing via 5'-UTR of psbB
226	LISC	Lipoyl synthase, chloroplastic	1.377127754	0.040	Protein lipoylation
227	CTH1	Mg-protoporphyrin IX monomethyl ester	1.377127754	0.008	Photosynthesis, chlorophyll biosynthesis
228	RSSA	40S ribosomal protein SA	1.377127754	0.062	Translation
229	CFA45	Cilia- and flagella-associated protein 45	1.404947596	0.052	Cell motility
230	YPTC5	GTP-binding protein YPTC5	1.404947596	0.237	GTPase activity, protein transport
231	CF251	Cilia- and flagella-associated protein 251	1.404947596	0.406	Cilium movement
232	RS14	40S ribosomal protein S14	1.404947596	0.012	Translation
233	SODM	Superoxide dismutase [Mn], mitochondrial	1.41906754	0.624	Destroys superoxide anion radicals
234	URM1	Ubiquitin-related modifier 1 homolog	1.41906754	0.009	Endonuclease activity, Intron homing
235	RS3A	40S ribosomal protein S3a	1.433329435	0.871	Translation
236	MUT11	Protein Mut11	1.433329435	0.019	Involved in 'Lys-4' histone H3 methylation
237	TEKT	Tektin	1.433329435	0.478	Assembly of flagella and cilia
238	RPOB2	DNA-directed RNA polymerase beta	1.433329435	0.327	Transcription, DNA-templated
239	DYI3	Dynein, 70 kDa intermediate chain	1.476980773	0.502	Cilium movement
240	IFT57	Intraflagellar transport protein 57	1.476980773	0.561	Maintenance and formation of cilia
241	CP12	Calvin cycle protein CP12, chloroplastic	1.476980773	0.024	Assembly of a core complex PRK/GAPDH
242	KTU	Protein kintoun	1.50681778	0.510	Axonemal dynein complex assembly
243	ODA1	Outer dynein arm protein 1	1.50681778	0.309	Cilium movement involved in cell motility
244	RTCB	RNA-splicing ligase RtcB homolog	1.50681778	0.442	tRNA splicing
245	GBLP	Guanine nucleotide- protein subunit beta	1.521961536	0.590	Transcription initiation and termination
246	PSBS1	Photosystem II protein PSBS1	1.537257535	0.330	Nonphotochemical quenching

247	NU5M	NADH-ubiquinone oxidoreductase chain 5	1.568312167	0.039	ATP synthesis
248	PSAB	PSI P700 chlorophyll a apoprotein A2	1.599994191	0.019	Primary electron donor of PSI
249	PSBB	Photosystem II CP47 RC protein	1.648721271	0.110	PET in photosystem II
250	SULP1	Sulfate permease 1, chloroplastic	1.648721271	0.081	Uptake and assimilation of sulfate
251	RSP6	Flagellar radial spoke protein 6	1.665291179	0.262	Cilium assembly
252	CP100	Cilia- and flagella-associated protein 100	1.750672504	0.464	Cell motility, Cilium movement
253	ATPX	ATP synthase subunit b', chloroplastic	1.768267039	0.008	ATP synthesis coupled proton transport
254	CALM	Calmodulin	1.768267039	0.351	Calcium-mediated signalling
255	PSBA	Photosystem II protein D1	1.840431425	0.003	Photosynthetic electron transport in PSII
256	WDR12	Ribosome biogenesis protein	1.840431425	0.471	LSU-rRNA from tricistronic rRNA
257	CATR	Caltractin	1.840431425	0.730	Cell cycle, Cell division
258	PSBC	PS II CP43 reaction center protein	1.858928051	0.006	PET in photosystem II
259	YPTC6	Ras-related protein YPTC6	1.993715528	0.265	GTPase activity
		Up-regulated proteins (FC ≥ 2.0)			
260	ALB32	ALBINO3-like protein 2, chloroplastic	2.095935534	0.818	Assembly and activity of LHC I and II.
261	H31	Histone H3 type 1	2.117000017	0.732	DNA binding, Chromosome stability
262	DRC8	Dynein regulatory complex protein 8	2.159766213	0.572	Regulates microtubule sliding
263	RK22	50S ribosomal protein L22, chloroplastic	2.203396474	0.034	Translation, Response to antibiotic
264	PSBR	Photosystem II protein PSBR, chloroplastic	2.944679677	0.862	Energy-dependent quenching-NPQ
265	HS22C	Heat shock 22 kDa protein, chloroplastic	3.455613498	0.001	Protein maturation and degradation
266	SC61B	Protein transport protein Sec61 beta	4.903749092	0.009	Intracellular protein transport
267	CYB6	Cytochrome b6	7.53832479	0.365	CEF around PSI and state transitions.
268	ATPD	ATP synthase delta chain, chloroplastic	69.40783595	0.048	ATP synthesis coupled proton transport
		Control specific protein			
269	RK16	50S ribosomal protein L16, chloroplastic	Control_W50		Translation
270	RK16	50S ribosomal protein L16, chloroplastic	Control_W50		Mitochondrial translation
271	FER	Ferredoxin, chloroplastic	Control_W50		electron transfer activity
272	RR19	30S ribosomal protein S19, chloroplastic	Control_W50		Translation
273	PSBE	Cytochrome b559 subunit alpha	Control_W50		PETC, Charge separation
274	RTL	Reverse transcriptase-like protein	Control_W50		cDNA synthesis
275	RL31	60S ribosomal protein L31	Control_W50		Translation, Ribosomal assembly
276	CYC	Cytochrome c	Control_W50		Final protein carrier in mitochondrial ETC
277	PETM	Cyt b6f complex subunit 7, chloroplastic	Control_W50		CEF around PSI and state transitions.
278	DYL2	Dynein 11 kDa light chain	Control_W50		Microtubule-based process
279	FLTOP	Protein Flattop homolog	Control_W50		Regulator of cilium basal body docking
280	YCX5	Uncharacterized trnR-chlB intergenic	Control_W50		Tag-protein stability
281	ATPF	ATP synthase subunit b, chloroplastic	Control_W50		ATP synthesis coupled proton transport
282	RK5	50S ribosomal protein L5, chloroplastic	Control_W50		Translation
		Treated specific protein			
283	UFM1	Ubiquitin-fold modifier 1	Treated_P5_500		Protein ufmylation
284	RR8	30S ribosomal protein S8, chloroplastic	Treated_P5_500		Translation
285	rpl36	50S ribosomal protein L36, chloroplastic	Treated_P5_500		Translation
286	MOC2A	Molybdopterin synthase sulfur subunit	Treated_P5_500		Mo-molybdopterin cofactor biosynthetic
287	H2A-II	Histone H2A	Treated_P5_500		Chromosomal stability
288	LI637	Group 1 truncated hemoglobin LI637	Treated_P5_500		Oxygen carrier activity
289	ARL3	ADP-ribosylation factor-like protein 3	Treated_P5_500		Cytokinesis and cilia signalling
290	GP1	Vegetative cell wall protein gp1	Treated_P5_500		Major component of outer cell wall W6
291	ND2	NADH-ubiquinone oxidoreductase chain 2	Treated_P5_500		NADH dehydrogenase activity
292	PSBL	Photosystem II reaction center protein L	Treated_P5_500		Photosynthesis, Correct PSII assembly
293	TBB	Tubulin beta-1/beta-2 chain	Treated_P5_500		Microtubule-based process
294	YCF4	Photosystem I assembly protein Ycf4	Treated_P5_500		Assembly of the photosystem I complex

Table 5.4 List all proteins expressed in *pgrl1_500* vs *pgrl1_50* (treated vs control) of *C. reinhardtii*

S.No.	Abbreviation	Description	Fold Change	p-value	GO Description
		Down-regulated protein (FC ≤ 0.5)			
1	PSBE	Cytochrome b559 subunit alpha	0.298197268	0.953	PETC, Charge separation
2	NU1M	NADH-ubiquinone oxidoreductase chain 1	0.418951547	0.453	NADH dehydrogenase activity
3	DYL1	Dynein 8 kDa light chain, flagellar outer arm	0.467666431	0.285	Microtubule-based process
4	LPAT	acyl-glycerol-phosphate acyltransferase	0.50157607	0.529	CDP-diacylglycerol biosynthetic
		Differential regulated protein (≤ 2.0 FC ≥ 0.5)			
5	RR2B	Putative 30S ribosomal S2-like protein	0.511708569	0.244	Transferase activity
6	TRXM	Thioredoxin M-type, chloroplastic	0.522045789	0.491	Cell redox homeostasis
7	SULP1	Sulfate permease 1, chloroplastic	0.532591804	0.249	Uptake and assimilation of sulfate
8	CALM	Calmodulin	0.543350861	0.276	Calcium-mediated signalling
9	IPYR1	Soluble inorganic pyrophosphatase 1	0.548811623	0.615	Phosphate-metabolic process
10	PETD	Cytochrome b6-f complex subunit 4	0.554327299	0.240	Transferring electrons within the CET
11	RR18	30S ribosomal protein S18, chloroplastic	0.559898376	0.579	Translation
12	YCX5	Uncharacterized trnR-chlB intergenic region	0.571209062	0.047	Tag-protein stability
13	DAW1	Dynein assembly WDR repeat domains 1	0.576949804	0.169	Intraciliary transport and assembly
14	SAC1	Sulfur deprivation response regulator	0.576949804	0.022	Potassium ion transport
15	SULT2	Proton/sulfate cotransporter 2	0.58274824	0.339	Sulfate transporter activity
16	PSBK	Photosystem II reaction center protein K	0.588604987	0.050	Photosynthesis
17	RBS2	RuBP carboxylase small chain 2, chloroplastic	0.60653066	0.569	Photosynthesis, Carbon fixation
18	MDHM	Malate dehydrogenase, mitochondrial	0.618783398	0.464	Carbohydrate metabolic process
19	RL11	60S ribosomal protein L11	0.625002269	0.789	Translation
20	GP1	Vegetative cell wall protein gp1	0.63128364	0.809	Major component of cell wall W6 layer
21	RL401	Ubiquitin-60S ribosomal protein L40	0.637628159	0.508	Translation
22	CB29	Chlorophyll a-b binding protein CP29	0.65050909	0.789	LHC Facilitates the State 1 to State 2
23	RSP4	Flagellar radial spoke protein 4	0.65050909	0.497	Cell motility, cilium assembly
24	RK16	50S ribosomal protein L16, chloroplastic	0.657046828	0.076	Mitochondrial translation
25	RBS1	RuBisCO small chain 1, chloroplastic	0.657046828	0.723	Photosynthesis, Photorespiration
26	PSBP	Oxygen-evolving enhancer 2, chloroplastic	0.657046828	0.331	Photosynthesis, Oxygen evolution
27	RSP3	Flagellar radial spoke protein 3	0.677056884	0.743	Cilium-dependent cell motility
28	UCRIA	Cyt b6-f complex Fe-S subunit, chloroplastic	0.677056884	0.876	Mediates LET and CET around PSI
29	POB15	Proteome of basal body protein 15	0.690734327	0.273	Motility and sensing stimuli
30	PSAE	PSI RC subunit IV, chloroplastic	0.690734327	0.016	Photosynthesis
31	PHOT	Phototropin	0.690734327	0.137	Blue light pathway, NPQ induction
32	H32	Histone H3 type 3	0.697676316	0.858	Nucleosome remodelling
33	RL403	Ubiquitin-60S ribosomal protein L40	0.704688094	0.397	Translation
34	RSP1	Flagellar radial spoke protein 1	0.704688094	0.346	Flagellar bending
35	G3PC	G3P dehydrogenase, cytosolic	0.704688094	0.295	Carbohydrate metabolism
36	CALR	Calreticulin	0.704688094	0.331	Protein folding
37	GLNA2	Glutamine synthetase, chloroplastic	0.71177032	0.978	Glutamine biosynthetic process
38	DYL2	Dynein 11 kDa light chain	0.71177032	0.225	Microtubule-based process
39	H2B2	Histone H2B.2	0.718923724	0.744	Nucleosome remodelling
40	RPC2B	DNA-directed RNA polymerase subunit beta	0.718923724	0.419	Transcription, DNA-templated
41	DNE1	DNA endonuclease I-Crel	0.726149042	0.955	Endonuclease activity, Intron homing
42	PSBN	Protein PsbN	0.726149042	0.228	Role in PSI and II biogenesis
43	SFAS	SF-assemblin	0.726149042	0.683	Striated microtubule fibres assembly
44	CYC6	Cytochrome c6, chloroplastic	0.733446954	0.635	Electron carrier between cyt b6f and PSI
45	PLD6	Mitochondrial cardiolipin hydrolase	0.740818212	0.173	Lipid catabolic process
46	RR11	30S ribosomal protein S11, chloroplastic	0.740818212	0.895	Translation
47	CFA77	Cilia- and flagella-associated protein	0.740818212	0.058	Cell motility
48	1433	14-3-3-like protein	0.740818212	0.879	Flagellar arrangement and assembly
49	RK23	50S ribosomal protein L23, chloroplastic	0.740818212	0.301	Translation
50	EZY1	Ezy-1 protein	0.748263574	0.413	Uncharacterized protein
51	CFA61	Cilia- and flagella-associated protein 61	0.748263574	0.363	Cilium movement, Cilium organization
52	FENR	Ferredoxin-NADP reductase, chloroplastic	0.755783741	0.052	Regulating the cyclic and non-CEF
53	CFA20	Cilia- and flagella-associated protein 20	0.755783741	0.715	Axoneme, Cilium assembly

54	HS22C	Heat shock 22 kDa protein, chloroplastic	0.763379486	0.305	Protein maturation and degradation
55	PSAC	Photosystem I iron-sulfur center	0.763379486	0.144	Required for binding of PsaD, PsaE to PSI
56	RK2	50S ribosomal protein L2, chloroplastic	0.763379486	0.532	Mitochondrial translation
57	DAAF1	Leucine-rich repeat- protein ODA7	0.771051593	0.537	Cell motility and localization to organelle
58	GPX1	Glutathione peroxidase 1, mitochondrial	0.771051593	0.230	Arachidonic acid metabolic process
59	RS27	40S ribosomal protein S27	0.771051593	0.940	Microtubule-based process
60	PSBJ	Photosystem II reaction center protein J	0.778800783	0.680	Photosynthesis, Charge separation
61	CFA70	Cilia- and flagella-associated protein 70	0.778800783	0.051	Axoneme-protein regulates ciliary motility
62	GLE	Autolysin	0.786627865	0.126	Cell wall organization
63	YCF3	Photosystem I assembly protein Ycf3	0.786627865	0.138	Chaperone-like assembly of PSI subunits
64	CP100	Cilia-flagella-associated protein 100	0.794533599	0.402	Cell motility, Cilium movement
65	NU4M	NADH-ubiquinone oxidoreductase 4	0.794533599	0.428	Electrons from NADH to respiratory chain
66	PSAA	PSI P700 chlorophyll a apoprotein A1	0.794533599	0.263	Photosynthesis
67	RS14	40S ribosomal protein S14	0.794533599	0.842	Translation
68	CMD1	5-methylcytosine- enzyme 1	0.802518799	0.101	5-methylcytosine catabolic process
69	RSP6	Flagellar radial spoke protein 6	0.802518799	0.084	Cilium assembly
70	SLT3	Probable sodium/sulfate cotransporter 3	0.802518799	0.231	Potassium ion transport, Sulfate transport
71	ANM1	Protein arginine N-methyltransferase 1	0.810584251	0.341	Asymmetric dimethylation of a flagellum
72	DES	Acyl-lipid omega-13 desaturase	0.810584251	0.485	Linoleic acid metabolic process
73	RL10A	60S ribosomal protein L10a	0.810584251	0.586	Translation
74	TOC34I	Translocase of chloroplast 34, chloroplastic	0.810584251	0.790	Transport from cytoplasm to chloroplast
75	DRC4	Dynein regulatory complex subunit 4	0.810584251	0.008	Axonemal dynein complex assembly
76	DRC3	Dynein regulatory complex subunit 3	0.818730751	0.531	Cilium-dependent cell motility
77	KPPR	Phosphoribulokinase, chloroplastic	0.818730751	0.113	Reductive pentose-phosphate cycle
78	NU2M	NADH-ubiquinone oxidoreductase chain 2	0.818730751	0.843	NADH dehydrogenase activity
79	DRC11	Dynein regulatory complex protein 11	0.818730751	0.670	Regulator of ciliary/flagellar motility
80	IFT22	Intraflagellar transport protein 22	0.826959136	0.653	Cellular availability of IFT particles
81	DRC1	Dynein regulatory complex protein 1	0.826959136	0.649	Dynein complex assembly
82	CHLB	Light-independent protochlorophyllide	0.826959136	0.206	Light-independent chl biosynthesis
83	YCX1	12.2 kDa protein in 16S rRNA region	0.835270205	0.204	Conserved phylogeny
84	EZY2	Ezy-1 protein	0.835270205	0.987	Uncharacterized protein
85	IPYR2	Soluble inorganic pyrophosphatase 2	0.835270205	0.966	Phosphate-containing metabolism
86	SRGT1	Peptidyl serine alpha-galactosyltransferase	0.843664815	0.331	Transferase activity
87	ASNA2	ATPase ARSA2	0.843664815	0.241	Tail-anchored protein insertion into ER
88	PETO	Cyt b6f complex subunit petO, chloroplastic	0.843664815	0.055	LET between PSII and I, CET around PSI
89	DYI3	Dynein, 70 kDa, flagellar outer arm	0.852143792	0.039	Cilium movement involved in cell motility
90	HEM2	aminolevulinic acid dehydratase, chloroplastic	0.852143792	0.363	Chlorophyll biosynthetic process
91	CFA74	Cilia- and flagella-associated protein 74	0.852143792	0.226	Cilium movement, Cell motility
92	CAH1	Carbonic anhydrase 1	0.852143792	0.550	Carbonate dehydratase activity
93	RSSA	40S ribosomal protein SA	0.852143792	0.190	Translation
94	RTL	Reverse transcriptase-like protein	0.860707971	0.436	cDNA synthethesis
95	TBG	Tubulin gamma chain	0.860707971	0.579	Cytoplasmic microtubule organization
96	DRC9	Dynein regulatory complex protein 9	0.860707971	0.316	Regulator of ciliary/flagellar motility
97	RAA3	Trans-splicing factor Raa3, chloroplastic	0.860707971	0.262	mRNA processing, RNA splicing
98	CATR	Caltractin	0.860707971	0.543	Cell cycle, Cell division
99	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	0.869358235	0.705	Unsaturated and fatty acid biosynthetic
100	CIAO1	Cytosolic Fe-S protein assembly, CIAO1	0.869358235	0.232	Required for iron-sulfur cluster assembly
101	BOP1	Ribosome biogenesis protein BOP1 homolog	0.878095435	0.440	Maturation of 5.8S rRNA
102	ATPD	ATP synthase delta chain, chloroplastic	0.878095435	0.059	ATP synthesis coupled proton transport
103	METK	S-adenosylmethionine synthase	0.878095435	0.036	S-adenosylmethionine biosynthesis
104	STT7	Serine/threonine-protein kinase, chloroplastic	0.878095435	0.361	State transition
105	GSA	Glutamate-semialdehyde-aminomutase	0.878095435	0.057	Chlorophyll biosynthetic process
106	ARF1	ADP-ribosylation factor 1	0.878095435	0.155	Vesicle-mediated transport
107	ENO	Enolase	0.886920439	0.229	Glycolytic process
108	RAA2	Trans-splicing factor Raa2, chloroplastic	0.886920439	0.468	RNA splicing, mRNA processing
109	FEN1	Flap endonuclease 1	0.895834136	0.387	DNA replication, Base-excision repair
110	YPTC4	GTP-binding protein YPTC4	0.895834136	0.040	Protein transport and vesicular traffic
111	CCD40	Coiled-coil domain-containing protein 4	0.904837417	0.027	Assembly of dynein regulatory complex
112	H2A	40S ribosomal protein S3a	0.913931182	0.303	Translation
113	RR7	Histone H2A	0.913931182	0.516	Chromosomal stability
114	CF157	Cilia- and flagella-associated protein 157	0.913931182	0.046	Cilium movement, Cell motility
115	H32	Histone H3 type 2	0.913931182	0.852	Chromosomal stability.
116	RUBA	RuBisCO large subunit-alpha, chloroplastic	0.923116348	0.161	Assembly of the enzyme oligomer
117	RR3	30S ribosomal protein S3, chloroplastic	0.923116348	0.175	Translational
118	EZY3	Ezy-1 protein1	0.923116348	0.522	Uncharacterized protein

119	YCF78	membrane protein ycf78	0.923116348	0.191	Cell growth
120	CF300	Cilia- and flagella-associated protein 300	0.923116348	0.277	Axonemal organization and motility
121	IFT25	Intraflagellar transport protein 25	0.923116348	0.003	Intraciliary transport
122	RL13	60S ribosomal protein L13	0.923116348	0.836	Translation
123	DRC10	Dynein regulatory complex protein 10	0.93239382	0.287	Regulates microtubule sliding
124	CFA65	Cilia- and flagella-associated protein 65	0.93239382	0.407	Cell projection organization
125	CHLL	Light-independent protochlorophyllide	0.93239382	0.697	Photosynthesis, Dark reaction
126	ADT	ADP,ATP carrier protein	0.941764535	0.209	Catalyzes the exchange of ADP and ATP
127	ATPE	ATP synthase epsilon chain, chloroplastic	0.941764535	0.209	ATP synthesis coupled proton transport
128	POC16	Centriole proteome protein 16	0.951229424	0.948	Flagellum assembly and maintenance
129	UFM1	Ubiquitin-fold modifier 1	0.951229424	0.003	Protein ufmylation
130	RSP14	Radial spoke protein 14	0.951229424	0.143	Cell motility, Endocytosis
131	EFTU	Elongation factor Tu, chloroplastic	0.951229424	0.532	Translational elongation
132	ATPX	ATP synthase subunit b', chloroplastic	0.951229424	0.171	ATP synthesis coupled proton transport
133	EFTS	Elongation factor Ts, mitochondrial	0.951229424	0.210	Translation elongation factor activity
134	ARLY	Argininosuccinate lyase	0.951229424	0.356	Arginine biosynthesis via ornithine
135	WDR12	Ribosome biogenesis protein	0.951229424	0.151	LSU-rRNA from tricistronic rRNA transcript
136	DYL3	Dynein 18 kDa light chain	0.951229424	0.440	Motor activity, Calcium ion binding
137	ZYS3	Zygote-specific protein 3	0.951229424	0.034	Cell development
138	ALB3.2	ALBINO3-like protein 2, chloroplastic	0.96078944	0.410	Assembly and activity of LHC I and II.
139	SLT2	Sodium/sulfate cotransporter 2	0.96078944	0.021	Potassium ion transport
140	CFA43	Cilia- and flagella-associated protein 43	0.96078944	0.282	Flagellum organization and function
141	CTH1	Mg-protoporphyrin IX monomethyl ester	0.96078944	0.395	Photosynthesis, chlorophyll biosynthesis
142	RSP5	Flagellar radial spoke protein 5	0.970445534	0.324	Regulation of flagellar bending
143	CFA91	Cilia- and flagella-associated protein 91	0.970445534	0.407	Cilium movement
144	RL12	60S ribosomal protein L12	0.970445534	0.095	Translation
145	RSP2	Flagellar radial spoke protein 2	0.970445534	0.027	Flagellar bending
146	CFA45	Mg-protoporphyrin IX monomethyl ester,	0.970445534	0.016	Photosynthesis, Chl biosynthetic process
147	CYC	Cytochrome c	0.970445534	0.000	Final protein carrier in mitochondrial ETC
148	CFA73	Cilia- and flagella-associated protein 73	0.970445534	0.687	Regulation of microtubule motor activity
149	SLT1	Sodium/sulfate cotransporter 1	0.970445534	0.048	Potassium ion transport
150	PSBD	Photosystem II D2 protein	0.980198674	0.046	Photosynthetic, Assembly of PSII complex
151	CFA45	Cilia- and flagella-associated protein 45	0.980198674	0.016	Cell motility
152	PESC	Pescadillo homolog	0.980198674	0.181	Maturation of 5.8S rRNA
153	H2B1	Histone H2B.1	0.980198674	0.658	Protein heterodimerization activity
154	PCDP1	Cilia- and flagella-associated protein 221	0.980198674	0.572	Cilium assembly and movement
155	LHR32	LHC stress-related protein 3.2, chloroplastic	0.980198674	0.758	Nonphotochemical quenching
156	DYI2	Dynein, 78 kDa intermediate chain	0.980198674	0.811	Motor activity, Arm assembly
157	CP12	Calvin cycle protein CP12, chloroplastic	0.980198674	0.164	Assembly core complex of PRK/GAPDH
158	BSD2	BUNDLE SHEATH DEFECTIVE 2, chloroplastic	0.980198674	0.725	Chaperone-mediated protein folding
159	IFT56	Intraflagellar transport protein 56	0.990049834	0.897	Intraciliary transport
160	IFT74	Intraflagellar transport protein 74	0.990049834	0.570	Cilium assembly
161	DC2L	Cytoplasmic dynein 2 intermediate chain 1	0.990049834	0.335	Regulates flagellar dynein activity
162	TBB	Tubulin beta-1/beta-2 chain	0.990049834	0.550	Microtubule-based process
163	PSBS1	Photosystem II protein PSBS1	0.990049834	0.476	Nonphotochemical quenching
164	TBA2	Tubulin alpha-2 chain	1	0.079	Alters the microtubule cytoskeleton
165	PSBB	Photosystem II CP47 RC protein	1	0.282	PET in photosystem II
166	SULP2	Sulfate permease 2, chloroplastic	1	0.733	Sulfate transport
167	HSP70	Heat shock 70 kDa protein	1	0.081	Protein maturation and degradation
168	HSTC	Homogentisate solanesyltransferase	1	0.263	Carotenoid and PQ biosynthetic process
169	PSBS2	Photosystem II protein PSBS2	1	0.271	Nonphotochemical quenching
170	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	1.010050167	0.055	Photosynthesis
171	IFT81	Intraflagellar transport protein 81	1.010050167	0.993	Transport of tubulin within the cilium
172	CHLB	Light-independent protochlorophyllide	1.010050167	0.357	Light-independent chl biosynthesis
173	SUVH3	Histone-lysine N-methyltransferase	1.010050167	0.111	Repressed chromatin functions
174	ASNA1	ATPase ARSA1	1.010050167	0.328	ATPase activity
175	RR12	30S ribosomal protein S12, chloroplastic	1.02020134	0.618	Translation
176	KLP1	Kinesin-like protein KLP1	1.02020134	0.270	Microtubule-based movement
177	RR2A	30S ribosomal protein S2, chloroplastic	1.02020134	0.798	Translation
178	GLNA1	Glutamine synthetase cytosolic isozyme	1.030454533	0.446	Glutamine biosynthetic process
179	TRXH	Thioredoxin H-type	1.030454533	0.733	Cell redox homeostasis
180	PSBW	PSII reaction center W protein, chloroplastic	1.030454533	0.347	Stabilizes dimeric photosystem II
181	CAPP2	Phosphoenolpyruvate carboxylase 2	1.030454533	0.063	Carbon fixation, Tricarboxylic acid cycle
182	CAO	Chlorophyllide a oxygenase, chloroplastic	1.030454533	0.644	Chlorophyll b biosynthetic process
183	RPOB1	DNA-directed RNA polymerase subunit beta	1.030454533	0.265	Transcription, DNA-templated

184	PSAK	PSI reaction center subunit psaK, chloroplastic	1.040810773	0.149	Photosynthesis
185	RCA	RuBP carboxylase/oxygenase activase,	1.040810773	0.463	Activation of RuBisCO
186	RTCB	RNA-splicing ligase RtcB homolog	1.040810773	0.785	tRNA splicing
187	HAP2	Hapless 2	1.040810773	0.172	Protein insertion into the membrane
188	DRC8	Dynein regulatory complex protein 8	1.051271097	0.741	Regulates microtubule sliding
189	CCD39	Coiled-coil domain-containing protein 39	1.051271097	0.286	Inner dynein arm assembly
190	P0DO19	LHC stress-related protein 3.1, chloroplastic	1.051271097	0.758	Nonphotochemical quenching
191	RL44	60S ribosomal protein L44	1.051271097	0.654	Translation, Response to cycloheximide
192	CFA44	Cilia- and flagella-associated protein 44	1.051271097	0.185	Cilium-dependent cell motility
193	TBA1	Tubulin alpha-1 chain	1.061836545	0.114	Microtubule-based process
194	CFA58	Cilia- and flagella-associated protein 58	1.061836545	0.168	Cilium-dependent cell motility
195	RL31	60S ribosomal protein L31	1.061836545	0.041	Translation
196	FLA10	Kinesin-like protein FLA10	1.061836545	0.040	Intraciliary anterograde transport
197	FLA10	Light-independent protochlorophyllide	1.061836545	0.285	Light-independent chl biosynthesis
198	PSAD	PSI RC subunit II, chloroplastic	1.061836545	0.629	Ferredoxin-docking protein
199	ATPB	ATP synthase subunit beta, chloroplastic	1.061836545	0.418	ATP synthesis coupled proton transport
200	IDLC	Inner dynein arm light chain, axonemal	1.061836545	0.308	Cilium movement involved in cell motility
201	CEMA	Chloroplast envelope membrane protein	1.061836545	0.676	Proton transporter activity
202	RK5	50S ribosomal protein L5, chloroplastic	1.061836545	0.245	Translation
203	LISC	Lipoyl synthase, chloroplastic	1.072508182	0.223	Protein lipoylation
204	HSTC	Homocysteine methyltransferase	1.072508182	0.372	Methionine biosynthetic process
205	CYB6	Cytochrome b6	1.072508182	0.362	CEF around PSI and state transitions.
206	DRC2	Dynein regulatory complex subunit 2	1.072508182	0.102	Axonemal dynein complex assembly
207	CYF	Cytochrome f	1.072508182	0.079	CEF around PSI
208	DRC7	Dynein regulatory complex subunit 7	1.083287066	0.467	Regulation of flagellar motility
209	CAPP1	Phosphoenolpyruvate carboxylase 1	1.083287066	0.081	Carboxylation of PEP into oxaloacetate
210	YPTC5	GTP-binding protein YPTC5	1.083287066	0.332	GTPase activity, protein transport
211	DAAF3	Dynein assembly factor 3, axonemal homolog	1.094174288	0.372	Axonemal dynein complex assembly
212	PSBA	Photosystem II protein D1	1.094174288	0.222	Photosynthetic electron transport in PSII
213	TEKT	Tektin	1.094174288	0.576	Assembly of flagella and cilia
214	RPOB2	DNA-directed RNA polymerase subunit beta C	1.094174288	0.331	Transcription, DNA-templated
215	YCX3	14.4 kDa protein in 16S rRNA region	1.10517092	0.362	Determine phylogeny
216	IFT57	Intraflagellar transport protein 57	1.10517092	0.243	Maintenance and formation of cilia
217	COX1	Cytochrome c oxidase subunit 1	1.10517092	0.750	Oxidative phosphorylation
218	ATPA	ATP synthase subunit alpha, chloroplastic	1.10517092	0.530	ATP synthesis coupled proton transport
219	RBL	Ribulose biphosphate carboxylase large chain	1.11627807	0.244	Carboxylation RuBP in PPP
220	GBLP	Guanine nucleotide-binding protein beta	1.127496849	0.656	Transcription initiation and termination
221	YPTC6	Ras-related protein YPTC6	1.138828378	0.069	GTPase activity
222	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	1.138828378	0.219	Protein urmylation
223	PGKH	Phosphoglycerate kinase, chloroplastic	1.1502738	0.250	Glycolytic process, Reductive PPP
224	CLPP	ATP-dependent Clp protease subunit	1.1502738	0.343	chymotrypsin-control misfolded protein
225	RUBB	RuBisCO large subunit protein-beta-1	1.1502738	0.167	Assembly of the enzyme, Protein refolding
226	MOT1	Molybdate transporter 1	1.1502738	0.341	Mo-ion transporter activity
227	CCSA	Cytochrome c biogenesis protein CcsA	1.1502738	0.013	Cyt- complex assembly, heme transport
228	DRC6	Dynein regulatory complex subunit 6	1.1502738	0.544	Regulator of ciliary/flagellar motility
229	RK14	50S ribosomal protein L14, chloroplastic	1.1502738	0.346	Translation
230	NAC2	PsbD mRNA maturation factor, chloroplastic	1.16183425	0.146	mRNA processing- 5' UTR of psbD mRNA
231	RPC1A	DNA-directed RNA polymerase subunit beta	1.16183425	0.072	Transcription by RNA polymerase II
232	S17P	Sedoheptulose-1,7-bisphosphatase,	1.16183425	0.705	Starch and Sucrose biosynthetic process
233	CHLI	Magnesium-chelatase subunit, chloroplastic	1.173510867	0.133	Insertion of Mg into protoporphyrin ring
234	IF172	Intraflagellar transport protein 172	1.185304853	0.442	Multicellular organism development
235	PSAF	PSI reaction center subunit III, chloroplastic	1.185304853	0.157	Electron transfer from PC to P700
236	NRT22	Nitrate transporter 2.2	1.197217372	0.094	Nitrate transporter 2.2
237	PF20	Flagellar WD repeat-containing protein Pf20	1.197217372	0.412	Inter-microtubule bridges in flagella
238	PR46B	Coiled-coil domain-containing protein 103	1.209249595	0.089	Cilia motility
239	RUBC	RuBisCO large subunit- beta-2	1.209249595	0.212	RuBisCO assembly, Protein refolding,
240	ARL3	ADP-ribosylation factor-like protein 3	1.209249595	0.213	Cytokinesis and cilia signalling
241	RK22	50S ribosomal protein L22, chloroplastic	1.209249595	0.724	50S ribosomal protein L22, chloroplastic
242	CB2	Chl a-b binding of LHCII type I, chloroplastic	1.209249595	0.062	Light-harvesting as a light receptor
243	RR9	30S ribosomal protein S9, chloroplastic	1.221402762	0.548	Translation
244	NU5M	NADH-ubiquinone oxidoreductase chain 5	1.221402762	0.136	ATP synthesis
245	YPTC1	GTP-binding protein YPTC1	1.221402762	0.968	Protein transport, vesicular traffic
246	DNAL1	Dynein light chain 1, axonemal	1.221402762	0.867	Motor activity for flagellar motility
247	ALFC	Fructose-bisphosphate aldolase 1	1.246076729	0.140	Glycolytic process
248	IFT27	Intraflagellar transport protein 27	1.246076729	0.428	GTPase activity, Form IFT complex B

249	MOC1	Holliday junction resolvase, chloroplastic	1.246076729	0.962	DNA recombination
250	LHSR1	LHCstress-related protein 1, chloroplastic	1.258600015	0.096	Non-photochemical quenching
251	CCS1	Cytochrome c biogenesis, chloroplastic	1.258600015	0.146	Cytochrome complex assembly
252	CFA53	Cilia- and flagella-associated protein 53	1.258600015	0.216	Cilium assembly and movement
253	CFA99	Cilia- and flagella-associated protein 99	1.284025417	0.211	Ciliary/flagellar motility
254	PSAB	PSI P700 chlorophyll a apoprotein A2	1.284025417	0.092	Primery electron donor of PSI
255	CFA52	Cilia- and flagella-associated protein 52	1.284025417	0.220	Cell motility
256	RR4	30S ribosomal protein S4, chloroplastic	1.284025417	0.137	Translation
257	YCX2	Uncharacterized protein in 16S rRNA region	1.296930074	0.939	Phylogenetic relationship
258	PURA	Adenylosuccinate synthetase, chloroplastic	1.296930074	0.172	"de novo" AMP biosynthetic process
259	FER	Ferredoxin, chloroplastic	1.309964465	0.278	Iron-sulfur protein's metabolic reactions
260	ARS	Arylsulfatase	1.309964465	0.104	Phenol-containing metabolic process
261	POR	Protochlorophyllide reductase, chloroplastic	1.309964465	0.391	Chlorophyll biosynthetic process
262	ODA1	Outer dynein arm protein 1	1.309964465	0.236	Cilium movement for cell motility
263	THI4	Thiamine thiazole synthase, chloroplastic	1.323129814	0.000	Thiamine biosynthetic process
264	NRT21	Nitrate transporter 2.1	1.336427477	0.092	Nitrate assimilation
265	PSBO	Oxygen-evolving enhancer 1, chloroplastic	1.349858824	0.034	Primary site of water splitting
266	RPOA	DNA-directed RNA polymerase subunit alpha	1.363425117	0.220	Transcription, DNA-templated
267	YCF4	Photosystem I assembly protein Ycf4	1.363425117	0.283	Assembly of the PSI complex
268	KTU	Protein kintoun	1.377127754	0.271	Axonemal dynein complex assembly
269	IFT46	Intraflagellar transport protein 46	1.377127754	0.291	Cilium-dependent cell motility
270	YCX6	12.3 kDa petA-petD intergenic region	1.377127754	0.329	Chloroplast based function
271	PSBC	Photosystem II CP43 reaction center protein	1.390968147	0.149	PET in photosystem II
272	CF251	Cilia- and flagella-associated protein 251	1.390968147	0.357	Cilium movement
273	URM1	Ubiquitin-related modifier 1 homolog	1.404947596	0.200	Endonuclease activity, Intron homing
274	ATPBM	ATP synthase subunit beta, mitochondrial	1.41906754	0.174	ATP synthesis coupled proton transport
275	PSAH	Photosystem I RC subunit VI, chloroplastic	1.447734622	0.135	Photosynthesis, Docking for LHC I antenna
276	CAH2	Carbonic anhydrase 2	1.447734622	0.229	CCM, Carbonate dehydratase activity
277	TBC2	Tbc2 translation factor, chloroplastic	1.462284582	0.202	PsbC mRNA for translation initiation
278	H2B4	Histone H2B.4	1.462284582	0.013	Protein heterodimerization activity
279	LIAS	Lipoyl synthase, mitochondrial	1.462284582	0.366	Lipoylation peptidyl-N6-lipoyl-L-lysine.
280	ARL13	ADP-ribosylation factor-like protein 13B	1.476980773	0.192	Control ciliary axoneme structure
281	PSBR	Photosystem II protein PSBR, chloroplastic	1.476980773	0.063	Essential for energy-dependent quenching
282	DIP13	13 kDa deflagellation-inducible protein	1.476980773	0.082	Protein stability
283	MUT11	Protein Mut11	1.50681778	0.427	Involved in 'Lys-4' histone H3 methylation
284	ATPG	ATP synthase gamma chain, chloroplastic	1.521961536	0.349	ATP synthesis coupled proton transport
285	ALB31	ALBINO3-like protein 1, chloroplastic	1.521961536	0.286	Assembly and activity of LHC I and II
286	DRC5	Dynein regulatory complex subunit 5	1.568312167	0.062	Ciliary/flagellar motility
287	FLTOP	Protein Flattop homolog	1.584073998	0.219	Regulator of cilium basal body docking
288	PSAG	Photosystem I RC subunit V, chloroplastic	1.616074385	0.516	Photosynthesis
289	G3PA	G3P dehydrogenase A, chloroplastic	1.632316236	0.352	Glucose metabolic process
290	SC61B	Protein transport protein Sec61 subunit beta	1.648721271	0.080	Intracellular protein transport
291	RR14	30S ribosomal protein S14, chloroplastic	1.665291179	0.448	Translation
292	CYB	Cytochrome b	1.716006899	0.694	Respiratory electron transport chain
293	ACT	Actin	1.716006899	0.967	Cytoplasmic streaming
294	PFL	Formate acetyltransferase	1.716006899	0.232	Activity to form pyruvate and coA
295	ATPF	ATP synthase subunit b, chloroplastic	1.716006899	0.165	ATP synthesis coupled proton transport
296	IFT43	Intraflagellar transport protein 43	1.768267039	0.698	Cell projection organization
297	RLA1	60S acidic ribosomal protein P1	1.840431425	0.020	Translational elongation
298	RS18	40S ribosomal protein S18	1.87761057	0.141	Translation
		Up-regulated proteins (FC ≥ 2.0)			
299	SODM	Superoxide dismutase [Mn], mitochondrial	2.117000017	0.292	Destroys superoxide anion radicals
300	H31	Histone H3 type 1	2.410899695	0.022	DNA binding, Chromosome stability
301	H4	Histone H4	2.718281828	0.011	Nucleosome remodelling
302	RR19	30S ribosomal protein S19, chloroplastic	3.455613498	0.138	Translation
303	atpH	ATP synthase subunit c, chloroplastic	4.220696059	0.201	ATP synthesis coupled proton transport
304	PLAS	Plastocyanin, chloroplastic	4.392945765	0.012	Electron transfer from cyt b ₆ f to P70
305	MOC2A	Molybdopterin synthase sulfur carrier	4.392945765	0.016	Mo-molybdopterin cofactor biosynthesis
306	ND6	NADH-ubiquinone oxidoreductase chain 6	5.640654016	0.011	NADH dehydrogenase activity
		Control specific protein			
307	PsbM	Photosystem II reaction center protein M	Contol_P_50		photosynthesis, light reaction

308	UFM1	Ubiquitin-fold modifier 1	Control_P_50		protein ufmylation
309	ATPI	ATP synthase subunit a, chloroplastic	Control_P_50		Proton-transporting ATP synthase activity
		Treated specific protein			
310	RK20	50S ribosomal protein L20, chloroplastic	Treated_P_500	.	Translation
311	PETM	Cyt b6f complex subunit 7, chloroplastic	Treated_P_500		CEF around PSI and state transitions

Table 5.5 List of proteins expressed in *pgr5_500* vs *pgr5_50* (treated vs control) of *C. reinhardtii*.

S.No.	Protein	Description	Fold change	P-Value	GO Description
Down-regulated protein (FC ≤ 0.5)					
1	atpl	ATP synthase subunit a, chloroplastic	0.037253851	0.224	ATP synthesis coupled proton transport
2	RR3	30S ribosomal protein S3, chloroplastic	0.208045171	0.411	Translation
3	SULP2	Sulfate permease 2, chloroplastic	0.225372653	0.082	Sulfate transport
4	YPTC5	GTP-binding protein YPTC5	0.304221247	0.010	protein transport
5	YCX3	14.4 kDa protein in 16S rRNA region O	0.313486191	0.288	Determine phylogeny
6	FENR	Ferredoxin--NADP reductase, chloroplastic	0.491644208	0.334	Photosynthesis
Differential regulated protein (≤ 2.0 FC ≥ 0.5)					
7	IFT27	Intraflagellar transport protein 27	0.50157607	0.029	GTPase activity, IFT complex B
8	RR11	30S ribosomal protein S11, chloroplastic	0.543350861	0.031	Translation
9	SRGT1	Peptidyl serine alpha-galactosyltransferase	0.559898376	0.410	Transferase activity
10	ACT	Actin	0.576949804	0.188	Cytoplasmic streaming
11	IFT25	Intraflagellar transport protein 25	0.58274824	0.051	Intraciliary transport
12	RR9	30S ribosomal protein S9, chloroplastic	0.618783398	0.208	Translation
13	HS22C	Heat shock 22 kDa protein, chloroplastic	0.625002269	0.038	Protein maturation and degradation
14	LHR32	LHC stress-related protein 3.2, chloroplastic	0.625002269	0.115	Non-photochemical quenching
15	LHR31	LHC stress-related protein 3.1, chloroplastic	0.644036423	0.119	Non-photochemical quenching
16	RSP6	Flagellar radial spoke protein 6	0.663650253	0.633	Cilium assembly
17	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	0.663650253	0.192	Protein urmylation
18	GPX1	Glutathione peroxidase 1, mitochondrial	0.670320042	0.139	Arachidonic acid metabolic process
19	DRC9	Dynein regulatory complex protein 9	0.704688094	0.543	Regulator of ciliary/flagellar motility
20	NU4M	NADH-ubiquinone oxidoreductase chain 4	0.71177032	0.027	Electrons NADH to the respiratory chain
21	RUBB	RuBisCO large subunit-binding protein beta-1	0.733446954	0.254	Assembly of the enzyme oligomer
22	PF20	Flagellar WD repeat-containing protein Pf20	0.733446954	0.221	Inter-microtubule bridges in flagella
23	RAA3	Trans-splicing factor Raa3, chloroplastic	0.740818212	0.177	mRNA processing, RNA splicing
24	ALFC	Fructose-bisphosphate aldolase 1, chloroplastic	0.748263574	0.055	Glycolytic process
25	IPYR1	Soluble inorganic pyrophosphatase 1	0.755783741	0.171	Phosphate metabolic process
26	PSAA	Photosystem I P700 chlorophyll a apoprotein A1	0.763379486	0.602	Photosynthesis
27	YCF3	Photosystem I assembly protein Ycf3	0.763379486	0.009	Chaperone assembly of the PSI subunits
28	CHLL	Light-independent protochlorophyllide reductase	0.771051593	0.396	Photosynthesis, Dark reaction
29	CP12	Calvin cycle protein CP12, chloroplastic	0.778800783	0.176	Assembly of core complex PRK/GAPDH.
30	PR46B	Coiled-coil domain-containing protein 103	0.786627865	0.056	Cilia motility
31	SAC1	Putative sulfur deprivation response regulator	0.786627865	0.036	Potassium ion transport
32	GSA	Glutamate semialdehyde aminomutase	0.794533599	0.419	Chlorophyll biosynthetic process
33	RR7	30S ribosomal protein S7, chloroplastic	0.802518799	0.278	Translation
34	BSD2	Protein BUNDLE SHEATH DEFECTIVE 2	0.802518799	0.015	Chaperone-mediated protein folding
35	ATPE	ATP synthase epsilon chain, chloroplastic	0.802518799	0.007	ATP synthesis coupled proton transport
36	ARS	Arylsulfatase	0.818730751	0.402	Phenol-containing metabolic process
37	PSAE	PSI reaction center subunit IV, chloroplastic	0.852143792	0.020	Photosynthesis
38	RTCB	RNA-splicing ligase RtcB homolog	0.852143792	0.404	tRNA splicing
39	DRC3	Dynein regulatory complex subunit 3	0.860707971	0.131	Cilium-dependent cell motility
40	TBC2	Tbc2 translation factor, chloroplastic	0.869358235	0.035	PsbC mRNA for translation initiation
41	ENO	Enolase	0.869358235	0.029	Glycolytic process
42	PSAD	PSI reaction center subunit II, chloroplastic	0.869358235	0.247	Photosynthesis, Ferredoxin-docking site
43	NRT21	Nitrate transporter 2.1	0.878095435	0.102	Nitrate assimilation
44	PGKH	Phosphoglycerate kinase, chloroplastic	0.886920439	0.269	Glycolytic process, Reductive PPP
45	CRD1	Mg-protoporphyrin IX monomethyl ester	0.886920439	0.218	Photosynthesis, Chl biosynthetic process
46	TEKT	Tektin	0.895834136	0.013	Assembly of flagella and cilia
47	SLT1	Sodium/sulfate cotransporter 1	0.895834136	0.593	Potassium ion transport
48	CAH2	Carbonic anhydrase 2	0.895834136	0.023	CCM, Carbonate dehydratase activity
49	DRC4	Dynein regulatory complex subunit 4	0.895834136	0.475	Axonemal dynein complex assembly
50	METK	S-adenosylmethionine synthase	0.913931182	0.101	S-adenosylmethionine biosynthetic
51	DRC5	Dynein regulatory complex subunit 5	0.941764535	0.132	Ciliary/flagellar motility
52	CTH1	Mg-protoporphyrin IX monomethyl ester	0.941764535	0.217	Photosynthesis, chlorophyll biosynthesis
53	CFA70	Cilia- and flagella-associated protein 70	0.941764535	0.272	Axoneme- regulates ciliary motility
54	RL44	60S ribosomal protein L44	0.941764535	0.427	Translation, Response to cycloheximide
55	RR2A	30S ribosomal protein S2, chloroplastic	0.941764535	0.194	Translation
56	ALB32	ALBINO3-like protein 2, chloroplastic	0.951229424	0.193	Assembly and activity of LHC I and II

57	UCRIA	Cyt b6-f complex Fe-S subunit, chloroplastic	0.980198674	0.135	CET around PSI, State transitions.
58	CYC6	Cytochrome c6, chloroplastic	0.980198674	0.204	Electron carrier between cyt b6-f and PSI
59	MUT11	Protein Mut11	0.990049834	0.168	Involved 'Lys-4' histone H3 methylation
60	IPYR2	Soluble inorganic pyrophosphatase 2	0.990049834	0.084	Phosphate-containing metabolic process
61	DNE1	DNA endonuclease I-Crel	1	0.339	Endonuclease in group I intron homing
62	DYI2	Dynein, 78 kDa intermediate chain	1	0.253	Motor activity, Arm assembly
63	PFL	Formate acetyltransferase	1	0.044	Activity to form pyruvate and coA
64	HAP2	Hapless 2	1	0.058	Protein insertion into membrane
65	EFTU	Elongation factor Tu, chloroplastic	1.010050167	0.005	Translational elongation
66	URM1	Ubiquitin-related modifier 1 homolog	1.010050167	0.738	Endonuclease activity, Intron homing
67	RK20	50S ribosomal protein L20, chloroplastic	1.010050167	0.544	Translation
68	ARF1	ADP-ribosylation factor 1	1.010050167	0.286	Vesicle-mediated transport
69	CYF	Cytochrome f	1.040810773	0.152	CEF around PSI
70	LIAS	Lipoyl synthase, mitochondrial	1.051271097	0.333	Lipoylation peptidyl-N6-lipoyl-L-lysine
71	BOP1	Ribosome biogenesis protein BOP1 homolog	1.072508182	0.028	Maturation of 5.8S rRNA
72	CAPP2	Phosphoenolpyruvate carboxylase 2	1.072508182	0.050	Carbon fixation, Tricarboxylic acid cycle
73	SLT2	Sodium/sulfate cotransporter 2	1.083287066	0.292	Potassium ion transport
74	CCSA	Cytochrome c biogenesis protein CcsA	1.083287066	0.002	Cytochrome complex assembly
75	CCSA	Intraflagellar transport protein 43	1.083287066	0.109	Cell projection organization
76	LHSR1	LHC stress-related protein 1, chloroplastic	1.094174288	0.920	Non-photochemical quenching
77	H4	Histone H4	1.094174288	0.018	Nucleosome remodelling
78	CEMA	Chloroplast envelope membrane protein	1.094174288	0.365	Proton transmembrane transporter
79	ASNA1	ATPase ARSA1	1.094174288	0.612	ATPase activity
80	CFA74	Cilia- and flagella-associated protein 74	1.11627807	0.100	Cilium movement, Cell motility
81	SFAS	SF-assemblin	1.127496849	0.149	Striated microtubule- fibres assembly
82	ZYS3	Zygote-specific protein 3	1.138828378	0.010	Cell development
83	GLNA2	Glutamine synthetase, chloroplastic	1.1502738	0.519	Glutamine biosynthetic process
84	RSP3	Flagellar radial spoke protein 3	1.1502738	0.245	Cilium-dependent cell motility
85	METE	Homocysteine methyltransferase	1.1502738	0.009	Methionine biosynthetic process
86	CCD39	Coiled-coil domain-containing protein 39	1.16183425	0.133	Cilium movement
87	RUBA	RuBisCO large subunit- alpha, chloroplastic	1.173510867	0.326	Assembly of the enzyme oligomer
88	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	1.173510867	0.780	Photosynthesis
89	KPPR	Phosphoribulokinase, chloroplastic	1.173510867	0.022	Reductive pentose-phosphate cycle
90	NU5M	NADH-ubiquinone oxidoreductase chain 5	1.173510867	0.000	NADH dehydrogenase activity
91	CCD40	Coiled-coil domain-containing protein 40	1.173510867	0.224	Assembly of dynein regulatory complex
92	CAO	Chlorophyllide a oxygenase, chloroplastic	1.173510867	0.079	Chlorophyll b biosynthetic process
93	ARL13	ADP-ribosylation factor-like protein 13B	1.185304853	0.139	Control ciliary axoneme structure
94	ODA1	Outer dynein arm protein 1	1.185304853	0.155	Cilium movement in cell motility
95	TBA2	Tubulin alpha-2 chain	1.197217372	0.661	Alters the microtubule cytoskeleton
96		Dynein regulatory complex subunit 7	1.197217372	0.100	Regulation of flagellar motility
97	SUVH3	Histone-lysine N-methyltransferase	1.197217372	0.148	Methyltransferase- repressed chromatin
98	STT7	Serine/threonine-protein kinase, chloroplastic	1.209249595	0.535	State transition
99	CAPP1	Phosphoenolpyruvate carboxylase 1	1.209249595	0.011	TCA, Carboxylation PEP to oxaloacetate
100	IDLC	28 kDa inner dynein arm light chain, axonemal	1.209249595	0.039	Cilium movement in cell motility
101	HSTC	Homogentisate solanesyltransferase	1.209249595	0.013	Carotenoid and PQ biosynthetic process
102	RSSA	40S ribosomal protein SA	1.209249595	0.091	Translation
103	TBB	Tubulin beta-1/beta-2 chain	1.209249595	0.340	Microtubule-based process
104	PHOT	Phototropin	1.209249595	0.014	Blue light signalling, NPQ induction
105	CFA58	Cilia- and flagella-associated protein 58	1.221402762	0.209	Cilium-dependent cell motility
106	EZY1	Ezy-1 protein	1.221402762	0.209	Uncharacterized protein
107	YCF78	membrane protein ycf78	1.221402762	0.510	Cell growth
108	KLP1	Kinesin-like protein KLP1	1.221402762	0.434	Microtubule-based movement
109	ARLY	Argininosuccinate lyase	1.221402762	0.006	Arginine biosynthetic via ornithine
110	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	1.221402762	0.275	Unsaturated and fatty acid biosynthetic
111	TBA1	Tubulin alpha-1 chain	1.233678052	0.853	Microtubule-based process
112	NU2M	NADH-ubiquinone oxidoreductase chain 2	1.233678052	0.156	NADH dehydrogenase activity
113	ASNA2	ATPase ARSA2	1.233678052	0.001	Tail-anchored protein insertion into ER
114	HSP70	Heat shock 70 kDa protein	1.233678052	0.085	Protein maturation
115	PSAC	Photosystem I iron-sulfur center	1.233678052	0.067	Binding of Psd and Psae to PSI
116	DRC11	Dynein regulatory complex protein 11	1.233678052	0.107	Regulator of ciliary/flagellar motility
117	CFA61	Cilia- and flagella-associated protein 61	1.246076729	0.049	Cilium movement, Cilium organization
118	SLT3	Probable sodium/sulfate cotransporter 3	1.246076729	0.054	Sulfate transport
119	POB15	Proteome of basal body protein 15	1.258600015	0.144	Motility and sensing stimuli
120	PSAG	Photosystem I RC subunit V, chloroplastic	1.284025417	0.019	Photosynthesis
121	DRC10	Dynein regulatory complex protein 10	1.284025417	0.017	Maintains microtubule sliding

122	PETO	Cytochrome b6-f subunit petO, chloroplastic	1.284025417	0.001	LET between PSII and I, CET around PSI
123	HEM2	Aminolevulinic acid dehydratase, chloroplastic	1.296930074	0.601	Chlorophyll biosynthetic process
124	RSP2	Flagellar radial spoke protein 2	1.296930074	0.030	Flagellar bending
125	CALM	Calmodulin	1.296930074	0.256	Calcium-mediated signalling
126	CLPP	ATP-dependent Clp protease	1.323129814	0.111	Control misfolded synthesized proteins
127	DAAF3	Dynein assembly factor 3	1.323129814	0.185	Axonemal dynein complex assembly
128	TBG	Tubulin gamma chain	1.336427477	0.099	Cytoplasmic microtubule organization
129	YPTC1	GTP-binding protein YPTC1	1.336427477	0.015	Protein transport, vesicular traffic
130	NRT22	Nitrate transporter 2.2	1.336427477	0.014	Nitrate assimilation, Nitrate transport
131	MBB1	PsbB mRNA maturation factor, chloroplastic	1.349858824	0.085	mRNA processing via 5'-UTR of the psbB
132	KTU	Protein kintoun	1.363425117	0.425	Axonemal dynein complex assembly
133	LISC	Lipoyl synthase, chloroplastic	1.363425117	0.450	Protein lipoylation
134	RCA	RuBisCO/oxygenase, chloroplastic	1.377127754	0.149	Activation of RuBisCO
135	CF300	Cilia- and flagella-associated protein 300	1.377127754	0.181	Axonemal organization and motility
136	IFT56	Intraflagellar transport protein 56	1.390968147	0.980	Intraciliary transport
137	CMD1	5-methylcytosine-modifying enzyme 1	1.390968147	0.058	5-methylcytosine catabolic process
138	PSAH	Photosystem I RC subunit VI, chloroplastic	1.390968147	0.021	Photosynthesis, Docking LHC I antenna
139	RBL	Ribulose biphosphate carboxylase large chain	1.390968147	0.134	Carboxylation of RuBP in PPP
140	CFA44	Cilia- and flagella-associated protein 44	1.404947596	0.000	Cilium-dependent cell motility
141	CIAO1	Cytosolic Fe-S protein assembly CIAO1 homolog	1.404947596	0.140	Assembly of extramitochondrial Fe/S
142	POC16	Centriole proteome protein 16	1.41906754	0.341	Flagellum assembly and maintenance
143	DRC2	Dynein regulatory complex subunit 2	1.41906754	0.235	Axonemal dynein complex assembly
144	CFA99	Cilia- and flagella-associated protein 99	1.433329435	0.403	ciliary/flagellar motility
145	RBS2	RuBisCO small chain 2, chloroplastic	1.433329435	0.312	Photosynthesis, Carbon fixation
146	CFA45	Cilia- and flagella-associated protein 45	1.433329435	0.040	Cell motility
147	CCS1	Cytochrome c biogenesis protein, chloroplastic	1.433329435	0.005	Cytochrome complex assembly
148	S17P	Sedoheptulose-bisphosphatase, chloroplastic	1.433329435	0.101	Starch and Sucrose biosynthetic process
149	CHLI	Magnesium-chelatase subunit, chloroplastic	1.433329435	0.130	Insertion of Mg into protoporphyrin ring
150	YCX2	Uncharacterized protein in 16S rRNA region	1.447734622	0.108	Phylogenetic relationship
151	PCDP1	Cilia and flagella associated protein 221	1.447734622	0.349	Cilium assembly and movement
152	RSP1	Flagellar radial spoke protein 1	1.462284582	0.353	Flagellar bending
153	DAAF1	Leucine-rich repeat-containing protein ODA7	1.462284582	0.291	Cell motility and localization to organelle
154	CFA91	Cilia- and flagella-associated protein 91	1.462284582	0.024	Cilium movement
155	IFT74	Intraflagellar transport protein 74	1.462284582	0.097	Cilium assembly
156	RR14	30S ribosomal protein S14, chloroplastic	1.462284582	0.123	Translation
157	IFT46	Intraflagellar transport protein 46	1.462284582	0.062	Cilium-dependent cell motility
158	POR	Protochlorophyllide reductase, chloroplastic	1.462284582	0.064	Chlorophyll biosynthetic process
159	DNAL1	Dynein light chain 1, axonemal	1.462284582	0.216	Motor activity for flagellar motility
160	RPC2B	DNA-directed RNA polymerase subunit beta	1.462284582	0.102	transcription, DNA-templated
161	RUBC	RuBisCO large subunit- beta-2	1.491824707	0.628	RuBisCO assembly, Protein refolding,
162	RS3A	40S ribosomal protein S3a	1.491824707	0.111	Translation
163	RL10A	60S ribosomal protein L10a	1.491824707	0.013	Translation
164	RR2B	Putative 30S ribosomal S2-like protein	1.491824707	0.081	Translation
165	LPAT	1-acyl-sn-glycerol-3-phosphate acyltransferase	1.50681778	0.241	CDP-diacylglycerol biosynthetic process
166	MDHM	Malate dehydrogenase, mitochondrial	1.521961536	0.190	Carbohydrate metabolic process
167	IFT57	Intraflagellar transport protein 57	1.521961536	0.879	Maintenance and formation of cilia
168	DRC1	Dynein regulatory complex protein 1	1.521961536	0.084	Dynein complex assembly
169	PSAF	Photosystem I RC subunit III, chloroplastic	1.521961536	0.223	Electron transfer from PC to P700
170	TRXH	Thioredoxin H-type	1.537257535	0.024	Cell redox homeostasis
171	RAA2	Trans-splicing factor Raa2, chloroplastic	1.537257535	0.059	RNA splicing, mRNA processing
172	THI4	Thiamine thiazole synthase, chloroplastic	1.537257535	0.365	Thiamine biosynthetic process
173	RBS1	RuBisCO small chain 1, chloroplastic	1.537257535	0.258	Photosynthesis, Photorespiration
174	CF157	Cilia- and flagella-associated protein 157	1.552707215	0.276	Cilium movement, Cell motility
175	TOC34	Translocase of chloroplast 34, chloroplastic	1.552707215	0.086	Protein from the cytoplasm to chloroplast
176	RPOA	DNA-directed RNA polymerase subunit alpha	1.552707215	0.908	Transcription, DNA-templated
177	RPC1A	DNA-directed RNA polymerase subunit beta	1.552707215	0.352	Transcription by RNA polymerase II
178	IF172	Intraflagellar transport protein 172	1.568312167	0.243	Multicellular organism development
179	FEN1	Flap endonuclease 1	1.568312167	0.062	DNA replication, Base-excision repair
180	DRC6	Dynein regulatory complex subunit 6	1.584073998	0.013	Regulator of ciliary/flagellar motility
181	GBLP	Guanine nucleotide-protein subunit beta	1.584073998	0.157	Transcription initiation and termination
182	IFT22	Intraflagellar transport protein 22	1.599994191	0.145	Cellular availability of IFT particles
183	FLA10	Kinesin-like protein FLA10	1.599994191	0.523	Intraciliary anterograde transport
184	IFT81	Intraflagellar transport protein 81	1.616074385	0.021	Transport of tubulin within the cilium
185	CFA73	Cilia- and flagella-associated protein 73	1.616074385	0.043	Regulation of microtubule motor activity
186	CFA52	Cilia- and flagella-associated protein 52	1.632316236	0.054	Cell motility

187	ARL3	ADP-ribosylation factor-like protein 3	1.632316236	0.759	Cytokinesis and cilia signalling
188	RK14	50S ribosomal protein L14, chloroplastic	1.632316236	0.003	Translation
189	CALR	Calreticulin	1.632316236	0.154	Protein folding
190	GLNA1	Glutamine synthetase cytosolic isozyme	1.648721271	0.038	Glutamine biosynthetic process
191	YPTC4	GTP-binding protein YPTC4	1.648721271	0.320	Protein transport and vesicular traffic
192	RK2	50S ribosomal protein L2, chloroplastic	1.648721271	0.067	Mitochondrial translation
193	SULP1	Sulfate permease 1, chloroplastic	1.665291179	0.162	Uptake and assimilation of sulfate
194	RLA1	60S acidic ribosomal protein P1	1.682027618	0.234	Translational elongation
195	RL13	60S ribosomal protein L13	1.682027618	0.946	Translation
196	G3PC	G3P- dehydrogenase, cytosolic	1.69893226	0.024	Carbohydrate metabolism
197	CHLN	Light-independent protochlorophyllide	1.716006899	0.215	Light-independent chl biosynthetic
198	CHLB	Light-independent protochlorophyllide	1.733253039	0.001	Light-independent chl biosynthetic
199	CHLB	Light-independent protochlorophyllide	1.733253039	0.820	Light-independent chl biosynthetic
200	NAC2	PsbD mRNA maturation factor, chloroplastic	1.750672504	0.246	mRNA processing- 5' UTR of psbD mRNA
201	ANM1	Protein arginine N-methyltransferase 1	1.768267039	0.349	Asymmetric dimethylation of a flagellum
202	ATPX	ATP synthase subunit b', chloroplastic	1.768267039	0.042	ATP synthesis coupled proton transport
203	DRC8	Dynein regulatory complex protein 8	1.803988368	0.954	Regulates microtubule sliding
204	PESC	Pescadillo homolog	1.803988368	0.349	Maturation of 5.8S rRNA
205	CFA65	Cilia- and flagella-associated protein 65	1.803988368	0.218	Cell projection organization
206	WDR12	Ribosome biogenesis protein	1.803988368	0.337	LSU-rRNA from tricistronic rRNA
207	GLE	Autolysin	1.840431425	0.452	Cell wall organization
208	DC2L1	Cytoplasmic dynein 2 intermediate chain 1	1.840431425	0.459	Regulates flagellar dynein activity
209	CF251	Cilia- and flagella-associated protein 251	1.840431425	0.057	Cilium movement
210	SULT2	Proton/sulfate cotransporter 2	1.840431425	0.085	Sulfate transmembrane transporter
211	RPOB1	DNA-directed RNA polymerase subunit beta N	1.840431425	0.276	Transcription, DNA-templated
212	CATR	Caltractin	1.858928051	0.104	Cell cycle, Cell division
213	RL12	60S ribosomal protein L12	1.87761057	0.072	Translation
214	ADT	ADP,ATP carrier protein	1.87761057	0.628	Catalyzes the exchange of ADP and ATP
215	CB29	Chlorophyll a-b binding protein CP29	1.896480852	0.359	LHC Facilitates the State 1 to 2 transition
216	UFM1	Ubiquitin-fold modifier 1	1.915540783	0.044	Protein ufmylation
217	PSBD	Photosystem II D2 protein	1.915540783	0.016	Photosynthetic, PSII complex assembly
218	PSBA	Photosystem II protein D1	1.915540783	0.005	Photosynthetic electron transport in PSII
219	PSBP	Oxygen-evolving enhancer 2, chloroplastic	1.915540783	0.879	Photosynthesis, Oxygen evolution
220	CAH1	Carbonic anhydrase 1	1.954237353	0.073	Carbonate dehydratase activity
221	PETD	Cytochrome b6-f complex subunit 4	1.954237353	0.015	Transferring electrons within the CET
222	DYL3	Dynein 18 kDa light chain	1.973877746	0.086	Motor activity, Calcium ion binding
223	CFA77	Cilia- and flagella-associated protein 77	1.973877746	0.202	Cell motility
224	PURA	Adenylosuccinate synthetase, chloroplastic	1.993715528	0.038	"de novo" AMP biosynthetic process
225	CHLB	Light-independent protochlorophyllide	1.993715528	0.945	Light-independent chl biosynthetic
Up-regulated proteins (FC ≥ 2.0)					
226	PSBS1	Photosystem II protein PSBS1	1.993715528	0.184	Nonphotochemical quenching
227	DIP13	13 kDa deflagellation-inducible protein	2.013752683	0.150	Protein stability
228	CP100	Cilia- and flagella-associated protein 100	2.033991215	0.523	Cell motility, Cilium movement
229	ATPBM	ATP synthase subunit beta, mitochondrial	2.033991215	0.023	ATP synthesis coupled proton transport
230	RSP14	Radial spoke protein 14	2.054433269	0.216	Cell motility, Endocytosis
231	G3PA	G3P- dehydrogenase, chloroplastic	2.054433269	0.003	Glucose metabolic process
232	PSBS2	Photosystem II protein PSBS2	2.075080647	0.399	Nonphotochemical quenching
233	ATPG	ATP synthase gamma chain, chloroplastic	2.159766213	0.086	ATP synthesis coupled proton transport
234	CFA53	Cilia- and flagella-associated protein 53	2.225540955	0.617	Cilium assembly and movement
235	RSP5	Flagellar radial spoke protein 5	2.247907992	0.018	Regulation of flagellar bending
236	PSAB	PSI P700 chlorophyll a apoprotein A2	2.435129616	0.184	Primary electron donor of PSI
237	PSBB	Photosystem II CP47 RC protein	2.459603053	0.373	PET in photosystem II
238	ATPB	ATP synthase subunit beta, chloroplastic	2.459603053	0.163	ATP synthesis coupled proton transport
239	DES	Acyl-lipid omega-13 desaturase	2.459603053	0.412	Linoleic acid metabolic process
240	DAW1	Dynein assembly WDR repeat domains 1	2.611696417	0.406	Intraciliary transport and assembly
241	RPOA	DNA-directed RNA polymerase subunit beta C	2.664456293	0.439	Transcription, DNA-templated
242	RSP4	Flagellar radial spoke protein 4	2.745600989	0.966	Cell motility, cilium assembly
243	PLD6	Mitochondrial cardiolipin hydrolase	2.801065755	0.343	Lipid catabolic process
244	RR12	30S ribosomal protein S12, chloroplastic	2.829216906	0.711	Translation
245	EFTS	Elongation factor Ts, mitochondrial	2.857650982	0.725	Translation elongation factor activity
246	CFA20	Cilia- and flagella-associated protein 20	2.857650982	0.007	Axoneme, Cilium assembly
247	DYI3	Dynein, 70 kDa intermediate chain	3.034358438	0.033	Outer dynein arm assembly
248	ATPD	ATP synthase delta chain, chloroplastic	3.095656485	0.281	ATP synthesis coupled proton transport
249	SODM	Superoxide dismutase [Mn], mitochondrial	3.12676832	0.126	Destroys superoxide anion radicals

250	ATPA	ATP synthase subunit alpha, chloroplastic	3.525421454	0.478	ATP synthesis coupled proton transport
251	PSBO	Oxygen-evolving enhancer 1, chloroplastic	3.669296493	0.070	Primary site of water splitting
252	PLAS	Plastocyanin, chloroplastic	4.137120263	0.558	Electron transfer from P700 and cyt b ₆ f
253	PSBC	Photosystem II CP43 reaction center protein	4.349235265	0.034	PET in photosystem II
254	YCF4	Photosystem I assembly protein Ycf4	4.437095561	0.397	Assembly of the PSI complex
255	DYL4	Dynein 14 kDa light chain	5.584528624	0.216	Outer dynein arm assembly
256	RL401	Ubiquitin-60S ribosomal protein L40	160.7740437	0.283	Translation
257	CYB6	Cytochrome b6	281.4626809	0.310	CEF around PSI and state transitions.
Control specific protein					
258	PSBZ	Photosystem II reaction center protein Z	Control_P5_50		Photosynthesis, PS II stabilization
259	RK16	50S ribosomal protein L16, chloroplastic	Control_P5_50		Mitochondrial translation
260	FER	Ferredoxin, chloroplastic	Control_P5_50		Fe-S proteins for metabolic reactions
261	PSAJ	Photosystem I reaction center subunit IX	Control_P5_50		Organization of Psal and Psaf subunit
262	PSBJ	Photosystem II reaction center protein J	Control_P5_50		Photosynthesis, Charge separation
263	PSBE	Cytochrome b559 subunit alpha	Control_P5_50		PETC, Charge separation
264	RTL	Reverse transcriptase-like protein	Control_P5_50		cDNA synthethesis
265	RL31	60S ribosomal protein L31	Control_P5_50		Translation
266	NU1M	NADH-ubiquinone oxidoreductase chain 1	Control_P5_50		NADH dehydrogenase activity
267	COX1	Cytochrome c oxidase subunit 1	Control_P5_50		Oxidative phosphorylation
268	PSBN	Protein PsbN	Control_P5_50		Role in photosystem I and II biogenesis
269	CYC	Cytochrome c	Control_P5_50		Last protein carrier in mitochondrial ETC
270	PETM	Cytochrome b6-f complex 7, chloroplastic	Control_P5_50		CEF around PSI, and state transitions.
271	DYL2	Dynein 11 kDa light chain	Control_P5_50		Microtubule-based process
272	RS27	40S ribosomal protein S27	Control_P5_50		Translation
273	FLTOP	Protein Flattop homolog	Control_P5_50		Regulator of cilium basal body docking
274	PSBW	Photosystem II RC W protein, chloroplastic	Control_P5_50		Stabilizes dimeric photosystem II
275	YCX5	Uncharacterized trnR-chlB intergenic region	Control_P5_50		Tag-protein stability
276	ATPF	ATP synthase subunit b, chloroplastic	Control_P5_50		ATP synthesis coupled proton transport
277	RK5	50S ribosomal protein L5, chloroplastic	Control_P5_50		Translation
Treated specific protein					
278	UFM1	Ubiquitin-fold modifier 1	Treated_P5_500		Protein ufmylation
279	H31	Histone H3 type 1	Treated_P5_500		DNA binding, Chromosome stability
280	PSBI	Photosystem II reaction center protein I	Treated_P5_500		Photosynthesis
281	PSBR	Photosystem II protein PSBR, chloroplastic	Treated_P5_500		Energy-dependent quenching
282	H32	Histone H3 type 2	Treated_P5_500		DNA replication, chromosomal stability.
283	RS18	40S ribosomal protein S18 O	Treated_P5_500		Translation

Table 5.6 List of proteins expressed in WT_500 vs *pgr5_50* (treated vs control) of *C. reinhardtii*

S.No.	Abbreviation	Description	Fold Change	P-value	GO Description
		Down-regulated protein (FC ≤ 0.5)			
1	CHLL	Light-independent protochlorophyllide	0.013568556	0.010	Photosynthesis, Dark reaction
2	RK14	50S ribosomal protein L14, chloroplastic	0.298197268	0.012	Translation
3	IPYR2	Soluble inorganic pyrophosphatase 2	0.343008499	0.010	Phosphate-containing metabolic process
4	PSBO	Oxygen-evolving enhancer 1, chloroplastic	0.382892894	0.007	Primary site of water splitting
5	PSAA	PSI P700 chlorophyll a apoprotein A1	0.382892894	0.399	Photosynthesis
6	PR46B	Coiled-coil domain-containing protein	0.390627836	0.005	Cilia motility
7	CYC6	Cytochrome c6, chloroplastic	0.390627836	0.184	Electron carrier between cyt b6-f and PSI
8	IFT25	Intraflagellar transport protein 25	0.410655759	0.034	Intraciliary transport
9	RAA3	Trans-splicing factor Raa3, chloroplastic	0.418951547	0.324	mRNA processing, RNA splicing
10	RUBB	RuBisCO large subunit-protein beta-1	0.427414922	0.162	Assembly of the enzyme oligomer
11	RL44	60S ribosomal protein L44	0.440431658	0.273	Translation, Response to cycloheximide
12	CB2	Chl a-b binding protein, LHCII type I	0.440431658	0.415	Photosynthesis, LHC as light receptor
13	RR2B	Putative 30S ribosomal S2-like protein	0.458406024	0.234	Translation
14	RL11	60S ribosomal protein L11	0.463013077	0.053	Translation
15	CEMA	Chloroplast envelope membrane protein	0.463013077	0.056	Proton transporter activity
16	ATPI	ATP synthase subunit a, chloroplastic	0.467666431	0.567	ATP synthesis coupled proton transport
17	CCS1	Cytochrome c biogenesis protein	0.472366553	0.392	Cytochrome complex assembly
18	RS18	40S ribosomal protein S18	0.481908981	0.006	Translation
19	SRGT1	Peptidyl serine-galactosyltransferase	0.486752242	0.426	Transferase activity
20	H33	Histone H3 type 3	0.491644208	0.586	Nucleosome remodelling
21	RUBC	RuBisCO large subunit- beta-2	0.49658531	0.431	RuBisCO assembly, Protein refolding,
22	H32	Histone H3 type 2	0.49658531	0.015	Chromosomal stability
		Differential regulated protein (≤ 2.0 FC≥0.5)			
23	CRD1	Mg-protoporphyrin IX monomethyl ester	0.511708569	0.062	Photosynthesis, Chl biosynthetic process
24	RR2A	30S ribosomal protein S2, chloroplastic	0.532591804	0.296	Translation
25	PSAG	Photosystem I RC subunit V, chloroplastic	0.543350861	0.647	Photosynthesis
26	IDLC	28 kDa inner dynein arm light chain	0.543350861	0.474	Cilium movement involved in cell motility
27	S17P	Sedoheptulose-1,7-bisphosphatase	0.548811623	0.003	Starch and Sucrose biosynthetic process
28	RL401	Ubiquitin-60S ribosomal protein L40	0.554327299	0.267	Translation
29	RR12	30S ribosomal protein S12, chloroplastic	0.554327299	0.086	Translation
30	METK	S-adenosylmethionine synthase	0.554327299	0.413	S-adenosylmethionine biosynthesis
31	EFTS	Elongation factor Ts, mitochondrial	0.559898376	0.019	Translation elongation factor activity
32	IPYR1	Soluble inorganic pyrophosphatase 1	0.571209062	0.176	Phosphate-containing metabolic process
33	CF300	Cilia- and flagella-associated protein 300	0.58274824	0.000	Axonemal organization and motility
34	DRC11	Dynein regulatory complex protein 11	0.58274824	0.384	Regulator of ciliary/flagellar motility
35	DNE1	DNA endonuclease I-Crel	0.594520559	0.915	Endonuclease activity, Intron homing
36	IFT27	Intraflagellar transport protein 27	0.600495585	0.008	GTPase activity, Form IFT complex B
37	ZYS3	Zygote-specific protein 3	0.600495585	0.181	Cell development
38	SULT2	Proton/sulfate cotransporter 2	0.600495585	0.092	Sulfate transmembrane transporter
39	RL403	Ubiquitin-60S ribosomal protein L40	0.618783398	0.304	Translation
40	CB29	Chlorophyll a-b binding protein CP29	0.625002269	0.046	LHC Facilitates the State 1 to 2 transition
41	PSAC	Photosystem I iron-sulfur center	0.637628159	0.032	Required for binding of PsaD, PsaE to PSI
42	THI4	Thiamine thiazole synthase, chloroplastic	0.637628159	0.800	Thiamine biosynthetic process
43	PSBI	Photosystem II reaction center protein I	0.644036423	0.007	Stability and assembly of PSII
44	NRT21	Nitrate transporter 2.1	0.644036423	0.177	Nitrate assimilation
45	RL10A	60S ribosomal protein L10a	0.657046828	0.129	Translation
46	DES	Acyl-lipid omega-13 desaturase	0.670320042	0.588	Linoleic acid metabolic process
47	RR14	30S ribosomal protein S14, chloroplastic	0.677056884	0.094	Translation
48	IFT43	Intraflagellar transport protein 43	0.677056884	0.412	Cell projection organization
49	CMD1	5-methylcytosine-modifying enzyme 1	0.683861412	0.020	5-methylcytosine catabolic process
50	DNAL1	Dynein light chain 1, axonemal	0.683861412	0.001	Motor activity for flagellar motility
51	ADT	ADP, ATP carrier protein	0.690734327	0.635	Catalyzes the exchange of ADP and ATP

52	PGKH	Phosphoglycerate kinase, chloroplastic	0.697676316	0.587	Glycolytic process
53	CCSA	Cytochrome c biogenesis protein CcsA	0.697676316	0.001	Cytochrome complex assembly
54	ACT	Actin	0.697676316	0.188	Cytoplasmic streaming
55	YCF3	Photosystem I assembly protein Ycf3	0.704688094	0.218	Chaperone-like assembly of PSI subunits
56	DAAF3	Dynein assembly factor 3, axonemal	0.71177032	0.098	Axonemal dynein complex assembly
57	KLP1	Kinesin-like protein KLP1	0.718923724	0.434	Microtubule-based movement
58	ALFC	Fructose-bisphosphate aldolase 1	0.726149042	0.181	Glycolytic process
59	LHR31	LHC stress-related protein 3.1	0.726149042	0.240	Nonphotochemical quenching
60	DYI2	Dynein, 78 kDa intermediate chain	0.726149042	0.021	Motor activity, Arm assembly
61	ATPB	ATP synthase subunit beta, chloroplastic	0.726149042	0.181	ATP synthesis coupled proton transport
62	YCX3	14.4 kDa protein in 16S rRNA	0.733446954	0.504	Determine phylogeny
63	RSP3	Flagellar radial spoke protein 3	0.733446954	0.030	Protein localization to organelle
64	CFA61	Cilia- and flagella-associated protein 61	0.733446954	0.123	Cilium movement, Cell organization
65	DRC2	Dynein regulatory complex subunit 2	0.733446954	0.461	Axonemal dynein complex assembly
66	EFTU	Elongation factor Tu, chloroplastic	0.740818212	0.236	Translational elongation
67	IFT74	Intraflagellar transport protein 74	0.740818212	0.603	Cilium assembly
68	HSP70	Heat shock 70 kDa protein	0.740818212	0.039	Protein maturation and degradation
69	TBA2	Tubulin alpha-2 chain	0.748263574	0.348	Alters the microtubule cytoskeleton
70	TBA1	Tubulin alpha-1 chain	0.748263574	0.290	Microtubule-based process
71	DAAF1	Leucine-rich repeat-containing protein	0.748263574	0.727	Cell motility and localization to organelle
72	D4FAD	Acyl-lipid (7-3)-desaturase, chloroplastic	0.748263574	0.141	Unsaturated and fatty acid biosynthetic
73	DRC4	Dynein regulatory complex subunit 4	0.748263574	0.508	Axonemal dynein complex assembly
74	FEN1	Flap endonuclease 1	0.755783741	0.314	DNA replication, Base-excision repair
75	GSA	Glutamate semialdehyde aminomutase	0.755783741	0.869	Chlorophyll biosynthetic process
76	G3PA	G3P-dehydrogenase A, chloroplastic	0.755783741	0.713	Glucose metabolic process
77	RL13	60S ribosomal protein L13	0.755783741	0.495	Translation
78	RSP2	Flagellar radial spoke protein 2	0.763379486	0.028	Flagellar bending
79	DRC9	Dynein regulatory complex protein 9	0.763379486	0.373	Regulator of ciliary/flagellar motility
80	PHOT	Phototropin	0.763379486	0.696	Blue light signalling, NPQ induction
81	CCD39	Coiled-coil domain-containing protein 39	0.771051593	0.592	Cilium movement
82	PCDP1	Cilia- and flagella-associated protein 221	0.771051593	0.170	Cilium assembly and movement
83	IFT46	Intraflagellar transport protein 46	0.771051593	0.013	Cilium-dependent cell motility
84	CFA20	Cilia- and flagella-associated protein 20	0.771051593	0.094	Axoneme, Cilium assembly
85	RCA	RuBisCO/oxygenase activase, chloroplastic	0.778800783	0.420	Activation of RuBisCO
86	PSAF	PSI RCsubunit III, chloroplastic	0.786627865	0.069	Electron transfer PC to P700
87	1433	14-3-3-like protein	0.794533599	0.155	Flageller arrangement and assembly
88	DRC7	Dynein regulatory complex subunit 7	0.802518799	0.325	Regulation of flagellar motility
89	YCF78	membrane protein ycf78	0.802518799	0.538	Cell growth
90	MOC1	Holliday junction resolvase, chloroplastic	0.802518799	0.340	DNA recombination
91	RR9	30S ribosomal protein S9, chloroplastic	0.810584251	0.308	Translation
92	RPOB1	DNA-directed RNA polymerase beta	0.810584251	0.385	Transcription, DNA-templated
93	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	0.818730751	0.005	Protein urmylation
94	NU4M	NADH-ubiquinone oxidoreductase chain 4	0.818730751	0.017	Transfer from NADH to respiratory chain
95	PLAS	Plastocyanin, chloroplastic	0.835270205	0.827	Electron transfer from P700 and cyt b6f
96	RK20	50S ribosomal protein L20, chloroplastic	0.835270205	0.461	Translation
97	POC16	Centriole proteome protein 16	0.843664815	0.350	Flagellum assembly and maintenance
98	IFT22	Intraflagellar transport protein 22	0.843664815	0.776	Cellular availability of IFT particles
99	PLD6	Mitochondrial cardiolipin hydrolase	0.843664815	0.224	Lipid catabolic process
100	RR3	30S ribosomal protein S3, chloroplastic	0.843664815	0.281	Translation
101	ATPE	ATP synthase epsilon chain, chloroplastic	0.843664815	0.243	ATP synthesis coupled proton transport
102	RUBA	RuBisCO large subunit-alpha, chloroplastic	0.860707971	0.199	Assembly of the enzyme oligomer
103	CF157	Cilia- and flagella-associated protein 157	0.860707971	0.016	Cilium movement, Cell motility
104	UCRIA	Cyt b6-f complex Fe-S subunit	0.860707971	0.320	Mediates LET and CET around PSI
105	ARLY	Argininosuccinate lyase	0.860707971	0.014	Arginine biosynthetic via ornithine
106	RAA2	Trans-splicing factor Raa2, chloroplastic	0.860707971	0.469	RNA splicing, mRNA processing
107	CYF	Cytochrome f	0.860707971	0.211	CEF around PSI
108	RSP1	Flagellar radial spoke protein 1	0.869358235	0.831	Flagellar bending
109	DRC3	Dynein regulatory complex subunit 3	0.869358235	0.357	Cilium-dependent cell motility
110	CFA43	Cilia- and flagella-associated protein 43	0.869358235	0.588	Flagellum organization and function
111	LHR32	LHC stress-related protein 3.2	0.869358235	0.192	Nonphotochemical quenching
112	PSBS2	Photosystem II protein PSBS2	0.869358235	0.649	Nonphotochemical quenching
113	PETO	Cyt b6f complex subunit, chloroplastic	0.869358235	0.256	LET between PSII and I, CET around PSI
114	UFM1	Ubiquitin-fold modifier 1	0.878095435	0.012	Protein ufmylation
115	POR	Protochlorophyllide reductase	0.878095435	0.602	Chlorophyll biosynthetic process
116	TOC34l	Translocase of chloroplast 34,	0.878095435	0.058	Transport cytoplasm to chloroplast

117	PSBD	Photosystem II D2 protein	0.886920439	0.053	Photosynthetic, Assembly of PSII complex
118	IF172	Intraflagellar transport protein 172	0.886920439	0.369	Multicellular organism development
119	DRC10	Dynein regulatory complex protein 10	0.886920439	0.102	Regulates microtubule sliding
120	PSAD	PSI RC subunit II, chloroplastic	0.886920439	0.183	Ferredoxin-docking protein
121	SUVH3	Histone-lysine N-methyltransferase	0.886920439	0.698	Repressed chromatin functions
122	RPOA	DNA-directed RNA polymerase alpha	0.886920439	0.255	Transcription, DNA-templated
123	HAP2	Hapless 2	0.886920439	0.194	Protein insertion into the membrane
124	BOP1	Ribosome biogenesis protein homolog	0.895834136	0.116	Maturation of 5.8S rRNA
125	CLPP	ATP-dependent Clp protease proteolytic	0.895834136	0.106	chymotrypsin-control misfolded proteins
126	DRC1	Dynein regulatory complex protein 1	0.895834136	0.033	Dynein complex assembly
127	KPPR	Phosphoribulokinase, chloroplastic	0.904837417	0.020	Reductive pentose-phosphate cycle
128	RPC1A	DNA-directed RNA polymerase beta	0.904837417	0.958	Transcription by RNA polymerase II
129	ASNA1	ATPase ARSA1	0.904837417	0.461	ATPase activity
130	YCX2	Protein in 16S rRNA region	0.913931182	0.174	Phylogenetic relationship
131	DYL3	Dynein 18 kDa light chain	0.913931182	0.013	Motor activity, Calcium ion binding
132	CAPP2	Phosphoenolpyruvate carboxylase 2	0.913931182	0.116	Carbon fixation, Tricarboxylic acid cycle
133	POB15	Proteome of basal body protein 15	0.923116348	0.089	Motility and sensing stimuli
134	CFA58	Cilia- and flagella-associated protein 58	0.923116348	0.248	Cilium-dependent cell motility
135	DRC5	Dynein regulatory complex subunit 5	0.923116348	0.196	Ciliary/flagellar motility
136	ARL13	ADP-ribosylation factor-like protein 13B	0.923116348	0.582	Control ciliary axoneme structure
137	CAO	Chlorophyllide a oxygenase, chloroplastic	0.923116348	0.097	Chlorophyll b biosynthetic process
138	CFA52	Cilia- and flagella-associated protein 52	0.93239382	0.112	Cell motility
139	CFA65	Cilia- and flagella-associated protein 65	0.93239382	0.423	Cell projection organization
140	PF20	Flagellar WD repeat-containing Pf20	0.93239382	0.425	Inter-microtubule bridges in flagella
141	PURA	Adenylosuccinate synthetase, chloroplastic	0.951229424	0.030	"de novo" AMP biosynthetic process
142	LPAT	Acyl-sn-glycerol-phosphate acyltransferase	0.951229424	0.228	CDP-diacylglycerol biosynthetic process
143	FLA10	Kinesin-like protein FLA10	0.951229424	0.422	Intraciliary anterograde transport
144	YPTC4	GTP-binding protein YPTC4	0.96078944	0.254	Protein transport and vesicular traffic
145	TBC2	Tbc2 translation factor, chloroplastic	0.970445534	0.062	PsbC mRNA for translation initiation
146	METE	Homocysteine methyltransferase	0.970445534	0.518	Methionine biosynthetic process
147	ARS	Arylsulfatase	0.980198674	0.100	Phenol-containing metabolic process
148	STT7	Serine/threonine- kinase, chloroplastic	0.980198674	0.864	State transition
149	PSAH	Photosystem I RC subunit VI, chloroplastic	0.990049834	0.287	Docking for LHC I antenna
150	ARF1	ADP-ribosylation factor 1	0.990049834	0.105	Vesicle-mediated transport
151	CFA99	Cilia- and flagella-associated protein 99	1	0.777	Ciliary/flagellar motility
152	LHSR1	LHC stress-related protein 1, chloroplastic	1	0.163	Non-photochemical quenching
153	RLA1	60S acidic ribosomal protein P1	1	0.141	Translational elongation
154	CAPP1	Phosphoenolpyruvate carboxylase 1	1	0.122	Carboxylation of PEP into oxaloacetate
155	CFA73	Cilia- and flagella-associated protein 73	1	0.389	Regulation of microtubule motor activity
156	ATPG	ATP synthase gamma chain, chloroplastic	1.010050167	0.003	ATP synthesis coupled proton transport
157	RR11	30S ribosomal protein S11, chloroplastic	1.010050167	0.085	Translation
158	HEM2	Aminolevulinic dehydratase, chloroplastic	1.02020134	0.561	Chlorophyll biosynthetic process
159	CHLN	Light-independent protochlorophyllide	1.030454533	0.360	Light-independent chl biosynthesis
160	RPC2B	DNA-directed RNA polymerase beta	1.030454533	0.304	Transcription, DNA-templated
161	PSBP	Oxygen-evolving enhancer 2, chloroplastic	1.030454533	0.004	Photosynthesis, Oxygen evolution
162	RR7	30S ribosomal protein S7, chloroplastic	1.040810773	0.319	Translation
163	SAC1	Putative sulfur deprivation regulator	1.040810773	0.414	Potassium ion transport
164	CFA44	Cilia- and flagella-associated protein 44	1.040810773	0.138	Cilium-dependent cell motility
165	G3PC	G3P dehydrogenase, cytosolic	1.051271097	0.234	Carbohydrate metabolism
166	ENO	Enolase (Fragment)	1.061836545	0.334	Glycolytic process
167	CHLB	Light-independent protochlorophyllide	1.061836545	0.372	Light-independent chl biosynthesis
168	HSTC	Homogentisate solanesyltransferase	1.061836545	0.007	Carotenoid, Plastoquinone biosynthetic
169	ASNA2	ATPase ARSA2	1.072508182	0.596	Tail-anchored insertion ER membrane
170	DRC6	Dynein regulatory complex subunit 6	1.072508182	0.339	Regulator of ciliary/flagellar motility
171	RBS2	RuBP carboxylase small chain 2	1.083287066	0.894	Photosynthesis, Carbon fixation
172	TRXH	Thioredoxin H-type	1.083287066	0.188	Cell redox homeostasis
173	TRXM	Thioredoxin M-type, chloroplastic	1.094174288	0.026	Cell redox homeostasis
174	TBG	Tubulin gamma chain	1.094174288	0.119	Cytoplasmic microtubule organization
175	BSD2	BUNDLE SHEATH DEFECTIVE 2	1.094174288	0.711	Chaperone-mediated protein folding
176	RBL	RuBisCO carboxylase large chain	1.094174288	0.029	Carboxylation of RuBP in PPP
177	SLT3	Probable sodium/sulfate cotransporter 3	1.10517092	0.106	Sulfate transport
178	DIP13	13 kDa deflagellation-inducible protein	1.10517092	0.059	Protein stability
179	RK2	50S ribosomal protein L2, chloroplastic	1.11627807	0.727	Mitochondrial translation
180	PESC	Pescadillo homolog	1.127496849	0.809	Maturation of 5.8S rRNA
181	NAC2	PsbD mRNA maturation factor	1.127496849	0.648	mRNA processing- 5' UTR of psbD mRNA

182	CCD40	Coiled-coil domain-containing protein 40	1.127496849	0.389	Assembly of dynein regulatory complex
183	PFL	Formate acetyltransferase	1.127496849	0.014	Form pyruvate and coA
184	SFAS	SF-assemblin	1.1502738	0.100	Striated microtubule-associated assembly
185	CAH1	Carbonic anhydrase 1	1.1502738	0.330	Carbonate dehydratase activity
186	CIAO1	Probable cytosolic Fe -S protein assembly	1.1502738	0.392	Required iron-sulfur cluster assembly
187	SULP2	Sulfate permease 2, chloroplastic	1.16183425	0.944	Sulfate transport
188	CFA70	Cilia- and flagella-associated protein 70	1.16183425	0.887	Axoneme-regulates ciliary motility
189	PETD	Cytochrome b6-f complex subunit 4	1.16183425	0.004	Transferring electrons within the CET
190	GLNA2	Glutamine synthetase, chloroplastic	1.173510867	0.171	Glutamine biosynthetic process
191	CFA74	Cilia- and flagella-associated protein 74	1.173510867	0.327	Cilium movement, Cell motility
192	CFA74	Dynein 14 kDa light chain	1.173510867	0.174	Outer dynein arm assembly
193	CFA77	Cilia- and flagella-associated protein 77	1.173510867	0.722	Cell motility
194	CAH2	Carbonic anhydrase 2	1.173510867	0.253	CCM, Carbonate dehydratase activity
195	CHLB	Light-independent protochlorophyllide	1.173510867	0.379	Light-independent chl biosynthesis
196	PSBQ	Oxygen-evolving enhancer 3, chloroplastic	1.185304853	0.369	Photosynthesis
197	PSAE	PSI RC subunit IV, chloroplastic	1.185304853	0.241	Photosynthesis
198		Light-independent protochlorophyllide	1.185304853	0.004	Light-independent chl biosynthetic
199	MDHM	Malate dehydrogenase, mitochondrial	1.197217372	0.099	Carbohydrate metabolic process
200	GPX1	Glutathione peroxidase 1, mitochondrial	1.197217372	0.104	Arachidonic acid metabolic process
201	CFA53	Cilia- and flagella-associated protein 53	1.197217372	0.727	Cilium assembly and movement
202	CALR	Calreticulin	1.197217372	0.011	Protein folding
203	ANM1	Protein arginine N-methyltransferase 1	1.209249595	0.740	Asymmetric dimethylation of a flagellum
204	SLT1	Sodium/sulfate cotransporter 1	1.209249595	0.989	Potassium ion transport
205	GLNA1	Glutamine synthetase cytosolic isozyme	1.221402762	0.370	Glutamine biosynthetic process
206	YPTC1	GTP-binding protein YPTC1	1.221402762	0.698	Protein transport, vesicular traffic
207	ATPBM	ATP synthase subunit beta, mitochondrial	1.221402762	0.022	ATP synthesis coupled proton transport
208	RL12	60S ribosomal protein L12	1.233678052	0.237	Translation
209	LIAS	Lipoyl synthase, mitochondrial	1.246076729	0.230	Lipoylation peptidyl-N6-lipoyl-L-lysine.
210	RSP5	Flagellar radial spoke protein 5	1.258600015	0.010	Regulation of flagellar bending
211	EZY1	Ezy-1 protein	1.258600015	0.384	Uncharacterized protein
212	GLE	Autolysin	1.258600015	0.225	Cell wall organization
213	RSP14	Radial spoke protein 14	1.271249144	0.046	Cell motility, Endocytosis
214	RSP4	Flagellar radial spoke protein 4	1.271249144	0.272	Cell motility, cilium assembly
215	SLT2	Sodium/sulfate cotransporter 2	1.284025417	0.288	Potassium ion transport
216	CHLI	Magnesium-chelatase subunit	1.296930074	0.280	Insertion of Mg into protoporphyrin ring
217	IFT81	Intraflagellar transport protein 81	1.309964465	0.268	Transport of tubulin within the cilium
218	H4	Histone H4	1.309964465	0.218	Nucleosome remodelling
219	FENR	Ferredoxin--NADP reductase, chloroplastic	1.309964465	0.102	Regulating the cyclic and non-CEF
220	IFT56	Intraflagellar transport protein 56	1.323129814	0.479	Intraciliary transport
221	DC2L	Cytoplasmic dynein 2 intermediate chain 1	1.323129814	0.152	Regulates flagellar dynein activity
222	DAW1	Dynein assembly WDR repeat domains 1	1.336427477	0.053	Intraciliary transport and assembly
223	CFA91	Cilia- and flagella-associated protein 91	1.336427477	0.681	Cilium movement
224	RBS1	RuBisCO small chain 1, chloroplastic	1.336427477	0.100	Photosynthesis, Photorespiration
225	MBB1	PsbB mRNA maturation factor	1.349858824	0.802	mRNA processing via 5'-UTR of psbB
226	LISC	Lipoyl synthase, chloroplastic	1.377127754	0.040	Protein lipoylation
227	CTH1	Mg-protoporphyrin IX monomethyl ester	1.377127754	0.008	Photosynthesis, chlorophyll biosynthesis
228	RSSA	40S ribosomal protein SA	1.377127754	0.062	Translation
229	CFA45	Cilia- and flagella-associated protein 45	1.404947596	0.052	Cell motility
230	YPTC5	GTP-binding protein YPTC5	1.404947596	0.237	GTPase activity, protein transport
231	CF251	Cilia- and flagella-associated protein 251	1.404947596	0.406	Cilium movement
232	RS14	40S ribosomal protein S14	1.404947596	0.012	Translation
233	SODM	Superoxide dismutase [Mn], mitochondrial	1.41906754	0.624	Destroys superoxide anion radicals
234	URM1	Ubiquitin-related modifier 1 homolog	1.41906754	0.009	Endonuclease activity, Intron homing
235	RS3A	40S ribosomal protein S3a	1.433329435	0.871	Translation
236	MUT11	Protein Mut11	1.433329435	0.019	Involved in 'Lys-4' histone H3 methylation
237	TEKT	Tektin	1.433329435	0.478	Assembly of flagella and cilia
238	RPOB2	DNA-directed RNA polymerase beta	1.433329435	0.327	Transcription, DNA-templated
239	DYI3	Dynein, 70 kDa intermediate chain	1.476980773	0.502	Cilium movement
240	IFT57	Intraflagellar transport protein 57	1.476980773	0.561	Maintenance and formation of cilia
241	CP12	Calvin cycle protein CP12, chloroplastic	1.476980773	0.024	Assembly core complex of PRK/GAPDH
242	KTU	Protein kintoun	1.50681778	0.510	Axonemal dynein complex assembly
243	ODA1	Outer dynein arm protein 1	1.50681778	0.309	Cilium movement involved in cell motility
244	RTCB	RNA-splicing ligase RtcB homolog	1.50681778	0.442	tRNA splicing
245	GBLP	Guanine nucleotide- protein subunit beta	1.521961536	0.590	Transcription initiation and termination
246	PSBS1	Photosystem II protein PSBS1	1.537257535	0.330	Nonphotochemical quenching

247	NU5M	NADH-ubiquinone oxidoreductase chain 5	1.568312167	0.039	ATP synthesis
248	PSAB	PSI P700 chlorophyll a apoprotein A2	1.599994191	0.019	Primary electron donor of PSI
249	PSBB	Photosystem II CP47 RC protein	1.648721271	0.110	PET in photosystem II
250	SULP1	Sulfate permease 1, chloroplastic	1.648721271	0.081	Uptake and assimilation of sulfate
251	RSP6	Flagellar radial spoke protein 6	1.665291179	0.262	Cilium assembly
252	CP100	Cilia- and flagella-associated protein 100	1.750672504	0.464	Cell motility, Cilium movement
253	ATPX	ATP synthase subunit b', chloroplastic	1.768267039	0.008	ATP synthesis coupled proton transport
254	CALM	Calmodulin	1.768267039	0.351	Calcium-mediated signalling
255	PSBA	Photosystem II protein D1	1.840431425	0.003	Photosynthetic electron transport in PSII
256	WDR12	Ribosome biogenesis protein	1.840431425	0.471	LSU-rRNA from tricistronic rRNA
257	CATR	Caltractin	1.840431425	0.730	Cell cycle, Cell division
258	PSBC	PS II CP43 reaction center protein	1.858928051	0.006	PET in photosystem II
259	YPTC6	Ras-related protein YPTC6	1.993715528	0.265	GTPase activity
		Up-regulated proteins (FC ≥ 2.0)			
260	ALB32	ALBINO3-like protein 2, chloroplastic	2.095935534	0.818	Assembly and activity of LHC I and II.
261	H31	Histone H3 type 1	2.117000017	0.732	DNA binding, Chromosome stability
262	DRC8	Dynein regulatory complex protein 8	2.159766213	0.572	Regulates microtubule sliding
263	RK22	50S ribosomal protein L22, chloroplastic	2.203396474	0.034	Translation, Response to antibiotic
264	PSBR	Photosystem II protein PSBR, chloroplastic	2.944679677	0.862	Energy-dependent quenching-NPQ
265	HS22C	Heat shock 22 kDa protein, chloroplastic	3.455613498	0.001	Protein maturation and degradation
266	SC61B	Protein transport protein Sec61 beta	4.903749092	0.009	Intracellular protein transport
267	CYB6	Cytochrome b6	7.53832479	0.365	CEF around PSI and state transitions.
268	ATPD	ATP synthase delta chain, chloroplastic	69.40783595	0.048	ATP synthesis coupled proton transport
		Control specific protein			
269	RK16	50S ribosomal protein L16, chloroplastic	Control_W50		Translation
270	RK16	50S ribosomal protein L16, chloroplastic	Control_W50		Mitochondrial translation
271	FER	Ferredoxin, chloroplastic	Control_W50		electron transfer activity
272	RR19	30S ribosomal protein S19, chloroplastic	Control_W50		Translation
273	PSBE	Cytochrome b559 subunit alpha	Control_W50		PETC, Charge separation
274	RTL	Reverse transcriptase-like protein	Control_W50		cDNA synthesis
275	RL31	60S ribosomal protein L31	Control_W50		Translation, Ribosomal assembly
276	CYC	Cytochrome c	Control_W50		Final protein carrier in mitochondrial ETC
277	PETM	Cyt b6f complex subunit 7, chloroplastic	Control_W50		CEF around PSI and state transitions.
278	DYL2	Dynein 11 kDa light chain	Control_W50		Microtubule-based process
279	FLTOP	Protein Flattop homolog	Control_W50		Regulator of cilium basal body docking
280	YCX5	Uncharacterized trnR-chlB intergenic	Control_W50		Tag-protein stability
281	ATPF	ATP synthase subunit b, chloroplastic	Control_W50		ATP synthesis coupled proton transport
282	RK5	50S ribosomal protein L5, chloroplastic	Control_W50		Translation
		Treated specific protein			
283	UFM1	Ubiquitin-fold modifier 1	Treated_P5_500		Protein ufmylation
284	RR8	30S ribosomal protein S8, chloroplastic	Treated_P5_500		Translation
285	rpl36	50S ribosomal protein L36, chloroplastic	Treated_P5_500		Translation
286	MOC2A	Molybdopter synthase sulfur subunit	Treated_P5_500		Mo-molybdopter cofactor biosynthetic
287	H2A-II	Histone H2A	Treated_P5_500		Chromosomal stability
288	LI637	Group 1 truncated hemoglobin LI637	Treated_P5_500		Oxygen carrier activity
289	ARL3	ADP-ribosylation factor-like protein 3	Treated_P5_500		Cytokinesis and cilia signaling
290	GP1	Vegetative cell wall protein gp1	Treated_P5_500		Major component of outer cell wall W6
291	ND2	NADH-ubiquinone oxidoreductase chain 2	Treated_P5_500		NADH dehydrogenase activity
292	PSBL	Photosystem II reaction center protein L	Treated_P5_500		Photosynthesis, Correct PSII assembly
293	TBB	Tubulin beta-1/beta-2 chain	Treated_P5_500		GTPase activity

Summary

Summary

Light is an essential component in regulating the growth of plants and algae. If the light increases beyond the organism's assimilation capacity, it goes through multiple photoprotective mechanisms, and alternative CET pathways adjust the photosynthesis. CET is one of the alternative electron transport pathways for energy generation in algae that require PGR5 and PGRL1 proteins, but the role of these proteins in PSI photochemistry and photoprotection under high light is unclear. I have taken and grown *pgrl1* and *pgr5* mutants of *C. reinhardtii* to investigate how these mutants are involved in the photoprotection under high light.

In the first objective, I have grown the WT and mutant strain in TAP (supplying acetate, photoheterotrophic) and HS (absence of acetate, photoautotrophic) media. I have seen that the modification in the supply of carbon substrate impacts the growth and photosynthetic. In high light, mutants showed slower growth and diminished photosynthetic activity. Under high light, the *pgr5* mutant failed to grow in autotrophic conditions, whereas the presence of acetate has rescued growth to some extent, but it's growing well in a photoheterotrophic medium. It is interesting to observe the reduced photosynthetic activity and growth of *pgr5*, showing its importance under high light.

The high ETR of PSII and reduced NPQ in HS medium than the TAP indicate that photoautotrophic growth is better than photoheterotrophic but provides the least photoprotection. Further, I have checked the NPQ and Chl fluorescence and observed that the reduced NPQ and fluorescence in the mutants was due to the over-reduction of the cofactor downstream of the PQ pool.

The reduced NPQ and higher $Y(NO)$ of PSII have linked phenomena vital in acclimation and photoprotection. The NPQ mechanisms provide sinks for removing extra excitation energy, and multiple electron transport pathways potentially serve as alternative routes for electrons or ‘safety valves for photosynthesis in excess light conditions. The PGR5 and PGRL1 are crucial for lumen acidification (indirectly related to NPQ induction, i.e., photoprotection) and supply a high amount of ATP required for the progression of the Calvin-Benson Cycle.

Based on the result of the first objective, I want to establish the relationship between the NPQ, *pmf*, and ATP synthase activity in the absence of one of the proteins of CET pathways under high light. In the second objective, I observed higher energy quenching (qE, pH-dependent quenching) in WT than in mutants. Subsequently, the slower relaxation in the mutant than in WT, a phenomenon called inhibitory quenching (qI), which might be related to pH-independent NPQ, i.e., “sustained quenching (qH)” delivers photoprotection and permits their survival.

Another critical aspect of the CET is the generation of *pmf*, and dynamic fine-tuning between the two components of *pmf* remains controversial but is essential for regulating photosynthesis. However, the energy components of the *pmf*, ΔpH were reduced in *pgr5* and transiently increased the electrical potential difference component's ($\Delta\psi$) contribution, which might accelerate the photosynthetic acclimation.

The regulation of these *pmf* components maintains the integrity of the thylakoid membrane by balancing the counter ions. The *pgr5* mutant can indirectly balance the energy partition at PSI by substituting donor side limitation Y(ND) and increasing acceptor side Y(NA) under high light. Here, the Cyt *b₆f* complex balances these two mechanisms at PSI by selective electron transfer as photosynthetic control.

In the third objective, I want to check how the whole proteome and macromolecular organization are regulated under high light in relationship with our biophysical and biochemical data. The nLC-MS/MS method of whole-cell proteome analysis unravels the up and down-regulation of essential proteins responsible for high light tolerance in *C. reinhardtii*. We have compared Chlamydomonas proteome treated versus control.

Overall, 302 proteins from many functional categories were significantly affected together in the treatments and control, out of which 14 (4.64%) proteins were associated with PSII, 12 (3.97%) were involved in PSI, 11 (3.64%) were in Cyt b_6f complex, 10 (3.31%) were engaged in ATPase assembly, and 17 (5.62%) were involved in photosystems (PSI and PSII) assembly. The up and down-regulated proteins are mainly involved in photosynthesis, metabolic pathway, glycolysis, protein synthesis, cilia and flagella-associated proteins, and proteins involved in cytoskeleton assembly.

This study shows that the CET mutation affects the OEC assembly by inhibiting the oxygen uptake under the high light condition. High light-induced prevalent thylakoid destacking allows the mingling of PSII with PSI core and LHCs. BN-PAGE and circular dichroism studies indicate that CET mutation alters macromolecular assembly under high light stress. The CD data show that limited interaction between protein and pigment could prevent excitation energy transfer from the LHCII antenna to the PSII reaction center.

Furthermore, we observed that the Cyt b_6 expression is increased ~280 times more in *pgr5* mutant to regulate the development of *pmf* and ΔpH across the thylakoid membrane. The increased Cyt b_6 function in *pgr5* could be due to the compromised function of (cp) ATP synthase subunits for energy generation and photoprotection under high light.

Further, I have immunoblotted all the PSII, PSI, and associated light-harvesting complexes proteins compared with the total cell proteome and obtained exciting results that support our western blot data. The proteins representing PSII core PsbA (D1) and PsbD (D2) accumulated more in *pgrl1* and *pgr5* than the WT in high light.

In high light, CP 43 and CP47 showed a reduced amount in *pgr5* high light due to changes in chlorophyll and carotenoid content around the PSII protein, which coordinates with the cofactors for efficient energy transfer from the light-harvesting antenna to the photosystem core. Likewise, I can see that high light-induced photoprotective protein LHCSR3 is accumulated more in WT and *pgrl1*, but *pgr5* lacks complete expression of LHCSR3. In addition, the time point expression levels of PSBS protein continuously increased in *pgr5* compared to the WT and *pgrl1*, indicating that PSBS aids in *pgr5* for sustainable growth and further contributes to the photoprotection under high light.

Additionally, our proteome data shows that the PSBS protein isoform (PSBS1 and PSB2) expressed more in *pgr5* than WT and *pgrl1* under high light agrees with the protein data. After this, I conclude that the photoprotection can be promoted by LHCSR3; despite the absence of LHCSR3, the PSBS can work together or independently compensate each other in PGRL1 and PGR5 cyclic electron transport during high light photoacclimation and photoprotection.

Based on this study, I can say that this is an excellent aid to the scientific community in understanding the role of CET in photoprotection under high light. On the other hand, based on this, we can engineer some essential traits associated with LHCSR3 and PSBS proteins to generate high light-resistant varieties of plant systems.

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Publications

List of Publications

- 1- **Yadav R.M**, Aslam, S.M., Madireddi, S.K. Subramanyam R. Role of cyclic electron transport mutations *pgrl1* and *pgr5* in acclimation process to high light in *C. reinhardtii*. *Photosynth Res.* **146**, 247–258 (2020)

- 2- **Rath J.R.^{1#}**, **Pandey J.^{1#}**, **Yadav R.M.^{1#}**, Ramachandran, P., Zamal Y.M, MeKala N.R Subramanyam, R., Temperature-induced reversible changes of photosynthesis efficiency and organization of thylakoid membranes from pea (*Pisum sativum*). *Plant Physiology and Biochemistry*. 28 Feb 2022. (# - Equal contribution)

- 3-. Nama S, Madireddi SK, **Yadav R.M**, Subramanyam R. non-photochemical quenching- dependent acclimation and thylakoid organization of *Chlamydomonas reinhardtii* to high light stress. *Photosynth Res.* 2019 Mar;139(1-3):387-400

- 4- Chouhan N, Devadasu E, **Yadav R.M**, Subramanyam R. Autophagy Induced Accumulation of Lipids in *pgrl1* and *pgr5* of *C. reinhardtii* Under High Light. *Front Plant Sci.* 2022 Jan 25; 12:752634

- 5- Dhokne K, Pandey J, **Yadav R.M**, Ramachandran P, Rath JR, Subramanyam R. Change in the photochemical and structural organization of thylakoids from pea (*Pisum sativum*) under salt stress. *Plant Physiology and Biochemistry* 2022 Feb 0981-9428, 2022.02.004

- 6- Chouhan N¹, **Yadav R.M¹**, Pandey J¹, Subramanyam R* Macromolecular structural changes of thylakoids in high light from *pgrl1* and *pgr5* mutants of *Chlamydomonas reinhardtii*. Submitted in *Biochimica et Biophysica Acta -Bioenergetics* 15 Feb 2022.

7- **Yadav RM**¹, Nisha Chouhan¹, Sai Kiran Meddireaddi¹ and Rajagopal Subramanyam* Interplay between LHCSR3 and PSBS proteins play a photoprotective role in *pgr5* of *Chlamydomonas reinhardtii* under high light. Submitted in *Plant Journal* 28 Feb 2022.

8- Sai Kiran Madireddi¹, **Yadav RM**¹, Pushan Bag^{1 2}, , Mohammad Yusuf Zamal¹, Rajagopal Subramanyam*. Distinctive mechanism of LHCSR3 expression and function under osmotic stress *Chlamydomonas reinhardtii* (under review).

9-**Yadav RM**,¹ Sureshbabu Marriboina and Rajagopal Subramanyam^{1*} High light-induced changes in whole-cell proteomic profile and supercomplex organization in *pgrl1* and *pgr5* mutants (under review).



Role of cyclic electron transport mutations *pgrl1* and *pgr5* in acclimation process to high light in *Chlamydomonas reinhardtii*

Ranay Mohan Yadav¹ · Sabit Mohammad Aslam¹ · Sai Kiran Madireddi¹ · Nisha Chouhan¹ · Rajagopal Subramanyam¹

Received: 4 February 2020 / Accepted: 15 April 2020
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Abstract

Light is crucial for photosynthesis, but the amount of light that exceeds an organism's assimilation efficacy can lead to photo-oxidative damage and even cell death. In *Chlamydomonas (C). reinhardtii* cyclic electron flow (CEF) is very important for the elicitation of non-photochemical quenching (NPQ) by controlling the acidification of thylakoid lumen. This process requires the cooperation of proton gradient regulation (PGR) proteins, PGRL1 and PGR5. Here, we compared the growth pattern and photosynthetic activity between wild type (*137c*, *t222+*) and mutants impaired in CEF (*pgrl1* and *pgr5*) under photoautotrophic and photoheterotrophic conditions. We have observed the discriminative expression of NPQ in the mutants impaired in CEF of *pgrl1* and *pgr5*. The results obtained from the mutants showed reduced cell growth and density, Chl *a/b* ratio, fluorescence, electron transport rate, and yield of photosystem (PS)II. These mutants have reduced capability to develop a strong NPQ indicating that the role of CEF is very crucial for photoprotection. Moreover, the CEF mutant exhibits increased photosensitivity compared with the wild type. Therefore, we suggest that besides NPQ, the fraction of non-regulated non-photochemical energy loss (NO) also plays a crucial role during high light acclimation despite a low growth rate. This low NPQ rate may be due to less influx of protons coming from the CEF in cases of *pgrl1* and *pgr5* mutants. These results are discussed in terms of the relative photoprotective benefit, related to the thermal dissipation of excess light in photoautotrophic and photoheterotrophic conditions.

Keywords *Chlamydomonas reinhardtii* · Chlorophyll fluorescence · Cyclic electron transport · High light · Non-photochemical quenching · Photosystems

Introduction

Photosynthesis is the process that can harness solar energy to fix it as chemical energy in a biologically available form. This is a highly regulated process that involves different electron transfer pathways for the generation of ATP and NADPH. The major portion of electron flux required for the generation of ATP and NADPH is provided by linear electron flow (LEF). This process involves the transfer of

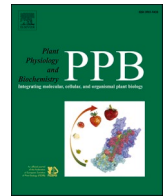
electrons from water, the primary electron donor, via photosystem (PS) II, several redox carriers, and PSI to the terminal electron acceptor NADP⁺. These electron transfer reactions are coupled with proton translocation from stroma to thylakoid lumen that generates proton motive force for the production of ATP by ATP synthase. The ATP and NADPH produced in this process are utilized by the Calvin–Benson cycle and other downstream metabolic reactions.

The stoichiometric proportions of 3ATP to 2NADPH required for the carbon fixation is slightly imbalanced towards NADPH in LEF. Therefore, the additional demand for ATP is compensated by the cyclic electron flow (CEF) (Alric 2010). CEF involves the reoxidation of NADPH and/or ferredoxin (FD) through plastoquinone (PQ), cytochrome (Cyt) *b₆f* complex and PSI. This process generates the transmembrane proton gradient utilized in the ATP synthesis (Kramer et al. 2004a, b). In vascular plants and green algae, the CEF occurs through two pathways, antimycin-sensitive

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11120-020-00751-w>) contains supplementary material, which is available to authorized users.

✉ Rajagopal Subramanyam
srgsl@uohyd.ernet.in

¹ Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad, Telangana 500046, India



Temperature-induced reversible changes in photosynthesis efficiency and organization of thylakoid membranes from pea (*Pisum sativum*)

Jyoti Ranjan Rath^{a,1}, Jayendra Pandey^{a,1}, Ranay Mohan Yadav^{a,1}, Mohammad Yusuf Zamal^a, Pavithra Ramachandran^a, Nageswara Rao Mekala^a, Suleyman I. Allakhverdiev^b, Rajagopal Subramanyam^{a,*}

^a Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, India

^b K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya St. 35, Moscow, 127276, Russia

ABSTRACT

High temperature can induce a substantial adverse effect on plant photosynthesis. This study addressed the impact of moderately high temperature (35 °C) on photosynthetic efficiency and thylakoid membrane organization in *Pisum sativum*. The Chl *a* fluorescence curves showed a significant change, indicating a reduction in photosynthetic efficiency when pea plants were exposed to moderate high-temperature stress. The pulse-amplitude modulation measurements showed decreased non-photochemical quenching while the non-regulated energy dissipation increased in treated compared to control and recovery plants. Both parameters indicated that the photosystem (PS)II was prone to temperature stress. The PSI donor side limitation increased in treated and recovery plants compared to control, suggesting the donor side of PSI is hampered in moderate-high temperature. Further, the PSI acceptor side increased in recovery plants compared to control, suggesting that the cyclic electron transport is repressed after temperature treatment but revert back to normal in recovery conditions. Also, the content of photoprotective carotenoid pigments like lutein and xanthophylls increased in temperature-treated leaves. These results indicate the alteration of macro-organization of thylakoid membranes under moderately elevated temperature, whereas supercomplexes restored to the control levels under recovery conditions. Further, the light harvesting complex (LHC)II trimers, and monomers were significantly decreased in temperature-treated plants. Furthermore, the amount of PSII reaction center proteins D1, D2, PsbO, and Cyt *b6* was reduced under moderate temperature, whereas the content of LHC proteins of PSI was stable. These observations suggest that moderately high temperature can alter supercomplexes, which leads to change in the pigment-protein organization.

1. Introduction

Plants are subjected to various biotic and abiotic stress factors during their growth period. Several abiotic stress factors affect plant growth, yield, and productivity. Among the abiotic stress factors, the role of temperature stress on the plant photosynthetic machinery cannot be overlooked. The remarkable growth, yield, and productivity of a plant depend on proper functioning of its photosynthetic machinery. Plants are exposed to fluctuations in temperatures throughout their growth period. Temperatures above the optimum levels that can induce any morphological, physiological, molecular, or biochemical changes to the exposed plant can be designated as heat stress (Wahid et al., 2007).

Several studies have revealed the physiological mechanisms involved in heat stress responses and their tolerance mechanism developed in plants. Since plants are sessile, they have to endure and develop specific preventive measures that help them adhere to this situation (Sattari et al., 2020). The productivity of a plant is affected in several

ways under high-temperature stress (Allakhverdiev et al., 2008). Although several physiological, biochemical, and molecular processes contribute to plant growth and productivity, photosynthesis is the major pathway that affects these processes directly or indirectly (Mathur et al., 2014). Temperature stress primarily inhibits plant photosynthesis before impairing other cell functions (Berry and Bjorkman, 2003). Any form of stress is initially perceived by the plant's photosynthetic machinery that includes both the photosystems (PSII and PSI) along with the oxygen-evolving complex (OEC), ATP synthase, and the enzymes involved in the dark reaction process (Aro et al., 1993; Mohanty et al., 2007).

Plant chloroplast pigments play an essential role in capturing light energy. They are also a constituent of the reaction centers (RC) and light-harvesting complexes (LHCs). The electron excitation from pigments leads to photochemical reactions, including the photolysis of water that mainly drives the photosynthesis process. Temperature stressed plants have reduced chlorophyll biosynthesis (Efeoglu and Terzioglu, 2009). This decrease is correlated with the inactivation of chlorophyll

* Corresponding author. Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, Telangana, India.

E-mail address: srgsl@uohyd.ac.in (R. Subramanyam).

¹ These authors contributed equally.



Autophagy Induced Accumulation of Lipids in *pgrl1* and *pgr5* of *Chlamydomonas reinhardtii* Under High Light

Nisha Chouhan, Elsinraju Devadasu, Ranay Mohan Yadav and Rajagopal Subramanyam*

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India

OPEN ACCESS

Edited by:

Milton Lima Neto,
São Paulo State University, Brazil

Reviewed by:

Fantao Kong,
Dalian University of Technology, China
Ayse Kose,
Ege University, Turkey
Justin Findinier,
Carnegie Institution for Science,
United States

*Correspondence:

Rajagopal Subramanyam
srgsl@uohyd.ernet.in

Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 03 August 2021

Accepted: 20 December 2021

Published: 25 January 2022

Citation:

Chouhan N, Devadasu E,
Yadav RM and Subramanyam R
(2022) Autophagy Induced
Accumulation of Lipids in *pgrl1*
and *pgr5* of *Chlamydomonas*
reinhardtii Under High Light.
Front. Plant Sci. 12:752634.
doi: 10.3389/fpls.2021.752634

Chlamydomonas (*C.*) *reinhardtii* is a potential microalga for lipid production. Autophagy-triggered lipid metabolism in microalgae has not been studied so far from a mutant of proton gradient regulation 1 like (PGR1) and proton gradient regulation 5 (PGR5). In this study, *C. reinhardtii* cells (wild-type CC124 and cyclic electron transport dependant mutants *pgrl1* and *pgr5*) were grown photoheterotrophically in high light 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, where *pgr5* growth was retarded due to an increase in reactive oxygen species (ROS). The lipid contents were increased; however, carbohydrate content was decreased in *pgr5*. Further, the Nile Red (NR) fluorescence shows many lipid bodies in *pgr5* cells under high light. Similarly, the electron micrographs show that large vacuoles were formed in high light stress despite the grana stacks structure. We also observed increased production of reactive oxygen species, which could be one reason the cells underwent autophagy. Further, a significant increase of autophagy ATG8 and detections of ATG8-PE protein was noticed in *pgr5*, a hallmark characteristic for autophagy formation. Consequently, the triacylglycerol (TAG) content was increased due to diacylglycerol acyltransferases (DGAT) and phospholipid diacylglycerol acyltransferase (PDAT) enzymes' expression, especially in *pgr5*. Here the TAG synthesis would have been obtained from degraded membrane lipids in *pgr5*. Additionally, mono, polyunsaturated, and saturated fatty acids were identified more in the high light condition. Our study shows that the increased light induces the reactive oxygen species, which leads to autophagy and TAG accumulation. Therefore, the enhanced accumulation of TAGs can be used as feedstock for biodiesel production and aqua feed.

Keywords: *Chlamydomonas reinhardtii*, cyclic electron transport, lipid bodies, Nile Red fluorescence, triacylglycerol

INTRODUCTION

Light is vital for microalgae for efficient photosynthesis. CO_2 fixation by Calvin Benson cycle occurs through photosynthesis that primarily synthesizes carbohydrates, leading to the synthesis of lipid stored as triacylglycerols (TAG) (Mondal et al., 2017). Microalgal species do not accumulate increased amounts of neutral lipids under normal growth conditions. Neutral lipid is accumulated under unfavorable conditions like nutrient, light, salt, and temperature stresses. Under normal light



Non-photochemical quenching-dependent acclimation and thylakoid organization of *Chlamydomonas reinhardtii* to high light stress

Srilatha Nama¹ · Sai Kiran Madireddi¹ · Ranay Mohan Yadav¹ · Rajagopal Subramanyam¹

Received: 3 April 2018 / Accepted: 30 June 2018 / Published online: 7 July 2018
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Abstract

Light is essential for all photosynthetic organisms while an excess of it can lead to damage mainly the photosystems of the thylakoid membrane. In this study, we have grown *Chlamydomonas reinhardtii* cells in different intensities of high light to understand the photosynthetic process with reference to thylakoid membrane organization during its acclimation process. We observed, the cells acclimatized to long-term response to high light intensities of 500 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with faster growth and more biomass production when compared to cells at 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. The ratio of Chl *a/b* was marginally decreased from the mid-log phase of growth at the high light intensity. Increased level of zeaxanthin and LHCSR3 expression was also found which is known to play a key role in non-photochemical quenching (NPQ) mechanism for photoprotection. Changes in photosynthetic parameters were observed such as increased levels of NPQ, marginal change in electron transport rate, and many other changes which demonstrate that cells were acclimatized to high light which is an adaptive mechanism. Surprisingly, PSII core protein contents have marginally reduced when compared to peripherally arranged LHCII in high light-grown cells. Further, we also observed alterations in stromal subunits of PSI and low levels of PsuG, probably due to disruption of PSI assembly and also its association with LHCI. During the process of acclimation, changes in thylakoid organization occurred in high light intensities with reduction of PSII supercomplex formation. This change may be attributed to alteration of protein–pigment complexes which are in agreement with circular dichroism spectra of high light-acclimatized cells, where decrease in the magnitude of psi-type bands indicates changes in ordered arrays of PSII–LHCII supercomplexes. These results specify that acclimation to high light stress through NPQ mechanism by expression of LHCSR3 and also observed changes in thylakoid protein profile/supercomplex formation lead to low photochemical yield and more biomass production in high light condition.

Keywords *Chlamydomonas reinhardtii* · Energy transfer · High light stress · Light-harvesting complexes · Photosystems · Reaction centers · Thylakoid organization

Introduction

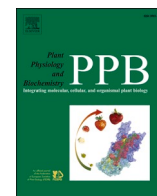
Light energy is the major source for the photosynthetic process in which carbon fixation occurs for food production. Light-dependent reactions occur in thylakoid membrane that consists of multi-protein membrane complexes namely

photosystem (PS) I and II that are connected by cytochrome (Cyt) *b6/f* complex. Both complexes are composed of core proteins that are responsible for charge separation and electron transport where an outer antenna increases the absorption capacity of photons. PSII and PSI complexes were functionally connected with each other and to their electron donor/acceptor and their capacity for absorbing photons increases by light-harvesting antenna complexes (LHCs) which binds with chlorophyll and carotenoid molecules. The LHCs have a conserved structure with three transmembrane helices and organized with a maximum of 14 chlorophylls (Chls) per monomer (5–9 Chl *a* and 4–6 Chl *b*). LHCs also have two carotenoid binding sites i.e., for lutein (Lut) and violaxanthin (Vio) while some LHCs have additionally two sites N1 for neoxanthin (Neo) and V1 for Vio/Lut located

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11120-018-0551-7>) contains supplementary material, which is available to authorized users.

✉ Rajagopal Subramanyam
srgsl@uohyd.ernet.in; rajagopal98@gmail.com

¹ Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, Telangana, India



Change in the photochemical and structural organization of thylakoids from pea (*Pisum sativum*) under salt stress

Kunal Dhokne^{a,b}, Jayendra Pandey^a, Ranay Mohan Yadav^a, Pavithra Ramachandran^a, Jyoti Ranjan Rath^a, Rajagopal Subramanyam^{a,*}

^a Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, India

^b Department of Botany, Shri Vitthal Rukmini College, Sawana, Yavatmal, 445001, India

ARTICLE INFO

Keywords:

Chl *a* fluorescence
Non-photochemical quenching
Pigment-protein complexes
Photosystem
Pisum sativum
Proton motive force
Salt stress
Thylakoid organization

ABSTRACT

Salt can induce adverse effects, primarily on the photosynthetic process, ultimately influencing plant productivity. Still, the impact of salt on the photosynthesis process in terms of supercomplexes organization of thylakoid structure and function is not understood in Pea (*Pisum sativum*). To understand the structure and function in the leaves and thylakoids under salt (NaCl) treatment, we used various biophysical and biochemical techniques like infrared gas analyzer, chlorophyll *a* fluorescence, circular dichroism, electron microscopy, blue native gels, and western blots. The net photosynthetic rate, transpiration rate, and stomatal conductance were reduced significantly, whereas the water use efficiency was enhanced remarkably under high salt conditions (200 mM NaCl). The photochemical efficiency of both photosystem (PS) I and II was reduced in high salt by inhibiting their donor and acceptor sides. Interestingly the non-photochemical quenching (NPQ) is reduced in high salt; however, the non-regulated energy dissipation (NO) of PSII increased, leading to inactivation of PSII. The obtained results exhibit inhibition of NAD(P)H dehydrogenase (NDH) mediated pathway-dependent cyclic electron transport under salinity caused a decrease in proton motive force of ΔpH and $\Delta \psi$. Further, the electron micrographs show the disorganization of grana thylakoids under salt stress. Furthermore, the macro-organization and super-complexes of thylakoids were significantly affected by high salt. Specifically, the mega complexes, PSII-LHCII, PSI-LHCI, and NDH complexes were notably reduced, ultimately altering the electron transport. The reaction center proteins of oxygen-evolving complexes, D1 and D2 proteins were affected to high salt indicating changes in photochemical activities.

1. Introduction

Salinity stress is a major environmental threat to the world by restricting plant growth, leading to ultimately reduced crop yield. Since photosynthesis is one of the most important metabolic processes in plants, the adverse impact of salinity generally caused by developing osmotic stress and ion-specific toxicity (Na^+) limiting the crop productivity (Pandolfi et al., 2012; Roy et al., 2014). This dual stress condition induces significant changes in photosynthetic activities by inhibiting the quantum yield of photosystem (PS)II electron transport and disorganizing the thylakoid membrane.

Photosynthetic efficiency often decreased under the salinity stress (Yuan et al., 2014; Shu et al., 2019). PSII is susceptible to salinity stress causes the photoinhibition of PSII, which leads to the generation of reactive oxygen species (Gururani et al., 2015). Salt stress damages the oxygen evolving complex (OEC), blocks the electron from photo-oxidized water to PSII donor side and acceptor side of PSII and inactive the PSII reaction center (Lu et al., 2020; Demetriou et al., 2007; Goussi et al., 2018). In higher plants, the OEC 23 kDa protein, which is extrinsically bound to PSII, was dissociated under salt stress (Murata et al., 1992). The previous reports exhibit that both PSI and PSII were inactivated by osmotic stress (Allakhverdiev et al., 2000). Other reports

Abbreviations: β -DM, n-dodecyl β -D-maltoside; Chl, chlorophyll; F_0 , initial fluorescence; F_m , maximum fluorescence; kDa, kilodaltons; LHCI, light-harvesting chlorophyll-protein of PSI; LHCI, light-harvesting chlorophyll-protein of PSII; PAGE, polyacrylamide gel electrophoresis; PSI, photosystem I; PSII, photosystem II; NPQ, non-photochemical quenching; OEC, oxygen-evolving complex; PPFD, photosynthetic photon flux density; SDS, sodium dodecyl sulfate; WUE, water use efficiency; Y(NA), acceptor side limitation of PSI; Y(ND), donor side regulation of PSI.

* Corresponding author. Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046, Telangana, India.

E-mail address: srgsl@uohyd.ac.in (R. Subramanyam).

<https://doi.org/10.1016/j.plaphy.2022.02.004>

Received 17 January 2022; Received in revised form 2 February 2022; Accepted 7 February 2022

Available online 12 February 2022

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***Acclimation and Organization of
Photosynthetic Apparatus to High Light
in PGRL1 and PGR5 mutants of
Chlamydomonas reinhardtii: A
Functional and Proteomic Approach***

by Ranay Mohan Yadav

Submission date: 04-July-2022, 04:19 PM (UTC+ 0530)

Submission ID: 1866530927

File name: Thesis_Ranay_Mohan_Yadav_plagiarism_16LPPH14.docx (202.06K)

Word count: 34947

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