

Light intensity-dependent changes in the photochemistry of photosystem II, cell constituents, and lipid accumulation in the Wild-type (WT) and DCMU-tolerant mutant of green alga *Scenedesmus vacuolatus*

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Abstract

Microalgae utilize light energy for photosynthesis; however, variations in light intensities can have antagonistic and synergistic effects on its growth and cell constituents. In the present experimental study, photodynamic effects of light intensity on the growth, cell constituents, photochemistry of PS II, and lipid accumulation were studied in wild-type (WT) and DCMU-tolerant mutant strains of *S. vacuolatus*. Microalgal algal cells (WT and DCMU-tolerant mutant) were exposed to light intensities ranging from 10 to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and cultured in a BG-11 nutrient growth medium. Overall results showed higher light intensity tolerance in the mutant strain (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than the WT (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The photosynthetic parameters derived from chlorophyll fluorescence induction kinetics (OJIP), non-photochemical quenching (NPQ), and Light curve (rETR) revealed better photosynthetic performance by the mutant strain than the WT under the high light stress. Thus, it was inferred that, unlike the WT, a better photosynthetic efficiency coupled with an improved photo-protection mechanism in the mutant strain at higher light intensities might be contributing to an enhanced level of cell constituent and lipid accumulation in the mutant strain.

Highlight

- Photodynamic effects of light growth, cell constituents, photochemistry and lipid
- Higher light intensity tolerance in the mutant strain than the WT
- Photosynthetic parameters OJIP, NPQ and rETR give better performance by Mutant strain
- A comparative FTIR and FC analysis showed higher lipid in the mutant strain

1. Introduction

Microalgae are photosynthetic microorganisms that are characterized by photoautotrophic metabolic pathways and exist predominantly in the aquatic ecosystem. Microalgae play a significant role in carbon dioxide fixation and drive the food chain of aquatic ecosystems. Algal biomass is a source of valuable bioproducts that can be used directly or indirectly in various sectors such as food, cosmetics, pharmaceutical, energy, etc. Large-scale algal biomass cultivation is a major challenge due to the fluctuating nutritional and environmental conditions (temperature, irradiation). A drastic change in the nutrient condition, pH, light intensity (low or high), temperature, and salinity can severely affect the total biomass productivity and quality of bioproducts (Brindhadevi et al., 2021). In general, the optimum growth of many photosynthetic organisms is observed at photosynthesis saturating light intensity (6000 lux) (Mandotra et al., 2016). An excess of light energy irradiance leads to photo-destruction of the photosynthetic components due to a variety of photosynthetically generated reactive oxygen radicals (ROS) (Nishiyama et al., 2001). An increase in the level of intracellular ROS causes extensive cellular damage including DNA and membrane lipids (Niyogi, 1999). Fortunately, many microalgae are known to have very strong antioxidative defense systems such as the presence of carotenoids, superoxide dismutase, and ascorbate-glutathione peroxidase enzyme systems, which can offset the adverse effects of ROS generated under the high-intensity light irradiance (Shi et al., 2017; Garg and Manchanda, 2009). Glycollate production is also considered to be an antioxidative system that can minimize the negative effect of oxidative stress (Pope, 1975). Among the photoautotrophs, another mechanism to protect themselves against excess light intensity irradiance includes the down-regulation of photosynthetic machinery by photo-inhibition of the PSII reaction center (Vonshak and Novoplansky, 2008). However, the molecular mechanism of PSII photo-inhibition is not yet fully understood.

Microalgae are also considered to be the most promising source of biofuel production due to an easier method of cultivation, photoautotrophic nature, and faster growth, which facilitate large-scale production of biomass with higher lipid content (Cordara et al., 2018). Wahidin et al. (2013) reported that high light intensity triggers the accumulation of neutral lipids in the microalgae *Scenedesmus abundance*, *Nanochloropsis sp.*, and *Botryococcus braunii*. It has been suggested that high-energy irradiation has the potential to influence both carbon sequestration and its allocation by altering cell metabolism (He et al., 2015; Jungandreas et al., 2014). A deeper understanding of the molecular basis of light use efficiency and cellular defense strategy under varying light intensities is seminal to optimizing microalgal cultivation and biofuel production.

Thus, the present investigation aims to study the effect of high light stress on the growth and photodynamic effect in wild-type (WT) and DCMU-tolerant mutant strain of microalga *Scenedesmus vacuolatus* under controlled culture conditions. Further, efforts have been made to correlate the high light tolerance and efficiency of the photosynthetic machinery of microalgal cells with the lipid accumulation in both strains.

2. Materials And Methods

2.1. Cultivation and experimental conditions

The microalgal culture was isolated from Pangong Lake in Jammu and Kashmir, India's Ladakh (or Leh) region. The culture was grown in a BG-11 medium, which has a pH of (7.4 ± 0.2) (Stanier et al., 1971). and purified by spreading method on agar plates. *Scenedesmus vacuolatus* was identified as the microalgal strain (wild-type) by the National Collection of Industrial Microorganisms (NCIM; CSIR-NCL, Pune, India) using 18S rRNA gene sequencing. The sequence was submitted to the NCBI (National Center for Biotechnology Information) (NCBI GenBank accession number MH459062). In the current study, DCMU-tolerant mutant cells (isolated by chemical mutagenesis using ethyl methanesulphonate (EMS) in presence of DCMU (75 M)) were originally inoculated into a monoclonal culture of wild-type (WT) and DCMU-tolerant mutant cells. (0.05 OD) in BG-11 media at 25°C for 7.0 days, as determined by a white fluorescent tube with a 16 / 8 h light/dark cycle. The experiment was done in the presence of light intensity ($10\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$) exposed to WT and mutant cells growing in the BG-11 medium. The light intensity is provided by the LED Floodlight (Philips, India Ltd). The light source is provided beneath the culture flask by fixing the light intensity position of each flask by solarimeter (KM-SPM-530). The culture flask inoculated the exponential phase of both WT and mutant cells of approx. (0.1 OD) in each flask, maintained $25 \pm 2^\circ\text{C}$ temperature, 16/8 h light/dark cycle. The experiment was observed in triplicate under similar conditions for 20 days. The culture flask was shaken five times a day for avoiding sticking and settling of cells.

2.2. Measurement of growth, biochemical constituents, and pigment analysis

The growth rate, pigment, and other cell constituents in both the wild-type (WT) and mutant strain of *S. vacuolatus* were measured at regular intervals for 0–20 days in response to different light intensities ($10\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$).

A double-beam UV-visible spectrophotometer was used to calculate the specific growth rate (μ , d⁻¹) of the microalgal cell solution at 680 nm (Yu et al., 2017). (Shimadzu, Japan 1601). The pigment was extracted by using acetone (80%, v/v), Chlorophyll and carotenoid contents were quantified as described by Lichtenthaler, (1987).

2.3. Flow cytometer analysis

WT and mutant cells' stationary phase cultures were centrifuged at 5000 g for 10 min., and the debris was then removed by washing with PBS (Phosphate buffered saline). By using a flow cytometer (BD FACS Influx™, Becton Dickinson, San Jose, CA, USA), the lipid content was determined. Data were then analyzed using BD FACSTM software 1.2.0.87. For the experiment, microalgal cells suspension was taken in the test tube followed by DMSO (dimethyl sulfoxide) 10% (v/v) then supplemented with Nile red (0.1 mg mL^{-1}) and incubated for 15 min at room temperature under darkness each sample had at least 10,000 events recorded in total, and the lipid content was calculated as the percentage of total fluorescence emission value corrected for autofluorescence given off by cells that had not been stained with Nile red. Excitation/emission maxima (488 nm and 580 nm, respectively) were recorded using the flow cytometer solid-state blue laser (Satpati and Pal, 2014).

2.4. Measurement of RNO bleaching

The exponentially grown cells of the WT and mutant strain of *S. vacuolatus* were centrifuged at $5000 \times g$ for 10 min and washed with HEPES buffer (pH 7.5, 20 mM). The pellet was suspended in the same buffer before ultrasonic breakage of the cells for 5 min. The unbroken cell was removed by low-speed centrifugation ($1000 \times g$ for 5 min). The supernatant was supplemented with RNO (N, N Diethyl p-nitrosoaniline) ($10 \mu\text{g/mL}$) prepared and incubated under different light intensities for

15 min. The light-induced bleaching of RNO was recorded at 440 nm in a UV-Visible spectrophotometer (UV-A 1601 Shimadzu, Japan) as described by Joshi and Pathak, (1984). The rate of RNO bleaching was expressed as $\Delta OD_{440\text{nm}}/\text{mg protein}/\text{min}$.

Radical quenchers such as histidine, sodium formate, and sodium azide (5 mM each) were added to the cell suspension before adding the RNO. The reaction mixture was incubated at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity for 15 min. The rate RNO bleaching was recorded at 440 nm as described above. A control set of RNO bleaching without radical quenchers was run in parallel.

2.5. Measurement of glycollate activity

The glycollate content was measured in the WT and mutant cells of *S. vacuolatus* by the method of Takahashi, (1972). The cell suspension 150 μL was mixed with 3 mL of reagent (0.1% (w/v) solution of 2,7-dihydroxy naphthalene) (Sigma, USA), in conc. H_2SO_4 . The reaction mixture was kept in a boiling water bath for 20 min. After cooling down the mixture at room temperature and collecting the condensed water vapors inside the walls of the test tube, the reaction mixture was centrifuged at $5000 \times g$ for 5 min to remove the turbidity. The absorbance of clear supernatant was read at 540 nm in a double beam UV-Visible spectrophotometer (UV-A 1601 Shimadzu, Japan) against an appropriate blank. Total glycollate content was expressed as $\mu\text{mol glycollate}/\text{mg protein}$.

2.6. Measurement of superoxide dismutase (SOD) activity

The method was used to analyze the SOD activity (Nishikimi and Rao, 1972). Based on the suppression of NADPH-Phenazine methosulfate-nitroblue tetrazolium formazon production, the SOD enzyme test was developed. A double-beam UV-visible spectrophotometer was used to measure the color creation after the reaction at 560 nm. The amount of enzyme needed to prevent 50% of NBT decrease in one minute is considered one unit of enzyme activity.

2.7. Measurement of chlorophyll fluorescence induction kinetics (OJIP), non-photochemical quenching (NPQ), and light curve (LC)

- Chlorophyll a fluorescence transient was measured by using Pulse Amplitude Modulated (PAM) (Aquapen-C AP 110-C, Photon Systems Instrument, Czech Republic). For the measurement of the chlorophyll fluorescence induction curve (OJIP), the cells were dark-adapted for 10 min at room temperature. The induction kinetics of chlorophyll fluorescence was recorded at an excitation wavelength of 450 nm. The fluorescence parameters (quantum yield F_v/F_m , ET_0/RC , TR_0/RC , ABS/RC , and RC/ABS) were determined as described by Strasser et al., (2000).
- Non-photochemical quenching (NPQ) is based on a flash of measuring light to acquire a minimal level of fluorescence (F_0). Thereafter, a short saturating flash of light is applied to achieve maximum fluorescence (F_m) level in the dark-adapted cells. After a short dark period, the sample is again exposed to actinic irradiance for 20 seconds to elicit a transient of the Kautsky effect. Moreover, a series of saturating flashes is applied on top of the actinic light to probe the NPQ and effective quantum yield (QY) of photosynthesis in the light-adapted state. The NPQ value under low and high light in both strains was determined Lichtenthaler et al., (2005).
- The light-Response Curve relating to the rate of photosynthesis to photon flux density was based on the successive exposure of the samples to a stepwise increase in light intensity (100, 200, 300, 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The relative photosynthetic electron transport rate (rETR) value through PSII was determined in exposed cells of WT and mutant strain in low and high light (Malapascua et al., 2014).

2.8. Fourier Transform Infrared (FTIR)

In stationary phase culture, the dried biomass of WT and mutant cells were mixed with IR grade KBr (1:10, w/w) after being air dried. Both samples were converted into pellets using a vacuum and a Spectra lab pelletizer before being scanned (4000 – 400 cm^{-1}). Using the software Omnic 8.0.342, the data were baseline corrected for background absorbance by KBr (Thermo scientific).

2.9. Statistical analysis

The experiment was completed in triplicate, and the results were presented as mean SD. To determine whether there was a significant difference between the treatments for WT and mutant, the statistical analysis paired sample Student 't'-test (p0.05) was used. Using SPSS version 21.0, the statistical analysis was carried out (IBM Co., Armonk, NY, USA)

3. Results

3.1. Effect of light intensity on growth

The specific growth rate of both the WT and mutant strain of *S. vacuolatus* was measured throughout 0-20 d in response to different light intensities ($10\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). The maximum specific growth rate of WT was achieved at $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity ($0.079\pm0.007\text{ d}^{-1}$) whereas the mutant strain showed optimum specific growth rate at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ($0.135\pm0.12\text{ d}^{-1}$) (Table 1). The results showed about 50% higher light tolerance in the mutant strain than in the WT. However, the lowest specific growth rate of the WT ($0.022\pm0.002\text{ d}^{-1}$) and mutant ($0.051\pm0.003\text{ d}^{-1}$) strain was recorded at $100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. The overall growth of the mutant strain under varying light intensities was higher than the WT.

3.2. Pigments analysis

The result on total chlorophyll content in the WT was found to be maximum at $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ grown cells ($2.06\pm0.18\ \mu\text{g mL}^{-1}$), followed by the cells grown at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity grown cells ($1.51\pm0.12\ \mu\text{g mL}^{-1}$). Whereas total chlorophyll contents in the mutant strain were higher at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity ($3.62\pm0.33\ \mu\text{g mL}^{-1}$), followed by $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity grown cells ($2.93\pm0.26\ \mu\text{g mL}^{-1}$). The carotenoid content in the WT strain was found to be the highest at $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ($2.99\pm0.26\ \mu\text{g mL}^{-1}$), followed by the cells grown at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ($2.40\pm0.20\ \mu\text{g mL}^{-1}$). Similarly, the carotenoid content in the mutant strain was maximum at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity ($5.60\pm0.52\ \mu\text{g mL}^{-1}$), followed by the cells grown at $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity ($4.56\pm0.41\ \mu\text{g mL}^{-1}$) (Table 2). The level of photosynthetic pigments in both strains was found to be the lowest in the cells grown at $100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity. The statistical analysis also showed a significant difference in value between WT and mutant strains. The overall results indicated a higher level of pigments and other cell constituents in the mutant strain, despite its higher level of high light tolerance.

3.3. Flow cytometer analysis

Flow cytometer analysis of neutral lipid of the WT and mutant cells was grown under various light intensities and exhibited a light intensity-dependent increase in the total lipid content of both the strains, depending upon their respective metabolic response. Initially, the level of lipid increased in an intensity-dependent manner in both the strains up to their respective growth saturating light intensity. The results revealed a maximum percentage of lipid yield in the WT ($37.40\pm2.5\%$) at $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ as compared to unstained cells (without the Nile red) (Fig. 1). However, the mutant cells grown at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ showed maximum lipid content ($46.87\pm3.0\%$) as in (Fig. 2). An increase in the light intensity beyond the tolerance limit of both the WT and mutant strain ($80\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) exhibited intensity dependent declining pattern.

3.4. Effect of RNO bleaching, glycollate, and SOD activity on light intensity

The rate of RNO bleaching in the cells grown under varying light intensities ($10\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) demonstrated a light intensity-dependent increase in the rate of RNO bleaching in the WT and mutant strain (Fig. 3 a). The highest rate of RNO bleaching was recorded at $100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity in both the strains (8.34 ± 0.70 , $3.94\pm0.21\text{ ng/mg protein/min}$ respectively). The results revealed a two-fold higher rate of RNO bleaching in the WT than in the mutant cells. This suggested that photo-induced production of oxyradicals was about two-fold higher in the WT than in the mutant.

The RNO bleaching in both the WT and mutant cells incubated in the presence of different radical quenchers like histidine, sodium formate, sodium azide (5 mM, each) at $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light intensity showed a maximum inhibitory effect of histidine (38.8 and 40.6%, respectively), followed by the effect of formate (59.7 and 65.5%, respectively) and sodium azide (86.1 and 87.5%, respectively) (Table 3). The inhibitory effect of radical quenchers was compared with the control (100%, RNO bleaching

without radical quenchers). Since the histidine and formate are quenchers of superoxide and hydroxyl radicals, the results suggested photooxidative damage in both strains, mainly caused by the superoxide radicals, followed by hydroxyl radicals.

The result on glycollate content in both the strains incubated under varying light intensities showed the maximum glycollate content in the WT ($153 \pm 11.7 \mu\text{M}$ glycollate/mg protein/h) grown at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and the mutant strain ($273 \pm 16.6 \mu\text{M}$ glycollate/mg protein/h) grown at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3 b). A further increase in the light intensity beyond the light intensity tolerance limit showed a gradual decrease in the glycollate content. However, the overall glycollate content in the mutant was relatively higher than the WT throughout the range of light intensity used.

The result on SOD activity in both the WT and mutant strain exhibited the highest rate of SOD activity in the WT (223 ± 16.2 Unit/g) at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and in the mutant (414 ± 35.6 Unit/g) at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity (Fig. 3 c). Further increase in the light intensity beyond the tolerance limit showed a gradual decreasing pattern in the activity of SOD enzyme. However, the overall rate of SOD activity was higher in the mutant strain than in the WT.

3.5. FTIR analysis

When the WT and mutant strains of cells were grown under different light irradiances, FTIR spectra of the cell biomass revealed variations in the IR absorption bands over the wavelength range $4000\text{--}400 \text{ cm}^{-1}$. The associated functional groups and each IR absorption band allocated to a particular molecular group of the biochemical contents IR bands attributed to stretching of amide I (C=O) (1655 cm^{-1}), (N–H) bending of amide II (1545 cm^{-1}), (C=O) of the ester group of lipid and fatty acid (1740 cm^{-1}), (C–O–C) stretching associated with polysaccharides/carbohydrate (1040 cm^{-1}), and lipid at 2920 cm^{-1} and 3011 cm^{-1} were considered for interpretation of results (Stehfest et al., 2005; Murdock and Wetzel, 2009). The compositional changes analyzed in the WT and mutant strain of *S. vacuolatus* was in terms of total lipid ($2920/3011 \text{ cm}^{-1}$), lipid/carbohydrate (L/C) ($1740/1040 \text{ cm}^{-1}$), carbohydrate/protein (C/P) ($1040/1650 \text{ cm}^{-1}$), and lipid/protein (L/P) ($1740/1650 \text{ cm}^{-1}$) ratio (Dean et al., 2010; Laurens et al., 2011) (Table 4 a, b). The result observed that the total lipid ratio in both the WT and mutant strain was grown in the presence of varying light intensities ($10\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and initially showed an intensity-dependent gradual increase, followed by a decline at a higher light intensity ($80\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The maximum level of total lipid in the WT (1.3 ± 0.1) and mutant (1.8 ± 0.08) cells was observed at their growth-saturating light intensities (40 and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). Similar observations were made for the result of C/P and L/P ratio in the WT cells was found to be the highest at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4 a) and in mutant cells at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity (Table 4 b). Together, these findings showed considerable compositional changes in both strains' lipid, carbohydrate, and protein contents, which were correlated with their capacities for light tolerance.

3.6. Effect of light intensity on chlorophyll fluorescence induction kinetics (OJIP), non-photochemical quenching (NPQ), and light curve (LC)

The impact of light intensity ($10\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$) on the PSII photochemistry of *S. vacuolatus* cells was analyzed in terms of Chlorophyll fluorescence induction kinetics, non-photochemical quenching (NPQ) and light curve (LC). Data about changes in the OJIP parameter are presented in Table 5. Different parameters such as F_v/F_m , ET_0/RC , TR_0/RC , ABS/RC , and RC/ABS derived from the OJIP curve showed an increasing trend with increasing light intensity in both the WT mutant cells up to their respective light tolerance limits (40 and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). A maximum increase in the quantum yield (F_v/F_m) of PSII of WT (0.762 ± 0.05) was observed at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$, while the quantum yield in the mutant cells (0.911 ± 0.05) was the highest at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4). The ABS/RC , denoting absorption of energy flux per active reaction center, showed the highest value in the WT cells (1.34 ± 0.13) at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas ABS/RC value in the mutant strain (1.52 ± 0.10) was maximum at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similarly, the values of TR_0/RC and ET_0/RC , denoting respective efficiency of electron transport leading to reduction of QA and beyond in the photosystem II, were found to be maximum in both the WT and mutant strain at 40 and $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, (0.92 ± 0.04 , 1.07 ± 0.10 respectively).

These results suggested that the photosynthetic yield and electron transport in both strains were maximum at their respective light-tolerance limit. In both strains, the light intensities ($80\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) beyond their respective photosynthesis-saturating light intensities showed down-regulation of the photosynthetic system. However, the photosynthetic parameters derived from the OJIP curve showed better light utilization efficiency and higher quantum yield in the mutant strain than the WT throughout the range of applied light intensities. Further, as evident from the OJIP curves, it was observed that a shorter time was taken by the mutant strain than the WT to reach the fluorescence maxima (F_m), indicating an efficient electron transfer in the mutant strain on the reducing side of PSII, which might be due to reduced probability of closure of PSII reaction center. A longer duration of time taken by the WT to reach F_m value and reduced quantum yield (F_v/F_m) might be contributed to sluggish electron transfer on the donor side of PS II reaction center.

The results on non-photochemical quenching (NPQ) measured at two light intensities i.e., low light (LL) $10\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ and high light (HL) $60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ showed relatively higher NPQ values in WT than the mutant strain. But the NPQ value in the high light ($60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) grown cells of both the WT and mutant strain (0.36 ± 0.03 and 0.22 ± 0.02 , respectively) registered a significant decline as compared to corresponding NPQ values obtained in the low light grown cell (0.44 ± 0.031 and 0.29 ± 0.017 , respectively) (Fig. 5). However, overall NPQ value in the mutant strain was significantly lower than the WT. The results indicated greater dissipation of absorbed light energy in the WT than in the mutant strain.

The light response curve of both the WT and mutant strains was recorded in the cells, particularly grown under two light conditions i.e., high light ($60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) and low light conditions ($10\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). The relative electron transport rate (rETR), was calculated from the light curve (LC) obtained under different actinic light intensities ($100, 200, 300$, and $500\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). The rETR values in both the WT and mutant strains exhibited a different level of photosynthetic yield under the range of light irradiance used for the light curve. The rETR value for the WT showed its maxima at ($200\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) which was about 33% lower than that recorded for the mutant strain ($300\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) (Fig. 6).

The rETR values of both strains indicated their respective photosynthetic performance, depending upon their light tolerance and light harvesting efficiencies. Beyond their photosynthesis saturating light intensities, both the WT and mutant dissipate the excess light energy in the form of heat. Thus, all the photosynthetic parameters indicated better photosynthetic performance by the mutant strain than the WT even at a higher light intensity.

4. Discussion

In general increase in light intensity has been found to increase the growth of alga up to a certain level, however, it varies with microalgal species. Acclimation of microalgae to high light intensity is often accompanied by an improved mechanism for handling excess energy that involves different strategies for the regulation of photosynthesis and photoprotection to avoid the damage induced by higher/ stressful light intensity (Erickson et al., 2015). In the present study, the DCMU-tolerant mutant strain showed relatively greater tolerance to high light intensity ($60\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) than the WT ($40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). Further, an increase in the light intensities ($80\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) beyond their respective tolerance limits caused a drastic decrease in the growth and cell constituents of both the strains except the lipid. Liu et al. (2018) found that the maximum growth of *C. vulgaris* under a light intensity of 60 and $80\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. They also suggested that the light intensities beyond the growth saturating limit caused a drastic decrease in the light use efficiency of the microalgal cells. It has been observed that varying light irradiances induce remarkable changes in the gross chemical composition, pigments, and photosynthetic activity of the microalgal cells (Nzayisenga et al., 2020).

The data on FTIR analysis showed changes in the protein, carbohydrate, and lipid content of both strains as suggested earlier. Both FTIR and flow cytometer analysis showed light intensity-dependent variation in the lipid content. However, the overall level of biochemical constituents including lipids was higher in the mutant strain than the WT throughout the range of light irradiances. Thus, it was imperative to know how the mutant strain processes the light energy and translates the energy which favors the growth and synthesis of cell constituents. Earlier workers have reported that photosynthetic microalgae have developed many strategies to handle the excess light energy such as down-regulation of the photosynthetic process by

photoinhibition (Murata et al., 2007), anti-oxidative defense system (Yilancioglu et al., 2014; Snoeijs et al., 2012) and storage of excess light energy into high energy compounds like lipid (He et al., 2015). The metabolic strategy of the algal cells to divert the photosynthetic carbon flux towards fatty acid synthesis can be useful for biofuel production technology (Liu et al., 2018).

Several workers have reported that photo-damage to the photosynthetic cells is always proportional to the intensity of incident light and excess of absorbed photo-energy in the photosynthetic organisms can induce an increase in the level of ROS (Menon et al., 2013). The results on the photobleaching of RNO and the effect of radical quenchers exhibited a greater level of ROS generation and oxidative stress experienced by the WT than the mutant strain. However, a prominent effect of histidine- a quencher of superoxide ($O_2^{\cdot -}$) radical (Matheson et al., 1975), on RNO bleaching indicated a predominant role of superoxide radicals in both the strains. But, the DCMU-tolerant mutant strain was better equipped than the WT with anti-oxidative defense systems such as higher levels of carotenoids, SOD activity, and glycollate production (Janknegt et al., 2007; Nishiyama et al., 2001). Earlier reports have also shown that SOD activity (Rossa et al., 2002), glycollate, and lipid production (Shi et al., 2017; Han and Eley, 1973) in the algal system are associated with the mitigation of photo-oxidative stress.

In the present study, the overall level of all the pigments was higher in the mutant strain than in the WT throughout the range of light intensities. It has been suggested that the processing of light energy and its conversion into biomass depends upon the photochemistry of PSII which is the most sensitive component of the electron transport chain (Roach and Krieger-Liszkay, 2014). Many physiological and biochemical studies have led to the conclusion that photosynthetic organisms protect themselves from high light stress through the down-regulation of photosystem II (Gururani et al., 2015).

A lack of consistency and uniformity in the experimental results requires a thorough investigation of parameters related to photosynthetic attributes. The photosynthetic parameters (F_v/F_m , ET_0/RC , TR_0/RC , ABS/RC , and RC/ABS) (Stirbet and Govindjee, 2012) studied in *S. vacuolatus* indicated better photosynthetic performance and electron transport in the mutant strain than the WT even under high light intensity. The quantum yield (F_v/F_m), and overall photosynthetic performance (RC/ABS) of the mutant strain, suggested better light utilization efficiency in the mutant strain than in the WT. The captured photons in the case of the WT were found to be largely dissipated in terms of heat (Muller et al., 2001) as evidenced by the higher value of NPQ in the WT as compared to mutant strain. A higher value of $rETR$ with higher photosynthesis saturating light intensity in the mutant strain supported the hypothesis that mutant was metabolically well adapted to handle the excess photons due to its altered photosynthetic system, which supports better light tolerance and photosynthetic efficiency in the mutant than the WT (Malapascua et al., 2014). Thus, it is inferred that high light intensity tolerance of the mutant strain than the wild-type was contributed by the altered photosynthetic system, better photosynthetic efficiency, robust anti-oxidative system, glycollate production, and lipid synthesis- a high energy storage compound.

5. Conclusion

The present study provided new insight into the influence of light intensity on the growth and biochemical composition of Wild-type (WT) and DCMU-tolerant mutant alga *Scenedesmus vacuolatus*. DCMU tolerant-mutant strain showed higher light intensity ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) tolerance than the WT ($40 \mu\text{mol m}^{-2} \text{s}^{-1}$). The photodynamic effect under the light intensity stress was greater in the WT than the mutant, which was mediated by the superoxide radical. A comparison of the data on FTIR and flow cytometer analysis showed a higher accumulation of lipids in the mutant strain. Therefore mutant strain can be used as a potential feedstock for biodiesel production. Photosynthetic parameters obtained from OJIP, NPQ, and Light curve, measured by PAM fluorometer under light stress, indicated better photosynthetic efficiency in the mutant strain than in the WT. Thus, a greater light-intensity tolerance, better photosynthetic performance, and lipid accumulation in the mutant strain might be attributed to its efficient cellular antioxidative defense system and changes in the photosynthetic attributes of PSII. The mutant strain of alga can be cultured in outdoor cultivation where light intensities varies enormously. As it possesses a high extent of photosynthetic efficiency it can also be recommended for wastewater treatment. Further investigations are required for the growth study of DCMU tolerant mutant algal strain under the stress conditions of nutrients and carbon sources.

Abbreviations

PAM: Pulse amplitude modulation

RC: reaction center

QY: quantum yield

F_v/F_m : maximum quantum yield of PSII

ET_0 /RC: electron transport flux per reaction center for reduction of electron carriers beyond QA TR_0 /RC: trapping of energy flux leading to reduction of QA

ABS/RC: absorption of energy flux per reaction center

RC/ABS: performance index

NPQ: non-photochemical quenching

LC: light curve

LED: light-emitting diode

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Declarations

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Conflict of interest

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

Author contribution

Neha Gupta: Visualization, Writing – original draft, Writing – review & editing, **Nisha Yadav**: Writing – review & editing Investigation, conceptualization, supervision, **D.P. Singh**: Writing – Review **Vinyak V Pathak**: Writing – review & editing. **Shamshad Ahmad**: Writing – review & editing

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Brindhadevi K., Mathimani T., Rene E.R., Shanmugam S., Chi N.T.L., and Pugazhendhi A., (2021), Impact of cultivation conditions on the biomass and lipid in microalgae with an emphasis on biodiesel, *Fuel*, 284, p.119058.

2. Cordara A., Re A., Pagliano C., Alphen P.V., et al. (2018), Analysis of the light intensity dependence of the growth of *Synechocystis* and the light distribution in a photobioreactor energized by 635 nm light, *PeerJ*, 6, e5256.
3. Dean A.P., Sigee D.C., Estrada B., Pittman J.K., (2010), Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae, *Bioresource Technology*, 101, 4499-4507.
4. Erickson E., Wakao S., and Niyogi K.K., (2015), Light stress and photoprotection in *Chlamydomonas reinhardtii*, *The Plant Journal*, 82, 449-465.
5. Garg N., Manchanda G., (2009), ROS generation in plants: Boon or bane? *Plant Biosystem*, 143, 81-96.
6. Gururani M.A., Venkatesh J., Son L., Tran P., (2015), Regulation of Photosynthesis during Abiotic Stress-Induced Photoinhibition, *Molecular Plant*, 8, 1304-1320.
7. Han T.W., and Eley J. H., (1973), Glycolate excretion by *Anacystis nidulans*: effect of HCO_3^- concentration, oxygen concentration and light intensity, *Plant & Cell Physiology*, 14, 285-291.
8. He Q.N., Yang H.J., Wu L., Hu C.X., (2015), Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae, *Bioresource Technology*, 191, 219-228.
9. Janknegt P.J., Rijstenbil J.W., et al. (2007), A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae, *Journal of Photochemistry and Photobiology B: Biology*, 87, 218-226.
10. Joshi P.C., Pathak M.A., (1984), The role of active oxygen ($^1\text{O}_2$ and O_2^-) induced by crude coal for its ingredients used in photo chemotherapy of skin disease, *Journal of Investigative Dermatology*, 82, 67-63.
11. Jungandreas B., Costa S., Jakob T., Bergen M.V., Baumann S., Wilhelm C., (2014), The acclimation of *Phaeodactylum tricornutum* to blue and red light does not influence the photosynthetic light reaction but strongly disturbs the carbon allocation pattern, *PLOS One*. 9, e99727.
12. Laurens L.M.L., Wolfrum E.J., (2011), Feasibility of spectroscopic characterization of algal lipids: Chemometric correlation of NIR and FTIR spectra with exogenous lipids in algal biomass, *Bioenergy Research*, 4, 22-35.
13. Lichtenthaler H.K., Buschmann C., Knapp M., (2005), How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer, *Photosynthetica*, 43, 379-393.
14. Lichtenthaler H.K., (1987), Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymology*, 148, 350-382.
15. Liu X., Yingd K., Chenc G., Taoc Y., Caid Z., Wanga J., (2018), Influence of light intensity on microalgal growth, nutrients removal and capture of carbon in the wastewater under intermittent supply of CO_2 Influence of light on wastewater treatment, *Journal of Chemical Technology & Biotechnology*, 93, 12.
16. Malapascua Jose, R.F., Jerez Celia G., Sergejevova M., Figueroa Felix L., Masojidek J., (2014), Photosynthesis monitoring to optimize growth of microalgal mass cultures: application of chlorophyll fluorescence techniques, *Aquatic Biology*, 22, 123-140.
17. Mandotra S.K., Kumar P., Suseela M.R., Nayaka S., Ramteke P.W., (2016), Evaluation of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under the influence of phosphorus, pH and light intensities, *Bioresource Technology*, 201, 222-229.
18. Matheson I.B.C., Etheridge R.D., Kratowich N.R., Lee J., (1975), The quenching of singlet oxygen by amino acids and protein, *Photochem Photobiology*, 21, 165-171.
19. Menon K.R., Balan R., Suraishkumar G.K., (2013), Stress induced lipid production in *Chlorella vulgaris*: relationship with specific intracellular reactive species levels, *Biotechnology Bioengineering*, 110, 1627-1636.
20. Muller P., Li X.P. and Niyogi K.K., (2001), Non-photochemical quenching. A response to excess light energy, *Plant Physiology*, 125, 1558-1566.
21. Murata N., Takahashi S., Nishiyama Y., Allakhverdiev S.I., (2007), Photoinhibition of photosystem II under environmental stress, *Biochimica et Biophysica Acta*, 6, 414-421.

22. Murdock J.N., and Wetzel D.L., (2009), FT-IR Microspectroscopy Enhances Biological and Ecological Analysis of Algae, *Applied Spectroscopy Reviews*, 44, 335-361.
23. Nishiyama Y., Yamamoto H., Allakhverdiev S.I., Inaba M., Yokota A., et al. (2001), Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery, *EMBO Journal*, 20, 5587-5594.
24. Nishikimi M., Rao N.A., (1972), The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen, *Biochemical Biophysical Research Communications*, 48, 849-854.
25. Niyogi K.K., (1999), Photoprotection revisited: genetic and molecular approaches, *Annual Review of Plant Physiology*, 50, 333-359.
26. Pope D.H., (1975), Effect of light intensity, oxygen concentration and carbon dioxide concentration on photosynthesis in algae, *Microbial ecology*, 2, 1-16.
27. Nzayisenga J.C., Farge X., Groll S.L. and Sellstedt A., (2020), Effects of light intensity on growth and lipid production in microalgae grown in wastewater, *Biotechnology for Biofuels*, 13, pp.1-8.
28. Roach T., and Krieger-Liszkay A.K., (2014), Regulation of Photosynthetic Electron Transport and Photoinhibition, *Current Protein Peptide Science*, 15, 351-362.
29. Rossa M.M., Oliveira M.C.D., et al. (2002), Effect of visible light on superoxide dismutase (SOD) activity in the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta), *Journal of Applied Phycology*, 14, 151-157.
30. Satpati G.G., Pal R., (2014), Rapid detection of neutral lipid in green microalgae by flow cytometry in combination with Nile red staining—an improved technique, *Annals Microbiology*, 65, 937-949.
31. Shi K., Gao Z., Shi T.Q., et al. (2017), Reactive Oxygen Species-Mediated Cellular Stress Response and Lipid Accumulation in Oleaginous Microorganisms: The State of the Art and Future Perspectives, *Frontiers Microbiology*, 8, 793.
32. Snoeijs P., Sylvander P., Häubner N., (2012). Oxidative Stress in Aquatic Primary Producers as a Driving Force for Ecosystem Responses to Large-Scale Environmental Changes, *Oxidative Stress in Aquatic Ecosystem*, John Wiley & Sons, pp. 72-88.
33. Stanier R.Y., Kunisawa R., Mandel M., Cohen-Bazire G., (1971), Purification and properties of unicellular blue-green algae (Order Chroococcales), *Bacteriological Reviews* 35, 171-205.
34. Stehfest K., Toepel J., Wilhelm C., (2005), The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae, *Plant Physiology and Biochemistry*, 43, 717-726.
35. Stirbet A., Govindjee., (2012), Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the O- J-I-P rise, *Photosynthesis Research*, 113, 1-3.
36. Strasser R.J., Srivastava A., Tsimilli-Michael M., (2000), *The fluorescence transient as a tool to characterize and screen photosynthetic samples*, In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), *Probing Photosynthesis: Mechanisms, Regulation and Adaption*, Taylor & Francis, London, pp. 445-483.
37. Takahashi K., (1972), A colorimetric method for quantitative determination of glycolic acid with 2, 7- dihydroxy naphthalene, *Journal of Bacteriology*, 71, 563-565.
38. Vonshak A., Novoplansky N., (2008), Acclimation to low temperature of two *Arthrospira platensis* (cyanobacteria) strains involves down-regulation of PSII and improved resistance to photoinhibition, *Journal of Phycology*, 44, 1071-1079.
39. Wahidin S., Idris A., Raehanah S., Shaleh M., (2013), The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis sp.* *Bioresource Technology*, 129, 7-11.
40. Yilancioglu K., Cokol M., Pastirmaci I., Erman B., Cetiner S., (2014), Oxidative Stress Is a Mediator for Increased Lipid Accumulation in a Newly Isolated *Dunaliella salina* Strain, *PLOS ONE*, 9, e91957.
41. Yu Ze., Song M., Peia H., Hana F., Jianga L., Houa Q., (2017), The growth characteristics and biodiesel production of ten algae strains cultivated in anaerobically digested effluent from kitchen waste, *Algal Research*, 24, 265-275.

Tables

Table 1. Comparison of the specific growth rates of the wild-type (WT) and mutant strains of *S. vacuolatus* under various light conditions (10–100 mol m⁻² s⁻¹). Student 't' test with *<p0.05, **<p0.01, ***<p0.001 as the significance difference threshold. The information is displayed as meanSD.

Specific growth rate (μ) (d ⁻¹)		
Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Wild-type	Mutant
10	0.042±0.004	0.060±0.004**
20	0.061±0.005	0.092±0.006***
40	0.079±0.007	0.110±0.010**
60	0.064±0.005	0.135±0.012**
80	0.041±0.003	0.081±0.005***
100	0.022±0.002	0.051±0.003**

Table 2. Total chlorophyll and carotenoids content of WT and mutant strain of *S. vacuolatus* in response to varying light intensities (10-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Student 't' test showing significance difference level, *p<0.05,**p<0.01,***p<0.001. The data are presented as mean±SD.

Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total Chlorophyll ($\mu\text{g mL}^{-1}$)		Carotenoids ($\mu\text{g mL}^{-1}$)	
	Wild-type	Mutant	Wild-type	Mutant
10	0.816±0.065	1.60±0.133**	1.44±0.105	2.76±0.208**
20	1.16±0.065	2.24±0.215**	2.23±0.217	3.46±0.321**
40	2.06±0.180	2.93±0.265*	2.99±0.0260	4.56±0.416**
60	1.51±0.120	3.62±0.335**	2.40±0.200	5.60±0.529***
80	0.816±0.06	1.68±0.135**	1.23±0.100	2.76±0.216*
100	0.313±0.025	0.913±0.08***	0.66±0.493	1.51±0.110**

Table 3. Effect of radical quenchers on RNO bleaching in the WT and mutant cells incubated under 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity for one hour. The values given in the parenthesis represent percent activity of RNO bleaching. The data are presented as mean±SD.

Name of radicals quenchers	Rate of RNO bleaching (ΔOD_{440} nm/mg protein/min)	
	Wild-type	Mutant
Control (without quencher)	7.2 $\pm$ 0.40 (100)	3.2 $\pm$ 0.24 (100)
Histidine (5mM)	2.8 $\pm$ 0.25 (38.8)	1.3 $\pm$ 0.10 (40.6)
Sodium formate (5mM)	4.3 $\pm$ 0.41 (59.7)	2.1 $\pm$ 0.20 (65.6)
Sodium azide (5mM)	6.2 $\pm$ 0.40 (86.1)	2.8 $\pm$ 0.17 (87.5)

Table 4. (a) Total lipid, Carbohydrate/Protein (C/P), Lipid/Carbohydrate (L/C) and Lipid/Protein (L/P) ratios derived from the FTIR spectra of cell biomass of wild-type of *S. vacuolatus* under varying light irradiances. The data are presented as mean $\pm$ SD.

Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total lipid	Carbohydrate/Protein (C/P)	Lipid/Carbohydrate (L/C)	Lipid/Protein (L/P)
10	1.0 $\pm$ 0.07	0.51 $\pm$ 0.04	0.7 $\pm$ 0.03	0.5 $\pm$ 0.04
40	1.3 $\pm$ 0.1	0.81 $\pm$ 0.05	1.1 $\pm$ 0.08	0.81 $\pm$ 0.05
60	0.7 $\pm$ 0.05	0.7 $\pm$ 0.03	0.91 $\pm$ 0.05	1.3 $\pm$ 0.10
100	0.51 $\pm$ 0.04	0.3 $\pm$ 0.02	0.61 $\pm$ 0.04	0.8 $\pm$ 0.03

Table 4. (b) Total lipid, Carbohydrate/Protein (C/P), Lipid/Carbohydrate (L/C) and Lipid/Protein (L/P) ratios derived from the FTIR spectra of cell biomass of mutant strain of *S. vacuolatus* under varying light irradiances. The data are presented as mean $\pm$ SD.

Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total lipid	Carbohydrate/Protein (C/P)	Lipid/Carbohydrate (L/C)	Lipid/Protein (L/P)
10	1.0 $\pm$ 0.07	0.51 $\pm$ 0.04	0.7 $\pm$ 0.03	0.5 $\pm$ 0.04
40	1.3 $\pm$ 0.1	0.81 $\pm$ 0.05	1.1 $\pm$ 0.08	0.81 $\pm$ 0.05
60	0.7 $\pm$ 0.05	0.7 $\pm$ 0.03	0.91 $\pm$ 0.05	1.3 $\pm$ 0.10
100	0.51 $\pm$ 0.04	0.3 $\pm$ 0.02	0.61 $\pm$ 0.04	0.8 $\pm$ 0.03

Table 5 shows changes in the OJIP parameters for wild-type and mutant *S. vacuolatus* cells that have been dark-adapted and subjected to light intensities ranging from 10 to 100 $\text{mol m}^{-2} \text{s}^{-1}$. Student 't' test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as the significance difference threshold. The information is displayed as mean $\pm$ SD.

Light intensity	Wild-type (WT)				Mutant			
	ET ₀ /RC	TR ₀ /RC	ABS/RC	RC/ABS	ET ₀ /RC	TR ₀ /RC	ABS/RC	RC/ABS
10	0.62±0.02	0.64±0.05	1.03±0.06	17.6±1.5	0.77±0.59 [*]	0.74±0.06 ^{**}	1.20±0.10 [*]	23.4±1.5 ^{***}
20	0.81±0.03	0.83±0.04	1.20±0.09	20±1.8	0.99±0.09 [*]	0.959±0.07 [*]	1.326±0.10 ^{**}	25.4±1.8 ^{***}
40	0.919±0.09	0.92±0.04	1.31±0.13	23.3±1.9	1.0±0.10 [*]	1.03±0.06 [*]	1.452±0.11 [*]	27.2±1.0 [*]
60	0.87±0.08	0.86±0.05	1.34±0.11	20.6±2.0	1.1±0.08 ^{***}	1.07±0.10 [*]	1.52±0.10 ^{**}	29.1±1.5 ^{**}
80	0.61±0.03	0.63±0.06	1.16±0.10	14.0±1.4	0.81±0.03 ^{**}	0.87±0.06 ^{***}	1.37±0.11 ^{**}	18.1±1.7 ^{**}
100	0.48±0.04	0.48±0.06	0.73±0.05	6.4±0.8	0.70±0.02 [*]	0.75±0.06 ^{**}	1.15±0.12 [*]	12.2±1.5 ^{**}

Figures

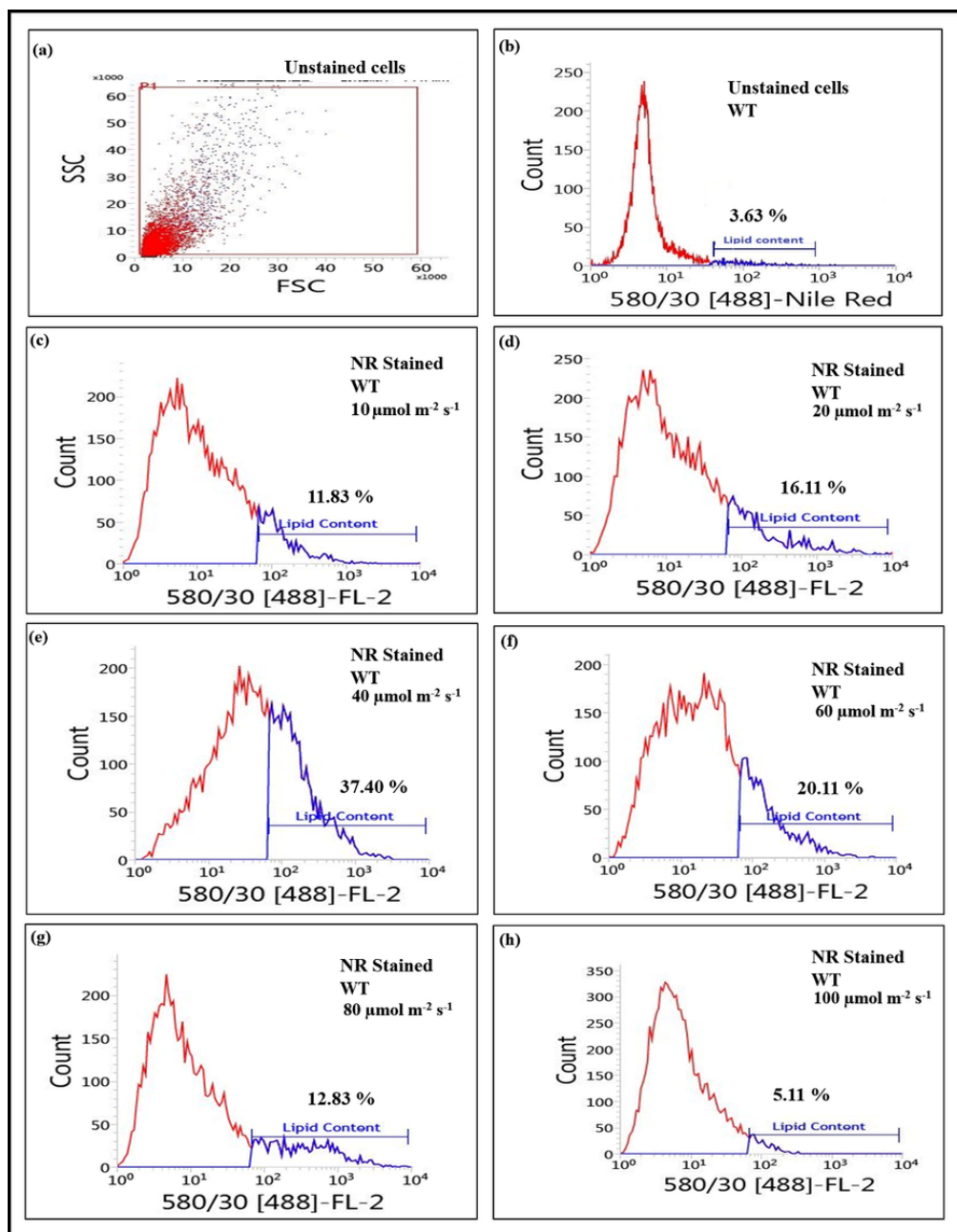


Figure 1

Flow cytometer analysis of lipid content of wild-type (WT) cells of *S. vacuolatus* exposed to different light intensity. Graph (a, b) represents the unstained cells of WT (without Nile red).

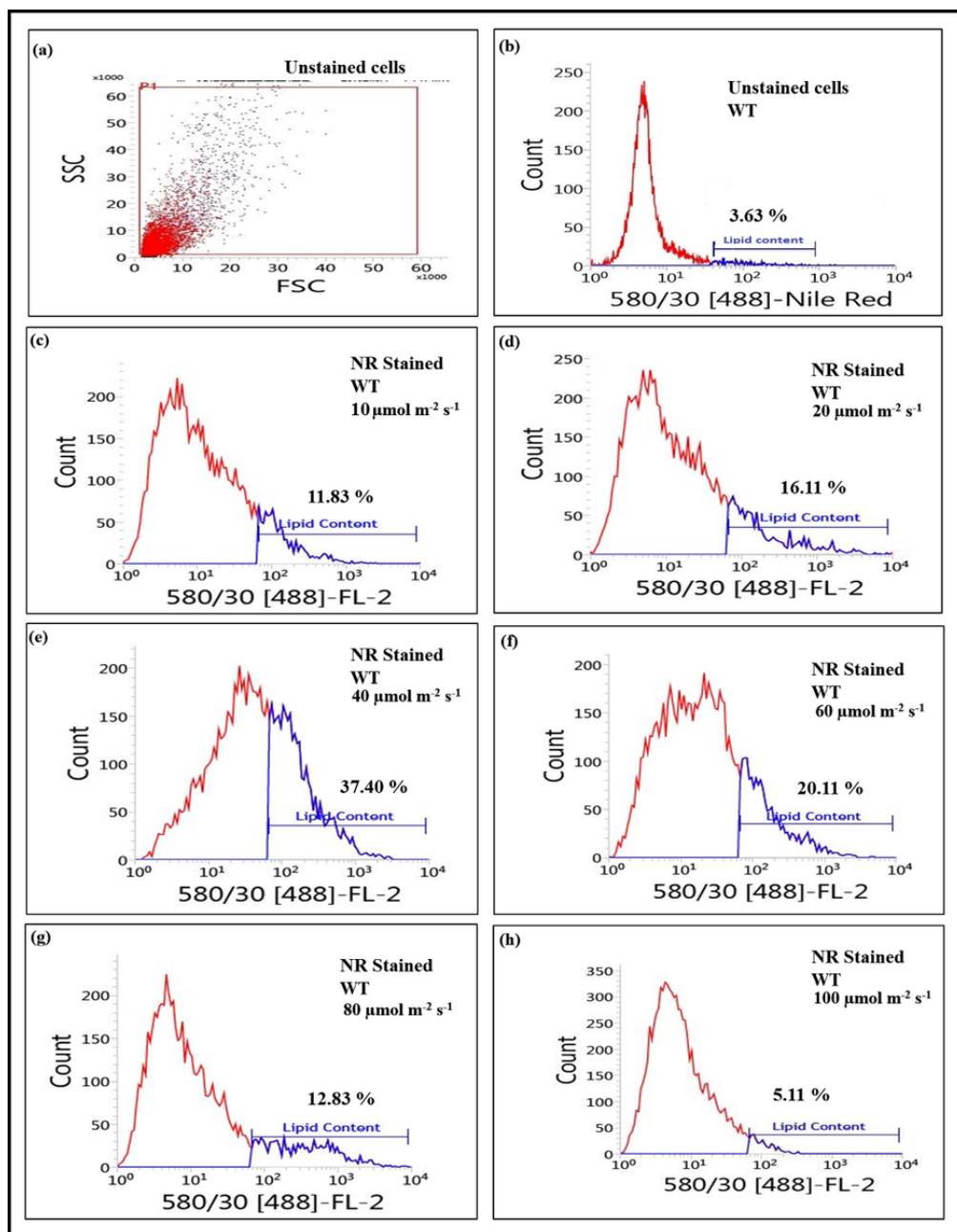


Figure 2

Flow cytometer analysis of lipid content of mutant cells of *S. vacuolatus* exposed to different light intensity. Graph (a, b) represents the unstained cells of WT (without Nile red).

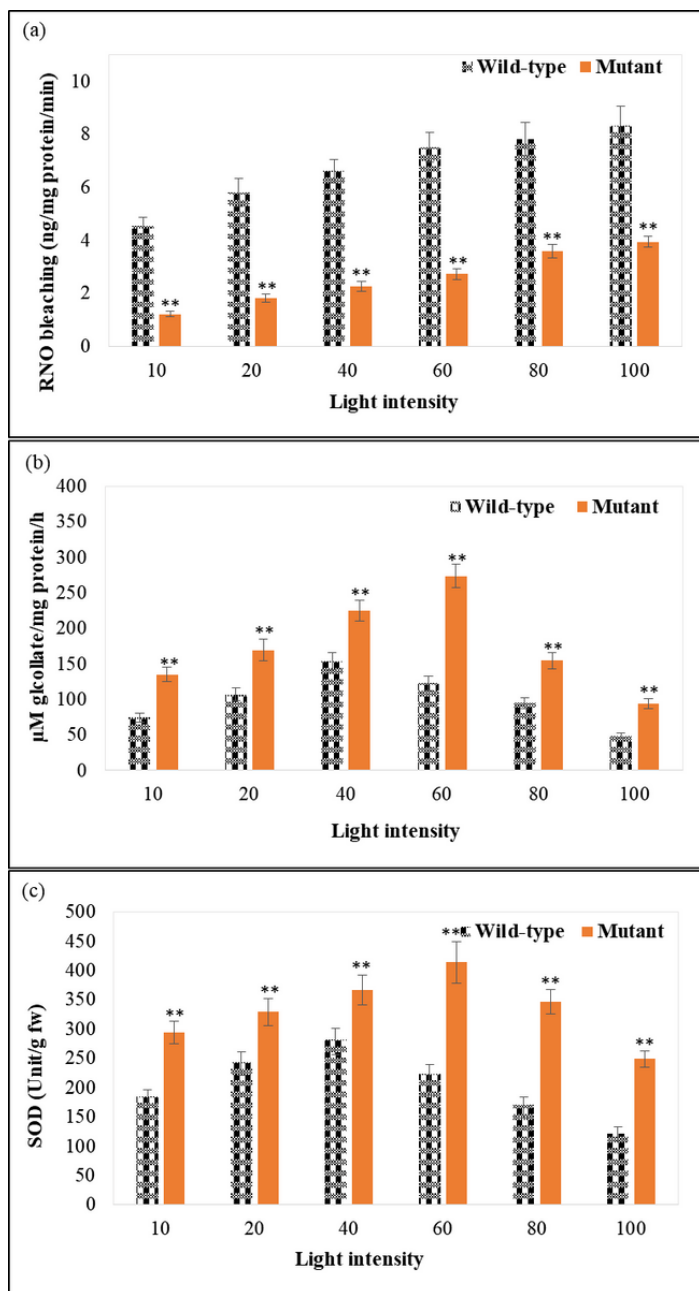


Figure 3

Effect of different light irradiance (10-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) on the (a) RNO bleaching (b) glycollate activity (c) Superoxide dismutase (SOD) activity in the WT and mutant strain of *S. vacuolatus*. Student's 't' test showing significance difference level, *p<0.05, **p<0.01, ***p<0.001. Error bars show the mean $\pm$ SD.

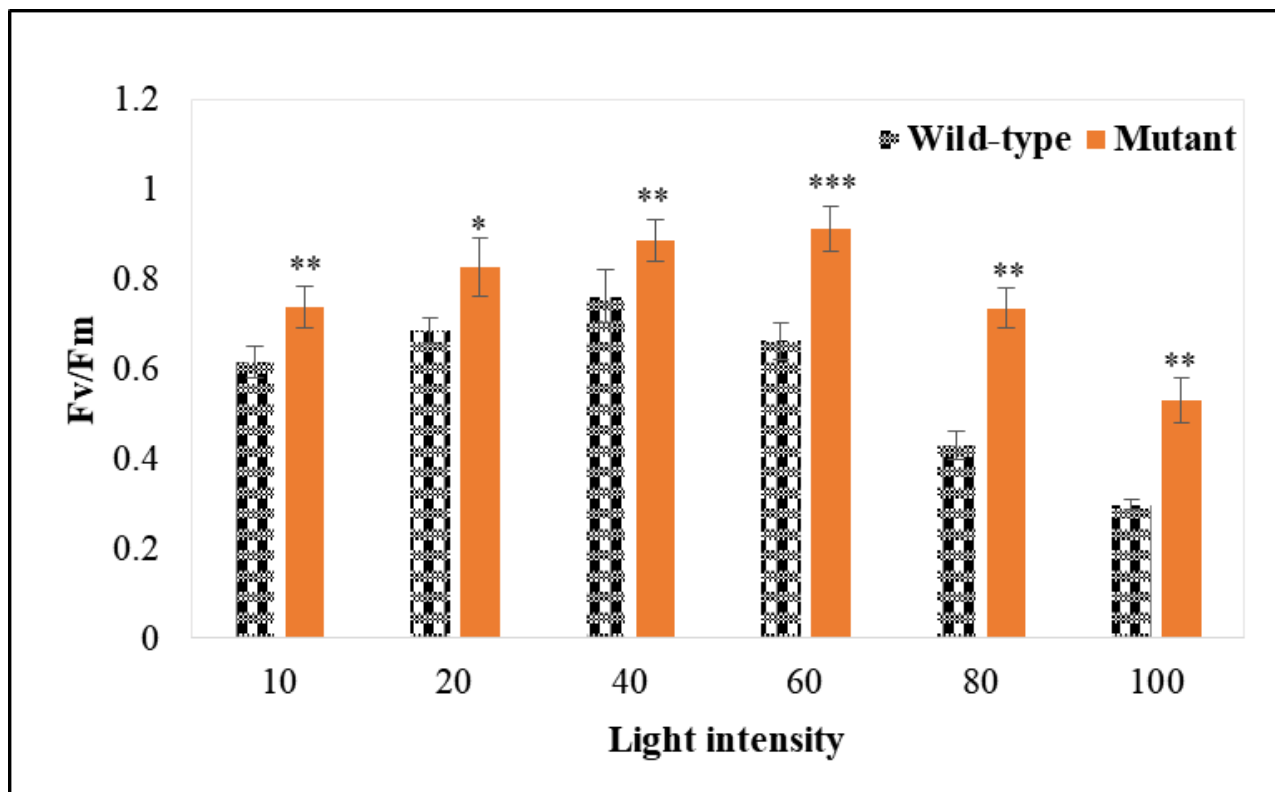


Figure 4

Changes in the PS II photosynthetic yield (F_v/F_m) of *S. vacuolatus* cells of WT and mutant strain exposed to varying light intensity (10-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Student 't' test showing significance difference level, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Error bars show the mean $\pm$ SD.

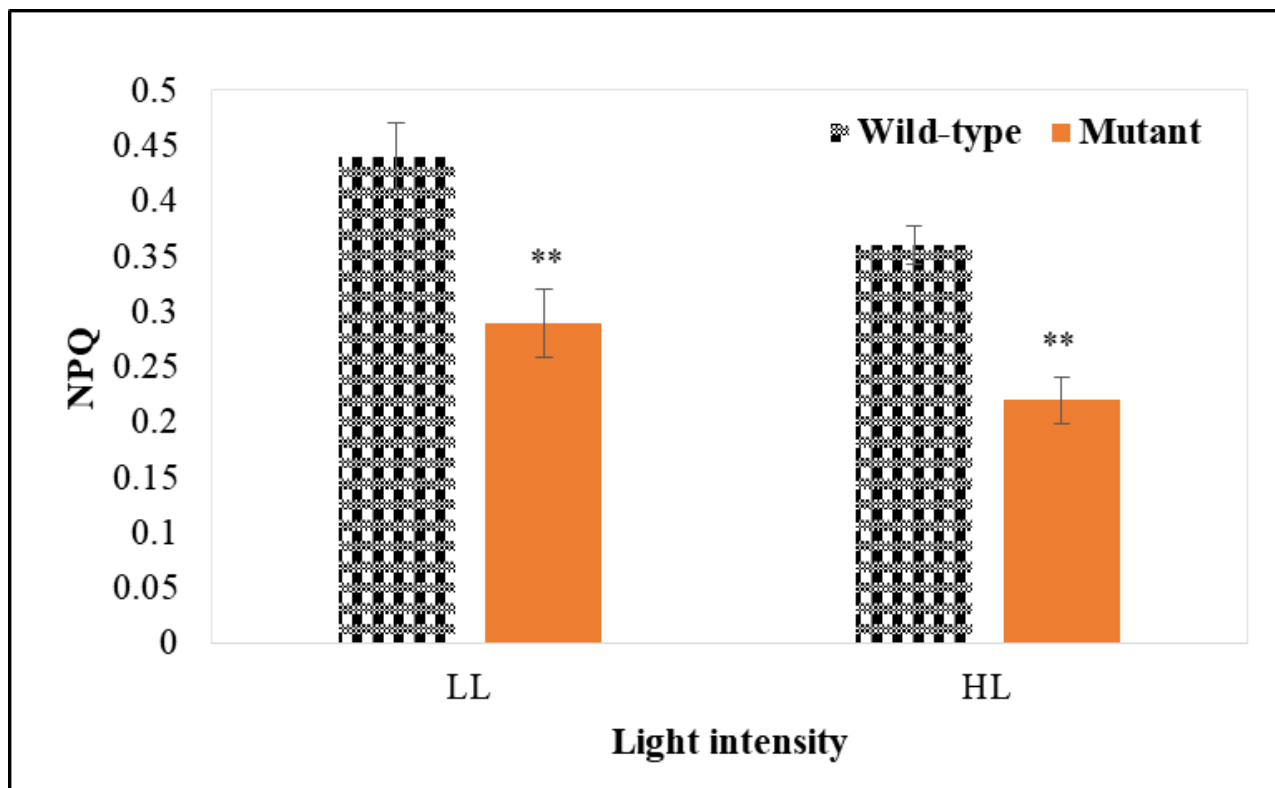


Figure 5

Non-photochemical quenching (NPQ) of *S. vacuolatus* cells of WT and mutant strain exposed to low light (LL) ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and high light (HL) ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) intensities. Student 't' test showing significance difference level, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars show the mean $\pm$ SD

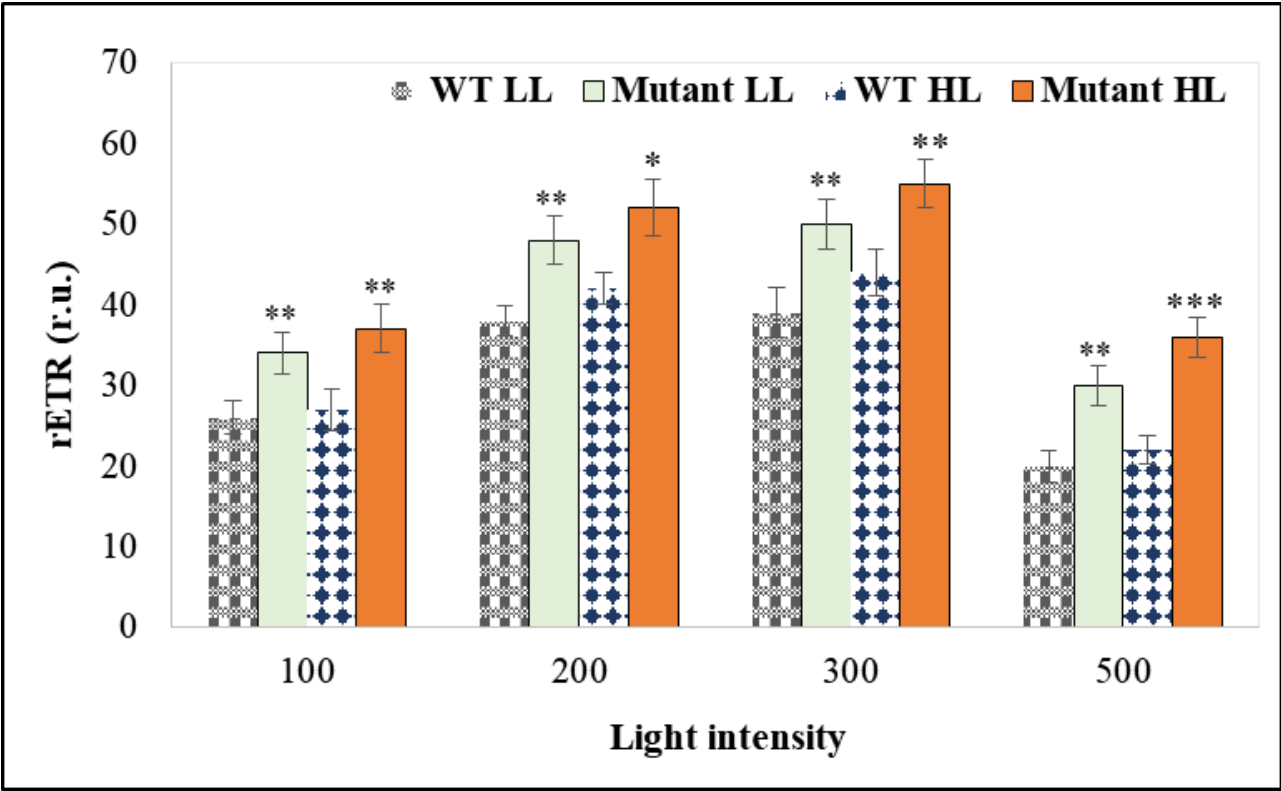


Figure 6

Response light curve (LC) effect on WT and mutant strain of *S. vacuolatus* cells exposed to varying irradiance (100-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Both strains were adapted to low light (LL) ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and high light (HL) ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) intensities. Student 't' test showing significance difference level, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars show the mean $\pm$ SD.