Probing the effects of high-light stress on pigment and lipid metabolism in nitrogen-starving microalgae by measuring chlorophyll fluorescence transients: Studies with a Δ5 desaturase mutant of *Parietochloris incisa* (Chlorophyta, Trebouxiophyceae)

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**A B S T R A C T**

We investigated effects of irradiance on the relationships between chlorophyll fluorescence transients (OJIP), carotenoid-to-chlorophyll ratio, and fatty acids in a nitrogen-deprived *Parietochloris incisa* (Chlorophyta, Trebouxiophyceae) Δ5 desaturase mutant accumulating valuable LC-PUFA dihomo-γ-linolenic acid (DGLA). High light (270 μE·m⁻²·s⁻¹ PAR) and nitrogen starvation brought about a decrease in maximum quantum yield of photosystem II (ΦPSII) and electron transport (ΦET) but enhanced the quantum yield of thermal dissipation (ΦNPQ) and induced non-photochemical quenching (NPQ) in an irradiance-dependent manner. Under high irradiance a decline in the rate of total fatty acid accumulation and DGLA percentage in comparison with the cultures grown under 130 μE·m⁻²·s⁻¹ PAR was recorded. Increasing irradiance from 130 to 270 μE·m⁻²·s⁻¹ enhanced total fatty acid accumulation only within the first week of nitrogen starvation and negatively affected DGLA production. Regardless of irradiance, ΦPSII, ΦET, and ΦNPQ exhibited tight (r² = 0.8–0.9) relationships with the stress-induced changes of total fatty acid and DGLA content and the carotenoid-to-chlorophyll ratio. The applicability and limitations of OJIP and its derived parameters for on-line monitoring of physiological condition and accumulation of value-added products in microalgal cultures grown in photobioreactors are discussed.

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1. Introduction

In various groups of microalgae environmental stresses such as high PAR irradiance and/or nitrogen deficiency bring about profound changes in photosynthetic apparatus (PSA) structure and function as well as in lipid and pigment metabolism [1]. In many cases a build-up of storage triacylglycerols (TAG) coordinated with a decline in chlorophylls (Chl) and accumulation of secondary carotenoids (Car) takes place [2,3]. These phenomena have important biotechnological implications since Car and fatty acids (FA) accumulated by certain microalgae under the stress condition are high-value nutraceuticals and antioxidants; neutral lipids are considered as a promising feedstock for biodiesel [4,5].

The high-light acclimation of microalgal cells involves engagement of protective mechanisms decreasing the absorption of light by the PSA and enhancing thermal dissipation of the absorbed light energy within PSA [1]. In mass culture of microalgae, understanding the relationships of the adaptive re-arrangements of the PSA with lipid metabolism is essential for the estimation of stress intensity of oleaginous species. It is also important for timely adjustment of cultivation conditions enabling the organism to cope with the stress, to achieve high yields of target products, and to make the whole process more sustainable.

Analysis of chlorophyll fluorescence (CF) is a powerful tool for revealing physiological state of photoautotrophic organisms [6,7]. Light-induced thermal energy dissipation in PS II antenna can be examined by measuring non-photochemical CF quenching with a Pulse Amplitude Modulated (PAM) fluorometer. Even more information can be obtained by recording high-resolution light-induced kinetics of Chl fluorescence (OJIP transients) [8]. OJIP transient reflects changes in electron transport in PS II over a wide range of time from microseconds to seconds that allows to evaluate such important characteristics of PS II as energy trapping, electron transport, and ΔpH-dependent dissipation of excitation energy into heat in the antenna complex [6]. It provides a valuable insight into the transformation of the absorbed light energy within PSA, efficiency of its photochemical utilization and engagement of photoprotective
mechanisms [9]. Other CF-related parameters such as NPQ related with non-photochemical dissipation of the absorbed light and $F_{v}/F_{m}$, the maximum quantum yield of photosystem (PS) II photochemistry [10], are commonly utilized in monitoring of the growth [11] and physiological condition of microalgae as well.

A major practical advantage of using CF is that the measurements are non-destructive, rapid, and easy to perform. These characteristics make CF analysis a particularly attractive method for on-line monitoring of the physiological condition of planktonic [8,12] and cultivated microalgae [13]. There are many reports on application of CF-based parameters for in situ monitoring of productivity and adaptation to light in mass cultured microalgae ([13] and references therein). However it is difficult to link the CF-derived parameters with remote to primary photochemistry characteristics of a culture such as cell dry weight or FA content and composition since they are affected by many environmental and intrinsic factors which are not directly related with PSA.

In spite of these difficulties, CF-based approach for on-line monitoring of key parameters of an algal culture would be welcomed by microalgal biotechnologists developing processes for the production of high value bioproducts and biofuels as well as biomitigation of wastes. An emphasis should be put to the assessment of photosynthetic capacity of cultivated algae in situ under nitrogen starvation conditions since it increases the vulnerability of the culture to photooxidative damage under higher irradiances.

Towards this end, we made an attempt to map selected CF-based parameters to the performance (biomass, total FA, DGLA, and Car accumulation) of a green oleaginous microalg strain cultivated under conditions of different stress intensity. A Δ5 desaturase mutant of P. incisa (P127) obtained in the Laboratory of Microalgal Biotechnology (Ben-Gurion University, Israel), served as an object in the present work. Due to the nonsense mutation in the Δ5 desaturase gene the strain is almost incapable of desaturation of dihomo-γ-linolenic acid (DGLA, 20:3ω−6) to arachidonic acid (AA, 20:4ω−6) [14]. Under nitrogen starvation conditions the mutant accumulates high contents of DGLA that is mainly incorporated in triacylglycerols (TAG) up to 39% of total fatty acids (TFA) and 14% of dry weight (DW) [3]. In fungi and algae and invertebrates DGLA normally occurs only as an intermediate in AA biosynthesis and does not accumulate in any appreciable amounts. DGLA has a pharmacological significance related with its anti-inflammatory activity such as for treating atopic eczema, psoriasis, asthma and arthritis [15]. Recent studies suggest that DGLA possesses antiproliferative properties and is unique among the ω−6 polynsaturated FA family members in its potential to suppress tumor growth and metastasis [16]. Therefore, the mutant could serve as a potential source of the nutraceutically important LC-PUFA.

In this context it was important to study the relationships between FA and pigment composition, FA accumulation and parameters of OJIP curves characterizing photosynthetic performance of the mutant strain under conditions favoring DGLA accumulation (nitrogen deficiency and high irradiance). Previously we have described in detail the major patterns in biomass and fatty acid accumulation by this strain grown on N-replete and N-depleted media under different irradiance levels and revealed tight correlations between FA accumulation, changes in pigment composition, and optical absorbance of the algal cell suspension [3]. In this paper we focus on OJIP curves in the stressed mutant cells grown under nitrogen starvation and different PAR irradiances. To the best of our knowledge, this is the first report of relationships between changes in selected OJIP-based parameters, and total FA (TFA) accumulation, a qualitative parameter of neutral lipid accumulation.

2. Materials and methods

2.1. Cultivation conditions

The Δ5 desaturase mutant of P. incisa, P127 [14] was obtained in the Microalgal Biotechnology Laboratory, J. Blaustein Institutes for Desert Research, and was cultivated on BG-11 medium [17] in 1 L glass columns (6 cm ID) under constant illumination (by daylight fluorescent lamps) of three different intensities (35, 130, and 270 μE·m$^{-2}$·s$^{-1}$ PAR as measured in the center of the empty column) and with constant bubbling of CO$_2$:air mixture (1:99, v/v) at 25 °C. Prior to the nitrogen-starvation experiment, cultures were daily diluted to maintain logarithmic growth. In all cases, initial chlorophyll content and DW upon transferring to nitrogen-free medium were maintained at 30 mg·L$^{-1}$ and 1 g·L$^{-1}$, respectively, to prevent photodamage of the cultures at high irradiance [3]. Before inoculation of the columns, cells were washed three times with sterilized distilled water and resuspended in nitrogen-free BG-11. The sampling for determination of DW, TFA content, pigment composition, and fluorescence measurements was performed at d 0 and following the 3rd, 7th, 10th and 14th d of nitrogen deprivation.

2.2. Fatty acid analysis

Capillary gas–chromatography was used for fatty acid quantification; the analysis was performed according to Cohen et al. [18]. The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analyzed in duplicate.

2.3. Pigment extraction and analysis

In routine measurements total Chl and Car were extracted from P127 biomass with dimethyl sulfoxide (DMSO) for 5 min at 70 °C with 5 mL per ca. 3.5 mg DW. The pigment concentrations were determined in DMSO extracts spectrophotometrically with a Cary 50 Bio spectrophotometer (Varian, Walnut Creek, CA, USA) [3]. Individual Car were analyzed in whole cells, isolated thylakoids and oil bodies (OB) using HPLC according to earlier published protocols [19]. Pigments were identified and quantified using pure pigment standards (Sigma-Aldrich, St. Louis, MO, USA; Fluka, Taufkirchen, Germany).

2.4. CF measurements and treatment of the data

Induction curves of CF (OJIP curves) were recorded using a Fluorpen FP100s portable Pulse Amplitude Modulated fluorometer (Photon Systems Instruments, Drasov, Czech Republic) according to the manufacturer’s protocol. To increase the signal to noise ratio and to prevent irregularities related with cell sedimentation in the course of measurements the cells were concentrated on glass fiber filters GF/F (Whatman, UK). Pilot experiments were carried out to ensure that deposition on GF/F filters does not affect the shape or amplitude of OJIP curve as well as to find a suitable period of dark adaptation of the microalgae. Under our experimental conditions, a dark adaptation of 15 min allowed the reliable recording of OJIP curves.

The analysis of the recorded OJIP curves was carried out according to Strasser et al. [6]; the employed JP-test parameters are detailed in Table 1 and in Fig. 1. Maximal quantum yield of PS II photochemistry, $\Phi_P$ [10], and coefficient of non-photochemical Chl fluorescence quenching, NPQ [20], were also estimated using the FP100s fluorometer.

2.5. Fatty acid analysis

Capillary gas–chromatography was used for fatty acid quantification; the analysis was performed according to [18]. The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analyzed in duplicate.

2.6. Statistical treatment

The experiments were carried out in two biological replications with three analytical replications for each of them. In figures average
values together with standard deviations are presented. The significance of differences was tested using ANOVA from the Analysis ToolPak of the Microsoft Excel spreadsheet software.

3. Results

3.1. Stress-induced changes in growth rate, pigment and lipid composition

The pattern of the changes in total Chl and Car, their ratio and Car composition is shown in Fig. 2a, b; the data on accumulation of biomass, TFA and FA composition are summarized in Table 2 and Fig. 2c, d. Under low light, [LL], intensity (35 μE·m⁻²·s⁻¹), the cultures displayed relatively slow biomass increase (0.16 mg DW d⁻¹) and attained dry weight (DW) of ca. 3.0 g·L⁻¹ by the end of cultivation period (14 d). Under medium light, [ML] (130 μE·m⁻²·s⁻¹ PAR), cultures reached a higher DW of ca. 4.3 g·L⁻¹ at the average rate of 0.29 mg DW d⁻¹. There was no increase in final biomass and growth rate under high light, [HL] (270 μE·m⁻²·s⁻¹ PAR), conditions in comparison with [ML] or [LL] (Table 2).

In all cases studied both TFA percentage of dry biomass and its volumetric content increased with time along with biomass accumulation (Fig. 2c, Table 2). In general, the cultures grown under [ML] or [HL] conditions featured higher TFA accumulation in comparison with the [LL] cultures. The DGLA percentage of DW was linearly related (r² ≈ 0.90) with the percentage of TFA (see Table 2). At the same time volumetric content of DGLA in the [ML] cultures became higher than in [HL] cultures after the 7th d of cultivation (Fig. 2c, d). Notably, the higher the irradiance the higher the proportion of oleic acid (C18:1) mainly at the expense of DGLA and other polysaturated FA was detected in the FA profiles of the cultures (Table 2).

The Chl content declined, after a moderate increase during the first 3 d of cultivation, as in the case of [LL] and [ML] (Fig. 2a, curves 1 and 2, respectively), or from the beginning of the experiment ([HL]; Fig. 2a, curve 3). Carotenoid content did not exceed 11 mg·L⁻¹ in all cases. A pronounced (up to four-fold under HL) irradiance-dependent rise in Car/Chl ratio was recorded in the absence of N: the higher the PAR irradiance, the higher the rate of the linear increase in Car/Chl with cultivation time (Fig. 2b). As could be seen from Fig. 2, the Car/Chl ratio rapidly increased along with the decline in Chl following similar non-linear trend regardless of the irradiance level. The Chl a/b ratio, as determined spectrophotometrically (not shown), tended to decrease slightly (from ca. 2.4 to 2.1) but these changes turned to be statistically insignificant.

The proportions of the individual carotenes and xanthophylls were determined at the beginning and at the end of the experiment (day 14; see pie charts in Fig. 2b). Regardless of irradiance, the Car profile of P127 cells included neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and β-carotene with lutein and β-carotene as the major Car. Among the changes recorded in the Car profile of P127 under the stressful conditions, the most distinct was an irradiance-dependent decrease in lutein and an increase in β-carotene; the bulk of the latter (ca. 70%) was localized in cytoplasmic OB by the end of cultivation (the 14th d). The proportions of the other Car decreased along with the decrease in Chl content.

3.2. Chl a fluorescence transients

The amplitude and shape of the OJIP curves recorded in the N-starving P127 cells were dependent on the irradiance level during cultivation (Fig. 1). The higher the PAR irradiance during cultivation, the lower the amplitude of the OJIP curve in comparison with the semi-diluted nitrogen-replete culture used as inoculum (Fig. 1, curves 1 and 2–3). Under irradiances of 35 (low light, LL) and 130 μE·m⁻²·s⁻¹ (medium light, ML) the O-J rise was faster than in non-stressed P127 cells; this was not the case in the cultures grown under high light (HL, 270 μE·m⁻²·s⁻¹). One should also note a pronounced decline in the intensity of fluorescence after 100 ms in all cases studied. At the same time the OJIP curves recorded for the ML and HL cultures became increasingly flat in the time domain 1500–10,000 μs (Fig. 1, curves 3–4); thus, in HL cultures 1 step was hardly discernible after 14 d of N-deprivation (Fig. 1, curve 4). All the cultures studied displayed a prominent decline after P peak in their OJIP curves.

3.3. Time-course of changes in the chlorophyll fluorescence parameters

The time-course of the changes in selected CF-based parameters (Table 1) is plotted on Fig. 3. In spite of overall decline in Chl (Fig. 2a), nitrogen starvation brought about an increase in the effective antenna size (ABS/RC; Table 1) calculated on the base of the recorded OJIP in all variants studied (Fig. 3a). The higher the irradiance during cultivation, the more pronounced the increase (ca. two-fold in the [HL] cultures). A notable feature of the ABS/RC

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Table 1

<table>
<thead>
<tr>
<th>Characteristic points of OJIP and derivation of selected JIP-test parameters ([8], see also Fig. 1).</th>
</tr>
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<tbody>
<tr>
<td>F₀, Fₛ, Fₐₐₙ, Fₐₓ</td>
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<tr>
<td>φₒₒ = Fₒₒ / Fₐₒₒ – 1</td>
</tr>
<tr>
<td>Vₒₒ = (Fₒₒ – Fₐₒₒ) / Fₐₒₒ</td>
</tr>
<tr>
<td>ψₒₒ = 1 – Vₒₒ</td>
</tr>
<tr>
<td>Mₒₒ = φₒₒ / (Fₒₒ – Fₐₒₒ)</td>
</tr>
<tr>
<td>ψₒₒ = φₒₒ (1 – Fₐₒₒ / Fₒₒ)</td>
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<td>ABS/RC = Mₒₒ / (1 – Fₐₒₒ / Fₒₒ)</td>
</tr>
<tr>
<td>TRₒₒ/RC = Mₒₒ / (1 – Fₐₒₒ / Fₒₒ)</td>
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Fig. 1. Effect of cultivation on N-free BG11 medium under (2) low, (3) medium or (4) high light conditions on OJIP curves in the P127 mutant of P. incisa; curve 1—OJIP curve recorded for the inoculum (0 d). Fluorescence values are expressed as F/F₀.
kinetics was the transient decline of the parameter which was observed at the 3rd day of cultivation under the [LL] conditions but disappeared at the 5th day (Fig. 3a, curve 1); it was hardly detectable in the [ML] cultures (Fig. 3a, curve 2) and lacked in the [HL] cultures (Fig. 3a, curve 3).

The analysis of OJIP also revealed that the increase in ABS/RC mentioned above was accompanied by a rise of absorbed light energy trapping (TR0/RC; Table 1) by the reaction centers of the cultivated cells (Fig. 3b). As in the case of the ABS/RC parameter, the overall increase in TR0/RC was preceded by a decline which was most significant

Table 2
Changes in fatty acid profile and DW in the cultures of the Parietochloris incisa P127 mutant grown under different irradiances.

<table>
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<tr>
<th>Cultivation time, d</th>
<th>Irradiance</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>18:3</th>
<th>20:3</th>
<th>% of total fatty acids</th>
<th>TFA</th>
<th>DGLA</th>
<th>DW</th>
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<tr>
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<td>2.3</td>
<td>14.9</td>
<td>2.6</td>
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<td>11.6</td>
<td>15.9</td>
<td>10.9</td>
<td>1.7</td>
<td>1.1</td>
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<td>LL</td>
<td>13.9</td>
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<td>15.0</td>
<td>2.1</td>
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<td>21.3</td>
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<tr>
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Fig. 2. The dynamics of (a) total chlorophyll, (b) carotenoid to chlorophyll ratio, (c) total fatty acids and (d) DGLA in the cells of the P127 grown on N-free medium at low (1), moderate (2) and high (3) irradiance. Pie charts in (b): carotenoid composition for the inoculum (d 0) and for the cultures grown at different irradiances for 14 d. Neo—neoxanthin, Vio—violaxanthin, Ant—antheraxanthin, Lut—lutein, Zea—zeaxanthin, β-Car—β-carotene localized in thylakoids, β-Car (OB)—β-carotene localized in oil bodies (the secondary carotenoid).
in the [LL] culture (Fig. 3b, curve 1) and less pronounced at higher irradiances (Fig. 3b, curves 2 and 3). Notably, after seven days of cultivation the increase in TR0/RC ceased in all variants and did not change significantly thereafter as in the case of the ABS/RC parameter (excluding the [HL] cultures).

The time-course of non-photochemical quenching (NPQ, Fig. 3c) and ϕD0 (Fig. 3d) levels under different irradiances displayed, for the first seven days, the increase at an irradiance-dependent rate. In the cultures grown under LL, sustaining nearly constant Chl content, only a modest increase in ϕD0 and NPQ was detected (curves 1 in Fig. 3c, d). However, in the [ML] and [HL] cultures, a pronounced irradiance-dependent rise of these parameters took place (curves 2 in Fig. 3c, d). In the [HL] cultures ϕD0 increased continuously till the last day of cultivation (Fig. 3c, d; curve 3) whereas NPQ essentially ceased to increase after the 5th day of cultivation. Notably, the kinetics of ϕD0 and ABS/RC followed a similar pattern (cf. Fig. 3a and d).

On the contrary, the magnitude of the photochemistry-related parameters, ϕE0 and ϕP0, in the cultures decreased with time in an irradiance-dependent manner (Fig. 3e, f). It should be noted that the initial values of ϕD0 and ϕE0 were close and comprised ca. 0.25. As the result of the changes described above, only a modest change in these parameters were detected under LL (curves 1 in Fig. 3e, f). Under ML conditions ϕD0 increased and ϕE0 decreased nearly two times. High PAR irradiance brought about a three-fold increase in ϕD0 (curves 2 in Fig. 3e, f) which was associated with a drastic (ca. five times) decline in ϕE0 (curves 3 in Fig. 3e, f).

3.4. Relationships of thermal dissipation and photochemical utilization of the absorbed light energy with key characteristics of the P127 cultures

The relationships between the changes in quantum yields of thermal dissipation of the absorbed light energy (ϕD0), electron transport (ϕE0), photosystem II photochemistry (ϕP0) with Car/Chl ratio and TFA accumulation are presented in Fig. 4. As could be seen from Fig. 4a, b, ϕD0 increased along with Car/Chl ratio and TFA percentage of DW in cultures grown under different irradiance levels following a similar trend. On the contrary, the values of the photochemistry-related parameters, ϕE0 and ϕP0, decreased with an increase in Car/Chl and TFA content in the cells of the alga exerting strong, uniform relationships with Car/Chl ratio (Fig. 4c, d) and TFA percentage of biomass (Fig. 4e, f) as well as with DGLA content (see Table 2 and Fig. 2d) regardless of the PAR irradiance during cultivation.

4. Discussion

This work represents an attempt to relate stress responses of pigment apparatus and lipid metabolism with the changes in OJIP curves and calculated on its basis parameters characterizing the condition of PSA in a green microalga of biotechnological importance, the DGLA-producing mutant of P. incisa [14]. The evidence obtained in this work (Fig. 2) confirmed our previous finding that the DGLA-producing mutant of P. incisa, P127, similarly to its parent strain [21–23] responds to elevated irradiances (represented by the [HL] and [ML] treatments in the present

![Fig. 3. Time course of the changes in the fluxes of energy (a) absorbed or (b) trapped per RC, (c) NPQ level as well as in quantum yields of energy dissipation (d), electron transport (e), and maximal quantum yield of primary photochemistry (f) in the cells of P127 mutant of P. incisa cultivated on N-free BG11 medium under (1) low, (2) medium or (3) high light.](image)
The adaptive rearrangements in pigment and lipid metabolisms revealed in the P127 cultures under conditions unfavorable for the microalga growth but favoring accumulation of PUFA-enriched TAG and secondary carotenoid deposition in OB. These rearrangements were accompanied by irradiance-dependent changes in the functioning of the PSA manifesting itself as a decline in overall CF intensity (likely due to an overall decline in Chl, at least in the cases of [ML] and [HL]) and profound alteration of the OJIP curves (Fig. 1). Generally, the slope of the initial CF rise in OJIP curve reflects photochemical reduction of Qo, which is limited by the rate of exciton formation in the PS II antenna [30] depending predominantly on the excitation light intensity and on the PS II antenna absorption cross-section. It is commonly accepted that the shape of the initial fluorescence rise depends on the energetic connectivity or probability of exciton exchange between PS II units [6]. In higher plants and green algae cultivated under optimal conditions this part of the OJIP possesses a sigmoid shape [8] that was explained by the high connectivity. Under our experimental conditions, increased rate of the initial fluorescence rise starting from the point O without the lag was found in N-starving P127 (Fig. 1). One may speculate that this effect could be related with high absorption cross-section of PS II (Fig. 3a) or lower segregation of photosystems reducing energetic connectivity between single PS II units [6,8]. The OJIP curves recorded in N-starved P127 cells displayed decreased amplitude of J step and a slightly increased dip after the J step. Then, the J–I–P rise declined significantly (both the I/O and P/O ratios) indicating impairment of electron flow from PS II to the PQ pool [31]. This effect progressively increased with irradiance; it may reflect a progressive impairment of electron transport between QA and plastoquinone pool and accumulation of ‘closed’ PS II centers in the N-deprived P127 cells. A prominent decline of the curve after P peak was likely due to the influence of non-photochemical quenching [32].

For further characterization of the condition and functioning of the PSA of the strain under the stress we used selected parameters derived from the recorded OJIP curves [6] (Table 1). Notably, ABS/RC tended to increase in irradiance-dependent manner in the course of cultivation (Fig. 3a). The trapped energy flux exhibited a more complex kinetics with a drop at 3rd day (most prominent in the LL and ML cultures) followed by the increase which ceased after the 7th day of cultivation. The magnitude of this drop displayed a dependence on the PAR irradiance indicating that slower rates of TFA accumulation at LL were associated with light-limiting conditions and reduced energy flux to RC. This suggestion was confirmed by steady NPQ levels which did not increase till the late starvation period (Fig. 3c, curve 1). These changes were unexpectedly followed by an increase in ABS/RC and TR0/RC which occurred on the background of overall decline in Chl content (Fig. 2a). It is difficult to infer the exact reason of this phenomenon: the relative increase in antenna size is unlikely since the decrease in Chl a/b ratio proved to be insignificiant. One may also think that the dismantling of antenna during the reduction of PSA characteristic e.g. of acclimation of the P. incisa WT to N deprivation [23] and high light [3] proceeds at a rate somewhat slower in comparison to the reaction center dismantling rate. This process could effectively decrease the number of reaction centers and correspondingly increase the effective size of the antenna. At the same time cultivation under N-starvation was accompanied with continuous FA formation requiring photosynthetic carbon assimilation to supply the precursors for the de novo FA synthesis. So it is quite possible that the observed increase in ABS/RC and TR0/RC is also related with an elevated carbon flux towards FA production to meet the increasing demand in FA for TAG formation occurring under the stressful conditions which likely allowed the N-starving cells of the microalga to cope with the long-term nutrient deprivation [33,34].

Notably, the kinetics of the ABS/RC and TR0/RC featuring a prominent fall occurring by the 3rd day under nitrogen starvation condition closely resembled an inverse trend of the accumulation of
transcripts of LC-PUFA biosynthesis genes, namely desaturases and PUFA elongase [14] as well as some other genes engaged in carbon supply for FA synthesis (I. Khozin-Goldberg, N. Shtaida, unpublished), which is characterized by a peak around the 3rd day of cultivation. Indeed, the initial period of 2–3 days upon transferring to the N-depleted medium is characterized by the highest daily rates of FA accumulation (Table 2 and Fig. 2c, d) as well as the fastest increase in the proportion of the major LC-PUFA in both mutant and WT strains of P. incisa. The rate of following biomass and TFA accumulation stage was irradiance-dependent (Fig. 2c; see also [24]). It is important to note that, under elevated PAR irradiances, the increased carbon fixation rates likely facilitate the incorporation of de novo produced oleic acid (18:1) into TAG. Probably, this could explain the decline of the DGLA proportion on the background of the accumulation TAG with less unsaturated FA.

The analysis of quantum yield of thermal dissipation showed that in the time-course of P127 cultivation under stress increasing part (more than one half under HL) of the absorbed light energy was dissipated into heat (Fig. 4d). A corresponding increase in NPO level was recorded under these conditions (Fig. 3c). Obviously, a considerable if not dominant contribution to NPQ was made by energy-dependent quenching (qE) mechanisms such as violaxanthin cycle [35,36]. At the same time, under our experimental conditions a significant amount of the violaxanthin remained in de-epoxidized state even after prolonged (several hours) dark adaptation (not shown) as in our previous studies with the WT [22] and the mutant [24]. This phenomenon could, at least in part, explain the permanently high NPQ levels recorded in the HL cultures at advanced stages of cultivation (curve 3 in Fig. 3c). Close levels of permanent NPQ were discovered in the wild type P. incisa [22] with the carotenoid profile similar to that of P127. Furthermore, one cannot rule out engaging other mechanisms such as photo-inhibitory quenching [30].

Remarkably, in spite of the different irradiance-dependent kinetics (Fig. 3d), the relationships between $\phi_{qE}$, Car/Chl ratio (which was found to be a marker of stress in P. incisa [37]), and accumulation of TFA were uniform (Fig. 4a and b). Similarly tight but opposite relationships were found between the biochemical parameters and $\phi_{qE}$ (Fig. 4c, d) or $\phi_{qE}$ (Fig. 4e, f). On one hand, these relationships could arise due to simultaneous increase in the proportion of ther- mally dissipated absorbed light energy under saturating PAR fluxes and induction of storage lipid and secondary Car biosyntheses. This is especially possible under combined stress (e.g. nitrogen starvation and strong irradiation) conditions [33]. On the other hand, quantum yield of PS II photochemistry decreases under conditions that reduce the rate of carbon fixation (including N-deprivation) and hence slow down the rate of dissipation of excitation energy through photochemistry. The enhanced rate of energy dissipation through non-photochemical pathways diminishes the possibility of photooxidative damage of the microalgal cells by reactive oxygen species which may be formed in antenna and electron-transport chain of chloroplast under over-excitation conditions.

Taking into account the results obtained, one may speculate that under all conditions studied in this work there is a single law by which the balance of light energy absorbed, utilized or dissipated by PSA determines the productivity of the microalgal cells. Still, under high fluxes of PAR (the [HL] conditions in our case) on the background of nitrogen starvation the energy-dependent quenching-based photoprotective mechanism though induced to the maximum possible extent (as suggested by the saturation of NPQ under [HL] conditions, see Fig. 3c, curve 3) cannot cope with the excessive excitation pressure leading to a decrease in biomass and FA (including DGLA which is the important target product for this alga) yields. Therefore the conditions conducive to the build-up of extremely high NPQ levels should be avoided in mass cultivation of microalgae. One may think that the CF-based methods would be helpful for the early detection of the onset of such conditions.

5. Conclusions

The results obtained in the present work suggest the existence, at least under conditions studied, of strong relationships between OJIP-derived parameters of CF characteristic of the state of the PSA of the P127 mutant as well as of remote from the primary photosynthetic reactions but biotechnologically important parameters such as Car/Chl ratio or TFA content. Taking into account advantages of CF-based techniques (simplicity, sensitivity, robustness, high throughput) and increasing availability of fluorometers optimized for measurements of microalgae, the insight obtained in this work could be useful for the development of algorithms for on-line monitoring of physiological condition of microalgae cultivated under stressful conditions for obtaining of high value products. However, there are many environmental factors influencing OJIP curves about which little is known. Therefore more basic research on relationships between fluorescence, photosynthesis, pigment and lipid metabolism under stress needs to be done in order to complement current methods for monitoring of microalgal cultures with reliable CF-based techniques.

Disclosure statement

The authors declare no conflict of interest.

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