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Effects of propyl gallate on photosystem II efficiency in *Dunaliella bardawil* under high illumination as investigated by chlorophyll fluorescence measurements

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Abstract Effects of n-propyl gallate, a plastid terminal oxidase inhibitor involved in chlororespiration, on photosystem II efficiency in *Dunaliella bardawil* under low or high illumination was investigated. Rapid chlorophyll *a* fluorescence transients were recorded and analyzed according to JIP-test, which can quantify the photosystem II performance. The fluorescence transients O-J-I-P drastically decreased and almost reached a plateau when low light-grown cells were exposed for 96 h to 1, 2 and 4 mM n-propyl gallate. Very similar reductions in the efficiency of quantum yield of primary photochemistry (Φ_{po}), the quantum yield for electron transport (Φ_{Eo}) and the inferred water-splitting complex activity (F_v/F_o) were found in the same inhibitor concentrations. However, no statistically significant change in fluorescence intensity and photosystem II efficiency was found when algal cells were exposed to the inhibitor concentrations up to 2 mM under high light intensity. The results indicated that inhibitory effects of n-propyl gallate on photosystem II electron flow in *D. bardawil* cells are dependent on environmental conditions. It is also demonstrated that n-propyl gallate is a multi-target inhibitor of growth kinetics as well as photosynthesis. In addition, we found that the donor side of photosystem II acts as main target place of the inhibitor.

Keywords Chlorophyll fluorescence · *Dunaliella bardawil* · JIP-test · High light intensity · n-Propyl gallate · Photosystem II

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1 Introduction

Photosynthesis in chloroplasts involves a vectorial electron transfer from water in the lumen to $NADP^+$ in the stroma, by means of redox carriers. High illumination is a complex stress in which the inactivation of photosystem II (PSII) reactions is manifested as a decrease in the quantum yield of photochemistry. Exposure of plants to excess light frequently results in PSII photoinactivation due to reactions on both the PSII acceptor and donor sides and also in the degradation of the reaction center D1 protein (Powles 1984; Barber and Andersson 1992; Prasil et al. 1992; Aro et al. 1993; Asada 1996, 1999; Niyogi 2000). Under such environmental conditions, plants have developed several mechanisms to dissipate excess absorbed energy (Quiles 2006; Diaz et al. 2007; Miyake et al. 2004, 2005). Thus, PSII electron flow can change to cope with stress (Quiles 2006; Diaz et al.

2007; Gamboa et al. 2009; Ibanez et al. 2010) which is easily detectable by fluorescence measurements.

Chlorophyll (Chl) *a* fluorescence kinetic is an informative tool for investigating PSII electron transport (For review see e.g. Krause and Weis 1991; Govindjee 1995; Strasser et al. 2000, 2004; Fricke and Peters 2002). When dark-adapted photosynthetic samples are excited with light, the fluorescence intensity rises rapidly from an initial low value, Fo (the O level), to a high value, F_p (the P level). Two intermediary steps designated as F_J (the J level) and F_I (the I level) normally appear under high light illumination conditions. The sequence of phases is labeled as O, J, I and P (Strasser et al. 1995). The Chl *a* fluorescence rise from minimum fluorescence level (F_o) to J level (F_J) at around 2 ms is due to reduction of Q_A by PSII. The fluorescence rise is followed to the I level (F_I) at around 30 ms, due to the filling up of the PQ pool. Finally, there is a rise from F_I to P level, due to traffic jam on the electron acceptor side of PSI (Strasser et al. 2004). The performance of PSII is quantified through a series of functional and structural parameters derived from the fluorescence transients (O-J-I-P) according to JIP-test (Strasser and Strasser 1995; Stirbet et al. 2001; Strasser and Stirbet 2001). The JIP-test offers simple equations expressing the equilibrium between the inflow and outflow of the entire energy flux within PSII. Therefore, this analysis enables us to understand the relationships between PSII activity, fluorescence signals, and their analytical expressions in various photosynthetic organisms under different stress conditions (Strasser et al. 2000; Tsimilli-Michael et al. 2000; Appenroth et al. 2001; Lu and Vonshak 2002; Bussotti et al. 2007).

n-Propyl gallate (PG), a free radical scavenger with antioxidant properties, displays wide biological effects in plants (Elich et al. 1997; Nagata et al. 2004; Raghavan and Hultin 2005; Chaudhuri and Kar 2008; Zurita et al. 2007). It is widely used as a specific inhibitor of plastid terminal oxidase (PTOX) involved in chlororespiration, in photosynthetic studies (Cournac et al. 2000; Kuntz 2004; Gamboa et al. 2009; Einali and Shariati 2012). Previous studies have demonstrated that PG concentration of 1 mM inhibits PTOX and decreases PSII quantum yield in stressed plants, but has no effect on PSII under favorable conditions (Cournac et al. 2000; Rizhsky et al. 2002; Aluru and Rodermel 2004; Quiles 2006; Diaz et al. 2007; Gamboa et al. 2009; Ibanez et al. 2010). Thus, the inhibitor might affect photosynthetic electron flow within PSII (Einali and Shariati 2012). However, most of these studies have focused on the effects of PG, as a chlororespiratory inhibitor in stressed higher plants, on PSII electron transport capacity and relatively few have examined these effects in algal classes. Furthermore, direct effects of different PG concentrations on PSII electron flow and growth kinetic under both stable and unstable conditions have not yet been investigated extensively.

Our recent study displayed that responses of *Dunaliella salina* cells to different PG concentrations is dependent on stress conditions (Einali and Shariati 2012). Therefore, PG-induced changes of PSII electron transport activity is influenced by stress situation. Due to the fact, however, the hypothesis can be developed if the dependence of PG effects to stress situation actually applies in other species. In the present study, *Dunaliella bardawil*, a unicellular and halotolerant green alga, was used to examine the effects of PG on PSII function as probed by rapid Chl *a* fluorescence measurement and JIP-test analysis. Additionally, we determined the inhibitory site of PG on PSII electron transport chain.

2 Materials and methods

2.1 Algal cultures and experimental conditions

Dunaliella bardawil Ben-Amotz *et* Avron, UTEX 2538 was obtained from UTEX, The Culture Collection of Algae at the University of Texas at Austin. The cells were grown in a culture medium (pH = 7.5) with concentration of 1 M NaCl as described before (Shariati and Lilley 1994). Cultures were incubated in a culture room at 25 °C and 70 µmol photons m^{-2} s^{-1} of light under a 16 h/8 h light/dark photoperiod with continuous shaking (100 rpm). Exponentially growing cultures were transferred into the aseptic 250-ml Erlenmeyer flasks in final volume of 100 ml where initial cell density was approximately 5×10^6 cells ml^{-1}. The algal samples (in triplicate) were treated with n-propyl gallate (PG) (purchased from Fluka company) at five levels of 0.1, 0.5, 1, 2 and 4 mM. PG-treated cultures were exposed for 96 h to 70 µmol photons m^{-2} s^{-1} as low light (LL) and 400 µmol photons m^{-2} s^{-1} as high light (HL)

conditions. Cultures at the low or high light in the absence of inhibitor were used as control.

2.2 Cell growth and pigment determination

The cell number was determined using a hemocytometer under a light microscope (Schoen 1988). For total Chl and β-Car extraction, an aliquot (1 ml) of algal suspension was precipitated by centrifugation (10,000 × *g* for 5 min) followed by the addition of 1 ml of 80 % (v/v) acetone and recentrifuged (10,000 × *g* for 2 min) after vortexing. Chl content was spectrophotometrically determined by method of Arnon (1949*g*). β-Car was assayed according to Ben-Amotz and Avron (1983). $E^{1\%}_{1cm}$ of 2,273 at 480 nm has been used to calculate of β-Car concentration.

2.3 Measurement of Chl fluorescence

To evaluate the effects of PG on the electron transport of PSII, the fast Chl *a* fluorescence transients in *D. bardawil* exposed 96 h to different inhibitor concentrations under LL and HL conditions were measured. Chl *a* fluorescence induction was measured using the Plant Efficiency Analyzer (Handy PEA fluorimeter, Hansatech Instruments Ltd., Pentney, King's Lynn, Norfolk, England). The cell suspensions (10 μg Chl *a* ml^{-1}) were pipetted into the glass vials and predarkened for 10 min at room temperature. Rapid Chl *a* fluorescence induction from 10 μs to 1,000 ms was measured when the dark-adapted cells were exposed to a strong light pulse (3,500 μmol photons m^{-2} s^{-1}). The data were analyzed and the so-called JIP-test parameters was calculated using BiolyzerHP3 software (Laboratory of Bioenergetics, University of Geneva, Switzerland) (Strasser et al. 2000). The JIP-test parameters used in the study is listed in Table 1. The fluorescence intensity at 50 μs was considered as O value ($F_{50\ \mu s}$) and the maximum fluorescence intensity was attained as P(F_m), according to Strasser et al. (2004). The PSII water-splitting complex activity was determined by the ratio between variable fluorescence (F_v, fluorescence intensity between O and P transients) and minimal (F_o, fluorescence intensity at O transient), F_v/F_o, according to Schreiber et al. (1994) and Pereira et al. (2000). The Performance index (PI_{ABS}) was calculated according to Strasser et al. (2000). The parameter describes overall photosynthetic performance, combines several parameters that depict three main functional characteristics of PSII

Table 1 OJIP test parameters and expressions

Parameters	Description
ABS/RC	Light absorption flux (for PSII antenna chlorophylls) per reaction center (RC)
dV/dt_o	The initial slope at the beginning of the relative variable fluorescence transients
$ET_o/(TR_o-ET_o)$	Electron transport beyond Q_{A^-}, $ET_o/(TR_o - ET_o) = (F_m - F_J)/(F_J - F_o)$
F_o	Fluorescence intensity at 50 μs (O step)
F_J	Fluorescence intensity at 2 ms (J step)
F_I	Fluorescence intensity at 30 ms (I step)
F_m	Maximal fluorescence intensity (P step)
F_v	Maximal variable fluorescence, $F_V = F_m - F_o$
F_v/F_o	A value that is proportional to the activity of the water-splitting complex on the donor side of the PSII
O–J–I–P	Transient fluorescence induction defined by the names of its intermediate steps (O is for 'origin' and 'P' is for peak, whereas, J and I are intermediate steps)
PI_{ABS}	Performance index, $PI = (RC/ABS)\cdot(TR_o/DI_o)\cdot[ET_o/(TR_o - ET_o)]$
RC/ABS	Density of reaction centers per PSII antenna chlorophyll
TR_o/DI_o	Flux ratio trapping per dissipation, $TR_o/DI_o = F_v/F_o$
Φ_{Po}	Maximum quantum yield for primary photochemistry
Ψ_o	Probability that an electron moves further than Q_A
Φ_{Do}	Thermal dissipation yield
Φ_{Eo}	Quantum yield for electron transport (ET)

reaction center, namely density of reaction centers per PSII antenna chlorophyll (RC/ABS), ratio of trapping and dissipation fluxes (TR_o/DI_o) and efficiency of the conversion of excitation energy to electron transport ($ET_o/(TR_o - ET_o)$) (Strasser et al. 2000).

2.4 Statistical analysis

The experiments were done in three independent replicates for all treatments. Means and standard deviations (SD) were calculated for each treatment. Statistically significant differences between control and PG-treated samples at $P < 0.05$ were determined using Analysis of Variance (ANOVA) with a Holm-Sidak post hoc test. The ANOVA information for all physiological and JIP-test parameters has been addressed in Table 2. Correlation coefficients and significance level of some physiological and JIP-test parameters was determined. All statistical analyses were performed using SigmaStat 3.0, Systat Software, San Jose, California.

3 Results

3.1 Effects of PG on cell density and pigment content

Cell mortality was severe in the *Dunaliella* cultures in the presence of PG concentrations higher than 0.1 mM, so that about 90 % of the cells died in LL-grown cells subjected 96 h to 4 mM of the inhibitor (Fig. 1a). No significant change was found in cell number when algal cultures were exposed for 96 h to PG concentrations up to 1 mM under HL conditions. However, cell density was decreased in these cultures at 2 and 4 mM PG by 23 and 33 % as compared to the control, respectively (Fig. 1a).

Pigment content showed a pattern similar to each other in PG-treated cells subjected 96 h to both LL and HL conditions (Fig. 1b, c). Chl and β-Carotene (β-Car) content increase in LL-grown cells treated with 0.1 mM PG but decreased significantly under higher PG concentrations. HL-grown cells exhibited a significant decrease in pigment content in the presence of the inhibitor concentrations up to 2 mM. However, pigment content of these cells increased pronouncedly at 4 mM PG (Fig. 1b, c).

3.2 Effects of PG on Chl *a* fluorescence induction curves

Dunaliella bardawil exhibited a typical polyphasic rise of fluorescence induction (O-J-I-P) (Fig. 2). Fluorescence transient curves represent no change in rise of fluorescence induction (O-J-I-P) in LL-grown cells subjected to PG concentrations of 0.1 and 0.5 mM when compared to the control (absence of PG) (Fig. 2a). However, these transients almost reached a plateau when these cells subjected to PG concentrations of 1, 2 and 4 mM (Fig. 2a). In fact, very low PSII electron transport capacity was detectable due to exposure of *Dunaliella* cells to the concentrations more than 0.5 mM PG. Experiments on HL-grown cells revealed a lesser polyphasic rise of fluorescence when compared to LL conditions (Fig. 2). Nevertheless, no significant change in photosynthetic electron transport was detectable between HL-grown cells incubated with PG concentrations up to 2 mM (Fig. 2b). In addition, electron transport capacity in these cultures subjected to 4 mM PG was appreciable although that is much lower than other fields.

3.3 Measurement of electron transport capacity between PSII components

The efficiency of quantum yield of primary photochemistry (Φ_{po}) declined significantly when LL-grown cells were exposed for 96 h to the inhibitor concentrations higher than 0.5 mM (Fig. 3a). Similar results were obtained in the oxidation content of Q_A^- to Q_A (Ψ_o) as well as the quantum yield of electron transport (Φ_{Eo}) (Fig. 3a). In contrast to LL, these parameters started to increase in HL-grown cells treated with 1 and 2 mM PG (Fig. 3b). However, a significant decline in all parameters was found for HL-grown cells incubated with 4 mM PG (Fig. 3b).

The efficiency of the water-splitting complex on the donor side of PSII (as inferred from F_v/F_o) decreased pronouncedly in LL-grown cells subjected 96 h to PG concentrations higher than 0.5 mM (Fig. 4a). However, no significant change in F_v/F_o parameter was found when HL-grown cells were incubated with PG concentrations up to 2 mM as compared to the control, though it declined at 4 mM PG (Fig. 4a). LL-grown cells incubated with PG concentrations higher than 0.5 mM revealed an evident increase in thermal dissipation yield (Φ_{Do}), while HL-grown cells

Table 2 ANOVA table for all physiological and JIP-test parameters used in the study

	Source	DF	SS	MS	F	P
C.D (LL)	Treatments	5	276.651	55.330	235.197	<0.001
	Error	12	2.823	0.235		
	Total	17	279.474			
C.D (HL)	Treatments	5	16.225	3.245	5.148	0.009
	Error	12	7.564	0.630		
	Total	17	23.789			
Chl (LL)	Treatments	5	324.506	64.901	1186.412	<0.001
	Error	12	0.656	0.0547		
	Total	17	325.162			
Chl (HL)	Treatments	5	21.0009	4.202	271.145	<0.001
	Error	12	0.186	0.0155		
	Total	17	21.195			
β-Car (LL)	Treatments	5	19.232	3.846	1633.326	<0.001
	Error	12	0.0283	0.00235		
	Total	17	19.260			
β-Car (Hl)	Treatments	5	1.102	0.220	164.519	<0.001
	Error	12	0.0161	0.00134		
	Total	17	1.118			
Φ_{po} (LL)	Treatments	5	0.986	0.197	186.056	<0.001
	Error	12	0.0127	0.00106		
	Total	17	0.998			
Φ_{po} (HL)	Treatments	5	0.135	0.0271	138.548	<0.001
	Error	12	0.00235	0.000195		
	Total	17	0.138			
Ψo (LL)	Treatments	5	0.0709	0.0142	11.766	<0.001
	Error	12	0.0145	0.00120		
	Total	17	0.0853			
Ψo (HL)	Treatments	5	0.0269	0.00538	12.835	<0.001
	Error	12	0.00503	0.000419		
	Total	17	0.0319			
Φ_{Eo} (LL)	Treatments	5	0.768	0.154	1262.308	<0.001
	Error	12	0.00146	0.000122		
	Total	17	0.769			
Φ_{Eo} (HL)	Treatments	5	0.109	0.0218	136.231	<0.001
	Error	12	0.00192	0.000160		
	Total	17	0.111			
F_v/F_o (LL)	Treatments	5	23.333	4.667	533.225	<0.001
	Error	12	0.105	0.00875		
	Total	17	23.438			
F_v/F_o (HL)	Treatments	5	2.964	0.593	161.502	<0.001
	Error	12	0.0440	0.00367		
	Total	17	3.008			
Φ_{Do} (LL)	Treatments	5	0.986	0.197	186.056	<0.001
	Error	12	0.0127	0.00106		
	Total	17	0.998			
Φ_{Do} (HL)	Treatments	5	0.135	0.0271	138.548	<0.001

Table 2 conitnued

	Source	DF	SS	MS	F	P
	Error	12	0.00235	0.000195		
	Total	17	0.138			
$dVdt_o$ (LL)	Treatments	5	3.707	0.741	158.513	<0.001
	Error	12	0.0561	0.00468		
	Total	17	3.763			
$dVdt_o$ (HL)	Treatments	5	0.0641	0.0128	2.778	0.068
	Error	12	0.0554	0.00462		
	Total	17	0.120			
ABS/RC (LL)	Treatments	5	38.068	7.614	175.596	<0.001
	Error	12	0.520	0.0434		
	Total	17	38.588			
ABS/RC (HL)	Treatments	5	1.396	0.279	2.377	0.102
	Error	12	1.410	0.117		
	Total	17	2.806			
PI_{ABS} (LL)	Treatments	5	34521.503	6904.301	48.903	<0.001
	Error	12	1694.186	141.182		
	Total	17	36215.689			
PI_{ABS} (HL)	Treatments	5	2598.827	519.765	11.196	<0.001
	Error	12	557.098	46.425		
	Total	17	3155.925			
RC/ABS (LL)	Treatments	5	163.655	32.731	34.357	<0.001
	Error	12	11.432	0.953		
	Total	17	175.087			
RC/ABS (HL)	Treatments	5	66.661	13.332	15.133	<0.001
	Error	12	10.572	0.881		
	Total	17	77.233			
TRo/DIo (LL)	Treatments	5	23.255	4.651	535.797	<0.001
	Error	12	0.104	0.00868		
	Total	17	23.359			
TRo/DIo (HL)	Treatments	5	2.964	0.593	161.502	<0.001
	Error	12	0.0440	0.00367		
	Total	17	3.008			
ETo/(TRo-ETo) (LL)	Treatments	5	28.044	5.609	21.828	<0.001
	Error	12	3.084	0.257		
	Total	17	31.128			
ETo/(TRo-ETo) (HL)	Treatments	5	2.752	0.550	14.431	<0.001
	Error	12	0.458	0.0381		
	Total	17	3.210			

N = 18. For more details, see Sect. 2

LL low light, *HL* high light, *C.D* cell density

manifested such increase only for 4 mM PG-treated cells (Fig. 4b). Furthermore, the initial slope at the beginning of the relative variable fluorescence transients (dV/dto) and the light absorption flux (for PSII antenna chlorophylls) per reaction center (ABS/RC) increased considerably in LL-grown cells exposed 96 h to the inhibitor concentrations higher than 0.5 mM, while they remained roughly unchanged when PG-treated algal cells were exposed to HL intensity (Fig. 4c, d).

In LL-grown cells exposed to the inhibitor concentrations higher than 0.5 mM, a pronounced decline in

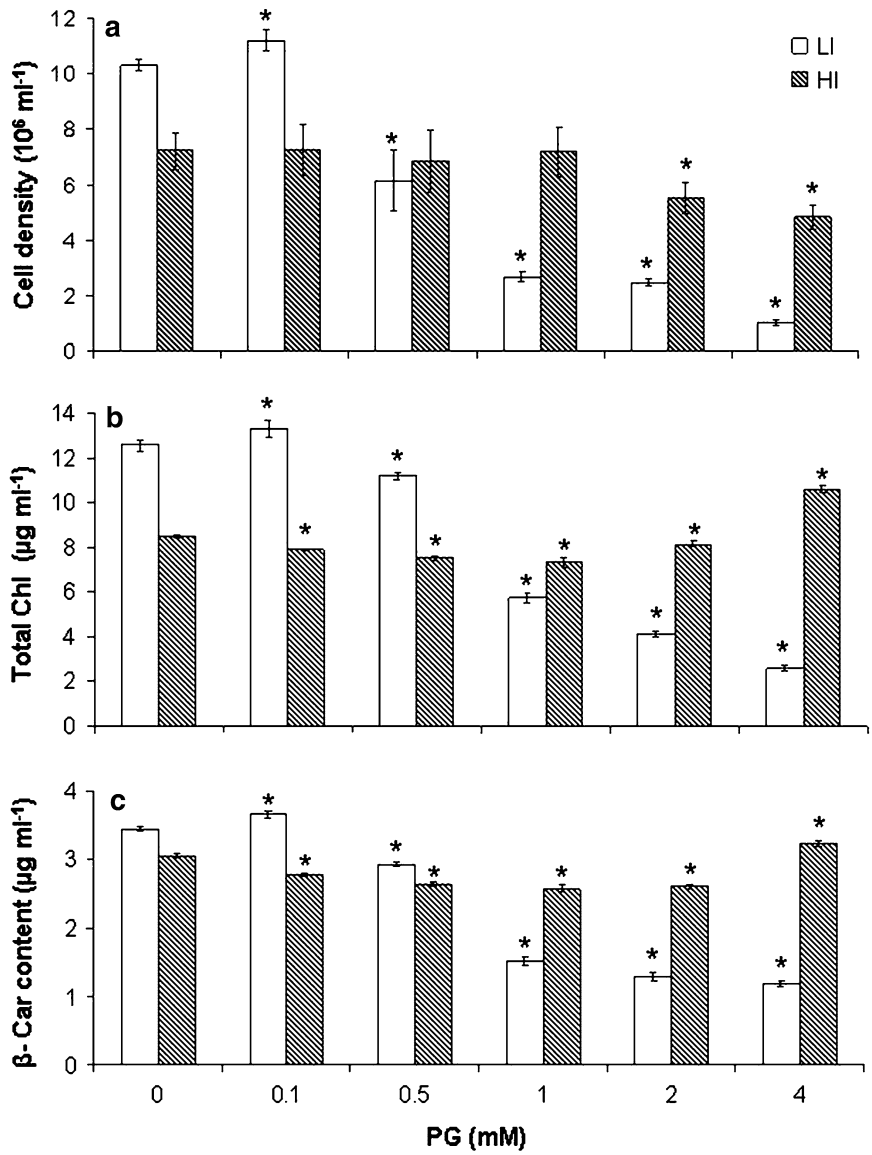

Fig. 1 Effect of PG on cell density (**a**), Total Chl (**b**) and β-Car (**c**) of *D. bardawil* suspensions under LL and HL conditions after 96 h of incubation with the inhibitor. Suspensions grown without PG were taken as control. Each point represents the mean value of three separate samples ± SD. *Asterisks* represent significant difference from control sample (absence of PG) at $P < 0.05$

values of PI_{ABS} and all its components including RC/ABS, TR_o/DI_o and $ET_o/(TR_o - ET_o)$ was detectable (Fig. 5a, c). However, PG had no negative effect on these parameters when algal cells were incubated with PG up to 2 mM under HL conditions (Fig. 5b, d).

3.4 Correlation of growth kinetic with PSII electron transport capacity

There was significant positive correlation between cell density and pigment content with PI_{ABS} and F_v/F_o in LL-grown algae, while no significant or a negative association was observed between these traits in HL-grown cells. The F_v/F_o parameter showed significant correlation with Φ_{po}, Φ_{Eo}, and PI_{ABS} parameters but negative correlation with Φ_{Do} in both LL and HL-grown cells (Table 3).

4 Discussion

Dunaliella dark-adapted cells exhibited a polyphasic Chl *a* fluorescence rise, as previously reported for higher plants, green algae and cyanobacteria (Strasser

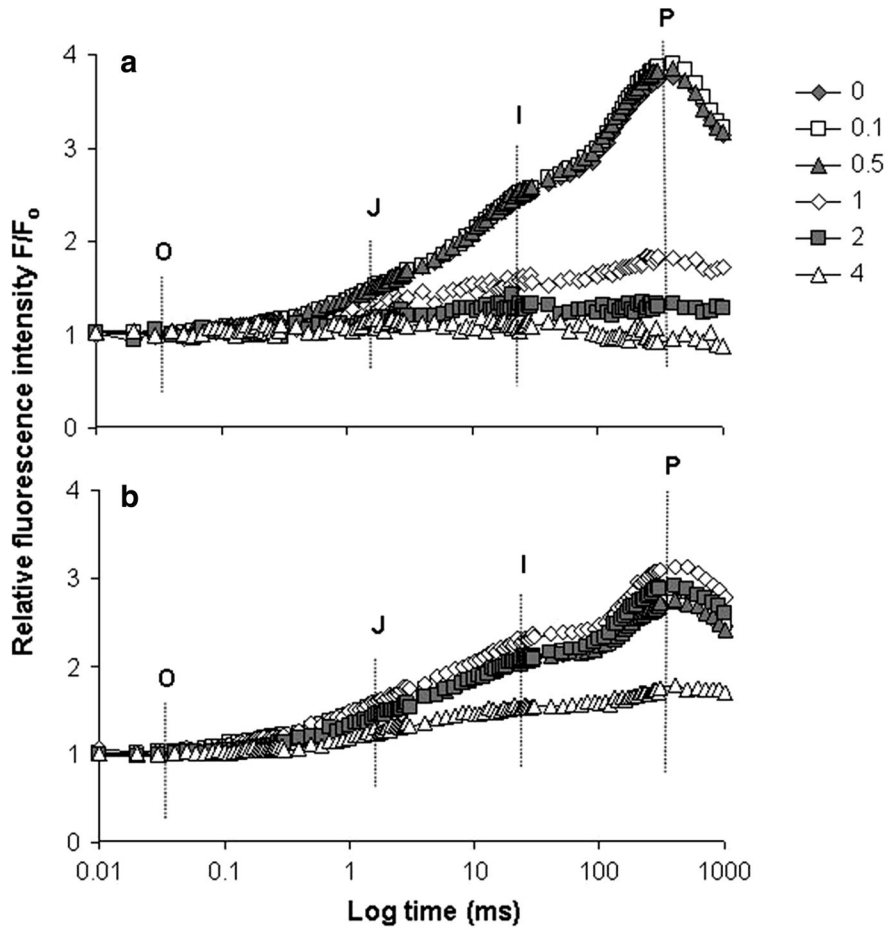

Fig. 2 Chl *a* fluorescence rise in *D. bardawil* cultures exposed for 96 h to 0.1, 0.5, 1, 2 and 4 mM PG under LL (**a**) and HL (**b**) conditions. Suspensions grown without PG under LL or HL conditions were taken as control. O, J, I and P indicate PSII rapid fluorescence transients

et al. 1995; Appenroth et al. 2001; Lu and Vonshak 2002; Xia et al. 2004). The OJIP curves showed that PSII electron flow drastically decreases in LL-grown cells incubated with PG concentrations of 1, 2 and 4 mM (Fig. 2a). It is consistent with Forti and Caldiroli (2005), demonstrating that PG inhibits progressively PSII fluorescence in *Chlamydomonas reinhardtii*. Such drastic decrease of fluorescence intensity at all fluorescence transients in these cultures (Fig. 2a) can show a diminished PSII capacity for electron transport from water-splitting system toward to PSI. Very similar reduction induced by PG concentrations more than 0.5 mM was also found for the efficiency of quantum yield of primary photochemistry (Φ_{po}), quantum yield for electron transport (Φ_{Eo}) (Fig. 3a) and F_v/F_o, a value that is proportional to activity of the water-splitting complex on the donor side of the PSII (Schreiber et al. 1994; Pereira et al. 2000, Kalaji et al. 2011) (Fig. 4a). It has been previously described that the efficiency of the water-splitting complex on the donor side of PSII (inferred from F_v/F_o) is the most sensitive component in the photosynthetic electron transport chain (Kalaji et al. 2011). A decrease in this ratio results from photosynthetic electron transport impairment (Pereira et al. 2000). Correlation analysis showed that there were positive correlation between F_v/F_o with Φ_{po}, Φ_{Eo}, and PI_{ABS} (Table 3). Therefore, it may be assumed that the decrease of the PSII activity can be induced by PG inhibition of water-splitting system feeding electron transport.

Previous studies on higher plants have shown that PG decreases PSII quantum yield under stress conditions (Quiles 2006; Diaz et al. 2007; Gamboa et al. 2009). However, we found that PG did not have any negative effect on electron transport yield and oxygen evolving complex in HL-grown cells (Figs. 2b, 3b, 4a). We suggest that PTOX does not affect PSII electron flow in

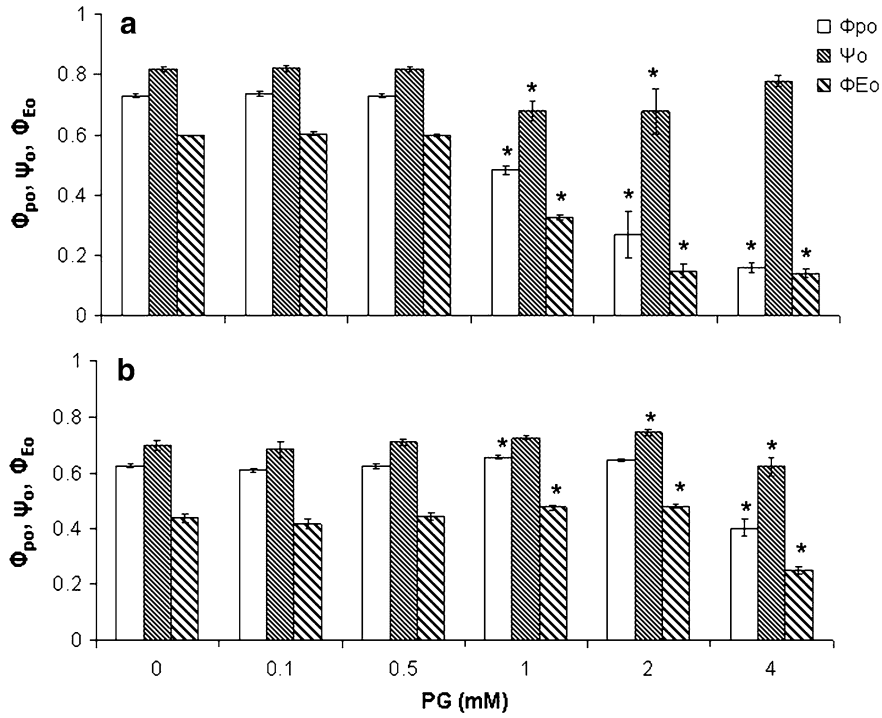

Fig. 3 Effects of PG on the quantum yield of primary photochemistry (Φ_{Po}), the efficiency of electron transfer from Q_{A^-} to Q_B (Ψ_o), and the quantum yield for electron transport (Φ_{Eo}) in *D. bardawil* cells under LL (**a**) or HL (**b**) conditions. Cultures grown without PG under LL or HL conditions were taken as control. Data are the means of three separate experiments ± SD. *Asterisks* indicate result significantly different from control sample ($P < 0.05$)

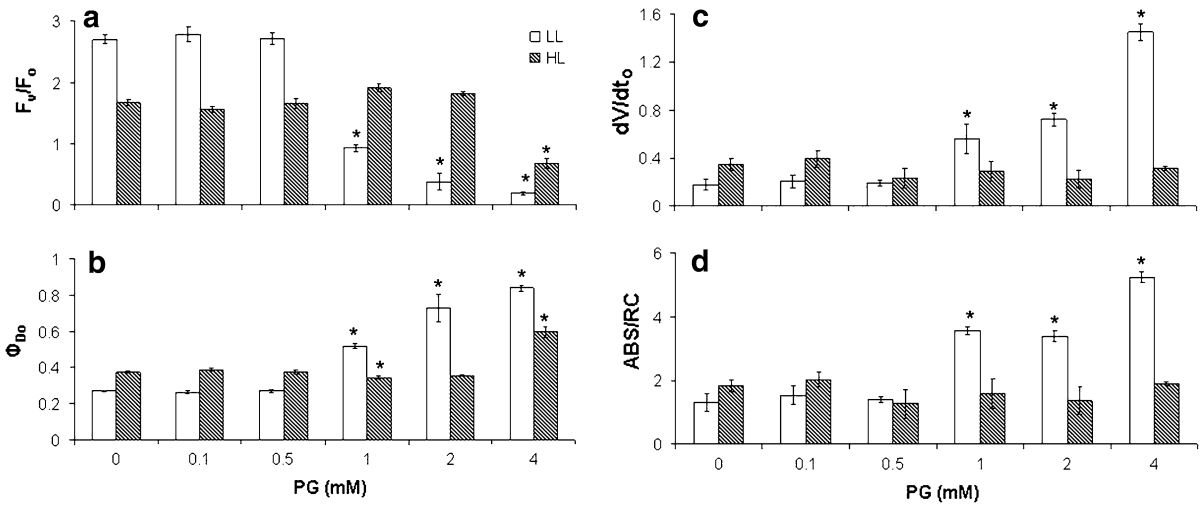

Fig. 4 Changes in **a** water-splitting complex activity (F_v/F_o), **b** thermal dissipation yield (Φ_{Do}), **c** the initial slope at the beginning of the relative variable fluorescence transients (dV/dt_o) and **d** the amount of light absorption per total number of active reaction center (ABS/RC) in *D. bardawil* cultures subjected 96 h to different PG concentrations under LL and HL conditions. Suspensions grown without PG under LL or HL conditions were taken as control. Values are means of three separate experiments ± SD. *Asterisks* represent significant difference from control sample ($P < 0.05$)

D. bardawil cells under HL intensity. Evidence in support of this suggestion comes from a study showing that PTOX does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus in *Arabidopsis* (Rosso et al. 2006). In agreement to the suggestion, Ibanez et al. (2010) discovered that in *Chrysanthemum morifolium*, a sun species, chlororespiratory pathway is not stimulated in response to stress

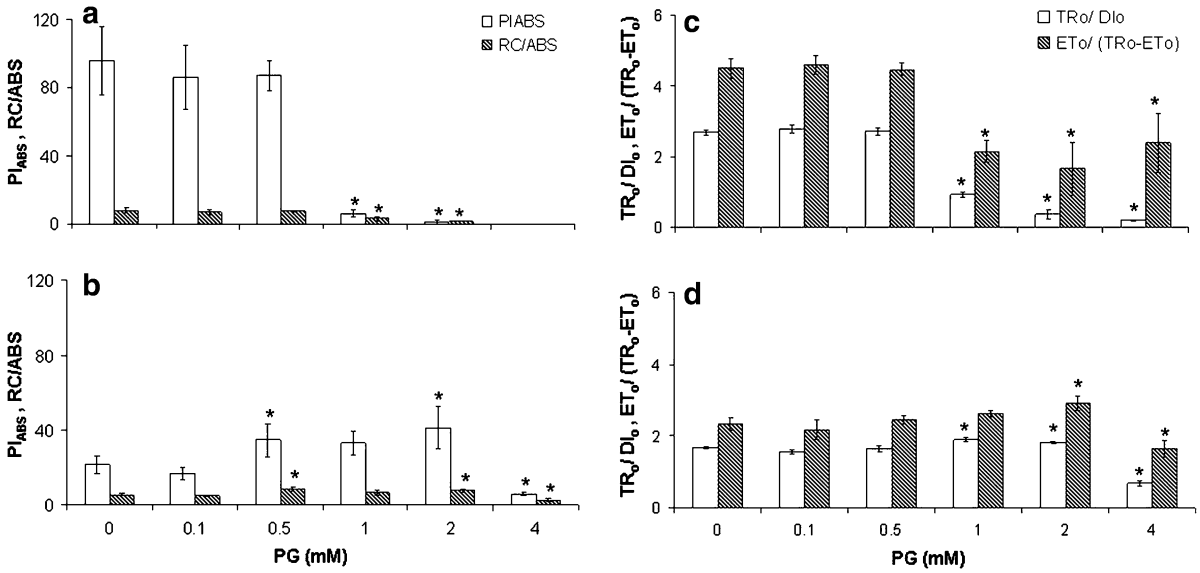

Fig. 5 Effects of different PG concentrations on performance index (PI_{ABS}) and density of reaction centers per PSII antenna chlorophyll (RC/ABS) (**a**, **b**) in *D. bardawil* cells under LL (**a**) and HL (**b**) conditions. Effect of the inhibitor on flux ratio trapping per dissipation (TR_o/DI_o) and electron transport beyond Q_{A^-} ($ET_o/(TR_o\text{-}ET_o)$) (**c**, **d**) in the cells under LL (**c**) and HL (**d**) conditions. Cultures grown without PG under LL or HL conditions were used as control. The *bars* show standard deviation of the means of the measured parameters of three separate experiments. *Asterisks* represent result significantly different from control sample ($P < 0.05$)

Table 3 Correlation coefficients of cell density, Chl, β-Car, the activity of oxygen evolving complex (Fv/Fo) and performance index (PI_{ABS}) with different JIP-test parameters

	Cell density	Chl	β-Car	F_v/F_o	PI_{ABS}
Φ_{po}					
LL	0.877*	0.970*	0.927*	0.976*	0.929*
HL	0.720 ns	−0.940*	−0.809 ns	0.994*	0.807 ns
Ψ_o					
LL	0.708 ns	0.724 ns	0.793 ns	0.761 ns	0.831*
HL	0.426 ns	−0.836*	−0.858*	0.951*	0.953*
Φ_{Eo}					
LL	0.895*	0.980*	0.956*	0.993*	0.964*
HL	0.631 ns	−0.919*	−0.839*	0.996*	0.870*
Φ_{Do}					
LL	−0.877*	−0.970*	−0.927*	−0.976*	−0.929*
HL	−0.720 ns	0.940*	0.809 ns	−0.994*	−0.807 ns
PI_{ABS}					
LL	0.917*	0.972*	0.975*	0.984*	
HL	0.237 ns	−0.757 ns	−0.876*	0.838*	
F_v/F_o					
LL	0.916*	0.989*	0.974*		0.984*
HL	0.679 ns	−0.931*	−0.830*		0.838*

* Significance at $P < 0.05$, *ns* non-significant

LL low light, *HL* high light, N = 6

and importance of this way in photosynthesis may differ in each plant species. In addition, our recent work on *D. salina* also showed that PG-induced PTOX inhibition did not affect PSII and PSI quantum yields under HL conditions (Einali et al. 2013), which further confirm our suggestion. Therefore, the supposition of existence of

another alternative pathway(s) rather than PTOX in *Dunaliella* cells for oxidizing of the electron transport chain to protection of the photosynthetic systems against reactive oxygen species (ROS) generated during high illumination does not seem unlikely. The latter suggestion might be supported by a study showing that acclimation to high light enhance the Mehler reaction in wheat (Savitch et al. 2000), an alternative method of keeping the electron transport chain oxidized. Although we could recently detect presence of PTOX genes in *Dunaliella* cells (submitted on Genbank, PCR results not shown), however, role of PTOX in *Dunaliella* cells remains to be elucidated by molecular and biochemical instruments.

In addition to PG effects on PSII electron flow, we found a severe increase in cell mortality and a pronounced decrease in pigment content in LL-grown cells subjected 96 h to 4 mM of the inhibitor (Fig. 1). This event might be due to a formation of ROS because of high PG concentration. Therefore, PG inhibition of PSII activity may also be related to its photoinhibitory effects. It has been previously determined that inhibition of water-splitting complex increases the susceptibility of PSII to photoinhibition (Wang et al. 1992) and it can accelerate degradation of the D1 protein in PSII (Jegershold et al. 1990). It has also been confirmed that PG inhibits phosphorylation of light-harvesting Chl *a*/*b* proteins (LHCII) (Elich et al. 1997; Georgakopoulos and Argyroudi-Akoyunoglou 1998). Inhibition of LHCII phosphorylation could be resulted in imbalance in excitation rates of PSII and PSI (Allen and Forsberg 2001) and oxidative stress occurrence. Thus, it seems that high concentration of PG could affect PSII by direct influence on photosynthetic components. Interestingly, it has also been demonstrated that PG inhibits high light-induced D1 degradation (Georgakopoulos and Argyroudi-Akoyunoglou 1998) and protects PSI against photoinhibition (Sonoike 1995). Therefore, the protective effect of PG on D1 protein might be due to resistance of *Dunaliella* cells against the inhibitor under HL conditions. It is also likely that PG could affect nature of PSII and or nature of cell membrane and chloroplast envelope under LL conditions. However, PG could not photodegrade under HL conditions because our experiments on algal cultures under salinity stress showed that high PG concentration does not also have any negative effect on PSII electron transport (Einali and Shariati 2012).

As mentioned above, Chl *a* fluorescence transients showed a very low potential of electron transport activity in LL-grown cells exposed to 1, 2 and 4 mM PG (Fig. 2a). The described differences in the shape of OJIP curves were reflected on values of performance index (PI_{ABS}) in LL-grown cells exposed to different concentrations of the inhibitor (Fig. 5a). Under this conditions, the main decreasing contributions of performance index (PI_{ABS}) were lowering of RC/ABS and TR_o/DI_o than $ET_o/(TR_o - ET_o)$ (Fig. 5). In addition, under LL conditions, PG resulted in an increase of the light absorption flux per reaction center (ABS/RC), which was associated with the increase in the antenna size or a decrease in the number of active reaction centers (Fig. 4d). A significant increase in dV/dto parameter (Fig. 4c), suggests a decrease in the number of active reaction centers. This means that amount of closed reaction centers has enhanced. The increase in ABS/RC together decrease in RC/ABS, indicate the presence of non-Q_A reducing reaction centers (Lepedus et al. 2011) and can also be related to irreversible PSII damage (Kalaji et al. 2011). Such non-Q_A reducing reaction centers are also called silent reaction centers and act as heat sinks (Strasser et al. 2004). The accumulation of inactive reaction centers is associated with the increased efficiency of dissipation of absorbed light as heat, as shown by the high values of thermal dissipation yield (Φ_{Do}) (Fig. 4b). The increase in dV/dto (Fig. 4c) and more decrease in TR_o/DI_o than $ET_o/(TR_o\text{-}ET_o)$ parameters (Fig. 5c) in PG-treated cells under LL conditions, further indicate PG inhibitory effects on the donor side of PSII. However, different inhibitor concentrations had no significant negative effect on none of dV/dto, ABS/RC and PI_{ABS} parameters under HL conditions (Figs. 4c, d, 5b), suggesting that algal cells respond to PG depending on environmental conditions.

In conclusion, data of the present study suggest that algal cells display different responses to PG under stress or non-stress conditions. It was found for PG to induce inhibitory effects on water-splitting complex on the donor side of PSII, decreasing its electron transport capacity. Therefore, PG-induced inhibition of the water-splitting complex on the donor side of PSII affects the conversion of light energy from antenna complex to electron transport within photosystem II. It was also demonstrated here that PG is a multi-target inhibitor of algal growth as well as photosynthesis. Indeed, PG in concentrations higher

than 0.5 mM act as an inhibitor in LL-grown cells, while in such concentrations it can play a protective role as an antioxidant in HL-grown cells. These two different responses can be attributed to molecular basis of direct effect of PG on PSII components although the direct evidence as to how PG inhibits PSII components requires to be further studied.

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