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Photosynthesis and Respiration under Low Temperature Stress in Two *Dunaliella* Strains

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Abstract: The photosynthetic and respiratory behaviors of two *Dunaliella salina* strains (IR-1 and Gh-U) were investigated at two temperature treatments and two assay temperatures. Net photosynthetic rate (P_N), photon-saturated net photosynthetic rate (P_{Nmax}), irradiance required to Saturate Photosynthetic (SI), respiration and pigment changes in *D. salina* strains, containing different chlorophyll (Chl) contents, shifted from 28 to 13°C for 24 h were compared with controls kept at 28°C, at two assay temperatures (28 and 13°C). Cold stress produced a decrease in total Chl content of IR-1 (higher Chl strain) whereas, Gh-U (lower Chl strain) did not exhibit significant changes under low temperature condition in total Chl. Carotenoid showed a larger decrease in IR-1 compared to that in Gh-U under treatment conditions. P_N, P_{Nmax}, SI and respiration rate were higher in IR-1 than those in Gh-U, which had a higher carotenoid/total chlorophyll ratio than IR-1, at all conditions. Transferring the cells to low temperature affected P_N, P_{Nmax}, SI and respiration decreasingly in both strains with a larger decrease in P_{Nmax} and SI in IR-1, when measuring the photosynthesis and respiration carried out at 13°C. Both strains exhibited an increase in P_N and respiration when chilled samples assayed at 28°C.

Key words: *Dunaliella salina* · low temperature stress · net photosynthetic rate · photon-saturated net photosynthetic rate · respiration · saturation irradiance

INTRODUCTION

Photosynthetic process is a thermosensitive function in higher plants [1] and green algae [2]. The primary effect of chilling and none freezing temperatures (e.g. 0-13°C), on photosynthesis are apparent within some important processes. Some of these effects were observed as inactivation of Calvin-cycle enzymes through oxidation in the stroma [3], delay in circadian rhythm of sucrose phosphate synthase activity in the cytosol [4] and oxidative damage to PSI and PSII reaction centers [5, 6].

Upon ideal conditions, high irradiance (full sunlight) may be required to cause an accelerated damage to photosynthesis, but under environmental conditions, a stress, which reduces CO_2-fixing capacity (e.g. Chilling), will result in a hypersensitivity to light [7]. Under normal turnover conditions, oxidative damage to D1 (reaction-center protein of PSII) comes along with the repair process. When the rate of repair can keep pace with the rate of damage, no inhibition of electron flow is observed. Under such conditions there is a maximum potential for photosynthesis (light-saturated rates). Chilling can also decrease the D1 repair rates. Any decrease in CO_2-fixing or in the repair rate of D1 protein can shift the maximum light intensities needed for photosynthesis saturation to a much lower intensities [7].

Respiration rates of plant cells are also affected by temperature [8] and there is a linear increase in O_2 uptake with temperature [9]. In addition to short-term responses, species grown at low temperatures often show higher rates of respiration than species grown at higher temperatures when measured at the same temperature [10]. This stimulation of respiration was reported as adaptive behavior of plants grown at cold conditions compared with related species from warmer conditions [11].

The increased rate of respiration at low temperatures in mitochondria involves two energy dissipating systems, Alternative Oxidase (AOX) pathway [12] and Plant Uncoupling Mitochondrial Protein (PUMP) [13]. This stimulation of respiration causes a decrease in the yield of ATPsynthase which is linked to an increase in heat dissipation [14]. These systems are also involved in removal of Reactive Oxygen Species (ROS), because they do not generate a proton electrochemical gradient [15].

Dunaliella, a wall-less unicellular green alga, is a dominant photosynthetic organism in many extreme conditions and it has high tolerance under altering environmental factors [16]. *Dunaliella* algae can tolerate low temperature stress conditions (personal observations). There are some research about *Dunaliella* photosynthetic response to salt stress and solar radiation,

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but there appears to be no information about *Dunaliella* response to chilling stress regarding photosynthetically and respiratory activities. The aim of this research was, to study the short-term effects of chilling treatment on the photosynthesis and respiration rate aspects with respect to chlorophyll and carotenoid levels in two *Dunaliella salina* strains.

MATERIALS AND METHODS

Algal cultures, growth and treatments: Algae used for this experiment were two strains of *Dunaliella salina* (IR-1 and Gh-U) having different Chl contents. IR-1 was isolated from Gave-Khooni-salt marsh in southeast of Isfahan-Iran and the other strain, Gh-U, was obtained from the Biotechnology Research Center of Gheshm Island. The cultures were grown in a Johnson modified medium, [17] at a NaCl molarity of 1.5 M. The algal cultures were inoculated as pure suspended cultures into 500 ml Erlenmeyer flasks, containing 250 ml of fresh medium, to give ~24×10^4 cells per ml. All flasks were placed in an incubator at 90-100 µmol photon m^{-1} s^{-2} provided by cool-white fluorescent lamps, under a 16/8 h light-dark (28±0.5°C) cycle with shaking at 96 rpm. The cultures were harvested in the exponential growth phase and transferred to the following continuous light and temperature conditions for 24 hours: 13°C/100 µmol photon m^{-1} s^{-2} (13/100) and 28°C/100 µmol photon m^{-1} s^{-2} (28/100). For pigment assays including total Chl (Chl a plus b) and carotenoids, 10 ml aliquots of cultures were sampled at 0 h and 24 h after transfer to the designed (temperature and light) treatments. Three replicate samples were taken for each assay.

Pigment measurements: Pigments were extracted with 95% acetone from 10 ml of cell cultures, which were spun down at 12,000 g (EPPENDORF-5415C) for 3 min. Pigment content was assayed spectrophotometrically [18].

Photosynthesis and respiration measurements

Harvesting the experimental cultures for photosynthesis measurement: The cultures were harvested by centrifugation at 500 g (SANYO MSE MISTRAL 3000i) for 5 minutes. The algal cells were resuspended in a phosphate buffer containing 0.25 mM KH_2PO_4, 0.25 mM K_2HPO_4, 0.2 mM $MgCl_2$, 5 mM $NaHCO_3$, 1.5 M NaCl. pH was adjusted to 7.5. The temperature was set-up according to the treatment. One ml of algal suspension was transferred to the measuring vessel of the O_2 electrode.

Measurement of O_2 evolution and uptake: Photosynthesis (oxygen evolution) and respiration (oxygen uptake) were measured polarographically using Clark-type O_2 electrode (Hansatech Ltd, UK) in water-jacketed reaction vessels [19]. The O_2 electrode was connected to a chart recorder. Measurements of photosynthesis and respiration were carried out at 13 and 28°C depending on the experiment. A slide projector with a 100-Watt lamp was used for illumination, when the white light beam was projected through a red filter (100 to 2000 µmol photon m^{-1} s^{-2}). The red-light beam was projected through a spherical focusing lens (a round-bottom flask filled with water) as required, to obtain high light intensity.

Statistical analysis: Statistical comparisons were made using ANOVA with Tukey test and independent-samples T test (p values<0.05).

RESULTS AND DISCUSSION

Pigment content in *Dunaliella* strains: *Dunaliella* strains used for these experiments were different in Chl content. As is shown in Table 1, the total Chl content expressed per fresh weight (f.wt) basis and per cell basis, before stress, in control cultures (grown at 28±0.5°C under 16/8 h light-dark cycle), was significantly higher in IR-1 than Gh-U.

Pigment content after treatment conditions in *Dunaliella* strains: Strain IR-1, having higher Chl content than Gh-U, exhibited a reduction in total Chl under low-temperature stress (Fig. 1.1-C). Carotenoid also decreased under both continuous conditions (light and cold stress) in this strain (Fig. 1.2-B, C). Chl and carotenoid degradation might be occurred by oxidative stress, as it was observed in chilling-sensitive maize (*Zea mays*, variety Penjalinan), exposed to low temperature stress [20]. However, there is

Table 1: Comparison of total chlorophyll content before stress in two *Dunaliella salina* strains (grown at 28±0.5°C under 16/8 h light-dark cycle) on µg mg^{-1}f.wt and µg 10^{-6} cells basis. Values are mean±S.E. of nine replicates. Significant differences (p<0.001) between two strains according to independent-Samples T test, are shown by asterisk

	Total Chl	
D. salina strains	(µg mg^{-1} f.wt)	(µg 10^{-6} cells)
IR-1	7.2±0.164*	1.67±0.05*
Gh-U	3.2±0.175	0.80±0.03

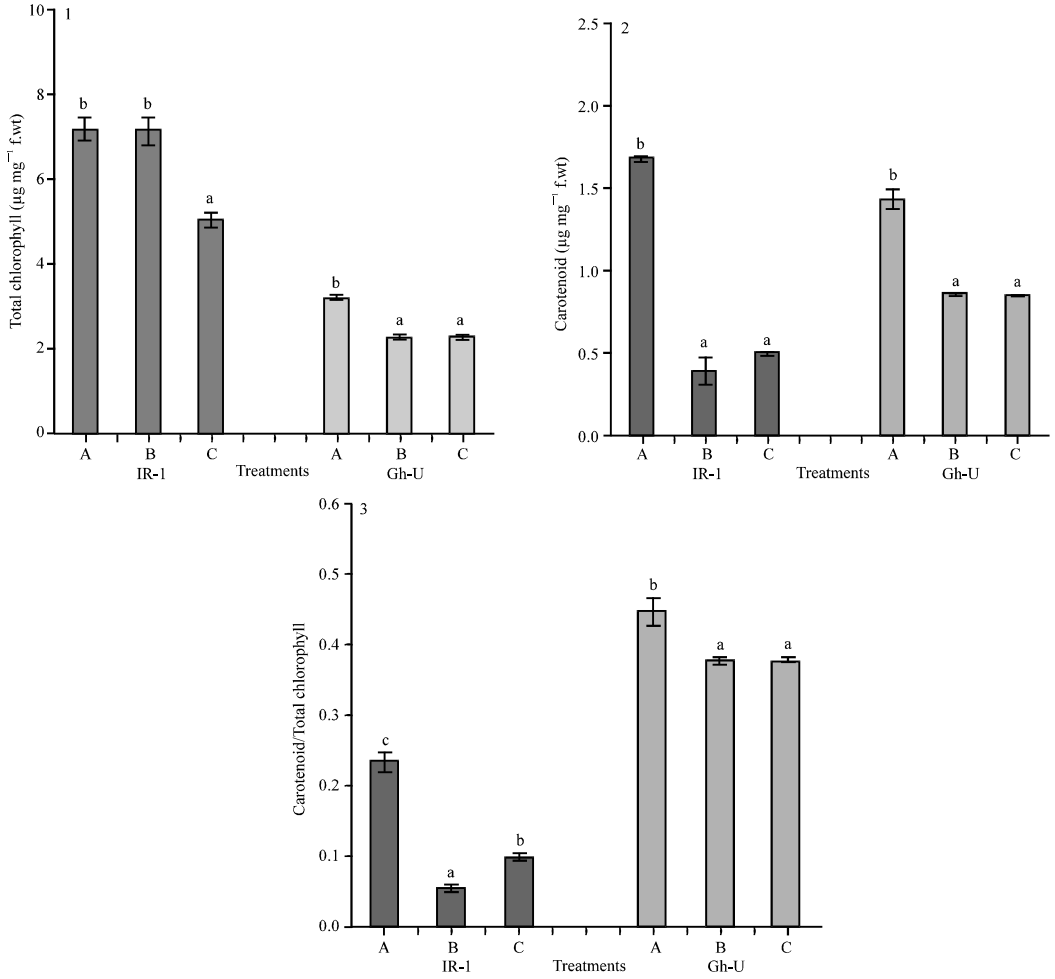

Fig. 1: Comparison of 1) total Chl (µg mg^{-1} f.wt), 2) carotenoid contents (µg mg^{-1} f.wt) and 3) carotenoid/total Chl ratio of two *D. salina* strains (IR-1 and Gh-U), at three conditions. (A) Before stress at 28±0.5°C, 16/8 h light-dark cycle, (B) 24 h at 28°C/continuous light 100 µmol photon m^{-1} s^{-2}, (C) 24 h at 13°C/continuous light 100 µmol photon m^{-1} s^{-2}. Data are mean±S.E. from three independent experiments. Different letters indicate significant differences between various treatment conditions in each strain at $p<0.05$ (according to Tukey test)

a compensative and dynamic response of photosynthetic system to light intensities, known as irradiance-dependent adjustment of the Chl antenna size [21]. It has been shown that, this adjustment reflects a photoacclimatory response to excitation pressure than a response to light perþ se [22]. Low temperature mimics the effect of high light on generating a high excitation pressure in photosynthetic system [23]. Thus, photostasis will be attained upon exposure of green algae to high excitation pressure generated by high light or low temperature through a decline in pigment and size and as a result in the efficiency of light harvesting [22].

Gh-U (having less Chl) did not show any difference in Chl and carotenoid content between 28/100 and 13/100 conditions (Fig. 1.1, 1.2-B and C) but both B and C decreased as compared to A (periodic condition). This might be related to exposure to continuous light condition for 24 hours. It seems that continuous light for 24 h has more effect on pigment degradation than low temperature in this strain. Gh-U had higher carotenoid/total chlorophyll ratios than IR-1 under all conditions (Fig. 1.3). The higher carotenoid/total chlorophyll ratio in Gh-U, compared with that in IR-1, is due to lower chlorophyll content and also a smaller decrease in carotenoids in this strain under stress conditions. Carotenoids protect pigment-protein complexes and chl against photooxidation [24]. An increased carotenoid/total chlorophyll ratio has been observed in chilling-tolerant *Zea maiz* variety (Z7) leaves in response to low temperature [20].

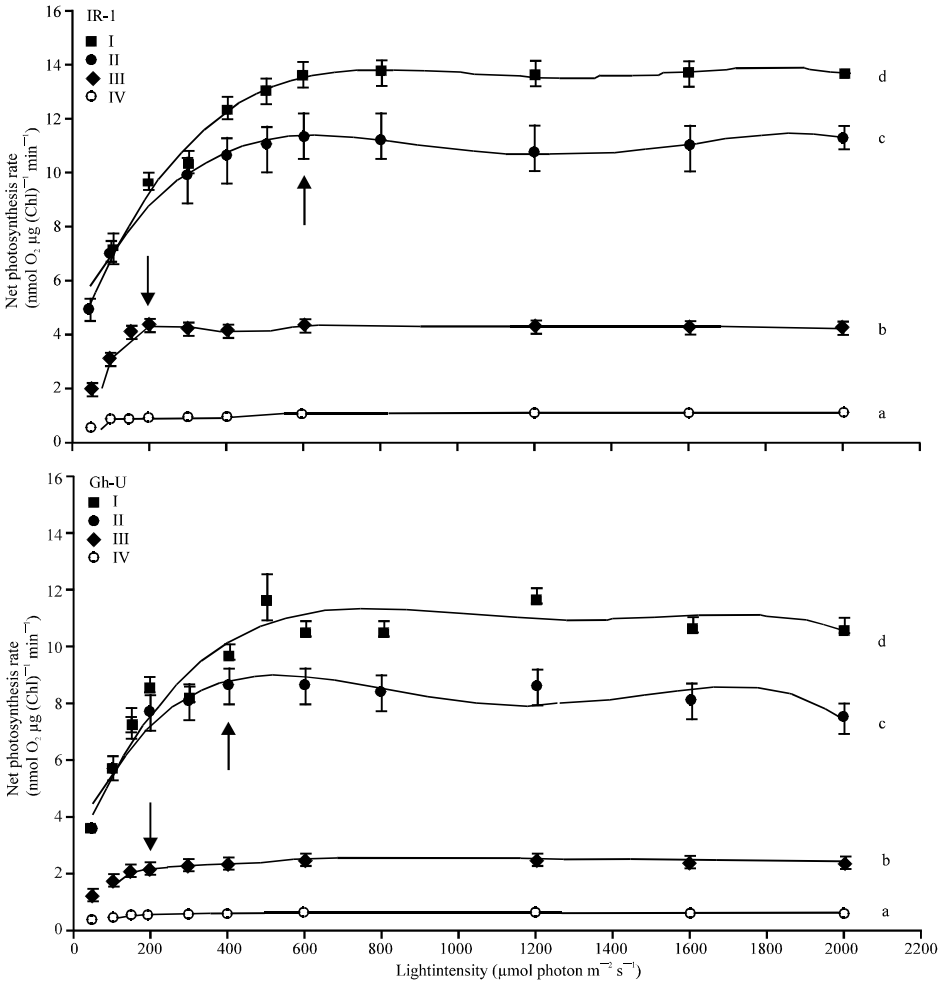

Fig. 2: PFD response curves of net photosynthesis rate (P_N) on nmol evolution O_2 µg $Chl^{-1}min^{-1}$ and photon-saturated net photosynthetic rate (P_{Nmax} is shown by arrow) in two *Dunaliella salina* strains, IR-1 and Gh-U, treated at four conditions; (I) 24 h under 13°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 28°C; (II) 24 h under 28°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 28°C; (III) 24 h under 13°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 13°C; (IV) 24 h under 28°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 13°C. Data are mean±S.E. from three independent experiments. Different small letters indicate significant differences between various treatment conditions in each strain at $p<0.05$ (according to Tukey test).

Light response curves of the net photosynthesis: To study short-term responses of photosynthesis and respiration to low temperature, measurements in treated cells at 28 and 13°C for 24 hours, were done at two assay temperatures (28°C, 13°C) and results were compared (Fig. 2 and 3). As shown in Fig. 2 and 3, P_N, P_{Nmax} and respiration amounts were significantly higher in IR-1 (having higher Chl content) than Gh-U under all conditions. It has been suggested that plants grown at high light for a long time had higher contents of electron transfer and photosynthetic enzymes and therefore higher P_{Nmax}, in comparison with plants grown at lower light [25]. Similar results were obtained when the effect of different irradiance on photosynthesis in *Mosla* species was investigated [26]. Photosynthetic characteristics of *Dunaliella* strains are nearly similar to plants grown at higher and lower light intensities. Therefore the difference in P_{Nmax} between two strains might be related to existence the different amount of photosynthetic component and enzyme activities in their photosynthetic systems.

P_N, P_{Nmax} were affected by the low temperature treatment in both strains decreasingly (Fig. 2, III) compared to treated cells at 28/100 (Fig. 2, II). Such results have been observed in chilling sensitive maize plants [20].

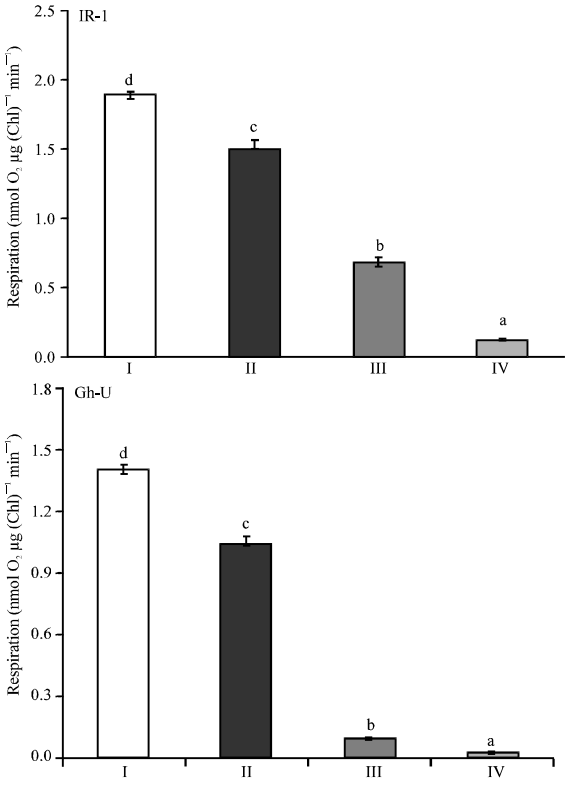

Fig. 3: Respiration rate (nmol O_2 uptake µg Chl^{-1} min^{-1}), in two *Dunaliella salina* strains, IR-1 and Gh-U, treated at four conditions; (I) 24 h under 13°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 28°C; (II) 24 h under 28°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 28°C; (III) 24 h under 13°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 13°C; (IV) 24 h under 28°C/continuous light 100 µmol photon m^{-1} s^{-2} assayed at 13°C. Data are mean±S.E. from three independent experiments. Different small letters indicate significant differences between various treatment conditions in each strain at $p<0.05$ (according to Tukey test)

The maize varieties exhibited a decrease in their P_N on the first day of chilling treatment (5°C). These results showed chilling sensitivity of photosynthesis in both *Dunaliella* strains.

Low temperature treatment also led to a decrease in light intensities required to saturate photosynthetic (saturation irradiance, SI) from 600 µmol photon m^{-1} s^{-2} to 200 µmol photon m^{-1} s^{-2} in IR-1 (having higher Chl content) and from 400 µmol photon m^{-1} s^{-2} to 200 µmol photon m^{-1} s^{-2} in lower Chl content strain (Gh-U), (Fig 2, II compared to III). It has been showed that *D. parva* exhibits the highest photosynthetic rates at a moderate light intensity (600 µmol photon m^{-1} s^{-2}) at 31°C. Above this light intensity a clear photoinhibition of the photosynthesis was found [27]. But no photoinhibition of photosynthesis was appeared after saturation of photosynthesis in chilled treated or untreated *Dunaliella* strains, at least up to irradiance 2000 µmol photon m^{-1} s^{-2} (Fig. 2). This can show more resistance of photosynthetic apparatus to photoinhibition in *D. salina* strains compared to that in *D. parva*. Decrease of SI in assayed *Dunaliella* strains at low temperature confirms chilling sensitivity of photosynthesis in strains. However this could also limit excitation pressure of reaction centers to protect the cells, especially in strain containing higher Chl (IR-1).

Our results also showed a decline in respiration when it was measured at 13°C (Fig. 3, III and IV) compared to those at 28°C (Fig. 3, I and II).

To examine the photosynthetic capacity of two strains, other measurements were made in chilled samples at optimal temperature (28°C), (Fig.2, I). Low temperature treatment of both strains for 24 h produced an increase in photosynthetic capacity, which was showed by increased P_{Nmax}. These increases probably contain three adaptive behaviors during cold conditions; increase in P_i availability [28], elevated activities of Calvin cycle enzymes [29] and also enzymes responsible for sucrose synthase [30].

Measuring the respiration rate of chilled treated cells at 28°C showed similar results to photosynthesis (Fig. 3, I compared to II). The stimulation of respiration by low temperature treatment in *Dunaliella* cells could be an acclimatory response to cold conditions. It has been suggested that although most plants, with the exception of thermogenic plants, do not produce enough metabolic heat to raise the temperature of bulk tissue, but this produced heat may have a physiological effect at subcellular level and localized increase in temperature around the individual mitochondria, which may have physiological importance [31].

CONCLUSIONS

In conclusion these results have shown that the photosynthesis and respiration activities of both strains are sensitive to low temperature stress and that some of acclimatory responses were differed in detail between two stains. Short-term exposure of cells to low temperature can

affect P_N, P_{Nmax}, SI and respiration decreasingly in both strains at cold assay temperature. But the capacity of photosynthesis and respiration were slightly increased during cold treatment in cells. Chl degradation also may consider as an acclimatory response to low temperature condition, in particular in strain containing higher chl content (IR-1). Higher carotenoid/total Chl ratio in Gh-U and also having lower Chl content can help this strain to cope with stress condition. Higher Chl strain (IR-1), had higher P_N, P_{Nmax} and also respiration rates under all conditions compared to those in Gh-U. It seems that a larger decrease in Chl content, SI and P_{Nmax} in IR-1 under cold stress, compared to those in Gh-u and also a small increase in carotenoid/total Chl ratio might be important to tolerate cold condition in this strain.

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