Correlation between lipid and carotenoid synthesis and photosynthetic capacity in *Haematococcus pluvialis* grown under high light and nitrogen deprivation stress

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SUMMARY: Recently, *H. pluvialis* has been demonstrated to have significant potential for biofuel production. To explore the correlation between total lipid content and other physiological parameters under stress conditions, the responses of *H. pluvialis* to high light intensity (HL), nitrogen deprivation (-N), and high light intensity with nitrogen deprivation (HL-N) were investigated. The total lipid content in the control cells was 12.01% dw, whereas that of the cells exposed to HL, -N, and HL-N conditions was 56.92, 46.71, and 46.87% dw, respectively. The fatty acid profile was similar under all conditions, with the main components including palmitic acid, linoleic acid, and linolenic acid. A good correlation was found between individual carotenoid and total lipids, regardless of culture conditions. Photosynthetic parameters and lipid content were also found to be well-correlated.

KEYWORDS: *Carotenoid;* Haematococcus pluvialis*; Lipid production; Photosynthetic capacity; Stress conditions*

RESUMEN: ***Correlación entre lípidos, síntesis de carotenoides y capacidad fotosintética de* Haematococcus pluvialis *desarrollado bajo luz intensa y deficiencia de nitrógeno.*** Recientemente, *H. pluvialis* ha demostrado tener un gran potencial para la producción de biocombustibles. Para explorar la correlación entre el contenido total de lípidos y otros parámetros fisiológicos en condiciones de estrés, se investigaron las respuestas de *H. pluvialis* a una alta intensidad de luz (HL), una privación de nitrógeno (-N), y ambos, alta intensidad de la luz con privación de nitrógeno (HL-N). El contenido total de lípidos de las células control fue de 12,01% dw, mientras que el de las células expuestas a HL, N, y condiciones de HL-N fue de 56,92, 46,71, y 46,87% dw, respectivamente. El perfil de ácidos grasos fue similar para todas las condiciones, cuyos componentes principales fueron los ácidos palmítico, linoleico y linolénico. Se encontró una buena correlación entre carotenoides y lípidos totales individuales, independientemente de las condiciones de cultivo. También se encontró una buena correlación entre los parámetros fotosintéticos y el contenido de lípidos.

PALABRAS CLAVE: *Capacidad fotosintética; Carotenoides; Estrés*; Haematococcus pluvialis; *Producción de lípidos*

1. INTRODUCTION

Energy has become a crucial factor for humans' economic growth and high standard of living, especially after the industrial revolution in the late eighteenth and early nineteenth centuries (Atabani *et al.*, 2012). The global energy crisis has stimulated the need to find alternative energy resources (González-Fernández *et al.*, 2012). Microalgae are a promising source of biofuel due to their simple cellular structure, higher growth rate, and higher lipid content than conventional oleaginous vegetable crops (Halim, 2012). It has been reported that microalgae are the more sustainable source of biodiesel in terms of food security and environmental impact, when compared with palm oil (Ahmad *et al.*, 2011).

Algae species and culture conditions have been found to be the two most important factors contributing to high oil yield (Ghasemi *et al.*, 2012; Xu *et al.*, 2013; Mou *et al.*, 2012). The freshwater microalga *Neochloris oleoabundans* and the marine microalgae *Nannochloropsis* sp. are considered to be suitable for biofuel production because of their high oil content (29.0 and 28.7%, respectively) (Gouveia and Oliveira, 2009). The average lipid production in microalgae has been found to vary between 1 and 70%; however, under certain conditions, some species have been noted to exhibit a lipid content of up to 90% dry weight (dw) (Chisti, 2007; Li *et al.*, 2008). It has been reported that the oil content of *Botryococcus braunii* can reach 75% dw under nitrogen-deficient condition; however, the alga has been found to exhibit low productivity (Ghasemi *et al.*, 2012). Recently, Damiani *et al.* (2010) assessed the potential use of the unicellular green alga *Haematococcus pluvialis* as a biodiesel feedstock, and analyzed the lipid content and composition of *H. pluvialis* under both control and stress conditions. It was observed that nitrogen is quantitatively the most important nutrient affecting the biomass growth and lipid productivity of various microalgae (Griffiths and Harrison, 2009). Furthermore, optimized light intensities have been reported to improve the lipid content in microalgae (Rosenberg *et al.*, 2008). The lipid production of the most common algae ranged between 20 and 50%, and high light intensity has been observed to stimulate higher productivities (Mata *et al.*, 2010).

H. pluvialis is an important commercial microalga due to its significant ability to accumulate ketocarotenoid-astaxanthin (Sarada *et al.*, 2006). Many studies have examined the content, synthesis, and biological activity of fatty acids and astaxanthin in *H. pluvialis* (Damiani *et al.*, 2010; Cifuentes *et al.*, 2003; Cerón *et al.*, 2007). Stress conditions such as nutrient limitation and high light intensity induced lipid accumulation during cyst formation in *H. pluvialis* (Zhekisheva *et al.*, 2002). These conditions are also conducive to enhancing astaxanthin synthesis and changing other physiological performance (Sarada *et al.*, 2006; Cifuentes *et al.*, 2003). A significant inverse correlation between photosynthetic efficiency and cellular neutral lipid yields has been found in the freshwater microalgae *Chlorella* sp. (White *et al.*, 2011). Furthermore, Solovchenko *et al.* (2010) demonstrated that there was a tight, nonlinear relationship between the car/chl ratio and TFA contents per dw, regardless of the cultivation conditions.

Although astaxanthin in *H. pluvialis* has been intensively investigated, studies examining its lipid accumulation strategy under various conditions are still limited. To explore the association between the synthesis of lipids and carotenoids in *H. pluvialis*, the lipid production and carotenoid accumulation in *H. pluvialis* under conditions of nitrogen deprivation and high light intensity were investigated in this study. In addition, the PSII photosynthetic characteristics were synchronously analyzed by PAM fluorometry technology to determine whether there was any relationship between them.

2. MATERIALS AND METHODS

2.1. Algal strain and culture conditions

The microalga *H. pluvialis* used in this study was provided by the Laboratory of Phycology, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Science. The strain was cultivated in an MCM medium at 20 °C under 50 µmol photons·m^{-2}s^{-1} with a 12:12-h light/dark photoperiod (Boussiba and Vonshak, 1991). The algae in exponential growth phase were used for the experiments. The algal cells were harvested by centrifugation (8000× g for 3 min), washed twice in double-distilled water, and re-suspended in the corresponding culture conditions: (1) MCM medium, under the same conditions as those indicated earlier (control); (2) MCM medium, under 350 µmol photons·m^{-2}s^{-1} light intensity (HL); (3) nitrogen-free medium, under optimal light intensity (-N); and (4) nitrogen-free medium, under 350 µmol photons·m^{-2}s^{-1} light intensity (HL-N). Each experiment was performed for 14 days and at least three independent repetitions for each treatment were carried out. The *H. pluvialis* cells were sampled every 2 days for the subsequent analyses.

2.2. Lipid extraction

H. pluvialis cells were harvested and lyophilized using a freezer dryer for analyzing the total lipid content. A total of 50 mg of freeze-dried samples were treated with 1000 µL of methanol at 4 °C for 15 min. The extraction was repeated three times to obtain all the neutral lipids. Subsequently, the methanol extract was treated with peroxide-free diethyl ether

(containing 0.01% butylhydroxytoluene (BHT), hexane, and water) up to a final ratio of 1:1:1:1 (v/v/v/v). After centrifugation of the mixture (3000 g for 5 min), the upper phase was collected. The pH of the lower phase was adjusted to 3–4 with acetic acid, and was subjected to re-extraction with a mixture of diethyl ether and hexane (at a ratio of 1:1, v/v). Subsequently, the combined phases were evaporated to dryness in nitrogen and stored at −20 °C. Four different treatments were performed after 14 days and at least three independent repetitions for each extraction were carried out.

2.3. Analysis of fatty acid composition

The fatty acid analysis was performed as described by An *et al.* (2013). A total amount of 20 mg of each lyophilized sample was added to a conical flask containing 30 mL of petroleum ether. The solution was placed in an ultrasound bath (40.0 kHz, 600 W) for 30 min at 50 °C, and this operation was repeated twice. Then, the solvent was moved from a rotary vacuum evaporator at 50 °C until the weight was unchanged. The total fatty acids were transmethylated to fatty acid methyl esters (FAMEs) with 5 mL of 0.4 M KOH:Methanol (v/v) at room temperature (25 °C). The analysis of the resulting FAMEs was carried out using a Finnigan Trace GC-MS (Agilent Technologies, USA). The FAMEs were identified by comparison with authentic standards (Sigma Chemicals Co., USA), and peaks were integrated with DPS software Version 7.05 (Zhejiang University, China).

2.4. Measurements of growth and PSII photosynthetic parameters

Cell growth was determined by counting the cell numbers using a hemocytometer. Photosynthetic capability was determined by means of the DIVING-PAM (Walz, Effeltrich, Germany) connected to a PC with WinControl software, using the pulse–amplitude modulated method. Before measurement, the samples were kept in the dark for 15 min and the original fluorescence (F_0) was determined under a low measuring light. A saturation light pulse was applied to obtain maximum fluorescence (F_m) in the dark-adapted samples. The F_m yield in the illuminated samples was denoted as F_m', and the real-time fluorescence yield was indicated as F_t. The maximal PSII quantum yield (F_v/F_m) was calculated according to the equation: $F_v/F_m = (F_m - F_0)/F_m$. The effective PSII quantum yield ($Y(II)$) was calculated as follows: $Y(II) = (F_m' - F_t)/F_m'$. The non-photochemical quenching of chlorophyll fluorescence (NPQ) was calculated based on the following equation: $NPQ = (F_m - F_m')/F_m'$. All the measurements were performed at room temperature.

2.5. Pigment analysis

For pigment analysis, 50 mL of the *H. pluvialis* culture were harvested at different intervals of stress induction and ultrasonic decomposition. The pigment was extracted from the algal cells by adding dimethyl sulfoxide (DMSO) at 70 °C for 5 min. The total carotenoids and chlorophyll contents were determined by UV–Vis spectrophotometers (Purkinje General, China) using the coefficients mentioned by Solovchenko *et al.* (2010). The chlorophyll and carotenoid concentrations were expressed in mg of chlorophyll per liter and mg of carotenoid per liter, respectively.

2.6. Statistical analysis

Each experiment was repeated three times. All the observations and calculations were made separately for each set of experiments. The data were expressed as means with standard deviation (SD). Statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). Variance among treatments was tested using a one-way ANOVA. The significance level was $p < 0.05$ for all tests unless otherwise indicated.

3. RESULTS

3.1. Growth measurements

In the control culture, the sample showed a maximum cell concentration of 9.01×10^5 cells·mL^{-1} after 14 days and exhibited an exponential phase from the 2nd to the 10th day. The cells exposed to the control culture retained their flagella for longer periods compared to that under stress conditions. In the [HL] cultures, after being cultured for 4 days, the algae entered the exponential growth phase with the highest cell concentration of 3.94×10^5 cells·mL^{-1} after 14 days. In the [-N] cultures and in the [HL-N] cultures, the maximum cell concentrations were 2.15×10^5 cells·mL^{-1} and 1.67×10^5 cells·mL^{-1}, respectively. Although as the stress advanced, all the treatments showed an increase in biomass yield; the cultures stressed with treatments showed lower cell concentrations than the control cultures. The cells under nitrogen-deficiency treatment and the high light combined with the nitrogen-deficiency treatment did not exhibit an obvious exponential phase (Figure 1). The culture stressed with high-light produced significantly more biomass than the cultures under other treatments.

3.2. Total lipid and rates of TFA synthesis

Figure 2 shows the contents in total lipid in the cultures. Under control condition, the total lipid content of *H. pluvialis* gradually increased with
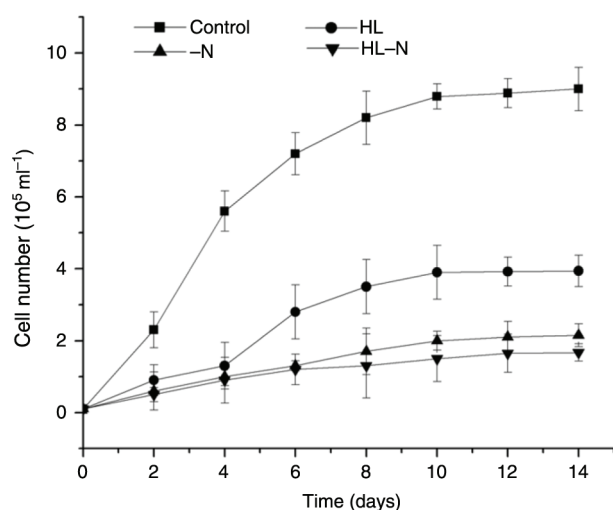
FIGURE 1.   Growth of *H. pluvialis* in the control, HL, -N, and HL-N cultures. Error bars denote standard deviations among the replicates. HL refers to the cells under high light (350 μmol photons $m^{-2} s^{-1}$ of continuous light); -N refers to the cells under nitrogen starvation stress; HL-N refers to the cells under high light (350 μmol photons $m^{-2} s^{-1}$ of continuous light) and nitrogen starvation stress.
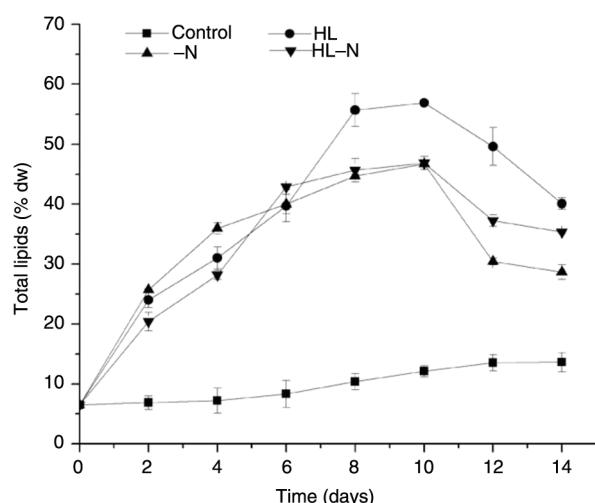

FIGURE 2.   Total lipid content (in % dw) in *H. pluvialis* grown in the control, HL, -N, and HL-N cultures.

increasing cultivation time, the maximum total lipid content was 13.60% (percentage dry weight = % dw) at the 10th day. The total lipid content in the [-N] cultures increased sharply in the first 6 days and raised slowly in the following 4 days, then declined after the 10th day. A similar result was observed in the [HL-N] cultures. Thus, the highest lipid productivities were obtained after 10 days, allowing for a production of up to 46.71% and 46.87%, respectively. In the [HL] culture, a sharp increase in total lipid content in the first 8 days was observed and a similar increase, although less pronounced, was observed in

the following 2 days. The highest amount of total lipid content was obtained after 10 days' cultivation, which was significantly higher than that in the longer cultivated culture (Figure 2). The maximum productivity for total lipid accumulation in high-light-exposed cells was 56.92%, which was almost six-fold over the control cells. The cellular contents of total lipid declined after 10 days regardless of any culture condition (Figure 2). The results suggest that the 10th stress day may be the optimal time for lipid production.

3.3. Effect of different stress conditions on fatty acid composition

To identify the change in the fatty acid composition of the *H. pluvialis* cells grown under stress, a typical profile of lipids extracted from the lyophilized cells of *H. pluvialis* was developed, as shown in Table 1. The major fatty acids in *H. pluvialis* were palmitic acid (C16:0), linoleic acid (C18:2n6), and linolenic acid (C18:3n3), which altogether were found to represent about 60–70% of the TFA. Under stress conditions, all of them increased, especially the palmitic acid content (C16:0) under HL-N. Additionally, the oleic acid (C18:1) increased by 2-fold, 1.2-fold and 1.4 fold, respectively, under HL, -N, and HL-N culture conditions, accompanied by a decrease in C16:2, C16:4 and EPA fatty acids. The percentage of saturated fatty acids (SFA) was shown to be significantly higher in cultures grown under the [HL] culture (30.80%), the [-N] culture (29.11%) and the [HL-N] culture (30.79%) conditions compared to the control (27.81%). In general, the polyunsaturated fatty acid (PUFA) content presented a downward trend, whereas the monounsaturated fatty acid (MUFA) content increased under stress conditions.

3.4. Analysis of pigment content

The influence of stress on carotenoid accumulation and chlorophyll was examined in the present study in order to understand whether the accumulation of lipid and carotenoid was synchronized. The dynamic changes in chlorophyll and carotenoids content are presented in Figure 3. The cellular content of total carotenoids in the control culture did not change appreciably. In the [-N] culture, the total carotenoid content increased moderately. In the [HL] culture, the content in total carotenoids increased slightly on the first 6 days, continued to increase sharply between day 6 and day 10, and subsequently remained stable (Figure 3A). The carotenoid content peaked on the 10th day, to 36.12 mg·L^{-1} in the [HL] cultures and to 27.21mg·L^{-1} in the [-N] cultures, respectively. The carotenoid content in the [HL-N] culture was more than two- times higher than that observed in the control culture (Figure 3A).

TABLE 1.   Fatty acid profile (% of TFA) in the control, HL, -N, and HL-N cultures

Fatty acids	Control	HL	-N	HL-N
C14:0	0.49±0.04	0.72±0.02	0.45±0.02	0.60±0.04
C16:0	24.5±0.51	26.62±0.00	26.41±0.10	27.70±0.62
C16:2	2.30±0.13	0.64±0.02	0.53±0.03	0.00±0.00
C16:3	3.58±0.08	1.23±0.06	0.90±0.06	0.58±0.02
C16:4	9.92±0.07	7.47±0.08	8.09±0.05	7.94±0.01
C18:0	3.26±0.01	3.40±0.09	2.44±0.06	3.09±0.01
C18:1	3.09±0.02	9.55±0.17	6.83±0.34	7.52±0.00
C18:2n6	19.09±0.03	19.25±0.04	20.14±0.56	21.30±0.45
C18:3n6	1.35±0.04	1.15±0.23	1.17±0.22	0.92±0.11
C18:3n3	19.59±0.50	19.80±0.34	20.48±0.22	20.04±0.01
C18:4	3.71±0.04	3.73±0.10	3.07±0.16	3.70±0.06
C20:4n6 (ARA)	2.77±0.05	1.54±0.05	1.38±0.12	1.40±0.04
C20:5n3 (EPA)	1.86±0.03	1.36±0.18	1.23±0.00	1.36±0.01
ΣSFA[b]	28.31±0.55	30.73±0.07	29.29±0.19	31.38±0.60
ΣMUFA[b]	3.09±0.02	9.55±0.17	6.83±0.34	7.52±0.00
ΣPUFA[b]	64.17±0.44	56.17±0.26	59.99±1.25	57.26±0.60

[a]Values are means ± SD of three determinations
[b]SFA Saturated fatty acids, MUFA Monounsaturated fatty acids, PUFA Polyunsaturated fatty acids
HL refers to the cells under high light (350 μmol photons·m^{-2} s^{-1} of continuous light); -N refers to the cells under nitrogen starvation stress; HL-N refers to the cells under high light (350 μmol photons m^{-2} s^{-1} of continuous light) and nitrogen starvation stress.

In the [HL] culture and [-N] culture, the chlorophyll content increased sharply after the first several days of incubation and then moderately increased, reaching a maximum value of 18.95 mg·L^{-1} on day 6 and 19.94 mg·L^{-1} on day 8, respectively. After 14 days, the chlorophyll content decreased to a similar level in both the cultures (Figure 3B). On the other hand, the [HL-N] culture exhibited the highest chlorophyll content, which reached a value of approximately 17.31 mg·L^{-1} on the fourth day of cultivation and declined slightly thereafter (Figure 3B).

3.5. PSII photosynthetic characteristics

Since the relationship between the change in photosynthetic efficiency and the production of lipids has not been widely studied in algae, we've paid special attention to the changes in the PSII photosynthetic characteristics. F_v/F_m, $Y(II)$ and NPQ were measured to evaluate changes in the photosynthesis efficiency. The F_v/F_m, $Y(II)$ and NPQ differed among the four samples during the course of incubation. The control showed no distinct changes in F_v/F_m, $Y(II)$ and NPQ. F_v/F_m of the [HL] cultures; the [-N] cultures and the [HL-N] cultures decreased by 47.6%, 55.9% and 55.67% after a treatment of 10 days (Figure 4A); $Y(II)$ of the stress treatments decreased by 45.2%,30.4% and 63.6% (Figure 4B); and NPQ of the three stress cultures was found

11.09, 14.2 and 6.9 times higher, respectively, than the initial value (Figure 4C).

3.6. Relationships between total lipids and carotenoid synthesis and photosynthetic capacity

A good correlation was found between contents of individual carotenoid and total lipid. As shown in Table 2, it provided high correlation factors ranging from R=0.786 to R=0.862. In stress conditions, the positive correlation of carotenoid and total lipid presents a more obvious trend compare to the control condition.

The parameters of the PAM Flourometer (F_v/F_m, $Y(II)$, NPQ) recorded significant physiological stress induced by different stress conditions. (Figure 5 A, B, C)

The algal samples in the control exhibited a tight, nonlinear relationship with total lipid content whether F_v/F_m (R^2=0.586), $Y(II)$ (R^2=0.289), NPQ (R^2=0.580) (Figure 5; Table 2). In the [HL] cultures, the [-N] cultures and the [HL-N] cultures, NPQ were well correlated with content of total lipids.

4. DISCUSSION

In the present work, attempts were made to compare the changes in lipid content and pigment profile with photosynthesis efficiency in *H. pluvialis* under
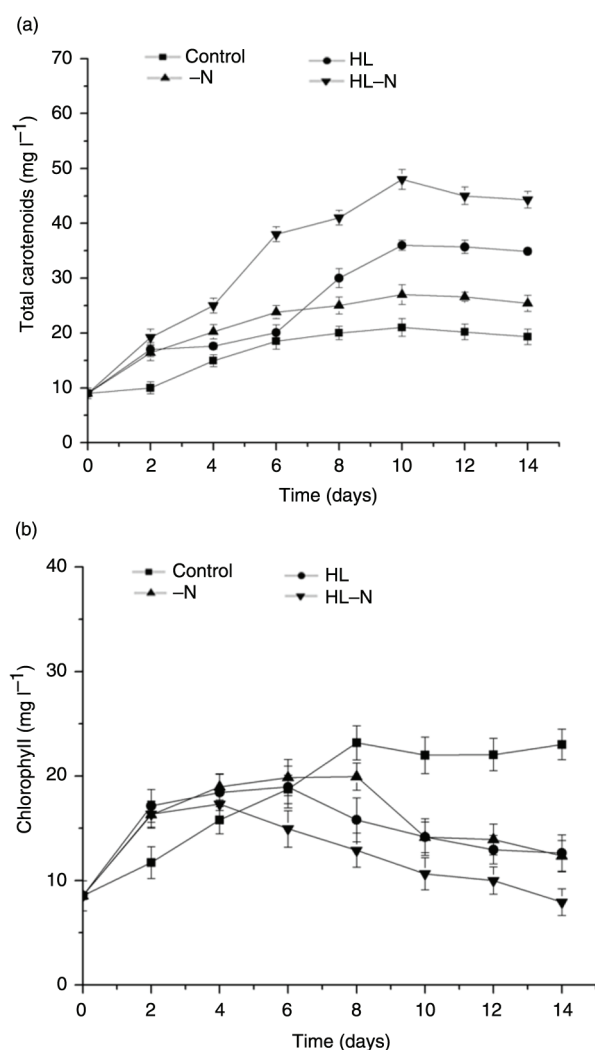
FIGURE 3. Dynamics of (A) total carotenoid and (B) chlorophyll contents in *H. pluvialis* cells grown in the control, HL, -N, and HL-N culture.

various stress conditions. The patterns of biomass accumulation recorded in *H. pluvialis* (Figure 1) are compatible with previous observations under similar conditions (Damiani *et al.*, 2010). The accumulation of fatty acids under nitrogen starvation is a widely known phenomenon (Ahmad *et al.*, 2011; Gouveia and Oliveira, 2009; Chisti, 2007), although the effect of high light intensity or high light and nitrogen starvation on fatty acid content has not been studied extensively. Thus, we were particularly interested in examining the relationship between lipid accumulation and high light intensity in *H. pluvialis* cultures with or without nitrogen. Stress conditions induced a sharp increase in the content of lipid of *H. pluvialis*. The data on biomass increase and lipid accumulation (Figures 1 and 2) were identified with the conclusion that when microalgae were cultured under stress condition, preferential degradation of the nitrogen
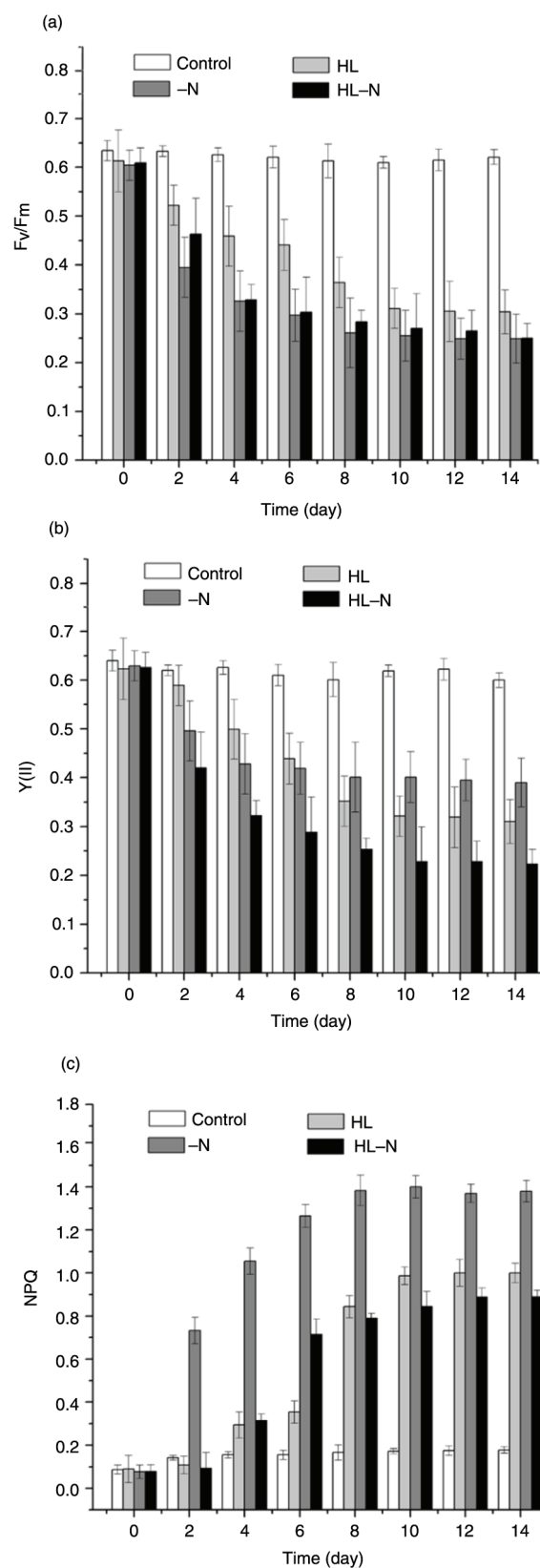

FIGURE 4. Changes in the three photosynthesis fluorescence parameters in the control, HL, -N, and HL-N cultures: (A) F_v/F_m, (B) $Y(II)$, and (C) NPQ.

TABLE 2. Correlation indices (R^2) of photosynthetic parameters and total lipid contents in the control, HL, -N, and HL-N cultures

Parameter	Control	HL	-N	HL-N
F_v/F_m	0.59	0.87	0.71	0.84
Y(II)	0.29	0.87	0.72	0.84
NPQ	0.58	0.77	0.74	0.78
Total carotenoids	0.79	0.81	0.81	0.86

Note: HL refers to the cells under high light (350 μmol photons· m^{-2}s^{-1} of continuous light); -N refers to the cells under nitrogen starvation stress; HL-N refers to the cells under high light (350 μmol photons m^{-2} s^{-1} of continuous light) and nitrogen starvation stress.

containing macromolecules occurs as a result in the change in cellular C/N balance, which apparently shifted toward lipid accumulation (White *et al.*, 2011). According to the previous study, Zhekisheva *et al.* (2002) found no significant differences in the fatty acid composition in cultures of the German strain of *H. Pluvialis* subjected to either high light intensity or nitrogen deprivation. And the study also showed that the accumulation of oleic acid was correlated with an increase in astaxanthin esters when *H. pluvialis* was grown under nitrogen starvation or high light intensity conditions. Our data were consistent with the conclusion. The data in Table 1 show that oleic acid content increased sharply in the cells exposed to stress conditions, but it is not the major composition of fatty acid. However, Cerón *et al.* (2007) showed that oleic acid was the major fatty acid present in cysts. The differences in oleic acid content observed in this study could be attributed to differences in growing conditions since the strain cultures were not supplemented with CO_2 (Damiani *et al.*, 2010). The de novo pathway produced most of the 18:1 and 16:0 at the expense of the PUFAs 16:2, 16:4 and EPA (Recht *et al.*, 2012). In our study, we also found that PUFAs 16:2, 16:4 and EPA decreased when *H. pluvialis* cells were cultivated under stress conditions. EPA is a group of fatty acids located in the chloroplast membrane under nutritional limitations, such as nitrogen, and cells are unable to resynthesize them and/or even keep the concentration of these components constant (Solovchenko *et al.*, 2010). Thus, we inferred that stresses would influence the photosystem. However, the relationship between the change in photosynthetic efficiency and the production of lipids has not been widely studied in the algal realm.

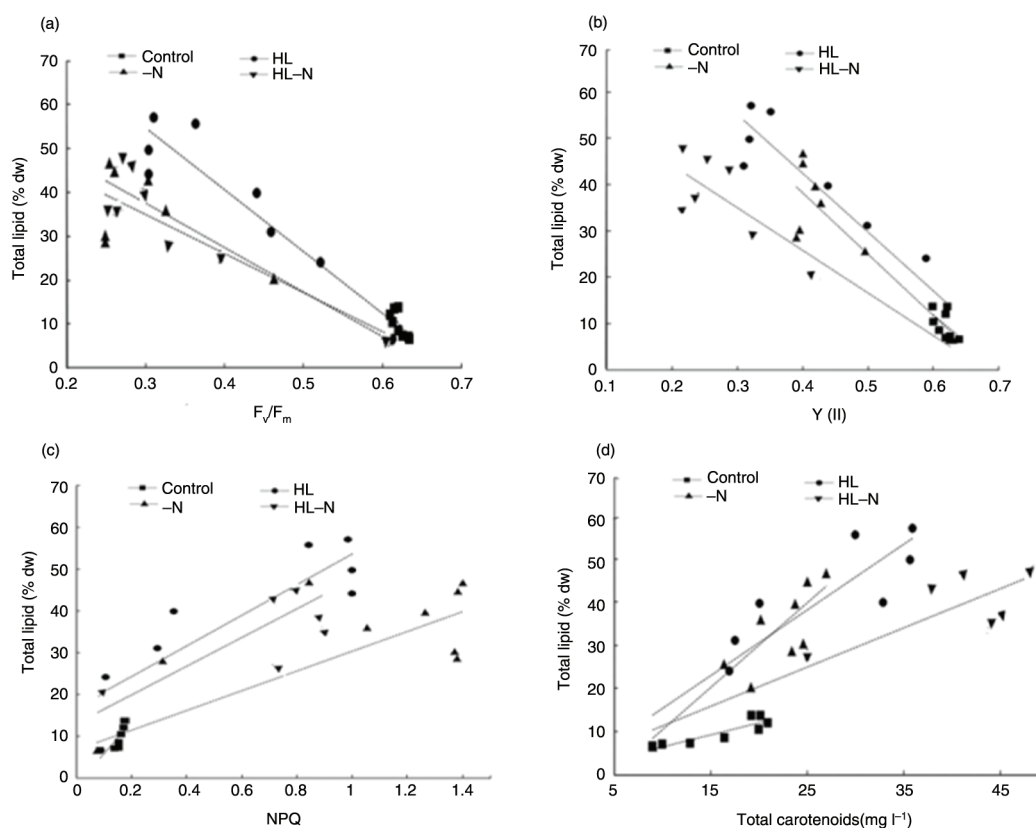The PAM fluorometry has been well used by ecologists to determine phytoplankton photosynthetic

FIGURE 5. Correlation analysis between total lipid content and photosynthetic parameters: (A) Between total lipid content and F_v/F_m; (B) Between total lipid content and Y(II); (C) Between total lipid content and NPQ; and (D) Between total lipid and total carotenoids.

efficiency (Petrou *et al*., 2008; Gustavs *et al*., 2010). Under environmental stress conditions, data showed significant changes in the physiological parameters (F_v/F_m, $Y(II)$ and NPQ) measured (Figure 4). Previous studies (Petrou *et al*., 2008) have shown that nutrient starved algae redirect energy from photosynthetic processes towards maximizing nutrient uptake upon nutrient addition. This redirection of energy leads to a net decrease in the capacity of cells to dissipate energy photochemically. This resulted (Figure 4C) in an increased NPQ value, suggesting a means of photoprotection. The maximum quantum efficiency F_v/F_m is used to estimate nutrient limitation and F_v/F_m value decreased in the stress culture. A significant inverse correlation was shown between both F_v/F_m and $Y(II)$ and cellular neutral lipid yields. Moreover, NPQ value and lipid content presented a positive correlation (Figure 5C). Oxborough *et al*. (2000) showed that fluorescence provides an extremely sensitive tool for examining energy metabolism in photosynthetic cells and the interactions between carbon and nutrient assimilation to be in the form of lipids. The synthesis of neutral lipids has been found to be a protective mechanism for cells against stressful conditions (Courchesne *et al*., 2009). PAM fluorometry can be used to increase the yields of lipids by identifying the extent of stress induced by environmental factors.

The influence of stress on carotenoid accumulation and chlorophyll was also studied in order to understand whether the accumulations of lipids and carotenoid synchronized. In our results, it was found that the cultivation of *H. pluvialis* in the [HL] cultures and the [-N] cultures was accompanied by significant changes in its pigment content and composition (Figure 3). It has been reported that under stress conditions, such as high light irradiance or nitrogen limitation, *H. pluvialis* formed clusters of globules containing carotenoids at the cell center (Zhekisheva *et al*., 2002). Carotenoids appeared mostly as mono- and di-esters of various fatty acids and total secondary carotenoids consist of astaxanthin up to 95% (Sarada *et al*., 2006). Thus, *H. pluvialis* had the primary carotenoid composition of the astaxanthin. After exposed to stress conditions, these clusters underwent a reversible spreading so as to shield a larger surface area of the chloroplast (Yong and Lee, 1991). It was suggested that astaxanthin may act as an antioxidant, inhibiting lipid peroxidation (Cifuentes *et al*., 2003). Our data represent the same trends of changes in total lipid and carotenoid contents. The relationship between lipid and carotenoids under stress conditions perfectly matched the above-mentioned point of view (Figure 5D), providing correlation indexes (R^2) from 0.786 to 0.862. It was thus reasonable to assume that the fatty acid metabolism under conditions inductive to pigment accumulation would be one of the key factors controlling astaxanthin

biosynthesis in this alga. Therefore, our results indicated that *H. pluvlialis* could be a potential microalga for synchronizing the production of biofuel and carotenoids.

5. CONCLUSIONS

This study described the favorable condition for lipid production by *H. pluvialis* grown under high light intensity and nitrogen deprivation. A significant correlation between carotenoid and lipid content revealed the possibility of using this alga for combined high-value production of biofuel and carotenoids. The strong correlation was also observed between the photosynthetic parameters and lipid accumulation. Future studies should consider different culture conditions, such as CO_2 supplementation or the use of a different nitrogen source to obtain an adequate lipid yield.

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