



# Effect of different light intensity on physiology, antioxidant capacity and photosynthetic characteristics on wheat seedlings under high CO<sub>2</sub> concentration in a closed artificial ecosystem

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## Abstract

The growth of plants under high carbon dioxide (CO<sub>2</sub>) concentrations ( $\geq 1000$  ppm) is explored for the climate change and the bioregenerative life support system (BLSS) environment of long-duration space missions. Wheat (*Triticum aestivum* L.) is a grass cultivated for cereal grain—a global staple food including astronauts. Light and CO<sub>2</sub> are both indispensable conditions for wheat seedlings. This study provides insights on the physiology, antioxidant capacity and photosynthetic characteristics of wheat seedlings under a range of photosynthetic photon flux densities in a closed system simulating BLSS with high CO<sub>2</sub> concentration. We found that the  $F_v/F_m$ ,  $F_v/F_0$ , chlorophyll content, intrinsic water use efficiencies (WUE<sub>i</sub>), membrane stability index (MSI), and malondialdehyde (MDA) of wheat seedlings grown under an intermediate light intensity of 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  environment were all largest. Interestingly, the high light intensity of 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  treatment group exhibits the highest net photosynthetic rate but the lowest MDA content. The stomatal conductance and  $F_0$  of high light intensity of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  treatment group were both significantly higher than that of other groups. Our study provides basic knowledge on the wheat growth in different environments, especially in a closed ecosystem with artificial lights.

**Keywords** Wheat · High CO<sub>2</sub> concentration · Light intensity · Photosynthetic characteristics · Physiology · Antioxidant capacity

## Abbreviations

HL	High light
IL	Intermediate light
LL	Low light
PPFD	Photosynthetic photon flux density
LED	Light-emitting diodes

LP1	Lunar palace 1
BLSS	Bioregenerative life support system
MSI	Membrane stability index
RWC	Relative water content
POD	Peroxidase
MDA	Malondialdehyde
A	Net photosynthetic rate
gs	Stomatal conductance
WUE <sub>i</sub>	Intrinsic water use efficiencies
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl a + b	Total chlorophyll content
PS II	Photosystem II
$F_0$	Minimum fluorescence
$F_m$	Maximum fluorescence
$F_v$	Variable fluorescence
$F_v/F_m$	Maximum quantum yield of PSII efficiency
$F_v/F_0$	Potential activity of PS II
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide

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## Introduction

Wheat (*Triticum aestivum* L.) is a globally important grain crop cultivated as a staple food, accounting for 20% of the calories consumed by human beings (Brenchley et al. 2012). It is also an important crop in bioregenerative life support system (BLSS) to provide food, oxygen, and necessary psychological well-being for spacecraft crews (Poughon et al. 2009; Wheeler 2017; Ushakova et al. 2018; Larrabee 2018).

Growth of wheat crops is affected by carbon dioxide (CO<sub>2</sub>) level (Prasad et al. 2002). Increasing CO<sub>2</sub> concentration can improve photosynthesis in C<sub>3</sub> plants such as wheat via reduction of photorespiration which can increase wheat yield (Eshghizadeh et al. 2018). Due to the global warming effect, the atmospheric CO<sub>2</sub> concentrations have risen from about 280 to 370 ppm since 1800 (Prasad et al. 2002; Griggs and Noguer 2002) and may reach 500–900 ppm by the end of the century (Joos et al. 1999; Bloom et al. 2002). The CO<sub>2</sub> levels in closed systems such as growth chambers and greenhouses can readily exceed 1000 ppm (Wang et al. 2015). Research has shown that CO<sub>2</sub> concentration which was controlled between 500 and 5500 ppm in a ground-based comprehensive experimental facility of BLSS called Chinese Lunar Palace 1 (LP1) could reach to a relatively steady-state level of approximately 1000 ppm (Fu et al. 2016). Although terrestrial CO<sub>2</sub> concentrations are not expected to exceed 1000 ppm in near future, it is necessary to explore the growth of plants under high CO<sub>2</sub> concentration to meet the future climates and the needs of BLSS environments. Controlled environmental studies showed that when wheat was cultivated under low CO<sub>2</sub> concentration (210 ppm), increasing CO<sub>2</sub> concentration was favorable to wheat growth (Dong et al. 2014a). In particular, 1000 ppm CO<sub>2</sub> was more favorable for growth and development of wheat (Bender et al. 1999; Taub et al. 2008; Dong et al. 2016a, b). High CO<sub>2</sub> concentration (1500 ppm) in closed BLSS can make carbon cycle time (residence in atmosphere) much shorter than natural (350 ppm) (Nelson et al., 2003). Therefore, it is essential for us to understand and control (Leong et al. 2020) the growth of wheat seedlings in high CO<sub>2</sub> concentration environments although which are not ideal on earth.

Light source is also an indispensable condition for plant (Schneider et al. 2006). The change of light intensity has a significant impact on physiology of wheat, such as the time to ear emergence and tillering of wheat, the adaptability to changing environmental conditions and the yield (Evtushenko and Chekurov 2004; Page and Feller 2016). Artificial lightings such as light-emitting diodes (LED) facilitate energy conservation compared to traditional light sources, which is also used to increase plant productivity per unit

of cultivation area (Dong et al. 2014a; Loconsole et al. 2019). In high CO<sub>2</sub>, higher growth rates may be obtainable as light intensity increases. If adequate energy inputs are available, the size of the food production component can be greatly reduced in a controlled environment life support system (Bugbee and Salisbury 1988).

In this study, we investigated the physiology, antioxidant capacity and photosynthetic characteristics of wheat (*Triticum aestivum* L.) seedlings under a range of photosynthetic photon flux densities (PPFD) in the controlled artificial closed ecosystem of Lunar Palace 1 (LP1), simulating BLSS. Specifically, we measured the plant growth and leaf water status by Membrane Stability Index (MSI) and relative water contents (RWC), respectively. We also understand the antioxidant capacity by measuring the contents of peroxidase, malondialdehyde, and proline in the plants. This study aims to reveal potential mechanisms for the physiological and developmental responses to different light intensities on wheat seedlings in a high CO<sub>2</sub> concentration environment.

## Materials and methods

### Plant material and cultivation conditions

Chinese Lunar Palace 1 (LP1) located at the Institute of Environmental Biology and Life Support Technology, Beihang University, Beijing, China, was used for plant cultivation in this study. It has one comprehensive cabin and two enclosed plant cabins (Fig. S1), with a total area of 160 m<sup>2</sup> and a volume of 500 m<sup>3</sup>. The comprehensive cabin hosts four bed cubicles, a bathroom, an animal-raising room, a common room, a storage room, and a waste-treatment room, which is the main activity area of spacecraft crew members. Each plant cabin has two divisions in which environmental conditions could be independently control, such as oxygen levels, water contents, and food production. Spring wheat plants (*Triticum aestivum* L. cv. 'Dwarf') were cultivated in vermiculite using a porous tube nutrient delivery system (PTNDS) with water supply on demand in plant cabin of LP1 (Wang et al. 2016; Dong et al. 2018). For this study, the wheat was planted at a density of 1200 seeds m<sup>-2</sup> (Fig. S1) in a plant cabin with continuous lighting (24/0 h light/dark) and 1000 ppm CO<sub>2</sub>. Temperature and relative humidity of the plant cabin were maintained at 21 ± 1.3 °C and 70 ± 4.6%, respectively. Growth period of the wheat seedling was 20 days. The basic culture medium was the modified Hoagland nutrient solution (Hoagland and Arnon 1950) (Table S1).

## Light intensity treatments

LEDs used for this study have uniform spectra of white (350–750 nm) and red (peak at 658 nm) light (W:R = 1:1) measured by a spectroradiometer (Avaspec-2048-UA, Avantes B.V., Netherlands) (Fig. S1). The LED light source was set at 70 cm on top of the wheat planting surface to ensure the unity of light intensity in the initial canopy of wheat. Wheat plants were exposed to 200, 400, 600, 800, 1000 and 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD, measured by a quantum sensor (Li-250A, Li-Cor, USA) (20 cm under the light). They were referred as low light (LL-200, 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; LL-400, 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), intermediate light (IL-600, 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; IL-800, 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and high light (HL-1000, 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; HL-1200, 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) treatment groups, respectively. PPFD levels were measured every two days at the top of the plant canopy with the quantum sensor. Five samples of those wheat seedlings were randomly chosen for each measurement.

## Straw height analyses

The height of wheat seedlings was measured every two days using a straight scale and vernier caliper. The straw height was estimated from the vermiculite surface to the tip of the last leaf. Five samples were randomly chosen for each plant height measurement.

## Membrane Stability Index (MSI) measurements

The leaves' MSI of wheat seedlings was measured with a conductivity meter (HI8733, Hanna instruments, Italy). Samples were excised from the leaves of 10 wheat seedlings at the first leaf at the terminal bud for each treatment. The samples were divided into two commensurate parts (about 100 mg) and soaked in 10 ml double-distilled water. The first part was then heated at 40 °C for 30 min and conductivity  $C_1$  was determined. The second part was heated at 100 °C for 10 min and conductivity  $C_2$  was determined. The MSI was calculated as:

$$\text{MSI} = \left(1 - \frac{C_1}{C_2}\right) \times 100\%$$

## Relative water content (RWC) measurements

To determine RWC, samples were excised from the leaves of 10 wheat seedlings at the first leaf at the terminal bud for each treatment during the wheat seedling period. About 0.5 g ( $m_1$ : initial weight of the leaves) of fresh leaves were

soaked in double-distilled water at room temperature for 4 h and immediately weighed again as  $m_2$ . The leaves were then dried at 65 °C for 48 h and weighed again as  $m_3$ . RWC was calculated using the following equation:

$$\text{RWC} = \frac{m_1 - m_3}{m_2 - m_3} \times 100\%$$

## Peroxidase (POD) activity determination

POD activity of wheat seedlings was analyzed following the change of absorbance at 470 nm due to guaiacol oxidation (Díaz et al. 2001). The POD activity was assayed for 3 min in a reaction solution composed of 0.15 ml of 4% (v/v) guaiacol, 0.15 ml of 1% (v/v)  $\text{H}_2\text{O}_2$ , 2.66 ml of 0.1 M phosphate buffer (pH 7.0) and 40  $\mu\text{l}$  of enzyme extract. Blank sample was made of the same mixture without enzyme extract.

## Malondialdehyde (MDA) estimation

Based on the thiobarbituric acid reactive substances (TBARS), the accumulation of lipid peroxide in tissues was determined by estimating the content of MDA (Stewart and Bewley 1980). The supernatant was mixed with 10 ml of 0.1% trichloroacetic acid (TCA). Pestled homogenate was used to centrifuge at 4000 rpm for 10 min and 2 ml supernatant was added to 4 ml 5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95 °C for 30 min, rapidly cooled on ice and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated with its extinction coefficient of 155  $\text{mM}^{-1} \text{cm}^{-1}$ .

## Proline content extraction

Samples were excised from the leaves of ten wheat seedlings at the first leaf at the terminal bud for each treatment. Seedling leaves were rapidly frozen in liquid nitrogen, freeze-dried and stored until lab use. Proline content was extracted and the content analyzed spectrophotometrically according to the method of Bates et al. (1973).

## Chlorophyll analysis

The contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) were analyzed with an ultraviolet spectrophotometer (SP-75, Shanghai Spectrum Instruments Co., Ltd, Shanghai, China). Seedling leaves were frozen in liquid nitrogen and stored at  $-80$  °C until measured.

## Photosynthetic efficiency measurement

Photosynthetic characteristics were measured using a portable photosynthesis instrument (LI-6400XT, Li-Cor, USA). Leaf gas-exchange parameters including net photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ) were determined from the first leaf in the seedling's terminal bud under saturating quantum flux densities ( $> 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at ambient temperature and relative humidity. Leaf temperatures were  $24 \pm 1.3 \text{ }^\circ\text{C}$  during the measurements. The intrinsic water use efficiencies ( $\text{WUE}_i$ ) were calculated by dividing  $A$  by  $g_s$  (Meloni et al. 2003).

## Determination of chlorophyll fluorescence

Chlorophyll fluorescence was measured simultaneously with  $\text{CO}_2$  gas exchange on the first leaves using a portable photosynthesis system (LI-6400XT, Li-Cor, USA). All measurements were carried out using the standard fluorescence leaf chamber ( $2 \text{ cm}^2$ ). The seedling leaves were dark-adapted for 20 min prior to measurements. The minimum fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) were obtained by the method described by Fu et al. (2012). The maximum quantum yield of PS II efficiency ( $F_v/F_m$ ) and potential activity of PS II  $\text{ssF}_v/F_0$  was calculated based on methods reported earlier (Maxwell and Johnson, 2000; Fu et al. 2012).

## Statistics

All measurements were performed in triplicate and randomly arranged in each block. Data were subjected to one-way analysis of variance (ANOVA) using Statistica Software SPSS 20.0 (SPSS Inc., Chicago, Illinois). A significant level of 0.05 was used for all statistical tests by Tukey's multiple range test.

## Results

### The impact on plant height, light intensity on the canopy, RWC and MSI

Figure 1a shows the average plant height under different light intensities (i.e. 200, 400, 600, 800, 1000 and  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) as a function of the light exposure time. The plant height increases with exposure time, for all light intensities under test. Although the plant treated by  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  is generally taller than others for the first 18 days and the light intensity on canopy greatly changes with the treatment time (Fig. 1b), all plants turn out to be of similar height after 23 days of growth regardless of the light intensities exposed to. Furthermore, the RWC and MSI contents of the plants do not vary much as the light intensity

increases (Fig. 1c, d). Within the small variations, minimum values were both found in LL-400, while maximum values were found in LL-200 and IL-600, respectively.

## Morphological characteristics antioxidant system

Figure 2a and b shows the plants' POD activity and MDA content as a function of light intensity. Among all groups, the POD activity of LL-400 scores the highest, which is nearly 74% and 36% larger than the LL-200 and HL-1200 treatment groups, respectively. The POD activity also fluctuates as the light intensity increases. For example, the POD activity of HL-1000 is higher than that of IL-800 and HL-1200. On the other hand, the MDA content exhibits a Gaussian distribution with the light intensity. In Fig. 2b, the MDA content initially increases with the light intensity and declines for light intensity larger than  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ , with the lowest content ( $3.06 \pm 0.03$ ) observed in the HL-1200 group.

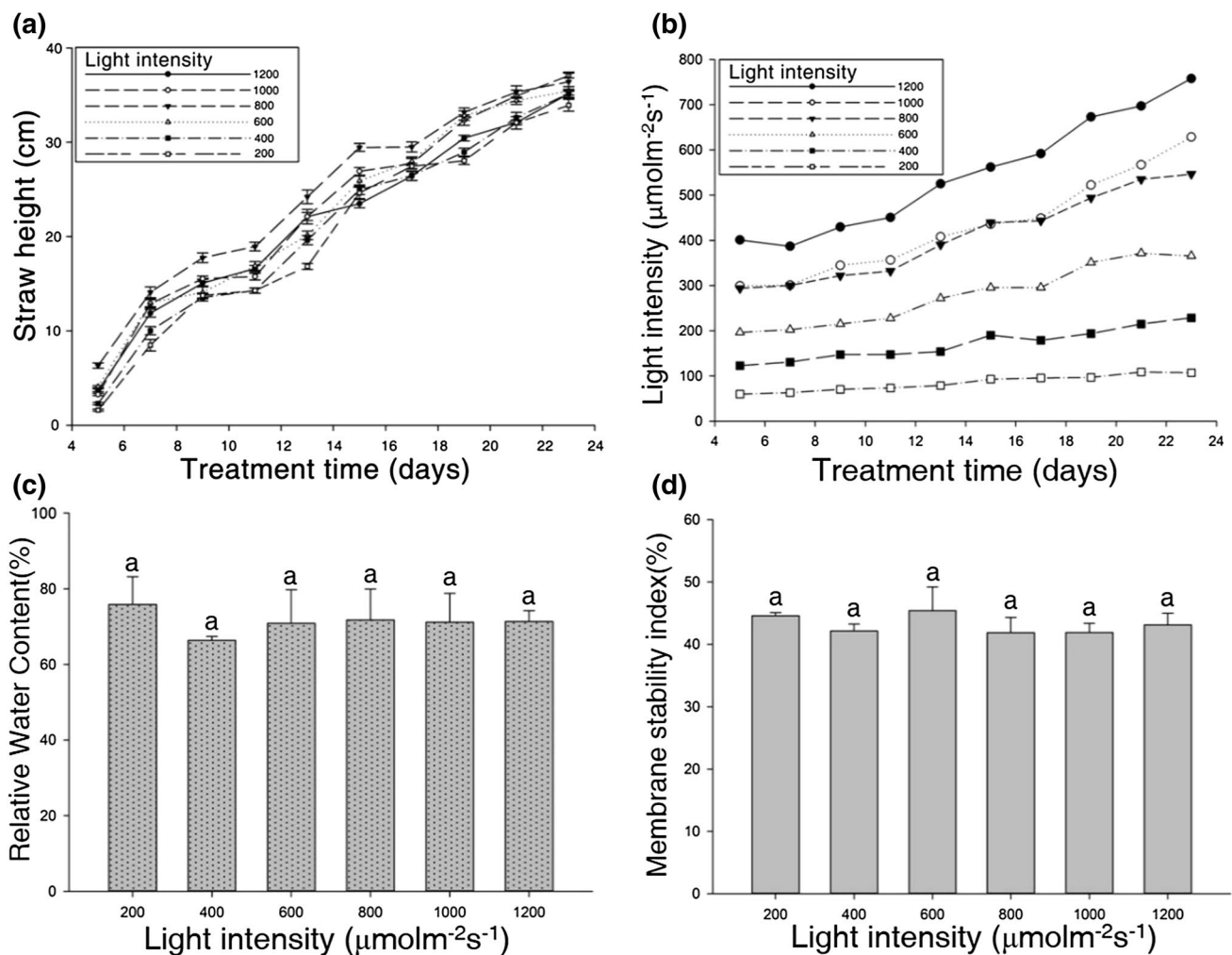
## Photosynthetic characteristics

Responses of net photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), intrinsic water use efficiency ( $\text{WUE}_i$ ), Chl a, Chl b, and Chl (a + b) to different light intensities are plotted in Fig. 3. Compared to LL-200, the net photosynthetic rate ( $A$ ) of LL-400, IL-600, IL-800, HL-1000 and HL-1200 is larger by 1.82, 3.19, 3.52, 3.48 and 4.12 times, respectively (Fig. 3a). The stomatal conductance ( $g_s$ ) of HL-1000 treatment group is 0.11, which is 18.1% and 15.5% higher than IL-800 and HL-1200 groups, respectively, and much larger than LL-200, LL-400 and IL-600 groups (Fig. 3b). Similarly, the  $\text{WUE}_i$  exhibits large variations across different light intensities treatments. The  $\text{WUE}_i$  increases initially from LL-200 to IL-600, with a maximum value of  $68.91 \pm 1.36 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}^{-1}$  at IL-600, and then decreases with light intensity (Fig. 3c). It rises again at HL-1200 under high  $\text{CO}_2$  concentration.

## Chlorophyll fluorescence

The increase of minimal fluorescence ( $F_0$ ) level is clearly visible in the HL-1000 group, which is  $70.3 \pm 4.8$  and significantly higher than that of LL-200 and HL-1200 (Table 1). The stability of  $F_0$  found among LL-400, IL-600 and IL-800 treatments indicates that IL caused negligible changes in the reaction centers. HL-1200 treatment does not impede the PS II reaction center under high  $\text{CO}_2$  concentration owing to the reduced  $F_0$  of HL-1200 (Table 1), which can be related to the inhibition of photorespiration. The trends of fluorescence ( $F_v$ ) and maximum fluorescence ( $F_m$ ) are similar to the  $F_0$  (Table 1). When the light intensity was too high, both  $F_v$  and  $F_m$  reduces.





**Fig. 1** The response of straw height (a) and light intensity (b) of wheat seedlings to different treatments; the values are means  $\pm$  SE ( $n=10$ ). The response of relative water content (RWC) (c) and membrane stability index (MSI) (d) of wheat seedlings to different treat-

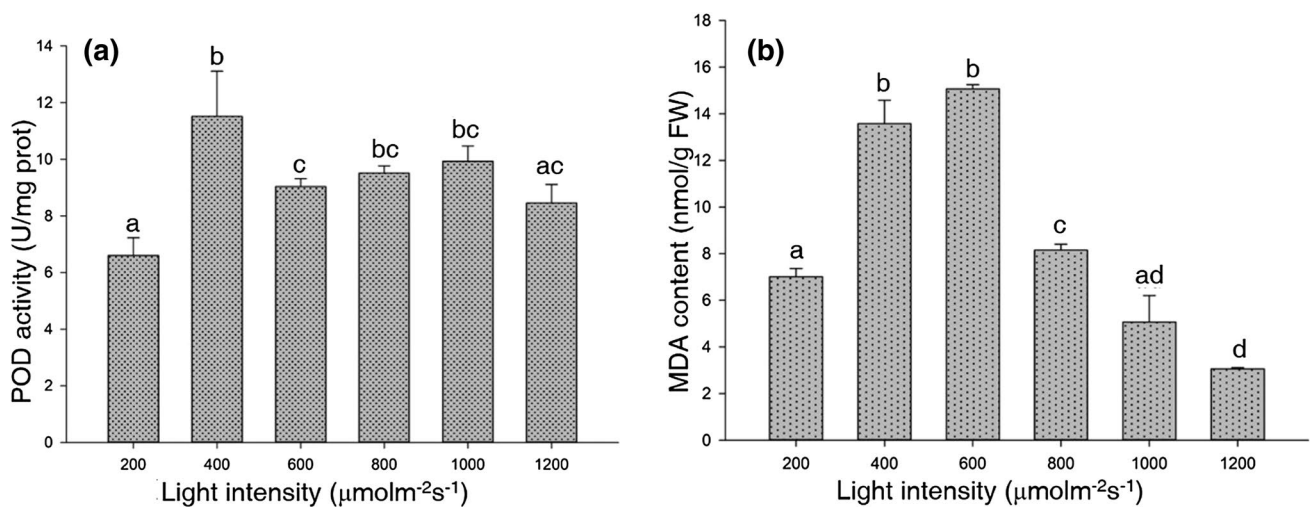
ments; the values are means  $\pm$  SE ( $n=3$ ). Different lowercase letters indicate significant differences among different light intensity treatments ( $p < 0.05$ )

Figure 4a shows that  $F_v/F_m$  of LL-400, HL-1000 and HL-1200 treatment groups is significantly lower than the IL-600 treatment group. Figure 4b clearly shows that the  $F_v/F_0$  of the LL-400, HL-1000 and HL-1200 groups is much lower than the IL-600 group, by about 8.5%, 10.7% and 6.5%, respectively. The largest  $F_v/F_0$  value is 5.029 in the IL-600 group. Meanwhile, the highest content of chlorophyll (Chl a, Chl b, Chl a + b) is in the IL-600 group (Fig. 3d–f). The decrease of high light intensity (HL-1000 and HL-1200) is observed in Fig. 4b, which has a similar condition to the change of chlorophyll content. Meanwhile, LL-400 has not enough light and chlorophyll content to sufficiently photosynthesize. Considering the chlorophyll fluorescence analysis performed in real growing conditions (Fig. 4c–f), the heat dissipative quantum ratio ( $F_0/F_m$ ) and steady-state fluorescence yield ( $F_s$ ) are invariant to the different growing

conditions. The maximum fluorescence yield ( $F'_m$ ) and electron transport rate (ETR) exhibit similar trend, where the minimum values were both recorded in the LL-200 group. Meanwhile, the ETR is significantly higher in LL-1200.

## Discussion

As a major scavenger of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), peroxidase (POD) catalyzes the dismutation of superoxide to  $\text{H}_2\text{O}_2$  and oxygen (Fu et al. 2012). The level of POD reflects the status of physiological activity of plants and has the function of oxidase IAA. The growth of overground of wheat, especially for the elongation growth, could be promoted in a low level of POD (Dong et al. 2014c). Our findings show that the light intensity has a substantial impact in tuning

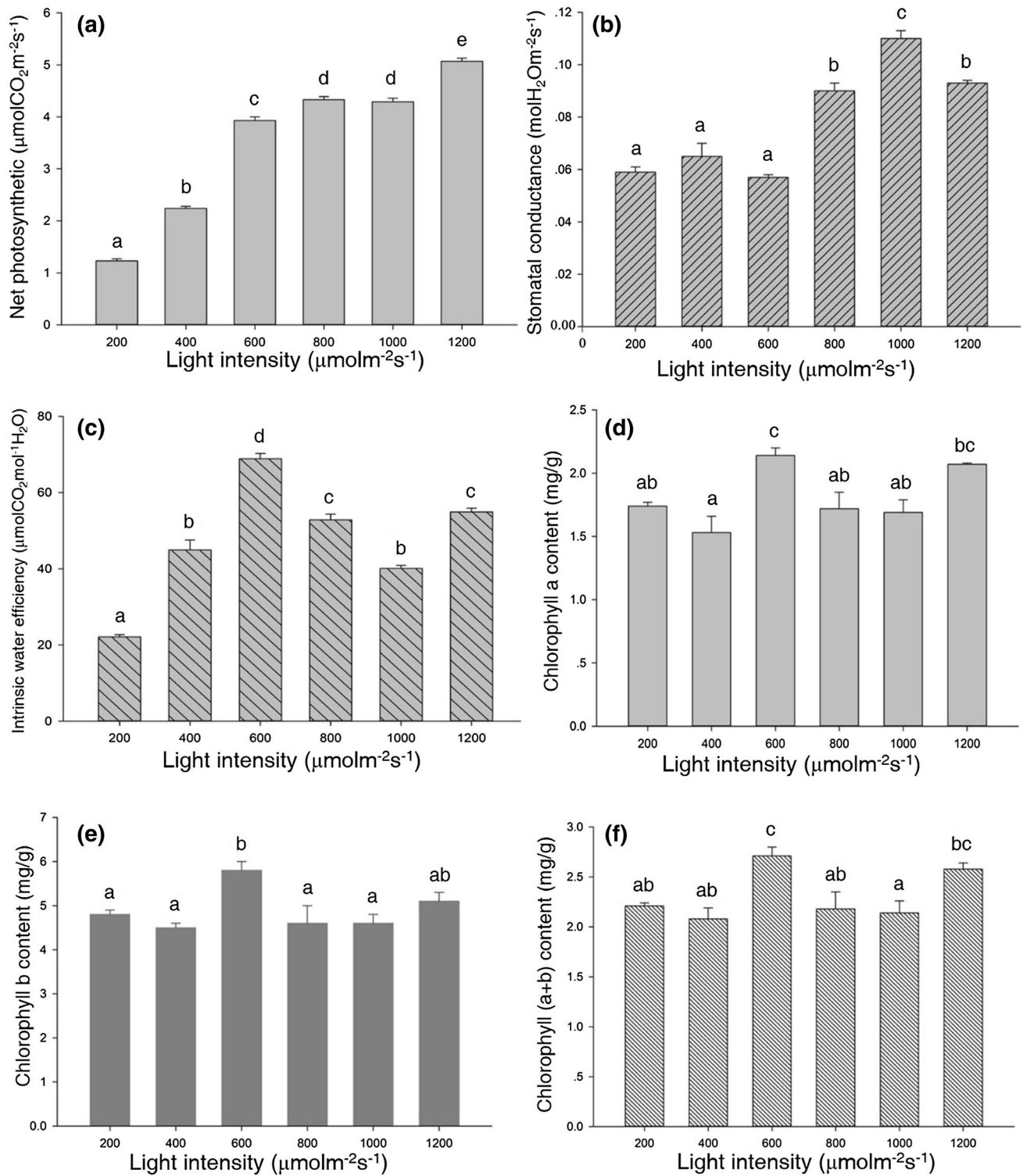


**Fig. 2** The response of POD (a) and MDA (b) of wheat seedlings to different treatments. The values are means  $\pm$  SE ( $n=3$ ). Different lowercase letters indicate significant differences among different light intensity treatments ( $p < 0.05$ )

the POD activity in wheat, similar to previous reports in romaine lettuce (*Lactuca sativa* L.), *Phalaenopsis* orchids, and *Lithocarpus litseifolius* (Hance) Chun. (Ali et al. 2005; Fu et al. 2012; Li et al. 2016). The POD activity of our low-light intensity group (LL-400) scores the highest. This indicates that excess energy light could damage photosynthetic organism and reactive oxygen species (ROS), although the photosynthetic capacity is stronger in high light intensity samples (Li et al. 2016). With the increase of light intensity, the activity of POD does not vary much, which is mainly affected by the genetic characteristics of the variety itself (Ashraf and Harris 2004). The increment of ROS causes membrane lipid peroxidation, leading to the damage of cell membranes (Li et al. 2016) and the production of MDA. The MDA content measures degree of damage and stress in plant (Abdollahi et al. 2004; Dong et al. 2014a), and which formation is associated with the POD in leaves (Dong et al. 2014a, b). Previous work demonstrated that diverse responses of antioxidant enzymes to light-stressed plantlets, implying that oxidative stress might be one of the major component of light stress on *Phalaenopsis* (Ali et al. 2005). In our study, we speculate that the photorespiration was restrained by high  $\text{CO}_2$  concentration from LL-600 to HL-1000, so that plants can efficiently utilize light energy for photosynthesis and ETR. Meanwhile, the damage caused by ROS reduces significantly. As a result of the reduction in POD activity from LL-600 to HL-1000 groups, more and more ROS was accumulated, damaging the chloroplast envelope and increasing the MDA content (Li et al. 2016).

Our net photosynthetic rate ( $A$ ) results agree well with previous findings that the stronger the light intensity, the higher the net photosynthetic rate (Dong et al. 2014a, b, c). The  $A$  of the HL-1200 is significantly greater than others,

indicating possibly that the former has a wide and long wheat leaf area (Jiang et al. 2003). Unlike the previous work, the differences observed between IL-800 and HL-1000 treatment groups are not significant (Fig. 3a), which could be due to the fact that Chl a, Chl b, and Chl (a + b) are not statistically significant between IL-800 and HL-100 (Fig. 3d–f). To a certain extent, Chlorophyll content determines the photosynthetic rate, and the change of chlorophyll content is closely related to the attenuation of A (Kura-Hotta et al. 1987; Spano et al. 2003). It has already been proposed that  $g_s$  increases with light intensity (Wang et al. 2017; Xiong et al., 2018). In this study, the max  $g_s$  was found in HL-1000 level because plants reduce their stomatal conductance to resist strong light (Dong et al. 2014a). However, the  $g_s$  of IL and HL groups is significantly larger than that of LL. Low-light intensity may decrease the  $g_s$  to  $\text{CO}_2$ , thus inhibiting photosynthesis (Loconsole et al. 2019). Zhang et al. (2017) and Li et al. (2015) also reported that  $g_s$  varies linearly with A. The intrinsic water use efficiency ( $\text{WUE}_i$ ), an index of potential resource use efficiency was used to facilitate the comparisons among leaves under similar conditions (Dong et al. 2016a). Our results prove that the  $\text{WUE}_i$  of different light intensities treatments has significant differences. This is consistent with earlier reports (Zhang et al. 2016). However, significant changes are not observed between IL-800 and HL-1200 treatment groups, indicating that the light intensity is excessive for the plant needs (Loconsole et al. 2019), due to the change in  $g_s$ . At the same time, it can also be seen that water-use efficiency does not solely depend on the amount of water intake of plants, but is a comprehensive evaluation index of resource utilization efficiency that represents  $\text{CO}_2$  concentration, humidity, and temperature or light in the environment (Dong et al. 2014a, 2016a; Medrano et al.



**Fig. 3** The response of net photosynthetic rate (a), stomatal conductance (b) and intrinsic water efficiency (c) of wheat seedlings to different treatments; the values are means  $\pm$  SE ( $n=10$ ). The response of chlorophyll a (d) and chlorophyll b (e) and chlorophyll a + b (f) of

wheat seedlings to different light intensity treatments; the values are means  $\pm$  SE ( $n=6$ ). Different lowercase letters indicate significant differences among different light intensity treatments ( $p < 0.05$ )

**Table 1** The response of potential activity of  $F_0$ ,  $F_m$  and  $F_v$  of wheat seedlings to different treatments

Light intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$F_0$	$F_m$	$F_v$
200	48.8 ± 2.9a	280.9 ± 16.1a	232.1 ± 13.3a
400	57.4 ± 4.8ab	318.3 ± 21.8ab	260.9 ± 17.1ab
600	63.1 ± 1.0ab	380.3 ± 5.7b	317.1 ± 5.2b
800	58.9 ± 3.2ab	340.9 ± 16.3ab	282.0 ± 13.2ab
1000	70.3 ± 4.8b	386.8 ± 26.7b	316.5 ± 22.2b
1200	53.8 ± 3.4a	333.9 ± 9.2a	242.3 ± 17.1a

The values are means ± SE ( $n=9$ ). Different lowercase letters indicate significant differences among different light intensity treatments ( $p < 0.05$ )

2015). Lighting system with different light intensities gives different responses to chlorophyll synthesis and chlorophyll degradation in wheat seedling leaves (Dong et al. 2014a). The chlorophyll content of wheat leaves was found to be increased quickly in high  $\text{CO}_2$  concentrations (700 ppm) (Gutiérrez et al. 2013). Chlorophyll concentrations increase when the light intensity rises from 210 to 420  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during seedling stage (Dong et al. 2014a), similar to our observations. The decline of Chlorophyll concentrations under high light intensity observed in our study can be attributed to three reasons. First, the response of chlorophyll to light intensity is species-dependent. Second, the light intensity and  $\text{CO}_2$  concentration both have impacts on the chlorophyll content, and their function was mutual and indivisible. Third, high  $\text{CO}_2$  concentrations induce sugar accumulation and decrease nitrogen content, causing senescence and the loss of chlorophyll in leaves (Lotfiomran et al. 2016; Agüera and Haba 2018).

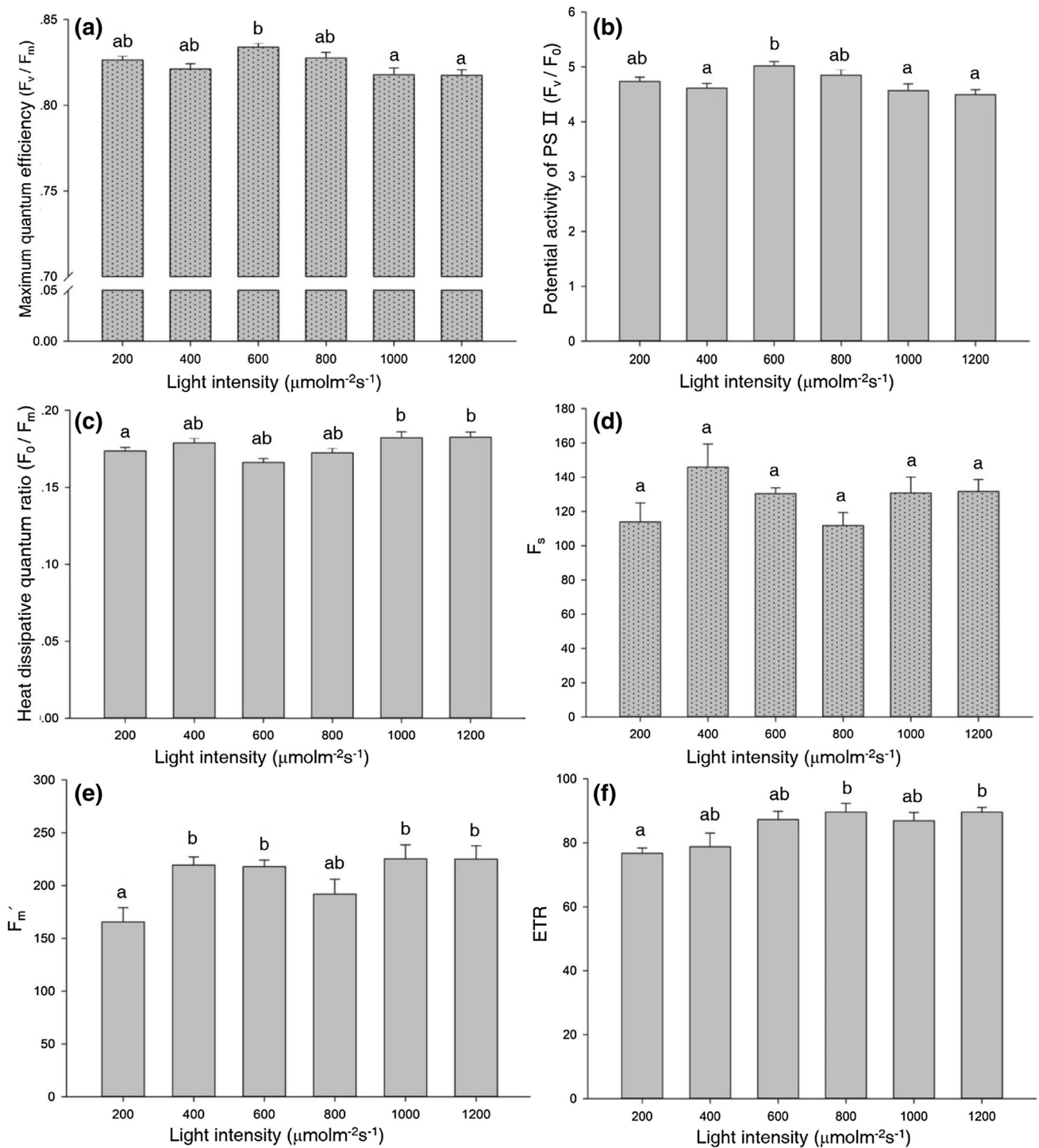
The change of fluorescence ( $F_0$ ) level observed in this work is supported by a previous study (Li et al. 2015). Increased  $F_0$  represents an impaired energy trapping efficiency in PS II reaction centers because of the stronger light intensity (Paunov et al. 2018). Decreased efficiency of the energy transfer from the antenna to the reaction center was demonstrated by Somersalo and Krause (1990). However, we found that HL-1200 treatment did not cause the destruction of the PS II reaction center under high  $\text{CO}_2$  concentration. This may be related to the reduced  $F_0$  of HL-1200 and the inhibition of photorespiration. To a certain extent, the high light intensity can cause them to rise, which means the reduction of electron accepting plastoquinone ( $Q_A$ ) is reduced under low light intensity and super high light intensity. In brief, the increase in light intensity can increase the urgency of  $Q_A$  (Van Eerden et al. 2017).  $F_v/F_m$  reflects the original light energy efficiency of PS II (Li et al. 2015). Under non-environmental stress conditions, this parameter rarely changes and it is not affected by species and growth conditions. It is always used as a good indicator to reflect the

degree of light inhibition (Wang et al. 2015; Mathur et al. 2016). According to Björkman and Demmig (1987), the optimal value of  $F_v/F_m$  was around 0.83 for healthy plants, and if  $F_v/F_m$  was higher than 0.8, the potential efficiency of PS II was not affected. In the present study, the largest  $F_v/F_m$  value was 0.834 in IL-600 treatment, which indicates that the plant is healthy and not suffering from photosynthetic stress (Li et al. 2015). The  $F_v/F_m$  value decreased with increasing light intensity. Wang et al. (2015) also reported that  $F_v/F_m$  also decreased significantly with super-elevated  $\text{CO}_2$  concentration in soybean and maize leaves. The results showed that  $F_v/F_m$  of HL treatment groups were significantly lower than IL treatment group in the range (0.817–0.821, higher than 0.8), which might indicate that the photosynthetic stress was prevented under high  $\text{CO}_2$  concentration.  $F_v/F_0$  indicates the potential activity or the size and number of PS II in the photoreaction center in the chloroplast, which is always used to represent photosynthetic strength of the plant (Li et al. 2015). The change  $F_v/F_0$  was similar to  $F_v/F_m$  in our study. The results strongly suggest that the original light energy efficiency of PS II and potential activity of PS II in the photoreaction center were not inhibited under IL-600, but they were significantly inhibited under LL-400, HL-1000 and HL-1200, relatively. However, the ETR was significantly higher in LL-1200, further indicating that higher light intensity caused a loss in the performance of the PSII under high  $\text{CO}_2$  concentration. Similar results were found in different light intensities under normal atmospheric (Loconsole et al. 2019).

## Conclusion

In this study, wheat seedlings were cultivated under different light intensities (200–1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in high carbon dioxide concentration, and characterized by appropriate morphological characteristics, antioxidant capacity, photosynthesis parameters and chlorophyll fluorescence parameters. Our results show that wheats planted under 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  exhibit the best characteristics in terms of  $F_v/F_m$ ,  $F_v/F_0$ , chlorophyll content,  $\text{WUE}_i$  and MSI. Hence, 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  is the most suitable light intensity for wheat seedlings growth under high carbon dioxide concentration. Interestingly, the net photosynthetic rate always increased, however, the content of MDA increased first and then decreased gradually with increasing light intensity. The stomatal conductance and  $F_0$  of HL-1000 treatment group were both significantly higher than that of other groups, and the  $\text{WUE}_i$  also showed a trend of increasing first and then decreasing with the increasing light intensity. Our study provides insights on the role of light intensity on wheat growth and serves as a foundation to understand and predict the





**Fig. 4** The chlorophyll fluorescence indexes measured on leaves of wheat seedlings to different light intensity treatments. The maximum quantum efficiency of PS II ( $F_v/F_m$ ) (a), the potential activity of PS II ( $F_v/F_0$ ) (b), the heat dissipative quantum ratio ( $F_0/F_m$ ) (c), the steady-

state fluorescence yield ( $F_s$ ) (d), the maximum fluorescence ( $F'_m$ ) (e), and the electron transport rate (ETR) (f). The values are means  $\pm$  SE ( $n=9$ ). Different lowercase letters indicate significant differences among different light intensity treatments ( $p < 0.05$ )

yield of wheat growths in different environments, especially in a closed ecosystem.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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