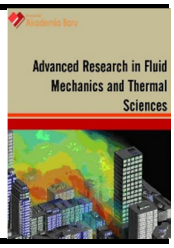
Penerbit Akademia Baru

Journal of Advanced Research in Fluid Mechanics and Thermal Sciences

Journal homepage: www.akademiabaru.com/arfmts.html
ISSN: 2289-7879



The Influence of Fluctuating Light Condition on Photosynthetic Acclimation in *Arabidopsis Thaliana*

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ARTICLE INFO

Article history:
Received 2 December 2017
Received in revised form 30 January 2018
Accepted 10 February 2018
Available online 18 February 2018

ABSTRACT

Photoacclimation is a process by which photosynthetic capacity is regulated in response to environmental adjustments in terms of light regime. Photoacclimation is essential in determining the photosynthetic capacity to optimize light use and to avoid potentially damaging effects. Previous work in our laboratory has identified a gene, *gpt2* (At1g61800) that is essential for plants to acclimate to an increase and decrease of growth irradiance, separately. To investigate the photoacclimation ability towards fluctuating natural light condition in *Arabidopsis thaliana*, photosynthetic capacity was measured in plants of the accession Wassileskija (WS) and in plants lacking expression of the gene At1g61800 (*WS-gpt2*). The experiment was carried out over a time span from early Autumn to early Spring season in 2010-2011 and 2011-2012. The seedlings were grown in an unheated greenhouse in Manchester, UK without supplementary lighting. Gas exchange measurements, chlorophyll fluorescence analysis and chlorophyll content estimation were performed on WS and *WS-gpt2* and it showed that both sets of plants could acclimate to fluctuating natural light condition. Therefore, it is suggested that the mechanisms of acclimation in a separate growth light condition is mechanistically distinct than the mechanism under fluctuating natural light condition.

Keywords:
Acclimation, Arabidopsis, Photosynthesis, WS, *WS-gpt2*

1. Introduction

Sunlight availability changes through the year. Light can fluctuate over long as well as short time periods. When the amount of light available changes, the plant needs to use the light efficiently to sustain life. Therefore, plants have evolved to overcome the problem by performing acclimation. Acclimation takes up to several days and can involve changes in pigments such as chlorophylls, carotenoids and anthocyanins, as well as of different enzymes involved in photosynthesis and other processes [1]. Changes in irradiance could lead to harmful effects on plants. Therefore, plants

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respond to changes by altering their light capture capacity and at the same time limit potentially damaging effects, such as photoinhibiton and the production of reactive oxygen species (ROS) [2].

As shown by Naramoto *et al*.,[3] *Fagus crenata* (Japanese beech) plants exposed to changing conditions are able to acclimate dynamically. *F.crenata* was grown under low light (LL) condition before being exposed to 2 different light intensities of medium light (ML) and high light (HL). Photochemical efficiency of photosystem II (Φ PSII) (Fv/Fm) and photosynthetic capacity were measured and it was found that *F.crenata* experienced more photoinhibition under HL. The HL acclimated plant was unable to increase its photosynthetic capacity compared to the ML. Therefore, it was concluded that a slow increase of the light intensity plays a key role to have a successful photosynthetic acclimation.

Arabidopsis thaliana is a model plant that has been extensively used in experiments for light acclimation [4-7]. Work by Athanasiou *et al*.,[7] showed that *Arabidopsis* grown at a low light intensity (100 µmol $m^{-2}s^{-1}$) had the ability to change their photosynthetic capacity when being transferred to a higher light intensity (400 µmol $m^{-2}s^{-1}$). It was also found that the gene *At1g61800,* which encodes a Glucose-6-P/phosphate translocator (GPT2) is essential for this type of acclimation. GPT2 has a primary function of translocating sugar and phosphates across the chloroplast [8].

As suggested by Bowsher *et al*., [9] the primary function of GPT is that it imports G6P into plastids of heterotrophic tissue as a precursor for starch biosynthesis. The GPTs (GPT1 and GPT2) belong to the phosphate translocator family, which contains six functional phosphate translocators (PTs) in *Arabidopsis*. These are a triose phosphate/PT (TPT) [10], two phosphoenolpyruvate (PEP)/PT [8], a xylulose-5-phosphate (Xul5P)/PT [11] and two glucose-6-phosphate (Glc6P)/PT [12]. The genes of Glc6P/PT, *gpt1* and *gpt2*, were demonstrated to have different effects on vegetative and generative development. Plants lacking the *gpt1* gene have retarded development of both pollen and embryo sac development [13]. However, GPT1 is known not to have an effect in starch biosynthesis. Thus, pollen and embryo sac development do not require starch for development [14]. The *gpt2* gene has been shown to be essential in the higher irradiance acclimation in *Arabidopsis thaliana,* as the *gpt2*- mutant plants did not acclimate photosynthesis when transferred to high light conditions [7]. To confirm the impairment in acclimation of photosynthesis of *gpt2* mutants, mutants were complemented with a copy of the *gpt2* gene and it was shown that plants could acclimate. Therefore, it was concluded that GPT2 is important in dynamic acclimation to increased light in *Arabidopsis*. Previous studies also have shown that the *gpt2* gene is also induced during sugar-feeding and sugar-induced senescence [15-17].

According to Yin *et al*., [4], many plants have been grown under separate and static light conditions, but few studies have been carried out when plants were grown under fluctuating light environments. The light environment variation ranges from seconds to hours and the light availability will greatly affect plants, especially for woodland plants. In the study by Yin *et al*., [4], *Arabidopsis thaliana, Digitalis purpurea and Silene dioica* were grown at different light intensities fluctuating between 100 µmol $m^{-2}s^{-1}$ and 475 µmol $m^{-2}s^{-1}$ or 810 µmol $m^{-2}s^{-1}$. It was found that the fluctuating light environment increased the maximum photosynthetic rate for all species. However, the extent of acclimation responses varied between species in terms of the cytochrome f content and Rubisco protein.

Besides affecting the maximum photosynthetic rate, growth of plants of *Arabidopsis* under short and long fluctuating light treatment may involve in the reorganization of pigment-protein complexes and enhancement of photoprotective mechanisms [18]. Seven ecotypes of *Arabidopsis* were treated with short and long sunflecks and it was found that all plants had an increased in non-photochemical quenching (NPQ). This shows that these plants were unable to utilize the light

efficiently, even under short sunflecks. Besides NPQ, the short and long sunflecks resulted in a decrease in chlorophyll content, an increase in the de-epoxidation of violaxanthin to zeaxanthin and antheraxanthin, upregulation of the amount of PsbS protein and of superoxide dismutase activity.

Thus, this study was carried out to study the acclimation responses of WS and *WS-gpt2* ecotypes of *Arabidopsis thaliana* under fluctuating natural light condition. This section of paper focuses on the responses of *A.thaliana* towards the changes in photosynthetic parameters and chlorophyll pigments.

2. Methodology

A. Plant Material

Wild type seeds of Wassilewskija-2 (WS-2) and mutant seeds of *WS-gpt2* were sown onto soil and then placed at 4^{o}C for two days before being transferred to 20ºC at low light (100 µmol m^{-2} s^{-1}). The seedlings were left in low light for 7 days before being transferred to the greenhouse.

The seedlings were grown in an unheated greenhouse in Manchester, UK without supplementary lighting during the periods of October 2010 to January 2011 and October 2011 to February 2012.

B. Maximum Photosynthetic Capacity Measurement (Pmax)

The maximum capacity for photosynthesis was measured as the rate of photosynthesis at 1600 µmol m^{-2} s^{-1} light and at 20^{o}C. Measurements were carried out at 2000 ppm CO_2. Immediately after the plant was removed from the greenhouse, it was placed into a CIRAS 1 standard broad leaf chamber (area 2.5 cm^2). The plants were left in the chamber for 5 min until a steady-state of gas exchange level was reached. Afterwards, the plant was illuminated with an actinic light for 20 min, after which the value of photosynthetic capacity was recorded.

C. Chlorophyll Fluorescence Measurements

Simultaneous to the photosynthetic capacity measurements, chlorophyll fluorescence analysis was performed using a PAM 101 chlorophyll fluorometer (Walz, Effeltrich, Germany). This analysis was performed to measure the chlorophyll fluorescence parameters of photosystem II efficiency (ΦPSII) and non-photochemical quenching (NPQ). Data were recorded on a PC using a National Instruments M series data acquisition card and running software written using Labview (National Instruments, Austin, US).

Prior to each chlorophyll fluorescence measurement, a full-size mature leaf was placed in the CIRAS 1 chamber. The leaf was left for 5 min in the chamber to equilibrate with the chamber environment. The fluorometer measuring beam was switched on to measure F_o and the leaf was exposed to a saturating flash of 7500 µmol m^{-2} s^{-1} to determine the value of F_m (Fig. 1). Afterwards, actinic light at 1500 µmol m^{-2} s^{-1} was given for the next 20 minutes. During the 20 min interval, a saturating flash was given to the leaf every 120 sec to measure changes in F_m' over time.

The data from the fluorescence analysis was calculated for Φ PSII and NPQ using Eqns. 1 and 2.

$$\Phi \text{ PSII} = (F_{m'} - F_t) / F_m \quad (1)$$

$$\text{NPQ} = (F_m - F_{m'}) / F_m \quad (2)$$

D. Chlorophyll Pigments Determination

After the measurements of photosynthesis, the same leaf was detached from the plant and the leaf area was measured by scanning using a Canon LiDE 20 scanner, with the leaf images being analysed using Scion Image (Scion Corp., Maryland, USA). The leaf was ground in a pestle and mortar in 80% (v/v) acetone. The extract centrifuged using a microfuge (Progen) at full speed (16,000 g) for 5 minutes. The absorbance of the supernatant was measured using a USB2000 spectrophotometer (Ocean Optics, Dunedin, USA) and the absorbance value at 646.6 nm, 663.5 nm and 750 nm were recorded. The chlorophyll content was calculated according to [19] as shown in Eqns. 3 and 4.

$$\text{Chl a } (\text{ng/cm}^2) = (13.71(A_{663.5\text{-}750}) - 2.85(A_{646.6\text{-}750})) / \text{Leaf area } (\text{cm}^2) \quad (3)$$

$$\text{Chl b } (\text{ng/cm}^2) = (22.39(A_{646.6\text{-}750}) - 5.42(A_{663.5\text{-}750})) / \text{Leaf area } (\text{cm}^2) \quad (4)$$

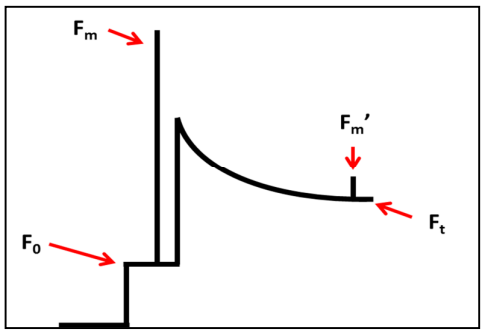

Fig. 1. An illustration of a typical fluorescence. The F_o was set 5 sec after the recording started. 10 sec later, the F_m was measured. A saturating flash was given at every 120 sec for 20 minutes to measure F_m'. Meanwhile, the F_t was recorded as the yield of fluorescence just before the saturating flash. F_m = maximum fluorescence, F_m' = fluorescence maximum in light, F_t = steady state fluorescence yield in light

3. Results

A. Photosynthetic acclimation of WS and WS-gpt2 under fluctuating light condition in Winter 2010-2011

In this experiment, WS and *WS-gpt2* plants were grown in an unheated greenhouse in Manchester. Photosynthetic capacity, Pmax, ΦPSII, NPQ, total chlorophyll and chl a/b were measured.

In the year of 2010-2011, plants were measured at one time-point only as the plants flowered earlier than expected. It was found that there were no significant changes between WS and *WS-gpt2* plants in maximum photosynthetic capacity (Fig. 2a) and ϕ PSII (Fig. 2b). Therefore, WS and *WS-gpt2* plants had equal capacity to survive under natural variable light. Furthermore, both plants of WS and *WS-gpt2* plants did not have the ability to quench excess excitation energy as there was

no significant change in NPQ (Fig. 2c). In terms of chlorophyll analysis, similarly there was no significant change in the total amount of chlorophyll (Fig. 3a) and the ratio of chlorophyll a to chlorophyll b (Fig. 3b).

B. Photosynthetic acclimation of WS and WS-gpt2 under fluctuating light condition in Winter 2011-2012

In the following year of 2011-2012, plants were measured at 5 different time-points which were at week 8, 9, 11, 12 and 13. The Pmax value was still low, however there was still no significant difference between WS and *WS-gpt2* plants during the course of experiment (Fig. 4a). Similarly, the value of ϕ PSII (Fig. 4b) had no difference but NPQ (Fig. 4c) decreased over the week.

As for the chlorophyll analysis the total value of chlorophyll was slightly lower than the previous year (Fig. 5a). The value of total chlorophyll content in this year did not significantly differ between plants and during the course of treatment. Meanwhile, the chl a/b ratio (Fig. 5b) of WS and *WS-gpt2* showed no difference over the course of measurement.

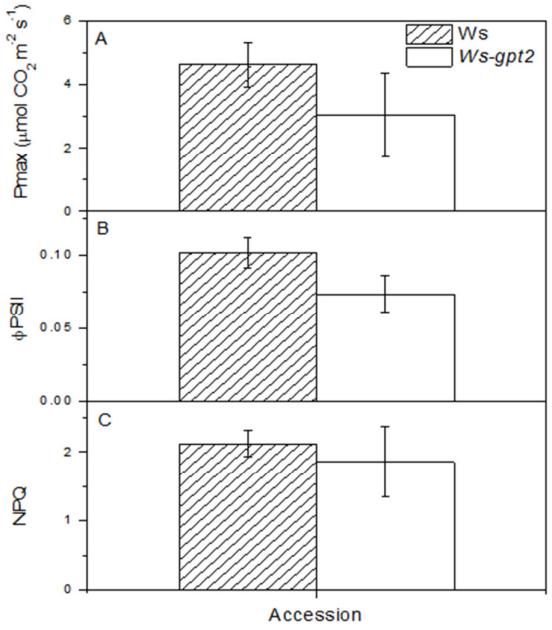

Fig. 2. Photosynthetic parameters measurement of (a) Pmax, (b) Φ PSII and (c) NPQ for WS and *WS-gpt2* in Winter 2010/2011. The plants were sowed in the lab and germinated in the greenhouse. After 12 weeks of growing in the greenhouse, the plants were taken to the lab for measurement. All data are mean ± SE for at least 5 biological replicates

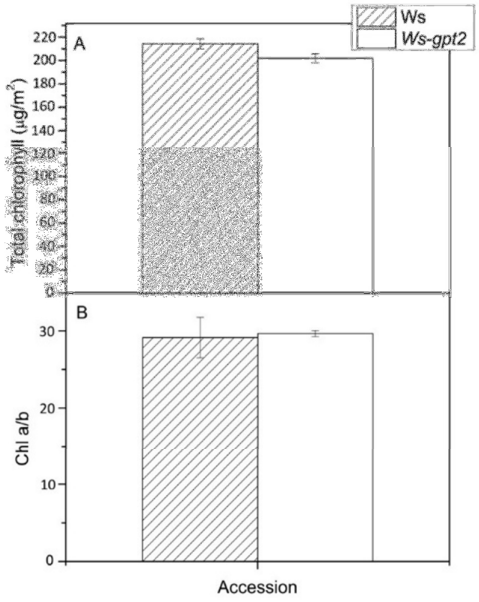

Fig. 3. Chlorophyll content measurement of (a) total chlorophyll and (b) chl a/b for WS and *WS-gpt2* plants during Winter of 2010 to 2011. The plants were sowed in the lab and germinated in the greenhouse. After 12 weeks of growing in the greenhouse, the plants were taken to the lab to be measured. The same leaf for photosynthetic measurement was used for this chlorophyll measurement. All data are mean ± SE for at least 5 biological replicates

4. Discussion

In this outdoor project, plants of WS and *WS-gpt2* were grown in an unheated greenhouse and without any lighting at the experimental ground in Manchester over the winter season. The project was carried out in two consecutive years of 2010 to 2011 (2010/2011) and 2011 to 2012 (2011/2012). In 2010/2011, only one measurement was successfully performed because the plants were already flowered by the time of the measurement. Flowering in plants marks the transition phase from vegetative phase to reproductive phase [20]. This transition is sensitive to any environmental stresses including chilling, drought and high light stresses. In 2010/2011, the mean temperature (^{o}C) during winter season was lower than the next year of 2011/2012. Therefore, it was shown that the winter of 2010/2011 was markedly colder than 2011/2012. Besides, vernalization which is plants exposure to a certain period of time to cold condition can promote flowering but this process is not required for *Arabidopsis thaliana* [21]. However, stratification which is seeds exposure to cold condition for a certain period of time can have similar effect on flowering to most but not all ecotypes of *Arabidopsis thaliana*. Therefore, plants of WS and *WS-gpt2* in 2010/2011 winter flowered earlier than 2011/2012 due to the chilling stress since the mean temperature were fluctuating.

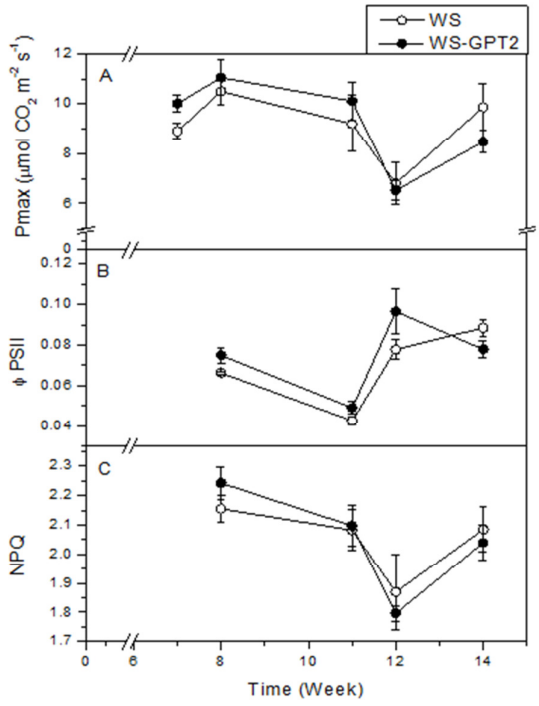

Fig. 4. Photosynthetic parameters measurement of (a) Pmax, (b) Φ PSII and (c) NPQ for WS and *WS-gpt2* plants during Winter of 2011 to 2012. The plants were sowed in the lab and germinated in the greenhouse. After the plants were mature, the plants were taken for measurement at week 7,8, 11, 12 and 14. All data are mean ± SE for at least 5 biological replicates

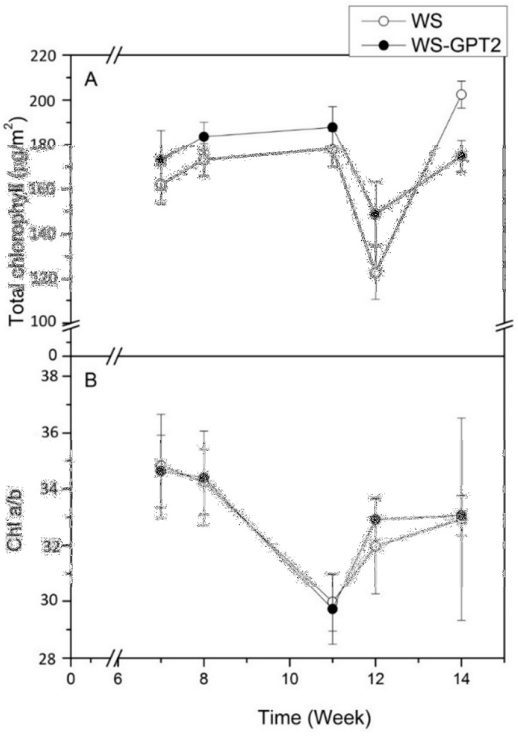

Fig.5. Chlorophyll content measurement of WS and *WS-gpt2* plants during Winter of 2011 to 2012 (a) total chlorophyll and (b) chl a/b for WS (open circle) and *WS-gpt2* (black circle) plants during Winter of 2011 to 2012. The plants were sowed in the lab and germinated in the greenhouse. After the plants were mature, the plants were taken for measurement at week 7,8, 11, 12 and 14. The same leaf for photosynthetic measurement was used for this chlorophyll measurement. All data are mean ± SE for at least 5 biological replicates

The reproductive stage of plants can influence the senescing stage in leaves or the whole plant [22]. Therefore, making the measurement on plants that are already flowered would not indicate an accurate value. This is why the Pmax value was very low compared to the value in 2011/2012. Besides, low temperature is one of the main important factors affecting plant performance, specifically photosynthesis [23]. However, *Arabidopsis* and other cold-hardy herbaceous species have the ability to acclimate to cold condition. Thus, plants WS and *WS-gpt2* had a low value of photosynthetic parameters (Pmax, ΦPSII, NPQ) due to the senescing factor but also those plants had the ability to survive under the fluctuating temperature and light. According to [7], the WS plants had the ability to acclimate to higher light intensities but *WS-gpt2* did not. However, in this experiment, it has been shown that both WS and *WS-gpt2* can acclimate to lower light intensities. However, in this fluctuating light condition, there was no difference between these plants of WS and *WS-gpt2*. Besides, according to metoffice, the mean temperature of winter season in 2010/2011 was even below the average. It was only two weeks before the measurement, the mean temperature rose above 0^{o}C but still below the average temperature. The fluctuating in temperature might indicate that the plants had a very limited sunlight and thus lowering the maximum photosynthetic capacity.

In the reproductive and senescing phase, chlorophyll breakdown is a very common event happening in plants [20]. Besides, during acclimation process, chloroplasts also undergo molecular re-arrangements involving the chloroplast composition. The changes in the chloroplast composition in terms of chl a/b shows a clear acclimation response along with the maximum photosynthetic capacity value [6]. Due to that, it was found that there was no difference in the total chlorophyll and chl a/b ratio in both WS and *WS-gpt2* plants. These data were consistent with the no significant changes in the Pmax as well. However, the value of chl a/b of WS and *WS-gpt2* plants were quite similar to the value of total chlorophyll of LL plants grown in the laboratory condition. In the laboratory condition, the chl a/b of LL plants were decreased indicating that the chl b was increased compared to chl a.

In 2011/2012 winter project, plants of WS and *WS-gpt2* were measured at 5 different time point starting at after 7 weeks of germination. At this stage, the leaves are mature enough to be measured. Similarly, in 2011/212, there was no significance difference in Pmax between the WS and *WS-gpt2*. Moreover, the value ϕPSII was also shown no difference in both WS and *WS-gpt2* indicating that there was no difference in the PSII efficiency since both plants had no difference in the capacity of light absorption. However, the value of Pmax in winter 2011/2012 was higher than the value in winter 2010/2011. Meanwhile, the NPQ was also shown no difference between WS and *WS-gpt2* but it showed that the NPQ tends to decrease over the course of measurement. The major contributor for NPQ is known to be a high energy state quenching (qE). qE is essential in plants in order to protect plants from damage due to strong light [24]. Thus, since the light availability to plants was very limited at this time of the year, the qE was less induced and eventually it lead to less excess energy quenching.

The total chlorophyll content and chlorophyll composition in terms of chl a/b were also had no difference between WS and *WS-gpt2*. When there was no difference in the chl a/b ration, it indicates that there was also no changes in the size of PSII light harvesting antennae [25], or the reaction centre content such as the number of PSII [26]. However, in week 14, there was a small difference in the total chlorophyll content between WS and *WS-gpt2*. This could be due to the temperature that started to rise. Therefore, under high light irradiance, plants of WS had more chlorophyll content to support the higher rate of photosynthesis [5].

Acknowledgement

We would like to thank The University of Manchester, MARA, short-term grant (U652) UTHM and others who have helped in this project.

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