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Photosynthetic Responses of *Arabidopsis thaliana* Acclimation to Decreases in Growth Irradiance

[1]Furzani Paee and [2]Giles Johnson
[1]Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia
[2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Abstract: In order to investigate the photosynthetic responses of photoacclimation in *Arabidopsis thaliana*, photosynthetic capacity was measured in plants of the accession Wassilew Skija (WS) and in plants lacking expression of the gene At1g61800 (WS-gpt2) during acclimation from high to low light. Plants of WS and WS-gpt2 were grown for 6 weeks under high light (400 μmol.m^{-2}.sec^{-1}) and then half of them were transferred to low light (100 μmol.m^{-2}.sec^{-1}) to start the treatment. Gas exchange measurements were performed in order to measure the maximum capacity for photosynthesis. Meanwhile, chlorophy ll fluorescence analysis was carried out to measure changes in the quantum efficiency of PSII (ΦPSII) and Non-Photochemical Quenching (NPQ) during acclimation. Besides, a chlorophy ll composition analysis was performed to estimate the total chlorophyll and chl a/b ratio. Acclimation to a decrease in light resulted in a decrease in the photosynthetic capacity in WS and WS-gpt2 plants. Meanwhile, ΦPSII decreased in both WS and WS-gpt2 plants showing that under low light, PSII is more saturated. However, it was found that there were no significant changes in NPQ level for either WS or WS-gpt2. There were no significant changes in the total chlorophyll for both WS and WS-gpt2. However, the chlorophy ll a/b ratio was seen to be decreased in low light plants representing an increase in light harvesting complexes relative to reaction centre core. It is concluded that acclimation from high to low light is not a simple reversal of acclimation from low to high light but is mechanistically distinct process.

Key words: Photosynthesis, Arabidopsis, acclimation, GPT2, light, chlorophy ll fluorescence, ΦPSII, NPQ, chlorophy ll, WS

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 acclimating to changing conditions. 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 (Wetzel *et al*., 2009).

Changes in irradiance for example could lead to harmful effects on plants. Therefore, plants 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) (Ballare, 1999). In the low to high light condition (Athanasiou *et al*., 2010) found that the maximum Photosynthetic capacity (P_{max}) showed a significant increase in the *Arabidopsis thaliana* WS ecotype and also a significant increase in the chl a/b ratio which marked a clear acclimation response. Therefore, these findings posed a question whether the reverse process in high to low light condition would also, give reversible result? In order to answer this question, the ecotypes of Arabidopsis used were the wild type, Wassilew Skija (WS) and WS-gpt2. Meanwhile, the WS-gpt2 plants were found to be not acclimating in the low to high light plants as it was found that gpt2 gene is essential in higher light intensity acclimation. As a result, the WS-gpt2 was also included in this high to low light experiment to investigate if the gpt2 gene is also, responsible and essential in lower light intensity acclimation.

MATERIALS AND METHODS

Plant materials: Wild type seeds of Wassilew Skija-2 (WS) and mutant seeds of WS-gpt2 were sown onto soil

Corresponding Author: Furzani Paee, Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

and then placed at 4°C for 2 days before being transferred to 20°C at low light (100 μmol.m^{-2}.sec^{-1}). The seedlings were left in low light for 7 days before being transferred to high light (400 μmol.m^{-2}.sec^{-1}). After germination, plants were grown in a growth cabinet (EJ Stieel, Glasgow, UK) with light being provided by high frequency fluorescent lamps. The plants were put under High Light (HL) condition (400 μmol.m^{-2}.sec^{-1}) for 6 weeks. All plants were grown under 8 h light at 20±2 °C and 16 h dark at 16±2°C. After 6 weeks, half of the plants were transferred to Low Light (LL) (100 μmol.m^{-2}.sec^{-1}). Control plants were kept at 400 μmol.m^{-2}.sec^{-1}.

Light response curve measurement: Photosynthesis measurements were carried out using a CIRAS 1 portable infra-red gas analyser (PP systems, Amesbury, MA, USA). In order to determine which light intensity is sufficient to saturate photosynthesis, a light response curve was measured. After 9 days of exposure to LL, the photosynthetic rate of plants was measured at 100, 200, 400, 800, 1200 and 1600 μmol.m^{-2}.sec^{-1}. Plants were illuminated at 1600 μmol.m^{-2}.sec^{-1} for the first 20 min and continued for 5 min at different light intensities which were at 100, 200, 400, 800 and 1200 μmol.m^{-2}.sec^{-1}. Measurements were performed at a CO_2 concentration of 2000 ppm. The actinic light used was provided by a red Luxeon LXHL-LD3C LED (Philips Lumileds, California) in a laboratory built lamp.

Photosynthetic capacity measurement: The maximum capacity for photosynthesis was measured as the rate of photosynthesis at 1500 μmol.m^{-2}.sec^{-1} light and at 20°C. Measurements were carried out at 2000 ppm CO_2. Immediately after the plant was removed from the growth cabinet 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.

Chlorophyll fluorescence measurement: Simultaneous to the photosynthetic capacity measurements, chlorophyll fluorescence analysis was performed using a PAM 101 chlorophyll fluorometer (Walz, Effeltrich, Germany). 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). 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}.sec^{-1} to determine the value of F_m. Afterwards, actinic light at 1500 μmol.m^{-2}.sec^{-1} was given for the next 20 min. 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 Eq. 1 and 2.

$$\text{ØPSII} = \frac{\left(F_m' - F_t\right)}{F_m'} \quad (1)$$

$$\text{NPQ} = \frac{\left(F_m' - F_m'\right)}{F_m'} \quad (2)$$

Chlorophyll extraction: Following 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 was centrifuged using a microfuge (Progen) at full speed (16,000 g) for 5 min. The absorbance of the supernatant was measured using a USB2000 spectrophotometer (Ocean Optics, Dunedin, USA) and the absorbance value at 646.6, 663.5 and 750 nm were recorded. The chlorophyll content was calculated according to Porra *et al.* (1989) as shown in Eq. 3 and 4.

$$\text{Chl a}\left(\frac{\text{ng}}{\text{cm}^2}\right) = \frac{13.71\,(A_{663.5\text{-}750}) - 2.85\,(A_{646.6\text{-}750}) \times 10\ \text{mL}}{\text{Leaf area (cm}^2)} \quad (3)$$

$$\text{Chl b}\left(\frac{\text{ng}}{\text{cm}^2}\right) = \frac{22.39\,(A_{646.6\text{-}750}) - 5.42\,(A_{663.5\text{-}750}) \times 10\ \text{mL}}{\text{Leaf area (cm}^2)} \quad (4)$$

Statistical analyses: A data management software and statistics package SPSS V15 (IBM Inc. Chicago, Illinois, USA) was used to conduct a statistical analysis on the data. To test the data significance, a simple t-test and one-way ANOVA analysis where appropriate were carried out. The one-way ANOVA result was then followed with a Tukey's post hoc test with a significance level at 0.05.

RESULTS AND DISCUSSION

Light intensity and determination from light response curve in WS: A light response curve was measured on the control (HL) and treated (LL) plants of WS. The actual rate of photosynthesis, PSII efficiency (Φ PSII) and

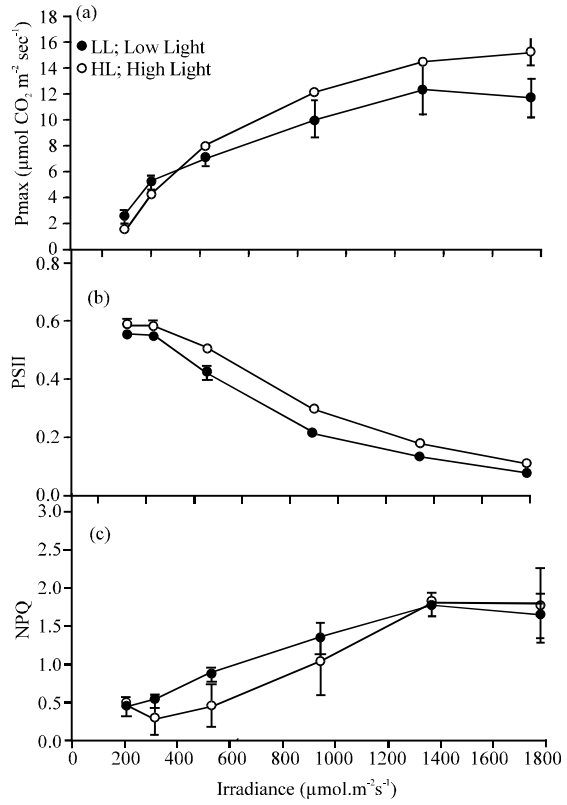

Fig. 1: A light response curve of WS: a) Maximum photosynthetic capacity of WS against irradiance in plants grown at 400 μmol.m^{-2}.sec^{-1} (High Light; HL; open circle) for 6 weeks after which half were transferred to low light at 100 μmol.m^{-2}.sec^{-1} (LL; black circle) for 7 days; b) The maximum quantum efficiency (PSII) of WS and c) Non-Photochemical Quenching (NPQ) of WS. All data are mean±SE for at least 5 biological replicates

Non-Photochemical Quenching (NPQ) were measured at different range of irradiances. At lower irradiance (100 and 200 μmol.m^{-2}.sec^{-1}) the LL plants had a higher photosynthetic rate compared to the HL plants (Fig. 1a). However, as the intensity increased, the HL plants increased their photosynthetic rate up to a point until it started to saturate at 1500 μmol.m^{-2}.sec^{-1}. In view of these data, a light intensity at 1500 μmol.m^{-2}.sec^{-1} was used for subsequent experiments as a saturating irradiance. Measurement of ΦPSII indicated that PSII is more efficient in utilizing the absorbed light for photochemistry processes at lower irradiance (Fig. 1b). As the irradiance increased, the PSII efficiency decreased. This is due to PSII reaction centres becoming saturated at higher light intensity. Meanwhile, the NPQ measurement increased when the irradiance increased (Fig. 1c).

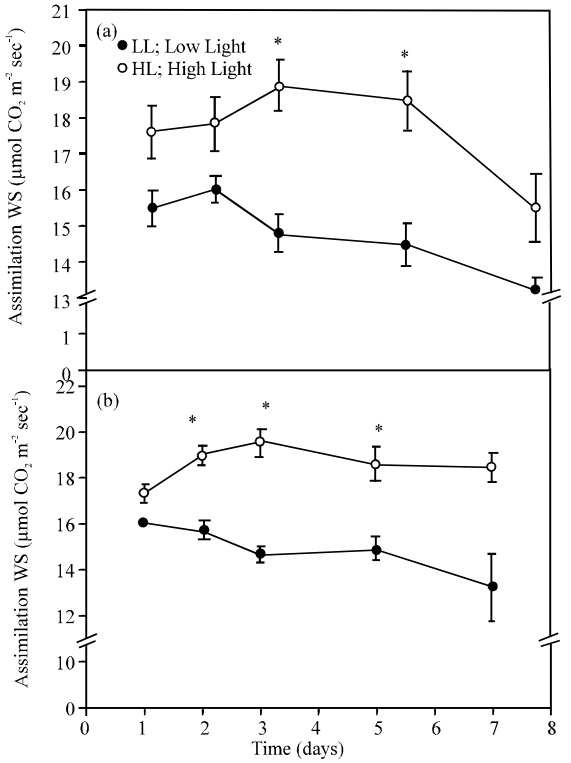

Fig. 2: A time-course acclimation of maximum photosynthetic capacity in a) WS and b) WS-gpt2. Plants were measured at different times following a transfer from HL-LL

Changes in maximum photosynthetic capacity in WS and WS-gpt2 during acclimation following transfer from high to low light: To investigate the changes in the maximum photosynthetic capacity during the acclimation process further, a time-course experiment was conducted by measuring WS plants at different times upon transfer to LL. Besides that microarrays have identified that GPT2 gene as the most up-regulated in arabidopsis leaves transferred from low to high light and it was shown that this is essential for dynamic acclimation to increased light (Athanasiou *et al*., 2010). Thus, to test whether GPT2, also, plays a role in acclimation from high to low light, plants of WS-gpt2 grown at HL and transferred to LL were analysed in terms of their photosynthetic capacity.

Both HL and LL plants of WS showed changes in Pmax through the experiment (Fig. 2a). The LL plants showed significant changes in Pmax starting on day 3 ($p<0.05$) compared to the HL. Therefore, WS plants had the ability to change their photosynthetic capacity when the growth condition was altered to low light. Both HL and LL plants of WS-gpt2 showed changes in P_{max}

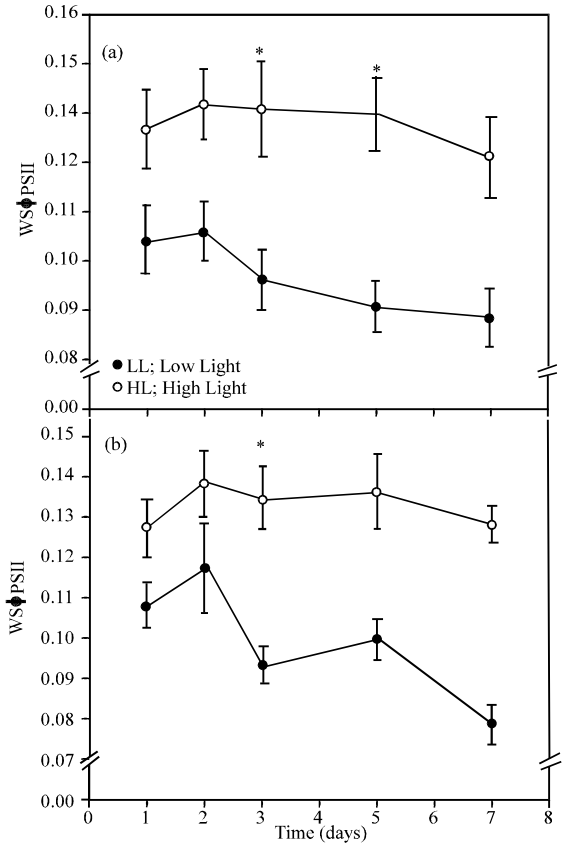

Fig. 3: A time-course acclimation of Photosystem II (PSII) efficiency (ΦPSII) in a) WS and b) WS-gpt2

(Fig. 2b) and ΦPSII (Fig. 3b) starting at day 1 showing that WS-gpt2 plants can acclimate to lower light. By the end of the acclimation period, changes between WS-gpt2 HL and LL plants were seen to be greatest in the maximum photosynthetic capacity and ΦPSII. At the same time, plants of WS HL and LL showed differences in the Φ PSII with the HL plants having a higher Φ PSII value (Fig. 3a). The NPQ was measured to give an idea on how much heat was dissipated in both HL and LL plants. The value of NPQ decreased towards day 7 in both WS and WS-gpt2 (Fig. 4a and 4b).

Photosynthetic capacity in WS using mature leaves was found to be decreased in low light plants. Initially, plants were grown under high light which is available for conducting their photochemistry processes. However, when light becomes a limiting factor (under low light condition) there is a limited energy to drive the photochemistry processes optimally. Since, photosynthesis is a light-dependent reaction, insufficient light limits the overall rate of photosynthesis.

When (Athanasiou *et al*., 2010) measured changes in the photosynthetic capacity following acclimation from

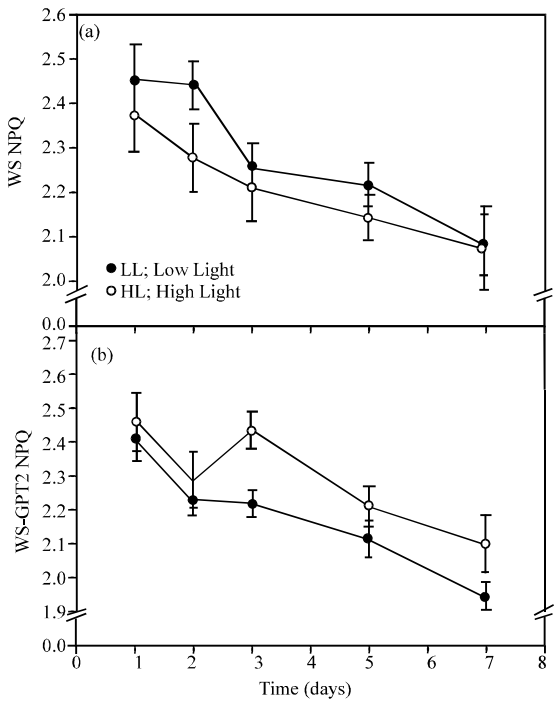

Fig. 4: A time-course acclimation of Non-Photochemical Quenching (NPQ) in a) WS and b) WS-gpt2

low to high light it was found that there was an increase in the arabidopsis accession WS. The extent of change and the values of photosynthetic rate obtained were similar to those seen here in plants transferred from HL to LL, suggesting the processes might simply be the reversal of one another. However, whilst the WS-gpt2 plants did not acclimate when moved from low to high light they did acclimate when transferred from HL-LL. The gpt2 mutants were complemented with a functional gene of gpt2 and it was found that it restored the ability to acclimate. Therefore, it was concluded that GPT2 is essential for acclimation from low to high light (Athanasiou *et al*., 2010). In the acclimation from high to low light, WS-gpt2 plants had the ability to acclimate. This suggests that the acclimation from high to low light in WS-gpt2 is partially but not completely inhibited.

Since, the low light plants have a larger antenna they possess more chlorophylls per reaction centre, so, the rate at which light energy arrives at the reaction centre is faster at any given light intensity. This indicates that reaction centres work more efficiently at low light but they are more vulnerable to an oversaturation of PSII. When PSII is oversaturated, the electron transport will be less efficient. PSII will also, be more vulnerable to photoinhibition. As a result, CO_2 fixation will be

decreased. The observation that ΦPSII is lower in low light acclimated plants is consistent with the idea that the antenna size of PSII increases when plants acclimate to low light. There is however, also, a decrease in overall photosynthetic capacity at low light. Previously, Athanasiou (2008) and Athanasiou *et al.* (2010) observed no consistent changes in ΦPSII during low to high light acclimation, suggesting that acclimation from high to low light is not simply the reverse of acclimation from low to high.

When plants are exposed to excess light, one of the short-term responses is Non-Photochemical Quenching (NPQ). This responseis switched on within seconds after the light exposure. When a low pH builds up in the thylakoid lumen it switches the antenna into heat dissipation rather than trying to utilize the excess light (Kulheim *et al.*, 2002). From the data (Fig. 4a, b, respectively), it shows that the low light acclimated plants in both WS and WS-gpt2 had no difference in the NPQ value indicating that both WS and WS-gpt2 plants had the same capacity to quench excitation energy under low light condition. Previously in the reverse acclimation to high light, no significant changes were found in terms of ΦPSII and NPQ (Athanasiou, 2008).

Plants were measured at different times following a transfer from HL to LL. Plants were grown at 400 $\mu mol.m^{-2}.sec^{1}$ (High Light; HL; open circle) for 6 weeks and half were transferred to low light at 100 $\mu mol.m^{-2}.sec^{ec-1}$ (LL; black circle). Plants were measured simultaneously with the maximum photosynthetic capacity measurement at an actinic light of 1500 $\mu mol.m^{-2}.sec^{-1}$ and CO_2 concentration at 2000 ppm. All data are mean±SE for at least 5 biological replicates. *p<0.05 compared to normal control (HL).

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Changes in chlorophyll content and composition during acclimation to low light in WS and WS-gpt2: Chlorophyll content analysis was performed to calculate the total chlorophyll and chl a/b ratio according to Porra *et al.* (1989). In terms of total amount of chlorophyll (chlorophyll a and b) there were no significant change

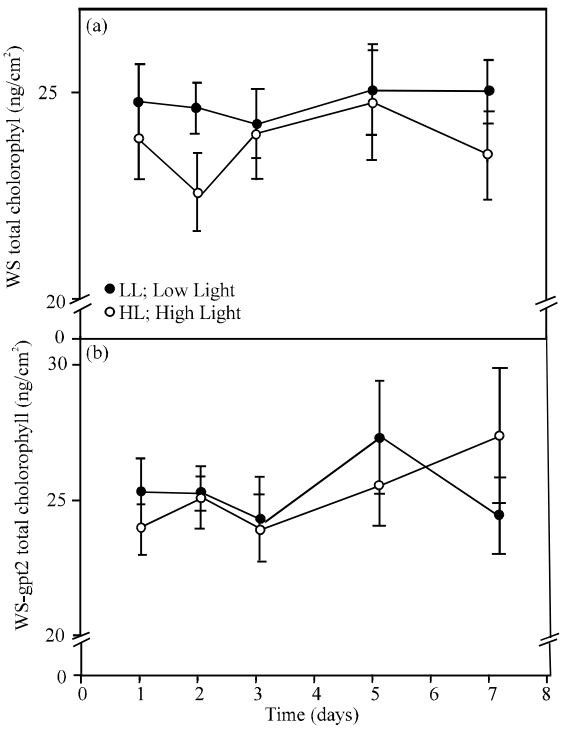

Fig. 5: Total chlorophyll content of a) WS and b) WS-gpt2 in a time-course experiment

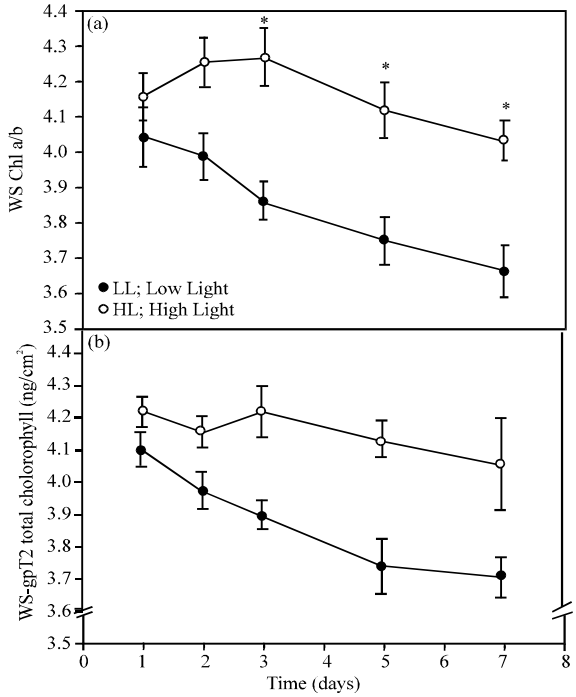

Fig. 6: Chl a/b of a) WS and b) WS-gpt2 in a time-course experiment

observed during acclimation to low light in both WS and WS-gpt2 plants (Fig. 5a, b). Meanwhile, the chl a/b ratio in this experiment showed a decrease over the week in LL plants as shown in Fig. 6a. The chl a/b ratio in WS-gpt2 plants showed significant ($p<0.05$) changes when measured during the acclimation period of 7 days (Fig. 6b). On the other hand, there was no significant change in chlorophyll content upon acclimation from low to high light (Athanasiou *et al*., 2010).

In many species, depending on the light condition, differences in chl a/b ratio have frequently been reported (Moharekar *et al*., 2007; Pantaleoni *et al*., 2009). Hence, it has been taken as an indicator of a simple light acclimation response (Ioannidou *et al*., 2004). In this study it was found that the chl a/b ratio decreased in plants transferred from high to low light, compared to plants kept in high light (Fig. 6a, b). This is most likely due to an increase in the light harvesting complexes relative to reaction center core. Reaction center cores contain only chlorophyll a. Associated with the reaction centers are the light harvesting complex which contain both chlorophyll a and b. Thus, the expansion of the complexes results in an increase in chlorophyll b and decrease in the chl a/b ratio in low light plants. The ability of plants to change the amount of light harvesting complexes has been claimed to determine the plant's ability to change in response to light environment (Ioannidou *et al*., 2004). In contrast, acclimation from low to high light resulted in only very marginal changes in chl a/b, suggesting that this form of acclimation involved only small changes in antenna size.

This is consistent with the observation that ΦPSII changes markedly during high to low but not low to high acclimation and reinforces the notion that these forms of acclimation are at least somewhat distinct processes.

The total chlorophyll content was calculated at different times after transfer to LL. Plants were grown for six weeks at 400 $\mu mol.m^{-2}.sec^{-1}$ (High Light; HL; open circle) and then half of the plants were transferred to a lower light intensity at 100 $\mu molm^{-2}.sec^{-1}$ (LL; black circle). The leaf used for maximum photosynthetic capacity measurement was used to estimate the chlorophyll content. The total chlorophyll content were calculated according to Porra *et al*. (1989). All data are mean ± SE for at least 5 biological replicates.

The chl a/b was calculated at different times after transfer to LL. Plants were grown for 6 weeks at 400 $\mu mol.m^{-2}.sec^{-1}$ (High Light; HL; open circle) and then half of the plants were transferred to a lower light intensity at 100 $\mu mol.m^{-2}.sec^{-1}$ (LL; black circle). The leaf used for maximum photosynthetic capacity measurement was used to estimate the chlorophyll content. The chl a/b were calculated according to Porra *et al*. (1989). All data are mean±SE for at least 5 biological replicates. *$p<0.05$ compared to normal control (HL).

CONCLUSION

The dynamic acclimation to light in arabidopsis was tested by growing WS plants under HL condition and transferring the plants to LL plants. Besides WS, plants lacking GPT2 expression in the background of WS (WS-gpt2) were also, grown to verify its ability to acclimate to LL condition. The acclimation responses to LL can be seen as early as day 1 upon transfer to low light. Similarly Athanasiou *et al*. (2010) observed that a small extent of acclimation occurred within the first day at HL in WS-gpt2. Therefore, it was concluded that arabidopsis of WS accession can photo acclimate when the light was increased and decreased. However, GPT2 was found to be non-essential in a decreasing light acclimation but essential in an increasing light acclimation. In a conclusion, this research was done in a hope to get a better understanding on how plants cope with changing conditions, especially, during this global warming issues. It is crucial for crop plants to find solution to survive during this harsh environment as crop plants are needed by the world population.

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