



TITLE:

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CITATION:

Maai, Eri ...[et al]. Light stress-induced chloroplast movement and midday depression of photosynthesis in sorghum leaves. *Plant Production Science* 2020, 23(2): 172-181

ISSUE DATE:

2020

URL:

<http://hdl.handle.net/2433/255884>

RIGHT:

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To cite this article: Eri Maai, Kazusa Nishimura, Rihito Takisawa & Tetsuya Nakazaki (2020) Light stress-induced chloroplast movement and midday depression of photosynthesis in sorghum leaves, *Plant Production Science*, 23:2, 172-181, DOI: [10.1080/1343943X.2019.1673666](https://doi.org/10.1080/1343943X.2019.1673666)

To link to this article: <https://doi.org/10.1080/1343943X.2019.1673666>



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Published online: 08 Oct 2019.



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# Light stress-induced chloroplast movement and midday depression of photosynthesis in sorghum leaves

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## ABSTRACT

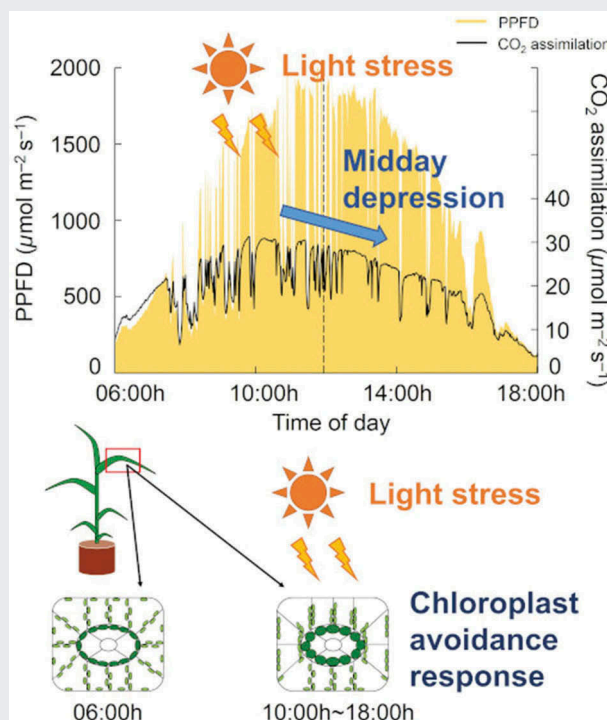
Plants are exposed to high light intensity, high leaf temperatures and high air-to-leaf water vapor pressure deficit (ALVPD) during the day. These environmental stresses cause stomatal closure and photoinhibitory damage, leading to midday depression of photosynthesis. Chloroplast positioning is essential for the efficient operation of photosynthesis. However, chloroplast behavior before, during, and even after the midday depression of photosynthesis remains unknown. We investigated changes in the intracellular positioning of chloroplasts and photosynthetic traits under a diurnal pattern of light. Sorghum leaves were exposed to a 12-h regime of light mimicking the natural light environment, with constant leaf temperature and ALVPD. Net photosynthetic rate ( $P_n$ ) showed a diurnal pattern, and midday depression in  $P_n$  was observed at 3.8 h of irradiation. Depression in  $P_n$  was attributed to stomatal limitation because the decrease in  $P_n$  was in accordance with the decrease in stomatal conductance. The maximum efficiency of photosystem II decreased with the increase in light intensity and remained low after 12 h of irradiation. Bundle sheath chloroplasts swelled after 8 h of irradiation, representing the accumulation of starch. Conversely, mesophyll chloroplasts exhibited avoidance response after 4 h of irradiation, and the avoidance position was maintained during the remainder of the daytime. These data suggest that chloroplasts are subject to light stress during and after the midday depression of photosynthesis. The intensity of natural light is excessive for most of the day and this light stress induces chloroplast avoidance response and depression of photosynthesis.

## ARTICLE HISTORY

Received 1 August 2019  
Revised 3 September 2019  
Accepted 18 September 2019

## KEYWORDS

Chloroplast movement; high light; mesophyll; midday depression of photosynthesis; photoinhibition; sorghum



## Introduction

The chloroplast is the site of photosynthesis, the process which converts sunlight to chemical energy in the form of energy-rich carbohydrates. Although exposure to sunlight is essential for optimal photosynthetic performance, excess light energy can cause photoinhibition of photosynthesis. Chloroplast avoidance response is considered to be one of the protective responses that mitigate photoinhibitory damage, in which chloroplasts move toward cell walls parallel to light direction under high light intensity (Haupt & Scheuerlein, 1990; Kasahara et al., 2002; Park, Chow & Anderson, 1996; Wada, Kagawa & Sato, 2003). Light-induced chloroplast movement is widely observed in various plant species, from green algae to seed plants (Suetsugu & Wada, 2012). Previous studies have found that chloroplasts of  $C_4$  plants such as finger millet and sorghum also rearrange their intracellular positions in response to light (Maai et al., 2011; Taniguchi, Taniguchi, Kawasaki & Miyake, 2003; Yamada, Kawasaki, Sugiyama, Miyake & Taniguchi, 2009).  $C_4$  plants have two types of photosynthetic cells, mesophyll (M) and bundle sheath (BS) cells, which contain functionally differentiated chloroplasts. A difference in the M chloroplast response under high light intensity among  $C_4$  species has been identified: M chloroplasts in maize move toward cell walls parallel to light direction whereas those in finger millet aggregate to the BS side (Maai et al., 2011; Yamada et al., 2009). The former chloroplast response is analogous to the avoidance response and the latter is considered to be a specific response in some  $C_4$  species. Kasahara et al. (2002) reported that *Arabidopsis* mutants deficient in chloroplast avoidance response are more susceptible to damage under high light intensity than wild-type plants, and that prolonged overexposure to light causes severe cell damage. Thus, chloroplast avoidance response is regarded as an important response for plants to survive in the natural environment.

In the natural environment, plants often experience high light levels, high temperatures and high vapor pressure deficits. These environmental stresses not only affect the severity of photoinhibition (Barber & Andersson, 1992; Medina, Souza, Machado, Ribeiro & Silva, 2002) but also reduce the photosynthetic rate of plants during midday (Schulze, Lange, Evenari, Kappen & Buschbom, 1980; Tenhunen, Percy & Lange, 1987). During the midday period when leaf temperatures and air-to-leaf water vapor pressure deficits (ALVPD) are greatest, stomata close to minimize water loss (Heroult, Lin, Bourne, Medlyn & Ellsworth, 2013; Iio, Fukasawa, Nose & Kakubari, 2004; Lange, Losch, Schulze & Kappen, 1971; Mott & Parkhurst, 1991). In addition, stomatal closure is also induced by

excessive light stress via the abscisic acid pathway (Devireddy, Zandalinas, Gomez-Cadenas, Blumwald & Mittler, 2018; Galvez-Valdivieso et al., 2009). Stomatal closure restricts  $CO_2$  uptake and decreases the photosynthetic rate by decreasing  $CO_2$  availability for the carbon fixation reaction involving ribulose 1,5-bisphosphate carboxylase (Crafts-Brandner & Salvucci, 2000; Salvucci & Crafts-Brandner, 2004). Thus, stomatal closure is regarded as a major physiological factor responsible for midday depression of photosynthesis (Hirasawa, Iida & Ishihara, 1989; Iio et al., 2004; Muraoka, Tang, Koizumi & Washitani, 1997). It is thought that stomatal closure raises susceptibility to photoinhibitory damage (Powles, 1984). Photoinhibitory damage generally represents inactivation of photosynthetic reactions associated with photosystem II (PSII) (Demmig-Adams & Adams, 1992; Powles, 1984). Serious damage to PSII often brings about a decrease in the light-saturated rate of  $CO_2$  assimilation (Tenhunen et al., 1987). Therefore, photoinhibitory damage is also responsible for the midday depression of photosynthesis.

To date, midday depression in photosynthesis has been commonly observed in many land plants including rice (Hirasawa, Tsuchida & Ishihara, 1992), maize (Hirasawa & Hsiao, 1999) and citrus species (Hu, Guo, Shen, Guo & Li, 2009; Jifon & Syvertsen, 2003; Medina et al., 2002). These studies focused mainly on physiological characteristics and did not mention the intracellular positioning of chloroplasts despite the potentially significant role that could play under light stress conditions. Chloroplast avoidance response can be observed under continuous high light ( $>160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of white light;  $>100 \mu\text{mol m}^{-2} \text{s}^{-1}$  of blue light) (Gotoh et al., 2018; Maai et al., 2011), though there is some variability depending on the plant species and growth environment (Higa & Wada, 2016). The light intensities referred to are lower than the maximum photosynthetic photon flux density (PPFD) in the summer over much of the Earth's surface, meaning that sunlight intensity is high enough to induce chloroplast avoidance response. Thus, it is assumed that excessive light during the daytime will cause chloroplast avoidance response. Williams, Gorton and Witiak (2003) investigated diurnal changes in light transmittance in leaves of *Alocasia* as an indicator of chloroplast movements in the field and found that there was an increase in light transmittance during the day, which indicated the occurrence of chloroplast avoidance response. However, the detailed intracellular positioning of chloroplasts and the diurnal responses of photosynthetic parameters remain unknown.

Since chloroplast positioning is essential for the efficient operation of photosynthesis and contributes to the protection of the photosynthetic apparatus against excess energy, it would be advantageous to know chloroplast

positioning before, during, and even after the midday depression of photosynthesis. The aim of this study was to reveal chloroplast behavior induced by light stress and its relationship with photosynthesis over the course of a day. We examined changes in the intracellular positioning of chloroplasts, and physiological traits such as gas exchange and chlorophyll fluorescence, with exposure to a diurnal regime of light that mimicked the natural light environment.

## Materials and methods

### Plant material and growth condition

Sorghum (*Sorghum bicolor* L. 'Meter Sorgo', Takii, Kyoto, Japan) seeds were sown in 8 cm diameter × 8 cm tall plastic pots filled with a mixture of vermiculite and a commercial substrate (Ikubyo baido, Takii) (1:1 v/v) and were grown for 3 weeks in a growth chamber under a 14-hr photoperiod at a PPFD of 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 28°C/20°C (day/night). The middle regions of the uppermost fully developed leaves were used for the experiments. Before the experiments, plants were kept in darkness for at least 6 h in order to exclude the effect of the preceding light exposure.

### Treatment with a short-term continuous light

The leaf was irradiated with a light-emitting diode (LED) lamp (LI-6400-02B, LI-COR, Lincoln, NE, USA) at PPFD of 200 or 1,200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 1 h. The lamp was composed of 665-nm red LEDs and 470-nm blue LEDs (maximum peak) in the ratio 9:1. Small segments (5 × 5 mm square) were excised from the treated leaf blade and fixed in 3% (v/v) glutaraldehyde in 50 mM sodium phosphate buffer (pH 6.8) at 4°C overnight. The fixed leaf segments were embedded into Super Cryo Embedding Medium (SCEM, Section Lab, Japan) such that the cutting plane for the region of interest was parallel with the bottom of the SCEM, and then frozen in dry ice-chilled hexane. The embedded blocks were sliced at 8–10  $\mu\text{m}$  thickness with a cryostat (CM3050S, Leica BioSystems, Germany) at –20°C, according to the procedure of Kawamoto (2003). Images of transverse sections were acquired with a light microscope (BX51, Olympus, Tokyo, Japan) on a charge-coupled device camera (DP70, Olympus).

### Treatment with a natural regime of light

The leaf was irradiated with an LED lamp (LI-6400-02B, LI-COR). Incident PPFD, supplied as natural sunlight to the leaf surface, was minutely reproduced from a natural light

regime recorded at the Experimental Farm of Kyoto University (34°44'N, 135°50'E) from 06:00 h to 18:00 h on a sunny day in August. The highest PPFD supplied by the lamp was adjusted to 2,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  so that it did not exceed the recorded *in situ* incident PPFD (2,002  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 10:53 h, 2,036  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 11:44 h and 2,019  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 11:45 h) due to the performance limit of LEDs.

After 0, 4, 8, and 12 h treatment, leaf segments were excised and fixed as described above. Transverse sections were observed with the light microscope. The number of BS chloroplasts per cell was counted. The transverse surface areas of the chloroplasts and the cell (3 BS cells per leaf section) were defined using the Easy PCC software (Guo et al., 2017), and the proportion of the total surface area of BS chloroplasts to BS cell surface area was calculated.

### Gas exchange and chlorophyll fluorescence during a natural regime of light

During the treatment with a natural regime of light, net photosynthetic rate ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) were recorded at 1-min intervals using a portable photosynthesis system (LI-6400, LICOR) (von Caemmerer & Farquhar, 1981). Leaf temperature and ALVPD were also calculated with the LI-6400 system. Measurements were conducted at 28°C with a relative humidity of 50–70% and an ambient  $\text{CO}_2$  concentration of 350–400  $\mu\text{mol mol}^{-1}$ . The flow rate was maintained at 500  $\mu\text{mol s}^{-1}$ .

To determine the maximum quantum efficiency of PSII, chlorophyll fluorescence was measured with a portable chlorophyll fluorometer Fluor Pen (FP110/S, Photon Systems Instruments, Brno, Czech Republic). The chlorophyll fluorescence parameter represents the maximum efficiency of PSII photochemistry. In general, the maximum efficiency of PSII is determined by measuring in the dark-adapted state after at least 30 min dark-adaptation. Meanwhile, it is possible that chloroplasts in  $C_4$  plants rearrange their intracellular positions in 30 min (Kobayashi et al., 2009). Chloroplast positions after short-term dark adaptation would be closer to those just after the light treatment. For this reason, after 0, 4, 8, and 12 h treatment, leaves were kept in the dark for 5 min and then the maximum efficiency of PSII (termed as  $F_v/F_m$ ) was measured. The degree of photoinhibition was evaluated from the maximum efficiency of PSII during the treatments relative to the value before treatments. Measurement of leaf gas exchange and subsequent chlorophyll fluorescence were conducted independently from observation of chloroplast arrangements.

## Statistical analysis

Statistical analyses of data were based on Dunnett tests. Calculations were performed on three independent biological replicates. For all tests, differences obtained at the level of  $P < 0.05$  were considered to be significant. Percentage data were subjected to arcsine transformation prior to statistical analysis.

## Results

### Chloroplast movement under continuous light

In sorghum leaves, chloroplasts in M cells randomly locate along the cell membranes while those in BS cells distribute centrifugally to the vascular tissue. To examine chloroplast response to light stress, sorghum leaves were irradiated with normal intensity light ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or high-intensity light ( $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 1 h. When leaves were exposed to normal intensity light, no rearrangement of the chloroplasts was observed (Figure 1(a)). By contrast, when leaves were irradiated with high-intensity light, M chloroplasts distributed to the anticlinal walls, parallel to the direction of the incident light (Figure 1(b)). This response of M chloroplasts is an avoidance response observed in many land plants under high-intensity light. BS chloroplasts did not change their intracellular positions under either light condition (Figure 1).

### Diurnal changes in photosynthetic parameters and chlorophyll fluorescence

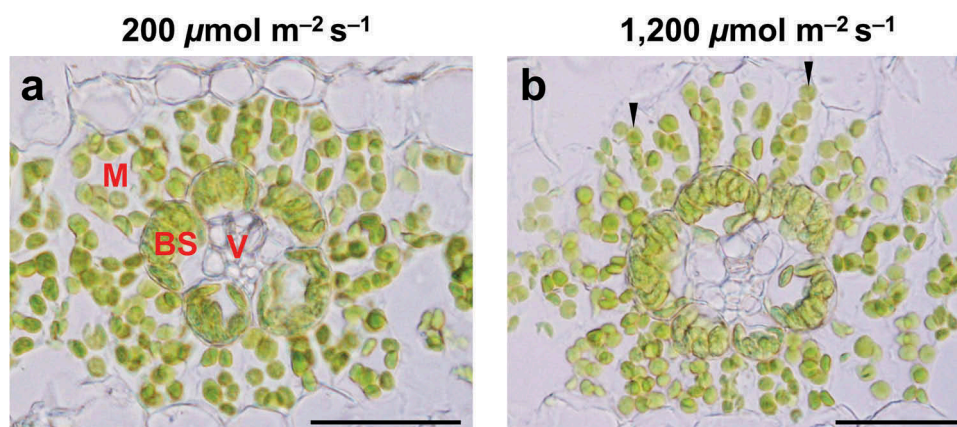
Leaves were subjected to a diurnal pattern of light intensity mimicking the natural light environment from 06:00 h to

18:00 h (Figure 2(a)). The maximum intensity of the incident light was  $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  at 4.9 h and 5.7 h, and the average intensity of the incident light was  $988 \mu\text{mol m}^{-2} \text{s}^{-1}$ . During the experimental period, both  $P_n$  and  $g_s$  responded rapidly to sudden fluctuations in light intensity.  $P_n$  was low at the beginning of irradiation, increasing with time to a maximum.  $P_n$  then decreased from the peak value at 3.8 h ( $31.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Figure 2(b)).  $g_s$  values corresponded closely to  $P_n$  and decreased from the peak values at 3.8 h ( $0.237 \text{ mol m}^{-2} \text{s}^{-1}$ ) (Figure 2(c)). Leaf temperature and ALVPD remained relatively constant during the treatment at  $25.7^\circ\text{C} - 27.9^\circ\text{C}$  and  $0.99 - 1.11 \text{ kPa}$ , respectively (Figure 2(d,e)). There was a strong positive correlation between  $P_n$  and  $g_s$  ( $r^2 = 0.9196$ ; Figure 2(f)).  $P_n$  values measured over a range of PPFD were almost always lower during hours 6 – 12 of the treatment than during hours 0 – 6 (Figure 3). The average value of  $P_n$  during 6 – 12 h treatment decreased by 13.2% compared to that during 0 – 6 h treatment.

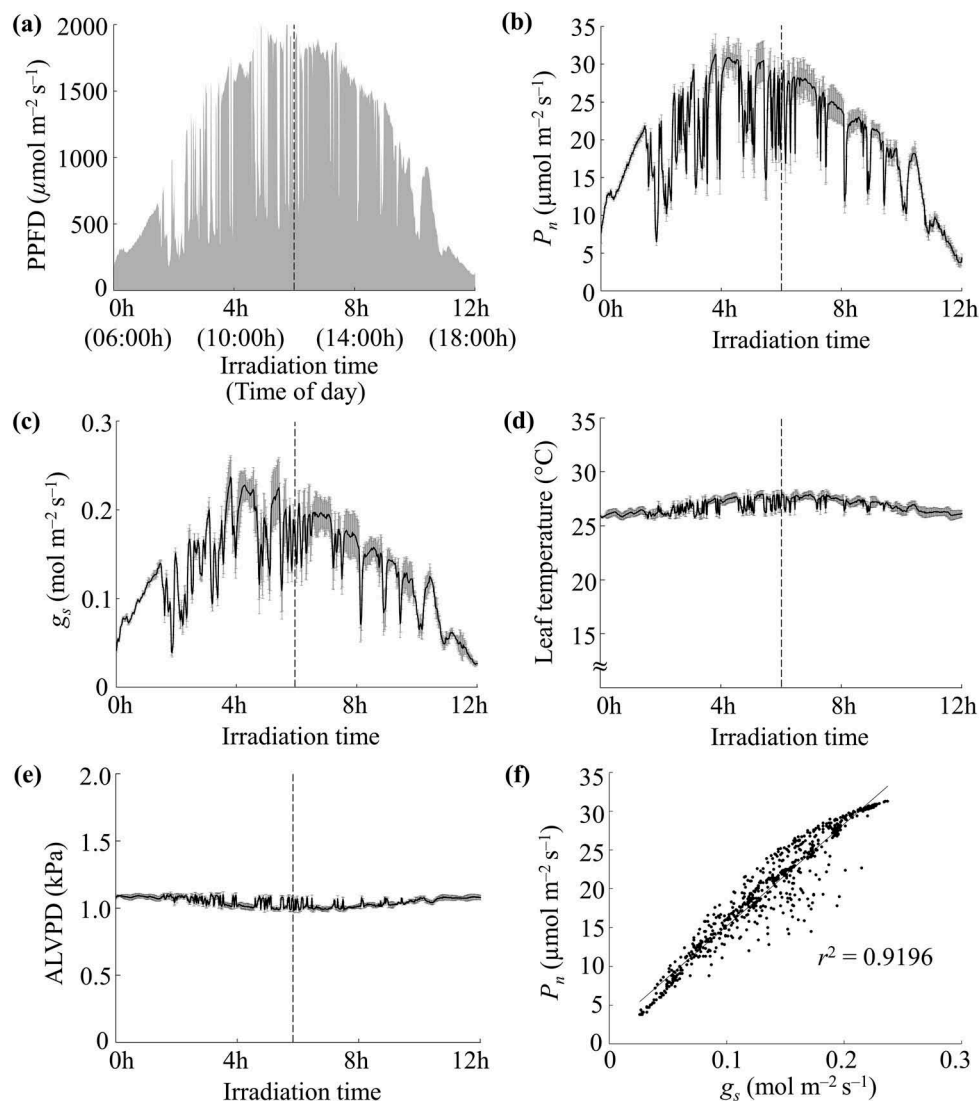
To evaluate the extent of photoinhibition, the maximum efficiency of PSII was measured at each time point of the light treatment. The efficiency value was 0.76 before treatment; afterward, it significantly decreased as PPFD increased (Figure 4). Although the efficiency value fell after 8 h treatment (0.55), it recovered to some extent after 12 h treatment (0.67).

### Diurnal changes in the positioning of chloroplasts

During the diurnal regime of light exposure, chloroplast positioning was periodically observed under the light microscope. Before the treatment, chloroplasts in M cells randomly located along the cell membranes whereas those in BS cells located in a centrifugal position (close



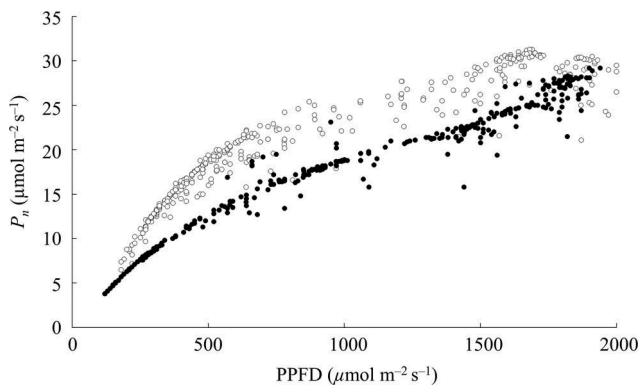
**Figure 1.** Effect of light intensity on the intracellular position of mesophyll and bundle sheath chloroplasts in sorghum. Leaves were continuously irradiated for 1 h with normal intensity ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (a) or high intensity ( $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light. (b) The adaxial side of each leaf section (upper side in the photograph) was illuminated. Transverse sections were observed with a light microscope. Typical chloroplasts which distribute parallel to the direction of the incident light (the avoidance response) are indicated by arrowheads. Scale bars =  $50 \mu\text{m}$ . BS, bundle sheath cell; M, mesophyll cell; V, vascular bundle.



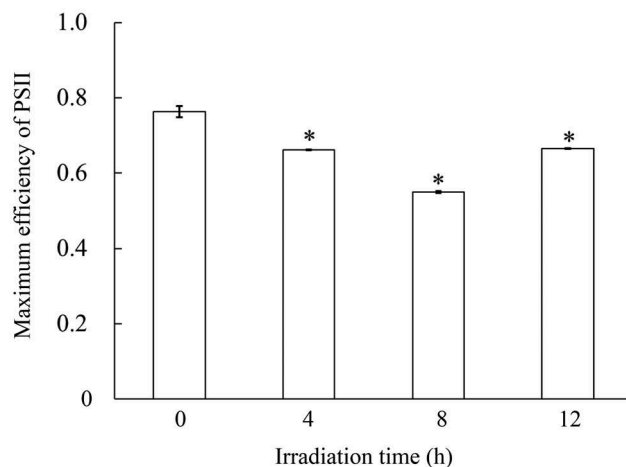
**Figure 2.** Diurnal measurements of gas exchange parameters under a natural light regime. Dotted lines in (a – e) indicate the middle time point of the experimental period. (a) The light regime for diurnal light treatment based on photosynthetic photon flux density (PPFD) recorded at Kyoto University on a sunny day in August. This PPFd data set from 06:00 h to 18:00 h was used to design the 12 h light irradiation treatments. The average light intensity during the treatment was  $988 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Net photosynthetic rate ( $P_n$ ) (b), stomatal conductance ( $g_s$ ) (c), leaf temperature (d) and air-to-leaf water vapor deficits (ALVPD) (e) were measured at 1-min intervals during the light treatment. The measurements were conducted at  $28^{\circ}\text{C}$  under ambient air of relative humidity 50%–70%. Error bars represent mean  $\pm$  SE,  $n = 3$ . (f) Relationship between  $P_n$  and  $g_s$  during the natural light regime. Results are the mean of three replicates per treatment.

to M cells) (Figure 5(a)). After 4 h treatment, soon after  $P_n$  reached maximum, we found M chloroplasts distributed to the anticlinal walls, parallel to the direction of the incident light (Figure 5(b)). This avoidance position of M chloroplasts continued until the end of the experimental period (Figure 5(c,d)). We could not decide whether the centrifugal position of BS chloroplasts was changed during the treatment because they swelled and covered the cell surface after 8 h and 12 h of the treatment (Figure 5(c,d)). The swelling of BS chloroplasts was confirmed by quantitative analysis of changes in the surface area of each BS chloroplast and the proportion of the total

surface area of BS chloroplasts to BS cell surface area (Table 1). During the treatment, there were no significant differences in the number of BS chloroplasts per cell and the cell surface area of a BS cell. Both the surface area of each BS chloroplast and the total surface area of BS chloroplasts per cell increased significantly after 8 h and 12 h treatment compared to that before treatment. The proportion of the total surface area of BS chloroplasts to BS cell surface area increased as irradiation time advanced, and there were significant differences in the proportion after 8 h and 12 h treatment compared to that before treatment. These results indicate that the



**Figure 3.** Relationship between net photosynthetic rate ( $P_n$ ) and incident photosynthetic photon flux density (PPFD) during the natural light regime shown in Figure 2(a). Data were collected either during 0 – 6 h (open symbols) or during 6 – 12 h (closed symbols) of the treatment. Results are the mean of three replicates per treatment.



**Figure 4.** Comparison of the maximum efficiency of photosystem II (PSII) during treatment with the natural light regime. Values are means  $\pm$  SE ( $n = 3$ ). Asterisks indicate statistically significant differences compared with  $t = 0$  according to Dunnett test ( $*P < 0.05$ ).

increased proportion of the total surface area of BS chloroplasts to BS cell surface area resulted from the increased total surface area of BS chloroplasts per cell due to chloroplast swelling.

## Discussion

### Response of chloroplasts under continuous light

When exposed to continuous high-intensity light ( $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), M chloroplasts redistributed to the anticlinal walls, parallel to the direction of the incident light (Figure 1(b)). This avoidance response of M chloroplasts was not observed under normal intensity light ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Figure 1(a)). These results indicate that

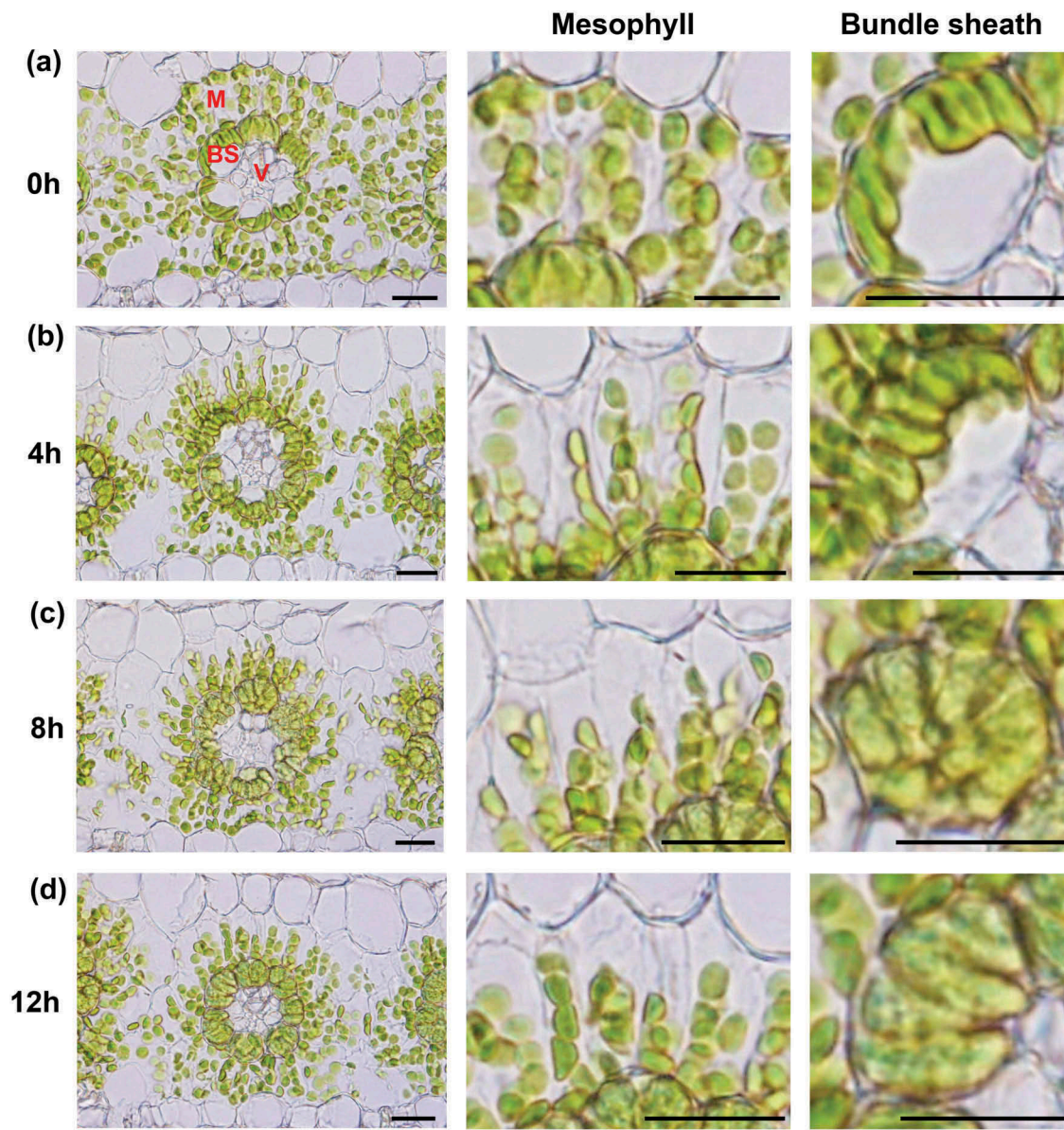
high-intensity light induces the avoidance response of M chloroplasts in sorghum as is the case in other plant species such as *Arabidopsis thaliana*. Sorghum is a  $C_4$  plant and its leaves typically contain two differentiated types of chloroplast, in M and BS cells. Intriguingly, only M chloroplasts changed their intracellular positions in response to high-intensity light. This result is consistent with previous studies exploring light-induced chloroplast movement in two other  $C_4$  species, finger millet and maize (Maai et al., 2011; Yamada et al., 2009). Yamada et al. (2009) pointed out that there was a difference in the direction of movement between those two species as follows. Under extremely strong light ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), finger millet chloroplasts moved toward the BS side without regard to the direction of the incident light while maize chloroplasts moved to the anticlinal walls, parallel to the direction of the incident light. Our result observed in sorghum M cells is similar to the latter response. The reason for this is unknown. Both maize and sorghum are classified as NADP-malic enzyme-type  $C_4$  plants whereas finger millet is an NAD-malic enzyme-type  $C_4$  plant (Gutierrez, Gracen & Edwards, 1974). Therefore, it is possible that chloroplast behavior in response to light varies with species or with  $C_4$  photosynthesis subtype.

### Response of chloroplasts and photosynthesis under a natural regime of light

During the regime of 12 h natural light, we found midday depression of photosynthesis.  $P_n$  and  $g_s$  showed a diurnal pattern and there was a positive correlation between these two photosynthetic parameters (Figure 2(b,c,f)). After the peak of  $P_n$  and  $g_s$  at 3.8 h of irradiation, they decreased against an increase in PPFD. Depression in  $P_n$  was attributed to stomatal limitation because the decrease in  $P_n$  was in accordance with the decrease in  $g_s$ . The average values of  $P_n$  over 0 – 6 h treatment were 13.2% lower than those during 6 – 12 h treatment. It has been reported that high light intensity, high leaf temperature and high ALVPD are important factors contributing to midday depression of photosynthesis (Chaves, Harley, Tenhunen & Lange, 1987; Correia, Chaves & Pereira, 1990; Kupperts, Wheeler, Kupperts, Kirschbaum & Farquhar, 1986; Raschke & Resemann, 1986; Roessler & Monson, 1985; Tenhunen et al., 1987). In our study, leaf temperature and ALVPD remained relatively constant while incident PPFD dynamically changed over the diurnal course of the treatment (Figure 2(a,d,e)). Therefore, it is suggested that midday depression in photosynthesis found in this experiment is attributable to high light rather than high leaf temperature or high ALVPD.

To the best of our knowledge, our study is the first report on a behavior of chloroplasts before and after midday depression of photosynthesis in  $C_4$  plants.





**Figure 5.** Diurnal changes in the intracellular positions of chloroplasts in sorghum under the natural light regime (see Figure 2(A)). Transverse sections were observed before (a), and 4 h (b), 8 h (c), and 12 h (d) after the start of light irradiation. The adaxial side of each leaf section (upper side in the photograph) was irradiated. Enlarged images of mesophyll and bundle sheath cells are shown in the center and the right side of each section photograph, respectively. Scale bars = 25  $\mu\text{m}$ . BS, bundle sheath cell; M, mesophyll cell; V, vascular bundle.

Although we could not decide whether BS chloroplasts changed their intracellular positions during the treatments, it is worth noting that they swelled to cover the cell surface after 8 h and 12 h of irradiation (Figure 5(c, d); Table 1). In  $C_4$  photosynthesis, atmospheric  $\text{CO}_2$  is assimilated by phosphoenolpyruvate to produce  $C_4$  acids.  $C_4$  acids are transported to BS cells and decarboxylated by  $C_4$  acid decarboxylase. Released  $\text{CO}_2$  is fixed again by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to produce carbohydrates in the Calvin cycle (Hatch, 1987). The enzymes involved in  $C_4$  photosynthesis are differentially located between the two types of cell, the BS cell and the M cell. For example,

Rubisco is exclusively located in BS chloroplasts, thereby accumulating carbohydrates in BS chloroplasts (Hatch, 1992). Many plants store the majority of their photoassimilates as starch during the day. An accumulation of starch granules leads chloroplasts to swell (Hasan, Ohnuki, Kawasaki, Taniguchi & Miyake, 2005; Ning, Yang, Li & Fritschi, 2018; Qian et al., 2011). In this study, we observed swelling chloroplasts in BS cells during the light treatment whereas chloroplasts in M cells did not seem to swell (Figure 5). According to the finding of Hatch (1992), we suggested that local accumulation of starch in the process of  $C_4$  photosynthesis in BS chloroplasts induced the BS chloroplast swelling. When

**Table 1.** Comparison of the number of bundle sheath (BS) chloroplasts, surface areas of chloroplasts and cells and the proportion of the total surface area of BS chloroplasts to BS cell surface area during the experimental period.

	Time of light treatment			
	0 hr	4 hr	8 hr	12 hr
Number of BS chloroplasts per cell	9.2 ± 0.2	9.0 ± 0.4	9.1 ± 0.3	9.7 ± 0.2
Surface area				
BS chloroplast (μm <sup>2</sup> per chloroplast)	30 ± 1	36 ± 5	43 ± 4*	45 ± 4*
BS cell (μm <sup>2</sup> per cell)	482 ± 6	464 ± 52	460 ± 57	469 ± 32
BS chloroplast (μm <sup>2</sup> per cell)	278 ± 11	318 ± 40	395 ± 34*	434 ± 31*
Proportion of the total surface area of BS chloroplasts to BS cell surface area (%)	57.7 ± 2.3	68.6 ± 2.0	85.9 ± 4.4*	92.5 ± 0.7*

Notes: Means±SE (n=3). Asterisks indicate significant differences at  $P < 0.05$  (Dunnett test) compared with  $t = 0$  hr.

carbohydrates accumulate in leaves, photosynthesis is often repressed (Araya, Noguchi & Terashima, 2010; Jeannette, Reyss, Gregory, Gantet & Prioul, 2000; Krapp, Quick & Stitt, 1991; Krapp & Stitt, 1995; Nakano, Muramatsu, Makino & Mae, 2000). Feedback inhibition of photosynthesis by carbohydrate accumulation is also a possible factor of midday depression of photosynthesis (Azcon-Bieto, 1986; Foyer, 1988). From these findings, it is suggested that the swelling of chloroplasts observed in this experiment represents the accumulation of starch, leading to a decrease in photosynthesis. On the other hand, before irradiation, sorghum M chloroplasts distributed along the cell membranes (Figure 5(a)). Under a natural regime of light, M chloroplasts showed the avoidance response, moving to the anticlinal cell wall at each time point from 4 h of irradiation onwards (Figure 5(b–d)). As irradiance increased, the efficiency of PSII decreased significantly after 4 h (Figure 4). This means that sorghum leaves showed photoinhibition due to excessive light energy in the daytime. It is well known that chloroplast avoidance response contributes to protect the photosynthetic apparatus from excess light and is important for survival under strong light conditions (Kasahara et al., 2002). Thus, it is likely that M chloroplasts in sorghum rearranged their intracellular positions to deal with excessive light energy. Leaves of sorghum showed a daily increase in  $P_n$ , with a maximum in the morning (at 3.8 h) and a midday depression. After 4 h of irradiation, when we observed the avoidance response of M chloroplasts, the depression of photosynthesis had already started. We could not ascertain whether the avoidance response occurred before the depression of photosynthesis. However, our data demonstrated that excess light leads not only to midday depression of photosynthesis but also to chloroplast avoidance response during the day. It is worth noting that this avoidance position of M chloroplasts was maintained throughout the midday depression of photosynthesis (Figure 2(a); Figure 5(b–d)).

Midday depression of photosynthesis has been found in citrus under high PPFD (Chen & Zhang, 1994), resulting in reduced plant growth, fruit yield

and quality. Some studies have reported that shading could alleviate midday depression of photosynthesis in grapevine (Cartechini & Palliotti, 1995), citrus (Jifon & Syvertsen, 2003; Medina et al., 2002) and tea (Mohotti & Lawlor, 2002). On the other hand, shading might decrease  $P_n$  when PPFD is low (i.e. in the early morning and late afternoon) and could even lead to reduced biomass productivity (Stampar, Hudina, Usenik, Sturm & Zadavec, 2001). Therefore, it is important to know whether plants are being exposed to excess light in order that lighting conditions may be adjusted at the correct time. There is a well-established relationship between leaf transmittance (or absorbance) and chloroplast position (Inoue & Shibata, 1974; Trojan & Gabryś, 1996; Walczak & Gabryś, 1980; Zurzycki, 1961); an increase in leaf transmittance indicates operation of chloroplast avoidance response to the anticlinal wall, whereas a decrease reflects operation of chloroplast accumulation response to the periclinal wall parallel to the plane of the leaf. Kasahara et al. (2002) reported that bleaching of leaf color accompanied by increased leaf transmittance is representative of the avoidance response. Our results revealed that the avoidance response of chloroplasts is a possible indicator of midday depression of photosynthesis. Thus, it would be of great interest to know whether bleaching of leaf color due to chloroplast avoidance response is a useful indicator for taking the opportunity to shade plants. For better understanding, further analysis of the interaction between chloroplast positioning and photosynthesis in crop production will be required.

## Acknowledgments

We thank Dr. Satoshi Ohkubo for his kindly assistance with manipulating LI6400.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was partly supported by the Kyoto University Foundation to E.M.

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