

博士論文（要約）

**Analysis of light spectrum effects on photosynthetic electron transport
based on excitation energy distribution between photosystem I and II**

(光合成電子伝達に光質が及ぼす影響の光化学系 I・II 間の励起エネルギー分配に基づく解析)

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Abbreviations

Symbol	Unit	Definition	Remark
ABS	$\mu\text{mol m}^{-2} \text{s}^{-1}$	flux density of absorbed photons	$\alpha \times \text{PFD}$
AEF	—	alternative electron flow	
AL	—	actinic light	
ANOVA	—	analysis of variance	
AS	—	artificial sunlight	
B	—	blue LED light	
BR	—	blue and red LED light	
C_a	Pa	ambient CO_2 partial pressure	
C_i	Pa	intercellular CO_2 partial pressure	
CEF-PSI	—	cyclic electron flow around PSI	
CEF-PSII	—	cyclic electron flow around PSII	
chl	—	chlorophyll	
EE	$\mu\text{mol m}^{-2} \text{s}^{-1}$	excitation energy	
ETR	$\mu\text{mol m}^{-2} \text{s}^{-1}$	whole-chain electron transport rate	
ETR_{I}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	electron transport rate through PSI	$E_{\text{I}} \times Y_{\text{I}}$
ETR_{II}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	electron transport rate through PSII	$E_{\text{II}} \times Y_{\text{II}}$
E_{I}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	excitation energy distributed to PSI	
E_{II}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	excitation energy distributed to PSII	
f	dimensionless	fraction of excitation energy distributed to PSII	$\frac{E_{\text{II}}}{E_{\text{I}} + E_{\text{II}}}$
Fd	—	ferredoxin	
FL	—	fluorescent lamps	
FR	—	far-red LED light	
F'	V	steady-state fluorescence in the light	
F_m	V	maximum fluorescence in the dark	
F'_m	V	maximum fluorescence in the light	
F_o	V	minimum fluorescence in the dark	
F'_o	V	minimum fluorescence in the light	

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Symbol	Unit	Definition	Remark
F_v	V	variable fluorescence in the dark	$F_m - F_o$
F'_v	V	variable fluorescence in the light	$F'_m - F'_o$
GL	—	growth light	
G_s	$\text{mol m}^{-2} \text{s}^{-1}$	stomatal conductance	
LED	—	light-emitting diode	
LEF	—	linear electron flow	
LHC	—	light-harvesting complex	
LMA	g m^{-2}	leaf mass per area	
n	—	number of replications, sample size	
NAD(P)H	—	nicotinamide adenine dinucleotide (phosphate)	
NDH	—	chloroplast NAD(P)H dehydrogenase-like complex	
NPQ	—	non-photochemical quenching	
PAM	—	pulse amplitude modulation	
PFD	$\mu\text{mol m}^{-2} \text{s}^{-1}$	photon flux density	
PPFD	$\mu\text{mol m}^{-2} \text{s}^{-1}$	photosynthetic photon flux density (400–700 nm)	
PQ	—	plastoquinone	
PSI	—	photosystem I	
PSII	—	photosystem II	
PSS	dimensionless	photostationary state of phytochrome	
PTOX	—	plastoquinone (or plastid) terminal oxidase	
P'	V	steady-state P700 absorbance signal in the light	
P_g	$\mu\text{mol m}^{-2} \text{s}^{-1}$	gross photosynthetic rate	$P_n + R_d$
P_m	V	maximum P700 absorbance signal in the dark	
P'_m	V	maximum P700 absorbance signal in the light	
P_n	$\mu\text{mol m}^{-2} \text{s}^{-1}$	net photosynthetic rate	
P_o	V	minimum P700 absorbance signal	
R	—	red LED light	

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Symbol	Unit	Definition	Remark
RC	—	reaction center complex	
RSD	—	relative spectral distribution	$\frac{SD}{\int SD_{\lambda} d\lambda}$
R_d	$\mu\text{mol m}^{-2} \text{s}^{-1}$	dark respiration rate	
SD	—	spectral distribution	
Y_I	dimensionless	quantum yield of PSI	$\frac{ETR_I}{E_I}, \frac{P'_m - P'_o}{P_m - P_o}$
Y_{II}	dimensionless	quantum yield of PSII	$\frac{ETR_{II}}{E_{II}}, \frac{F'_m - F'_o}{F_m - F_o}$
$Y_{\text{max, I}}$	dimensionless	maximum quantum yield of PSI	
$Y_{\text{max, II}}$	dimensionless	maximum quantum yield of PSII	
Y_{NA}	dimensionless	quantum yield of non-photochemical energy dissipation due to acceptor side limitation of PSI	$\frac{P_m - P'_m}{P_m - P_o}$
Y_{ND}	dimensionless	quantum yield of non-photochemical energy dissipation due to donor side limitation of PSI	$\frac{P' - P_o}{P_m - P_o}$
W	—	white LED light	
WWC	—	water-water cycle	
α	dimensionless	leaf absorptance	
ΔP_n	$\mu\text{mol m}^{-2} \text{s}^{-1}$	difference in P_n	
λ	nm	wavelength	

CHAPTER 1

General Introduction

本章の内容の一部が学術雑誌論文として出版される可能性があるため公表できない

5 年以内に出版予定

1 General introduction

CHAPTER 2

Quantification of Excitation Energy Distribution Between Photosystems Based on a Mechanistic Model of Photosynthetic Electron Transport

本章の内容を学術雑誌論文として出版する計画があるため公表できない

2 年以内に出版予定

submitted

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“Quantification of excitation energy distribution between photosystems based on a mechanistic model of photosynthetic electron transport”

2 Quantification of excitation energy distribution between photo-systems based on a mechanistic model of photosynthetic electron transport

CHAPTER 3

A Mathematical Model of Photosynthetic Electron Transport Based on Excitation Energy Distributed to Photosystems for Estimation of the Electron Transport Rate

本章の内容を学術雑誌論文として出版する計画があるため公表できない
2年以内に出版予定

to be submitted

Keach Murakami*, Ryo Matsuda*, and Kazuhiro Fujiwara.

3 A mathematical model of photosynthetic electron transport based on excitation energy distributed to photosystems for estimation of the electron transport rate

CHAPTER 4

Interaction Between the Spectral Photon Flux Density Distributions of Light During Growth and for Measurements in Net Photosynthetic Rates

Published as:

Keach Murakami*, Ryo Matsuda, and Kazuhiro Fujiwara.

“Interaction between the spectral photon flux density distributions of light during growth and for measurements in net photosynthetic rates of cucumber leaves”

Physiologia Plantarum, Vol. 158: pp. 213–224.

4 Interaction between the spectral photon flux density distributions of light during growth and for measurements in net photosynthetic rates

4.1 Introduction

In plants, the amounts of photosynthetic electron transport components in leaves change according to the relative spectral distribution (RSD) of photon flux density (PFD) of light during growth (Chow et al. 1990a, 1990b; Anderson et al. 1995; Matsuda et al. 2004, 2007, 2008). In particular, the stoichiometry between photosystem II and I complexes (PSII and PSI, respectively) responds sensitively to the RSD of growth light (GL) (Chow et al. 1990a, 1990b; Smith et al. 1993; Walters and Horton 1994, 1995a; Pfannschmidt et al. 1999; Wagner et al. 2008; Hogewoning et al. 2012). Within the chlorophyll (chl) absorption band (approximately 350–750 nm), longer wavelengths of light (> 680–690 nm) are estimated to be preferentially absorbed by PSI, and PSI is drastically overexcited (e.g. Evans 1986). Monochromatic light at shorter wavelengths (< 680–690 nm) is estimated to be preferentially absorbed by PSII (especially in the wavebands near 470 and 650 nm corresponding to the absorption peaks of chl *b*), or evenly absorbed by both photosystems (e.g. Evans 1986). Thus, light with a given RSD can be categorized as either PSII- or PSI-light according to whether the excitation energy (EE) is preferentially distributed to PSII or PSI, respectively. Because photosynthetic electron transport reactions occur in series, the electron transport rate (ETR) is limited by the slowest step (see also Chapter 3). In this respect, any imbalance in the distribution of EE between the two photosystems results in decreased light-use efficiency of photosynthesis (Pfannschmidt 2005; Walters 2005; Hogewoning et al. 2012). Moreover, such imbalances can harm leaves by generating reactive oxygen species, which cause oxidative damage to chloroplast components (Asada 1999).

In the short term, the distribution of EE is adjusted by the dynamic allocation of the light-harvesting antenna complex of PSII (LHCII) to PSI. When state transitions are insufficient to counterbalance the uneven distribution of EE, a long-term acclimation response occurs (see also ??). Under PSII-light, the relative amount of the reaction center complex of PSII (RCII) to that of PSI (RCI) (RCII/RCI ratio) in leaves decreases to balance the distribution of EE between the two photosystems. Conversely, under PSI-light, the RCII/RCI ratio increases to achieve a balance (Chow et al. 1990a, 1990b; Smith et al. 1993; Walters and Horton 1994, 1995a; Pfannschmidt et al. 1999; Wagner et al. 2008; Hogewoning et al. 2012). In this study, the terms ‘PSII-light’ and ‘PSI-light’ are used only in a relative context. That is, we state which should be more PSII-biased when referring to two (or more) RSDs. In their study on *Pisum sativum*, Chow et al. (1990b) reported that adjustments in photosystem stoichiometry allowed the plant to maintain a high quantum efficiency of photosynthesis. In that study, pea plants were grown under PSII- and PSI-light (PSII- and PSI-leaves, respectively) and the photosynthetic quantum yield of O₂ evolution was measured under PSII- and PSI-light. When measured under PSII-light, the yield was higher in PSII-leaves; when measured under PSI-light, the yield was

higher in PSI-leaves. A similar trend was observed in other studies on *Arabidopsis thaliana* (Walters and Horton 1995b) and *Cucumis sativus* (Hogewoning et al. 2012). These studies indicated that comparisons of leaf photosynthetic rates between plants grown under different RSDs of light can be biased depending on the RSD of actinic light (AL), as Walters (2005) pointed out. In other words, there must be an interaction between the RSDs of GL and AL in terms of the measured photosynthetic rate. Adjustments in photosystem stoichiometry and the resulting changes in the distribution of EE in response to the RSD of GL appeared to explain these observations. On the basis of this idea, supplemental far-red light (FR) was expected to trigger the interaction because it drastically decreases the proportion of EE distributed to PSII (e.g. Evans 1986), and drastically induces imbalanced excitation. However, it has not been experimentally validated whether supplemental FR during growth—the simplest and clearest trigger—induces the interaction between GL and AL that affects the measured photosynthetic rate.

Supplemental FR has attracted interest as a practical solution for controlling the R/FR ratio (i.e. the ratio of the PFD in the red waveband to that in the far-red waveband) of light, which regulates plant growth, development, and morphology (for a review, see Demotes-Mainard et al. 2016). Supplemental FR and/or a lower R/FR ratio has been reported to increase the fresh and dry weight of lettuce (Li and Kubota 2009), to enhance the water-use efficiency of cucumber seedlings (Shibuya et al. 2015), and to stimulate height convergence in densely grown cucumber seedlings, thereby equalizing their height (Shibuya et al. 2013). These potential advantages of supplemental FR have sometimes been contrasted with the low net photosynthetic rate (P_n) of the leaves measured under mixture of blue and red light supplied by light-emitting diodes (LEDs) (Shibuya et al. 2015). Because blue and red lights are typical PSII-biased lights, measuring P_n under that light source could give higher readings for PSII-leaves than for PSI-leaves because of the way that EE is distributed between the photosystems. If based on such a biased evaluation, the photosynthetic rates might be overestimated for PSII-leaves and underestimated for PSI-leaves compared with the case of the evaluation under PSI-light. Indeed, when P_n was measured under the broad waveband of FR-rich PSI-light provided by halogen vapor lamps, there was no significant difference between *Trifolium repens* leaves grown with and without supplemental FR (Heraut-Bron et al. 2000). However, the extent of the interaction has been poorly examined, and it remains unclear whether the interaction should be taken into account when evaluating leaf photosynthesis.

In this study, we examined the extent of the interaction between supplemental FR during growth and the RSD of AL in terms of measured P_n values. We cultivated cucumber seedlings under white LED light with or without supplemental FR and then measured P_n under each type of GL, light with sunlight-like RSD and blue and red LED lights. We found a significant interaction, and therefore we further investigated the mechanisms of the interaction by evaluating the changes in the distribution of EE under each light environment and photosystem stoichiometry.

4.2 Materials and methods

4.2.1 Plant material and growth conditions

Seeds of *C. sativus* (cv. Hokushin) were sown in rockwool (Grodan Multiblocks; Rockwool International A/S, Hedehusene, Denmark). The seedlings were grown in a temperature-controlled growth chamber (MIR-553; SANYO Electric Co. Ltd., Osaka, Japan) equipped with natural white fluorescent lamps (FPL55-EX-N; IWASAKI Electric Co. Ltd., Tokyo, Japan; Fig. 4-1a). The conditions in the chamber were as follows: a photosynthetic PFD (PPFD) of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the tops of plants, a 16-h light/8-h dark photoperiod, and 25/20°C day/night air temperatures. The PPFD was measured using a quantum sensor (LI-190SA with LI-250; LI-COR Inc., Lincoln, NE). Outside air was introduced into the chamber using an air pump at a flow rate of 4 L min^{-1} (air exchange rate: approximately 0.5 h^{-1}) to keep CO_2 concentrations close to atmospheric levels. The relative air humidity and CO_2 concentration inside the chamber were maintained at approximately 50% and higher than $400 \mu\text{mol mol}^{-1}$, respectively. The rockwool was continuously subirrigated with tap water until the seeds germinated (3–4 days after sowing) and subsequently with a commercial nutrient solution (Otsuka House A Prescription; Otsuka AgriTechno Co. Ltd., Tokyo, Japan) adjusted to an electrical conductivity of 130 mS m^{-1} . Plants were subjected to the treatments described in the following section from 8 days after sowing, when the first true leaves had emerged. The rockwool was subirrigated with the nutrient solution for 30 min d^{-1} during the treatments.

4.2.2 Treatments

Two treatments were prepared in which plants were grown under white LED (NSPW310DS; Nichia Chemical Industries Ltd., Tokushima, Japan; Fig. 4-1a) light (W) without or with supplemental FR provided by far-red LEDs (L735–36AU; Epitex, Inc., Kyoto, Japan; Fig. 4-1a) (W- and WFR-treatment, respectively). W was provided by custom-made printed wiring LED panels and FR was provided by laboratory-made line LED modules arrayed on transparent acrylic bars and hung below the panel. In the treatment for chl fluorescence and 830-nm absorbance measurements, FR was also provided by the LED panel. The white and far-red LEDs were arranged in a checkerboard pattern on the panel. The PFDs at the top of plants during the treatments are shown in Table 4-1. The PPFD of white LED light was measured with a quantum sensor and the PFD of FR was calculated from the SD measured with a spectroradiometer (MS-720; EKO Instruments Co. Ltd., Tokyo, Japan). The proportions of active phytochrome to total phytochrome (photostationary state) were calculated from phytochrome photochemical cross sections (Sager et al. 1988) and the RSD of light. Environmental settings other than SD were the same as those described above. Each treatment consisted of four plants, and experiments were replicated at least three times.

4.2.3 Gas exchange measurements

Gas exchange parameters of the first true leaves were measured using a portable gas exchange measurement system (LI-6400XT; LI-COR Inc.) at 14 and 15 days after sowing in a dark growth chamber (MIR-554; Panasonic Corp., Osaka, Japan) operated at 25°C. On day 14, measurements were made under each plant's respective GL provided by a laboratory-made small LED panel and under artificial sunlight (AS; Fig. 4-1b) provided by an LED-AS source system (Fujiwara et al. 2013) within a wavelength range of 380–950 nm. On day 15, gas exchange parameters were measured under blue and red LED light (BR; Fig. 4-1b) provided by an LED light source (6400-02B; LI-COR Inc.). A part of the leaf was sandwiched in a leaf cuvette and P_n , stomatal conductance (G_s), and intercellular CO₂ partial pressure (C_i) were measured. The measurements were made under three different combinations of PPFD and CO₂: (1) a PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and an atmospheric CO₂ partial pressure (C_a) of 40 Pa; (2) a PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and an C_i of 100 Pa; and (3) a PPFD of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and an C_i of 20 Pa. The measurements under the second and third conditions were made only under AS and BR. The leaf temperature was controlled at 25°C and the leaf-to-air vapor pressure deficit was controlled at < 1.0 kPa. The C_i was calculated according to von Caemmerer and Farquhar (1981). Gas exchange parameters under combination (1) were obtained approximately 15 min after the start of measurements, and then those under constant C_i conditions were obtained at 5- to 10-min intervals. To analyze quantitatively the interaction between the SDs of GL and AL, we calculated the difference in P_n between W- and WFR-leaves (ΔP_n ; P_n of W-leaves – P_n of WFR-leaves) from the rates measured under GL, AS and BR.

4.2.4 Chl fluorescence and 830-nm absorbance measurements

Chl fluorescence and changes in absorbance at 830 nm were measured simultaneously on the first true leaves using a fiber-type P700 and chl fluorescence measuring system (DUAL-PAM/F; Heinz Walz GmbH, Effeltrich, Germany) at 15 and 16 days after sowing in a dark room. A part of a dark-treated (> 60 min) leaf was sandwiched in the leaf cuvette of the gas exchange measurement system equipped with a fiber optics adapter (6400-06; LI-COR Inc.). The adapter was used to adjust the fiber optics in the cuvette to a defined angle and to a fixed distance from the leaf. The cuvette was maintained at an C_a of 100 Pa, a leaf temperature of 25°C and a leaf-to-air vapor pressure deficit of < 1.2 kPa. We used AL with three different RSDs: W at a PPFD of 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ without or with supplemental FR at a PFD of 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (W and WFR, respectively) and BR at a PPFD of 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The PPFD and PFD were lowered to measure the reactions under light-limited conditions. The laboratory-made small LED panel provided W and WFR, and BR was supplied by the LED light source (6400-02B; LI-COR Inc.). The incident angle of AL was 45° to illuminate the leaf surface uniformly. The C_i during measurement was approximately 100 Pa because the P_n was almost zero during the measurement. The parameters in the light-treated state were measured 10 min after the light irradiation commenced. The parameters (Y_{II} , Y_I , Y_{NA} , and Y_{ND}) were calculated as described in ??.

4.2.5 Leaf sampling and chl determination

On day 15, we sampled the first true leaves and measured their thickness, fresh weight and leaf area. An adjustable measuring force digimatic micrometer (CLM1-15QM; Mitutoyo Co., Kanagawa, Japan) and an area meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan) were used for leaf thickness and leaf area measurements, respectively. A leaf disk (approximately 10 mm in diameter) was punched out from the first leaf and chl was extracted by incubating the disk in 3 ml of *N,N*-dimethylformamide for 1 day in the dark at 4°C. The absorbance of the solution was then determined with a spectrophotometer (V-530; JASCO Corp., Tokyo, Japan) and the amounts of chl *a* and chl *b* were calculated from the absorbance values using the equations described by Porra et al. (1989). Then, the sampled leaves were dried at 100°C for 1 h, and subsequently at 80°C for 72 h before measuring dry weight. Leaf mass per area (LMA: leaf dry weight per leaf area) was calculated.

4.2.6 Statistical analyses

All analyses were conducted for mean values of three or four plants in each replicate, i.e. we obtained data using three or four plants in each treatment and the mean values were used as representative data from that replicate (i.e. $n = 1$). Then, we replicated the experiment at least three times to obtain the independent samples (i.e. $n \geq 3$) to avoid ‘pseudoreplication’ (Hurlbert 1984). The effects of supplemental FR during growth on gas exchange, chl fluorescence and 830-nm absorbance parameters, LMA, leaf thickness and chl contents in leaves were analyzed by Welch’s *t*-test. The main effects of supplemental FR during growth, the RSD of AL and the interaction between these two factors on gas exchange, chl fluorescence and 830-nm absorbance parameters were analyzed using two-way ANOVA. The effect of the RSD of AL on ΔP_n was analyzed by Tukey’s HSD test or Welch’s *t*-test. All analyses were conducted using the statistical software R (R Core Team 2016).

Table 4-1 Incident photon flux density (PFD) on the top of plants and calculated phytochrome photostationary state (PSS) and R/FR ratio of light from fluorescent lamps (FL) and during the treatments (W and WFR). PSS was calculated according to Sager et al. (1988). R/FR ratio was calculated as PFD of 600–700 nm/PFD of 700–800 nm.

Light source	PFD (400–700 nm) [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	PFD of FR [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	PSS	R/FR ratio
FL	300	–	0.82	5.5
W	300	–	0.83	6.7
WFR	300	70	0.67	1.0

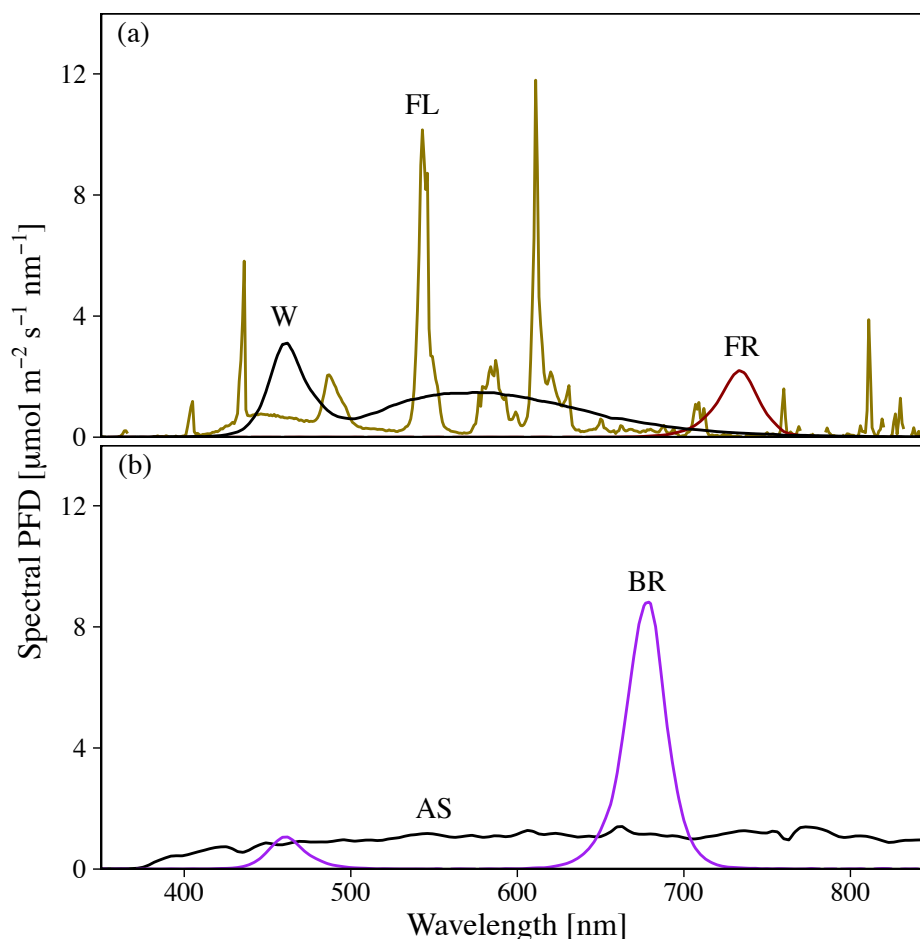


Fig. 4-1 Spectral distributions of photon flux density (PFD) of light provided by lamps used (a) until and (b) during the measurements of photosynthetic rates. Spectral distributions of light were measured at a photosynthetic PFD (400–700 nm) of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, except for that of FR, which was measured at a PFD of $70 \mu\text{mol m}^{-2} \text{s}^{-1}$. All spectral distributions were measured using an MS-720 spectroradiometer (EKO Instruments Co. Ltd.), except for that of FL, which was measured using an HR-2000 spectroradiometer (Ocean Optics Inc., Dunedin, FL) to detect its emission lines. Growth lights (W and W+FR) were also used for measurements, but are not shown in the lower panel to avoid duplication.

AS: artificial sunlight provided by an LED artificial sunlight source system (Fujiwara et al. 2013); BR: blue+red LED light provided by 6400-02B (LI-COR Inc.); FL: light from fluorescent tubes (FPL55-EX-N; IWASAKI Electric Co. Ltd.); FR: light from far-red LED (L735-36AU; Epitex Inc.); W: light from white LED (NSPW310DS; Nichia Chemical Industries Ltd.)

4.3 Results

When measured under BR, the P_n of WFR-leaves was lower than that of W-leaves (Fig. 4-2a). However, there was no significant difference in P_n between W- and WFR-leaves when measured under GL and AS (Fig. 4-2a). In terms of measured P_n values, there was a statistically significant interaction between supplemental FR during growth and the RSD of AL, while their main effects were not significant (Table 4-2). The ΔP_n calculated from the rates measured under BR was significantly greater than those calculated from the rates measured under GL and AS (Fig. 4-2b). There were no significant main effects or interaction effects on G_s although C_i was affected by the RSD of AL and tended to be lower when measured under BR (Table 4-2, 4-3). In addition to the ΔP_n measured under moderate PPFD and CO_2 conditions, the ΔP_n measured under BR was greater than that measured under AS in high PPFD and low C_i conditions (Fig. 4-3a), and tended to be greater under moderate light and a high C_i conditions (Fig. 4-3b). The Y_{II}/Y_I ratios tended to be lower in WFR-leaves than in W-leaves (Fig. 4-4c), irrespective of the RSD of AL. The differences in the Y_{II}/Y_I ratio between W- and WFR-leaves were mainly because of the lower Y_I of W-leaves (Fig. 4-4b). The lower Y_I of W-leaves resulted from a limitation at the acceptor-side (Y_{NA}) rather than the donor-side of PSI (Y_{ND}) (Fig. 4-5). The Y_{II}/Y_I ratios tended to be higher under WFR followed by BR and then W, irrespective of the GL (Fig. 4-4c). This trend resulted from the lower Y_{II} under W and the lower Y_I under WFR (Fig. 4-4a, b). The amounts of chl *a* and chl *b* was tended to be lower in WFR-leaves than in W-leaves, although the chl *a/b* ratio was comparable between W-leaves and WFR-leaves (Fig. 4-6). The fractions of the photons absorbed by WFR-leaves under AS and BR tended to be smaller than those by W-leaves, respectively (Fig. 4-7). There was no significant difference in LMA between W- and WFR-leaves, while W-leaves were thicker than WFR-leaves (Table 4-4), indicating that WFR-leaves had a greater volumetric density. Compared with plants in the W treatment, the plants in the WFR treatment were significantly taller and had paler leaves (Fig. 4-8).

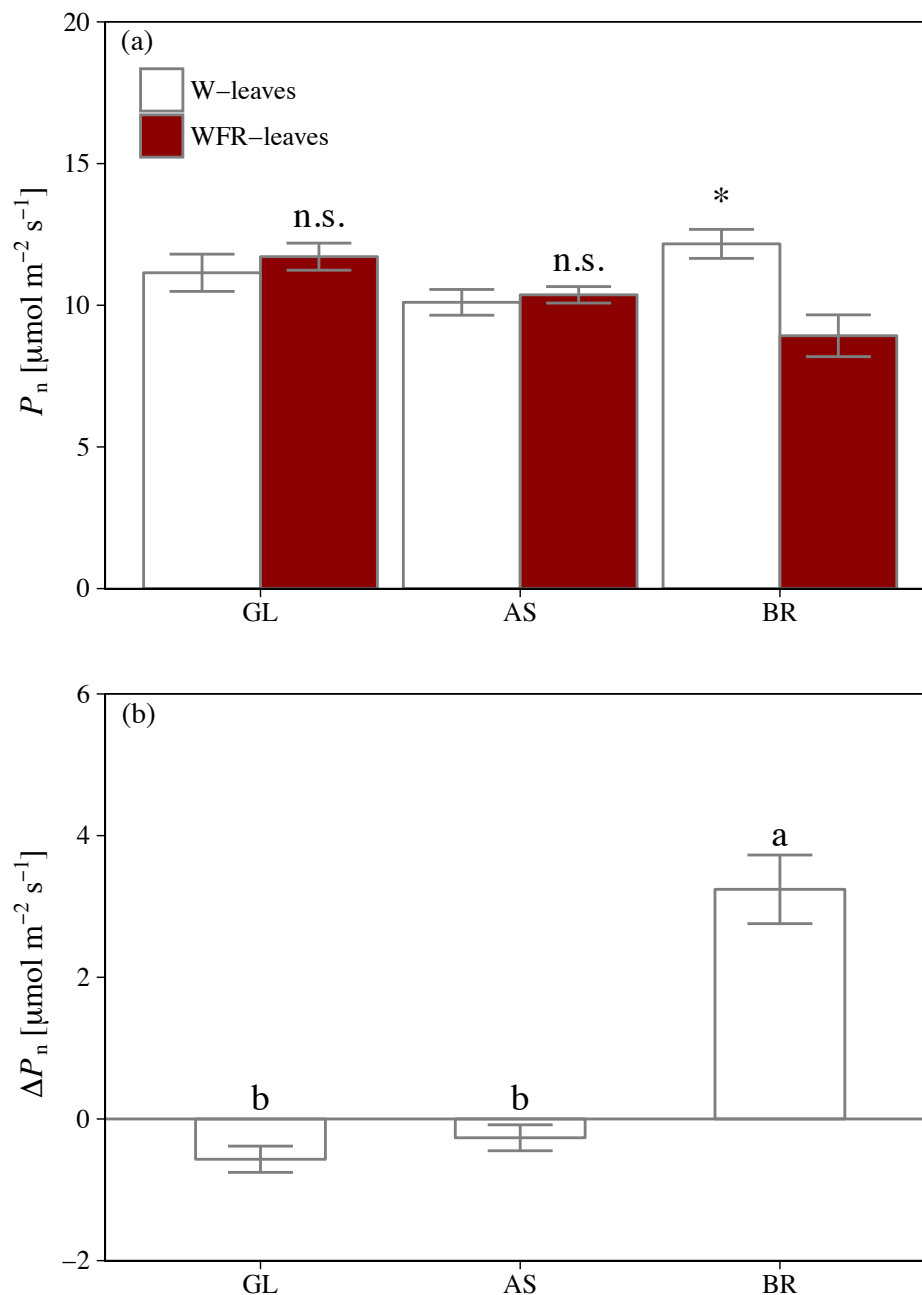


Fig. 4-2 (a) Net photosynthetic rates (P_n) of cucumber leaves grown under white LED light without and with supplemental far-red LED light (W- and WFR-leaves, respectively) and (b) calculated differences in P_n (ΔP_n) from the rates measured under their respective growth light (GL), artificial sunlight (AS) and blue and red LED light (BR; 6400–02B; LI-COR Inc., Lincoln, NE, USA). The measurements were made at a photosynthetic photon flux density of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and an atmospheric CO_2 partial pressure of 40 Pa. * in (a) indicates significant difference between the rates of W- and WFR-leaves (Welch's t -test, $P < 0.05$). Different small letters above the bars in (b) represent significant differences among treatments (Tukey's HSD test, $P < 0.05$). Means \pm standard errors are shown ($n = 3$).

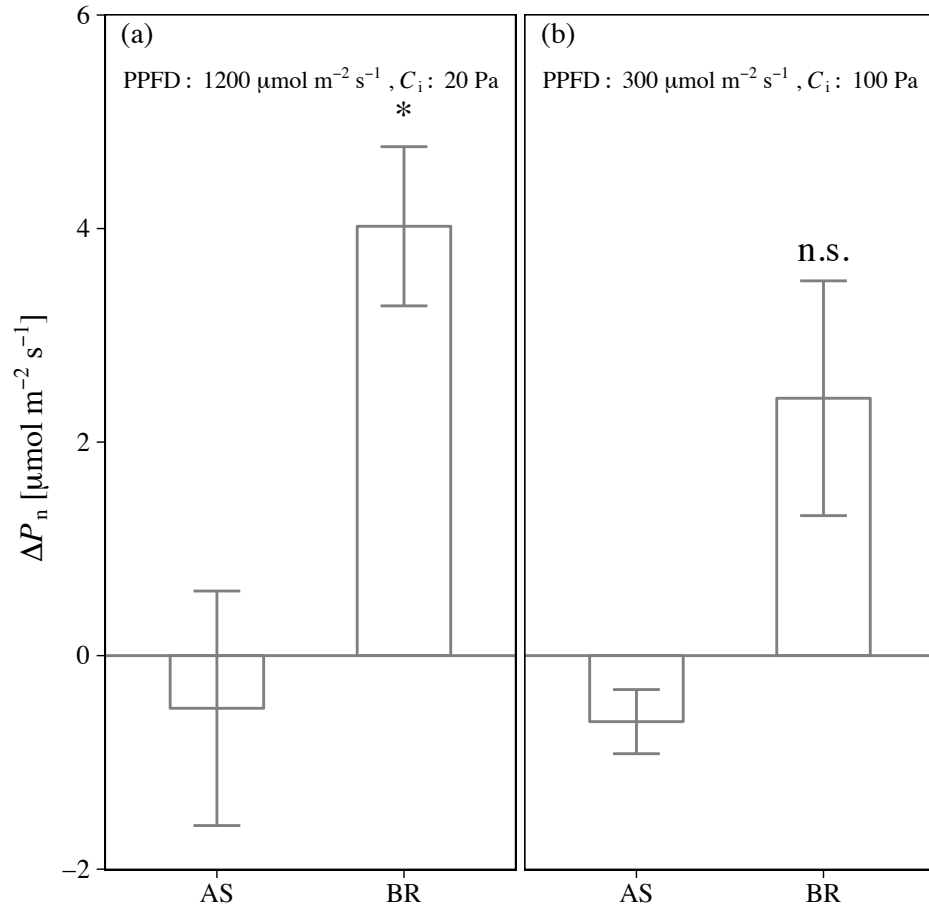


Fig. 4-3 Calculated differences in net photosynthetic rates (ΔP_n) of cucumber leaves grown under white LED light without and with supplemental far-red LED light (W- and WFR-leaves, respectively) measured under artificial sunlight (AS) and blue and red LED light (BR; 6400–02B; LI-COR Inc., Lincoln, NE, USA) at (a) a photosynthetic photon flux density (PPFD) of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and an intercellular CO_2 partial pressure (C_i) of 20 Pa and (b) a PPFD of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and an C_i of 100 Pa. * indicates significant difference between the AL (Welch's t -test, $P < 0.05$). Means \pm standard errors are shown ($n = 3$).

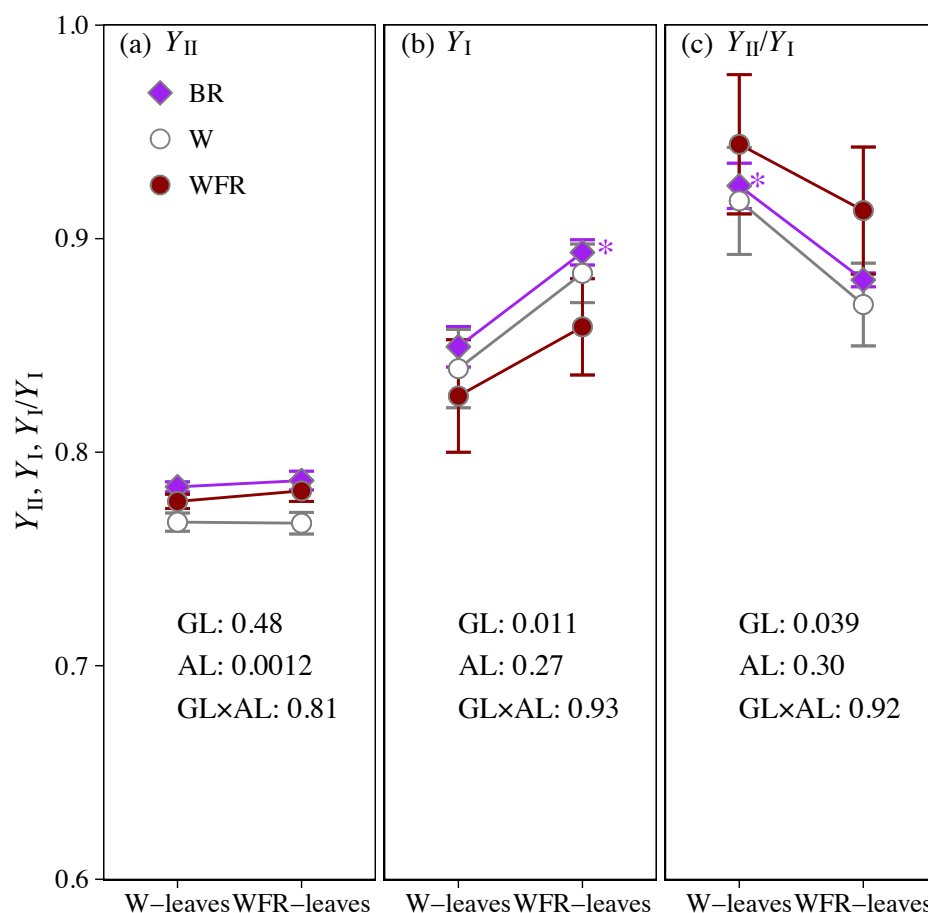


Fig. 4-4 Photochemical yield of (a) PSII (Y_{II}), (b) PSI (Y_I) and (c) the ratio (Y_{II}/Y_I) of cucumber leaves grown under white LED without and with supplemental FR light (W- and WFR-leaves, respectively) measured under W, WFR and blue and red LED light (BR; 6400–02B; LI-COR Inc., Lincoln, NE, USA). The measurements were made at an intercellular CO_2 partial pressure of approximately 100 Pa. The P -values obtained from two-way ANOVA with growth light (GL) and actinic light (AL) as independent variables and their interaction (GL \times AL) for each dependent variable are shown in each panel. * indicates significant difference between the values of W- and WFR-leaves (Welch's t -test, $P < 0.05$). Means \pm standard errors are shown ($n = 4$).

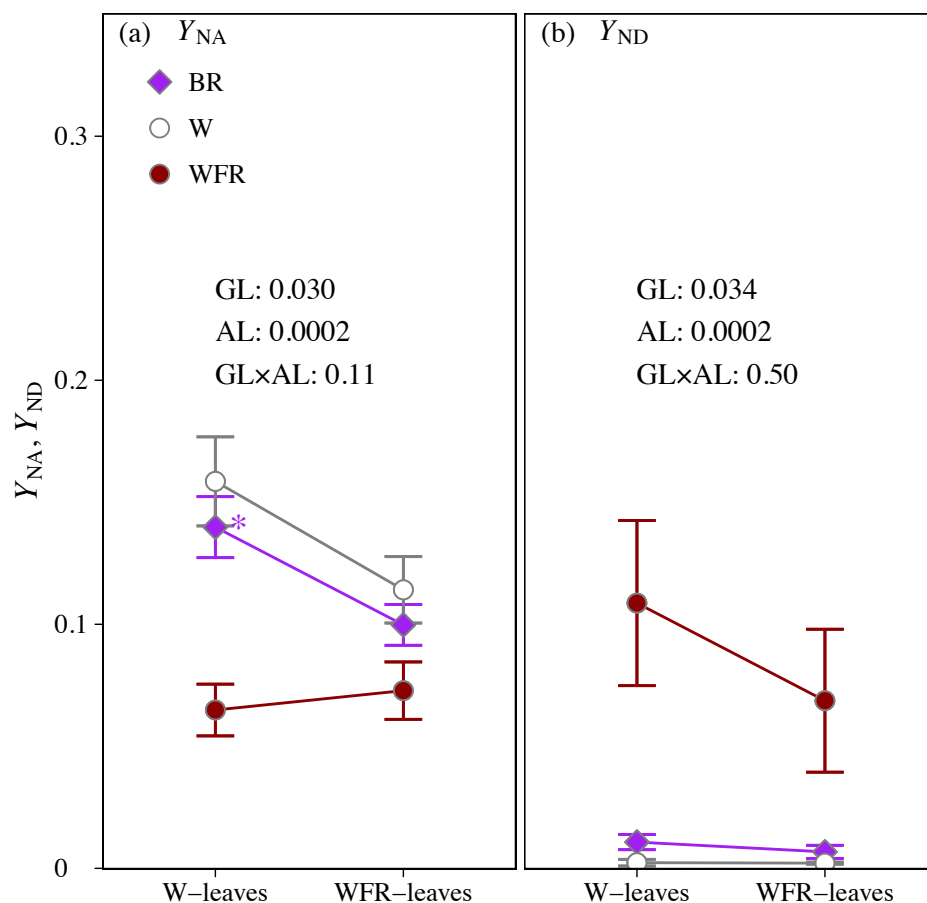


Fig. 4-5 (a) Acceptor-side (Y_{NA}) and (b) donor-side limitations (Y_{ND}) of photochemical reaction of PSI of cucumber leaves grown under white LED light without and with supplemental far-red LED light (W- and WFR-leaves, respectively) measured under W, WFR and blue and red LED light (BR; 6400-02B; LI-COR Inc., Lincoln, NE, USA). The measurements were made at an intercellular CO_2 partial pressure of approximately 100 Pa. The P -values obtained from two-way ANOVA with growth light (GL) and actinic light (AL) as independent variables and their interaction (GL \times AL) for each dependent variable are shown in each panel. * indicates significant difference between the values of W- and WFR-leaves (Welch's t -test, $P < 0.05$). Means \pm standard errors are shown ($n = 4$).

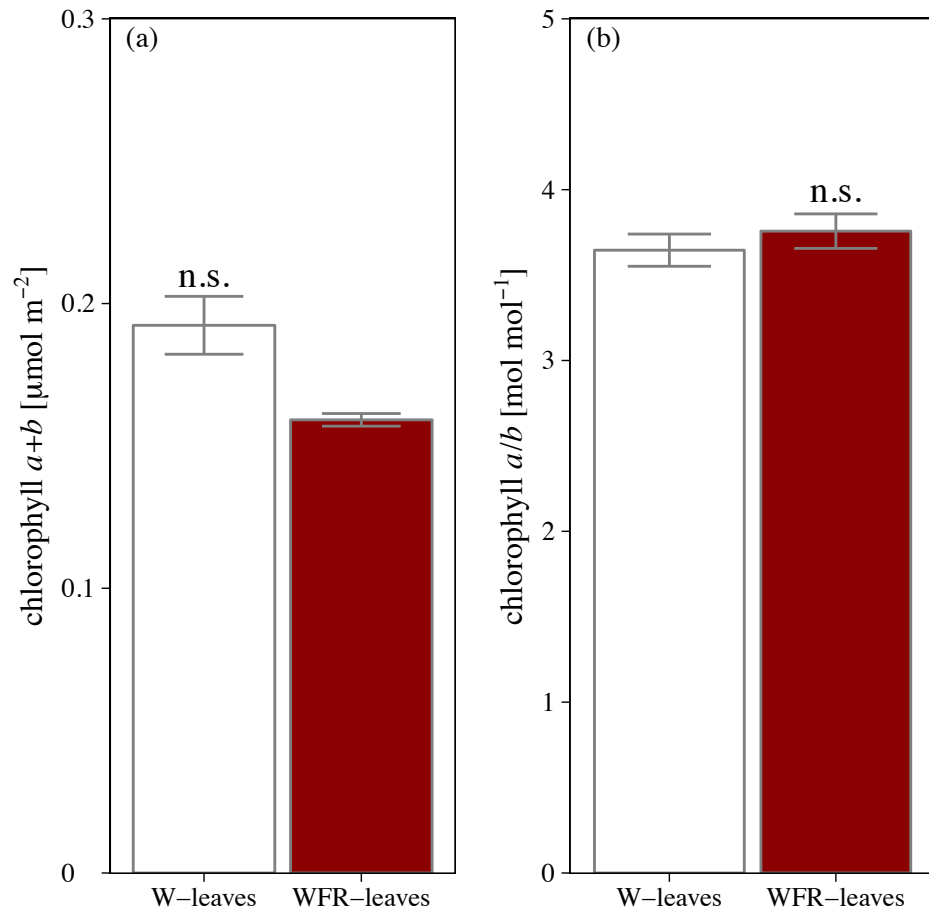


Fig. 4-6 (a) The amounts of chlorophyll a and chl b , and (b) chlorophyll a/b ratios of cucumber leaves grown under white LED without and with supplemental far-red light (W- and WFR-leaves, respectively). No significant difference was detected between the values of W- and WFR-leaves (Welch's t -test, $P < 0.05$). Means \pm standard errors are shown ($n = 3$).

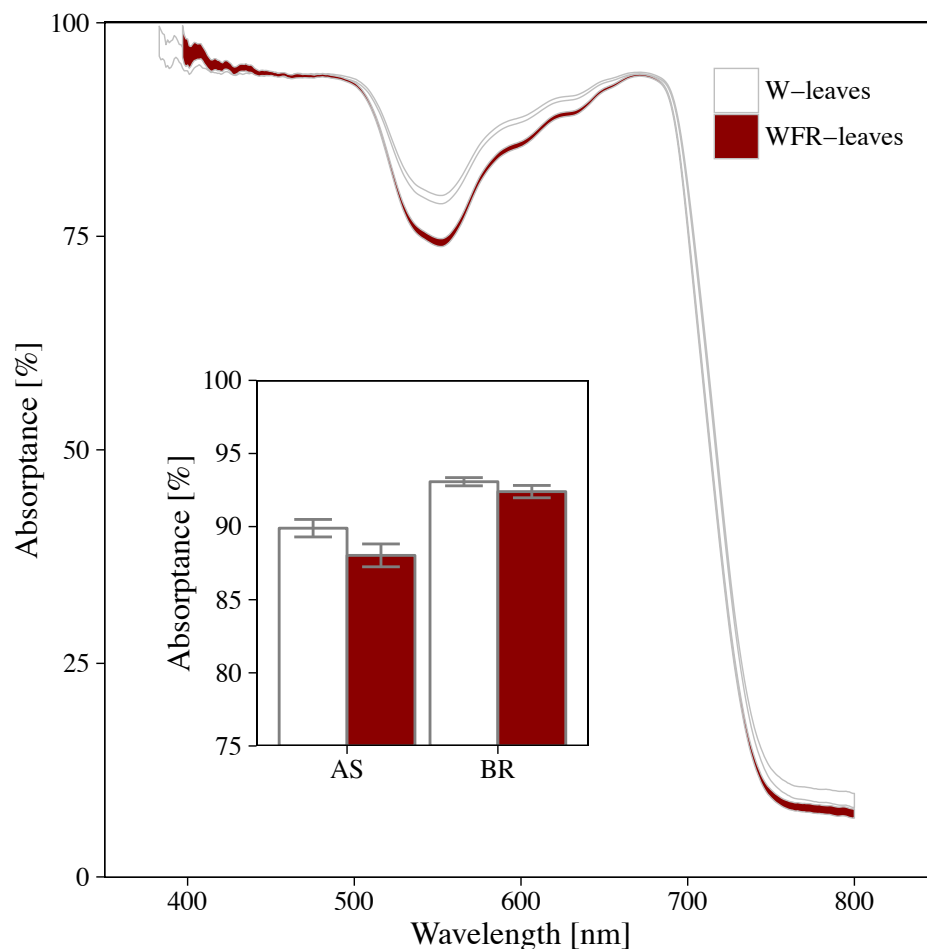


Fig. 4-7 Absorption spectrum of cucumber leaves grown under white LED light without and with supplemental far-red LED light (W- and WFR-leaves, respectively). The ranges between mean – standard error and mean + standard error are shown ($n = 3$). The inset shows estimated absorbed photons by W- and WFR-leaves under artificial sunlight (AS) and blue and red light (BR). Mean \pm standard errors are shown ($n = 3$). Absorbance at a given wavelength was calculated from reflectance and transmittance measured with integral spheres (ISP-REF and FOIS-1; Ocean Optics Inc., Dunedin, FL, USA) connected to a spectroradiometer (HR-2000, Ocean Optics).

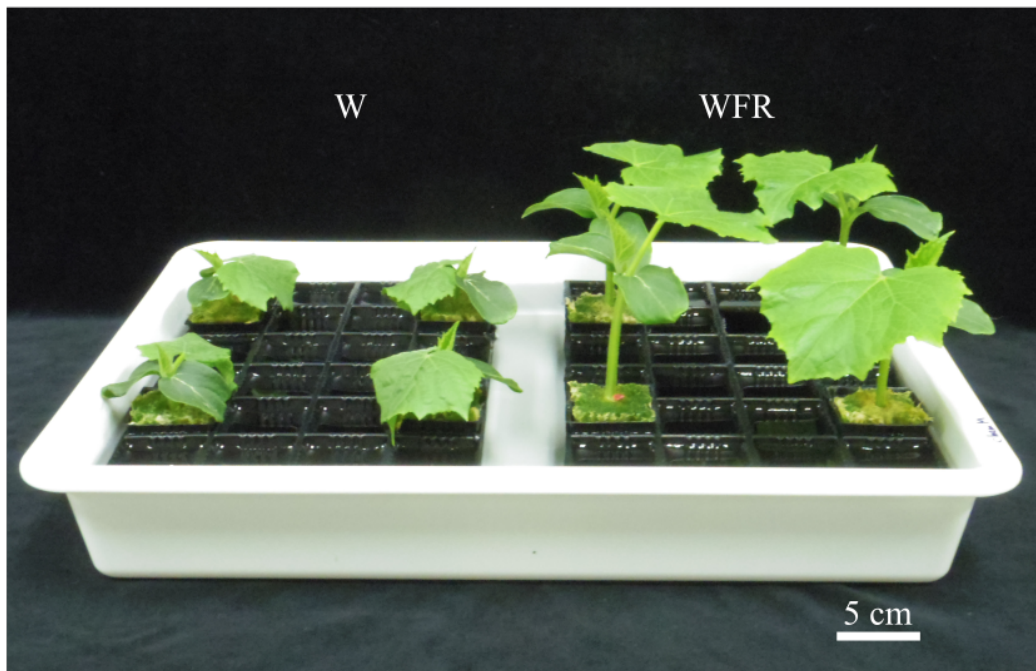


Fig. 4-8 Cucumber plants grown under white LED light without and with supplemental far-red LED light (W and WFR, respectively).

Table 4-2 Results of ANOVA to test the main effects of and the interaction between the spectral distribution of growth light (GL) and actinic light (AL) in net photosynthetic rate (P_n), stomatal conductance (G_s) and intercellular CO_2 partial pressure (C_i) of leaves. Means \pm standard errors are shown ($n = 3$). ** indicates significant difference between the treatments at $P < 0.01$.

Factor	df	P_n		G_s		C_i	
		F	P	F	P	F	P
GL	1	3.30	0.09	0.55	0.47	3.93	0.07
AL	2	2.63	0.11	2.66	0.11	7.32	0.008**
GL \times AL	1	7.66	0.007**	0.07	0.93	0.68	0.52

Table 4-3 Stomatal conductance (G_s) and intercellular CO_2 partial pressure (C_i) of cucumber leaves grown under white LED light without and with supplemental far-red LED light (W and WFR, respectively) measured under their respective growth light (GL), artificial sunlight (AS) and blue and red LED light (BR; 6400-02B; LI-COR Inc., Lincoln, NE, USA). Means \pm standard errors are shown ($n = 3$). No significant difference was detected between the values of W- and WFR-leaves.

Growth light	Actinic light	G_s [$\text{mol m}^{-2} \text{s}^{-1}$]	C_i [Pa]
W	GL	0.34 ± 0.10	32.6 ± 1.9
WFR		0.30 ± 0.12	30.1 ± 3.3
W	AS	0.33 ± 0.09	33.3 ± 1.8
WFR		0.30 ± 0.11	31.3 ± 2.9
W	BR	0.18 ± 0.03	27.6 ± 1.3
WFR		0.09 ± 0.02	20.7 ± 2.3

Table 4-4 Leaf mass per area (LMA) and thickness of cucumber leaves grown under white LED without and with supplemental far-red light (W and WFR, respectively). Means \pm standard errors are shown ($n = 4-7$). * indicates significant difference between the treatments (Welch's t -test, $P < 0.05$).

Growth light	LMA [g m^{-2}]	Leaf thickness [μm]
W	39.6 ± 2.9	$450 \pm 14^*$
WFR	40.6 ± 2.4	368 ± 14

4.4 Discussion

In this study, there was a significant interaction between supplemental FR during growth and the RSD of AL in terms of P_n (Fig. 4-2b, Table 4-2). In other words, we demonstrated that the effect of supplemental FR during growth on P_n was biased depending on the RSD of AL. In the following two sections, we discuss these results from the viewpoints of evaluating the photosynthetic characteristics of a leaf and the mechanisms of the interaction.

4.4.1 Biased evaluation of leaf photosynthesis

The greater ΔP_n under BR than under GL and AS (Fig. 4-2b) suggested that measurements under BR underestimate the P_n of WFR-leaves under certain growth conditions (i.e. *in situ* photosynthetic rate) and in sunlight. A similar trend was observed in an additional experiment using blue and red light with or without supplemental FR as the GL (Fig. 4-9). These results, therefore, strongly suggest that P_n should be measured under the same SD as those of the GL when evaluating photosynthesis *in situ*. In contrast to this simple method, it appears to be more complex to evaluate the photosynthetic characteristics (i.e. the responses of P_n to changes in PPFD and/or C_i). Several studies have reported that the RSD of the AL affected the P_n of leaves (McCree 1972; Inada 1976; Hogewoning et al. 2012; Murakami et al. 2013). To eliminate this direct effect, measurements of photosynthetic parameters should be made under identical RSD conditions, irrespective of the RSD of GL. However, even when the direct effect was removed, the comparisons of P_n were indirectly biased depending on the RSD of AL because of the interaction (Fig. 4-2b). The evaluation of P_n not only under moderate PPFD + moderate CO_2 conditions but also under high PPFD + low C_i and moderate PPFD + high C_i conditions was biased in comparisons between BR and AS (Fig. 4-3). Therefore, the P_n and photosynthetic characteristics of leaves must be discussed in connection with the RSD of AL. The RSD of AL must always be considered when evaluating leaf and whole-plant photosynthesis.

If researchers aim to describe the photosynthetic characteristics or the SD of light the leaves receive is not predetermined, then P_n should be measured under both PSII- and PSI-light and the interaction should be evaluated to detect any biases. Such biases can result in spurious estimates of photosynthetic parameters (e.g. initial slopes and compensation points of CO_2 and PPFD–photosynthetic rate curves) calculated from the measured P_n values using model-fitting methods (e.g. Sharkey et al. 2007). Biases in quantum yields of photosynthetic O_2 evolution and CO_2 fixation rates were reported in pioneering papers (Chow et al. 1990b; Walters and Horton 1995b; Hogewoning et al. 2012). When comparing values for such parameters among reports (i.e. in reviews and meta-analyses), particular care should be taken to consider the SD of AL. If the SD of light the leaves receive is predetermined, then measurements should be made under the same light conditions. For instance, commercial transplants are sometimes raised in a closed system with artificial lighting (e.g. Kozai 2007), and subsequently transferred to a greenhouse and cultivated under sunlight. Therefore, in comparisons of light sources with different RSDs for transplant production, P_n measurements should be made under sunlight to eliminate the need to consider the interaction. A basis for selecting the RSD of light for

evaluating leaf photosynthetic rates is also discussed in our recent letter (Murakami et al. 2017).

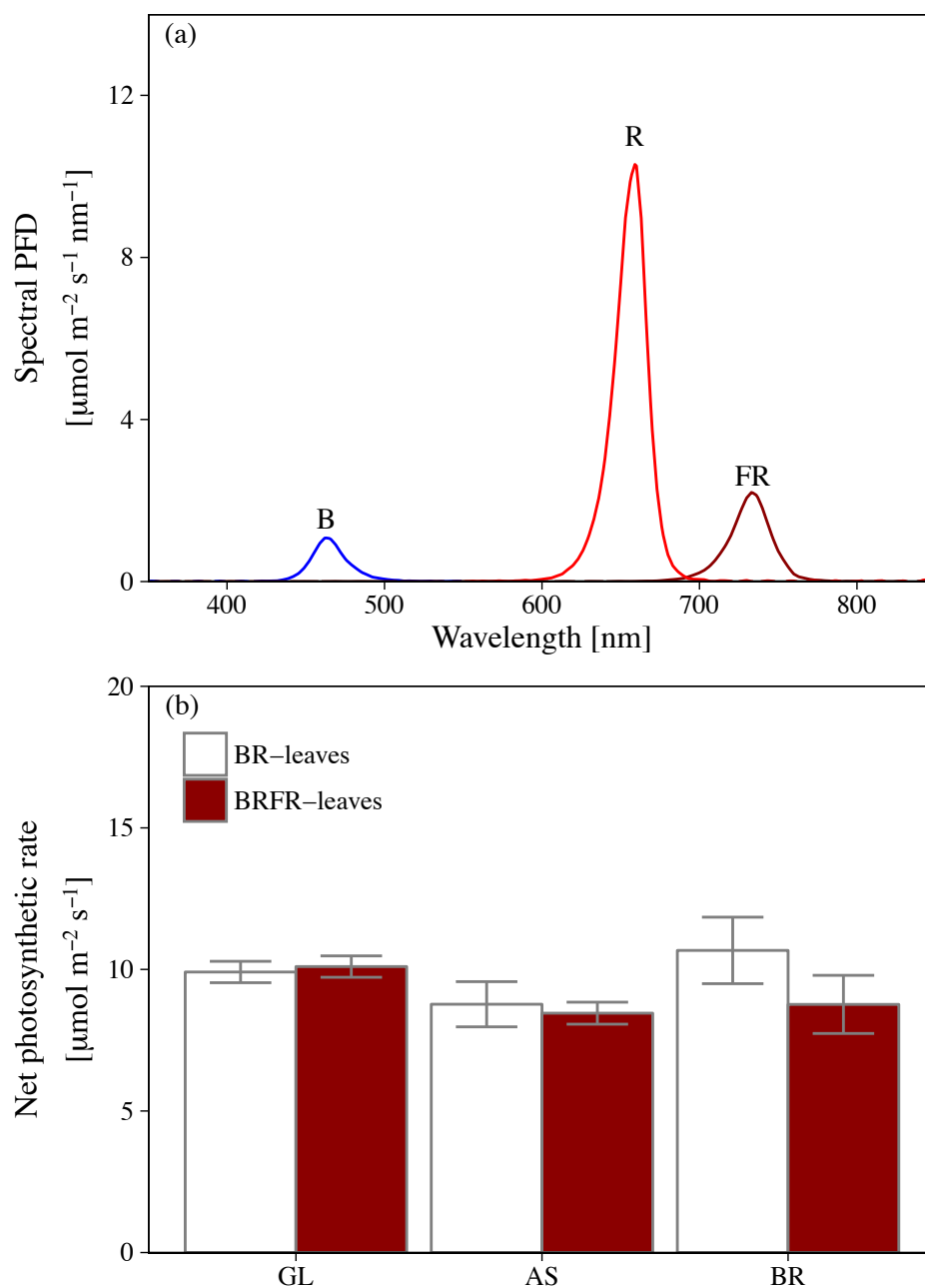


Fig. 4-9 (a) Spectral distributions of photon flux density (PFD) of light used for BR- and BRFR-treatment and (b) net photosynthetic rates of BR- and BRFR-leaves measured under their respective growth light (GL), artificial sunlight (AS), and blue and red LED light (BR; 6400–02B; LI-COR Inc., Lincoln, NE, USA). Measurements were made at a PPFD of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and an atmospheric CO_2 partial pressure of 40 Pa. Means \pm standard deviations of the rates for four plants are shown.

Blue (HBL3-3S55-LE; Toricon Co., Shimane, Japan), red (SRK3-3A80-LE; Toricon Co.), and far-red LEDs (L735-36AU; Epitex, Inc., Kyoto, Japan) were used for providing GL.

4.4.2 Origin of the interaction between spectral distributions of growth light and actinic light in terms of leaf photosynthesis measurements

The ΔP_n was smaller under GL than under BR (Fig. 4-2b). The gas exchange measurements under GL were made using W for W-leaves and W+FR for WFR-leaves. Because the FR contains a little PPFD (Fig. 4-1a) and the LHCII can slightly absorb shorter wavelengths of FR (approximately < 710 nm), the FR might drive both photosystems and decrease the ΔP_n , at least partly, when calculated from P_n measured under GL. Thus, based only on the results obtained under GL and BR, the interaction can be explained simply, i.e. the larger difference in ΔP_n under BR resulted from the lower photosynthetic rate of WFR-leaves and the increased PFD in the FR waveband and slightly increased PPFD under GL compensated for the gap. Also, the relatively lower amounts of chl (Fig. 4-6) and decreased thickness (Table 4-4) of WFR-leaves would have resulted in slightly lower light absorption (Fig. 4-7), and may partly explain their lower photosynthetic rate.

In the comparison of P_n measured under AS and BR, on the other hand, the evaluation was biased depending only on the RSD of AL (Fig. 4-2, 4-3). The absence of the significant interaction in C_i under ambient conditions (Table 4-2) and the difference in ΔP_n calculated from values measured under the constant C_i conditions (Fig. 4-3) suggest that the interaction in P_n originated from light-dependent reactions (i.e. electron transport) rather than CO_2 -dependent photosynthetic processes (i.e. Calvin cycle). One of the most plausible explanations for these phenomena was that there were adjustments in photosystem stoichiometry and the distribution of EE (see also 4.1). According to this idea, these results can be explained as follows: the RCII/RCI ratio increased in WFR-leaves during acclimation to PSI-light to maintain the excitation balance between the photosystems. Because of this change, the WFR-leaves were able to achieve a higher P_n under PSI-light at the expense of that under PSII-light. The opposite responses would occur in W-leaves, i.e. the RCII/RCI ratio decreased, and so they became capable of achieving higher P_n under PSII-light and lower P_n under PSI-light. Therefore, the ΔP_n under BR, which contained little FR and served as PSII-biased-light, was greater than that under AS, which contained much more FR and served as PSI-biased light. It appeared reasonable to explain the interaction in P_n based on these adaptive adjustments. Accordingly, we tested whether these changes in EE distribution and photosystem stoichiometry occurred under our experimental conditions. The change in the distribution of EE between the photosystems was evaluated by simultaneous measurements of the quantum yields of photochemical reactions at PSII and PSI, obtained by measuring chl fluorescence and absorbance at 830 nm. As long as the ETRs through PSII and PSI are equal, Y_{II}/Y_I is in proportion to E_I/E_{II} (i.e. the ratio of EE distributed to PSI and that distributed to PSII), as discussed in Chapter 2. The low PPFD ($< 20 \mu\text{mol m}^{-2} \text{s}^{-1}$) on the leaves during measurements may ensure the ETR balance in the present measurements (Fig. 4-4, 4-5).

The proportion of EE distributed to PSII was estimated to be highest under blue light, high under red light and low under FR in a relative context (e.g. Evans 1986). On the basis of these reports and the RSD of AL (Fig. 4-1), the ratio should be highest under W (which contains a large proportion of blue light), followed by BR and then WFR. Although not clear, the predicted trend was observed in the measured Y_{II}/Y_I values (Fig.

4-4c). The Y_{ND} under WFR was markedly higher than those under W and BR (Fig. 4-5b), suggesting that there was stronger PSI-donor-side limitation under WFR. This may have resulted from the shortage in EE distribution to PSII (i.e. E_{II}). This result also suggested that there was relatively smaller energy distribution to PSII under WFR than under W. We confirmed that supplemental FR made white light relatively PSI-biased.

The relatively lower Y_{II}/Y_I ratios of WFR-leaves than those of W-leaves irrespective of the RSD of AL (Fig. 4-4c) suggested that more EE was distributed to PSII than to PSI. This PSII-biased distribution property of WFR-leaves might enhance their light-use efficiency under PSI-light and explain why their P_n was comparable to that of W-leaves under AS (Fig. 4-2a). Under PSII-light, however, PSII-biased distribution will result in overexcitation of PSII and wasted light energy (Pfannschmidt 2005; Walters 2005; Hogewoning et al. 2012). The PSII-biased distribution property of WFR-leaves resulted in lower light-use efficiency and P_n (Fig. 4-2a) and greater ΔP_n under BR (Fig. 4-2b). These results support the idea that adjustments in the distribution of EE between photosystems caused the interaction in P_n . Notably, in the comparison between W- and WFR-leaves, the difference in Y_{II}/Y_I resulted mainly from the difference in Y_I because the values of Y_{II} were similar in both leaves (Fig. 4-4). Although it is unclear whether the photochemical reactions in PSI were regulated actively or passively, our data suggest that the regulation of Y_I is important for maintaining higher photosynthetic light-use efficiency.

We further analyzed the mechanism of the lower Y_{II}/Y_I in WFR-leaves, which suggested that a greater proportion of EE was distributed to PSII. A likely explanation was the change in photosystem stoichiometry resulting in a higher RCII/RCI ratio in PSI-leaves (i.e. WFR-leaves) than in PSII-leaves (i.e. W-leaves). Previous reports on interactions affecting P_n showed that the RCII/RCI ratios were higher in PSI-leaves than in PSII-leaves (Chow et al. 1990b; Walters and Horton 1995b; Hogewoning et al. 2012). Because a large fraction of chl b is bound to LHCII (Hogewoning et al. 2012; Laisk et al. 2014), a higher RCII/RCI ratio accompanies a lower chl *a/b* ratio (Fig. 4-10). We found that the chl *a/b* ratio of WFR-leaves was comparable to, or higher than, that of W-leaves (Fig. 4-6), suggesting that there was no increase in the RCII/RCI ratio. Note that in previous studies, emmental FR induced a slight (Chow et al. 1990a) or no increase (Walters and Horton 1995a) in RCII/RCI ratios. Considering these results reported in the literature, the interaction in P_n observed in this study might not be caused by changes in the RCII/RCI ratio. The distribution of EE may be adjusted by other mechanisms besides a simple change in the RCII/RCI ratio. Walters and Horton (1995b) suggested that changes in the LHCII/RCII ratio might be involved in RSD-dependent light acclimation. Recent studies have demonstrated that LHCII can serve as an efficient antenna for PSI (Galka et al. 2012; Wientjes et al. 2013) and that LHCII can mediate energy spillover from PSII to PSI (Yokono et al. 2015). emmental FR might act as a stimulus to trigger a change in the LHCII/RCII ratio, ultimately balancing the distribution of EE. Because of the dynamics of the LHCII allocation, the LHCII/RCII ratio obtained by dissolving the complexes may not always reflect the *in vivo* distribution of EE between the complexes. More detailed research on the compositions and functions of *in vivo* photosystems using non-destructive methods, such as the oxygen electrode and the fluorescence lifetime analyses, will elucidate details of the mechanisms of long-term light acclimation in

plants.

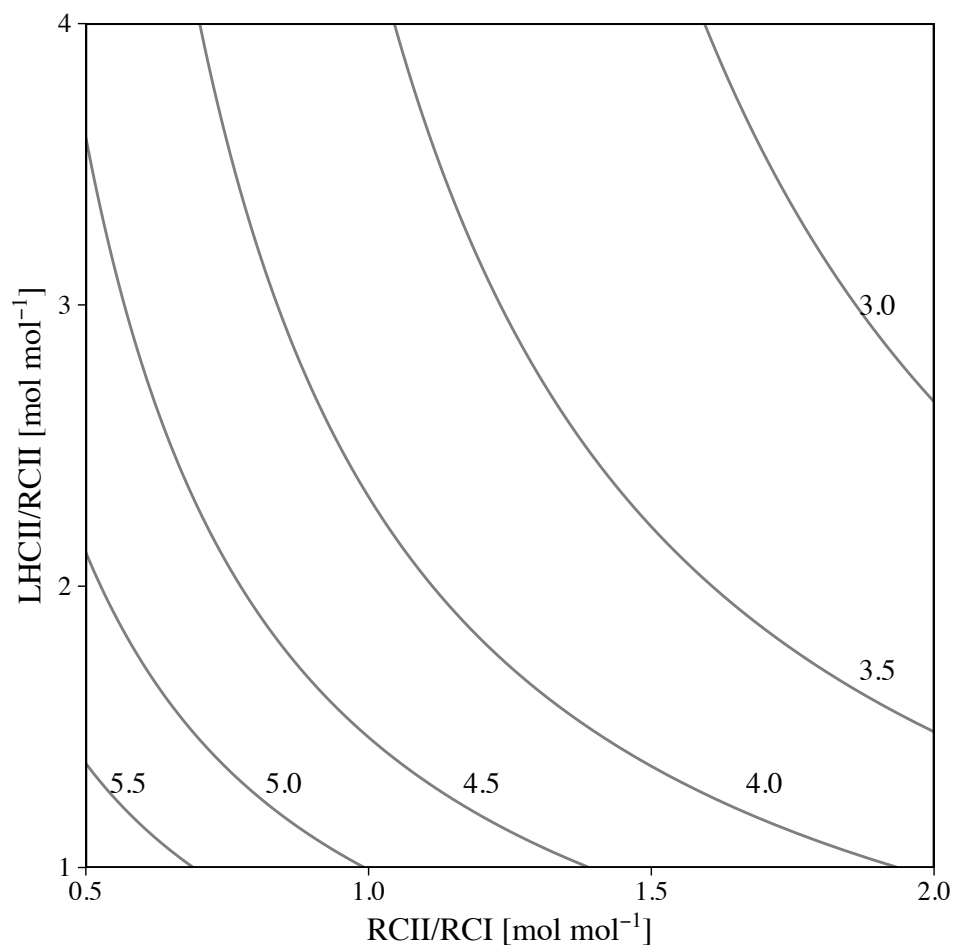


Fig. 4-10 Estimated chlorophyll a/b ratio from the photosynthetic membrane compositions.

RC: Reaction center; LHC: light-harvesting complex.

4.5 Conclusion

The results of this study showed that the effect of emental FR during growth on P_n was biased depending on the RSD of AL. Therefore, it is important to pay particular attention to the RSD of AL when evaluating the photosynthetic characteristics of leaves, especially when comparing plants grown under different PFDs of FR. In prospective studies focusing on plant growth after measurements, the evaluations should be made under the SD of light to which the plant will be subjected. In retrospective studies that aim to explain differences in growth brought about by the different growth conditions, the measurements should be made under the SDs of light that the plants received during the treatments. The origin of the interaction appears to be explained by the adjustments in the distribution of EE between the photosystems, as earlier reports proposed (Chow et al. 1990b; Walters and Horton 1995b; Hogewoning et al. 2012). However, the mechanisms discussed in these papers could not fully explain the adjustment in EE distribution that we observed in our experiments. Our results might suggest that other mechanisms may also be involved in adjusting the distribution of EE, e.g. changes in the composition of PSII. The interactions detected in this study suggest that it is necessary to conduct further detailed and circumspect research on photosynthesis. We can easily detect interactions between factors by using a two-way ANOVA. This fundamental but powerful technique will help to improve our understanding of leaf photosynthesis.

CHAPTER 5

Conclusions

学術雑誌論文として出版される計画がある章の内容を含むため公表できない

5 Conclusions

Abstract

Analysis of light spectrum effects on photosynthetic electron transport
based on excitation energy distribution between photosystem I and II
(光合成電子伝達に光質が及ぼす影響の光化学系 I ・ II 間の励起エネルギー分配に基づく解析)

Chapter 1. General introduction

The spectral distribution (SD) of light is a determinant of the light-dependent photosynthetic reaction rate, or electron transport rate (ETR). Even though the amount of absorbed photons is the same and under a strictly light-limited condition, the ETR depends on the SD (e.g. Emerson and Lewis 1943). That is, the relative SD (RSD) of light affects the intrinsic ETR. This RSD-dependency of the ETR is found to originate from the distribution of excitation energy (EE) between two photosystems (PSII and PSI) and the serial photosynthetic electron transport through these photosystems (e.g. Evans 1986).

Previous studies have provided qualitative knowledge on the distribution of EE and electron transport in response to the RSD of light. It is believed that a leaf maintains a higher ETR by functioning multiple systems in response to the RSD of light thereby adjusting the distribution of EE (e.g. Dietzel et al. 2008). However, little is known about the quantitative contributions of respective systems to the adjustments of the EE distribution. To elucidate the light acclimation of photosynthesis in response to the RSD of light, quantitative analyses of the adjustments of the distribution of EE in connection with their influence on the ETR are necessary.

The effects of the RSD of artificial lighting on plant growth and leaf photosynthesis for enhancing productivity of horticultural facilities have been intensively investigated. Owing to the acclimation response, leaves grown under different RSDs of light are expected to represent a different RSD-dependency of the ETR and, thus, photosynthetic characteristics. Therefore, evaluating photosynthesis of leaves grown under different RSDs of light using an identical RSD of light might result in a biased evaluation. Although this possible bias was pointed out (e.g. Walters 2005), its significance on photosynthetic evaluation has not yet been experimentally demonstrated considering the horticultural situations.

Therefore, quantitative analyses of the RSD-dependency of the ETR are essential for a comprehensive understanding of the light acclimation responses of a leaf and the evaluation of leaf photosynthesis toward efficient light use in horticultural plant production systems. The objective of this dissertation was to analyze the adjustments of the distribution of EE between the photosystems in response to the RSD of light and its influence on the ETR. In chapter 2, a novel method for estimating the EE distributed to PSII is proposed. In chapter 3, the electron transport based on the distribution of EE is illustrated as a mathematical model. Chapter 4 demonstrates that the acclimation response under different RSDs of growth light biased the evaluation

of the net photosynthetic rate (P_n) in a practical horticultural situation.

Chapter 2. Quantification of excitation energy distribution between photosystems based on a mechanistic model of photosynthetic electron transport

The fraction of the EE distributed to PSII (f) depends on the wavelength of light (e.g. Evans 1986). The SD of f appears to be adjusted by at least two mechanisms in response to the RSD of light on different timescales (e.g. Dietzel et al. 2008); the adjustment of the photosystem stoichiometry (long-term response) and reversible reallocation of the light-harvesting complex between the photosystems (state transitions; short-term response). A quantitative evaluation of the EE distributed to the photosystems is required to comprehend the functioning and physiological roles of these mechanisms in the acclimation of the photosynthesis to the light environment.

In this chapter, a non-destructive, quantitative, and mechanistic method for estimating the *in vivo* f values of a leaf was developed and validated. To estimate the f values, a mechanistic model, which illustrates the ratio of photochemical quantum yields of PSII and PSI (Y_{II} and Y_I) from the f values and photon flux densities (PFDs) of the two simultaneously provided RSDs of actinic lights (ALs), was developed. This model assumes that the EE from individual ALs is distributed additively to the respective photosystems and that the ETRs through PSII and PSI are equal. By fitting values of Y_{II} and Y_I , obtained by monitoring chlorophyll fluorescence and leaf reflectance, under ALs provided at several PFD combinations into the model using the least-squares (i.e. curve-fitting) method, f values for the ALs can be estimated. This method was tested by comparing f values for red and far-red LED lights (R and FR, respectively) of cucumber leaves presumably giving different f values owing to the long- and short-term responses. The leaves were grown under white LED light (W; $300 \mu\text{mol m}^{-2} \text{s}^{-1}$) with and without supplemental FR ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) for about 1 week to induce the long-term response. They were then pre-irradiated with R with and without supplemental FR for about 10 min to induce the short-term response, and then subjected to the f estimation. Irrespective of conditions of the long- and short-term responses, the quantified f values for R were clearly greater than those for FR. The values of the leaves subjected to 1-week supplemental FR (i.e. grown under W+FR) tended to be greater than those of the control (i.e. grown under W), presumably due to the long-term response. The values of the leaves subjected to 10-min of supplemental FR (i.e. pre-irradiated with R+FR) tended to be greater than those of the control (i.e. pre-irradiated with R), presumably due to the short-term response. These trends are consistent with those of earlier studies on the wavelength dependency of the f (e.g. Evans 1986), long-term response (e.g. Chow et al. 1990), and short-term response (e.g. Allen 1983). Furthermore, the fitted curve generated by the model was in agreement with the actual values in all experiments, supporting the validity of the model.

Chapter 3. A mathematical model of photosynthetic electron transport based on excitation energy distributed to photosystems for estimation of the electron transport rate

Simultaneously provided PSII- and PSI-light (light under which the whole-chain ETR is limited by photochemical reactions in PSII and PSI, respectively) produce a greater gross photosynthetic rate (P_g) than the sum of P_g under PSII-light and that under PSI-light (e.g. Emerson et al. 1957). This phenomenon, called the ‘enhancement effect’, led to the idea that electron transport occurs in series through the photosystems (Hill and Bendall 1960). It is generally accepted that the distribution of EE between the photosystems determines the photosynthetic quantum yield (e.g. Evans 1986). However, the electron transport in response to the EE distribution has not yet been illustrated as a mathematical model. Several available methods estimate the EE distribution from the SD of light (e.g. Evans 1986 and Chapter 2); therefore, developing a model may enable the estimation of the ETR in response to the SD of light.

In this chapter, a mathematical model, which illustrates the photosynthetic electron transport based on the EE distributed to the photosystems, was developed. This model assumes that 1) the whole-chain ETR is given as the minimum of potential ETR at either PSII or PSI, 2) the rate-limiting photosystem represents potential ETR by maintaining its maximum photochemical quantum yield, and 3) the photochemical quantum yield of the non-rate-limiting photosystem is passively down-regulated to equalize the actual ETRs through the two photosystems. To test the proposed model, Y_{II} , Y_I , and ETR of cucumber leaves under simultaneously provided R and FR were estimated from the EE distributed to the photosystems, which was calculated as described in Chapter 2, and compared to the actual values. Because the actual ETR could not be assessed directly, the estimated ETR was converted into P_g and compared to the actual P_g . The estimated values of Y_{II} , Y_I and P_g based on the model were in agreement with the actual values. The model explained the mechanisms determining the quantum yield of photosynthetic electron transport under light-limited conditions reasonably well.

Chapter 4. Interaction between the spectral photon flux density distributions of light during growth and for measurements in net photosynthetic rates

The P_n is often measured, compared, and evaluated among leaves of plants grown under different RSDs of light. Leaves adjust the distribution of EE in response to the RSD of growth light (GL) (e.g. Anderson 1986). Therefore, even when evaluated under light with the same RSD, the EE distributed to the respective photosystems will be modified depending on the RSD of GL. That is, the RSDs of GL and AL interact on the ETR and thus P_n through the EE distribution. When the effect of the interaction is considerable, the P_n of leaves compared using a single RSD of AL does not always reflect their relationship under other RSDs of light. Although some earlier physiological studies have already demonstrated the significance of this interaction (Chow et al. 1990, Walters and Horton 1995, Hogewoning et al. 2012), it has not yet been clarified that the interaction should be considered even in practical situations.

This chapter describes how the significance of the interaction was examined in a practical situation imitating seedling production. The effects of the RSD on seedling growth and photosynthetic characteristics have been intensively investigated (e.g. Shibuya et al. 2015). As the seedlings are grown under artificial light

sources and then under sunlight, the photosynthesis under both conditions should be discussed. However, most of recent measurements of P_n were made using blue and red LED light (BR) preinstalled in widely-used instruments. P_n of leaves of cucumber seedlings grown under W ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$) with and without supplemental FR ($70 \mu\text{mol m}^{-2} \text{s}^{-1}$) were measured and compared under three different RSDs of ALs: their respective GL, BR, and light with a RSD approximate to that of sunlight (artificial sunlight; AS). The P_n of W+FR-grown-leaves was lower than that of W-grown-leaves under BR, moderate PFD ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$ within 400–700 nm), and ambient CO_2 (40 Pa) conditions, whereas no significant difference was found between the leaves under GL and AS under the same PFD and CO_2 conditions. In short, the RSDs of GL and AL interacted on the P_n . Analyses of the photochemical yields of photosystems showed that the interactions in P_n were related, at least partly, to the distribution of EE. It was demonstrated that the evaluation of P_n of leaves grown under different RSDs of light could be biased depending on the RSD of AL even in practical situations. P_n should be discussed in connection to the RSD of AL especially when leaves of plants grown under different RSDs of GL are compared.

Chapter 5. Conclusions

This dissertation focused on the distribution of EE between PSII and PSI in response to the RSD of light and its influence on photosynthetic electron transport. A non-destructive and quantitative method for estimating *in vivo* distribution of EE was developed. The f estimation was performed based on a mechanistic model of electron transport, in which the EE distributed to respective photosystems are additive and the ETRs through PSII and PSI are equal. The contributions of mechanisms adjusting the distribution were compared based on an identical quantitative measure, i.e. the fraction of EE distributed to PSII or the f value. In addition, by assuming that the rate-limiting photosystem represents its maximum efficiency, the electron transport under light-limited conditions was illustrated as a mathematical model based on the distribution of EE. The interaction between the RSDs of light for measurement and during growth on leaf P_n was shown to be significant even in practical situations. The necessity of a circumspect consideration of the distribution of EE between the photosystems in evaluating leaf photosynthesis was emphasized.

謝辞

本博士論文は、筆者が東京大学 大学院農学生命科学研究科 生物・環境工学専攻 生物環境工学研究室において、日本学術振興会 (特別研究員奨励費, 課題番号: 26-9372) の助成を受けて行った研究をまとめたものです。本研究の進行および本論文の作成にあたり、多くの方々に支えていただきました。

生物環境工学研究室に所属して以来、富士原 和宏 教授ならびに松田 怜 准教授 (生物環境工学研究室) には、始終ご指導を賜りました。富士原先生には、研究の大綱を示していただくのみならず、細部にわたる丁寧なご指導をいただきました。松田先生には、実験の進め方、議論の仕方、論文の書き方から研究の進め方・考え方に至るまで、研究のいろはを教えていただきました。両先生に心より感謝いたします。

同研究科 芋生 憲司 教授 (生物機械工学研究室)、細井 文樹 准教授 (生物情報工学研究室)、ならびに大学院理学系研究科 寺島 一郎 教授 (植物生態学研究室) には、快く審査を引き受けていただき、多くの客観的かつ有益なご指摘・ご助言を賜りました。寺島 教授ならびに河野 優 博士 (植物生態学研究室) には、励起エネルギー分配定量に関する共同研究を進める機会をいただきました。期待通りの結果とは参りませんでしたが、多くのご助言を頂戴したことは、筆者にとって非常に貴重な経験となりました。荊木 康臣 教授 (山口大学 大学院創成科学研究科) ならびに渋谷 俊夫 准教授 (大阪府立大学大学院 生命環境科学研究科) には、学会などで度々お声かけいただき、議論する機会を頂戴しました。Sander Willem Hogewoning 博士 (Plant Lighting B.V., The Netherlands) には光化学系の吸光スペクトルデータをご提供いただきました。Wah Soon Chow 名誉教授 (Research School of Biology, The Australian National University) には、励起エネルギー分配定量に研究についてご助言をいただきました。皆様に厚くお礼を申し上げます。

生物環境工学研究室の研究員、学生諸氏および秘書の皆様には、日々お世話になりました。とくに藤内 直道 助教 (現 筑波大学 生物圏資源科学専攻 農林技術センター) ならびに地子 智浩 氏に感謝いたします。おふたりが博士課程に先輩として在籍し、多くの時間を過ごせたことは大きな刺激であり、楽しい記憶です。

2017.03.15

村上 貴一

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