学位論文（要約）

Responses of the photosynthetic electron transport system to fluctuating light

（変動光に対する光合成電子伝達系の応答）

平成 26 年 10 月　　博士（理学）　　申請

東京大学大学院理学系研究科
生物科学専攻
河野　優

ABSTRACT

Light energy absorbed by chloroplasts drives photosynthesis. When absorbed light is in excess, the thermal dissipation systems of excess energy are induced and the photosynthetic electron flow is regulated, both contributing to suppression of reactive oxygen species production and photodamages. Various regulation mechanisms of the photosynthetic electron flow and energy dissipation systems have been revealed. However, most of such knowledge has been obtained by the experiments conducted under controlled conditions with constant light, whereas natural light condition is drastically fluctuated. To understand photosynthesis in nature, we need to clarify not only the mechanisms that raise photosynthetic efficiency but those for photoprotection in fluctuating light. Although these mechanisms appear to be well balanced, regulatory mechanisms achieving the balance are little understood.

To assess roles of the cyclic electron flow around PSI (CEF-PSI) and O_2-dependent alternative pathways including the water-water cycle in fluctuating light (FL), I grew the wild type and *pgr5* mutant of *Arabidopsis thaliana* in continuous light for 8 h per day, and measured chlorophyll fluorescence and P700 absorbance changes in their leaves in the FL alternating between 240 (HL) and 30 μmol photon m^{-2} s^{-1} (LL) every 2 min. At 20% O_2, the photochemical quantum yield of PSII decreased, in particular in *pgr5*, soon after the start of the fluctuating light treatment. PSI of the *pgr5*

plants was markedly photoinhibited by this treatment for 42 min. Slight PSI photoinhibition was also observed in the wild type. I measured energy sharing between PSII and PSI and estimated the electron transport rates through PSII, ETR(II), and through PSI, ETR(I). *pgr5* showed larger energy allocation to PSI. In contrast to the wild type, the ratios of ETR(I) to ETR(II) in the *pgr5* plants were high in LL but lowered in HL at 20% O_2 due to the acceptor-side limitation on PSI. At 2.7 or 0% O_2, the CEF-PSI of the *pgr5* plants was enhanced, the acceptor-side limitation of PSI was released, and PSI photoinhibition was not observed. The results suggest that the light fluctuation is a potent stress to PSI and that the CEF-PSI is essential to protect PSI from this stress.

To assess the effects of short-term fluctuating light on photoinhibition of both PSII and PSI, and on regulation of the photosynthetic electron transport system, I measured chlorophyll fluorescence and P700 parameters of *A. thaliana* grown in the continuous light in three FLs alternating between the HL for 2 min and LL for 2min, the FL-240/30 (HL at 240 and LL at 30 μmol photons m^{-2} s^{-1}), FL-1200/30 (HL at 1200 and LL at 30 μmol photons m^{-2} s^{-1}) and FL-1200/240 (HL at 1200 and LL at 240 μmol photons m^{-2} s^{-1}). All of the FL caused PSI photoinhibition, but the degree of PSI photoinhibition was similar during three FL treatments. In response to the FL-1200/30, ETR(II) and ETR(I) kept pace with the changes in light intensity. In these FLs, photoprotective systems, such as the energy dissipation in the PSII antenna system and the down-regulation of electron flow by the photosynthetic control

at the cytochrome b_6/f complex, functioned to regulate the linear electron flow. However, the activities of these systems were insufficient in the FL-240/30. Thus, ETR(II) and ETR(I) in HL phases in the FL-240/30 decreased stepwise with the cycle. These results suggest that differences in modes of light fluctuation have distinct effects on regulation of the photosynthetic electron transport system. I examined the roles of photosynthetic alternative electron flows in response to the FL. The over-expression line of PGR5 showed the marked tolerance to the FL. In addition, continuous measurements of the changes in the electrochromic pigment shift showed that the rate of H^+ effluxes via the H^+-ATPase in chloroplasts did not decrease with the cycles. This may explain why PSI photoinhibition did not enhance PSII photoinhibition in the FL. I suggest that the alternative electron flows, especially the PGR5-mediated cyclic electron flow around PSI, contribute significantly to the compensation of electron flow through PSI, and consequently keep the whole electron transport safely.

Table of contents

Acknowledgements

I would like to express my sincere gratitude and appreciation to Professor Ichiro Terashima (The University of Tokyo), who gave thoughtful advice and continuous guidance throughout the study. I also wish to express my special thanks to Associate Professor Ko Noguchi for kind support and valuable guidance.

I would like to thank Professor Toshiharu Shikanai (Kyoto University) for providing the *pgr5*, over-expression lines of PGR5 and wild-type Columbia type *gl1* seeds.

I would like to thank Professor Kintake Sonoike (Waseda University) and Associate Professor Chikahiro Miyake (Kobe University) for helpful discussions.

I would like to thank Professor Masahiko Ikeuchi (The University of Tokyo), Professor Naoki Sato (The University of Tokyo), Professor Tatsuru Masuda (The University of Tokyo) and Associate Professor Masaki Tateno (The University of Tokyo) for critically examining my doctoral study and providing invaluable comments.

Finally, I wish to extend my appreciation to Associate Professor Yoshihiro Suzuki (Kanagawa University).

Abbreviations

ΔA_{max}	maximum level of P700 signal in the dark
ATP	adenosine triphosphate
CEF	cyclic electron flow
CEF-PSI	cyclic electron flow around PSI
Chl	chlorophyll
CL	continuous light
CL-240	continuous light at 240 μmol photons m^{-2} s^{-1}
CL-1200	continuous light at 1200 μmol photons m^{-2} s^{-1}
Cyt *b6/f*	cytochrome *b6/f*
DIRK	dark-interval relaxation kinetics
ECS	electrochromic pigment absorbance shift
ETR	electron transport rate
ETR(I)	electron transport rate through PSI
ETR(II)	electron transport rate through PSII
Excess energy	rate of excess energy production
F_0	minimal fluorescence
f_{PSII}	share of absorbed light energy allocated to PSII
FL	fluctuating light
FL-240/30	fluctuating light that altered between HL phase at 240 μmol photons m^{-2} s^{-1} for 2 min and LL phase at 30 μmol photons m^{-2} s^{-1} for 2 min
FL-1200/30	fluctuating light that altered between HL phase at 1200 μmol photons m^{-2} s^{-1} for 2 min and LL phase at 30 μmol photons m^{-2} s^{-1} for 2 min
FL-1200/240	fluctuating light that altered between HL phase at 1200 μmol photons m^{-2} s^{-1} for 2 min and LL phase at 240 μmol photons m^{-2} s^{-1} for 2 min
flg22	peptide derived from bacterial flagellin
FLP	fluctuating light photoinhibition

F_m	maximal fluorescence
FQR	ferredoxin-plastoquinone reductase
F_s'	steady-state fluorescence in light
F_v/F_m	maximum quantum yield of PSII
H^+	proton
H^+-ATPase	H^+-exporting ATP synthase
HL	high light
I_A	absorbed PPFD
k_{pi}	rate of photodamage
$k_{pi,PSII}$	rate of photodamage of PSII
$k_{rec.PSII}$	rate of PSII recovery from photodamage
LED	light-emitting diode
LEF	linear electron flow
LHCII	light harvesting chlorophyll-protein complex II
LL	low light
MAP	Mehler-ascorbate peroxidase
NDH	NADH dehydrogenase-like complex
NDH-mediated CEF	NDH-mediated cyclic electron flow around PSI
NPQ	non-photochemical quenching
P515	elechtrochromic pigment absorbance shiftvia dual-wavelength (550-515 nm) transmittance changes
P700	reaction center of PSI
PAMP	pathogen-associated molecular pattern
PC	plastocyanin
PGR5	PROTON GRADIENT REGULATION5
PGR5-mediated CEF	PGR5-mediated cyclic electron flow around PSI
ΔpH	proton gradient
ΔΨ	thylakoid membrane potential
pmf	proton motive force
PPFD	photosynthetically photon flux density
PQ	plastoquinone

PSI	photosystem I
PSII	photosystem II
PsbS	a specific PSII protein
PTOX	plastid terminal oxidase
q_0	coefficient of the increment of heat dissipation in the light
qE	energy-dependent quenching
qI	photoinhibitory quenching
qL	fraction of open PSII reaction centers
qN	coefficient of non-photochemical quenching
qP	coefficient of photochemical quenching
qT	state transition quenching
RC	reaction center
ROS	reactive oxygen species
WT	wild type
WWC	water-water cycle
Y(I)	photochemical quantum yield of PSI
Y(II)	photochemical quantum yield of PSII
Y(EX)	fraction of energy that is neither used for PSII photochemistry nor dissipated as heat in PSII
Y(NA)	non-photochemical quantum yield due to the acceptor-side limitation of PSI
Y(ND)	non-photochemical quantum yield due to the donor-side limitation of PSI
Y(NO)	quantum yield of non-regulated energy dissipation at PSII centers
Y(NPQ)	quantum yield of regulated energy dissipation at PSII centers

CHAPTER 1.

General introduction

Light is the ultimate resource for photosynthesis, and its intensity (irradiance) drastically changes with time in nature. Many factors including seasons, daily solar movement, cloud cover and canopy architecture combine to produce complex patterns of changes in irradiance in time and space (Pearcy 1990). Plants, therefore, experience dynamic fluctuations of irradiance even when they are in open habitats. Understory plants experience more frequent, short-term irradiance fluctuations due to the leaves and stems of other plants above them (Pearcy 1983; Pearcy 1990; Chazdon 1988; Pearcy et al. 1994; Vierling and Wessman 2000). Plants have to cope with such light fluctuations of various time scales employing several mechanisms (Grieco et al. 2012; Rochaix et al. 2012; Suorsa et al. 2012). These include mechanisms increasing efficiency of photosynthesis as well as those increasing efficiencies of photoprotection (Alter et al. 2012). The balances of these mechanisms should be of supreme importance for actual plant life in nature.

Efforts have been made to clarify how the photosynthetic machinery responds to short-term changes in irradiance. Our knowledge of the dynamics of photosynthesis has been advanced by these studies (Pearcy 1990; Kirschbaum et al. 1998; Külheim et al. 2002; Alter et al. 2012; Suorsa et al. 2012). However, the complex interactions between the fluctuating light

and the dynamics of photosynthesis have not been fully clarified yet. This chapter focuses on recent experimental approaches that have advanced our understanding of 'effects of fluctuating light on long-term response (acclimation) of electron transport system' and 'effects of fluctuating light on short-term response of the electron transport system'.

1.1. Controls of photosynthetic electron flow in fluctuating light

Plants are able to acclimatize their photosynthetic characteristics to their growth light environments. The acclimation of the photosynthetic apparatus, such as changes in the amount of antenna proteins, PSII/PSI stoichiometry, and the contents of electron transport components and enzymes (Anderson et al. 1995), requires several hours to a week. In the longer period encompassing many generations, adaptation occurs, which involves genetic changes leading to adjustments to the light environments although there is an exception. The photosynthetic characteristics brought about by acclimation and/or adaptation to growth irradiance levels were studied intensively (Chazdon et al. 1996; Le Roux et al. 2001; Rothstein and Zak 2001; Oguchi et al. 2005; Oguchi et al. 2006). In these studies, however, dynamic fluctuations of light environments were not paid much attention. In daytime, plants are exposed to changes in irradiance on various timescales, typically in the order of seconds to minutes or hours, but sometimes even milliseconds. Plants have to

cope with such changes in irradiance by various mechanisms that regulate light-harvesting capacity (for example, NPQ; non-photochemical quenching), electron flows, and enzymatic activities (Fig. 1).

The term 'sunfleck' has been used frequently in the literature to describe strong light pulses in natural environments. The duration and distribution of sunflecks are highly variable, and such sunflecks exert substantial effects on CO_2 assimilation and growth of plants (Kirschbaum and Pearcy 1988). The sunfleck light regime may be separated into the periods of multiple sunflecks and the periods with few or no sunflecks (Vierling and Wessman 2000). Leaves in the understory may receive only a few sunflecks or up to 300 or more sunflecks per day. Most of these sunflecks are shorter than 10 s (Pearcy 1983; Chazdon 1988). In the understory of a tropical rain forest, sunflecks longer than 120 s represent only 5% in number but contribute more than 75% of the total daily photosynthetic active photon flux density (PPFD) (Pearcy et al. 1994). In a deciduous forest in early spring, when tree branches have no leaves, irradiance on the forest floor changes more dynamically and contribution of longer sunflecks was greater compared with the situation in summer (Kono, personal observation).

In the 1980s, Pearcy and co-workers revealed that understory plants utilize sunflecks efficiently. They demonstrated that retention of the proton gradient (ΔpH) across the thylakoid membrane and the metabolites of Calvin-Benson cycle are essential for efficient post-illumination CO_2 assimilation (Pearcy 1990; Kirschbaum et al. 1998). In the high-light periods of the fluctuating light in the order of minutes, the electron transport system

is over-reduced and NPQ is developed. The qE-quenching dissipates excess light energy during the high-light periods. In the low-light periods, the qE-quenching can be inactivated within minutes to allow maximum photosynthetic electron transport (Demmig-Adams and Adams 1992; Demmig-Adams et al. 1996; Horton et al. 1996). Porcar-Castell et al. (2006) constructed a dynamic model of PSII quantum yield taking account of the adjustments of the NPQ processes, since steady-state models cannot describe dynamics of the photosynthetic electron flow in fluctuating light. The results showed that both the changes in irradiance on the timescales of seconds to minutes and those in the activation state of Calvin-Benson cycle enzymes influenced the partitioning of energy between NPQ and the photosynthetic electron flow. Although this approach is attractive, we need to prove the model prediction experimentally. The slow relaxation of NPQ after a high-light period would transiently limit CO_2 assimilation in the subsequent low-light period. The high activity of CEF-PSI would support the post-illumination CO_2 assimilation at a high rate via providing ATP and thereby alleviate this problem.

To understand photosynthetic responses of plants to fluctuating light we need to evaluate the responses to the consecutive sunflecks, which are observed in natural environments. Photosynthetic responses to the consecutive sunflecks of alternating low- and high-light are distinct from those to the continuous low- and high-light (Fietz and Nicklisch 2002; Hjelm and Ogren 2004; Nedbal et al. 2005; Porcar-Castell et al. 2006; Wagner et al. 2006). In other words, responses to fluctuating light could not be understood

by our knowledge of acclimation to continuous low- or high-light only (Fietz and Nicklisch 2002).

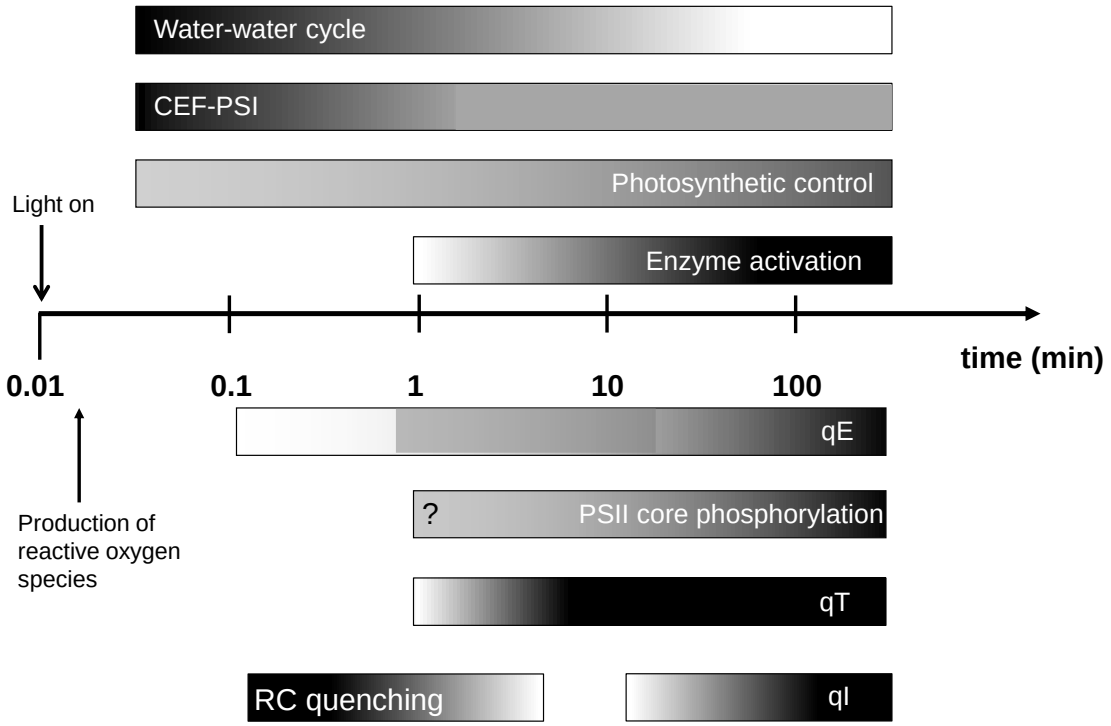

Figure 1. Major mechanisms that occur in response to irradiance in plants. The water-water cycle, Mehler-ascorbate peroxidase pathway (Asada cycle); CEF-PSI, cyclic electron flows around PSI; Photosynthetic control, control of the electron flow by the Cyt b_6/f complex; Enzyme activation, activation of key enzymes in the Calvin-Benson cycle by thioredoxin; qE, qE-quenching; PSII core phosphorylation, photoprotection by phosphorylation of PSII core proteins; qT, qT-quenching; RC quenching, quenching within active PSII reaction center (reaction center quenching); and qI, qI-quenching. ? in PSII core phosphorylation denotes ambiguity of the initiation time scale. Based on a diagram proposed by Eberhard et al. (2008) with modifications. The timescales adopted here are based on the data from various sources mostly cited in the text, but should not be regarded as solid values, because the timescales and the extents of contributions of these regulation mechanisms

differ depending on the experimental conditions.

1.2. Sun and shade-type chloroplasts

Differences between the sun- and shade-type chloroplasts have been well documented (Lichtenthaler 1981; Lichtenthaler et al. 1981; Lichtenthaler et al. 1982; Lichtenthaler 1984; Hjelm and Ogren 2004; Nedbal et al. 2005; Wagner et al. 2006). In shade-type chloroplasts, the content of the light harvesting complexes relative to core complexes is higher than in sun-type chloroplasts (Anderson 1986; Evans 1989; Hikosaka and Terashima 1995). In low light, it is more economical to increase light capture by the light harvesting complexes rather than to have core complexes (Hikosaka and Terashima 1995). The thylakoid membrane structure also differs. Shade-type chloroplasts typically show higher density of thylakoids per chloroplast sectional area, more extensive grana stacks, and thereby more granal thylakoids than sun-type chloroplasts. These features should influence the capacity of the photosynthetic electron transport.

Thylakoid membrane protein complexes distribute heterogeneously between the granal and stroma-exposed regions of the thylakoids. The photosystem II (PSII) complexes are mostly located in grana stacks, whereas photosystem I (PSI) and the H^+-ATP synthase are mostly located in the stroma-exposed thylakoids. The thylakoid architecture and protein distribution dynamically change according to light intensity (Rozak et al. 2002; Kirchhoff et al. 2011; Anderson et al. 2012; Herbstová et al. 2012; Kirchhoff 2013). A recent study, using spinach leaves grown in continuous

light, and kept in the dark, has revealed that most of the PSII subunits distributed in the grana thylakoids and grana margins rather equally, but low molecular mass subunits including the PsbS protein were found in the grana thylakoids (Suorsa et al. 2013). Intriguingly, PROTON GRADIENT REGULATION5 (PGR5) was distributed evenly between granal and stroma-exposed thylakoids, whereas PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1) was enriched in stroma-exposed thylakoids.

1.3. Non-photochemical quenching

1.3.1. Energy-dependent quenching

Non-photochemical quenching (NPQ) contributes to down-regulation of the photosynthetic electron transport in the chloroplast. NPQ of excess excitation plays a protective role, which prevents over-acidification of the lumen and decreases the damage to PSII in high irradiance. Various mechanisms contribute to NPQ, and there are three components on the basis of the time constants of the NPQ relaxation kinetics in the dark following a period of illumination: (i) qE, energy-dependent quenching, requires acidification of the thylakoid lumen and is relaxed within seconds to minutes; (ii) qT, state transition quenching, is caused by the changes in the relative size of the antennae associated with PSII and PSI; (iii) qI, photoinhibitory quenching, is

caused by photoinhibition of PSII and shows very slow relaxation (repair) kinetics in the range of hours.

The down-regulation of PSII activity by qE-quenching is reversible and flexible (Bianchi et al. 2010; Ruban et al. 2012), which is triggered by the light-induced acidification of the thylakoid lumen. Acidification of the lumen induces the NPQ through protonation of a specific PSII protein (PsbS) and activation of the xanthophyll cycle. PsbS, an integral membrane protein that does not appear to bind pigments (Bonente et al. 2008) functions as a sensor of lumen pH (Li et al. 2000; Niyogi 2000; Li et al. 2002; Li et al. 2004). Although the biochemical mechanism is not yet understood, protonation of PsbS seems to promote a rearrangement of the PSII supercomplex in grana. The rearrangement is necessary for rapid induction of NPQ (Betterle et al. 2009; Goral et al. 2012). Activation of the xanthophyll cycle is achieved through activation of violaxanthin de-epoxidase by acidic pH and results in de-epoxidation of violaxanthin to zeaxanthin (Demmig-Adams 1990; Demmig-Adams et al. 2012). The deepoxidation may induce conformational changes of PSII to a quenching mode. Alternative explanation is that zeaxanthin quenches excited state of chlorophyll and eventually dissipates as heat (Blankenship 2001). Collaboration of these two pH-induced processes, PsbS protonation and activation of xanthophyll cycle, allows the accomplishment of a maximal performance of NPQ (Muller et al. 2001; Ruban et al. 2012). According to a photodamage hypothesis claiming that photoinhibition is induced by excess light energy, namely the light energy reaching closed PSII reaction centers, these pH-induced processes contribute

to suppression of photoinhibition of PSII (Niyogi 2000; Vass 2011).

1.3.2. qE-quenching in fluctuating light

Physiological importance of qE-quenching for plant performance is suggested by the observation using *A. thaliana* mutants, *npq1* and *npq4*, with an impaired xanthophyll cycle and deficient in the PsbS protein, respectively. Fitness components, such as the seed number per plant, of these mutants were significantly reduced when grown outdoors under natural fluctuating light conditions or in an artificially fluctuating light in a growth chamber. However, when grown in constant light, these mutants showed no phenotypic defects. These demonstrate that dynamically regulated non-photochemical energy control is an important mechanism providing a strong fitness advantage under field conditions (Külheim et al. 2002; Kulheim and Jansson 2005). Field-grown mutants were photo-inactivated to a greater degree than wild type, whereas the mutant plants grown in the continuous light in the growth chamber showed no photoinhibition of PSII. These results demonstrate that qE-quenching confers an advantage to the wild-type plants through increasing the dynamic range of photosynthesis and thereby allowing the photosynthetic apparatus to utilize light energy optimally. Although it is generally believed that NPQ plays a role in photoprotection (Demmig-Adams and Adams 1992; Horton et al. 1996; Niyogi 1999), NPQ indirectly protects the repair process of photodamaged PSII from the oxidative stress by suppression of production of the reactive oxygen species. It is also worth noting that the direct role of qE-quenching in photoprotection has been

questioned in the two-step hypothesis of PSII photoinhibition (for the two-step hypothesis, see Sarvikas et al. 2006). NPQ may also contribute to the well-balanced excitation of the two photosystems (Peterson and Havir 2001).

Recently, interaction between the regulation of NPQ and plant-pathogen has been suggested. Plants sense potential pathogens by recognizing the conserved pathogen-associated molecular patterns (PAMPs). Gohre et al. (2012) demonstrated that a long-term treatment of *A. thaliana* plants with flg22, a peptide derived from bacterial flagellin, one of the PAMPs, triggered the increase in NPQ in chloroplasts and promoted expression of defense-related genes, but a receptor mutant, *flg22,* did not (Gohre et al. 2012). They have proposed that regulation of NPQ was an intrinsic component of the plant defense program. It may be important, therefore, to note that the plants grown in the field are exposed to more or less a variety of other biotic stresses, such as herbivory by insects and infection by pathogens. These stresses may explain the decrease in fitness of the *npq4* mutant in the field (Külheim et al. 2002).

1.3.3. Enhanced photoprotection and photosynthetic capacity

Depending on whether the same amount of photons is given as a short, bright 'pulse (sunfleck)' or as a longer continuous light, acclimation processes are different. Alter et al. (2012) demonstrated acclimation of *A. thaliana* to fluctuating light regimes of different duration, frequency, and/or intensity but

of the same total PPFD per day. Wild-type plants grown in a fluctuating light regime with short- (less than 12 min) and high- (above 650 μmol photons m^{-2} s^{-1}) lightflecks showed enhanced activities of photoprotection and energy dissipation, presumably because they were unable to utilize efficiently the strong light energy provided in this manner. These acclimation mechanisms involved reorganization of the pigment-protein complexes, resulting in faster light-induced NPQ and the increase in the NPQ capacity, as well as an enhanced activity of superoxide dismutase. Effective acclimation responses to the short lightflecks enabled these plants to cope with photo-oxidative stress induced by these lightflecks. On the other hand, the fluctuating light with longer (for example, 40 min) lightflecks at high light caused plants to up-regulate their electron transport capacity rather than NPQ.

1.3.4. Reaction center quenching

In addition to the dissipation of excess light energy occurring in the PSII antenna via the PsbS protonation and xanthophyll cycle, there is another ΔpH-dependent NPQ process within the PSII reaction center, sometimes referred to as 'reaction center quenching' (Weis and Berry 1987; Krause 1988; Krause and Weis 1991; Walters and Horton 1993). It has been proposed that the conversion of photochemically active, fluorescent, closed PSII reaction centers into photochemically inactive, non-fluorescent PSII reaction centers may serve as an effective mechanism for energy dissipation. The proportion

of the inactive PSII centers to the active centers is dependent on the ΔpH across the thylakoid membrane and the proportion of closed reaction centers measured as the relative reduction state of Q_A (Weis and Berry 1987; Krause and Weis 1991). Thus, over-reduction of Q_A (increase in E_m of Q_A/Q_A^-) has been suggested to be a major prerequisite for this quenching within the PSII reaction center (Krause 1988; Horton 1993; Bukhov et al. 2001; Öquist and Huner 2003). This reversible interconversion of PSII from the photochemical energy transducer to non-photochemical energy quencher could protect the photosynthetic apparatus from the environmental stresses, such as low temperature and high light (Ivanov et al. 2006), which potentially induce the high excitation pressure. Non-radiative charge recombination between Q_A^- and the donor side of PSII has been suggested as a mechanism for dissipating excess energy within the PSII reaction center (Ivanov et al. 2008). This quenching was shown to be extensive during the first several seconds of illumination of dark-adapted plants, even in low light, indicating that the reaction center quenching is triggered by the transient over-acidification of the thylakoid lumen. On the other hand, its disappearance would result from the relaxation of ΔpH across the thylakoid membrane and the activation of the Calvin-Benson cycle (Finazzi et al. 2004). This reaction center quenching would commonly occur in response to sudden increases in the irradiance, depending on the balance between the rate of electron flow and that of the Calvin-Benson cycle. Thus, we propose that the reaction center quenching may serve as an effective response to fluctuating light, especially when periods of extremely low light are long enough to inactivate the Calvin-

Benson cycle enzymes and convert zeaxanthin to violaxanthin.

In contrast to the reversible quenching within the PSII reaction center, the possible involvement of irreversibly photoinhibited PSII as a quencher has been also proposed. Importance of the irreversibly photoinactivated centers as the quencher, would increase with the severity of photoinhibition. This was suggested by the fact that the decline of the proportion of the active PSII did not follow the first-order kinetics (Lee et al. 2001; Chow et al. 2002). Further studies demonstrated that these damaged PSII complexes function as strong quenchers of excess light energy, thus effectively protecting the remaining active PSII reaction centers from photodamage (Matsubara and Chow 2004). However, recent studies (Sarvikas et al. 2010; Kou et al. 2012) have raised a question about the quenching by the photoinhibited PSII because, in these studies, photoinhibition of PSII followed the first-order kinetics (Tyystjärvi et al. 1994; Tyystjärvi and Aro 1996). These discrepancies should be solved. Beside the discrepancy concerning the quenching by the photoinhibited PSII, PSII photoinhibition protects photodamage of PSI, because electron flow to PSI via PSII decreases and production of reactive oxygen species is also suppressed (Sonoike 1996). Although we do not detail in this chapter, PSII photoihibition-repair cycle would be important process that indirectly regulates the photosynthetic electron flows, suppresses formation of reactive oxygen species, and protects PSI from photodamage (Sonoike 1996; Takahashi and Murata 2008; Tikkanen et al. 2013).

1.4 PSI in fluctuating light

1.4.1 Photosynthetic control

"Photosynthetic control" at the Cyt b_6f complex also down-regulate the electron transport (West and Wiskich 1968; Rumberg and Siggel 1969; Hall et al. 1971). This "photosynthetic control" works depending on the phosphate potential, [ATP]/([ADP] x [Pi]), where [ATP], [ADP] and [Pi] stand for concentrations of ATP, ADP and Pi, respectively (for reviews, see Kramer et al. 1999 and Tikhonov 2013). When ATP synthesis occurs intensively, protons are excreted from the lumen to stroma through the H^+-ATP synthase, preventing excessive acidification of the lumen. The moderately acidic pH allows high rate of electron transfer to PSI. However, when ADP and Pi are in shortage, production of ATP is suppressed, lumen pH decreases and thereby the electron transport is decelerated (Takizawa et al. 2008; Kiirats et al. 2009). This acidification of the lumen affects PSII and the Cyt b_6f complex (Takizawa et al. 2007). The pH-dependent modulation of PSII may be accomplished by decelerating the protolytic steps of PSII (Tikhonov et al. 1981). In the proton-coupled electron transport events in the Cyt b_6f complex, the oxidation of plastoquinol (PQH_2) at the Q_0 site is the rate-limiting step. A recent study using *Nicotiana tabacum* indicates that the H^+-ATPase also contributes to the photosynthetic control (Rott et al. 2011). Thus, the light-induced acidification of the lumen is the main factor of the feedback control

of the linear electron transport in chloroplasts. This photosynthetic control may function as photoprotective mechanism in fluctuating light, especially in relatively-prolonged sunflecks in combination with ΔpH-dependent NPQ. However, photoprotective mechanism due to the photosynthetic control is too slow to be active enough upon the extremely rapid increase in light intensity, since the light-induced acidification of the lumen has a lag.

1.4.2. PSI photoinhibition

How is PSI affected under fluctuating light? In contrast to PSII that is highly susceptible to photodamage, it was widely believed that PSI is efficiently protected against photodamage. While PSI is quite resistant to typical high light stress, it is very sensitive to photodamage under certain conditions such as chilling temperatures in certain plants. In *Cucumis sativus*, a chilling sensitive plant, chilling of leaves at moderate light irradiance gives damage to PSI with little damage to PSII (Terashima et al. 1994; Sonoike et al. 1995; Sonoike et al. 1997; Sonoike 2011). Photodamage to PSI has been also shown in other plants (Havaux and Davaud 1994; Ivanov et al. 1998; Tjus et al. 1999). Photoinhibition of PSI to an extent similar to that of PSII photoinhibition has been reported in chilling-tolerant plants such as *A. thaliana* (Zhang and Scheller 2004). PSI photoinhibition would stimulate PSII photodamage because electron flow from PSII is disrupted and electron transport chain is over-reduced, whereas PSII photoinhibition protects PSI from photodamage

(Sonoike 1996; Tikkanen et al. 2013).

1.4.3. The role of PGR5 protein in PSI protection in fluctuating growth light

A recent pioneering paper has proposed an important role of PGR5 in protection of PSI under the fluctuating growth light in *A. thaliana* (Suorsa et al. 2012). The *pgr5* mutant, isolated on the basis of its high chlorophyll fluorescence at high irradiance (Munekage et al. 2002), showed no growth when grown in the drastically fluctuating light, alternating low light for 5 min and high light for 1 min (Tikkanen et al. 2010; Suorsa et al. 2012). In more moderately fluctuating growth light, the plants grew to some extent but the PSI complex in this mutant was photodamaged. They also reported that, under the constant growth light, the *pgr5* mutant did not show any visible growth phenotype irrespective of the growth irradiance levels. The *pgr5* mutant is deficient in development of ΔpH across the thylakoid membrane with the increase in irradiance (Munekage et al. 2002), but under constant light conditions *pgr5* forms a normal ΔpH, in which ATP production is probably driven in a rate similar to that in wild type (Suorsa et al. 2012). In the absence of PGR5, not only rapid induction of qE-quenching but also the pH-dependent photosynthetic control of linear electron flow via the Cyt b_6f complex is suppressed. Therefore, the combination of suppression of NPQ and uncontrolled linear electron flow readily leads to an endangered state for PSI, as demonstrated in the *pgr5* under fluctuating growth light conditions (Suorsa et al. 2012). Inability of the *pgr5* to slow down electron flow via the Cyt b_6f complex upon increase in irradiance results in over-reduction of the

acceptor-side of PSI.

The photoprotection of PSI by PGR5 (Suorsa et al. 2012) and the excitation energy balance provided by the steady-state LHCII phosphorylation (Pesaresi et al. 2009; Tikkanen et al. 2010; Grieco et al. 2012) in fluctuating growth light are key factors for acclimation in that both of these contribute to maintenance of the activity of PSI but not PSII (Grieco et al. 2012). According to Suorsa et al. (2012), when plants were grown under the fluctuating light condition with alternating 5 min of low light (50 μmol photons m^{-2} s^{-1}) and 1 min of high light (500 μmol photons m^{-2} s^{-1}) during the photoperiod, the lack of PGR5 in the *pgr5* was not compensated for by up-regulation of the NDH-mediated CEF. The complete absence of the NDH complex (*ndho* mutant) did not cause growth suppression in the fluctuating growth light. Likewise, PTOX did not seem to play a crucial role under the fluctuating growth light. Although the over-expression of antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase in the *pgr5* plants decreased the level of reactive oxygen species, PSI was not protected against fluctuating light-induced stress. According to Grieco et al. (2012), the functionality of PSI and the response to fluctuating light are regulated by PGR5-dependent control of electron flow in cooperation with steady-state LHCII phosphorylation and NPQ-dependent electron flow control. In short-term response, the balanced excitation and redox balance provided by the steady-state phosphorylation of LHCII is essential to maintain PSI.

1.5 Aims of the study

In my studies for the doctoral thesis, I have done simultaneous measurements of chlorophyll fluorescence and P700 absorption changes at 830 nm with leaves of *Arabidopsis thaliana* plants.

In the experiments described in chapter 2, I have used *pgr5* mutant and measured PSII and PSI parameters at 20, 2.7 and 0% O_2 concentrations to assess the roles of the cyclic electron flow around PSI and O_2-dependent alternative pathway including the water-water cycle in response to short-term fluctuating light.

In the study for chapter 3, I have done photionhibition experiments to examine the effects of some modes of fluctuating light on the H^+-ATPase and especially on photoinhibition of two photosystems in chloroplasts. Further, I have measured the responses of PSII and PSI parameters to short-term fluctuating light to evaluate the effects of fluctuating light for 160 min on regulation of the photosynthetic electron transport system.

In chapter 4, I discuss the results of these results and propose several further studies.

CHAPTER 2

Roles of the cyclic electron flow around PSI (CEF-PSI) and O_2-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in *Arabidopsis thaliana*

2.1. Introduction

Even in open habitats, plants experience dynamic fluctuations of light because of clouds. Understory plants experience more frequent, short-term light fluctuations due to leaves and stems of other plants above them in addition to clouds. Plants have to cope with these light fluctuations of various time ranges. In constant low light, plants can use most of light energy in driving photochemistry. In contrast, in constant high light, energy transfer from antenna chlorophylls to the photosystem II (PSII) reaction center is suppressed and the excess energy is dissipated as heat. This process prevents photoinhibition. When the light intensity fluctuates between low and high levels very rapidly, however, it is not possible for chloroplasts to synchronously switch on and off the heat dissipation system with the light fluctuation because both induction and deactivation of the heat dissipation system require at least several minutes (Muller et al. 2001). Plants must have more rapid systems to cope with very rapid light fluctuations.

Photosynthetic electron transport primarily occurs via a linear pathway, in which electrons flow from water via PSII and cytochrome b_6f complex to PSI and reduce $NADP^+$ to NADPH. The linear electron flow (LEF) generates the transmembrane electrochemical potential difference of H^+, through water splitting by PSII in the thylakoid lumen and translocation of H^+ across the thylakoid membrane by the Q cycle. The electrochemical potential difference thus produced drives the H^+-ATP synthase to produce ATP. Low pH in the thylakoid lumen causes de-epoxidation of violaxanthin to

zeaxanthin via antheraxanthin and protonation of the PsbS protein, both of which contribute to the heat dissipation, which can be measured fluorometrically as the non-photochemical quenching (NPQ).

In addition to the LEF system, there are two PSI cyclic electron flow (CEF) systems (Shikanai 2007): the NADH dehydrogenase-like complex-dependent pathway (NDH-CEF, Burrows et al. 1998; Shikanai et al. 1998; Peng et al. 2011; Yamamoto et al. 2011) and the ferredoxin-plastoquinone reductase pathway (FQR-CEF, Munekage et al. 2002, 2004; DalCorso et al. 2008; Hertle et al. 2013). FQR-CEF involves cytochrome *b*$_6$*f* complex, plastocyanin, PSI, ferredoxin (Fd) and ferredoxin-plastoquinone reductase (FQR). PGR5 was identified as an essential component of the FQR-CEF (Munekage et al. 2002, see below). Very recently, PGRL1 has been proposed to be the elusive FQR (Hertle et al. 2013). In C_4 plants, the cyclic electron flows around PSI (CEF-PSI), particularly the NDH-CEF, operate to supply ATP to the CO_2 concentrating mechanism (Takabayashi et al. 2005) as well as the Calvin Benson cycle. It is noteworthy that the NDH-CEF also involves ferredoxin (Okegawa et al. 2008; Johnson 2011; Yamamoto et al. 2011). It is often claimed that the ATP and NADPH production by the LEF cannot meet the required ATP/NADPH ratio for the photosynthetic carbon fixation by the Calvin-Benson cycle. In particular, when photorespiration occurs at high rates, the required ratio shifts from 3ATP/2NADPH towards 3.5ATP/2NADPH, and thereby shortage of ATP may be more serious (Allen 2002; Shikanai 2007). The CEF-PSI would contribute to producing additional ATP. Another function of CEF-PSI is enhancement of the NPQ, through

generating the electrochemical potential difference of H^+ across the thylakoid membrane (Munekage et al 2002).

The pseudo-cyclic electron flow, also called the water-water cycle (WWC) (Asada 1999) or the Mehler-ascorbate peroxidase (MAP) pathway (Schreiber et al. 1995), is the electron flow from water via PSII, cytochrome *b6/f*, and PSI to molecular oxygen. Since the redox potentials of the electron acceptors on the acceptor side of the PSI complex are sufficiently low to reduce O_2, the electron flow to O_2 occurs, particularly when $NADP^+$ is not available. This results in the formation of reactive oxygen species (ROS), such as O_2^- and H_2O_2 (Asada 1999). Superoxide dismutase and ascorbate peroxidase in the WWC scavenge O_2^- and H_2O_2. NADPH is used to regenerate ascorbate from monodehydroascorbate or dehydroascorbate. Summing up these reactions, electrons are transferred from water to H_2O_2 to form water. Thus, the WWC acts as a large electron sink (Asada 2000). The WWC also generates the electrochemical potential difference of H^+ across the thylakoid membrane, which enhances the non-radiative dissipation of excess light energy observed as the increase in NPQ. Therefore, the WWC is also considered to play roles in dissipation of excess light energy (Osmond and Grace 1995; Osmond et al. 1997; Asada 1999, 2000; Foyer and Noctor 2000; Miyake 2010). The CEF-PSI and WWC are argued to protect plants from damages that occur due to the over-reduction of the thylakoids under stress conditions (Miyake 2010).

An *Arabidopsis thaliana* mutant, *pgr5* (*proton gradient regulation*), was reported to have the impaired electron transfer in FQR-CEF (Munekage et al. 2002). In the screening using the chlorophyll fluorescence imaging

technique, this mutant showed a phenotype similar to that of *npq* mutants (Shikanai et al. 1999). NPQ measurements with this mutant showed an almost complete absence of qE-quenching at high irradiance under steady-state photosynthesis (Munekage et al. 2002). Nandha et al. (2007), however, reported that the capacity of the cyclic electron transport in *pgr5* was comparable to that of the wild-type. They also showed that the electron transport system in *pgr5* was largely reduced under most conditions.

A recent paper has proposed an important role of PGR5 in protection of PSI under the fluctuating growth light in *Arabidopsis thaliana* (Suorsa et al. 2012). The *pgr5* mutant showed no growth when grown in the drastically fluctuating light, alternating low light for 5 min and high light for 1 min (Tikkanen et al. 2010; Suorsa et al. 2012). In a more moderately fluctuating growth light, the PSI complex in this mutant was found to be photodamaged. They also reported that, under the constant growth light, the *pgr5* mutant did not show any visible growth phenotype irrespective of the growth irradiance levels. From these, they argued that the *pgr5* could not maintain redox balance of the electron transfer reactions in the fluctuating light. However, how the redox imbalance occurs in *pgr5* and how the wild-type plants cope with the drastically fluctuating light are still unclear.

The aim of this study was to examine photosynthetic responses of the wild-type (WT) and *pgr5* plants, both grown in the continuous moderate light in the light period, to a fluctuating light using simultaneous chlorophyll fluorescence and P700 measurements under the precise control of gas concentrations. The fluctuating light adopted was alternation of low light for

2 min and high light for 2 min. Even for high light, a moderate level of photosynthetically active photon flux density (PPFD) was chosen. I examined whether photoinhibition of PSI occurred in the mature leaves of the *pgr5* plants in short-term experiments for up to 42 min. Next, I tried to elucidate which of the photosynthetic alternative electron flows was impaired in the *pgr5* plants through examining the effects of O_2 concentrations at various PPFDs.

2.2. Materials and methods

Plant materials

Arabidopsis thaliana wild-type (ecotype Columbia *gl1*) and *pgr5* mutant (Munekage et al. 2002) plants were pot grown in a growth cabinet with white fluorescent light at 90-100 μmol photons m^{-2} s^{-1} for 8-h photoperiod at room temperature of 23℃ and relative humidity of 60%. Plants were irrigated two to three times weekly and were fertilized with Hyponex 6-10-5 solution (Hyponex Japan, Osaka, Japan) diluted to the 1: 1000 strength every irrigation from two weeks after germination. Mature rosette leaves from 7- to 9-week-old plants were used in the experiments. An *A. thaliana* mutant, *crr2-2,* were also used. The growth conditions of these plants were the same as those for the wild type.

Chlorophyll fluorescence and 830 nm absorbance change measurements

Chlorophyll fluorescence and absorption changes at 830 nm were measured simultaneously using a Dual-PAM-100 (chlorophyll fluorescence and P700 absorption analyzer equipped with a P700-dual wavelength-emitter at 830 and 875 nm, Walz, Effeltrich, Germany) with the intact leaf in a hand-made leaf chamber. CO_2 concentration in the leaf chamber was monitored with a LI-6400 (Li-Cor, Lincoln, NE, USA). O_2 concentration in the air was controlled by mixing N_2 gas and O_2 gas using mass flow controllers. Saturation pulses

(SP) from red light-emitting diodes (LEDs; > 8000 μmol photons m^{-2} s^{-1}, 400 ms duration) were applied to determine the maximum chlorophyll fluorescence with closed PSII centers in the dark (F_m) and in the actinic light (F_m'). Maximum photochemical quantum yield of PSII (F_v/F_m) and effective quantum yield of PSII (Y(II)) were calculated as $(F_m - F_0)/F_m$ and $(F_m' - F_s')/F_m'$ (Genty et al. 1989), respectively, where F_s' is the steady-state chlorophyll fluorescence level in the actinic light from red LEDs with wavelength peak at 635 nm, in which chloroplast avoidance movement does not occur and has no effect on assessment of non-photochemical quenching components (Cazzaniga et al. 2013). The coefficient of non-photochemical quenching, qN, was calculated as $(F_m - F_m')/(F_m - F_0')$. F_0' is minimal fluorescence yield in the actinic light and was estimated using the approximation of Oxborough and Baker (1997) as $F_0/(F_v/F_m + F_0/F_m')$. Two other PSII quantum yields, Y(NPQ) and Y(NO) (Genty et al. 1996; Kramer et al. 2004a), which represent the regulated and non-regulated energy dissipation at PSII centers respectively and add up to unity with the photochemical quantum yield (i.e. Y(II) + Y(NPQ) + Y(NO) = 1), were also used. Y(NPQ) and Y(NO) were calculated as $F_s'/F_m' - F_s'/F_m$ and F_s'/F_m, respectively (Hendrickson et al. 2004; Klughammer and Schreiber 2008a). The coefficient of photochemical quenching, qL, a measure of the fraction of open PSII reaction centers, based on the "lake model" of PSII antenna pigment organization, was calculated as $(F_m' - F_s')/(F_m' - F_0')\cdot F_0'/F_s'$ (Kramer et al. 2004a).

In the Dual-PAM-100, $P700^+$ was monitored as the absorption

difference between 830 and 875 nm in a transmission mode. In analogy to chlorophyll fluorescence yield, the quantum yield of PSI was determined using the saturation pulse method (Klughammer and Schreiber 1994; Klughammer and Schreiber 2008b). Maximum level of P700 signal (P700 fully oxidized) in the dark, called P_m, was determined by application of a SP in the presence of far-red light at the wavelength of 720 nm. The zero P700 signal, P_0, was determined when complete reduction of P700 was induced after the SP in the absence of far-red light. P_m' is the maximal P700 signal in the presence of actinic light induced by the SP. The photochemical quantum yield of PSI, Y(I), was calculated from the complementary PSI quantum yields of non-photochemical energy dissipation, Y(ND) and Y(NA), respectively: $Y(I) = 1 - Y(ND) - Y(NA)$. Y(ND) corresponds to the fraction of P700 that is already oxidized by actinic light, and Y(NA) corresponds to the fraction of P700 that cannot be oxidized by a SP to the overall P700. These calculations were made according to Klughammer and Schreiber (2008b). To oxidize the inter-system electron carriers, far-red light was applied from 100 ms before the start of the SP to its cessation. As shown in Fig. 2, Y(I) did not decrease under the constant HL. My preliminary checks showed that the SP of 400 ms duration was enough to induce maximal $P700^+$ oxidation level and to obtain complete reduction level of P700 after the SP. Photodamage by the SP did not occur.

The proportions of the non-photochemical quenching components were determined from the relaxation kinetics of the variable fluorescence (F_v) in the absence of actinic light for 30 min (Quick and Stitt 1989; Walters and

Horton 1991). Relaxation of F_v was monitored with saturation pulses given every 100 s to the leaf. The intervals of 100 s were sufficient to eliminate an effect of saturation pulse on F_v relaxation. The fast-relaxing component of fluorescence quenching was assigned to the energy-dependent mechanism (qE), the intermediate relaxing component was assigned to the state transition (qT), and the slow relaxing component was assigned to the photoinhibitory processes (qI). For quantification of qE, qT and qI, the semi-logarithmic plot of F_v versus time was analyzed considering the relationship $(1 - qN) = (1 - qE) \times (1 - qT) \times (1 - qI)$.

I estimated the electron transport rate through PSI (ETR(I)) and PSII (ETR(II)) simultaneously. In this study, a source of artifacts should be considered for a comparison of ETR(I) with ETR(II). With the blue measuring light, chlorophyll fluorescence signal mainly emitted from the upper layer of the mesophyll cells closest to the emitter detector unit was detected, while the P700 signal detected was more generally from the whole leaf tissue. In high light, these upper cells would be prone to light saturation of photosynthesis and photoinhibition than the cells in the deeper layer (Terashima et al. 2009; Oguchi et al. 2011a and b). To effectively prevent this preferential light saturation of photosynthesis and photoinhibition near the leaf surface, red light at 635 nm of peak wavelength instead of blue light was used as the actinic light. The red actinic light at this wavelength reaches the deeper cell layers, and would cause more even light saturation of photosynthesis and photoinhibition than the blue actinic light.

PSI fluorescence may contribute to total leaf fluorescence (Pfundel

1998; Rappaport et al. 2007). In this study, blue light at 460 nm of peak wavelength was used as the measuring light, except for the data shown in Fig 5, see below. The blue measuring light excites PSII more than that from PSI. In addition, according to Pfundel et al. (2013), emission of PSI fluorescence by the *A. thaliana* leaves is low irrespective of growth PPFD levels.

The P700 signal can be interfered by the absorbance changes of plastocyanin. Up to 10% of the P700 difference absorption signal measured by the DUAL-PAM instrument may be attributable to that of plastocyanin, which shows considerable absorption at both 830 to 870 nm (Kirchhoff et al. 2004). Livingston et al. (2010) compared the results with the Dual-PAM system and those using the two-wavelength deconvolution method (ΔA of 820-950 nm) described by Oja et al. (2004) and concluded that absorbance changes from plastocyanin or other components may not substantially affect the P700 measurements.

Measurements of the share of absorbed light energy allocated to photosystem II

To estimate the share of absorbed light energy allocated to PSI and PSII, simultaneous measurements of O_2 evolution and chlorophyll fluorescence in the leaf were made at 23℃ using a leaf-disk oxygen-electrode system (LD2, Hansatech, Kings Lynn, UK) and a chlorophyll fluorometer (PAM-2500, Walz, Effeltrich, Germany).

Leaf segments were placed in the chamber of the leaf-disk O_2

electrode. When the steady-state rate of O_2 evolution was attained, the quantum yield of PSII photochemistry (Y(II)) was measured. Irradiance of actinic light was increased in a stepwise manner. A Björkman-type lamp equipped with a red color filter of the wavelength centered at 635 nm was used as the light source. The red light was used to mimic the spectrum of the actinic light of the Dual-PAM system. PPFD was altered with neutral density filters (Toshiba, Tokyo, Japan). The air in the chamber contained about 5% CO_2 and 15% O_2. The quantum yield of O_2 evolution ($Y(O_2)$) of the leaf was calculated by dividing the rate of gross O_2 evolution per leaf area (μmol O_2 m^{-2} s^{-1}) by absorbed PPFD. Absorptance of the leaf was measured with a handmade integrating sphere, whose inside was coated with $BaSO_4$, and a quantum sensor (LI-190SA, Li-Cor). When f_{PSII}, the share of absorbed light energy allocated to PSII is less than 0.5, the relationship between quantum yield of O_2 evolution at saturating CO_2 ($Y(O_2)$) and that of PSII electron transport (Y(II)) can be expressed as $Y(O_2) = I_A \times f_{PSII} \times Y(II)/4$, where I_A is the absorbed PPFD (Genty et al. 1989).

This equation, which compares gross O_2 evolution from the whole tissue with Y(II) obtained from the shallow part of mesophyll, may lead to uncertainty in f_{PSII}. The error in f_{PSII} gives rise to uncertainty in ETR(II). Since fluorometrically estimated ETR(II) tends to be underestimated compared with that calculated from the gross O_2 evolution rate, especially at high PPFDs, ETR(I)/ETR(II) ratio calculated with fluorometrical ETR(II) would be overestimated (Kou et al. 2013). To obtain chlorophyll fluorescence signal from the deeper mesophyll cells with the PAM-2500, I used red light

peaked at 630 nm as the measuring light. The use of the red measuring light, rather than blue light, would minimalize the error in estimation of ETR(II), particularly that at relatively low PPFDs. Thus, I used data points obtained at low PPFDs. Effects of fluorescence from PSI would be small at low PPFDs.

Determination of chlorophyll content

Chlorophyll *a* and *b* contents were determined according to Porra et al. (1989).

2.3. Results

Responses of PSII and PSI quantum yields to fluctuating and continuous light

Chlorophyll fluorescence and absorption changes at 830 nm in the intact leaf were measured simultaneously using a Dual-PAM-100 (Walt GmbH, Germany). Changes in the PSII quantum yield, Y(II), in mature leaves of the wild-type (WT) and *pgr5* plants were measured in the light regime that alternated between high light (HL) at 240 μmol photon m^{-2} s^{-1} for 2 min and low light (LL) at 30 μmol photon m^{-2} s^{-1} for 2 min, for a total of 42 min (Fig. 1). The leaf was kept in a small hand-made chamber, and CO_2 and O_2 gas concentrations in the chamber were regulated with mass-flow controllers. Unless otherwise stated, the CO_2 and O_2 concentrations were 390 ppm and 20%, respectively. In the leaves of WT plants grown in the constant light at 90 - 100 μmol photon m^{-2} s^{-1} for 8 h /day, Y(II) at the end of each LL-period decreased with the cycle, but Y(II) at the end of each HL-period did not change after attaining the steady value around 0.45. In *pgr5*, Y(II) in LL-period decreased with the cycle more markedly than in WT. Y(II) in HL-period also decreased after the fifth cycle. In the last cycle, Y(II) in LL-period became 0.4, approaching that in HL-period being around 0.3.

To compare the photosynthetic responses in the fluctuating light and those in the continuous light, I measured changes in Y(II) and the PSI quantum yield, Y(I), in the constant HL at 240 μmol photon m^{-2} s^{-1} or LL at

30 μmol photon m^{-2} s^{-1} for 42 min (Fig. 2). When the plants that had been kept in the dark for more than 30 min were exposed to the constant HL, Y(I) of WT increased for about 5 min and attained a steady level, while that in LL gradually decreased. Y(II) in HL once decreased and attained a steady level, while that in LL decreased and attained the peak value at around 5 min and slightly decreased. In the *pgr5* plants, Y(I) in LL showed a transient similar kinetic to that of WT. Y(I) in HL, however, once decreased, increased to the peak at around 10 min and then decreased very slightly. Changes in Y(II) in *pgr5* were similar to those in WT. In HL, both Y(I) and Y(II) in *pgr5* were considerably lower than those in WT.

Light responses of the steady-state PSI and PSII parameters at various PPFDs

Light responses of PSI and PSII parameters obtained from chlorophyll fluorescence and P700 signals were further analyzed (Fig. 3). For energy captured by PSI pigments, the quantum yield of the PSI photochemistry, Y(I), the quantum yield of non-photochemical energy dissipation due to the donor-side limitation, Y(ND), and that of the energy dissipation due to the acceptor-side limitation, Y(NA), were measured. The fluorescence parameters measured included the effective PSII quantum yield, Y(II), the quantum yield of regulated energy dissipation, Y(NPQ), and that of non-regulated energy dissipation, Y(NO).

Y(I) and Y(II) in both WT and *pgr5* decreased with the increase in PPFD. In WT, Y(NA) was greater than Y(ND) at PPFDs less than 250 μmol photon m^{-2} s^{-1}, while, above this level, Y(NA) decreased and Y(ND) increased. In WT, with the increase in PPFD, Y(NPQ) markedly increased, while Y(NO) increased only slightly. *pgr5* showed trends very different from those of WT. Y(NA) of *pgr5* was similar to that of WT up to 100 μmol photon m^{-2} s^{-1}, but it markedly increased with further increases in PPFD, causing the drastic decrease in Y(I) in *pgr5* at PPFDs above 150 μmol photon m^{-2} s^{-1}. Y(ND) in *pgr5* was nearly zero over the entire PPFD range. These results indicate that, at high PPFDs, the electron flow through PSI in *pgr5* was limited by the acceptor-side reactions. Furthermore, the increase in Y(NPQ) was much less than that in WT, while Y(NO) markedly increased.

Effects of fluctuating light on photoinhibition of photosystems and photosynthetic electron transport

Maximum level of P700 signal (full oxidation of P700) in the dark (ΔA_{max}) and maximum quantum yield of PSII (F_v/F_m) were measured before and after the treatment with the constant HL (240 μmol photon m^{-2} s^{-1}) or the fluctuating light (alternating between HL at 240 μmol photon m^{-2} s^{-1} for 2 min and LL at 30 μmol photon m^{-2} s^{-1} for 2 min), both for 42 min (Fig. 4). After the light treatments, plants were kept in the dark for 30 min and ΔA_{max} and F_v/F_m were measured. ΔA_{max} and F_v/F_m were unchanged after the constant HL treatment

for 42 min from the levels before the treatment (Fig. 4a, c), indicating that photoinhibition of photosystems hardly occurred. Y(NA) and 1 – qL were measured in LL at 30 μmol photon m^{-2} s^{-1} for 5 min before and after the 42-min light treatment. The data obtained at the end of 5-min LL are denoted as $Y(NA)_{30}$ and $1 – qL_{30}$, respectively. After the HL treatment, both $Y(NA)_{30}$ and $1 – qL_{30}$ in *pgr5* were significantly higher than before (Fig. 4b, d), indicating some damage to the acceptor side of PSI.

WT showed small decreases both in ΔA_{max} and F_v/F_m after the treatment with the fluctuating light treatment (Fig. 4e, g). In contrast, ΔA_{max} in the *pgr5* plants after the fluctuating light treatment decreased by 38%, while F_v/F_m decreased only slightly. Although there were only small decreases in ΔA_{max} and F_v/F_m in WT, $Y(NA)_{30}$ and $1 – qL_{30}$ after the fluctuating light treatment increased by 42% and 135% (Fig. 4f, h), respectively, indicating some damage to the acceptor-side of PSI and competence of PSI in oxidizing the intersystem chain. In *pgr5*, $Y(NA)_{30}$ and $1 – qL_{30}$ increased by 94% and 332% after the fluctuating light treatment.

Do the *pgr5* plants show the CEF-PSI activity in constant light?

To investigate whether the *pgr5* plants showed CEF-PSI activity in constant light, I estimated the electron transport rate through PSI (ETR(I)) and PSII (ETR(II)) simultaneously. The photochemical quantum yield of PSI, Y(I), may be expressed as $Y(I) = Y(L_I) + Y(WWC) + Y(CEF_I)$, where $Y(L_I)$, Y(WWC), and

$Y(CEF_I)$ are the quantum yields of the LEF through PSI, the WWC, and the CEF-PSI, respectively. Similarly, the photochemical quantum yield of PSII, Y(II), may be written as $Y(II) = Y(L_{II}) + Y(WWC)$, where $Y(L_{II})$ is the quantum yield of the LEF through PSII.

To obtain directly comparable ETR(I) and ETR(II) values, I measured leaf absorptance, and estimated the share of absorbed light energy allocated to PSII (f_{PSII}). Fig. 5A shows relationships between the gross O_2 evolution in the air containing 5% CO_2 and the absorbed PPFD at low PPFDs calculated with the absorptance values, measured in four leaves each of WT and *pgr5*, respectively. When compared at the same absorbed PPFD, the O_2 evolution rates for *pgr5* leaves was always lower than that for the WT leaves, indicating the quantum yield of O_2 evolution on absorbed quantum basis was lower in the *pgr5* leaves. However, F_v/F_m values in the leaves did not differ between WT and *pgr5*. Fig. 5B shows relationships between the quantum yield of gross O_2 evolution ($Y(O_2)$) and that of PSII photochemistry (Y(II)) in the same leaves used for Fig. 5A. The slope of the line should be proportional to the share of absorbed light energy allocated to PSII (f_{PSII}). In the *pgr5*, the slope was lower by 17.3% than in the WT. The share of absorbed light energy allocated to PSII was 35 ± 3.4% in *pgr5*, while the share in WT was 47 ± 4.1%. With these values, it would be possible to calculate the absolute rates of ETR(I) and ETR(II).

In the WT plants, the ETR(I)/ETR(II) ratio was close to 1 at PPFDs below 100 μmol photon m^{-2} s^{-1}, and subsequently increased with the increase in PPFD. In contrast, the ETR(I)/ETR(II) ratio in *pgr5* was high at low PPFDs

and decreased with the increase in PPFD and eventually became close to 1 (Fig. 6). In accordance with the view accepted widely, contribution of the CEF-PSI increased with the increase in PPFD in WT, while *pgr5* showed a contrasting trend: the contribution of CEF-PSI decreased with the increase in PPFD.

The ETR(I)/ETR(II) ratio would not be correct if there were large changes in the energy share between PSII and PSI due to the state transition. To examine the contribution of the state transition on the change in the ETR(I)/ETR(II) ratio, I determined the components of non-photochemical chlorophyll fluorescence quenching, qN, from the relaxation kinetics of qN in the dark. Fig. 7 shows the extents of energy-dependent quenching (qE), state transition quenching (qT) and photoinihibitory quenching (qI) after 20 min of constant light at PPFDs of 30, 240 and 470 μmol photon m^{-2} s^{-1}. Both WT and *pgr5* leaves exhibited substantial qE, although qE in the *pgr5* leaf was much less than that in WT plants as was reported previously (Munekage et al. 2002, 2008). qT components were small compared to qE in both plants. As the measurements were carried out with red actinic light, there was no effect of chloroplast avoidance movement on apparent state transition (Cazzaniga et al. 2013). Therefore, it is unlikely that the state transition affected the ETR(I)/ETR(II) very much.

Table 1 shows the chlorophyll contents on leaf area basis in WT and *pgr5* leaves. The chlorophyll content was higher in WT than in *pgr5*. There were some differences in absorptance, reflecting the difference in the chlorophyll content. The chlorophyll *a/b* ratio in the *pgr5* leaves was greater

than that in WT leaves by 0.4.

Does the WWC in the *pgr5* plants function in constant light?

Next, I investigated whether the *pgr5* plants were able to drive the WWC. In the WWC, the electron acceptor from PSI is O_2 and the extent of the electron flow to the WWC depends on O_2 concentration (Miyake and Yokota 2000). The CEF-PSI activity has been shown to increase at low O_2 concentration, indicating suppression of the WWC by low O_2 (Arnon and Chain 1975, 1979; Scheller 1996; Makino et al. 2002). If the *pgr5* plants possessed no WWC capacity, the activity of the CEF-PSI would not be enhanced even at low O_2 concentrations. I measured the light dependence of the ETR(I)/ETR(II) ratio on the O_2 concentration in the chamber, namely at 20, 2.7 and 0% O_2 (Fig. 8). In the *pgr5* plants, the ETR(I)/ETR(II) ratios at 2.7 and 0% O_2 were higher than that at 20% O_2 when PPFD was greater than 100 μmol photon m^{-2} s^{-1}. Both WT and *pgr5* exhibited the highest ETR(I)/ETR(II) ratios at 2.7% O_2 at any PPFD below 300 μmol photon m^{-2} s^{-1}. Therefore, it is likely that the *pgr5* plants possessed the WWC capacity.

Response of the ETR(I)/ETR(II) ratio to fluctuating light

Changes in the ETR(I)/ETR(II) ratio in the fluctuating light are shown in Fig.

9. The same fluctuating light regime used for the data in Fig. 1 was used. Being consistent with the data in Fig. 6, the ETR(I)/ETR(II) ratio in the *pgr5* plants was lower in HL-period than in LL-period, whereas WT plants showed the higher ETR(I)/ETR(II) ratio in HL-period than in LL-period. In WT, ETR(I)/ETR(II) ratio was maximum at 15 s after each transfer from LL-period to HL-period, and, within 2 min, decreased slowly toward the steady-state value. The maximal value at 15 s after the transfer gradually increased with the cycle, while the steady-state value slightly decreased. In contrast, in *pgr5*, the ETR(I)/ETR(II) ratio rapidly decreased to the minimum at 15 s after the transfer from LL-period to HL-period. The minimal value gradually increased with the cycles. Also the ratio at the last data point in the HL-period increased with the cycle. The peak value in the 2 min LL-period gradually increased. In each of the LL-periods, the ratio decreased.

Effects of O_2 concentration on responses of electron transport to fluctuating light

Responses of Y(II) to the fluctuating light were measured at 2.7 and 0% O_2 (Fig. 10). In *pgr5*, Y(II) at the end of the LL-period decreased only slightly with the cycle at 2.7% O_2. The decrease in Y(II) in LL was further smaller at 0% O_2. Moreover, in contrast to the gradual decrease in Y(II) in HL-period at 20% O_2, Y(II) in HL increased with the cycle at 2.7 and 0% O_2. Similarly, in WT, Y(II) at the end of the LL-period did not decrease with the cycle at 2.7 or

0% O_2, whereas Y(II) at the end of the LL-period decreased at 20% O_2. Y(II) in the HL-period at 2.7 and 0% O_2 continued to increase with the cycle and did not reach the steady-state values within 42 min.

I assessed the degrees of photoinhibition after the treatment with the fluctuating light for 42 min at 0, 2.7 or 20% O_2 and dark treatment for 30 min (Fig. 11) using the same experimental protocol that was used for the data shown in Fig. 4. The *pgr5* plants showed little photoinhibition of PSI at 2.7 and 0% O_2 compared with that at 20% O_2 (c.f. Fig. 4e and Fig. 11a and e). The marked increases observed in $Y(NA)_{30}$ and $1 - qL_{30}$ after the light treatment at 20% O_2 were much suppressed at low O_2, although the increases were consistently observed not only in *pgr5* but also in WT.

Responses of PSII and PSI quantum yields to fluctuating light in NDH-deficient mutant

I measured the responses of an NDH-deficient mutant, *crr2-2*, to the fluctuating light under the same conditions as those use for Fig.1 (Fig. 12). The results in the *crr2-2* were almost identical to those in WT. Y(II) of *crr2-2* decreased with the cycles. The light dependence of the ETR(I)/ETR(II) ratio exhibited trends similar to those in WT (Fig. 13). Moreover, at any PPFD, the ratios at 2.7 and 0% O_2 were higher than that at 0% O_2.

2.4. Discussion

Fluctuating light as a stress factor causing photodamage

How plants use sunflecks, pulses of light at high intensity, is the topic that has attracted attention of researchers for decades (Allee 1926; Evans 1956). There have been many laboratory-based mechanistic studies as well as field-oriented studies focusing on photosynthetic responses to the fluctuating light. For instance, photosynthetic responses of the plants grown in controlled-fluctuating light and those grown in constant light were compared (Yin and Johnson 2000; Alter et al. 2012; Suorsa et al. 2012). The responses were also compared between plants grown in natural fluctuating light in a forest understory and those grown in an open field site (Knapp and Smith 1989). In most of these studies, photosynthetic responses to the single light pulse were examined. However, in natural environments, such as the forest understory, light fluctuates more frequently, as many researchers quantified (Pearcy 1983, Chazdon 1988; Vierling and Wessman 2000). Although there have been some pioneering studies (Alter et al. 2012; Suorsa et al. 2012), our knowledge of the photosynthetic responses to the fluctuating light is still poor.

It is important to choose appropriate fluctuating light regimes for studying plant responses to the fluctuating light. The light environment in the forest understory drastically changes due to sunflecs. Most sunsflecks are less than several minutes in length, and have PPFD more than several-fold that of LL-periods (Pearcy 1983; Koizumi and Oshima 1993; Vierling and

Wessman 2000). Recently, Suorsa et al. (2012) grew several mutant lines of *Arabidopsis thaliana* in the light alternately changing from low light (LL) at 50 μmol photon m^{-2} s^{-1} for 5 min to high light (HL) at 500 μmol photon m^{-2} s^{-1} for 1 min and successfully elucidated a role of the PGR5 protein in acclimation to the fluctuating light. In the present study, I used a fluctuating light regime with the same durations of HL and LL. The duration I adopted was 2 min because the photosynthetic parameters most drastically change upon the change from the LL- to HL-period in the first 2 min in *A. thaliana* plants. Thus, the light fluctuation in the 2 min intervals would subject the plants to the most stressful situation. Next, I chose intensity of the HL. The HL at 240 μmol photon m^{-2} s^{-1} was strong enough, but induced no photoinhibition when given continuously. The present results indicate that the fluctuating light I adopted was suitable for analysis of the effects of fluctuating light on photoinhibition. The results clearly showed that light fluctuation itself is a very effective stress factor causing photodamage. I propose the term 'fluctuating light photoinhibition' and the target is mainly PSI as has been already indicated by the pioneering studies (Munekage et al. 2002, 2008; Suorsa et al. 2012).

Effects of short-term fluctuating light on photosynthetic electron transport system

The decreases in the photochemical quantum yield of PSII, Y(II), of the WT

and *pgr5* plants occurred soon after the start of the fluctuating light treatment. Y(II) of *pgr5* decreased more drastically (Fig. 1). Moreover, these plants showed photoinhibition of PSI by the fluctuating light treatment. In particular, the extent of PSI photoinhibition in *pgr5* was marked. It should be noted that this was not the result of the long-term effect of the fluctuating 'growth' light (Suorsa et al. 2012) but that of the treatment for a short period. It has been reported that PSI of *pgr5* was sensitive to light (Munekage et al. 2002, 2008). In the present study, I found that PSI activity in *pgr5* was limited by the acceptor-side reactions: Y(NA) was higher than Y(ND) over the entire PPFD range examined (Fig. 3).

What component/event in the PSI acceptor-side did limit photochemical reaction and thereby cause PSI photoinhibition? The crucial difference between the fluctuating light and constant light was that fluctuating light included LL-period, during which photosynthetic activities were lower than those at HL-period. When leaves were in the LL, various reactions that had occurred in response to HL, including the de-epoxidation of violaxanthin and protonation of the PsbS protein, would be relaxed to some extents. In HL-period, the thylakoid lumen acidification would not be enough for down-regulation of LEF via the photosynthetic control of plastoquinol re-oxidation at Cyt b_6f complex (Rott et al. 2011; Suorsa et al. 2012), particularly in *pgr5*. Therefore, especially in *pgr5*, when the every HL-period started, the thylakoids in the more or less relaxed state would cause a gush of electron flow to PSI leading to prompt reduction of electron acceptors, O_2 photoreduction, and formation of ROS. From the preceding studies (Sonoike

1996; Sonoike et al. 1997; Choi et al. 2002), it is clear that the photoinhibition of PSI involves ROS and thereby require O_2.

It is noteworthy that the PSI photoihibition occurred even in the WT plants by the fluctuating light; the photodamage by the fluctuating light is not a phenomenon specific only to *pgr5*.

The effective responses avoiding photoinhibition to the fluctuating light

The large Y(NA) means the high level of PSI acceptor-side limitation. This is not necessarily directly associated with photoinhibition of PSI. As clearly shown in Fig. 4, *pgr5* treated in continuous HL showed little photoinhibition of PSI, although $Y(NA)_{30}$ and $1 - qL_{30}$, measured at the end of the illumination of 30 μmol photon m^{-2} s^{-1} for 2 min, just after the constant HL for 42 min, were very high. On the other hand, when fluctuating light was applied for 42 min, PSI photoinhibition occurred in both WT and *pgr5*, in addition to the increases in $Y(NA)_{30}$ and $1 - qL_{30}$. The extent of the damage was markedly greater in *pgr5*. The large difference in the PSI photoinhibition between the WT and *pgr5* plants indicates that WT had mechanisms to cope with rapid light fluctuations. I hypothesized that this would be related to photosynthetic alternative electron flows interacting with PSI because PSI was firstly photoinhibited in the fluctuating light. Previous works reported that, in *pgr5*, the CEF-PSI via the putative ferredoxin-dependent quinone reductase (FQR)

was impaired (Munekage et al. 2002, 2004; DalCorso et al. 2008). However, some other studies reported that *pgr5* possessed CEF-PSI capacity (Nandha et al. 2007; Joliot and Johnson 2011). I tried to assess the activities of the alternative electron flows in *pgr5*.

Several methods have been used to quantify the rate of the CEF-PSI (for a review, see Kramer et al. 2004b). In LEF, the rates of electron transfer through PSII should equal that through PSI (Klughammer and Schreiber 1994) or the Cyt b_6f complex (Klughammer and Schreiber 1994; Sacksteder and Kramer 2000). Thus, the relationship between some factors associated with the electron flow and the LEF should be changed when the activity of the CEF-PSI becomes substantial. In turn, from the increased ratios of these factors to LEF, the rate of the CEF-PSI would be assessed. The possible factors would include the proton to electron stoichiometry (Sacksteder et al. 2000), electrochromic shift of carotenoid pigments due to the electric field formation across the thylakoid membrane (Joliot and Joliot 2002, 2005; Joliot et al. 2004; Sacksteder and Kramer 2000), and the proportion of overall photosynthetic energy storage assessed by the photoacoustic method (Herbert et al. 1990; Joet et al. 2002). More directly, measurements of post-illumination re-reduction kinetics of $P700^+$ after red + far-red actinic light (Fan et al. 2007), or after a far-red illumination (Maxwell and Biggins 1976; Joet et al. 2002; Chow and Hope 2004), have been conducted. The transient rise in the fluorescence level after turning off the actinic light has been also measured as a parameter reflecting the activity of CEF-PSI (Asada et al. 1993; Burrows et al. 1998). However, it is not feasible

to measure the absolute rate of the CEF-PSI in situ with these techniques. Instead, I used the ETR(I)/ETR(II) ratio as an indicator of the CEF-PSI activity (Fig. 6). Because the linear electron transport rate through PSII can be quantified (Genty et al. 1989), if the ratio is properly obtained, the rate through PSI may be quantified. For this purpose, I measured leaf absorptance and the share of absorbed light energy allocated to PSII (f_{PSII}) (Table 1). Very recently, Kou et al. (2013) estimated the activity of PSI-CEF at saturating CO_2 based on measurements of the O_2 evolution rate and PSI quantum yields. However, they did not measure f_{PSII}.

Solving the equation, $Y(O_2) = I_A \times f_{PSII} \times Y(II)/4$, where I_A is the absorbed PPFD (Genty et al. 1989), I obtained the share of absorbed light energy allocated to PSII. In spite of the fact that the plants were grown in constant illumination, the share in *pgr5* was 35%, while WT showed almost equal sharing of light energy between PSI and PSII (Fig. 5B). Furthermore, the contribution of the state transition to the energy share was small over the range from low- to high-PPFD in both plants (Fig. 7) in agreement with the studies reporting that the state transitions in higher plants were not marked (Pesaresi et al. 2011). These results indicate that the share of light energy allocation to PSI was much greater than that to PSII in *pgr5*. In the light response curve shown in Fig.3, Y(I) in *pgr5* started to decrease from very low PPFDs, whereas that in WT was relatively high up to PPFD of ca. 250 μmol photon m^{-2} s^{-1} and started to decrease with further increase in PPFD. In *pgr5*, a limitation of electron flow through PSI due to this decrease in Y(I) at low PPFD would be compensated by the increase in the share of absorbed light

energy allocated to PSI. This was also supported by the fact that the chlorophyll *a*/*b* ratio was greater in the *pgr5* leaves than that in WT by 0.4. The previous studies reported that growth of *pgr5* was similar to that of WT in both low light (Munekage et al. 2008) and moderate light (Suorsa et al. 2012). However, in fact, energy sharing between two photosystems and the composition of chlorophyll proteins would be markedly changed in *pgr5*. The calculation of ETR using properly measured f_{PSII} may be a useful method to estimate CEF-PSI.

When white light was used as the actinic light, maximum $Y(O_2)$ values for non-stressed leaves of C_3 plants approached 0.105 (Björkman and Demmig 1987). In this study, $Y(O_2)$ decreased with the absorbed PPFD at low PPFDs because red light was used for the actinic light. However, when the maximum $Y(O_2)$ was obtained by extrapolating the line in Fig. 5B to Y(II) of 0.81, the value for WT was 0.09, a value within the range of the data for C_3 species (Björkman and Demmig 1987).

From the light energy allocation to PSI and the changes in the ratio of ETR(I)/ETR(II) measured in the constant- and fluctuating-light, I suggest that *pgr5* plants possessed the CEF-PSI activities because the ratios at low PPFDs and LL in the fluctuating light were far above 1 (Figs. 6 and 9). These results also indicate that, under the growth light conditions at a PPFD of 100 μmol photon m^{-2} s^{-1}, *pgr5* drove CEF-PSI continuously (Nandha et al. 2007). On the other hand, the ETR(I)/ETR(II) ratio in WT increased with the increase in PPFD. This suggests that the CEF-PSI not only function during photosynthetic induction (Makino et al. 2002; Joliot and Joliot 2002, 2005

2006; Fan et al. 2007) but also at the steady-state conditions in high light.

There are some O_2-dependent pathways besides the WWC, and they may contribute to the photodamage by the fluctuating light. Photorespiration is one of the O_2-dependent pathways. When CO_2 and O_2 concentrations were 800 ppm and 20% in the leaf chamber, respectively, where the effect of photorespiration was suppressed to a considerable extent, Y(II) of WT and *pgr5* showed responses similar to those at 390 ppm and 20%. Plastid terminal oxidase (PTOX) is also proposed to be associated with O_2-consumption, in the reaction called chlororespiration. The PTOX is a plastoquinol oxidase, and is able to transfer electrons from PQ to O_2. Thus, chlororespiration can be a source of ROS generation. However, PTOX is suggested to play an important role in chloroplast biogenesis rather than in stress responses (Rosso et al. 2006). Furthermore, in plants grown under normal conditions, PTOX is present at about only 1% of the level of the D1 protein that houses the PSII reaction center (Lennon et al. 2003). Therefore, the contributions of photorespiration and chlororespiration to the photodamage caused by the fluctuating light would be small, if any.

In both the constant- and fluctuating-light, *pgr5* appeared to show WWC activities (Fig. 8). When light dependence of the ETR(I)/ETR(II) ratio was measured at 2.7 or 0% O_2, the ratios in *pgr5* at PPFD above 100 μmol photon m^{-2} s^{-1} were greater than those measured at 20% O_2. This suggests that, at least some fraction of electrons that flowed through the WWC at 20% O_2, would flow through the CEF-PSI at low O_2 concentrations, resulting in the increases in the ETR(I)/ETR(II) ratio. The ETR(I)/ETR(II) ratios in WT

and *pgr5* were highest at 2.7% O_2 rather than at 0% O_2 for all the PPFD levels examined (Fig. 8). Reasons for this are unknown.

For WT plants in the fluctuating light, the ETR(I)/ETR(II) ratio in the HL-periods rapidly increased immediately after each transition from LL- to HL-period, attained the maximal levels, then decreased and attained the steady-state values within HL-periods. Although the steady-state values decreased in a stepwise fashion with the cycle, the maximal levels were almost constant. In contrast, in *pgr5*, the ratio rapidly decreased immediately after each transition from LL- to HL-period and then attained the minimum levels within the HL-periods. Under these conditions, PSI of *pgr5* would be more sensitive to the damage due to the fact that PSI capacity was not able to manage the gush of the electron flow caused by the rapid increase in PPFD. However, at low O_2 concentrations, Y(II) in LL-period in fluctuating light did not decrease with the cycles (Fig. 10), and no photoinhibition of PSI occurred after the light treatment (Fig. 11). These results indicate that an increase in the activity of the CEF-PSI at low O_2 concentrations lead to relaxation of the acceptor-side limitation of PSI, resulting in acceleration of the linear and/or the other electron flows. Therefore, I conclude that the CEF-PSI is essential to efficiently cope with the rapid increase in PPFD and preventing photoinhibition of PSI caused by the fluctuating light. Furthermore, my data indicate that the CEF-PSI could be regulated by O_2. The enhancement of the CEF-PSI by low O_2 is probably attributable to suppression of the electron flow to O_2 at low O_2. As the activity of the CEF-PSI cannot be properly regulated in *pgr5*, considerable electrons inevitably flow to O_2, leading to ROS formation

and thereby PSI photoinhibition. From these, it is suggested that, in WT, electron flow to O_2 can be controlled by regulating engagement of alternative electron flows including CEF-PSI in a way that the photooxidative damage is minimized even at 20% O_2.

In *A. thaliana*, NDH-CEF has been suggested to play a complementary role, since the NDH-CEF is not essential for photosynthesis at least under ordinary laboratory conditions, and NDH-deficient mutants of *A. thaliana* grow similarly to WT (Munekage et al. 2002, 2004; Okegawa et al. 2008). I measured the responses of an NDH-deficient mutant, *crr2-2*, to the fluctuating light under the same conditions as those use for Fig.1 (Supplementary Fig. S3). The results in the *crr2-2* were almost identical to those in WT; Y(II) decreased with the cycles showing similar changes in the ETR(I)/ETR(II) ratio, and PSI was slightly photoinhibited by the fluctuating light treatment. The light dependence of the ETR(I)/ETR(II) ratio exhibited trends similar to those in WT. Moreover, at any PPFD, the ratios at 2.7 and 0% O_2 were higher than that at 0% O_2 (Supplementary Fig. S4). Thus, I conclude that the NDH-CEF would not contribute to response to the fluctuating light. It is noteworthy, however, that ETR(I)/ETR(II) ratios in WT and *pgr5* at lowest two PPFD levels were somewhat greater than those at 35 μmol m^{-2} s^{-1} (Fig. 8). This was not the case in *crr2-2*, although I did not measure the ratios at very low PPFDs for *crr2-2*. These differences may indicate that NDH-CEF in WT and *pgr5* operated at very low PPFDs as suggested for *Oryza sativa* (Yamori et al. 2011) and *Marchantia polymorpa* (Ueda et al. 2012). It is necessary to conduct detailed measurements including

f_{PSII} with *crr2-2*.

Concluding remarks and future scopes

PSI of the *pgr5* plant was sensitive as previously reported (Munekage et al. 2008) due to the large acceptor-side limitation of PSI. *pgr5* was particularly sensitive to the fluctuating light, and showed marked photoinhibition of PSI. In this study, I clearly elucidated that *pgr5* can drive CEF-PSI in low light. Namely, *pgr5* not only possesses the CEF-PSI capacity (Nandha et al. 2007) but actually drives the CEF-PSI at low PPFDs. However, its capacity dramatically decreases with the increase in the PPFD, supporting the view that the PGR5 protein is involved in the redox control of PSI (Nandha et al. 2007).

The general message of this study is that the CEF-PSI is essential for effective responses to the drastic light fluctuation. When plants are exposed to drastic fluctuation in PPFD in the field, the plant would activate the CEF-PSI more than the WWC to accommodate the electron flows and thereby avoid the risk of photo-oxidative damage. I also found that the fluctuation in PPFD is a potent stress factor, even when the PPFD level in the HL-periods is moderate.

Plants grown in the forest understory are exposed to drastic fluctuation in PPFD. If they are able to acclimate to such the fluctuating light conditions, one of the mechanisms would be an enhancement of the ability of

appropriate regulation of the activity of the CEF-PSI in response to light fluctuation. This would be achieved by the increase in the proportion of the PSI complex with the PGR5 protein. Plants may be able to avoid photodamage to PSI by altering the ratio of two photosystems in the thylakoid membranes (Suorsa et al. 2012, Yin and Johnson 2000, Jahns and Junge 1992) as observed in *pgr5* grown in the constant light in this study. I am currently examining whether the photosynthetic apparatus in WT acclimates to the drastically fluctuating growth light to actually become resistant to the fluctuating light.

2.5. Table

Table 1. Chlorophyll *a* + *b*, chlorophyll *a/b* in thylakoids and leaf absorptance in the WT and *pgr5* leaves.

Genotype	Chl *a* + *b* (mg m^{-2})	Chl *a*/*b*	Leaf absorption
WT	228 ± 23	3.38 ± 0.007	0.837 ± 0.0521
pgr5	190 ± 10 *	3.73 ± 0.009 *	0.823 ± 0.0742 *

Plants were grown at 90-100 μmol m^{-2} s^{-1} in a short-day photoperiod (8 h of light, 16 h of dark) for 55 d. Means ± SD (n = 3 to 5) are shown. *P < 0.005 (t-test, WT vs. *pgr5*). Light absorptance was measured with an integrating sphere. The light from the Björkman-type lamp passing through a 635 nm red filter was used.

2.6. Figures

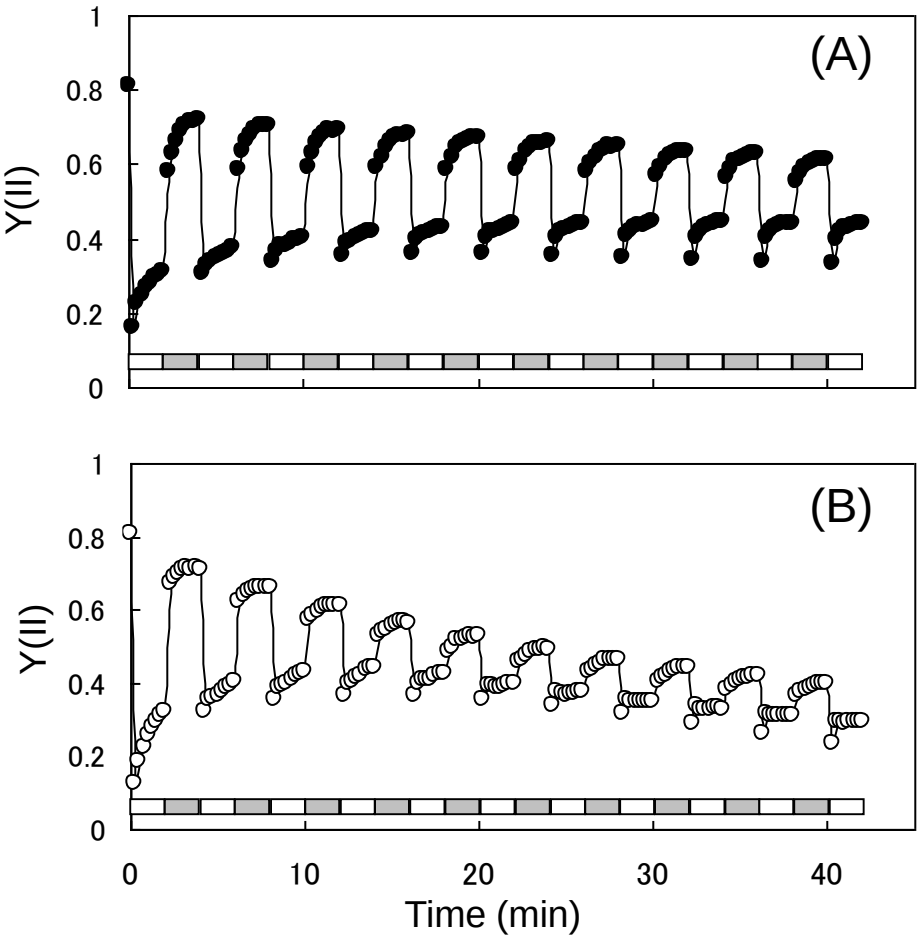

Figure 1. Response of photochemical quantum yield of PSII (Y(II)) of the WT (A) and *pgr5* (B) plants to the fluctuating light. The plants were grown in a constant moderate light (100 μmol photons m^{-2} s^{-1}) for 8 h per day. The light alternating between HL at 240 μmol photons m^{-2} s^{-1} for 2 min (open bars) and LL at 30 μmol photons m^{-2} s^{-1} for 2 min (grey bars) was applied to the leaf after the dark treatment for 30 min. The leaf lamina was sandwiched in a chamber. The air in the chamber contained 20% O_2 and 390 ppm CO_2. Each data point represents the mean (n = 5 to 6).

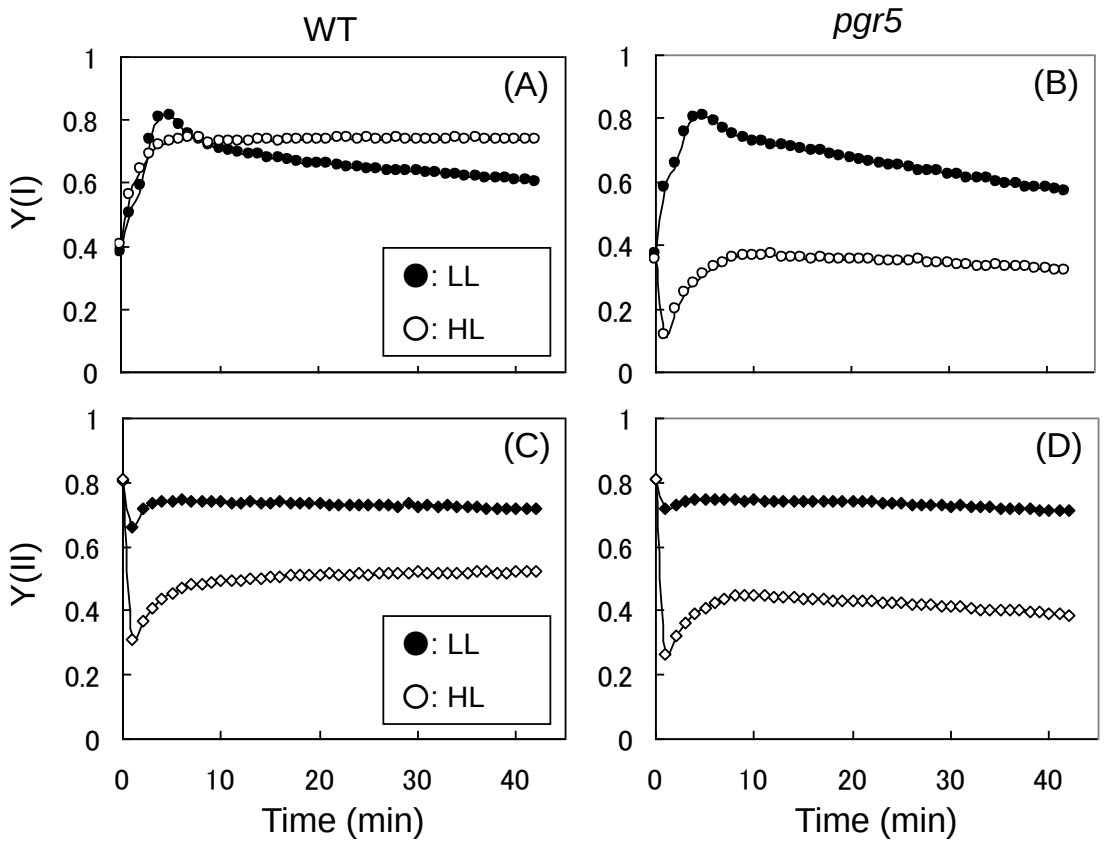

Figure 2. Changes in the quantum yield of PSI (Y(I)) and PSII (Y(II)). (A) and (B), Y(I); (C) and (D), Y(II). Continuous light at PPFD of 240 μmol photons m^{-2} s^{-1} (open symbols, HL), or at PPFD of 30 μmol photons m^{-2} s^{-1} (closed symbols, LL) was applied for 42 min after the 30 min dark treatment. Measurements were made at 20% O_2 and 390 ppm CO_2. Each data point represents the mean (n = 4).

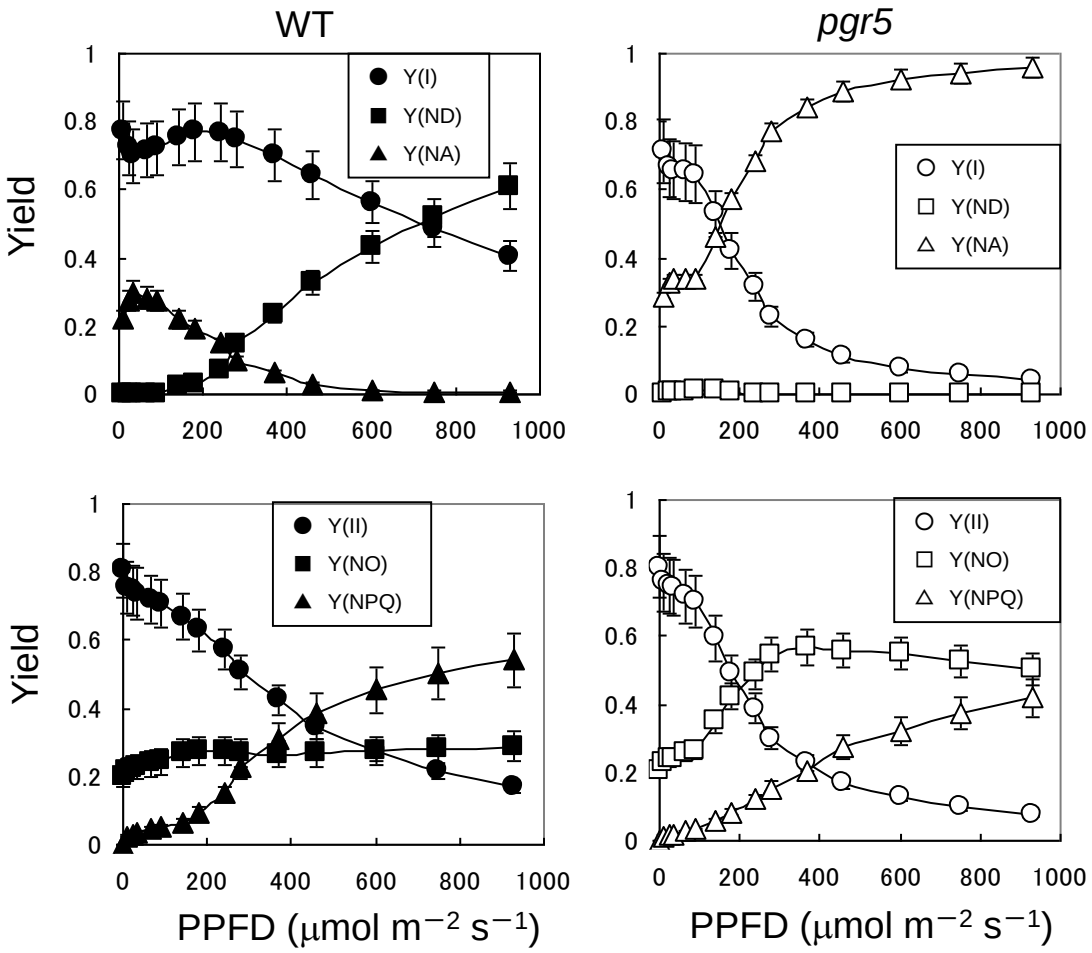

Figure 3. Changes in the photosynthetic quantum yields of PSI and PSII with PPFD of the constant light in WT (closed symbols) and *pgr5* (open symbols). For energy captured by PSI pigments, the quantum yield of the PSI photochemistry, Y(I) (circle), the quantum yield of non-photochemical energy dissipation due to the donor-side limitation, Y(ND) (square), and that of the energy dissipation due to the acceptor-side limitation, Y(NA) (triangle), are indicated. The fluorescence parameters, the effective PSII quantum yield, Y(II) (circle), the quantum yield of regulated energy dissipation, Y(NPQ) (triangle), and that of non- regulated energy dissipation, Y(NO) (square) are shown. Measurements were made at 20% O_2 and 390 ppm CO_2. The values represent the mean ± SD (n = 4 to 6).

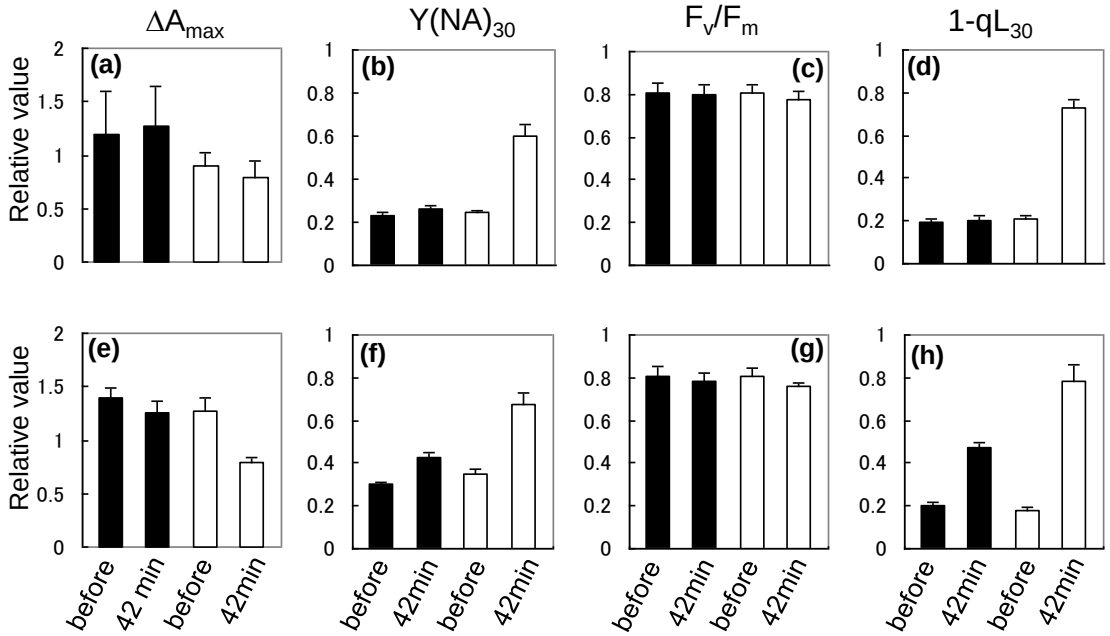

Figure 4. Effects of constant high light (a, b, c and d) and fluctuating light (e, f, g and h) on changes in photosynthetic parameters in leaves of WT (solid bars) and *pgr5* (open bars). Following the light treatments for 42 min and dark treatment for 30 min, functions of the PSI and PSII reaction centers were determined as ΔA_{max} and F_v/F_m. $Y(NA)_{30}$ and $1 - qL_{30}$ were measured at the end of the low light treatment at PPFD of 30 μmol photons m^{-2} s^{-1} for 2 min just after the light treatments for 42 min. Measurements were made at 20% O_2 and 390 ppm CO_2. Error bars represent the SD (n = 6 to 8).

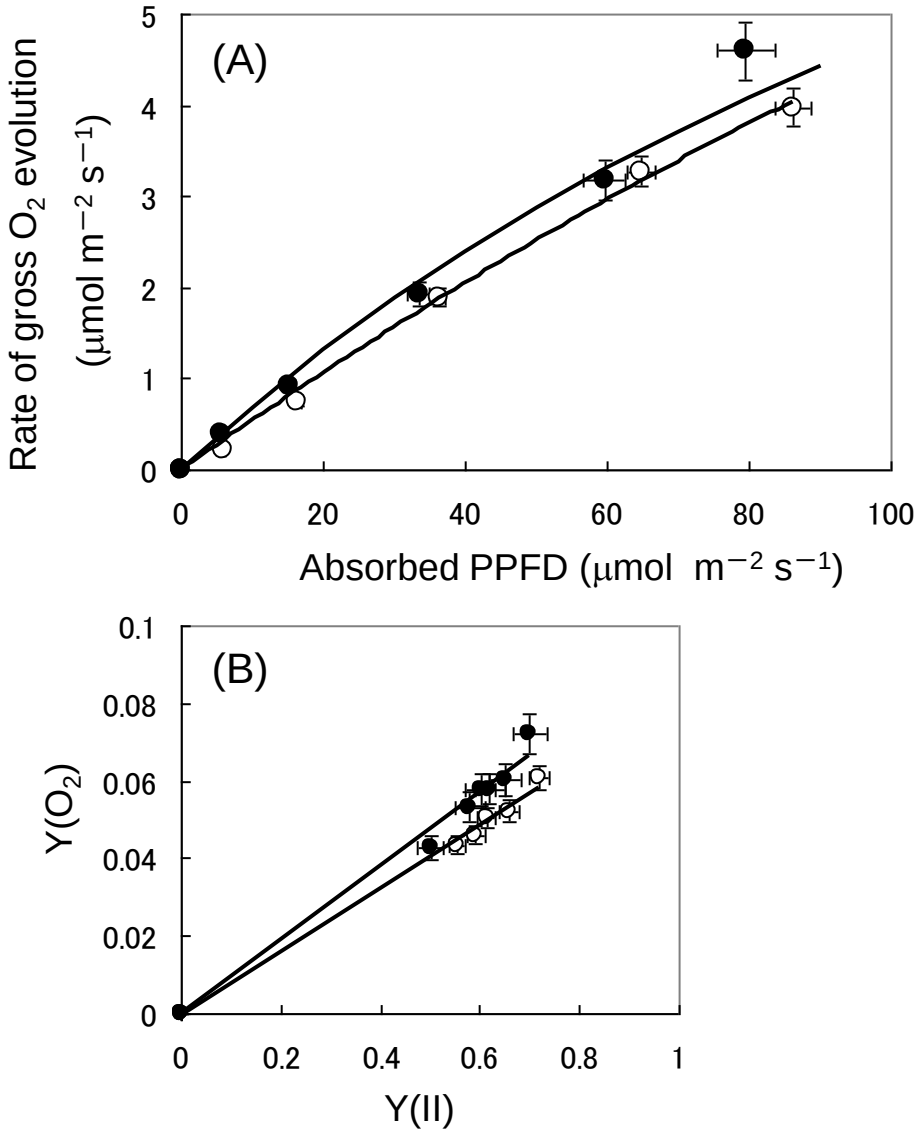

Figure 5. Estimation of the share of absorbed light energy to PSII. (A) Light-response curve of the photosynthetic O_2 evolution in the leaf discs at low PPFDs. The rate of gross O_2 evolution was plotted against absorbed PPFD Fitted hyperbolic functions through the origin are shown. (B) The relationship between the quantum yield of O_2 evolution, $Y(O_2)$, and the photochemical yield of PSII,Y(II). Closed circle; WT, open circle; *pgr5*. Error bars represent the SD (n = 4 to 6). Regression lines through the origin are shown.

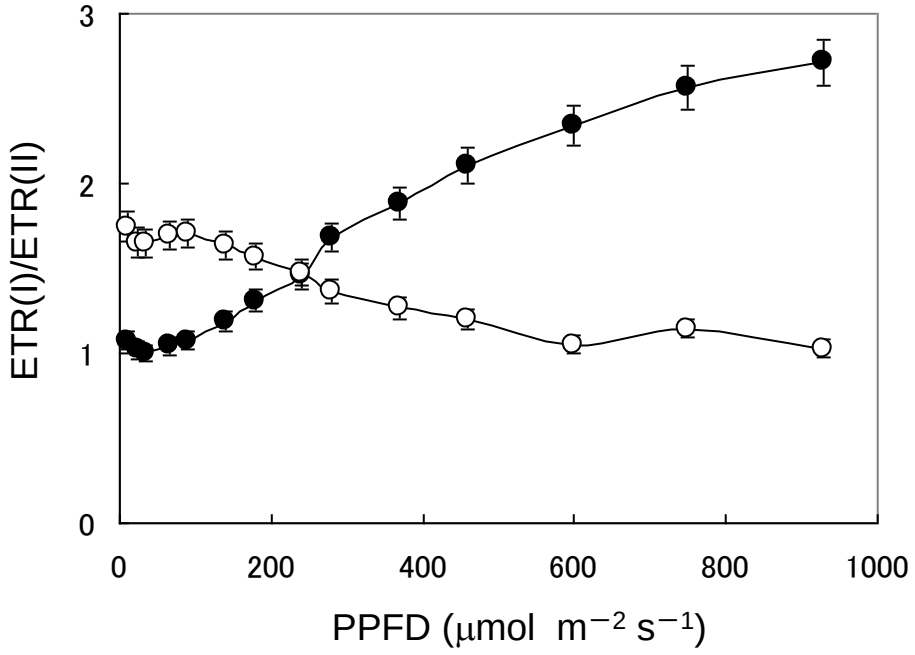

Figure 6. Changes in the ratio of ETR(I)/ETR(II) in WT (closed circle) and *pgr5* (open circle) leaves as a function of PPFD of the constant light. Y(I) and Y(II) were measured as in Figure 3. Measurements were made at 20% O_2 and 390 ppm CO_2. The values represent the mean ± SD (n = 4 to 6).

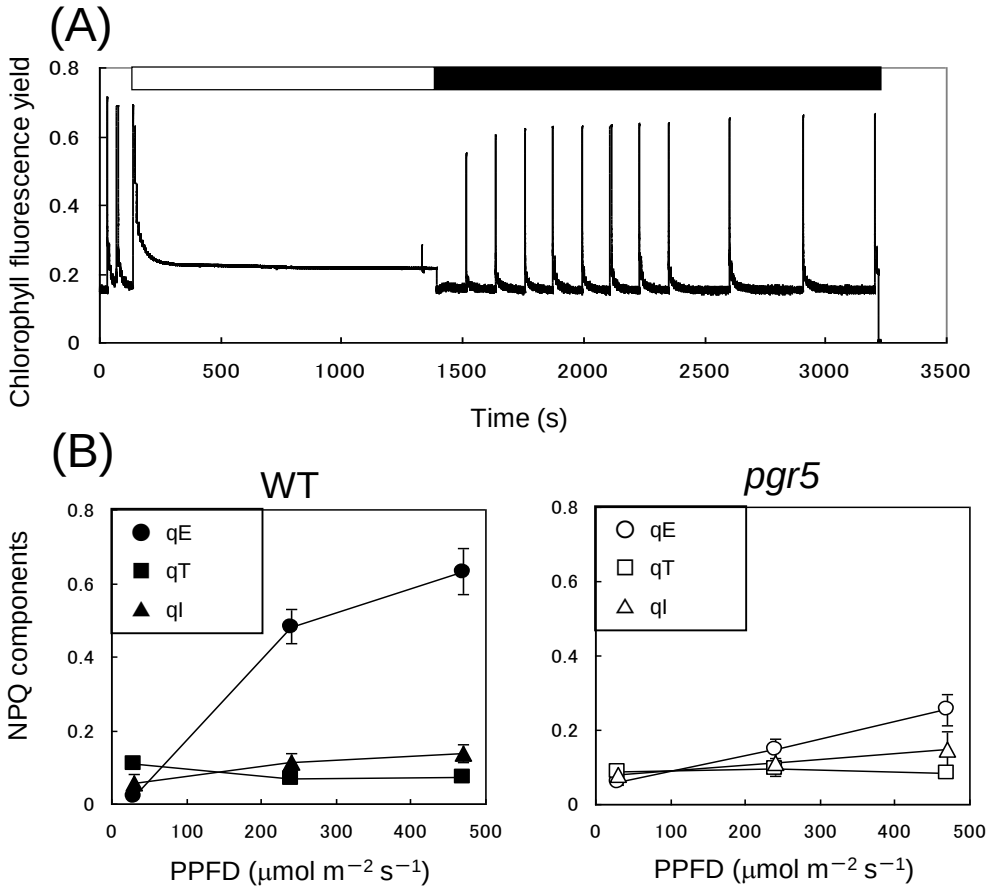

Figure 7. Dissection of NPQ into the energy-dependent quenching (qE), the state transition (qT) and the photoinhibition (qI). (A) A typical Chl fluorescence trace obtained with a WT leaf after 30 min dark adaptation. The trace shows the changes in fluorescence yield during (white bar), and after turning off the actinic light (black bar). (B) Components of non-photochemical chlorophyll fluorescence quenching in WT and *pgr5*. The different components of NPQ were derived from semi-logarithmic plots of the dark relaxation of F_v after the light treatment at three PPFDs of 30, 240 and 470 μmol photons m^{-2} s^{-1}. Energy-dependent quenching (qE; circle) was attributed to the fast phase, quenching by state transition (qT; square) to the medium phase and photoinhibitory quenching (qI; triangle) to the slow phase of relaxation. The quenching components were calculated from the amplitude of the respective phases considering the relationship $(1 - qN) = (1 - qE) \times (1 - qT) \times (1 - qI)$. Measurements were made at 20% O_2 and at 390 ppm CO_2. The values represent the mean ± SD ($n = 3$).

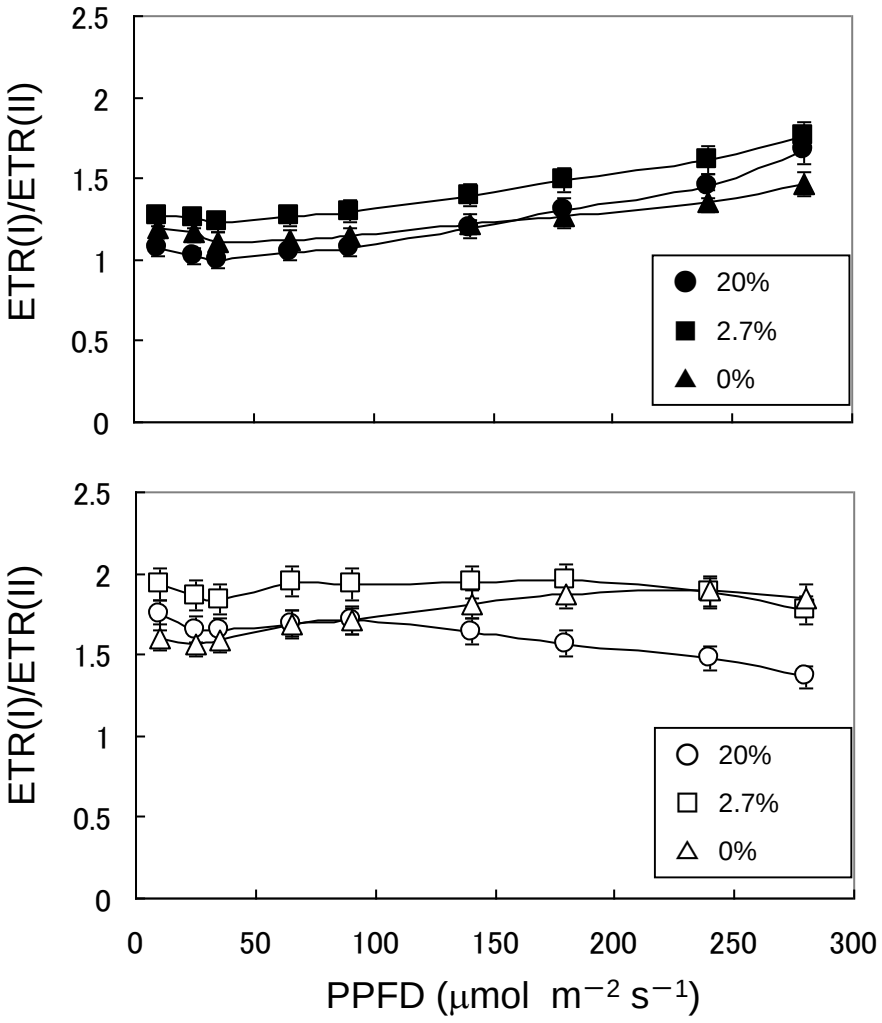

Figure 8. Light intensity dependence of the ETR(I)/ETR(II) ratio in 20, 2.7 and 0 % O_2 in the WT (closed symbols) and *pgr5* (open symbols) leaves. Measurements were made for the PPFDs ranging from 0 to 280 μmol photons m^{-2} s^{-1} and at CO_2 concentration of 390 ppm. The values represent the mean ± SD (n = 3).

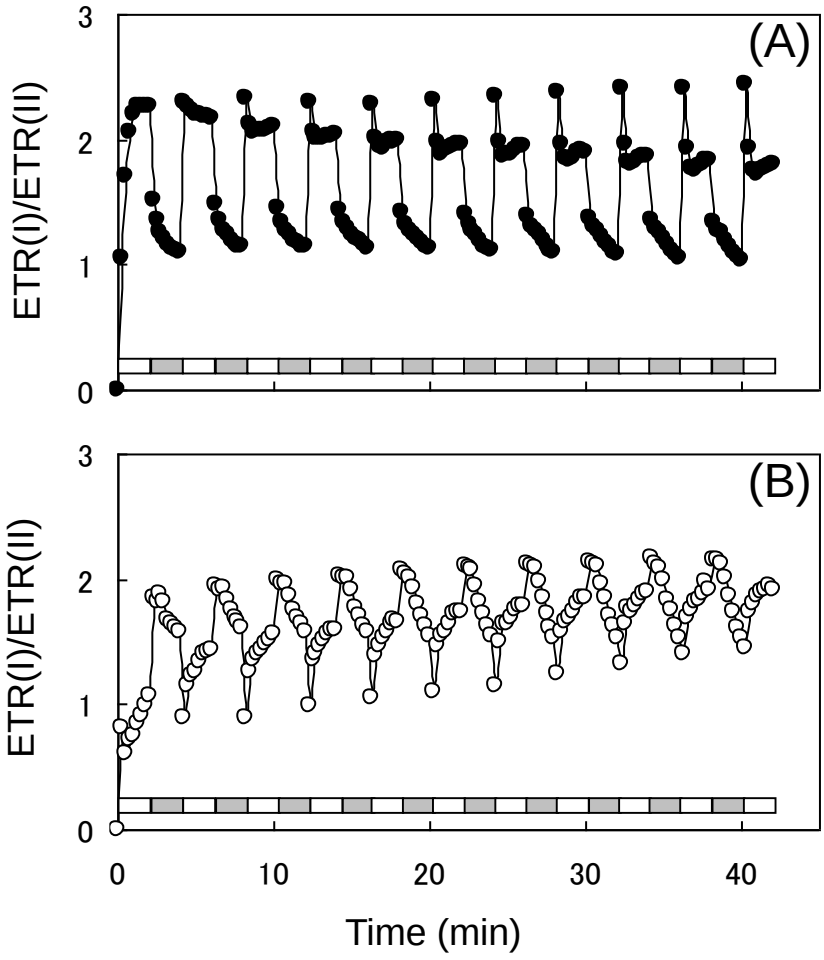

Figure 9. Changes in the ratio of ETR(I)/ETR(II) in the WT (A) and *pgr5* (B) leaves in the fluctuating light. The same light treatment protocol for Figure 1 was used. Measurements were made at 20% O_2 and 390 ppm CO_2. The values represent the mean ± SD (n = 5 to 6).

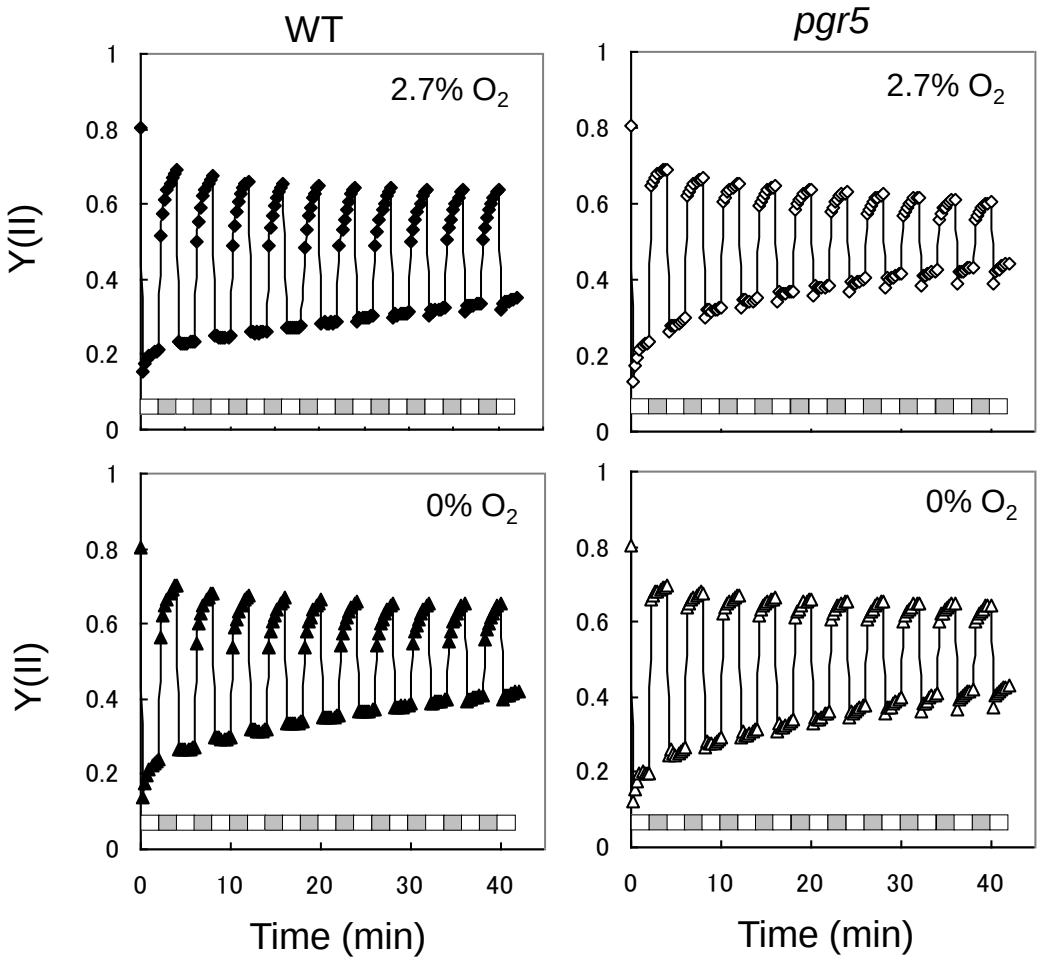

Figure 10. Effects of low O_2 concentrations (2.7%; diamond, 0% triangle) on responses of Y(II) to the fluctuating light in WT (closed symbol) and *pgr5* (open symbol). The fluctuating light treatment was the same that used for Figure 1. Measurements were made at 390 ppm CO_2. The values represent the mean, $n = 4$ to 6.

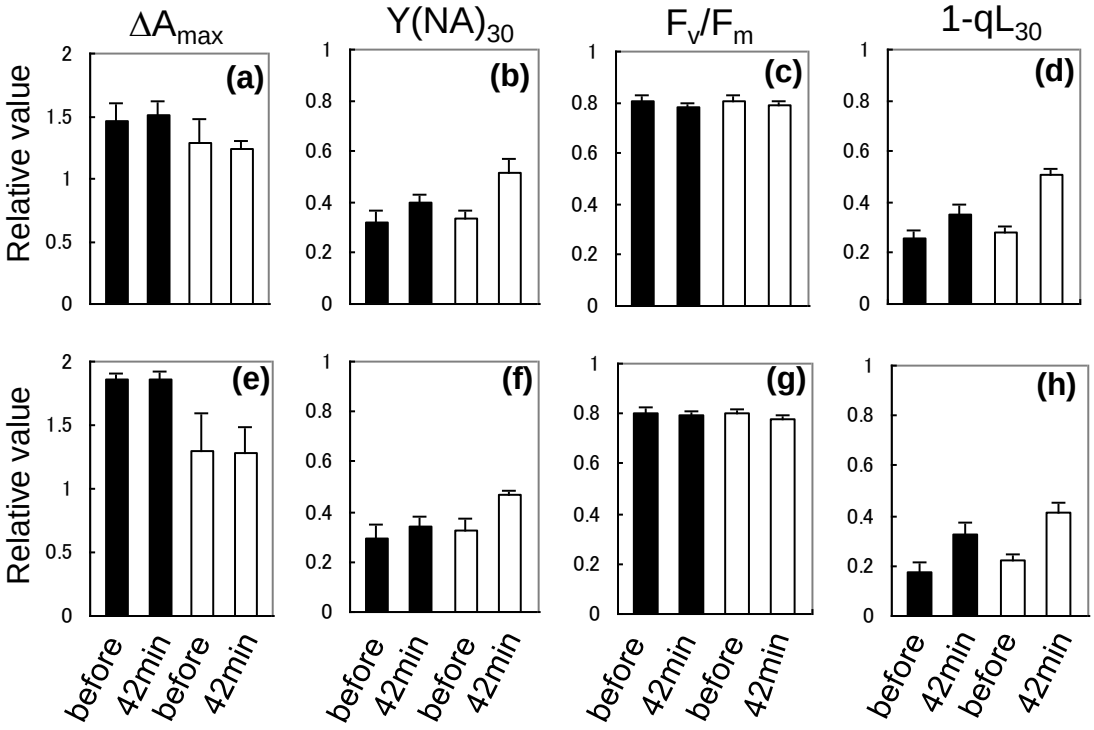

Figure 11. Effects of low O_2 concentrations (2.7%; a, b, c and d, 0%; e, f, g and h) on changes in the photosynthetic parameters after the fluctuating light treatment in WT (black bar) and *pgr5* (white bar). Measurements were made in the same manner that used for Figure 4 and at 390 ppm CO_2. Error bars represent the SD (n = 4 to 8).

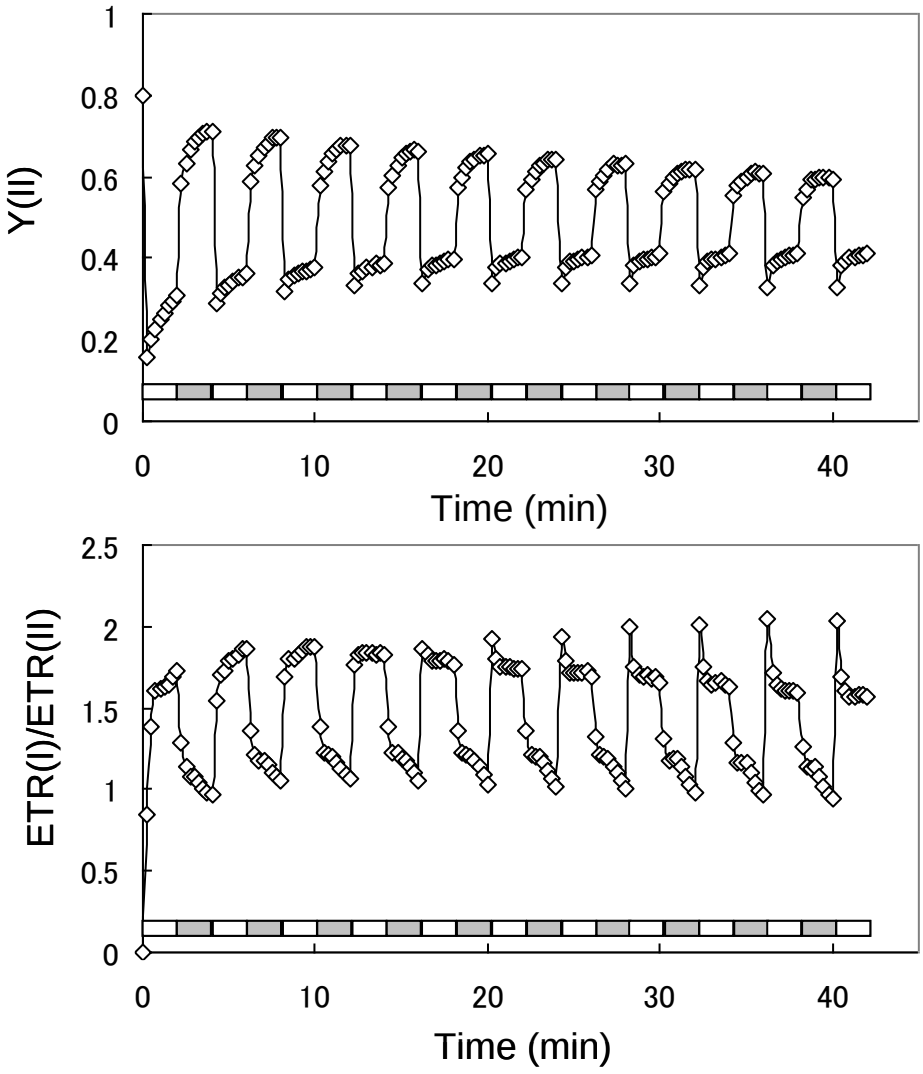

Figure 12. Response of Y(II) (A) and ETR(I)/ETR(II) ratio (B) in *crr2-2* leaves to the fluctuating light. The plants were grown in a constant moderate light (100 μmol photons m^{-2} s^{-1}) for 8 h per day. The same light treatment protocol for the data in Figure 1 was used. The air in the chamber contained 20% O_2 and 390 ppm CO_2. Value of 0.5 was used as the share of absorbed light energy allocated to PSII. The values represent the mean ± SD (n = 3).

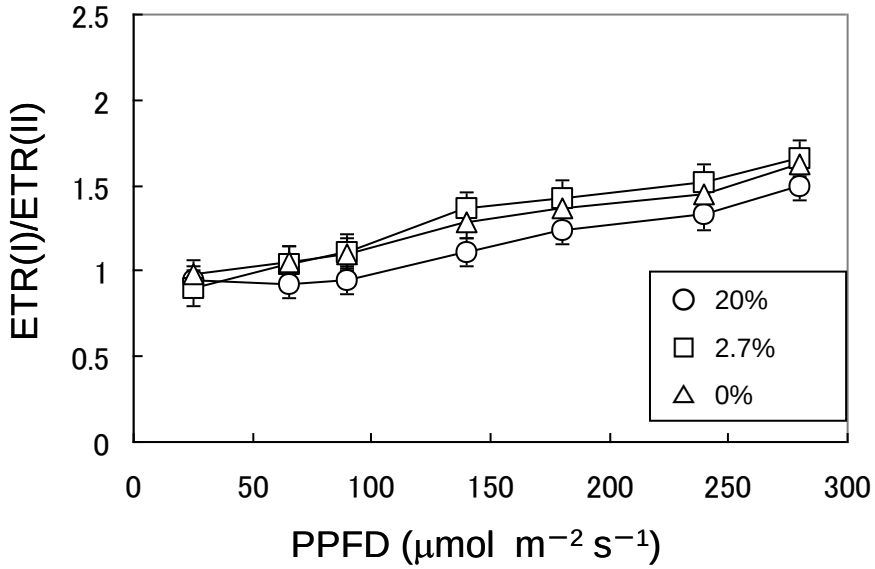

Figure 13. Light intensity dependence of the ETR(I)/ETR(II) ratio in 20, 2.7 and 0 % O_2 in the *crr2-2* leaves. Measurements were made for the PPFDs ranging from 0 to 280 μmol photons m^{-2} s^{-1} and at CO_2 concentration of 390 ppm. The values represent means ± SD (n = 3).

CHAPTER 3

Effects of fluctuating light on photoinhibition of photosystems I and II, and regulation of the photosynthetic electron transport system in *Arabidopsis thaliana*

一部内容が学術雑誌に掲載の形で刊行される予定のため、インターネット公表不可。

CHAPTER 4.

一部内容が学術雑誌に掲載の形で刊行される予定のため、インターネット公表不可。

References

Allee WC (1926) Measurement of Environmental Factors in the Tropical Rain-Forest of Panama. Ecology **7**: 273–302

Allen J (2002) Photosynthesis of ATP-electrons, proton pumps, rotors, and poise. Cell **110**: 273–6

Alter P, Dreissen A, Luo F-L, Matsubara S (2012) Acclimatory responses of Arabidopsis to fluctuating light environment: comparison of different sunfleck regimes and accessions. Photosynth Res **113**: 221–237

Anderson JM (1986) Photoregulation of the Composition, Function, and Structure of Thylakoid Membranes. Annu Rev Plant Physiol **37**: 93–136

Anderson JM, Chow WS, Park YI (1995) The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. Photosynth Res **46**: 129–139

Anderson JM, Horton P, Kim E-H, Chow WS (2012) Towards elucidation of dynamic structural changes of plant thylakoid architecture. Philos Trans R Soc Lond B Biol Sci **367**: 3515–3524

Arnon DI, Chain RK (1975) Regulation of ferredoxin-catalyzed photosynthetic phosphorylations. Proc Natl Acad Sci U S A **72**: 4961–4965

Arnon DI, Chain RK (1979) Regulatory electron transport pathways in cyclic photophosphorylation: Reduction of C-550 and cytochrome b6 by ferredoxin in the dark. FEBS Lett **102**: 133–138

Asada K (2000) The water-water cycle as alternative photon and electron sinks. Philos Trans R Soc Lond B Biol Sci **355**: 1419–1431

Asada K (1999) THE WATER-WATER CYCLE IN CHLOROPLASTS: Scavenging of Active Oxygens and Dissipation of Excess Photons. Annu Rev Plant Physiol Plant Mol Biol **50**: 601–639

Asada K, Heber U, Schreiber U (1993) Electron Flow to the Intersystem Chain from Stromal Components and Cyclic Electron Flow in Maize Chloroplasts , as Detected in Intact Leaves by Monitoring Redox Change of P700 and Chlorophyll Fluorescence. Plant Cell Physiol **34**: 39–50

Avenson TJ, Cruz J a, Kanazawa A, Kramer DM (2005) Regulating the proton budget of higher plant photosynthesis. Proc Natl Acad Sci U S A **102**: 9709–13

Avenson TJ, Cruz J a, Kramer DM (2004) Modulation of energy-dependent quenching of excitons in antennae of higher plants. Proc Natl Acad Sci U S A **101**: 5530–5

Betterle N, Ballottari M, Zorzan S, de Bianchi S, Cazzaniga S, Dall'osto L, Morosinotto T, Bassi R (2009) Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. J Biol Chem **284**: 15255–15266

De Bianchi S, Ballottari M, Dall'osto L, Bassi R (2010) Regulation of plant light harvesting by thermal dissipation of excess energy. Biochem Soc Trans **38**: 651–660

Bilger W, Schreiber U (1986) Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. Photosynth Res **10**: 303–8

Blankenship RE (2001) Molecular evidence for the evolution of photosynthesis. Trends Plant Sci **6**: 4–6

Bonente G, Howes BD, Caffarri S, Smulevich G, Bassi R (2008) Interactions between the photosystem II subunit PsbS and xanthophylls studied in vivo and in vitro. J Biol Chem **283**: 8434–8445

Bukhov NG, Heber U, Wiese C, Shuvalov VA (2001) Energy dissipation in photosynthesis: Does the quenching of chlorophyll fluorescence originate from antenna complexes of photosystem II or from the reaction center? Planta **212**: 749–758

Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid ndh genes. EMBO J **17**: 868–76

Cazzaniga S, Dall' Osto L, Kong S-G, Wada M, Bassi R (2013) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis. Plant J **76**: 568–79

Cazzaniga S, Dall'Osto L, Kong S-G, Wada M, Bassi R, Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, et al (2002) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Photosynth Res **110**: 361–71

Chazdon RL (1988) Sunflecks and their importance to forest understorey plants. Adv Ecol Res **18**: 1–63

Chazdon RL, Pearcy RW, Lee DW, Fetcher N (1996) Photosynthetic responses of tropical forest plants to contrasting light environments. Trop. For. Plant Ecophysiol. Springer US, pp 5–55

Chikahiro M, Akiho Y (2000) Determination of the rate of Photoreduction of O2 in the Water-Water Cycle in Watermelon Leaves and Enhancement of the Rate by Limitation of Photosynthesis. Plant Cell Physiol **41**: 335–343

Choi SM, Jeong SW, Jeong WJ, Kwon SY, Chow WS, Park Y-I (2002) Chloroplast Cu/Zn-superoxide dismutase is a highly sensitive site in cucumber leaves chilled in the light. Planta **216**: 315–24

Chow WS, Hope a B (2004) Electron Fluxes through Photosystem I in Cucumber Leaf Discs Probed by far-red Light. Photosynth Res **81**: 77–89

Chow WS, Lee H-Y, Park Y-I, Park Y-M, Hong Y-N, Anderson JM (2002) The role of inactive photosystem-II-mediated quenching in a last-ditch

community defence against high light stress in vivo. Philos Trans R Soc Lond B Biol Sci **357**: 1441–1450

Christine H. F, Graham N (2000) Tansley Review No . 112 Oxygen processing in photosynthesis : regulation and signalling. New Phytol **146**: 359–388

Cruz J a, Avenson TJ, Kanazawa A, Takizawa K, Edwards GE, Kramer DM (2005) Plasticity in light reactions of photosynthesis for energy production and photoprotection. J Exp Bot **56**: 395–406

Cruz JA, Sacksteder CA, Kanazawa A, Kramer DM (2001) Contribution of Electric Field (Δψ) to Steady-State Transthylakoid Proton Motive Force (pmf) in Vitro and in Vivo. Control of pmf Parsing into Δψ and ΔpH by Ionic Strength †. Biochemistry **40**: 1226–1237

DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schünemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. Cell **132**: 273–85

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. Biochim Biophys Acta - Bioenerg **1020**: 1–24

Demmig-Adams B, Adams III WW, Barker DH, Logan BA, Bowling DR, Verhoeven AS (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol Plant **98**: 253–264

Demmig-Adams B, Adams WW (1992) Photoprotection and Other Responses of Plants to High Light Stress. Annu Rev Plant Physiol Plant Mol Biol **43**: 599–626

Demmig-Adams B, Cohu CM, Muller O, Adams WW (2012) Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. Photosynth Res **113**: 75–88

Driever SM, Baker NR (2011) The water-water cycle in leaves is not a major alternative electron sink for dissipation of excess excitation energy when CO(2) assimilation is restricted. Plant Cell Environ **34**: 837–46

Eberhard S, Finazzi G, Wollman F-A (2008) The dynamics of photosynthesis. Annu Rev Genet **42**: 463–515

Endo T, Shikanai T, Takabayashi a, Asada K, Sato F (1999) The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. FEBS Lett **457**: 5–8

Evans GC (1956) AN AREA SURVEY METHOD OF INVESTIGATING THE OF LIGHT INTENSITY IN WOODLANDS , DISTRIBUTION WITH PARTICULAR REFERENCE TO SUNFLECKS. J Ecol **44**: 391–428

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia **78**: 9–19

Fan D-Y, Nie Q, Hope AB, Hillier W, Pogson BJ, Chow WS (2007) Quantification of cyclic electron flow around Photosystem I in spinach leaves during photosynthetic induction. Photosynth Res **94**: 347–57

Fietz S, Nicklisch A (2002) Acclimation of the diatom Stephanodiscus neoastraea and the cyanobacterium Planktothrix agardhii to simulated natural light fluctuations. Photosynth Res **72**: 95–106

Finazzi G, Johnson GN, Dall'Osto L, Dallosto L, Joliot P, Wollman F-A, Bassi R (2004) A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. Proc Natl Acad Sci U S A **101**: 12375–12380

Fisher N, Kramer DM (2014) Non-photochemical reduction of thylakoid photosynthetic redox carriers in vitro: Relevance to cyclic electron flow around photosystem I? Biochim Biophys Acta **1837**: 1944–1954

Foyer C, Furbank R, Harbinson J, Horton P (1990) The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosynth Res **25**: 83–100

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta - Gen Subj **990**: 87–92

Genty B, Harbinson J, Cailly A (1996) (1996)(10th FESPP Meeting)(Genty et al. Abstr. B. 10th FESPP Meet. p 86

Göhre V, Jones AME, Sklenář J, Robatzek S, Weber APM (2012) Molecular crosstalk between PAMP-triggered immunity and photosynthesis. Mol Plant Microbe Interact **25**: 1083–1092

Goral TK, Johnson MP, Duffy CDP, Brain APR, Ruban A V, Mullineaux CW (2012) Light-harvesting antenna composition controls the macrostructure and dynamics of thylakoid membranes in Arabidopsis. Plant J **69**: 289–301

Grieco M, Tikkanen M, Paakkarinen V, Kangasjärvi S, Aro E-M (2012) Steady-state phosphorylation of light-harvesting complex II proteins preserves photosystem I under fluctuating white light. Plant Physiol **160**: 1896–1910

Hall DO, Reeves SG, Baltscheffsky H (1971) Photosynthetic control in isolated spinach chloroplasts with endogenous and artificial electron acceptors. Biochem Biophys Res Commun **43**: 359–366

Harbinson J, Genty B, Baker NR (1990) The relationship between CO2 assimilation and electron transport in leaves. Photosynth Res **25**: 213–224

Havaux M, Davaud A (1994) Photoinhibition of photosynthesis in chilled potato leaves is not correlated with a loss of Photosystem-II activity : Preferential inactivation of Photosystem I. Photosynth Res **40**: 75–92

Hendrickson L, Furbank RT, Chow WS (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Photosynth Res **82**: 73–81

Herbert SK, Fork DC, Malkin S (1990) Photoacoustic measurements in vivo of energy storage by cyclic electron flow in algae and higher plants. Plant Physiol **94**: 926–34

Herbstová M, Tietz S, Kinzel C, Turkina M V, Kirchhoff H (2012) Architectural switch in plant photosynthetic membranes induced by light stress. Proc Natl Acad Sci U S A **109**: 20130–20135

Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. Mol Cell **49**: 511–23

Hikosaka K, Terashima I (1995) A model of the acclimation of photosynthesis in the leaves of C3 plants to sun and shade with respect to nitrogen use. Plant, Cell Environ **18**: 605–618

Hiroshi. Koizumi, Yasuyuki O (1993) Light environment and carbon gain of understory herbs associated with sunflecks in a warm temperate deciduous forest in Japan. Ecol Reserch **8**: 135–142

Hjelm U, Ogren E (2004) Photosynthetic responses to short-term and long-term light variation in Pinus sylvestris and Salix dasyclados. Trees **18**: 622–629

Horton P, Ruban a V, Wentworth M (2000) Allosteric regulation of the light-harvesting system of photosystem II. Philos Trans R Soc Lond B Biol Sci **355**: 1361–70

Horton P, Ruban A V., Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol **47**: 655–684

Ichiro T, Takashi F, Takeshi I, Wah Sonn C, Riichi O (2009) Green light drives leaf photosynthesis more efficiently than red light in strong

white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol **50**: 684–697

Ivanov A., Morgan R., Gray G., Velitchkova M., Huner NP. (1998) Temperature/light dependent development of selective resistance to photoinhibition of photosystem I. FEBS Lett **430**: 288–292

Ivanov AG, Hurry V, Sane P V., Öquist G, Huner NPA (2008) Reaction centre quenching of excess light energy and photoprotection of photosystem II. J Plant Biol **51**: 85–96

Ivanov AG, Sane P V, Krol M, Gray GR, Balseris A, Savitch L V, Oquist G, Hüner NPA (2006) Acclimation to temperature and irradiance modulates PSII charge recombination. FEBS Lett **580**: 2797–2802

Jahns P, Junge W (1992) Thylakoids from pea seedlings grown under intermittent light: biochemical and flash-spectrophotometric properties. Biochemistry **31**: 7390–7397

Joet Thierry, Cournac L, Peltier G, Havaux M (2002) Cyclic Electron Flow around Photosystem I in C 3 Plants . In Vivo Control by the Redox State of Chloroplasts and Involvement of the NADH-Dehydrogenase Complex. Plant Physiol **128**: 760–769

Johnson GN (2011) Physiology of PSI cyclic electron transport in higher plants. Biochim Biophys Acta **1807**: 384–389

Johnson MP, Ruban A V (2010) Arabidopsis plants lacking PsbS protein possess photoprotective energy dissipation. Plant J **61**: 283–9

Joliot P, Béal D, Joliot A (2004) Cyclic electron flow under saturating excitation of dark-adapted Arabidopsis leaves. Biochim Biophys Acta **1656**: 166–76

Joliot P, Johnson GN (2011) Regulation of cyclic and linear electron flow in higher plants. Proc Natl Acad Sci U S A **108**: 13317–22

Joliot P, Joliot A (2005) Quantification of cyclic and linear flows in plants. Proc Natl Acad Sci U S A **102**: 4913–8

Joliot P, Joliot A (2002) Cyclic electron transfer in plant leaf. Proc Natl Acad Sci U S A **99**: 10209–14

Joliot P, Joliot A (2006) Cyclic electron flow in C3 plants. Biochim Biophys Acta **1757**: 362–8

Jones L, Kok B (1966) Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. Plant Physiol **41**: 1037–1043

Kanazawa A, Kramer DM (2002) In vivo modulation of nonphotochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase. Proc Natl Acad Sci U S A **99**: 12789–94

Kangasjärvi S, Tikkanen M, Durian G, Aro E-M (2013) Photosynthetic light reactions - An adjustable hub in basic production and plant immunity signaling. Plant Physiol Biochem 1–7

Katharina B, Lars D, Tatjana K, Elke S, Dennis W, Karl-Josef D, Dörte R, Markus W, Rüdiger H, Peter D, et al (2009) Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. Plant Cell **21**: 2715–2732

Kato M, Hikosaka K, Hirose T (2002) Photoinactivation and recovery of photosystem II in Chenopodium album leaves grown at different levels of irradiance and nitrogen availability. Funct Plant Biol **29**: 787–795

Kato MC (2003) The Excess Light Energy that is neither Utilized in Photosynthesis nor Dissipated by Photoprotective Mechanisms Determines the Rate of Photoinactivation in Photosystem II. Plant Cell Physiol **44**: 318–325

Kiirats O, Cruz JA, Edwards GE, Kramer DM (2009) Feedback limitation of photosynthesis at high CO 2 acts by modulating the activity of the chloroplast ATP synthase. Funct Plant Biol **36**: 893–901

Kirchhoff H (2013) Architectural switches in plant thylakoid membranes. Photosynth Res **116**: 481–487

Kirchhoff H, Hall C, Wood M, Herbstová M, Tsabari O, Nevo R, Charuvi D, Shimoni E, Reich Z (2011) Dynamic control of protein diffusion within the granal thylakoid lumen. Proc Natl Acad Sci U S A **108**: 20248–20253

Kirchhoff H, Schöttler MA, Maurer J, Weis E (2004) Plastocyanin redox kinetics in spinach chloroplasts: evidence for disequilibrium in the high potential chain. Biochim Biophys Acta **1659**: 63–72

Kirschbaum MUF, Küppers M, Schneider H, Giersch C, Noe S (1998) Modelling photosynthesis in fluctuating light with inclusion of stomatal conductance, biochemical activation and pools of key photosynthetic intermediates. Planta **204**: 16–26

Kirschbaum MUF, Pearcy RW (1988) Gas Exchange Analysis of the Fast Phase of Photosynthetic Induction in Alocasia macrorrhiza. Plant Physiol **87**: 818–821

Klughammer C, Schreiber U (2008a) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. PAM Appl Notes **1**: 27–35

Klughammer C, Schreiber U (2008b) Saturation Pulse method for assessment of energy conversion in PS I. PAM Appl Notes **1**: 11–14

Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700+-absorbance changes at 830 nm. Planta **192**: 261–268

Klughammer C, Siebke K, Schreiber U (2013) Continuous ECS-indicated recording of the proton-motive charge flux in leaves. Photosynth Res **117**: 471–87

KNAPP AK, WILLIAM KS (1989) Influence of Growth from on Ecophysiological Responses to Variable Sunlight in Subalpine Plants. Ecology **70**: 1069–1082

Kok B (1956) On the inhibition of photosynthesis by intense light. Biochim Biophys Acta **21**: 234–244

Kornyeyev D (2003) Predicting the Extent of Photosystem II Photoinactivation Using Chlorophyll a Fluorescence Parameters Measured during Illumination. Plant Cell Physiol **44**: 1064–1070

Kou J, Oguchi R, Fan D-Y, Chow WS (2012) The time course of photoinactivation of photosystem II in leaves revisited. Photosynth Res **113**: 157–164

Kou J, Takahashi S, Oguchi R, Badger MR, Wah, Soon C (2013) Estimation of the steady-state cyclic electron fl ux around PSI in spinach leaf discs in white light , CO 2 -enriched air and other varied conditions. Fuctional Plant Biol **40**: 1018–1028

Kozaki A, Takeba G (1996) Photorespiration protects C3 plants from photooxidation. Nature **384**: 557–560

Kramer D, Sacksteder C, Cruz J (1999) How acidic is the lumen? Photosynth Res **60**: 151–163

Kramer DM, Avenson TJ, Edwards GE (2004a) Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions. Trends Plant Sci **9**: 349–57

Kramer DM, Cruz J a., Kanazawa A (2003) Balancing the central roles of the thylakoid proton gradient. Trends Plant Sci **8**: 27–32

Kramer DM, Johnson G, Kiirats O, Edwards GE (2004b) New fluorescence parameters for the determination of Q(A) redox state and excitation energy fluxes. Photosynth Res **79**: 209–18

Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant **74**: 566–574

Krause GH, Weis E (1991) Chlorophyll Fluorescence and Photosynthesis: The Basics. Annu Rev Plant Physiol Plant Mol Biol **42**: 313–349

Külheim C, Agren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. Science **297**: 91–93

Kulheim C, Jansson S (2005) What leads to reduced fitness in non-photochemical quenching mutants? Physiol Plant **125**: 202–211

Laasch H, Weis E (1989) Photosynthetic control, "energy-dependent" quenching of chlorophyll fluorescence and photophosphorylation under influence of tertiary amines. Photosynth Res **22**: 137–146

Lee H-Y, Hong Y-N, Chow WS (2001) Photoinactivation of photosystem II complexes and photoprotection by non-functional neighbours in Capsicum annuum L. leaves. Planta **212**: 332–342

Lemeille S, Rochaix J-D (2010) State transitions at the crossroad of thylakoid signalling pathways. Photosynth Res **106**: 33–46

Lennon AM, Prommeenate P, Nixon PJ (2003) Location, expression and orientation of the putative chlororespiratory enzymes, Ndh and IMMUTANS, in higher-plant plastids. Planta **218**: 254–260

Li X, Muller-Moule P, Gilmore AM, Niyogi KK (2002) PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. Proc Natl Acad Sci U S A **99**: 15222–15227

Li XP, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature **403**: 391–395

Li X-P, Gilmore AM, Caffarri S, Bassi R, Golan T, Kramer D, Niyogi KK (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. J Biol Chem **279**: 22866–22874

Lichtenhaler HK, Kuhn G, Prenzel U, Buschmann C, Meier D (1982) Adaptation of chloroplast-ultrastruture and of chlorophyll-protein levels to high-light and low-light growth conditions. Z Naturforsch **37c**: 464–475

Lichtenthaler HK (1984) Differences in morphology and chemical composition of leaves grown at different light intensities and qualities. Contro Leaf Growth **27**: 201–221

Lichtenthaler HK (1981) Adaptation of leaves and chloroplasts to high quanta fluence rates. Photosynthesis **6**: 273–287

Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf U (1981) Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynth Res **2**: 115–141

Lichtenthaler HK, Kuhn G, Prenzel U, Meier D (1982) Chlorophyll-protein levels and degree of thylakoid stacking in radish chloroplasts from high-light, low-light and bentazon-treated plants. Physiol Plant **56**: 183–188

Lichtenthalera HK, Meiera D, Buschmann C (1984) Development of chloroplasts at high and low light quanta fluence rates. Isr J Bot **33**: 185–194

Livingston AK, Cruz J a, Kohzuma K, Dhingra A, Kramer DM (2010) An Arabidopsis mutant with high cyclic electron flow around photosystem I (hcef) involving the NADPH dehydrogenase complex. Plant Cell **22**: 221–33

Makino A, Miyake C, Yokota A (2002) Physiological functions of the water-water cycle (Mehler reaction) and the cyclic electron flow around PSI in rice leaves. Plant Cell Physiol **43**: 1017–26

Matsubara S, Chow WS (2004) Populations of photoinactivated photosystem II reaction centers characterized by chlorophyll a fluorescence lifetime in vivo. Proc Natl Acad Sci U S A **101**: 18234–18239

Maxwell PC, Biggins J (1976) Role of cyclic electron transport in photosynthesis as measured by the photoinduced turnover of P700 in vivo. Biochemistry **15**: 3975–3981

Meier D, Lichtenthaler HK (1981) Ultrastructural development of chloroplasts in radish seedlings grown at high- and low-light conditions and in the presence of the herbicide bentazon. Protoplasma **107**: 195–207

Miyake C (2010) Alternative electron flows (water-water cycle and cyclic electron flow around PSI) in photosynthesis: molecular mechanisms and physiological functions. Plant Cell Physiol **51**: 1951–63

Miyake C (2002) Cyclic Electron Flow within PSII Functions in Intact Chloroplasts from Spinach Leaves. Plant Cell Physiol **43**: 951–957

Miyake C, Yokota a (2001) Cyclic flow of electrons within PSII in thylakoid membranes. Plant Cell Physiol **42**: 508–15

Muller P, Li X-P, Niyogi KK (2001) Non-Photochemical Quenching . A Response to Excess Light Energy. Plant Physiol **125**: 1558–1566

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature **429**: 579–82

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell **110**: 361–371

Munekage YN, Genty B, Peltier G (2008) Effect of PGR5 impairment on photosynthesis and growth in Arabidopsis thaliana. Plant Cell Physiol **49**: 1688–98

Murata N (1969) Control of excitation transfer in photosynthesis I. Light-induced change of chlorophyll a fluoresence in Porphyridium cruentum. Biochim Biophys Acta - Bioenerg **172**: 242–251

Nandha B, Finazzi G, Joliot P, Hald S, Johnson GN (2007) The role of PGR5 in the redox poising of photosynthetic electron transport. Biochim Biophys Acta **1767**: 1252–9

Nedbal L, Brezina V, Cervený J, Trtílek M (2005) Photosynthesis in dynamic light: systems biology of unconventional chlorophyll fluorescence transients in Synechocystis sp. PCC 6803. Photosynth Res **84**: 99–106

Nishiyama Y, Murata N (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. Appl Microbiol Biotechnol. doi: 10.1007/s00253-014-6020-0

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol **50**: 333–359

Niyogi KK (2000) Safety valves for photosynthesis. Curr Opin Plant Biol **3**: 455–460

Niyogi KK, Grossman AR, Björkman O (1998) Arabidopsis Mutants Define a Central Role for the Xanthophyll Cycle in the Regulation of Photosynthetic Energy Conversion. Plant Cell **10**: 1121–1134

Oguchi R, Douwstra P, Fujita T, Chow WS, Terashima I (2011a) Intra-leaf gradients of photoinhibition induced by different color lights: implications for the dual mechanisms of photoinhibition and for the application of conventional chlorophyll fluorometers. New Phytol **191**: 146–59

Oguchi R, Hikosaka K, Hirose T (2005) Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. Plant, Cell Environ **28**: 916–927

Oguchi R, Hikosaka K, Hiura T, Hirose T (2006) Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. Oecologia **149**: 571–582

Oguchi R, Terashima I, Kou J, Chow WS (2011b) Operation of dual mechanisms that both lead to photoinactivation of Photosystem II in leaves by visible light. Physiol Plant **142**: 47–55

Oja V, Bichele I, Hüve K, Rasulov B, Laisk A (2004) Reductive titration of photosystem I and differential extinction coefficient of P700+ at 810-950 nm in leaves. Biochim Biophys Acta **1658**: 225–34

Okegawa Y, Kagawa Y, Kobayashi Y, Shikanai T (2008) Characterization of factors affecting the activity of photosystem I cyclic electron transport in chloroplasts. Plant Cell Physiol **49**: 825–34

Okegawa Y, Long TA, Iwano M, Takayama S, Kobayashi Y, Covert SF, Shikanai T (2007) A balanced PGR5 level is required for chloroplast development and optimum operation of cyclic electron transport around photosystem I. Plant Cell Physiol **48**: 1462–1471

Olle B, Demmig B (1987) Planta characteristics at 77 K among vascular plants of diverse origins *. Planta **170**: 489–504

Öquist G, Huner NPA (2003) Photosynthesis of overwintering evergreen plants. Annu Rev Plant Biol **54**: 329–355

Osmond B, Badger M, Maxwell K, Bjorkman O, Leegood R (1997) Too many photons: photorespiration, photoinhibition and photooxidation. Trends Plant Sci **2**: 119–121

Osmond CB (1981) Photorespiration and photoinhibition. Biochim Biophys Acta - Rev Bioenerg **639**: 77–98

Osmond CB, Grace SC (1995) Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J Exp Bot **46**: 1351–1362

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv' / Fm' without measuring Fo'. Photosynth Res **54**: 135–142

Pearcy RW (1990) Sunflecks and Photosynthesis in Plant Canopies. Annu Rev Plant Physiol Plant Mol Biol **41**: 421–453

Pearcy RW (1983) The light environment and growth of C3 and C4 tree species in the understory of a Hawaiian forest. Oecologia **58**: 19–25

Pearcy RW, Chazdon RL, Gross LI, Mott KA (1994) Photosynthetic utilization of sunflecks: a temporally patchy resource on a time scale of seconds to minutes. Exploit Environ Heterog by plants 175–208

Peng L, Fukao Y, Fujiwara M, Takami T, Shikanai T (2009) Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in Arabidopsis. Plant Cell **21**: 3623–40

Peng L, Yamamoto H, Shikanai T (2011) Structure and biogenesis of the chloroplast NAD(P)H dehydrogenase complex. Biochim Biophys Acta **1807**: 945–53

Pesaresi P, Pribil M, Wunder T, Leister D (2011) Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. Biochim Biophys Acta **1807**: 887–96

Peterson R, Havir E (2001) Photosynthetic properties of an Arabidopsis thaliana mutant possessing a defective PsbS gene. Planta **214**: 142–152

Pfündel E (1998) Estimating the contribution of Photosystem I to total leaf chlorophyll fluorescence. Photosynth Res **56**: 185–195

Pfündel EE, Klughammer C, Meister A, Cerovic ZG (2013) Deriving fluorometer-specific values of relative PSI fluorescence intensity from quenching of F(0) fluorescence in leaves of Arabidopsis thaliana and Zea mays. Photosynth Res **114**: 189–206

Porcar-Castell A, Bäck J, Juurola E, Hari P (2006) Dynamics of the energy flow through photosystem II under changing light conditions: a model approach. Funct Plant Biol **33**: 229–239

Porra RJ, Thompson W a., Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification

of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta - Bioenerg **975**: 384–394

Quick WP, Stitt M (1989) An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim Biophys Acta - Bioenerg **977**: 287–296

Rappaport F, Béal D, Joliot A, Joliot P (2007) On the advantages of using green light to study fluorescence yield changes in leaves. Biochim Biophys Acta **1767**: 56–65

Rochaix J-D, Lemeille S, Shapiguzov A, Samol I, Fucile G, Willig A, Goldschmidt-Clermont M (2012) Protein kinases and phosphatases involved in the acclimation of the photosynthetic apparatus to a changing light environment. Philos Trans R Soc Lond B Biol Sci **367**: 3466–3474

Rosso D, Ivanov AG, Fu A, Geisler-Lee J, Hendrickson L, Geisler M, Stewart G, Krol M, Hurry V, Rodermel SR, et al (2006) IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of Arabidopsis during steady-state photosynthesis. Plant Physiol **142**: 574–585

Rothstein DE, Zak DR (2001) Photosynthetic adaptation and acclimation to exploit seasonal periods of direct irradiance in three temperate, deciduous-forest herbs. Funct Ecol **15**: 722–731

Rott M, Martins NF, Thiele W, Lein W, Bock R, Kramer DM, Schöttler M a (2011) ATP synthase repression in tobacco restricts photosynthetic electron transport, CO2 assimilation, and plant growth by overacidification of the thylakoid lumen. Plant Cell **23**: 304–321

Le Roux X, Walcroft a S, Sinoquet H, Chaves MM, Rodrigues A, Osorio L (2001) Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. Tree Physiol **21**: 377–386

Rozak PR, Seiser RM, Wacholtz WF, Wise RR (2002) Rapid, reversible alterations in spinach thylakoid appression upon changes in light intensity. Plant, Cell Environ **25**: 421–429

Ruban A V, Johnson MP, Duffy CDP (2012) The photoprotective molecular switch in the photosystem II antenna. Biochim Biophys Acta **1817**: 167–181

Rumberg B, Siggel U (1969) pH changes in the inner phase of the thylakoids during photosynthesis. Naturwissenschaften **56**: 130–132

Sacksteder C a, Kanazawa a, Jacoby ME, Kramer DM (2000) The proton to electron stoichiometry of steady-state photosynthesis in living plants: A proton-pumping Q cycle is continuously engaged. Proc Natl Acad Sci U S A **97**: 14283–8

Sacksteder C a, Kramer DM (2000) Dark-interval relaxation kinetics (DIRK) of absorbance changes as a quantitative probe of steady-state electron transfer. Photosynth Res **66**: 145–58

Sarvikas P, Hakala M, Pätsikkä E, Tyystjärvi T, Tyystjärvi E (2006) Action spectrum of photoinhibition in leaves of wild type and npq1-2 and npq4-1 mutants of Arabidopsis thaliana. Plant Cell Physiol **47**: 391–400

Sarvikas P, Hakala-Yatkin M, Dönmez S, Tyystjärvi E (2010a) Short flashes and continuous light have similar photoinhibitory efficiency in intact leaves. J Exp Bot **61**: 4239–47

Sarvikas P, Tyystjärvi T, Tyystjärvi E (2010b) Kinetics of prolonged photoinhibition revisited: photoinhibited Photosystem II centres do not protect the active ones against loss of oxygen evolution. Photosynth Res **103**: 7–17

Scheller H V. (1996) In Vitro Cyclic Electron Transport in Barley Thylakoids follows Two Independent Pathways. Plant Physiol **110**: 187–194

Schreiber U, Hormann H, Asada K, Neubauer C (1995) O2-dependent electron flow in spinach chloroplasts: properties and possible regulation of the Mehler- ascorbate peroxidase cycle. Photosynthesis **2**: 813–818

Schreiber U, Klughammer C (2008) New accessory for the Dual-PAM-100 : The P515 / 535 module and examples of its application. PAM Appl Notes **1**: 1–10

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10**: 51–62

Sejima T, Takagi D, Fukayama H, Makino A, Miyake C (2014) Repetitive short-pulse light mainly inactivates photosystem I in sunflower leaves. Plant Cell Physiol **55**: 1184–93

Shikanai T (2007) Cyclic electron transport around photosystem I: genetic approaches. Annu Rev Plant Biol **58**: 199–217

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota a (1998) Directed disruption of the tobacco ndhB gene impairs cyclic electron flow around photosystem I. Proc Natl Acad Sci U S A **95**: 9705–9

Shikanai T, Munekage Y, Shimizu K, Endo T, Hashimoto T (1999) Identification and Characterization of Arabidopsis Mutants with Reduced Quenching of Chlorophyll Fluorescence. Plant Cell Physiol **40**: 1134–1142

Shimizu H, Peng L, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2008) CRR23/NdhL is a subunit of the chloroplast NAD(P)H dehydrogenase complex in Arabidopsis. Plant Cell Physiol **49**: 835–42

Singsaas EL, Ort DR, DeLUCIA EH (2000) Diurnal regulation of photosynthesis in understory saplings. New Phytol **145**: 39–49

Sonoike K (1996a) Degradation of psaB gene product, the reaction center subunit of photosystem I, is caused during photoinhibition of

photosystem I: possible involvement of active oxygen species. Plant Sci **115**: 157–164

Sonoike K (2011) Photoinhibition of photosystem I. Physiol Plant **142**: 56–64

Sonoike K (1996b) Photoinhibition of Photosystem I: Its Physiological Significance in the Chilling Sensitivity of Plants. Plant Cell Physiol **37**: 239–247

Sonoike K, Kamo M, Hihara Y, Hiyama T, Enami I (1997a) The mechanism of the degradation of psaB gene product, one of the photosynthetic reaction center subunits of photosystem I, upon photoinhibition. Photosynth Res **53**: 55–63

Sonoike K, Kamo M, Hihara Y, Hiyama T, Enami I (1997b) The mechanism of the degradation of psa B gene product , one of the photosynthetic reaction center subunits of Photosystem I , upon photoinhibition. Photosynth Res **53**: 55–63

Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem I iron-sulfur centers in leaves of Cucumis sativus L. by weak illumination at chilling temperatures. FEBS Lett **362**: 235–238

Stefanov D, Terashima I (2008) Non-photochemical loss in PSII in high- and low-light-grown leaves of Vicia faba quantified by several fluorescence parameters including L(NP), F0/F'm, a novel parameter. Physiol Plant **133**: 327–38

Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzykowska M, Rantala M, Kangasjärvi S, Paakkarinen V, Tikkanen M, Jansson S, et al (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. Plant Cell **24**: 2934–2948

Suorsa M, Rantala M, Danielsson R, Järvi S, Paakkarinen V, Schröder WP, Styring S, Mamedov F, Aro E-M (2013) Dark-adapted spinach thylakoid protein heterogeneity offers insights into the photosystem II repair cycle. Biochim Biophys Acta. doi: 10.1016/j.bbabio.2013.11.014

Takabayashi A, Kishine M, Asada K, Endo T, Sato F (2005) Differential use of two cyclic electron flows around photosystem I for driving CO2-concentration mechanism in C4 photosynthesis. Proc Natl Acad Sci U S A **102**: 16898–903

Takahashi S, Murata N (2008) How do environmental stresses accelerate photoinhibition? Trends Plant Sci **13**: 178–182

Takizawa K, Cruz J a, Kanazawa A, Kramer DM (2007) The thylakoid proton motive force in vivo. Quantitative, non-invasive probes, energetics, and regulatory consequences of light-induced pmf. Biochim Biophys Acta **1767**: 1233–1244

Takizawa K, Kanazawa A, Kramer DM (2008) Depletion of stromal P(i) induces high "energy-dependent" antenna exciton quenching (q(E)) by decreasing proton conductivity at CF(O)-CF(1) ATP synthase. Plant Cell Environ **31**: 235–243

Terashima I, Funayama S, Sonoike K (1994) The site of photoinhibition in leaves of Cucumis sativus L. at low temperatures is photosystem I, not photosystem II. Planta **193**: 300–306

Tikhonov AN (2013) pH-dependent regulation of electron transport and ATP synthesis in chloroplasts. Photosynth Res **116**: 511–534

Tikhonov AN, Khomutov GB, Ruuge EK, Blumenfeld LA (1981) Electron transport control in chloroplasts. Effects of photosynthetic control monitored by the intrathylakoid pH. Biochim Biophys Acta - Bioenerg **637**: 321–333

Tikkanen M, Grieco M, Kangasjärvi S, Aro E-M (2010) Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. Plant Physiol **152**: 723–735

Tikkanen M, Mekala NR, Aro E-M (2013) Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage. Biochim Biophys Acta **1837**: 210–215

Tjus SE, Møller BL, Scheller HV (1999) Photoinhibition of photosystem I damages both reaction centre proteins PSI-A and PSI-B and acceptor-side located small photosystem I polypeptides. Photosynth Res **60**: 75–86

Tyystjarvi E, Ali-yrkko K, Kettunen R, Aro E (1992) Slow Degradation of the Dl Protein Is Related to the Susceptibility of Low-Light-Grown Pumpkin Plants to Photoinhibition. Plant Physiol **100**: 1310–1317

Tyystjärvi E, Aro E-M (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc Natl Acad Sci U S A **93**: 2213–2218

Tyystjärvi E, Mäenpää P, Aro EM (1994) Mathematical modelling of photoinhibition and Photosystem II repair cycle. I. Photoinhibition and D1 protein degradation in vitro and in the absence of chloroplast protein synthesis in vivo. Photosynth Res **41**: 439–449

Ueda M, Kuniyoshi T, Yamamoto H, Sugimoto K, Ishizaki K, Kohchi T, Nishimura Y, Shikanai T (2012) Composition and physiological function of the chloroplast NADH dehydrogenase-like complex in Marchantia polymorpha. Plant J **72**: 683–93

Vass I (2011) Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. Physiol Plant **142**: 6–16

Vierling LA, Wessman CA (2000) Photosynthetically active radiation heterogeneity within a monodominant Congolese rain forest canopy. Agric For Meteorol **103**: 265–278

Wagner H, Jakob T, Wilhelm C (2006) Balancing the energy flow from captured light to biomass under fluctuating light conditions. New Phytol **169**: 95–108

Waiters RG, Horton P (1991) Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. Photosynth Res **27**: 121–133

Walters RG, Horton P (1993) Theoretical assessment of alternative mechanisms for non-photochemical quenching of PS II fluorescence in barley leaves. Photosynth Res **36**: 119–139

Weis E, Berry JA (1987) Quantum efficiency of Photosystem II in relation to "energy"-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta - Bioenerg **894**: 198–208

West KR, Wiskich JT (1968) Photosynthetic control by isolated pea chloroplasts. Biochem J **109**: 527–532

Wünschmann G, Brand JJ (1992) Rapid turnover of a component required for photosynthesis explains temperature dependence and kinetics of photoinhibition in a cyanobacterium, Synechococcus 6301. Planta **186**: 426–33

Yamamoto H, Peng L, Fukao Y, Shikanai T (2011) An Src homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in Arabidopsis. Plant Cell **23**: 1480–93

Yamori W, Sakata N, Suzuki Y, Shikanai T, Makino A (2011) Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. Plant J **68**: 966–76

Yin ZH, Johnson GN (2000) Photosyhtetic acclimation of higher plants to growth in fluctuating light environments. Photosynth Res **63**: 97–107

Zhang S, Scheller HV (2004) Photoinhibition of photosystem I at chilling temperature and subsequent recovery in Arabidopsis thaliana. Plant Cell Physiol **45**: 1595–1602