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REGULAR PAPER

Is light quality involved in the regulation of the photosynthetic apparatus in attached rice leaves?

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Abstract The regulatory effect of light quality on the photosynthetic apparatus in attached leaves of rice plants was investigated by keeping rice plants under natural light, in complete darkness, or under illumination with light of different colors. When leaves were left in darkness and far-red (FR)-light conditions for 6 days at 30°C, there was an initial lag in chlorophyll (Chl) content, Chl *a*/*b* ratio, and maximum photosystem (PS) II photochemistry that lasted until the second day; these then rapidly decreased on the fourth day. In contrast, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) rapidly disappeared with no lag under low or zero light conditions. By using spectrophotometric quantitation, it was determined that the PSII and PSI reaction centers were regulated by light quality, but cytochrome (Cyt) *f* was regulated by light intensity. However, the PSII heterogeneity was also strongly modified by the light intensity; PSIIα with the large antenna decreased markedly both in content and in antenna size. Consequently, the PSIIα/PSI ratio declined under FR-light because the low intensity of FR-light dominated over its quality in the modulation of the PSIIα/PSI ratio. An imbalance between them induced the generation of reactive oxygen species (ROS), although the ROS were scavenged by stromal enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR). The activities of these stromal enzymes are also regulated by light quality. Thus, although the photosynthetic apparatus is regulated differently depending on light quality, light quality may play an important role in the regulation of the photosynthetic apparatus.

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Keywords Light quality · Light absorption balance · Photosystem stoichiometry · PSII heterogeneity · Reactive oxygen species · Rice (*Oryza sativa* L.)

Abbreviations

APX	Ascorbate peroxidase
C-550	Electrochromic band shift of pheophytin in the PSII reaction center complex
Chl	Chlorophyll
Cyt	Cytochrome
DCMU	3-(3,4-Dichlorophenyl)-1, 1-dimethylurea
Fv/Fm	Maximal photochemical efficiency of PSII in the dark-adapted leaves
GR	Glutathione reductase
GSH and GSSG	Reduced and oxidized glutathione, respectively
PS	Photosystem
Q_A	Primary quinone acceptor in PSII
Q_B	Secondary quinone acceptor in PSII
R- and FR-light	Red and far-red light, respectively
ROS	Reactive oxygen species
Rubisco	Ribulose 1,5-bisphosphate carboxylase/oxygenase
SOD	Superoxide dismutase

Introduction

Plants respond to two light factors, i.e., the light intensity and the spectral quality.

In upright plants, the light intensity decreases exponentially from the uppermost to the bottom layer due to

mutual shading in the canopies of plants (Monsi and Saeki 1953). Alterations in the photosynthetic properties and the thylakoid components in response to short term/growth irradiance have also been noted in several plant species. A decrease in growth irradiance resulted in significant decreases in photosystem (PS) II in peas (Leong and Anderson 1984; Chow and Anderson 1987; Evans 1987), barley (De la Torre and Burkey 1990) and rice (Yamazaki et al. 1999a, b), while PSI/chlorophyll (Chl) remained relatively constant, resulting in a low PSII/PSI ratio. In the previous study, although a low PSII/PSI ratio created uneven absorption of light between the two PSs, the imbalance was compensated for by the modulation of the two types of PSII centers (Yamazaki et al. 1999a). One PSII center has a large antenna and is functional in electron transport, and the other has a small antenna and is unable to transfer electrons from Q_A^- to Q_B. The former is referred to as PSIIα, and the latter as PSIIβ (Melis and Homann 1975, 1976). This indicates that changes in the stoichiometry of PSII and PSI are of importance in maintaining photosynthetic efficiency under light-limiting conditions.

Okada et al. (1992) found that Chl degradation was suppressed by short exposure of the leaves to red (R)-light, and the effect of R-light was suppressed by far-red (FR)-light. This result suggests that phytochrome is involved in this process. A field experiment with dense canopies of *Helianthus annuus* demonstrated that FR enrichment of the light penetrating the canopy enhanced the senescence of the lower leaves (Rousseaux et al. 1996).

In several reports regarding PS stoichiometries, Melis and his co-workers (for reviews see Melis 1991, 1996) examined the effects of light quality during plant and algal cell growth using spectro/kinetics methods, and they revealed a compensatory system that adjusts the two PS stoichiometries depending on light quality. Chow et al. (1990b) found that when pea plants were grown under light of a spectral quality designed to over-stimulate either PSII or PSI, changes in the PSII/PSI ratio were observed that appeared to compensate for the deficiency in excitation of one or the other PS. The redox state of the different components of the photosynthetic electron transport chain is modulated by the spectral quality as well as by the light intensity. In cyanobacteria, light quality would regulate the expression of *psbA* and *psaE* genes, which encode the D1-protein of the reaction center of the PSII and the subunit PsaE of the PSI involved in ferredoxin binding, respectively, by means of a redox sensory mechanism (Allen 1995; El Bissati and Kirilovsky 2001). Besides, light quality alters photosynthesis by affecting the activity of the photosynthetic apparatus in leaves and the expression and/or activity of the Calvin-Benson cycle enzymes in *Cucumis sativus* (Wang et al. 2009) and adjusts several cellular processes in cyanobacteria (Singh et al. 2009). Thus, light quality controls a part of the photosynthetic machinery. In the lower canopy, the decrease in the irradiance due to selective filtering by photosynthetic pigments is always accompanied by a low R/FR ratio (Yamazaki et al. 1999a). Yamazaki and Kamimura (2002) found that an imbalance in light absorption balance between PSII and PSI in the bottom leaves induced the accumulation of reactive oxygen species (ROS) to accelerate, generating a Mehler reaction on the reducing side of PSI and leading to the death of the leaves.

In plant cells, ROS, e.g., the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), are generated via the photosynthetic electron transport pathway and attack cellular molecules such as DNA, Rubisco, and Chl, leading to the acceleration of the plant cell senescence process (reviewed by Thompson et al. 1987; Buchanan-Wollaston 1997; del Río et al. 1998; Yamazaki and Kamimura 2002). ROS are safely scavenged by antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Asada 1999). In addition, Mullineaux et al. (2000) found that phytochrome regulates the expression of *APX1* and *APX3*, which encode stromal ascorbate peroxidase (sAPX).

Opinions are divided among investigators on the effects of phytochrome on photosynthesis and/or the two PSs. Okada and Katoh (1998) elucidated the evidence that phytochrome is involved in the stabilization of Chl, whereas the photoprotection of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a complex reaction including signal functions of light. On the other hand, shading with black cloth, which does not change the R/FR ratio of the light, has successfully mimicked the effects of natural shade on leaf characteristics in many shading experiments (Smith et al. 1993; Hikosaka 1996). Yamazaki et al. (1999a) showed using a juvenile rice canopy that the light intensity rather than the light quality mainly affects the longevity of the leaves placed in the bottom layer. Furthermore, Tinoco-Ojanguren and Pearcy (1995) compared the open field condition to the FR-enriched condition, which resembled the understory, and showed that light quality did not affect the photosynthetic characteristics.

To examine whether the composition and function of photosynthetic apparatus are regulated by light quality, this study monitored the photosynthetic properties and the thylakoid components under different color conditions. In addition, relationship between the stoichiometry of PSII and PSI and the activities of ROS-scavenging enzymes closely related to the photosynthetic electron transport were taken into consideration.

Materials and methods

Plant growth condition

Rice seedlings (*Oryza sativa* L. cv. Norin No. 8) were grown at the campus of Toho University, Funabashi, Chiba prefecture, Japan (35°41′N, 140°02′E; 20 m a.s.l.) using the same procedure described by Yamazaki et al. (2000). In brief, germinated seeds were planted in a plastic container at 3-cm intervals in an artificial granular soil, Bonsol No. 1 (Sumitomo Kagaku, Osaka, Japan), which contained sufficient levels of nutrients for the growth of rice seedlings during the period of this study. Rice plants were grown in a greenhouse under natural sunlight for 2–3 weeks and watered daily. Leaves were numbered in the order of their development. When the fourth leaves were fully expanded, three quarters of the plants were transferred to a light-controlled room kept at 30°C, and the rest remained in a greenhouse for six more days. In a light-controlled room, one-third of the plants were placed in complete dark, and two-thirds of the plants were irradiated under a 12 h photoperiod with R- or FR-light in a light-tight compartment. R-light was obtained by passing light from a fluorescent lamp (Plant lux FL20SS; Toshiba, Tokyo, Japan) through a 3-mm thick red acryl resin filter (Shinkolite #102; Mitsubishi Rayon Co., Tokyo, Japan). A combination of a fluorescent lamp (FL20SFR74; Toshiba, Tokyo, Japan) and a 3-mm thick acryl filter (Delaglass A-900; Asahi Kasei Chemical Co., Tokyo, Japan) was the source of FR-light. The relative spectral radiation energy distribution of R-light and FR-light is shown in Fig. 1. The intensities of R-light and FR-light were 0.5 and 0.62 μmol photons m^{-2} s^{-1}, respectively, at the level of the leaf surfaces. Light intensity in red region was determined with a Li-Cor quantum sensor (LI-190; Li-Cor Co., Lincoln, NE, USA) and that in far-red region measured with a Kipp & Zonen laboratory thermopile (CA2; Kipp & Zonen, Delft, the Netherlands) and converted into the photosynthetic photon flux density at 750 nm. The fourth leaves were used for analysis. In this experiment, ventilation in the dark room was improved in order to eliminate the advance in senescence induced by some phytohormones, e.g., ethylene, as much as possible.

Measurement of photosynthetic characteristics

Photosynthesis of leaves was measured with a gas-phase leaf-disk oxygen electrode (LD2/3; Hansatech, UK) at 30°C. Leaves were harvested between 10:00 and 11:00 (local time) and immediately measured. The gas phase consisted of air containing 4% CO_2. A saturating actinic light (2000 μmol photons m^{-2} s^{-1}) was provided by a 100-W halogen lamp. Leaf area was estimated by inputting leaf shapes into a computer with an image scanner. After photosynthesis measurement, Chl *a* fluorescence was measured with a portable fluorometer (MINI-PAM; Walz, Germany) at room temperature and under ambient CO_2 concentration after the leaves had been dark-adapted for at least 30 min. The standard Chl fluorescence nomenclature was followed as described by van Kooten and Snel (1990). Maximal photochemical efficiency of PSII was calculated as the ratio of variable to maximum fluorescence (Fv/Fm) for dark-adapted leaves (Yamazaki et al. 2007).

Assays of Chl and Rubisco

For Chl and Rubisco determination, leaf segments were homogenized in cold buffer containing 100 mM phosphate buffer (pH 7.0), 10 mM NaCl, 10 mM $MgCl_2$, and 5 mM iodoacetic acid. A portion (0.2 ml) of the homogenates was used for Chl determination. Chl was extracted with 80% (v/v) aqueous acetone and quantified spectrophotometrically (Porra et al. 1989). The rest of the homogenate was centrifuged at 35,000×*g* for 30 min, and the supernatant was used

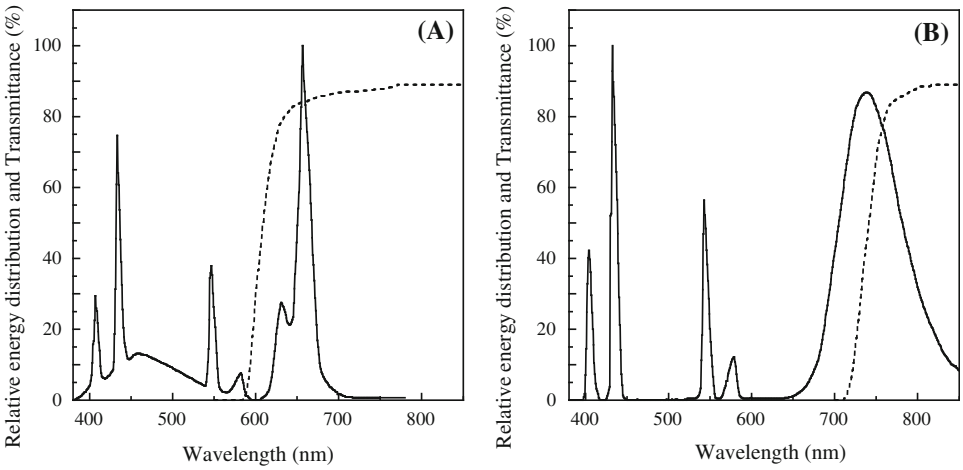

Fig. 1 Relative spectral radiation energy distribution of red light (**a**) and far-red light (**b**). The combination of fluorescent lamps and filters was described in "Materials and methods" in detail. *Solid* and *broken lines* represent the spectra of the fluorescent lamps and the transmittance of filters, respectively

for determination of the large subunit of Rubisco. The supernatant was denatured with 2.5% SDS, 8 M urea, and 5% (v/v) 2-mercaptoethanol for 2 h at room temperature, and then 5 µl of the sample solution was applied to 15% acrylamide gel. Electrophoresis was carried out according to the buffer system of Laemmli (1970). The gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 dissolved in 15% methanol and 7% acetic acid for 1 h and, after destaining, were then analyzed with ImageJ software (NIH, http://rsb.info.nih.gov/ij/).

Thylakoid membrane preparation and spectrophotometrical assays

The thylakoid membranes of rice leaves were isolated as described in Yamazaki et al. (1999a). In brief, leaves were homogenized in a cold buffer that contained 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 50 mM HEPES–NaOH (pH 7.5) for 30 s with a Waring blender. The homogenate was filtered through two layers of Miracloth (Calbiochem, USA), and the filtrate was centrifuged at 250×*g* for 5 min. The supernatant was centrifuged at 5500×*g* for 15 min, and the precipitate was suspended in the medium just described. The levels of P-700, C-550, and cytochrome (Cyt) *f* were measured with a Hitachi 556 spectrophotometer in accordance with Yamazaki et al. (1999a). The two types of PSII reaction centers, PSIIα and PSIIβ, were estimated by analyzing the growth of the area over the fluorescence induction curves in the presence of DCMU with a custom-made fluorometer as described in Yamazaki et al. (1999a).

Assays of active oxygen-scavenging enzymes

Measurements of active oxygen species-scavenging enzymes were carried out at 25°C, and the activity was expressed on the basis of leaf area. APX and GR activities were determined by the following method. Leaf segments were cut into small pieces, and these segments were homogenized in an ice-cold buffer containing 0.1 M phosphate buffer (pH 7.0), 1 mM sodium ascorbate, and 1 mM EDTA with a mortar and pestle. The homogenate was centrifuged at 10,000×*g* for 5 min. For the APX assay, the reaction mixture, which contained 50 mM phosphate buffer (pH 7.0), 1 mM sodium ascorbate, and 50 µl of the extract, was equilibrated for 3 min. The reaction was started by adding 0.5 mM H_2O_2, with the decrease in ascorbate being monitored at 300 nm and the extinction coefficient used being 0.49 mM^{-1} cm^{-1} (Amako et al. 1994), as the absorbance at 290 nm was too high due to proteins in the extract. For the GR assay, the reaction mixture contained 0.3 ml of 5 mM GSSG, 0.2 ml of 2 mM NADPH, and 0.3 ml of the extract (Jablonski and Anderson 1978). The consumption of NADPH was defined as a decrease in absorbance at 340 nm during the first minute of the reaction, and the extinction coefficient used was 6.2 mM^{-1} cm^{-1} for NADPH. SOD extracted from leaf segments was ground with a mortar and pestle in 50 mM Tris–HCl (pH 7.8), 0.1 mM EDTA, and 0.25 (w/v) Triton X-100. The homogenate was centrifuged at 13,000×*g* for 5 min. The supernatant was used for the SOD assay, and the SOD activity was estimated by the inhibition of nitroblue tetrazolium (NBT) reduction (Beyer and Fridovich 1987). The reaction mixture contained 0.3 ml each of 20 µM riboflavin, 150 mM L-methionine, 600 µM NBT, and extracts of 0.1, 0.2, 0.3, and 0.4 ml. The above buffer was added to a final volume of 3 ml. The reaction was started by adding riboflavin and was carried out for 17 min under an irradiance of 170 µmol photons m^{-2} s^{-1} provided by a white fluorescent lamp. The absorbance at 560 nm was determined regularly, and the extract volume causing a 50% inhibition of NBT reduction was taken as one unit of activity.

Results

Effects of light quality on the photosynthetic properties, Chl concentration and Rubisco contents

To examine whether the photosynthetic properties, Chl concentration, Chl *a*/*b* ratios and the relative amount of Rubisco are regulated by light quality, these parameters were determined in leaves that had been kept under illumination with various treatments of light for 6 days. The photosynthetic activities (Fig. 2a) and the relative amount of Rubisco (Fig. 2e) remaining relatively constant only under natural light conditions, while low light of any spectral quality led to an immediate decrease. On the other hand, the decline of the Fv/Fm ratio (Fig. 2b), Chl *a*/*b* ratios (Fig. 2e), and the degradation of Chl (Fig. 2c) were unchanged for up to 2 days, and were suppressed only after more than 2 days at low or zero light intensity, the suppression being greater in R-light than in FR-light. These results revealed that Chl degradation was regulated by the light quality, while that of Rubisco was regulated by the light intensity.

Effects of light quality on the levels of thylakoid components

C-550, which is the electrochromic band shift of pheophytin in the PSII reaction center complex, is known to be proportional to the amount of PSII reaction complex estimated from the Q_A photo-oxidation (McCauley and Melis 1986). The level of PSII reaction centers (measured as

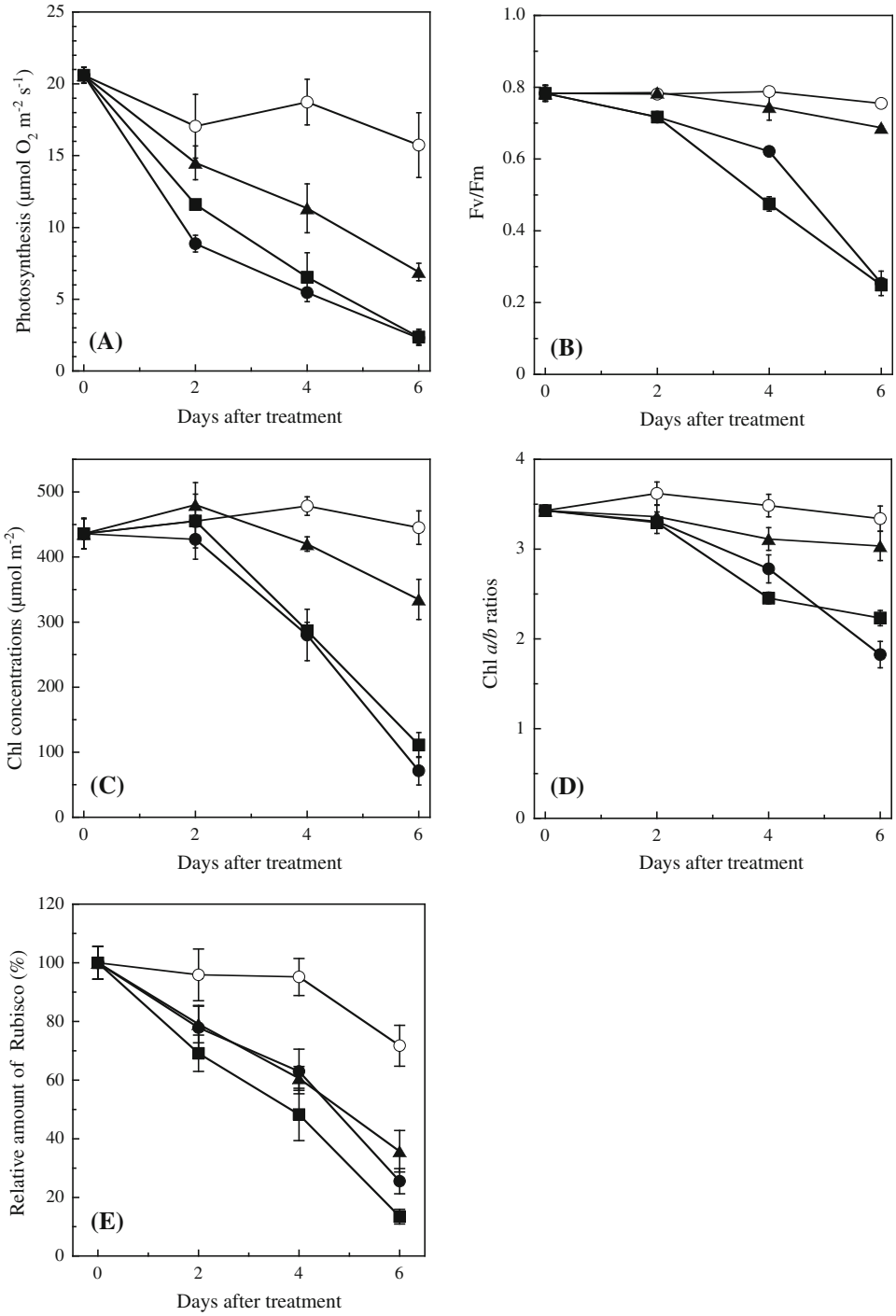

Fig. 2 Time courses of the light-saturated photosynthesis (**a**), maximal quantum yield of PSII in the dark-adapted leaves (Fv/Fm) (**b**), Chl contents (**c**), Chl *a/b* ratio (**d**), and relative amounts of Rubisco (**e**). *Open circles*, *closed circles*, *closed triangles*, and *closed squares* represent the natural light, dark, red light, and far-red light treatments over 6 days, respectively. In (**d**), the amount on day 0 was taken as 100%. *Bars* indicate SD ($n = 5$)

C-550) remained constant under the natural light and R-light but decreased in the dark and FR-light treatments, while the amount of P-700 based on Chl was constant under all light treatments. Consequently, the ratio of PSII to PSI reaction centers declined below one in the dark and FR-light treatments (Table 1). It is recognized that the degradation of Cyt *f* occurs in dark treatments or under natural shade conditions (Leong and Anderson 1984; Yamazaki et al. 1999a). The levels of Cyt *f*, however, also remained constant under all light treatments. Thus, this result indicates that the ratios of PSII to PSI are regulated by light quality, while the levels of Cyt *f* are not.

Effects of the light quality on the PSII antenna heterogeneity

In terms of both function and antenna size, the PSII reaction centers are heterogeneous (Melis and Homann 1975,

Table 1 Levels of thylakoid components and the PSII/PSI ratio under different light treatments over 6 days of incubation

Light treatments	C-550 (PSII) (mmol/mol Chl)	P-700 (PSI) (mmol/mol Chl)	Cyt *f* (mmol/mol Chl)	PSII/PSI
Day 0	4.50 ± 0.13^{a}	2.07 ± 0.32^{a}	1.51 ± 0.32^{a}	2.01 ± 0.17^{a}
Natural light day 6	4.44 ± 0.14^{a}	2.05 ± 0.13^{a}	1.34 ± 0.14^{a}	1.94 ± 0.28^{a}
Red day 6	4.07 ± 0.16^{a}	2.13 ± 0.22^{a}	1.52 ± 0.28^{a}	1.88 ± 0.09^{a}
Far-red day 6	2.44 ± 0.12^{b}	2.60 ± 0.37^{a}	1.39 ± 0.37^{a}	0.87 ± 0.08^{b}
Dark day 6	2.18 ± 0.24^{b}	2.17 ± 0.48^{a}	1.69 ± 0.07^{a}	0.88 ± 0.28^{b}

All experiments were repeated five times independently. About 300 leaves were used in each experiment. The values given are the means ± SD of five independent experiments. Letters indicate significant differences in the mean values ($P < 0.05$) according to Scheffe's multiple comparison tests

1976; Melis 1991, 1996). One has a large antenna and is functional in electron transport, and another has a small antenna and is unable to transfer electrons from Q_A^- to Q_B. The former is defined as PSIIα, and the latter as PSIIβ. Thus, the relative abundance of the PSII reaction centers can be kinetically distinguished as functional or non-functional in electron transport by measuring the rate constant of the photoreduction of Q_A through the growth of the area over the fluorescence induction curve.

Table 2 shows that the relative abundance of PSIIα was, on average, 64–65% of the total number of PSII centers on day 0 and under the natural light condition. The relative abundance of PSIIα centers decreased to 30% in the dark and 40% under FR-light over a six-day incubation. Kα and Kβ represent the rate constants of the PSIIα and the PSIIβ centers, respectively (Melis and Homann 1975, 1976), and they are proportional to the respective antenna size. There was also a small but significant decline in Kα at the low light intensity (Table 2). These results indicate that the proportion of the PSIIα and PSIIβ centers and the antenna size of PSIIα are regulated by the light intensity.

The total PSII/Cyt *f*/PSI ratio was 2.98:1:1.37 on day 0 and 1.29:1:1.28 in the dark condition (Table 3). When only the PSIIα centers that were functional in electron transport were taken into account, the PSIIα/Cyt *f*/PSI ratio was about 1.91:1:1.37 on day 0 and 0.40:1:1.28 in the dark condition. The tendencies under the R-light and FR-light treatments resembled those under natural light and complete darkness, respectively (Table 3). Thus, although the imbalance in light absorption of the two PSs would be partly compensated for by the modulation of the two types of PSII centers, the low light effect is dominant over the light quality effect (Chow et al. 1990a).

Effects of the light quality on the active oxygen-scavenging enzyme activities

The decrease in levels of Rubisco, which is the key enzyme in the Calvin-Benson cycle in chloroplasts (Fig. 1e), may reflect the degradation of many enzymes in stroma, including some ROS-scavenging enzymes under low light intensity. Among the enzymes responsible for the scavenging of ROS, SOD activity remained only under the natural light and R-light conditions (Table 4). We considered the performance of some enzyme activities from the perspective of H_2O_2 scavenging. APX, which is the first enzyme of this pathway, detoxifies H_2O_2 using ascorbate as a reductant (Nakano and Asada 1981). It was found that

Table 2 Relative abundances of PSIIα and IIβ and the rate constants of PSIIα and PSIIβ under different light treatments over 6 days of incubation

Light treatments	PSIIα (%)	PSIIβ (%)	Kα (s^{-1})	Kβ (s^{-1})
Day 0	64.1 ± 1.4^{a}	35.9 ± 1.4^{a}	16.8 ± 1.6^{a}	0.30 ± 0.07^{a}
Natural light day 6	64.9 ± 4.2^{a}	35.1 ± 4.2^{a}	16.2 ± 1.2^{a}	0.32 ± 0.04^{a}
Red day 6	52.3 ± 0.7^{b}	47.7 ± 0.7^{b}	10.1 ± 1.5^{b}	0.41 ± 0.01^{b}
Far-red day 6	43.2 ± 1.9^{c}	56.8 ± 1.9^{c}	7.5 ± 0.7^{b}	0.53 ± 0.01^{c}
Dark day 6	30.8 ± 7.7^{d}	69.2 ± 7.7^{d}	9.7 ± 1.0^{b}	0.43 ± 0.03^{b}

All experiments were repeated five times independently. About 300 leaves were used in each experiment. The values given are the means ± SD of five independent experiments. Letters indicate significant differences in the mean values ($P < 0.05$) according to Scheffe's multiple comparison tests

Table 3 Stoichiometries of the thylakoid components involved in the electron transport under different light treatments over 6 days of incubation

Light treatments	PSII/PSI	PSII/Cyt *f*/PSI	PSIIα/Cyt *f*/PSI
Day 0	2.01 ± 0.17^{a}	2.98:1:1.37	1.91:1:1.37
Natural light day 6	1.94 ± 0.28^{a}	3.31:1:1.52	2.15:1:1.52
Red day 6	1.88 ± 0.09^{a}	2.68:1:1.40	1.40:1:1.40
Far-red day 6	0.87 ± 0.08^{b}	1.76:1:1.87	0.76:1:1.87
Dark day 6	0.88 ± 0.28^{b}	1.29:1:1.28	0.40:1:1.28

All experiments were repeated five times independently. About 300 leaves were used in each experiment. The values given are the means ± SD of five independent experiments. Letters indicate significant differences in the mean values ($P < 0.05$) according to Scheffe's multiple comparison tests

Table 4 Activities of reactive oxygen species-scavenging enzymes under different light treatments over 6 days of incubation

Light treatments	SOD ($\times 10^3$ Units m^{-2})	APX (μmol m^{-2} s^{-1})	GR (μmol m^{-2} s^{-1})	SOD/APX
Day 0	85.5 ± 8.7[a]	107.9 ± 13.0[a]	2.3 ± 0.3[a]	0.79
Natural light day 6	88.8 ± 8.5[ab]	114.8 ± 16.4[a]	2.4 ± 0.3[a]	0.77
Red day 6	100.7 ± 12.6[b]	80.8 ± 6.5[a]	2.0 ± 0.4[a]	1.25
Far-red day 6	42.5 ± 17.9[c]	25.1 ± 9.5[b]	1.4 ± 0.0[b]	1.69
Dark day 6	55.2 ± 9.4[c]	51.7 ± 8.3[b]	1.0 ± 0.3[b]	1.07

All experiments were repeated five times independently. About 300 leaves were used in each experiment. The values given are the means ± SD of five independent experiments. Letters indicate significant differences in the mean values ($P < 0.05$) according to Scheffe's multiple comparison tests

APX activity remained at a higher level in the natural light and R-light conditions than in the dark and FR-light treatments (Table 4). We compared the SOD/APX ratio, which is the ratio of ROS-generating to ROS-scavenging enzymes, among the light treatments. Low light intensity seemed to suggest a proneness to the production of ROS (Table 4). GR catalyzes the rate-limiting last step of the ascorbate-GSH pathway. This enzyme consumes NADPH and GSSG and releases $NADP^+$ and 2GSH, respectively. GR also maintains a high ratio of GSH/GSSG, which is required not only for the regeneration of ascorbate but also for the activation of several Calvin-Benson cycle enzymes. The trend in GR activity resembled the trends of APX and SOD (Table 4).

Discussion

In this study, the regulatory effects of light quality on the photosynthetic characteristics, light absorption balance, and production of ROS in attached rice leaves were investigated. Therefore, this study was conducted under natural light, complete dark, and very low R- and FR-light conditions (less than 1 μmol photons m^{-2} s^{-1}). These conditions might not represent exactly processes that happen in the plants grown in lower canopy or understory. However, this is the fundamental and informative investigation to reveal the response of photosynthetic apparatus to light intensity and/or light quality. The results obtained are discussed in the following, taking the natural light environments of plants or leaves into consideration.

The Chl concentration and maximal photochemical efficiency of PSII in the dark-adapted leaves (Fv/Fm) decreased in leaves kept in the dark and in FR-light, but a notable feature of the decline kinetics of these parameters in the dark and FR-light treatments is the occurrence of an initial long lag (Fig. 2b, c). A long delay of dark degradation of Chl lasting less than one day was observed in detached leaves in some species (Mishra and Pradhan 1973; Thimann et al. 1977; Okada et al. 1992), but the lag in this study lasted until the second day, suggesting that the lag shorter than that observed in this study may be caused by an artificially induced senescence in the detached leaves. It was considered that a "long-lag" under dark and FR-light involves the synthesis, assembly, and insertion of new protein complexes and/or the degradation of already existing proteins (Anderson 1986; Anderson et al. 1995).

As demonstrated in Fig. 2b through d, Fv/Fm, Chl concentration, and the Chl *a*/*b* ratio keep constant under the natural light condition, whereas FR-light promotes declines in Chl concentration, the Chl *a*/*b* ratio, and Fv/Fm as rapidly as does the dark incubation. The R-light condition lies between the natural light and FR-light condition. Okada et al. (1992) showed that the degradation of Chl is regulated by phytochrome, and the loss of Chl was strongly retarded by continuous illumination with white light of intensity as low as 0.5 μmol photons m^{-2} s^{-1}, but this effect was counteracted by illumination with the FR-light.

In contrast to Chl, Rubisco underwent rapid degradation except under the natural light condition (Fig. 2e). The retarding effect of light quality on the decline in Rubisco was much lower than that on the breakdown of Chl (Fig. 2c). The result obtained here suggests that this difference between the effects on Rubisco and Chl is a manifestation of the different degrading mechanisms involved in the breakdown of the membrane and stroma proteins. Along with the degradation of Rubisco, a decline in the photosynthetic activities was observed (Fig. 2a). On the other hand, the behavior of the PSII photochemistry resembled the degradation of Chl (Fig. 2b).

A significant change in the Chl *a*/*b* ratio was observed during the loss of Chl in the dark and FR-light conditions (Fig. 2d). The decline in the Chl *a*/*b* ratio seems to be an acclimative response of leaves to changing light environments because plants or leaves grown at low light intensities have lower Chl *a*/*b* ratios than those grown at high light intensities (Anderson 1986). Yamazaki et al. (1999a) indicated that there is a positive correlation between the Chl *a*/*b* ratio and the PSII/PSI ratio. This finding indicates that the degradation kinetics of PSII and PSI are regulated

by the spectral quality (Table 1), creating uneven absorption of light between the two PSs. This imbalance is partly compensated for by the modulation of the ratio of PSIIα to PSIIβ (Table 2), although the PSII antenna heterogeneity is regulated by the light intensity under natural environmental conditions. However, under FR-light, PSIIα decreased with an increase in PSI, creating in the imbalance of the electron transport balance (Table 3). It is possible that the low light effect was dominant over the light quality effect (Chow et al. 1990a).

Yamazaki and Kamimura (2002) found that an imbalance in light absorption in the leaves under low light induced the accumulation of ROS to accelerate, generating a Mehler reaction on the reducing side of PSI, which is involved in the regulation of leaf longevity. This study also attempted to elucidate whether light quality regulates the activity of the ROS-scavenging system. Mullineaux et al. (2000) proposed that phytochrome regulates the expression of *APX1* and *APX3*, which encode stromal APX. As summarized in Table 4, the activities of three enzymes, which are the main players in the water–water cycle (Asada 1999), would be regulated by light quality. A robust estimation of the ratio of ROS-generating and -scavenging enzymes, the SOD/APX ratio, could support this consideration. These enzymes showed a parallel tendency in the breakdown of Chl, because the PSs were preserved in the natural light and R-light conditions.

Several studies have shown that light quality did not affect photosynthetic characteristics, but light quality plays an important role in photosynthesis (Tinoco-Ojanguren and Pearcy 1995; Chow et al. 1990b). The data obtained here indicate that the light quality does not regulate the degradation of Rubisco or the heterogeneity of PSII centers, but does regulate the degradation of Chl, the modulation of the PS stoichiometry, and the activities of the ROS-scavenging system. Thus, this study also indicates that light quality may play an important role in regulating the photosynthetic characteristics.

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