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Changes in the photosynthetic characteristics and photosystem stoichiometries in wild-type and Chl *b*-deficient mutant rice seedlings under various irradiances

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Abstract

By using a wild-type rice (*Oryza sativa* L. cv. Norin No. 8) and the chlorophyll (Chl) *b*-deficient mutant derived from Norin No. 8 (chlorina 11), the present study monitored the oxygen evolution, contents of Chl *a* and *b*, β-carotene, and lutein in leaf and the contents of cytochrome *f*, and the reaction centres of photosystem I (PSI) and photosystem II (PSII) in thylakoids. The oxygen evolution, maximal quantum yield of PSII (F_v/F_m) and Chl concentration remained constant in both Norin No. 8 and chlorina 11 under 5 and 2% of full sunlight for six days. On the other hand, on the thylakoid level, the PSII reaction centre of chlorina 11 was more stable even under high irradiance, while approximately 40% decrease in levels of the PSII reaction centre occurred under 2% of full sunlight for six days. However, under such conditions, by regulating the stoichiometry of active PSII and PSI centres, the light absorption balance in both rice types was adjusted between the two photosystems. The present study attempted to examine whether the light absorption balance between PSII and PSI is altered to effectively conduct photosynthesis in the wild-type and Chl *b*-deficient mutant rice seedlings.

Additional keywords: antenna size; Chl *b*-deficient mutant; photosystem stoichiometry; PSII heterogeneity; rice (*Oryza sativa* L.).

Introduction

It is well known that irradiance affects the modulation of the photosystem stoichiometry and the antenna size heterogeneity (Melis 1991). In terms of both function and antenna size, there are two types of PSII centres, namely PSIIα, which has a large antenna and is active in electron transport, and PSIIβ, which has a small antenna and is unable to reduce Q_B (Lavergne 1982, Melis 1991, Lazár 1999). Due to the large difference in antenna size, photoreduction of Q_A in PSIIα proceeds more rapidly than that in PSIIβ under irradiation with limited-intensity light in DCMU-poisoned thylakoids. Thus, the percentage of abundance of PSII reaction centres can be kinetically distinguished as active or inactive in electron transport by measuring the rate constant of photoreduction of Q_A through the growth of the area over the fluorescence induction curve.

In the previous study, leaves located in the bottom layer, where the light intensity is exponentially decreased in comparison to the uppermost layer, showed a low PSII/PSI ratio, resulting in a large imbalance in light absorption and electron transport capacity between PSII and PSI (Yamazaki *et al.* 1999a,b). Although this imbalance was partly and/or minimally compensated for due to an abundance of active PSII centres, designated as PSIIα (Melis 1991), in the bottom layer, the imbalance may have resulted in an over-reduction between the two photosystems, as the imbalance between them remained (Yamazaki *et al.* 1999a,b). If electrons jam in the PQ pool, surplus electrons could lead to photoreduction of molecular oxygen at the acceptor side of PSI and possible

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Abbreviations: C_{550} – electrochromic shift of pheophytin in the reaction centre complex of photosystem II; Chl – chlorophyll; Cyt – cytochrome; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v – variable Chl fluorescence yield in the dark-adapted leaves; F_m – maximal Chl fluorescence yield in the dark-adapted leaves; F_o – minimal Chl fluorescence yield in the dark-adapted leaves; chlorina 11 – Chl *b*-deficient mutant derived from Norin No. 8; LHCI – light-harvesting Chl-protein of PSI; LHCII – light-harvesting Chl-protein of PSII; Norin No. 8 – wild-type rice; P_{700} – the reaction centre complex in photosystem I; PPFD – photosynthetic photon flux density; PQ – plastoquinone; PSI – photosystem I; PSII – photosystem II; Q_A – primary quinone acceptor of PSII; Q_B – secondary quinone acceptor of PSII.

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formation of harmful reactive oxygen species (Asada 1999, Yamazaki *et al.* 2007).

There are several reports about Chl mutants in tomato (Melis and Thielen 1980), barley (Ghirardi *et al.* 1986), maize (Greene *et al*. 1988), and rice (Terao *et al*. 1985*a,b*, Terao and Katoh 1996). Chl *b* is found in the light-harvesting pigment-protein complex contained in the two photosystems and the Chl *b* is necessary for the stable integration of the LHC apoproteins and membrane stacking to occur (Droppa *et al*. 1988, Terao and Katoh 1989, Preiss and Thornber 1995). These mutants were classified on the basis of the amount of Chl *b*: *i.e.*, those with a complete lack of Chl *b*, termed Chl *b*-less mutants, and those with reduced Chl *b* content, termed Chl *b*-deficient mutants (Terao *et al*. 1985*a*, Falbel *et al*. 1996). These classifications of mutants provide a good model for elucidating the regulatory mechanisms and the roles of Chl *b* in the stacking and function of the thylakoid membrane.

Terao *et al*. (1985*a,b*) have characterized light-harvesting Chl-proteins of Chl *b*-deficient nuclear gene mutants derived from a cultivar of rice, Norin No. 8. Chl *b*-less or -deficient mutants of rice totally or partially lack a major light-harvesting Chl-protein of PSII (LHCII) (Terao *et al*. 1985*b*, Ghirardi and Melis 1986, Habash *et al*. 1994, Król *et al*. 1995, Preiss and Thornber 1995), because the apoproteins of LHCII are normally synthesized, but are rapidly degraded due to the absence or shortage of Chl *b* (Terao and Katoh 1989). The levels of a light-harvesting Chl-protein of PSI (LHCI) are also reduced but to a lesser extent than LHCII in the mutants. This suggests that the light-harvesting capacity of PSII is more strongly reduced than that of PSI in the Chl *b*-deficient mutants. Those researchers concluded that Chl *b* is needed to stabilize a major portion of newly synthesized apoproteins of LHCI and LHCII, and losses of LHCII are at least partly counterbalanced by increased ratios of PSII to PSI (Falbel *et al*. 1996, Terao and Katoh 1996). Similar conclusions have been reached on the basis of studies in tomato (Melis and Thielen 1980), barley (Ghirardi *et al*. 1986), maize (Greene *et al*. 1988), and rice (Terao *et al*. 1985*a,b*, Terao and Katoh 1996).

Terao *et al*. (1996) investigated the stoichiometry of PSI and PSII and the antenna heterogeneity of active and inactive components of PSII in the Chl *b*-less and Chl *b*-deficient mutants. The PSII to PSI ratio is maximally 1.3 in the wild-type rice cultivar, Norin No. 8, but increases to 1.8 in chlorina 2, which totally lacks Chl *b*, and to 2.0–3.0 in chlorina 11 and chlorina 14, which have Chl *a/b* ratios of 9 and 13, respectively. The elevated PSII to PSI ratios are considered to be a response of the plant to the imbalance in light absorption between PSI and PSII, because the inactive PSII centres determined by repetitive flash-induced oxygen evolution serve as an antenna for the active PSII centres so that there is a balance in light absorption between the two photosystems in the Chl *b*-deficient mutants (Terao *et al*. 1996). An elevated PSII/PSI ratio and altered antenna size heterogeneity can create additional problems in the photosystems. Baroli and Melis (1998) showed that a large Chl antenna size enhanced the rates of photon absorption and photodamage of PSII. However, Habash *et al*. (1994) reported that there are contrasting data regarding the role of antenna size in influencing the sensitivity of Chl *b* mutants to photodamage.

The present study examined whether Norin No. 8 and chlorina 11 show different responses under 5 and 2% of full sunlight. Little attention has so far been paid to this imbalance, which should prevail in Chl *b*-deficient mutants with high PSII to PSI ratios. To investigate the above question, this study determined the photosynthetic characteristics, levels of electron transport carriers, and photosystem stoichiometries.

Materials and methods

Plant materials and growth conditions: Wild-type rice seedlings (*Oryza sativa* L. cv. Norin No. 8) and Chl *b*-deficient mutant derived from Norin No. 8 (chlorina 11) 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). Germinated seeds were planted at 30-mm intervals in an artificial soil (*Bonsol No. 1, Sumitomo Kagaku*, Osaka, Japan) that contained sufficient levels of nutrients for the growth of rice seedlings during the period of this work. Rice plants were grown in a greenhouse for two to three weeks under natural condition [during summer at the campus, average irradiance cycle, *ca*. 9 h light/16 h dark; daily mean temperature 23°C; average relative humidity 70%; maximal PPFD, 2,000 μmol(photon) m^{-2} s^{-1}] and watered daily. Leaves were numbered in the order of their development, and the fully expanded fourth leaves were used for all analyses. Light intensity was determined with a quantum sensor (*LI-190; Li-Cor*, Lincoln, NE, USA). In this experiment, when the fourth leaves were fully expanded, the plants were divided into three equal groups and each group was placed under a different light treatment as follows for six more days: no shade cloth (high irradiance = HI; 100% full sunlight), three layers of shade cloth (middle irradiance = MI; 5% relative to full sunlight), five layers of shade cloth (low irradiance = LI; 2% relative to full sunlight). The shade cloth only decreased PPFD; it did not change the light quality (Fig. 1).

Photosynthetic properties: Oxygen evolution in the leaves was measured with an oxygen electrode (*LD2/2, Hansatech*, Norfolk, UK) at 30°C. The gas phase consisted of air containing 4% CO_2. A saturating actinic

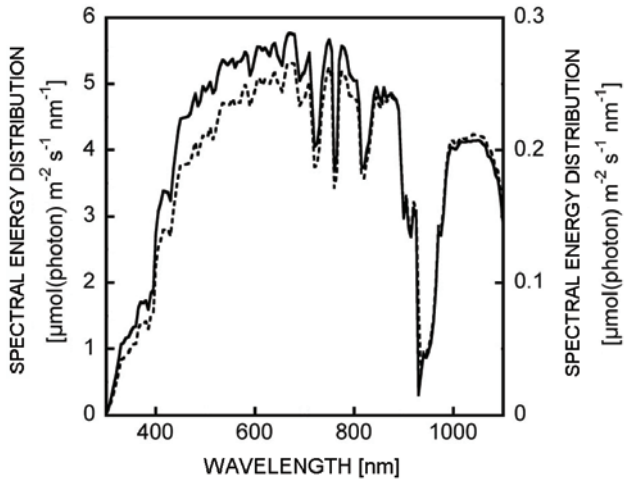

Fig. 1. Spectral profile of direct sunlight and shade (with cloth) used in this study measured at 12:00 h (local time). *Left* and *right y-axes* represent direct sunlight and shade condition, respectively. *Solid* and *dashed lines* represent the spectra of the direct sunlight and shade conditions, respectively.

light [2,000 μmol(photon) m^{-2} s^{-1}] from a 100-W halogen lamp was used. Leaf shapes were input into a computer (*PC-9801 BA, NEC*, Tokyo, Japan) with a flat bed scanner (*GT-6500, Seiko Epson*, Nagano, Japan), and the leaf area was calculated with a custom-made program. Chl *a* fluorescence was measured at room temperature with a portable fluorometer (*MINI-PAM, Walz*, Effeltrich, Germany) after the leaves had been dark-adapted for at least 30 min. The fluorometer was connected to a computer with data acquisition software (*WinCont 1.60, Walz*, Effeltrich, Germany). The maximal quantum yield of PSII in the dark-adapted leaves was calculated as $F_v/F_m = (F_m - F_o)/F_m$. F_o (minimal Chl fluorescence yield in the dark-adapted leaves) was measured at 0.6 kHz with the modulated weak measuring light [> 0.1 μmol(photon) m^{-2} s^{-1}]. F_m (maximal Chl fluorescence yield in the dark-adapted leaves) was measured at 20 kHz with an 800-ms pulse of 8,000 μmol(photon) m^{-2} s^{-1} of halogen light.

Pigment assays: Chl concentration was determined with a spectrophotometer for the wild type and with a HPLC for the mutant because the spectrophotometric method has sometimes underestimated Chl concentrations and the Chl *a*/*b* ratio in Chl *b*-deficient mutants. Pigments from the harvested leaves were separated and quantified by HPLC basically as described by Yamazaki *et al*. (2007) with a modification to the HPLC system. Briefly, an HPLC system (*Model 600; Waters*, Milford, MA, USA) equipped with a *TSK ODS-120A* column (4.6 mm × 250 mm; *Tosoh*, Tokyo, Japan) and a *Tosoh ODS* guard column and mobile phase was run under the following conditions. A linear gradient from 100% Solvent A [85:15 (v/v) acetonitrile:methanol] to 100% Solvent B [4:1 (v/v) methanol:hexane] was used for the first 16 min, followed by an isocratic elution with 100% Solvent B for the next 7 min. This procedure was followed by a 2-min linear gradient from 100% Solvent B to 100% Solvent A and an isocratic elution with 100% Solvent A for a further 10 min to allow the column to re-equilibrate with Solvent A before the next injection. All pigments were eluted from the column within about 30 min at a flow rate of 1.2 ml min^{-1}. The eluate was monitored at 440 nm, and the temperature was maintained at 20°C. The peak area was calculated automatically by a software module (*Model 740, Waters*, Milford, MA, USA). Known amounts of authentic pigments were used to construct standard curves.

Thylakoid membrane preparation and spectrophotometrical assays: The thylakoid membranes of rice leaves were isolated as described in Yamazaki *et al*. (1999b). 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 5,500 × *g* for 15 min, and the precipitate was suspended in the medium just described. The levels of P_{700} and C_{550} reaction centre complexes were quantified by measuring light-induced absorbance changes using a spectrophotometer (*Model 556, Hitachi*, Tokyo, Japan) in a dual-wavelength mode in accordance with Yamazaki *et al*. (1999b). McCauley and Melis (1986) demonstrated that C_{550} measured as the index of the PSII reaction centre is the electrochromic band shift of pheophytin at 550 nm, and this is in proportion to the amount of Q_A photoreduction. This method is a credible way to determine the PSII reaction centre (McCauley and Melis 1986, Hikosaka 1996, Yamazaki *et al*. 1999b, 2000). The levels of Cyt *f* were determined with a spectrophotometer (*Model 556, Hitachi*, Tokyo, Japan) in a double-beam mode in accordance with Yamazaki *et al.* (1999b).

Measurement of PSII antenna heterogeneity: The area over the fluorescence induction curve was measured with a laboratory-constructed apparatus. Green light obtained by passing light from a halogen lamp through a blue band-pass filter (*CS 4-96*, Corning, NY, USA) and an orange cut-off filter (*O-54, Toshiba*, Tokyo, Japan) was used for excitation. This region was used in order to excite equally both Chl *a* and Chl *b* molecules. Actinic light intensity was varied with neutral-density filters (*Toshiba*, Tokyo, Japan). Actinic light was illuminated diagonally to the sample cuvette and fluorescence was monitored at 690 nm by inserting a red cut-off filter (*R-64, Toshiba*, Tokyo, Japan) and a high-intensity *Bausch & Lomb* high-intensity grating monochromator (*model 33-86-02, Bausch & Lomb Inc*., NY, USA) between the sample cuvette and the photomultiplier (*R928, Hamamatsu Photonics*, Hamamatsu, Japan). Signals were stored in a transient recorder (*TCDC-12-8000(E), Riken Denshi*,

Tokyo, Japan) and analyzed with a computer. The two types of PSII reaction centres, the PSIIα and PSIIβ centres, were estimated by analyzing fluorescence induction curves in the presence of 10 μM DCMU dissolved in methanol as described in Yamazaki *et al*. (1999b).

Statistical analyses: A two-way analysis of variance followed by mean separation using *Scheffe*'s test was performed using software (*KaleidaGraph ver. 4.0, Hulinks*, Tokyo, Japan).

Results

Determination of pigment concentration and photosynthetic properties: Leaf oxygen evolution measured under saturating light (Fig. 2*A,B*) and the maximal quantum yield of PSII in the dark-adapted leaves (F_v/F_m) (Fig. 2*C,D*) remained irrespective of irradiance in both Norin No. 8 and chlorina 11 for six days, but the oxygen evolution in chlorina 11 was slightly lower than that in Norin No. 8. The Chl concentration in both Norin No. 8 and chlorina 11 also remained constant for six days under all irradiances, although chlorina 11 contained half the amount of Chl compared to Norin No. 8 (Fig. 2*E,F*). Also, as shown in Fig. 2*G,H*, there was no remarkable change observed in the Chl *a*/*b* ratio. β-carotene and lutein concentrations determined on the basis of Chl in chlorina 11 remained constant irrespective of irradiance (Fig. 3*B,D*), while those in Norin No. 8 were about 1.5 times higher for six days under LI than those in the other experimental groups (Fig. 3*A,C*).

Assay of thylakoid components: Table 1 shows the levels of the reaction centre complexes of the PSII (C_{550}) and PSI (P_{700}). As shown in Table 1, under the LI condition, the ratio of mmol C_{550} to mol Chl in Norin No. 8 and the mutants gradually decreased from 2.6 and 8.8 on day 0 to 2.3 and 5.3 on day 6, respectively, while, irrespective of irradiances, the levels of P_{700} determined on the basis of Chl in Norin No. 8 and chlorina 11 were approximately 1.7–1.8 and 4.2–4.4 mmol(P_{700}) mol^{-1}(Chl) in the wild type and in the mutant, respectively. Consequently, the PSII/PSI ratio in the wild-type

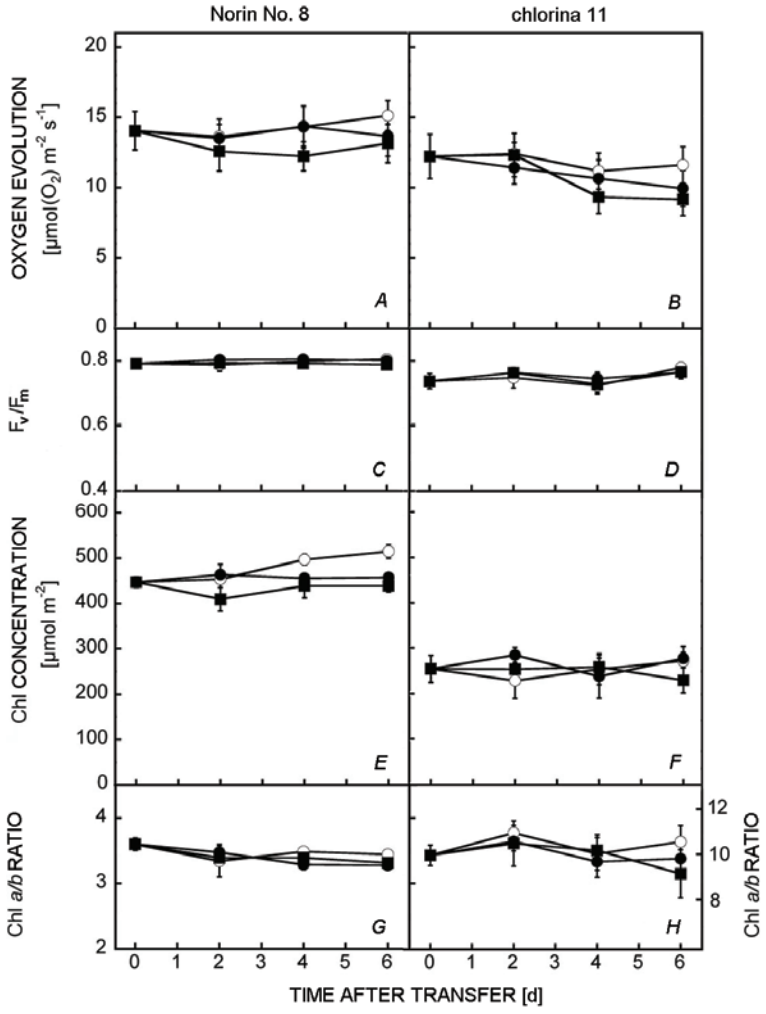

Fig. 2. Time courses of *A,B*: the light-saturated photosynthesis, *C,D*: the maximal quantum yield of PSII photochemistry in the dark-adapted leaves (F_v/F_m), *E,F*: Chl concentrations, and *G,H*: the Chl *a*/*b* ratio. *Open circles*, *closed circles* and *closed squares* represent the HI (100% full sunlight), MI (5% relative to full sunlight), and LI (2% relative to full sunlight) treatment for six days, respectively. Left side (*A,C,E,G*) and right side (*B,D,F,H*) showed the wild-type and chlorina 11, respectively. Means are shown and the *bars* indicate SD (n = 5).

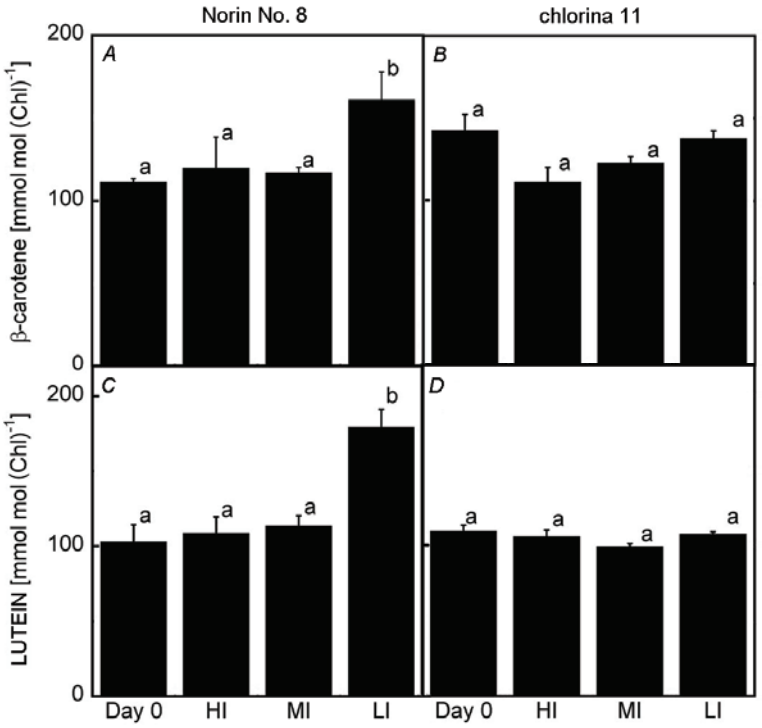

Fig. 3. Pigment concentrations in leaves of Norin No. 8 and chlorina 11 incubated for six days under different irradiances. *A*: β-carotene, *B*: lutein. Means and SD are shown (n = 5). Left side (*A,C*) and right side (*B,D*) showed the wild-type and chlorina 11, respectively. HI, MI, and LI represent 100% full sunlight, 5%, and 2% relative to full sunlight treatment for six days, respectively. *Different letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

Table 1. Levels of electron transport carriers in both Norin No. 8 and chlorina 11. All experiments were repeated five times independently. About 300 leaves were used in each experiment. HI, MI, and LI represent 100% full sunlight, 5%, and 2% relative to full sunlight treatment, respectively. The values given are the means ± SD of five independent experiments. *Different letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

	Treatment	Day	C_{550} [mmol mol(Chl)$^{-1}$]	P_{700} [mmol mol(Chl)$^{-1}$]	Cyt *f* [mmol mol(Chl)$^{-1}$]	PSII/PSI
Norin No. 8	-	0	2.6 ± 0.1^{a}	1.7 ± 0.2^{a}	2.0 ± 0.1^{a}	1.6 ± 0.3^{a}
	HI	6	2.7 ± 0.2^{a}	1.7 ± 0.2^{a}	1.9 ± 0.2^{a}	1.6 ± 0.2^{a}
	MI	6	2.3 ± 0.2^{b}	1.8 ± 0.1^{a}	1.2 ± 0.2^{b}	1.3 ± 0.2^{ab}
	LI	6	2.3 ± 0.1^{b}	1.8 ± 0.2^{a}	1.2 ± 0.2^{b}	1.3 ± 0.2^{b}
Chlorina 11	-	0	8.8 ± 1.4^{a}	4.2 ± 1.0^{a}	2.9 ± 0.5^{a}	2.2 ± 0.3^{a}
	HI	6	9.5 ± 1.1^{a}	4.4 ± 0.9^{a}	3.2 ± 0.8^{ab}	2.0 ± 0.3^{a}
	MI	6	8.7 ± 1.5^{b}	4.1 ± 1.0^{a}	2.5 ± 0.3^{ab}	2.2 ± 0.4^{a}
	LI	6	5.3 ± 1.7^{c}	4.2 ± 0.7^{a}	2.2 ± 0.3^{b}	1.2 ± 0.2^{b}

Table 2. Relative abundance of PSIIα and PSIIβ and the rate constant of Kα and Kβ in both Norin No. 8 and chlorina 11. All experiments were repeated five times independently. HI, MI, and LI represent 100% full sunlight, 5%, and 2% relative to full sunlight treatment, respectively. About 300 leaves were used in each experiment. The values shown are the means ± SD of five independent experiments. *Different letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

	Treatment	Day	PSIIα [%]	PSIIβ [%]	Kα [s^{-1}]	Kβ [s^{-1}]
Norin No. 8	-	0	68.3 ± 3.3^{a}	31.7 ± 3.3	6.2 ± 0.4^{a}	0.3 ± 0.1^{a}
	HI	6	66.1 ± 4.3^{a}	33.9 ± 4.3	6.0 ± 0.3^{a}	0.3 ± 0.1^{a}
	MI	6	62.2 ± 4.3^{ab}	37.8 ± 4.3	6.2 ± 0.3^{a}	0.3 ± 0.1^{a}
	LI	6	62.0 ± 2.7^{b}	38.0 ± 3.7	6.8 ± 0.2^{b}	0.3 ± 0.1^{a}
Chlorina 11	-	0	52.9 ± 1.3^{a}	47.1 ± 3.3	5.0 ± 0.2^{a}	0.5 ± 0.1^{a}
	HI	6	53.0 ± 2.8^{a}	47.0 ± 2.8	5.2 ± 0.4^{a}	0.5 ± 0.1^{a}
	MI	6	43.3 ± 4.0^{b}	56.7 ± 4.0	5.9 ± 0.4^{b}	0.4 ± 0.0^{a}
	LI	6	47.8 ± 2.2^{b}	52.2 ± 4.2	5.8 ± 0.2^{b}	0.4 ± 0.1^{a}

Table 3. PSII/PSI and PSIIα/PSI ratios in both Norin No. 8 and chlorina 11. All experiments were repeated five times independently. About 300 leaves were used in each experiment. HI, MI, and LI represent 100% full sunlight, 5%, and 2% relative to full sunlight treatment, respectively. The values shown are the means ± SD of five independent experiments. *Different letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

	Treatment	Day	PSII/PSI	PSIIα/PSI
Norin No. 8	-	0	1.6 ± 0.3^{a}	1.08 ± 0.23^{a}
	HI	6	1.6 ± 0.2^{a}	1.03 ± 0.15^{a}
	MI	6	1.3 ± 0.2^{ab}	0.82 ± 0.12^{b}
	LI	6	1.3 ± 0.2^{b}	0.83 ± 0.16^{b}
Chlorina 11	-	0	2.2 ± 0.3^{a}	1.05 ± 0.10^{a}
	HI	6	2.0 ± 0.3^{a}	0.93 ± 0.08^{b}
	MI	6	2.2 ± 0.4^{a}	0.91 ± 0.06^{b}
	LI	6	1.2 ± 0.2^{b}	0.55 ± 0.13^{c}

and the mutant slightly decreased from 1.6 and 2.2 on day 0 to 1.3 and 1.2 on day 6, respectively. Cyt *f* levels per unit of Chl in the wild type and the mutants decreased from 2.0 and 2.9 on day 0 to 1.2 and 2.2–2.5 on day 6, respectively.

Measurement of antenna size heterogeneity: Table 2 shows that the relative abundance of PSIIα in Norin No. 8 and chlorina 11 was, on average, 68% and 53%, respectively, of the total PSII centres on day 0. Therefore, when only the PSII centres that were active in electron transport are taken into account, the PSIIα/PSI ratio was 1.08 in Norin No. 8 and 1.05 in chlorina 11 on day 0 (Table 3). The percentage of PSIIα gradually decreased from day 0 to day 6 under shaded conditions (MI and LI). Thus, the degree of decline in the PSIIα/PSI ratio (Table 3) was larger than that in the total PSII/PSI ratio (Table 1). Kα and Kβ represent the rate constants of the PSIIα and PSIIβ centres, respectively, and the slope of the fast phase represents the rate constant of the α centre,

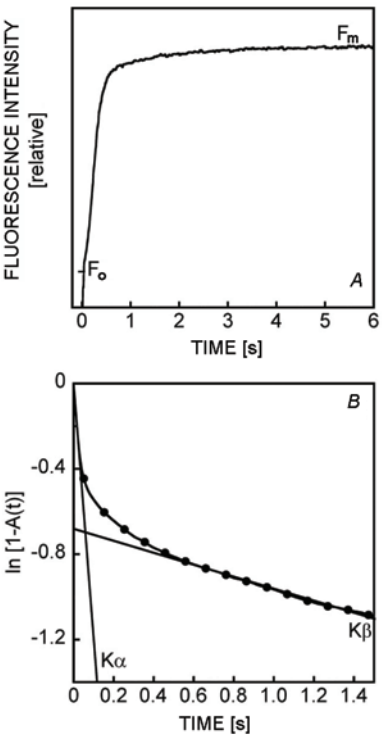

Fig. 4. *A*: The fluorescence induction curve of isolated chloroplasts in the presence of 10 μM DCMU. *B*: A semilogarithmic plot of the kinetics of the area over the fluorescence induction curve. Fluorescence was monitored at 690 nm. Kβ, which is the rate constant of the PSIIβ, was determined from the slope of the slow linear phase, whereas Kα, which is the rate constant of the PSIIα, was determined from the slope at zero time, which was obtained by subtracting the slow β-phase from the overall kinetic phenomenon.

namely Kα, while the slope of the slow phase represents the rate constant of the β centre, Kβ (Fig. 4; *see* Melis and Homann 1975, 1976). These rate constants are proportional to the size of each antenna. Kα increased slightly under LI, while Kβ remained constant irrespective of the irradiance (Table 2).

Discussion

Although photosynthetic activity is influenced by the irradiance, both Norin No. 8 and chlorina 11 showed no changes in their photosynthetic activities irrespective of irradiance (Fig. 2*A,B*). The values of F_v/F_m, which is a general indicator of photosynthetic function, remained constant also in both rice types for six days (Fig. 2*C,D*). It is well known that Chl is very stable even in low irradiance (Okada and Katoh 1998). The data regarding Chl concentration supported these results (Fig. 2*E,F*).

As depicted in Fig. 3, lutein, which is contained in the light-harvesting antenna, was at the same level under all irradiance conditions. There are several reports that, in the Chl mutants, carotenoids do not play a major role as light-harvesting pigments (Connelly *et al*. 1997, Havaux *et al*. 1998). β-carotene, which is contained in the reaction centre complex in both photosystems and might serve as the photoprotection from the active oxygen species, also remained constant except under the LI condition. Unfortunately, it remains unclear why the levels of both β-carotene and lutein in the wild-type only increased under the LI condition. Potential of the carotenoid biosynthesis in the wild-type would tend to resemble that in the chlorina mutant.

The observed declines in the levels of Cyt *f* indicate that the thylakoid components are influenced by irradiance (Table 1), as plants exposed to weak light had reduced levels of Cyt *f* than did plants exposed to stronger light (Leong and Anderson 1984, De la Torre

and Burkey 1990, Murchie and Horton 1998). Chow and Anderson (1987) suggested that the Cyt b_6/f complex and PQ limit photosynthesis. In this study, although the levels of Cyt *f* in both Norin No. 8 and chlorina 11 declined under LI, photosynthetic activity showed no change.

In the present study an irradiance of *ca*. 40 μmol(photon) m^{-2} s^{-1} was designated as the LI condition. Since this condition involves sufficient irradiance for the stabilization of Chl, the leaf level measurements (oxygen evolution and F_v/F_m) might not be affected. However, the components embedded in thylakoid membranes might be affected even in this condition.

The levels of the PSII reaction centre complex were also affected by the irradiance, while those of the PSI reaction centre complex remained unchanged (Leong and Anderson 1984, Lee and Whitmarsh 1989, De la Torre and Burkey 1990, Burkey and Wells 1996). As a result, the PSII/PSI ratio represented an imbalance between the two photosystems. These results indicate that the large number of electrons formed by the imbalance of light absorption might be prone to cause a photoinactivation such as an over-reduction of the photosynthetic electron transport pathway (Asada 1999, Yamazaki *et al*. 2007).

As stated in the Introduction, in DCMU-poisoned thylakoid membranes, the PSII is heterogeneous in the antenna size, which is classified into the active and large antenna size PSII centres (PSIIα) and the inactive and small antenna size ones (PSIIβ) (Melis and Homann 1975, 1976, Melis 1991, Lazár 1999). In this study, the abundance of PSIIα decreased (Table 2). In particular, under the LI condition, the decrease in PSIIα was greater in chlorina 11 than in Norin No. 8, but the degree of decline of PSIIα was approximately equal between chlorina 11 and Norin No. 8. Both rice types might extend their antenna sizes to provide more effective light capture under low-irradiance conditions. These results suggest that the decline in the PSII/PSI ratio under the LI condition is partly compensated by an increase in the size of the antennas attached to the PSIIα centres rather than by the abundance of PSIIα centres. This type of regulation would minimally mitigate the imbalance of light absorption between the two photosystems (Tables 1,3).

If photodamage depends on the balance between the rates of light absorption, the size of the light-harvesting Chl antenna of PSII may be relevant to the process of photodamage. Cleland and Melis (1987) revealed that the rate of photodamage depends on the rate of light absorption by the Chl pigment bed. In contrast, the Chl *b*-deficient mutants have small antennas due to the reduction of LHCII and LHCI (Ghirardi and Melis 1988, Terao *et al*. 1996).

By using the Chl *b*-less mutant, chlorina 2, Terao *et al*. (1996) demonstrated that half of the total PSII centres are active in electron transport by flash-induced oxygen evolution. By measuring with fluorescence induction curve using DCMU-poisoned thylakoid membranes, Tsurumi and Katoh suggested that the rest of the PSII centres, *i.e.* inactive PSII centres, serve as antennas for the active PSII centres (personal communication). Chlorina 11 also showed a sigmoidal shape fluorescence induction curve (data not shown). The fact indicates the existence of the connectivity between the PSII units (Joliot 1965, Lazár 1999). Therefore, since the antenna function of the inactive PSII and the connectivity between PSIIα might largely compensate the reduced light-harvesting capacity of the mutant PSII, the overall light-harvesting capacity of PSII is roughly comparable to that of PSI in the Chl *b*-deficient mutants. This contrasts with the wild-type rice, where the total antenna Chl number of PSII is about twice as large as that of PSI (Terao *et al*. 1996). Further improvements of light absorption imbalance include the spill-over of excitation energy from PSII to PSI (Murata 1969) or thermal energy dissipation from the antenna (Štroch *et al*. 2004). However, these two compensation mechanisms depend upon the presence of Chl *b* or LHC-II and hence should be mostly or totally inactive in the Chl *b*-deficient mutants.

In conclusion, the present study proposed that the relatively high resistance of the PSII of chlorina 11 to high irradiance is related to the regulation of the stoichiometry of PSIIα and PSI. In particular, the PSIIα/PSI ratio in both rice types adjusted to 1.0, which may be a good light absorption balance to effectively operate the photosynthetic electron transport. Chlorina 11 is needed for moderate irradiance to stabilize the photosystems because low irradiance rather than high irradiance might induce an imbalance between the two photosystems. These findings might also indicate that Chl *b* is needed for the stabilization of the photosystems and protection against the photodamage.

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