Changes in photosynthetic characteristics and photosystem stoichiometries in the lower leaves in rice seedlings

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Abstract

Changes in the Chl *a/b* ratio, electron transport, electron carriers, and photosystem stoichiometries were examined in rice leaves in the present study. The Chl *a/b* ratio is known to decrease gradually from the top to the bottom leaves, indicating an increase in the abundance of LHC II relative to the reaction center complexes of the two photosystems. We used juvenile rice canopy and obtained the following results: (1) the photosynthetic activity and Chl content per leaf area decreased from the top to the bottom leaves, the Chl *a/b* ratio also declined from 3.7 to 3.0; (2) when determined on the basis of Chl content, C-550 and Cyt *f* content decreased, but there was no loss of P-700. Consequently, the PS II/PS I ratio significantly decreased; (3) on the basis of mmol Chl, the levels of Cyt *f* dramatically decreased and, therefore, no loss was observed for whole chain electron transport per Cyt *f*; and (4) the percentage abundance of PS II α increased, but the rate constants of Q_A photoreduction and P-700 photooxidation gradually decreased. From these results, we hypothesize that there is a compensatory relationship between the decline in the Chl *a/b* ratio and that in the PS II/PS I ratio in the lower leaves in rice seedlings. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Antenna size of PS I and PS II; Chl *a/b* ratio; Light intensity; PS II heterogeneity; PS II/PS I ratio; Rice

1. Introduction

Light is an important factor for plant growth. In vertically vascular plants, because leaves that develop at the top of a canopy receive full sunlight, lower leaves receive very weak light. Accordingly, the light intensity decreases exponentially from the uppermost to the bottom layer made by mutual shading of leaves [1]. Hikosaka [2] and Okada and Katoh [3] made several important

statements on the aggravation of the light environment, especially the attenuation of the light intensity. They claimed that photosynthesis in lower leaves is limited by light intensity, and an excessive amount of the proteins related to CO₂ fixation are degraded rapidly. In contrast, the proteins involved in the harvesting and utilization of light are effectively protected in weak light [3], as in the example of the decline in the ratio of Chl *a* to *b* during leaf senescence [4]. A decline in the Chl *a/b* ratio indicates a more rapid degradation of the reaction center complexes of the photosystems than of LHC II [4–6]. Thus, lower leaves retain the capacity to drive photosynthesis maximally under the light-limiting conditions of shade. This indicates that a plant has the plasticity to adjust its photosynthetic apparatus in response to the appearance of mutual shading leaves that are inevitably associated with plant growth.

Abbreviations: C-550, electrochromic shift of pheophytin in the reaction center complex of photosystem II; Chl, chlorophyll; Cyt, cytochrome; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC I and II, the light-harvesting chlorophyll *a/b*-protein complex in PS I and II, respectively; P-700, the reaction center complex in photosystem I; PS, photosystem; Q_A , primary quinone acceptor of PS II.

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There are many reports that the spectral quality and/or the intensity of light affect the changes in the Chl *a/b* and the PS II/PS I ratios [5–11]. It should be noted that most of these findings were obtained from experiments in which plants were grown in artificially controlled light conditions. Little is known about these changes when plants were grown under natural conditions. Recently, the PS II/PS I ratio is less affected by natural shade compared with artificial conditions where the light intensity and spectral quality conditions can be extreme [10].

In several reports about photosystem stoichiometries, Melis and his associates [11,12] reported on the effects of light quality during plant and algal cell growth, and they revealed a compensatory system adjusting the two photosystem stoichiometries depending on light quality by using spectrophotometrical analysis. In contrast, a few reports examined the effects of light intensity using spectrophotometric measurement [7].

In terms of both function and antenna size, the PS II reaction center is heterogeneous [12,13]. One center has a large antenna and is functional in electron transport, and one has a small antenna and is unable to transfer electrons from Q_A^- to Q_B . The former is defined as PS II α , and the latter as PS II β . As a result of the large difference in antenna size, photoreduction of Q_A in PS II α proceeds more rapidly than it does in PS II β under irradiation with a limited-intensity green light in the DCMU-poisoned thylakoids.

In the present study, the Chl *a/b* ratio, levels of the reaction center complex of PS I and PS II and of Cyt *b₆/f* complex, and electron transport activity were determined at the different leaf positions of rice seedlings. In addition, the relative antenna sizes of PS I and PS II were determined by measuring the kinetics of photooxidation of P-700 and photoreduction of Q_A . Using the results obtained, we discuss herein the physiological significance of a decline in the Chl *a/b* ratio in the lower leaves.

2. Materials and methods

2.1. Plant growth conditions

Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a greenhouse for 4–5 weeks as described in [4]. Germinated seeds were planted at

3-cm intervals in an artificial granular soil, Bonsol No. 1 (Sumitomo Kagaku, Osaka, Japan) that contained sufficient levels of nutrients for the growth of rice seedlings. The plants were watered daily. Leaves were numbered in the order of their development. Monocotyledonous rice leaves successively developed with high synchronization, and a newly developed leaf blade was always longer than a lower leaf blade that had developed earlier. The 3rd–6th leaves were harvested immediately after full expansion of the 6th leaves had occurred. By this time, the 1st and 2nd leaves had withered and the tips of the 3rd leaves had turned yellow.

2.2. Light intensity and spectral quality measurements

Light intensity was measured at each leaf position with a Li-Cor model LI-189 light meter (Lincoln Co., Lincoln, NE, USA). Spectral photon distribution measurements were made for each leaf position using a Li-Cor model LI-1800 spectroradiometer. The sensor probe was placed on the adaxial surface of the center leaf blade to evaluate the transmission of light through the upper leaves. Each measurement was the average of three spectral scans taken from 300 to 800 nm at 2-nm intervals. Light intensity and spectral photon distributions were measured between 1300 and 1500 h on cloudless, clear days.

2.3. Measurement of photosynthesis

Photosynthesis of leaves was measured with a Hansatech leaf-disc oxygen electrode at 30°C. The gas phase consisted of air containing 4% CO₂. A saturating actinic light (2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) from a 100-W halogen lamp passed through a Hoya HA heat-absorbing filter. Leaf area was estimated by inputting leaf shapes into a microcomputer (NEC PC-9801 RX) with an image scanner (EPSON GT-6500).

2.4. Isolation of thylakoid membranes and their photosynthetic activities

The thylakoid membranes of rice leaves were isolated as described in [4]. In brief, leaves were homogenized in a cold buffer that contained 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl₂, 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 $250 \times g$ for 5 min. The supernatant was centrifuged at $5500 \times g$ for 15 min, and the precipitate was suspended in the medium just described. The rates of evolution or consumption of oxygen at light-saturation were measured with a Clark-type oxygen electrode at 25°C in a basal medium that contained 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl_2 , and 50 mM HEPES-NaOH (pH 7.5). PS II activity was measured by adding 1 mM phenyl-*p*-benzoquinone to a basal reaction medium. PS I activity was determined in the presence of 10 μM DCMU, 500 μM DCIP, 1 mM methyl viologen, and 2 mM sodium ascorbate. For whole chain electron transport, 1 mM methyl viologen and 1 mM NaN_3 were added to the basal medium. All mixtures contained 20 mM HCl-methylamine as an uncoupler, and thylakoid membranes were adjusted to 11 μM Chl. Chlorophyll was determined by the method of [14].

2.5. Determination of PS I and PS II reaction center contents

P-700 and C-550 were measured with a Hitachi 556 dual-wavelength spectrophotometer. P-700 was determined by measuring light-induced absorbance changes at 700 nm with a reference wavelength at 730 nm. The reaction medium contained 50 mM HEPES-NaOH (pH 7.5), 10 mM NaCl, 5 μM DCIP, 1 mM sodium ascorbate, 10 μM DCMU, 1 mM methyl viologen, 0.05% (w/v) Triton X-100, and thylakoid membranes equivalent to 11 μM Chl. Actinic light passed through a blue band-pass filter (Corning, CS 4–96), and the photomultiplier was protected with a red cut-off filter (Toshiba, R-66). The differential absorption coefficient of P-700 was taken to be $64 \text{ mM}^{-1} \text{ cm}^{-1}$ [15].

Light-induced absorbance changes of C-550, an electrochromic band shift of pheophytin that is associated with Q_A photoreduction [16], was determined at 550 nm with a reference wavelength at 540 nm as described previously [17]. Red actinic light was obtained by passing light through a 650-nm interference filter, with the photomultiplier guarded by a CS 4–96 filter. The differential absorption coefficient used was $5.2 \text{ mM}^{-1} \text{ cm}^{-1}$ [17]. The reaction medium contained 50 mM

HEPES-NaOH (pH 7.5), 10 mM NaCl, 15 μM DCMU, 20 μM gramicidin D, 2 mM ferricyanide, 0.1% (w/v) Triton X-100, and thylakoid membranes (82 μM Chl). The detergent treatment effectively removed the light-scattering, and we made sure that this concentration of the detergent did not influence the measurement of C-550. We also made sure that the actinic light used for P-700 and C-550 determination was sufficiently saturating for the measurement. Cyt *f* contents were determined with a Hitachi 556 spectrophotometer in a double-beam mode according to the method described in [18]. The reaction mixture contained 50 mM HEPES-NaOH (pH 7.5), 10 mM NaCl, 1% (w/v) Triton X-100, and thylakoid membranes (45 μM Chl). The differential absorption coefficient used was $17.7 \text{ mM}^{-1} \text{ cm}^{-1}$ [18].

2.6. Relative antenna size measurement

The area over the fluorescence induction curve was measured with a laboratory-constructed apparatus. Green light obtained by passing light from a halogen lamp (Hayashi Tokei Kogyo, LA-150SE) through a CS 4–96 filter and a Toshiba O-54 cut-off filter 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 Toshiba neutral-density filters. Fluorescence was monitored at 690 nm by inserting a Toshiba R-64 red cut-off filter and a high-intensity Bausch & Lomb grating monochromator between the sample cuvette and the photomultiplier (Hamamatsu Photonics, R928). Signals were stored in a transient recorder (Riken Denshi, TCDC-12-8000(E)) and analyzed with a computer. The two types of PS II reaction centers, the PS II α and PS II β centers, were estimated by analyzing fluorescence induction curves in the presence of DCMU as described in [19,20]. The reaction medium contained 50 mM HEPES-NaOH (pH 7.5), 10 mM NaCl, 5 mM MgCl_2 , 20 μM DCMU, and 0.4 M sucrose and thylakoid membranes (11 μM Chl).

The P-700 photooxidation kinetic measurements were performed according to [21] with the slight modifications described in [22]. In brief, the thylakoid membranes (110 μM Chl) were incubated in 50 mM HEPES-NaOH (pH 7.5) and 150 mM KCN for 2 h on ice, to block electron transport at plastocyanin. After incubation, the samples were

diluted tenfold in a medium contained 50 mM HEPES-NaOH (pH 7.5), 10 mM NaCl, 5 mM MgCl₂, 0.4 M sucrose, 20 μM DCMU, and 200 μM methyl viologen. The color and intensity of the actinic light were the same as that in a measurement of the fluorescence induction kinetics. Signals were stored in a transient recorder and analyzed with a computer.

3. Results and discussion

In this study, we investigated the changes in photosynthetic characteristics with depth of canopy in rice seedlings. Photosynthesis, Chl levels, and the Chl *a/b* ratio of the 3rd–6th leaves are shown in Table 1. The rates of light-saturated photosynthesis ($P_{\max}$) decreased with the depth of the canopy except for the 5th leaves, which showed a $P_{\max}$ similar to that of the 6th leaves. Chl levels were highest in the 5th leaves, and then decreased from the top to the bottom leaves. This may indicate that the 6th leaves contain some developing photosynthetic apparatus. As shown in Table 1, the Chl *a/b* ratio decreased gradually from 3.7 in the 6th leaves to 3.0 in the 3rd leaves. Although leaf senescence is primarily under genetic control, it is well established that the light environment of leaves is a major factor in the modulation of the Chl *a/b* and PS II/PS I ratios [2,3]. The changes in the Chl *a/b* and PS II/PS I ratios that occur during senescence of erect herbaceous canopy leaves must be caused mainly by shading of these leaves by upper leaves [2,3].

Levels of the PS II and PS I reaction center complexes were also affected by the light intensity [8,10,23,24]. These levels were quantified by measuring light-induced absorbance changes of

Table 1
Photosynthetic capacities, Chl *a/b* ratio and levels of Chl in the 3rd–6th leaves of the juvenile rice seedlings^a

Leaf position	$P_{\max}$ (μmol O ₂ m ⁻² s ⁻¹)	Chl (μmol m ⁻²)	Chl <i>a/b</i> ratio
6th	14.6 ± 1.2	333.3 ± 10.1	3.7 ± 0.1
5th	14.7 ± 1.9	371.7 ± 8.3	3.5 ± 0.1
4th	11.9 ± 0.4	268.4 ± 11.2	3.3 ± 0.1
3rd	6.0 ± 1.1	162.8 ± 10.6	3.0 ± 0.1

^a All experiments were repeated three times independently. These values are means ± S.D. of three independent experiments. We used about 300 leaves in one experiment.

Table 2
Levels of the thylakoid components correlated with electron transports and the PS II/ PS I ratios^a

Leaf position	C-550 mmol/mol Chl	P-700 mmol/mol Chl	PS II/PS I	Cyt <i>f</i> mmol/mol Chl
6th	3.15 ± 0.2	2.06 ± 0.2	1.54	2.07 ± 0.1
5th	2.80 ± 0.2	1.97 ± 0.1	1.42	1.66 ± 0.2
4th	2.30 ± 0.2	2.09 ± 0.1	1.10	1.52 ± 0.1
3rd	2.07 ± 0.3	2.11 ± 0.2	0.90	1.35 ± 0.1

^a All experiments were repeated three times independently. These values are means ± S.D. of three independent experiments. We used about 300 leaves in one experiment.

the C-550 and P-700 using thylakoid membranes isolated from the 6th to the 3rd leaves. McCauley and Melis [17] demonstrated that C-550 measured as the index of the PS II reaction center is the electrochromic band shift of pheophytin at 550 nm and this is in proportion to the amount of Q_A photoreduction. Recently, many studies have been published that C-550 is measured as amounts of the PS II reaction center [2,25,26]. The values obtained in this experiment are very high confidences because the value in the 6th leaves obtained here agreed with the literatures. The ratio of mmol C-550 to mol Chl decreased gradually from 3.2 in the 6th leaves to 2.1 in the 3rd leaves (Table 2). In contrast, irrespective of leaf position, the levels of P-700 determined on the basis of Chl were approximately 2 mmol P-700 per mol Chl (Table 2). Consequently, the PS II/PS I ratio decreased from 1.5 in the 6th leaves to 0.9 in the 3rd leaves (Table 2).

Hikosaka [2] showed that in a vine grown horizontally to avoid mutual shading, the Chl *a/b* and PS II/PS I ratios remained constant when leaves were grown without shading. This result strongly supports the hypothesis that both ratios are affected by light intensity. Light that reaches a leaf in natural shade is weak and enriched in the far-red component of light, relative to the red component of leaves that are exposed to sunlight [27].

The light environment of the juvenile rice canopy is presented in Fig. 1. The light environment of lower leaves, which is enriched in far-red light, indicates the absorption of red light by the upper leaves. The spectral photon distributions,

indicating an apparent increase in far-red light in the lower canopy, are shown in Fig. 1A. This region (over 750 nm) is not available for photosynthesis. However, we see from Fig. 1A that the red/far-red (R/FR) ratio (650/750 nm) dropped from 100% at the 6th to 19% at the 3rd leaves. Similarly, the light intensity also decreased from 100% at the 6th leaves to 30% at the 3rd leaves (Fig. 1B). Thus, because these two factors change simultaneously, it is difficult to decide which is the dominant factor on the changes in the photosynthetic apparatus.

Cyt *f* levels per unit of Chl decreased from 2.1 in the 6th leaves to 1.4 in the 3rd leaves (Table 2). Cyt *b₆* also declined, but the extent of the decline was a little slower than that of Cyt *f* (data not shown). This indicates that the two cytochromes degraded in the lower leaves. The observed declines in the levels of Cyt *f* also indicate that the thylakoid components are influenced by irradiance, as plants grown in weak light had reduced levels of Cyt *f* than did plants grown in stronger light [5,6,23,24,28].

Whole chain electron transport activity, determined on the basis of Chl content, slightly decreased from the upper to the lower leaves (Table 3). On the other hand, the rates of electron transport activity expressed on the basis of Cyt *f* remained fairly constant at all leaf positions (Table 3), implying that the Cyt *b₆/f* complexes are one of the limiting factors involved in the whole chain electron transport in the lower leaves [29]. More-

over, as shown in Table 3, there were also no significant decreases in PS II electron transport expressed on the basis of C-550 in the depth of the canopy. Chow and Anderson [30] suggested that Cyt *b₆/f* complex and plastoquinone limit $P_{\max}$. Consequently, because the degradation of the thylakoid components is complex, it is not easy to decide on the site of the limitation.

As stated in the Introduction, the PS II reaction center is heterogeneous. In terms of both function and antenna size, there are two types of PS II centers, namely PS II α , which has a large antenna and is active in electron transport, and PS II β , which has a small antenna and is unable to reduce Q_B [12,13]. As a result of the large difference in antenna size, photoreduction of Q_A in PS II α proceeds more rapidly than that in PS II β under irradiation with limited-intensity light in the DCMU-poisoned thylakoids. Thus, the percentage of abundance of PS II reaction centers can be kinetically distinguished as functional or non-functional in electron transport by measuring the rate constant of photoreduction of Q_A through growth of the area over the fluorescence induction curve.

$K\alpha$ and $K\beta$ stand for the rate constants of the PS II α and PS II β centers, respectively, and the slope of the fast phase represents the rate constant of the α center, namely $K\alpha$, and the slow phase represents that of the β center, $K\beta$ (Fig. 2; see refs. [19,20]). Table 4 shows that the relative abundance of PS II α was, on average, 60% of the total PS II centers in the newly developed 6th leaves. There-

Fig. 1. The light environments of the juvenile canopy of rice seedlings. (A) Spectral photon distribution. Solid and broken lines represent at the 6th leaves and at the 3rd leaves, respectively. (B) Relative intensity of light as 100% at the 6th leaves.

Table 3

Whole chain electron transport activities expressed on Chl and Cyt *f*, and PS II activities expressed on C-550^a

Leaf position	PS II+PS I [mmol O ₂ (mol Chl) ⁽¹⁾ s ⁽¹⁾]	Whole chain [mmol O ₂ (mol Cyt <i>f</i>) ⁽¹⁾ s ⁽¹⁾]	PS II activity [mmol O ₂ (mol C-550) ⁽¹⁾ s ⁽¹⁾]
6th	58.4 ± 5.5	28.4 ± 7.1	29.2 ± 8.2
5th	51.0 ± 4.3	31.2 ± 6.2	30.8 ± 5.8
4th	59.6 ± 5.0	39.4 ± 6.6	39.5 ± 3.5
3rd	43.4 ± 6.0	36.1 ± 7.9	34.9 ± 8.7

^a All experiments were repeated three times independently. These values are means ± S.D. of three independent experiments. We used about 300 leaves in one experiment.

fore, when only the PS II centers that are functional in electron transport are taken into account, the PS II α /PS I ratio is 0.9 in the 6th leaves. The percentage of PS II α gradually increased from the top to the bottom leaves and was 70% in the 3rd leaves. Thus, the decline in the PS II α /PS I ratio (Table 4) was smaller than that in the total PS II/PS I ratio (Table 2). There was also a small but significant decline in K α from the upper to the lower leaves. As shown in Fig. 3, K α can be used as a measure of antenna size because K α increased linearly with the intensity of excitation light. In contrast, K β did not increase linearly with light intensity but became saturated at about 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This implies that the rate constant of Q_A photoreduction can be used as a measure of the antenna size only in PS II α . The antenna size of PS II β may be estimated by measuring the initial slope of the light-response curve at a light intensity of zero, and hence the antenna size of PS II β determined at a definite intensity of light is an underestimate. Various hypotheses at present are submitted about inactive PS II centers. Hsu and Lee [31] observed that the rate constants of slow phases of Q_A photoreduction were independent of light intensity. In their experiment, however, actinic light might have already saturated for the measurement of the PS II β . On the other hand, by using Chl *b*-less rice mutant, chlorina-2, carrying only PS II β , Terao et al. [32] showed that a half of the total PS II centers are active in electron transport by flash induced oxygen evolution. Recently, Tsurumi and Katoh revealed that the rest of the PS II centers, i.e. inactive PS II, serve as an antenna of the active PS II centers (personal communication). Therefore, because PS II β is defined differently in various plant systems, we followed to the literatures of [32] and [33] in the present study.

The relative antenna size of PS I was determined by measuring the kinetics of P-700 photooxidation with the same excitation light as that used for fluorescence measurement (Fig. 2C, D). The rate constant of P-700 photooxidation (KI) was linear to the intensity of the excitation light (data not shown). KI decreased from the 6th to the 3rd leaves and it was smaller than K α , i.e. the antenna size of PS I is smaller than that of PS II α , at all leaf positions (Table 4).

Comparing K α with KI, both levels decreased in parallel with leaf order from upper to lower leaves (Table 4). K α and the levels of C-550 per unit of Chl decreased, while the abundance of only PS II α increased. These results suggest that the leaves increased the total abundance of active PS II centers to compensate for a decrease in the antenna size for acclimation under light-limiting conditions. Alternatively, KI also decreased, in spite of there being no losses of P-700 per mol Chl, suggesting that leaves cause antenna size to be smaller in order to avoid receiving excess light under weak light conditions. Burkey and Wells [10] indicated that the Chl distribution between LHC I and LHC II was influenced by plant density. No loss of P-700 but a decrease in CP1a were observed in the dense canopy. The large shade effects in the dense canopy involved both a reduction in LHC I and an increase in LHC II, as a consequence of the shift in the light-harvesting capacity from PS I to PS II in the weak light environment. In Burkey and Wells' experiment, a dense soybean canopy indicated a high FR/R ratio and low light intensity in the lower position. In spite of a high FR/R ratio, its influence was low, or it had no effect. Consequently, from both their result and Fig. 1, because changes in the both factors occur synchronously in this rice canopy, it would be difficult to decide which is a main deter-

minant of the arrangements of the photosynthetic apparatus.

The present work indicates that the Chl *a/b* and PS II/PS I ratios decreased gradually with the depth of the canopy. The most important part of this paper is that this experiment indicates that the decline in the PS II/PS I ratio resulting from the low-light environment is partly compensated for by an increase in the abundance of PS II α centers which are associated with a large amount of LHC II and which avoid minimally an imbalance of light absorption by the two photosystems. Thus, the decrease of the Chl *a/b* ratio paralleled the increase of PS II α centers. However, to drive electron transport effectively, both photosystems have to absorb light equally. Indeed, when we calculated the electron transport capacity between the two photosystems based on the equations described in [34], the capacity in the 6th leaves was 1.5-times larger than that in the 3rd leaves [35]. As a result, large imbalances occur between the two

photosystems. Nevertheless we considered a state transition at first, it did not involve in the energy deployment (data not shown). Accordingly, this apparent anomaly is currently being investigated.

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Fig. 2. (A) The fluorescence induction curve of isolated thylakoid membranes in the presence of DCMU. (B) A semilogarithmic plot of the area over the fluorescence induction curve. The slope, which defined the rate of light utilization, of slow phase represented for the rate constant of PS II β center (K_β) and of fast phase did K_α . (C) The time course of P-700 photooxidation with the KCN-poisoned thylakoids. (D) A semilogarithmic plot of the absorbance change of P-700 photooxidation.

Table 4

Relative abundances of PS II α and PS II β and the rate constants of PS II α , PS II β and PS I in the 3rd–6th leaves of the juvenile rice seedlings^a

Leaf position	PS II α (%)	PS II β (%)	K α (s ⁻¹)	KI (s ⁻¹)	PS II α /PS I
6th	60.1 $\pm$ 0.8	39.9 $\pm$ 0.8	15.7 $\pm$ 0.6	9.8 $\pm$ 0.2	0.92
5th	62.9 $\pm$ 0.4	37.1 $\pm$ 0.4	13.3 $\pm$ 0.8	8.8 $\pm$ 0.4	0.89
4th	66.2 $\pm$ 0.5	33.9 $\pm$ 0.5	12.8 $\pm$ 0.3	7.3 $\pm$ 0.3	0.73
3rd	69.9 $\pm$ 0.6	30.1 $\pm$ 0.6	11.1 $\pm$ 0.7	6.9 $\pm$ 0.2	0.69

^a All experiments were repeated three times independently. These values are means $\pm$ S.D. of three independent experiments. We used about 300 leaves in one experiment.

Fig. 3. Light-response curves of the rate constants of Q_A photoreduction. ●, the rate constants of PS II α ; ○, those of PS II β .

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