Changes in Photosynthetic Apparatus in the Juvenile Rice Canopy and a Possible Function of Photosystem I in the Bottom Leaves

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Changes in the photosynthetic apparatus and relative antenna sizes of photosystem (PS) I and PS II were measured in the rice canopy. We used juvenile rice seedlings to examine light utilization and its absorption in the bottom leaves and obtained the following results: (1) When referred to chlorophyll (Chl), levels of the electrochromic shift at 550 nm and cytochrome *f* decreased from the sixth to the third leaves, but there was no loss of pigment (P)-700. As a consequence, the PS II/PS I ratio significantly decreased from 1.5 in the sixth leaves to 0.9 in the third leaves. (2) The electron transport capacity in the sixth leaves was 1.5-times larger than that in the third leaves. (3) The levels of cytochrome b_6 referred to Chl were almost constant from top to bottom. (4) The photosynthetic performance of the leaf decreased concomitant with the depth, whereas the respiration was slightly increased. From these results, we hypothesize that there are maintenance mechanisms when the imbalances of light absorption and electron transport capacity occur in the bottom leaves.

Introduction

The PS II/PS I ratio decreased with the decline of the Chl *a*/*b* ratio resulting from the attenuation of light intensity (Leong and Anderson, 1984; De la Torre and Burkey, 1990; Burkey and Wells, 1996). A balanced light absorption by PS I and PS II is essential for the efficient operation of photosynthesis.

In experiments in which the light quality was altered, it has been shown that a change in the PS II/PS I ratio is a response for maintaining a light absorption balance between the two photosystems (Melis, 1991; Fujita, 1997). Melis and Anderson (1983) claimed that the share of far-red light increase relatively in the lower leaves region of the canopy. However, we found (unpublished results) that far-red light (longer than 750 nm) which is not effective in photosynthesis, showed a relative increase in the bottom leaves within the rice canopy. The PS II/PS I ratios decreased with the depth of the canopy in this experiment, contradicting a common result from the viewpoint of the light quality. Therefore, it is necessary to discuss the relevance of light intensity changes.

Usually, PS II is present 1.5 to 1.7-times more than PS I in terrestrial vascular plants (Melis, 1991). The PS II reaction center, however, is heterogeneous; there are two types of a PS II center, PS IIα and PS IIβ (Lavergne, 1982; Melis and Homann, 1976). The α center exists in the grana thylakoids, and is associated with most of the LHC II. From here, the light energy is transferred and electrons move from H_2O to plastoquinone by the photochemical reaction. In contrast, the β center exists in the stroma thylakoid and binds minor LHC II. It is thought that the β center may split H_2O to O_2, but it is not possible for an electron to be delivered directly to the plastoquinone (Lavergne, 1982; Melis, 1985). Thus, the two populations of PS II reaction centers can be kinetically distinguished with different antenna sizes by measuring the rate constant of Q_A photoreduction through the

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

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growth of the area over the fluorescence induction curve (Melis and Homann, 1975).

A study of the light absorption balance between the two photosystems from an estimation of the relative abundance of PS IIα, PS IIβ, and PS I, and each antenna size is therefore required. Furthermore, we must consider the possibility that the large amount of electrons formed by the imbalance of the light absorption causes a photoinhibition, such as "overreduction" of the photosystem. Thus, it is necessary to consider the balance among PS IIα, PS I and Cyt *f*, as well as that between PS IIα and PS I.

In the present study, the balances of light absorption and electron transport between the two photosystems were estimated, and the energy distribution mechanism of one excited photosystem was examined. With the results obtained, we suggest a working hypothesis about the involvement of a cyclic photophosphorylation and a respiration to provide ATP as energy for translocating the degradation products in the bottom leaves to the upper developing leaves.

Materials and Methods

Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a greenhouse for four to five weeks as described by Kura-Hotta *et al.* (1987). The third to sixth leaves were harvested immediately after full expansion of the sixth leaves. By this time, the first and second leaves had withered and the tips of the third leaves had turned yellow.

The thylakoid membranes of rice leaves were isolated according to Terao *et al.* (1986). The chlorophyll was determined by the method described by Porra *et al.* (1989).

The P-700 and C-550 were measured with a Hitachi 556 dual-wavelength spectrophotometer. The 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 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (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 was 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 the P-700 used was 64 mM^{-1} cm^{-1} (Hiyama and Ke, 1972).

The light-induced absorbance changes of C-550 were determined at 550 nm with a reference wavelength at 540 nm (McCauley and Melis, 1986). Red actinic light was obtained by passing light through a 650-nm interference filter. The absorbance change was measured with the photomultiplier guarded by a CS 4–96 filter. The differential absorption coefficient used was 5.2 mM^{-1} cm^{-1} (McCauley and Melis, 1986). 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 ensured that the concentration of the detergent did not influence the measurement of C-550.

Levels of Cyt *f* and Cyt b_6 were determined with a Hitachi 556 spectrophotometer in a double-beam mode according to the methods described by Bendall *et al.* (1971) and Stuart and Wasserman (1973), respectively. 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 mM^{-1} cm^{-1} for Cyt *f* (Bendall *et al.*, 1971) and 15 mM^{-1} cm^{-1} for Cyt b_6 (Stuart and Wasserman, 1973).

The area over the fluorescence induction curve was measured with a laboratory-constructed apparatus. The excitation light used was a weak broadband green light in order to excite both Chl *a* and Chl *b* equally (Ghirardi and Melis, 1983). This light was obtained by passing light from a halogen lamp through a CS 4–96 filter and a Toshiba O-54 cut-off filter. Fluorescence was monitored at 690 nm by inserting a Toshiba R-64 red cut-off filter and a High-Intensity Bausch & Lomb grating monochrometer between the sample cuvette and the photomultiplier (Hamamatsu photonics, R928). The two types of PS II reaction centers, PS IIα and PS IIβ, were estimated by analyzing the fluorescence induction curves in the presence of DCMU (Melis and Homann, 1975). 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 with the same apparatus used in the determination of the P-700 mode as described by Melis (1982) with slight modifications as described by Svensson *et al.* (1991). The thylakoids (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 the plastocyanin. After incubation, the samples were diluted 10-fold in a medium contained 50 mM HEPES-NaOH (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 0.4 M sucrose, 20 μM DCMU, 200 μM methyl viologen. The color and intensity of the actinic light were the same as that in the measurement of the fluorescence induction kinetics. Signals from Q_A photoreduction and P-700 photooxidation kinetics were stored in a transient recorder (Riken Denshi, TCDC-12–8000(E)) and analyzed by a computer.

Photosynthesis and dark respiration of the leaves were measured with a Hansatech leaf-disc oxygen electrode at 30 °C. The gas phase consisted of air containing 4% CO_2. A saturating actinic light (2000 μmol photons m^{-2} s^{-1}) from a 100-W halogen lamp was passed through a Hoya HA heat-absorbing filter for photosynthesis measurement. The plants were incubated for 1 h in the dark for measurement of the dark respiration. The leaf area was estimated by inputting the leaf shapes into a microcomputer (NEC PC-9801 RX) with an image scanner (EPSON GT-6500).

Results and Discussion

As shown in Table I, the Chl *a*/*b* ratios decreased moderately from 3.7 in the sixth leaves to 3.0 in the third leaves, also the levels of C-550 and Cyt *f* based on Chl decreased significantly from the sixth to the third leaves. In contrast, the levels of P-700 were approximately 2 mmol P-700 per mol Chl irrespective of leaf position. Consequently, the PS II/PS I ratios decreased from the sixth to the third leaves (see below). The changes in the Chl *a*/*b* and the PS II/PS I ratios that occur during growth of the erect herbaceous canopy leaves must be caused mainly by the shading of these bottom leaves by the upper ones.

Table I. The Chl *a*/*b* ratios and levels of the thylakoid components correlated with electron transports. These values are means ± S. D. of three independent experiments. We used about 300 leaves for one experiment.

Leaf position	Chl *a*/*b* ratio	C-550	P-700	Cyt *f*
		mmol / mol Chl		
6 th	3.7 ± 0.1	3.15 ± 0.2	2.06 ± 0.2	2.07 ± 0.3
5 th	3.5 ± 0.1	2.80 ± 0.2	1.97 ± 0.1	1.66 ± 0.3
4 th	3.3 ± 0.1	2.30 ± 0.2	2.09 ± 0.1	1.52 ± 0.2
3 rd	3.0 ± 0.1	2.07 ± 0.3	2.11 ± 0.2	1.35 ± 0.3

PS II heterogeneity was measured by fluorescence induction. Upon illumination with excitation light, the Chl *a* fluorescence yield rises with time from an initial level (Fo) to a steady-state level (Fm) within a few seconds (Fig. 1A). This process reflects the redox state from the open to the closed state in Q_A. In the presence of DCMU, the fluorescence induction curve showed a rapid increase from Fo to Fm because of the inhibition of the reoxidation of Q_A^- by Q_B. Therefore, the fluores-

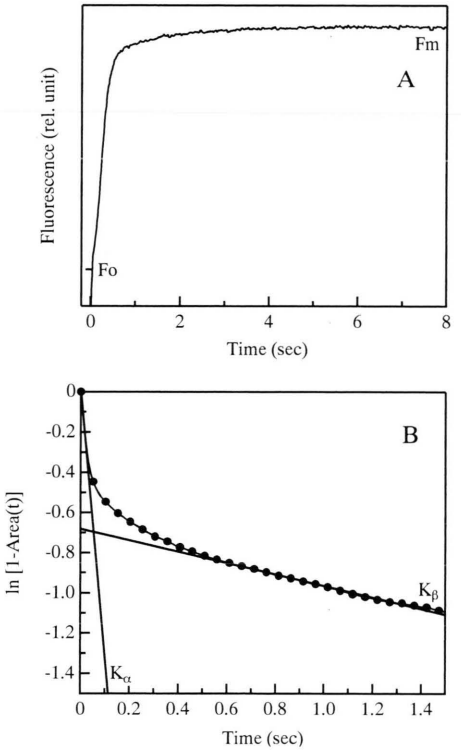

Fig. 1. A, The fluorescence induction curve of isolated chloroplasts in the presence of DCMU. B, A semilogarithmic plot of the kinetics of the area over the fluorescence induction curve. Fluorescence was monitored at 690 nm. Kβ which is the rate constant of the PS IIβ was determined from the slope of the slow linear phase, whereas Kα was determined from the slope at zero time, which was obtained by subtracting the slow β-phase from the overall kinetic phenomenon.

cence induction kinetics provides a measure of the accumulation of Q_A^- and hence a measure of the rate of light absorption by the PS II antenna. The time courses of Q_A photoreduction can be estimated by measuring the growth of the area over the fluorescence induction curve as described by Melis and Homann (1975). As presented in Figure 1B, semilogarithmic plots of the area growth showed it was biphasic. A fast steep phase and a slow gentle phase derived from the two types of PS II centers. The fast, steep phase derived from PS IIα, which has a large antenna and is active in electron transport, and the slow gentle phase derived from PS IIβ, which has a small antenna and is unable to reduce the plastoquinone pool (Lavergne, 1982; Melis, 1985).

A slower phase after 0.5 seconds defined the rate of Q_A photoreduction of the PS IIβ center (Fig. 1B). The relative proportion of PS IIβ was estimated by the intercept of the β-phase with the ordinate at zero time. Kα and Kβ, as shown in Figure 1B, represent the rate constants of the PS IIα and the PS IIβ center, respectively. The fast phase defines the rate constant of the PS IIα (Kα), and the slow phase defines the rate constant of the PS IIβ (Kβ) (Melis and Homann, 1975). Kβ was determined from the slope of the slow linear phase, whereas Kα was determined from the slope at zero time, which was obtained by subtracting the slow β-phase from the overall kinetic phenomenon (Fig. 1B).

The relative antenna size of PS I was estimated by measuring the time course of P-700 photooxidation kinetics which measured the same light conditions used to measure the fluorescence induction kinetics. In KCN-poisoned chloroplasts, the function of plastocyanin is inhibited (Izawa *et al.*, 1973), thus electron donation to $P700^+$ is prevented. Under these conditions, the kinetics of P-700 photooxidation provides a measure of the rate of light absorption by the antenna pigments of PS I. The photooxidation kinetics of P-700 with the thylakoids shows a first-order exponential function of time (Fig. 2A), as evidenced by the monophasic straight line in the semilogarithmic plot (Fig. 2B). The slope of this line defined the rate constant of light absorption by PS I (KI).

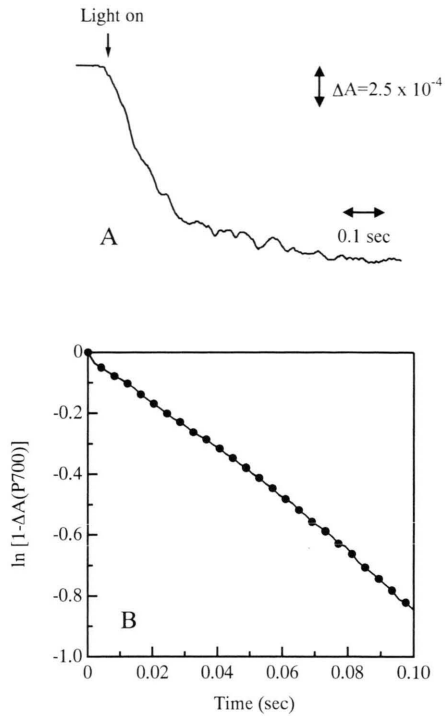

Fig. 2. A, The time course of the absorbance change at 700 nm monitoring with KCN-treated thylakoid membranes. B, A semilogarithmic plot of the P-700 photooxidation kinetics of the absorbance change at 700 nm (ΔAP700) showing the monophasic exponential function of time for P-700 photooxidation.

Table II. Rate constants of the PS IIα and PS IIβ and PS I with the isolated thylakoid membranes in different leaf positions. These values are means ± S. D. of three independent experiments. We used about 300 leaves for one experiment.

Leaf position	PS IIα (%)	PS IIβ	Kα	Kβ (photons/s)	KI
6 th	60.1 ± 0.8	39.9 ± 0.8	15.7 ± 0.6	0.5 ± 0.1	9.8 ± 0.2
5 th	62.9 ± 0.4	37.1 ± 0.4	13.3 ± 0.8	0.5 ± 0.1	8.8 ± 0.4
4 th	66.2 ± 0.5	33.9 ± 0.5	12.8 ± 0.3	0.4 ± 0.1	7.3 ± 0.3
3 rd	69.9 ± 0.6	30.1 ± 0.6	11.1 ± 0.7	0.4 ± 0.0	6.9 ± 0.2

Table II shows that the relative abundance of PS IIα was about 60% of the total PS II centers in the sixth leaves. The percentage of PS IIα markedly increased as leaves aged and was 70% in the third leaves. In contrast, the abundance of the PS IIβ decreased with the depth of the canopy. Kα and KI decreased to 29% and 30% at the third leaves compared with the sixth leaves, respectively. In contrast, Kβ was unaltered at all leaf positions.

These results indicate that an increased LHC II does not enlarge the antenna size of each PS II reaction center, but it does increase the abundance of PS IIα which are associated with a large amount of LHC II. Thus, an increase of the α center in the bottom leaves is concomitant with the decline of the Chl *a*/*b* ratio, i.e. the relative increase of LHC II.

Alternatively, KI also decreased (Table II), in spite of the fact that there were no losses of P-700 per mol Chl (Table I). It has been proposed that the large shade effects in the dense canopy involve 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 (Burkey and Wells, 1996). Under weak light conditions, these changes suggest the possibility that the bottom leaves cause the total abundance of the PS IIα to increase in order to harvest weak light actively and render PS I antenna size smaller in order to avoid receiving the excessive excitation energy transferred to the reaction centers.

Table III shows the PS II/PS I ratio, the PS IIα/PS IIβ/PS I ratios, and the PS IIα/Cyt *f*/PS I ratios. The total PS II/PS I ratios decreased from 1.5 in the sixth to 0.9 in the third leaves. When the PS II heterogeneity was taken into consideration (Table II), the PS IIα/PS I ratios decreased slightly from 0.9 in the sixth to 0.6 in the third leaves compared to the decline of the total PS II/PS I ratios.

Table III. Stoichiometries of the thylakoid components involved in the electron transport in different leaf positions.

Leaf position	PS II/PS I	PS IIα/PS IIβ/PS I	PS IIα/Cyt *f*/PS I
6 th	1.54	0.93 : 0.61 : 1	0.91 : 1 : 1.00
5 th	1.42	0.89 : 0.53 : 1	1.06 : 1 : 1.19
4 th	1.10	0.73 : 0.37 : 1	1.00 : 1 : 1.38
3 rd	0.90	0.63 : 0.27 : 1	1.07 : 1 : 1.56

The relative electron-transport capacity of each photosystem, which is the ratio of the total antenna size of PS II (N_{II}) to that of PS I (N_I), is obtained by multiplying the relative concentration of each reaction center by its antenna size (Melis and Anderson, 1983). The calculation of the electron-transport capacity ratio ($N_{II/I}$) was as follows:

$$N_{II/I} = \frac{[\text{PS IIα}]N\alpha + [\text{PS IIβ}]N\beta}{[\text{PS I}]NI} \quad (1)$$

$$K\alpha = aI\ N\alpha \quad (2)$$

$$K\beta = bI\ N\beta \quad (3)$$

$$KI = cI\ NI \quad (4),$$

where NI, Nα and Nβ are the antenna sizes or numbers of Chl associated with PS I, PS IIα and PS IIβ, respectively, *I* the actinic light intensity and *a*, *b* and *c* are the proportionality constants depending on the quantum yields of photochemistry at each photosystem. KI, Kα and Kβ are proportional to NI, Nα and Nβ, respectively. Therefore, when Eqn (2) through (4) are substituted for Eqn (1), it is shown as follows:

$$N_{II/I} = \frac{x K\alpha \cdot \text{PS IIα} + y K\beta \cdot \text{PS IIβ}}{z KI \cdot \text{PS I}} \quad (5),$$

where *x*, *y* and *z* are proportionality constants depending upon the quantum yield of the primary photochemistry of each reaction center. We may assume that the quantum yields for the photochemistry at PS IIα, PS IIβ and PS I have the same proportionality constant, i.e. $x = y = z$. The solution of Eqn (5) for the sixth leaves, where PS I : PS IIα : PS IIβ = 1.0 : 0.93 : 0.61, yields $N_{II/I}$ = 1.52, indicating that there is an approximately 1.5-times larger relative electron transport capacity in PS II compared with PS I in the sixth leaves. In the third leaves, PS I : PS IIα : PS IIβ = 1.0 : 0.63 : 0.27. Thus, $N_{II/I}$ = 1.03. The fourth and the fifth leaves have the PS I : PS IIα : PS IIβ ratios of 1.0 : 0.73 : 0.37 and 1.0 : 0.89 : 0.53, respectively. We obtained $N_{II/I}$ = 1.30 for the fourth leaves and $N_{II/I}$ = 1.38 for the fifth leaves. Furthermore, taking Cyt *f* into consideration, the PS IIα/Cyt *f* ratios remained almost at unity at all leaf positions. However, both the PS IIα/PS I and the Cyt *f*/PS I ratios in the third leaves also decreased in comparison with the sixth leaves (Table III), implying that large imbalances of light absorption occur in the bottom leaves.

The large numbers of electrons flow into the plastoquinone pool from water through PS II than are removed from the pool through PS I, resulting in an "overreduction" of the plastoquinone pool. Therefore, the mechanism is necessary to avoid photoinhibition.

There is a state transition that occurs as the mechanism for excessive energy allocation (Bonaventura and Myers, 1969; Murata, 1969). State 1 is induced when plants are exposed to an excessive light preferentially absorbed by PS I (PS I light, e.g. far-red light of 720 nm). In this state, the transfer of the absorbed light energy from PS II to PS I is reduced, resulting in an increased fluorescence yield from PS II. State 2 occurs when light preferentially absorbed by PS II is present (PS II light, e.g. red light of 640 nm). In this state, the transfer of the absorbed light energy from PS II to PS I is large, resulting in a decreased relative fluorescence yield (Bennet *et al.*, 1980; Allen *et al.*, 1981).

To ensure whether state transition occurs at each leaf position, LHC II was phosphorylated artificially with ATP (Allen *et al.*, 1981). In contrast, it is known that LHC I is not phosphorylated with ATP (Haworth and Melis, 1983). However, there is no effect of the phosphorylation with ATP at all leaf positions (data not shown). This result strongly suggests that a state transition has no relation to the leaf position in this rice canopy. The reason why the short-term effects in the rice leaves do not occur is that the rice seedlings develop erectly and the sunlight is well-penetrated into the rice canopy even in daytime.

The question we have to ask here is why levels of PS I referred to Chl are unchanged at all leaf positions? It is possible to establish a working hypothesis about the function of PS I. It has been recognized that massive protein degradations occurs in the bottom leaves, leading to a recycling process in which nitrogen and other mineral elements are translocated from the bottom to the upper leaves (Feller and Fischer, 1994; Gan and Amasino, 1997). We speculate the involvement of a cyclic photophosphorylation and a respiration in order to provide ATP as energy for the translocation. For the purpose of supporting this working hypothesis, the levels of Cyt b_6 referred to Chl were determined. Interestingly, the levels of Cyt b_6 remained at about 4 mmol per mol Chl at all leaf positions (Fig. 3). Furthermore, there is evidence for heterogeneity of the PS I unit as well as that of PS II, indicating that about 70% of the PS I unit may be connected with a cyclic electron transport (Albertsson *et al.*, 1990; Andreasson *et al.*, 1990; Malkin *et al.*, 1991). Thus, one of the PS I populations and Cyt b_6 may take part in a cyclic electron flow.

Moreover, we measured the rates of the respiration in each position (Fig. 4). The rates of the respiration in the lower leaves increased in inverse proportion to the rates of the light-saturated photosynthesis (Pmax). The third leaves were in the state of being at the end period of the senescence because they have a very short plastochron. This increase of respiration rate may be called "the climacteric" by Woolhouse (1967). Therefore, the in-

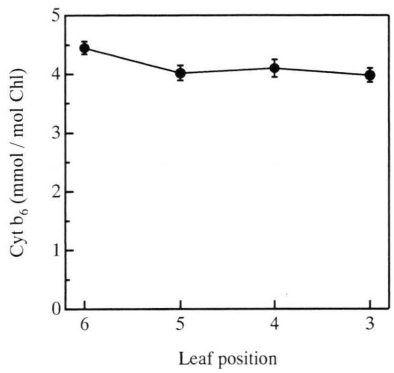

Fig. 3. Cyt b_6 contents with the isolated thylakoid membranes at different position of the rice leaves. Bars, S. D. n = 4–5.

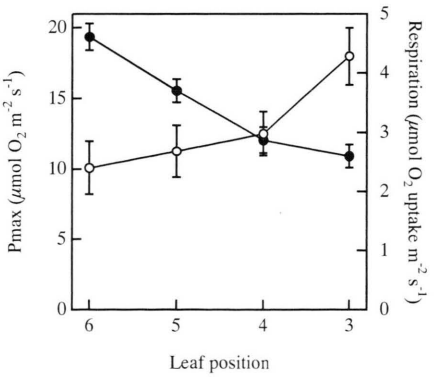

Fig. 4. Rates of the light-saturated photosynthesis (Pmax, ●) and the dark adapted respiration (○) with different position of the rice leaves. Bars, S. D. n = 4–5. P. photosynthetic oxygen evolution at light saturation.

volvement of a cyclic photophosphorylation and the respiration to formation of ATP which may be necessary to translocate the nutrients from the bottom withering leaves to the newly expanding leaves. But, this speculation must be proven by further examination.

The present study has shown that an increase in the abundance of the PS IIα center is partly compensated for by a decline in the total PS II/PS I ratio in order to avoid an imbalance of light absorption by the two photosystems as much as possible. However, the imbalances of light absorption were still occurring and did not result in a state transition.

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