Vol 12 No 10, October 2003
1009-1963/2003/12(10)/1159-07

Chinese Physics

© 2003 Chin. Phys. Soc.
and IOP Publishing Ltd

Research on the ultrafast fluorescence property of thylakoid membranes of the wild-type and mutant rice*

Ren Zhao-Yu(任兆玉)[a)†], Xu Xiao-Ming(许晓明)[b)], Wang Shui-Cai(王水才)[c)], Xin Yue-Yong(辛越勇)[d)], He Jun-Fang(贺俊芳)[c)], and Hou Xun(侯 洵)[a)c)]

[a)] *Institute of Photonics & Photo-Technology, Northwest University, Xi'an* 710068, *China*
[b)] *Laboratory of Photosynthesis Research, Nanjing Agricultural University, Nanjing* 210095, *China*
[c)] *State Key Laboratory of Transience Optics Technology, Xi'an Institute of Optics and Precision Mechanics, Chinese Academy of Sciences, Xi'an* 710068, *China*
[d)] *Laboratory of Photosynthesis Basic Research, Institute of Botany, Chinese Academy of Sciences, Beijing* 100093, *China*

(Received 9 January 2003; revised manuscript received 23 May 2003)

A high yielding rice variety mutant (*Oryza sativa* L., Zhenhui 249) with low chlorophyll b (Chl b) has been discovered in natural fields. It has a quality character controlled by a pair of recessive genes (nuclear gene). The partial loss of Chl b in content affects the efficiency of light harvest in a light harvest complex (LHC), thus producing the difference of the exciting energy transfer and the efficiency of photochemistry conversion between the mutant and wild-type rice in photosynthetic unit. The efficiency of utilizing light energy is higher in the mutant than that in the wild-type rice relatively. For further discussion of the above-mentioned difference and learning about the mechanism of the increase in the photochemical efficiency of the mutant, the pico-second resolution fluorescence spectrum measurement with delay-frame-scanning single photon counting technique is adopted. Thylakoid membranes of the mutant and the wild-type rice are excited by an Ar^+ laser with a pulse width of 120 ps, repetition rate of 4 MHz and wavelength of 514 nm. Compared with the time and spectrum property of exciting fluorescence, conclusions of those ultrafast dynamic experiments are: 1) The speeds of the exciting energy transferred in photo-system I are faster than that in photo-system II in both samples. 2) The speeds of the exciting energy transfer of mutant sample are faster than those of the wild-type. This might be one of the major reasons why the efficiency of photosynthesis is higher in mutant than that in the wild-type rice.

Keywords: rice, photosynthesis, ultrafast process, time constant, exciting energy transfer
PACC: 8725B, 8750E, 8780, 8750B

1. Introduction

Much research has been done, at home and abroad, on the ultrafast process of energy transferring, charge separating and charge recomposing in the photo-system (PS) of various plants by means of time-resolution fluorescence spectrum, absorption spectrum, resonance Raman spectrum, etc. Some significant accomplishments have been obtained.[1−5] As is well known, the mutant caused by the decrease in chlorophyll b (Chl b) is a very common phenomenon in nature. The chlorophyll mutants have been discovered in crops such as corn,[6] barley,[7] cotton,[8] rice,[9], etc. Scientists have carried out much research on their inherited character, microstructure, absorption spectrum, fluorescence property and physiological character.[10−12] The current-existing mutant caused by the decrease in Chl b of rice indicates that mutant is generally produced by physical and chemical mutagenic ways. As a result, its mutant characters are unstable and the efficiencies of photochemistry conversion of most mutant PS II are approximate to or lower than those of the wild-type rice.[13] The rice used in this experiment is Zhenhui 249, whose spontaneous mutant produced by the decrease in Chl b is controlled by a pair of recessive gene (nuclear gene).[14] The initial research showed that the lack or loss of Chl b has an effect on the light harvesting efficiency in light harvest complex, thus resulting in the difference between exciting energy transferring and efficiency of photochemistry conversion and bringing the mutant with a higher photochemical efficiency than the wild-type rice does. Briantais *et al*[4] have studied the chlorophyll fluorescence of the leaf of the wild-type and mutant barley, and its relations varying with the temperature when photochemistry reaction centre was

*Project supported by the State Key Development Program for Basic Research of China (Grant No G1998010100).
†E-mail: rzy@nwu.edu.cn

open by the method of pico-second resolution fluorescence spectrum with single photon counting technique. With the help of multi-exponential fitting and deconvolution, they have obtained three lifetime fluorescence components, 100, 400 and 1200 ps (at 23°C). In their opinions, the content of Chl b in light harvest complex II (LHC II) makes no difference from the above two lifetimes (100 and 400 ps). This makes them draw a conclusion that in PS II the exciton transfer from Chl b to the other antenna pigments in LHC II is irreversible. In order to discuss the difference between the mutant and the wild-type rice, photosynthetic apparatus in energy transfer and conversion, and find out the mechanism about the increased efficiency of photochemistry conversion in mutant resulting from the decrease in Chl b, we adopt the pico-second resolution fluorescence spectrum measurement with a delay-frame-scanning single photon counting technique. Comparing the time character and spectral character of fluorescence in the thylakoid membrane between the wild-type and mutant rice of Zhenhui 249, we have come to a conclusion, different from that of Briantais,[4] that the exciting energy transfer in the mutant sample caused by the decrease in Chl b is faster than that in the wild-type sample; in other words, the content of Chl b does make a difference in the lifetime of fluorescence — the exciting energy transfers being faster in the photosynthesis of the lower Chl b mutant rice might be one of the important reasons why the efficiency of photosynthesis in the mutant is higher than that in the wild-type rice.

2. Materials and Method

2.1. Experimental materials

Two types of field genotype rice — the wild-type (*Oryza sativa* L. var. Zhenhui 249) and the mutant with partial loss of Chl b, provided by Zhenjiang Agricultural Institute of Jiangsu Province, grow in the natural environments (from May to September) with a temperature of 25–40°C and the photon flux density of 1500 μmol/m^{2}s at noon. Preparations for the thylakoid membranes of the mutant and the wild-type rice can be referred to Refs.[14, 15]. To sop the rice leaves in the pre-cooled extracting solution (with the cane sugar of 0.4 mol/L, Tricine of 50 mmol/L and the pH of 7.6) with the ratio of 1:5 (V/V), we filtrate the mixture through the 4-layer gauze and the 16-layer gauze successively; after that, centrifuge the solution at the acceleration 500g for 2 min to eliminate the bigger fragments, and then centrifuge the supernate at the 3000g for 10 min. At this time, the deposit is the chloroplast. After that, the deposit is required to suspend twice in the suspending medium (with the tricin of 50 mmol/L, NaCl of 15 mmol/L and $MgCl_2$ of 5 mmol/L, and the pH of 7.6) and then centrifuge the solution at the 10000g for 10 min. The deposit will be the thylakoid membrane. Draw a small amount of the prepared thylakoid membrane solution and suspend it in the buffer solution, then add glycerine of 10% and freeze in liquid nitrogen for later use. The whole operation is carried out in a darkroom at 0–4°C.

2.2. Experimental method

The experimental set-up is shown schematically in Fig.1. The Ar^+ laser adopted in the experiment is an exciting light source, with the output wavelength of 514 nm, the pulse width of 120 ps and the repetition rate of 82 MHz. Because the laser source of a high repetitive frequency is apt to pile up charges in the organism and make the sample fatigue or even damage, so the first thing to do is to select the laser pulse of 82 MHz by Bragg diffraction modulation technique with the ideal output pulse frequency of 4 MHz for the experiment. The fluorescence to be measured will be collected by a collimating mirror with 30% collection efficiency and then input into the spectrometer (SPEX270M). The quasi-monochromatic fluorescence signal from the spectrometer is detected by an avalanche photodiode (APD)(SPCM-AQR-15) while the multi-channel signals are collected by a single photon counting template T914P, and then sent to the computer for data procession by MCS software. For the measurement principles one can refer to Ref.[16].

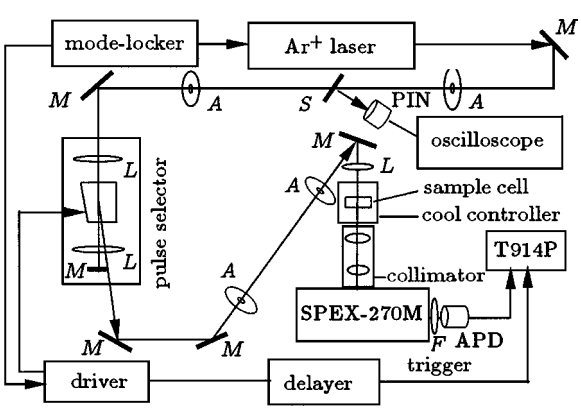


Fig.1. The fluorescence spectroscope of pico-second resolution with delay-frame-scanning single photon counting technique. M—mirror, S—splitter, A—aperture, F—filter, L—lens, APD—avalanche photodiode, PIN—positive intrinsic negative.

The experiment is required to be carried out in a dark state. The sample with a concentration of 10μg/ml is poured into the sample pool set in a room of constant temperature of 1–2°C. During measurement, the step used in the monochromator is 2 nm and the fluorescence measured ranges from 640 to 780 nm. In order to avoid irreversible damage and fluorescence quenching in the sample, the exciting light intensity is controlled within $(1.5\text{–}2.0)\times10^9$ photon/pulse, based on the experimental determination of the intensity relation between the exciting light and fluorescence (namely, the fluorescence is about saturated when the light intensity reaches 2.3×10^9 photon/pulse).

2.3. Data analysis

In measuring the fluorescence emitted from the fluorescence transmission system excited by the ultra short pulse laser, the fluorescence curve obtained from the experiment is not the real fluorescence curve, but a convolved result composed of exciting light pulses, the response function of the measured system and the real fluorescence curve, because the duration of the exciting light pulse and the response behaviour of the instruments have effect on the pulse signals. So the real fluorescence curve has to be obtained by deconvolving through the mathematical analogue beforehand.

Given $L(t)$, $R(t,\lambda)$ and $S(t,\lambda)$ which are the laser pulse, system response and real fluorescence signal respectively, and $F(t,\lambda)$ the measured fluorescence from the experiment, we have

$$F(t,\lambda) = R(t,\lambda) * L(t,\lambda) * S(t,\lambda). \tag{1}$$

If $M(t,\lambda) = R(t,\lambda) * L(t)$ is the system impact response function for the laser pulse, then

$$F(t,\lambda) = M(t,\lambda) * S(t,\lambda). \tag{2}$$

Here, "$*$ " is an operation of convolution. So, the real signal will be obtained by deconvolving the measured fluorescence signal and the system response function. Given S', the multi-exponent fitting function of the real signal,

$$S' = \sum_{j=1}^{N} C_j \times \exp(-t/\tau_j), \tag{3}$$

where j (j =1,2,3,$\cdots, N$) is the fitting number of terms, and τ the time constant. Nonlinear least-squares techniques are adopted here,

$$s^2 = \sum_{i}^{n} W_i[F_i - M_i * S_i']^2, \tag{4}$$

where i (i=1, 2, 3, $\cdots, n$) indicates the series number of wavelengths measured in the experiment, W_i is the weight factor often determined by the reciprocal of the best-fitting value $M_i * S_i'$ in the fluorescence attenuation curve; and generally, it is approximately represented by the reciprocal of F_i. Using the Nelder–Mead simplex method to optimize the search, the values of C_j and τ_j can be deduced when s^2 is minimal. (Two, three and four exponents were tried in our fitting work, and finally we found that the three-exponent fitting has the minimal fitting residual error.)

3. Experimental results and analyses

The differences of the pigment contents and their coefficients of pigment fluorescence in the 11th leaves (in the natural condition) of the wild-type and mutant rice are listed in Tables 1 and 2. The speeds of photosynthesis and coefficients concerning the two leaves are given in Table 3. Figure 2 shows the ultraviolet-visible absorption spectra of the high-yield wild-type and its natural mutant rice thylakoid membranes at room temperature. Figure 3 shows the fluorescence curves of the two thylakoid membranes measured under the zero-delaying condition. And their normal spectra are plotted in Fig.4. The time constants acquired by three-exponent fitting at 692 and 732 nm of the wild-type and at 686 and 714 nm of the mutant rice are listed in Table 4. The time character curves of ultrafast fluorescence at 692 nm (wild-type) and 686 nm (mutant) are respectively given in Figs.5 and 6. In order to show the experimental curve and real fluorescence curve in one figure, all curves were normalized. A_i in Table 4 denotes the ratio of fluorescence pigment at the time constant t_i to the total of the fluorescence pigments at the same wavelength. The formula[17] is as follows:

$$A_i = |C_i \times \tau_i| / \sum_i |C_i \times \tau_i|.$$

Here, C_i in the formula represents the coefficient before the exponent.

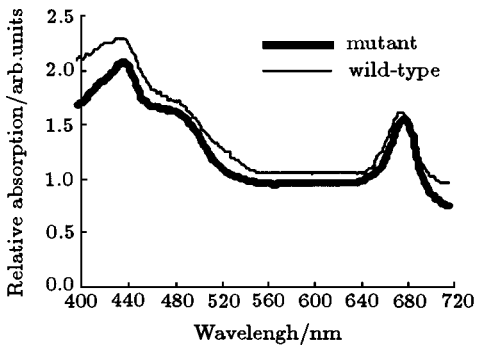


Fig.2. Ultraviolet-visible absorption spectra of the wild-type and mutant rice thylakoid membrane, at room temperature.

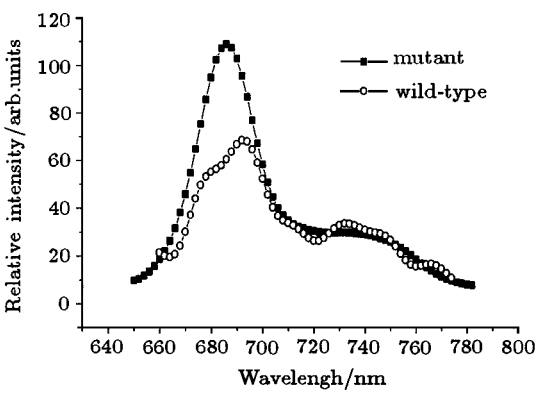


Fig.3. Fluorescence spectra of the wild-type and mutant rice thylakoid membranes.

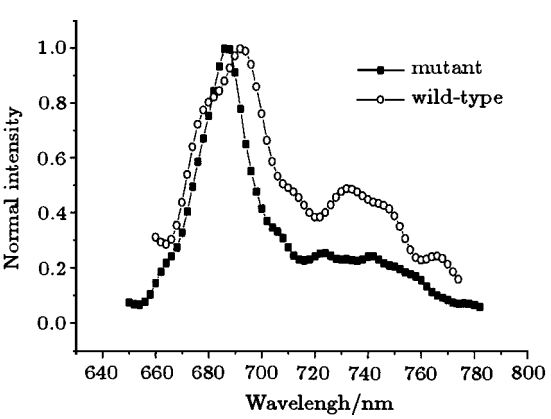


Fig.4. Normal fluorescence spectra of the wild-type and mutant rice thylakoid membranes.

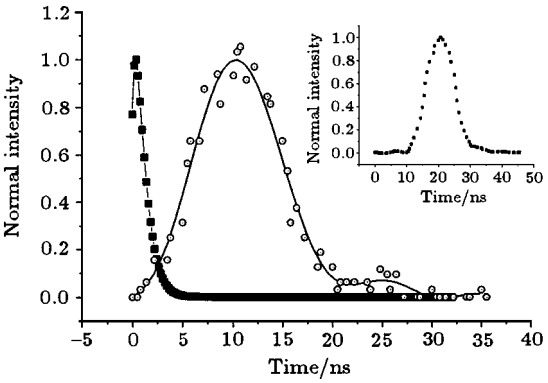


Fig.5. The normalized curves of fluorescence time of wild-type rice thylakoid membrane at 692 nm. Dot circlets are the experimental data; solid line is the fitting curve and square-line is the real curve of fluorescence time; the dotted curve at upper right corner is the system response.

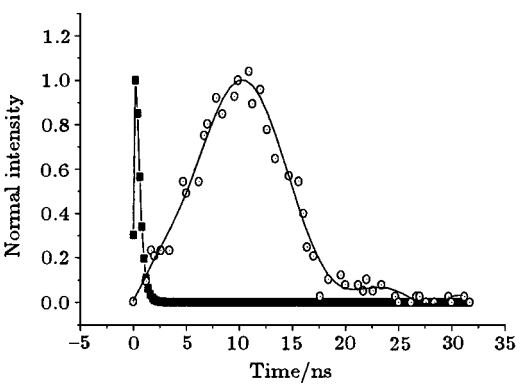


Fig.6. The normalized curves of fluorescence time of mutant rice thylakoid membrane at 686 nm. Dot circlets are the experimental data; solid line is the fitting curve and square-line is the real curve of fluorescence time.

3.1. Comparative analyses of different characters

Table 1 suggests that the total chlorophyll contents of Chl a, Chl b per unit fresh weight or per unit leaf area in the mutant are about 1/2 of that in the leaves of the wild-type rice. However, the content of Chl b decreases more dramatically, thus the mutant has a higher ratio of Chl a to Chl b. The decrease of chlorophyll affects the efficiency of light harvest of LHC II in PS II,[14] which brings down the light absorptivity of mutant (shown in Fig.2). In addition, the β-Car contents in both kinds of rice remain constantly, thus the mutant has a lower ratio of Chl to β-Car which is in favour of its higher endurance to strong light compared with the wild-type.

Table 1. The contents of pigments in the leaf of wild-type and mutant rice.

Material	Contents of pigments in the leaf				Chl a/Chl b
	Contents of Chl		Contents of β-Car		
	$C_w/\mu g\cdot g^{-1}$	$C_s/\mu g\cdot cm^{-2}$	$C_w/\mu g\cdot g^{-1}$	$C_s/\mu g\cdot cm^{-2}$	
Wild-type	3087.8± 91.4	45.75± 0.08	371.9± 25.0	5.51± 0.17	2.55
Mutant	1746.5± 38.8	22.10± 1.45	371.4± 0.1	4.70± 0.21	3.96

Table 2. The fluorescence coefficients.

Material	F_o	F_v/F_m	F_v/F_o	$\Phi_{PS\ II}$	q_N	$T_{1/2}$
Wild-type	0.120	0.801	4.030	0.365	0.625	317.2±2.5
Mutant	0.064	0.854	5.840	0.411	0.615	424.5±4.0

Note: F_o is the original fluorescence and the fluorescence level as well (namely Q_A is entirely oxidized) when the reaction centre is entirely opened. F_v/F_o is the photochemistry potential of PS II. F_v/F_m is the primeval photochemistry efficiency. $\Phi_{PS\ II}$ is the real photochemistry quantum productivity. q_N is the coefficient of nonphotochemical quench. $T_{1/2}$, the value of which indicates the relative size of PQ reservoir,[18] is half of the time that is required from original fluorescence F_o to maximum fluorescence F_m.

Table 3. The speed of photosynthesis and coefficients of the rice in the natural condition.

Material	$P_n/\mu mol\ (CO_2)\cdot m^{-2}s^{-1}$	$P_{max}/\mu mol\ (CO_2)\cdot m^{-2}s^{-1}$	Φ_e	Light absorption coefficient
Wild-type	25.0±0.8	27.2±0.6	0.024±0.0005	0.86±0.02
Mutant	21.8±0.5	28.3±0.8	0.031±0.0007	0.56±0.01

Note: P_n is the net speed of photosynthesis and P_{max} the saturation speed of photosynthesis. ϕ_e is the quantum efficiency calculated from the real absorbed optical energy, i.e. the mole number of O_2 released from photosynthesis organization after absorbing 1 mol light quantum, or the mole number of assimilation CO_2.

Table 4. Time constants by three-exponent fitting of thylakoid membranes at 692 and 732 nm (wild-type) and 686 and 714 nm(mutant).

Wavelength/nm	Component 1		Component 2		Component 3	
	τ_1/ps	A_1/%	τ_2/ps	A_2/%	τ_3/ps	A_3/%
692	337.9	25.6	373.9	27.7	597.8	46.7
732	165.4	60.2	195.7	22.4	231.3	17.4
686	117.9	30.9	252.3	33.3	333.0	35.8
714	114.8	21.7	130.4	50.1	145.4	28.2

Note: τ_i is the time constant and A_i is the ratio of the fluorescence pigment at time constant τ_i to the total fluorescence pigments at the same wavelength.

In Table 2, the initial fluorescence value F_o of the mutant is lower than that of the wild-type, and this may well be related to the lower content of chlorophyll in the mutant. F_v/F_o indicates that the original photochemical efficiency in the PS II of the mutant is higher than that of the wild-type, and the real photochemical quantum productivity ($\Phi_{PS\ II}$) of the mutant is higher than that of the wild-type. All of the above results show that the partial loss of Chl b in the mutant results in increasing the photochemical efficiency of the PS II. Besides, the data in Table 2 indicate that the PQ reservoir of the mutant is higher than that of the wild-type, which makes the PS II reaction centre of the mutant lie in the open state, thus reducing the exciting energy transferred from PS II to PS I and raising the photochemical efficiency in the PS II of the mutant.

From Table 3 we know that the net photosynthetic rate of the mutant in moderate light intensity is a little lower than that of the wild-type; however, the saturation photosynthetic rate (P_{max}) of the mutant in high light intensity is higher than that of the wild-type, which is indicative of the fact that the real quantum efficiency of the mutant is higher than that of the wild-type. The photon absorptivity of the mutant is lower than that of the wild-type, but the partial decrease in the Chl b, and moreover, the content of β-Car in the mutant is close to that of the wild-

type (see Table 1), all of which are favourable to increasing the lightfastness of the mutant and shelter it from the strong light.[14] So compared with the wild-type, it is obvious that the photochemical efficiency of the mutant in the intense light is higher than that of the wild-type and has a good quality of lightfastness, which lends it a potential of high yields, though the net photosynthetic rate of the mutant is lower.

3.2. Analysis of ultrafast fluorescence spectra

The thylakoid membrane of the high plant has two photosynthetic systems (PS II and PS I) in the state of photosynthesis. On being illuminated, the antenna pigments in both PS II and PS I absorb photons and transfer them to the reaction centres of P680 and P700, whose fluorescence ranges are 650–710 nm and 710–750 nm, respectively. The exciting light of 514 nm excites both the Chl b and β-Car in the thylakoid membrane. The Chl b will transit to the second singlet (S_2 state) after absorbing photons of 514 nm, and will first de-excite without radiation at a speed of $10^{12} - 10^{14}\,s^{-1}$ to the state S_1, and then transit to the ground state S_0 from S_1 state by emitting fluorescence. The exciting energy captured by the Chl b and β-Car is transferred from the peripheral and internal antenna pigment complex to the reaction centre of P680 (PS II) and P700 (PS II), where the reaction of photochemistry takes place.

3.2.1. Analysis of spectrum property

From Figs.3 and 4, we know that the peak value of fluorescence (692 nm) in the wild-type PS II has an Einstein shift about 4 nm compared with that of the mutant PS II (688 nm). In both samples, the fluorescence ranges from 720 to 760 nm (the wild-type) and from 710 to 760 nm (the mutant) have been measured, which contain the fluorescence band of PS I and the side fluorescence band of vibration and rotation energy decay. Judging from the normal spectrum, the fluorescence range of the wild-type is wider than that of the mutant, which might result from the higher contents of pigment in the wild-type, and the relative content of Chl b is higher than that in the mutant, thereby producing the "shoulder" of 670–678 nm in the fluorescence curves. Meanwhile, the mutant has a higher fluorescence intensity (Fig.3) though its photon absorptivity is lower than that of the wild-type (Fig.2). This result implies that the conversion of light–light in the mutant is more efficient than that in the wild-type.

3.2.2. Analysis of time property

In Table 4, the thylakoid membrane of the wild-type has three time constants of fluorescence decay at the wavelength of 692 nm (PS II), which are larger than those at the length of 732 nm (PS I). The ratios of the fluorescence pigment in the different time groups to the total fluorescence are different at 692 and 732 nm, namely, the ratio of the faster group (Group 1) becomes larger with increasing the wavelength, however, the case is quite opposite for the slower groups (Groups 2 and 3). In the wild-type sample, the time constants of the longer wavelengths are smaller than those of the shorter wavelengths, that is to say, the speed of exciting energy among pigment molecules in PS I transfers faster than that in PS II. As far as the mutant is concerned, the time constants of the fluorescence decay at 686 nm are respectively larger than those at 714 nm. In Groups 1 and 3, A_i becomes smaller with increasing the wavelength, while the case in Group 2 is quite opposite. In the mutant sample, the exciting energy of the longer-wavelength also transfers faster than that of the shorter wavelength.

The total time of fluorescence decay for the mutant is smaller than that of the wild-type by comparing the fluorescence pigment time constants of both the mutant and the wild-type rice. The two real fluorescence decay curves by deconvolution in Figs.5 and 6 indicate that the mutant decays faster than the wild-type, in other words, the exciting energy in the mutant transmits faster than that in the wild-type. This may well be one of the critical factors to make the photosynthesis in the mutant more efficient than in the wild-type.

4. Conclusions

This paper deals with the thylakoid membrane ultrafast fluorescence spectrum property of the wild-type and the mutant rice caused by decreasing Chl b by means of pico-second resolution fluorescence spectrum measurement with delay-frame-scanning single photon counting technique. After comparing the experimental data and results of both the time property and spectral property, conclusions have been made as follows:

i) The exciting energy of pigment molecules in PS I transfers faster than in PS II of both the wild-type and the mutant rice.

ii) The exciting energy in the mutant sample transfers faster than that in the wild-type.

iii) That the exciting energy transfers faster in

the photosynthesis of the mutant rice with lower Chl b may be one of the critical factors that make the mutant have a higher efficiency of photosynthesis than the wild-type.

References

[1] Pack S D *et al* 1998 *Opt. Lett.* **23** 1215
[2] Terjung F *et al* 1997 *Photosyn. Res.* **53** 29
[3] Gilmore A M *et al* 1996 *Photosyn. Res.* **48** 171
[4] Briantais J M *et al* 1996 *Photosyn. Res.* **48** 189
[5] Grondelle R V, Dekker J P, Gillbro T and Sundstrom V 1994 *Biochem. Biophys. Acta* **1187** 1
[6] Harpster M H, Mayfield S P and Taylor W C 1984 *Plant Mol. Biol.* **3** 59
[7] Bellemare G, Bartlett S G and Chua N H 1982 *J. Biol. Chem.* **257** 7762
[8] Killough D T and Horlacher W R 1993 *Genetics* **18** 329
[9] Yamashita A and Terao T 1983 *Jap. J. Breeding* **33** (Suppl 1) 92
[10] Terao T, Yamashita A and Katoh S 1985 *Plant Cell Physiol.* **26** 1361
[11] Terao T, Yamashita A and Katoh S 1985 *Plant Cell Physiol.* **26** 1369
[12] Hsu B D and Lee J Y 1995 *Aust. J. Plant Physiol.* **22** 195
[13] Lokstein H *et al* 1993 *J. Photochem. Photobiol.* **19** 217
[14] Dai X B *et al.* 2000 *Acta Botan. Sin.* **42** 1289
[15] Xin Y Y *et al.* 2000 *Acta Botan. Sin.* **42** 1278
[16] Ren Z Y *et al.* 2002 *Laser J.* **23** 42 (in Chinese)
[17] Govindjee V M V *et al* 1990 *Biochem. Biophys. Acta* **1015** 173
[18] Bohar-Nordenkampf H R *et al* 1989 *Funct. Ecol.* **3** 497