

BRIEF COMMUNICATION

High photosynthetic efficiency of a rice (*Oryza sativa* L.) *xantha* mutantZHOU Xiang-sheng^{*}, WU Dian-xing^{*}, SHEN Sheng-quan^{*}, SUN Jun-wei^{*,†}, and SHU Qing-yao^{*,***}*National Key Laboratory of Rice Biology and Ministerial Key Laboratory for Nuclear Agricultural Sciences, Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou 310029, China^{*}**College of Life Sciences, Zhejiang University, Hangzhou 310029, China^{**}**Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,**International Atomic Energy Agency, Wagramer Straase 5, P.O. Box 100, A-1400 Vienna, Austria^{***}***Abstract**

Comparative analysis revealed that a *xantha* rice mutant (cv. Huangyu B) had higher ratios of chlorophyll (Chl) *a/b* and carotenoids/Chl, and higher photosynthetic efficiency than its wild type parent (cv. II32 B). Unexpectedly, the mutant had higher net photosynthetic rate (P_N) than II32 B. This might have resulted from its lower non-photochemical quenching (q_N) but higher maximal photochemical efficiency (F_V/F_M), higher excitation energy capture efficiency of photosystem 2 (PS2) reaction centres (F_V'/F_M'), higher photochemical quenching (q_P), higher effective PS2 quantum yield (Φ_{PS2}), and higher non-cyclic electron transport rate (ETR). This is the first report of a chlorophyll mutant that has higher photosynthetic efficiency and main Chl fluorescence parameters than its wild type. This mutant could become a unique material both for the basic research on photosynthesis and for the development of high yielding rice cultivars.

Additional key words: chlorophyll *b*-deficiency; chlorophyll fluorescence; net photosynthetic rate; *Oryza sativa*; photosynthetic efficiency; *xantha* mutation.

Chlorophyll (Chl) mutations are common in both spontaneous and mutant populations (van Harten 1998). In rice, more than 70 Chl mutations (for review see Kurata *et al.* 2005) originating from physical, chemical, biological (transposon insertion), or spontaneous mutagenesis (Reddi and Reddi 1984, Terao *et al.* 1985, Dai *et al.* 2003, Lin *et al.* 2003) have been reported. These mutations result in defects in Chl synthesis and lead to *albino*, *xantha*, *chlorina*, strip, virescent, yellow-green, or zebra mutant phenotypes (van Harten 1998). Mutants defective in Chl *b* expression, *i.e.* *xantha* and *chlorina* have been frequently used in studies on the function of various photosynthetic components containing Chl *b*. Rice mutants

defective in Chl *b* expression have been classified into two types on the basis of Chl *a/b* ratios (Terao *et al.* 1985). Type I mutants (Chl *b*-less mutants) contain no detectable Chl *b* and normally contain approximately half the total amount of Chl as the normal plant. Type II mutants (Chl *b*-deficient mutants) contain somewhat reduced amount of Chl *b*, normally having a Chl *a/b* ratio >5 compared with values of approximately 3 for normal plants (Markwell *et al.* 1986).

In the progeny of γ -rays-irradiated seeds of a cytoplasmic male sterile (CMS) maintainer line, cv. II32 B, a *xantha* mutant line, cv. Huangyu B, was previously developed (Shu *et al.* 2001). Genetic analysis has already

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Abbreviations: AQY, apparent quantum yield; Car, carotenoids; Chl, chlorophyll; ETR, non-cyclic electron transport rate; F_M , maximum Chl fluorescence yield in the dark-adapted state; F_M' , maximum Chl fluorescence yield in the light-adapted state; F_0 , minimum Chl fluorescence yield in the dark-adapted state; F_0' , minimum Chl fluorescence yield in the light-adapted state; F_S , steady-state Chl fluorescence yield; F_V , variable Chl fluorescence; F_V/F_M , maximal photochemical efficiency; F_V'/F_M' , excitation energy capture efficiency of PS2 reaction centres; P_N , net photosynthetic rate; PPFD, photosynthetic photon flux density; PS2, photosystem 2; q_N , non-photochemical quenching; q_P , photochemical quenching; Φ_{PS2} , effective PS2 quantum yield.

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confirmed that this *xantha* mutation is controlled by a single recessive locus (Zhou *et al.* in press), which is consistent with other reported Chl *b*-deficient mutations. In the present study, the photosynthesis and Chl fluorescence properties of II32 B and Huangyu B were compared. Unexpectedly, we found that Huangyu B had higher photosynthetic efficiency than its wild type, a phenomenon never before reported for any of known Chl *b* mutants.

Seeds of II32 B and Huangyu B were first sown on seedling beds. Then the seedlings were individually transplanted to soil in pots, and grown outdoors in 2004. Ten leaf discs were cut from fully extended leaves of both lines and extracted in 5 cm³ of 80 % (v/v) aqueous acetone solution in the dark at 25 °C until fully blanched (*ca.* 48 h). The contents of Chl *a*, Chl *b*, and carotenoids (Car) were determined in leaves of seedlings and mature plants according to Wellburn (1994). Gas exchange was measured with a portable photosynthesis determination system (LICOR-6400, USA) at a constant airflow rate of 500 µmol s⁻¹, CO₂ concentration of 385±5 cm³ m⁻³, and the temperature of 39±2 °C. A gradient of photosynthetic photon flux density (PPFD) was set as follows: 0, 20, 50, 100, 200, 500, 800, 1 000, 1 200, 1 500, and 2 000 µmol m⁻² s⁻¹. Chl fluorescence was measured with an integrating fluorescence fluorometer (LI-6400-40 leaf chamber fluorometer, USA). After dark-adaptation of samples for 1 h, the minimum fluorescence in the dark-adapted state (*F*₀) was measured with weak modulated irradiation (<0.1 µmol m⁻² s⁻¹). A 600 ms saturating flash (>7 000 µmol m⁻² s⁻¹) was applied to determine the maximum Chl fluorescence yield in the dark-adapted state (*F*_M) and the *F*_V/*F*_M. Immediately, the leaf was continuously irradiated with red-blue actinic beams (1 400 µmol m⁻² s⁻¹) and equilibrated for 30 min to record the steady-state Chl fluorescence yield (*F*_S). Following this, another saturation flash (>6 000 µmol m⁻² s⁻¹) was applied and then the maximum Chl fluorescence yield in the light-adapted state (*F*_M') was determined. After the flash, actinic irradiation was replaced with far-red irradiation, and the minimum Chl fluorescence yield in the light-adapted state (*F*₀') was determined. Other fluorescence parameters were calculated according to Genty *et al.* (1989):

$$\begin{aligned} F_V/F_M' &= (F_M' - F_0')/F_M'; \\ \Phi_{PS2} &= (F_M' - F_S)/F_M'; \\ q_P &= (F_M' - F_S)/(F_M' - F_0'); \\ q_N &= (F_M - F_M')/(F_M - F_0). \end{aligned}$$

The non-cyclic electron transport rate (ETR) was calculated as (*F*_M' - *F*_S)/*F*_M'*fI* α_{leaf}, where *I* is incident PPFD, *f* is the fraction of absorbed quanta that is used by PS2, and α_{leaf} is leaf absorbance (Bilger and Björkman 1990). All the measurements were made in triplicate on the flag leaves of plants 3 d after heading.

The content of photosynthetic pigments varied among leaves at different growing stages, but the ratios of

Chl *a/b* and Car/Chl remained relatively stable both for the mutant and the wild type (data not shown). For the flag leaf samples, the mutation resulted in the significant reduction of contents of photosynthetic pigments in the mutant compared to its wild type parent, *i.e.* 57, 77, and 41 % for Chl *a*, Chl *b*, and Car, respectively (Table 1). Consequently, the ratios of Chl *a/b* and Car/Chl increased significantly (Table 1). Based on the reduction of Chl *b* content and the increased Chl *a/b* ratio (7.78 *versus* 4.15 of II32 B), Huangyu B can be classified as a Chl *b*-deficient mutant (Markwell *et al.* 1986). The Chl *a/b* ratio of Huangyu B was relatively higher than that of the Chl *b*-deficient mutant ZH249-m (*i.e.* 4.7) (Gong *et al.* 2001), but it was lower than those reported in the experiment of Terao *et al.* (1985).

Table 1. The contents of photosynthetic pigments and the chlorophyll (Chl) fluorescence parameters of the *xantha* mutant Huangyu B and the wild type II32 B. Measurements were made in triplicate on the flag leaves of plants 3 d after heading. Means±S.D.; those of which are followed by different letters on the same line were significantly different at *p*<0.01.

	Huangyu B	II32 B
Chl <i>a</i> [g kg ⁻¹ (FM)]	1.76±0.14B	4.05±0.25A
Chl <i>b</i> [g kg ⁻¹ (FM)]	0.23±0.02B	0.98±0.07A
Chl [g kg ⁻¹ (FM)]	1.99±0.15B	5.03±0.31A
Car [g kg ⁻¹ (FM)]	0.56±0.04B	0.95±0.06A
Chl <i>a/b</i>	7.78±0.12A	4.15±0.12B
Car/Chl	0.28±0.00A	0.19±0.01B
<i>F</i> ₀	63.4±1.0B	105.1±2.2A
<i>F</i> ₀ '	83.8±1.5B	115.7±1.6A
<i>F</i> _S	118.0±2.3B	148.7±5.2A
<i>F</i> _V / <i>F</i> _M	0.86±0.00A	0.83±0.00B
<i>F</i> _V / <i>F</i> _M '	0.47±0.01A	0.35±0.02B
Φ _{PS2}	0.25±0.01A	0.17±0.01B
q _P	0.54±0.02A	0.47±0.02B
q _N	0.76±0.01B	0.85±0.01A
ETR [µmol(e ⁻) m ⁻² s ⁻¹]	160.7±8.3A	106.4±7.7B

Net photosynthetic rate (*P*_N) of both Huangyu B and II32 B increased as PPFD was raised from 0 to about 2 000 µmol m⁻² s⁻¹ (Fig. 1). Both the mutant Huangyu B and the parent II32 B had saturation of *ca.* 1 500 µmol m⁻² s⁻¹, but *P*_N of the mutant was consistently higher (by 11–17 %) than that of the parent when irradiance was above 500 µmol m⁻² s⁻¹ (Fig. 1). The apparent quantum yield (AQY) of photosynthetic CO₂ fixation was similar for the mutant and its wild type parent (Fig. 1). This implied that the mutation did not affect the efficiency of photochemical energy transformation. On the other hand, the non-photochemical quenching (q_N) decreased by 11.6 % and the maximum photochemical efficiency (*F*_V/*F*_M) increased by 3.6 % in the mutant over its parent (Table 1). All these data indicated that the efficiency of gas exchange and photochemistry was maintained or improved in the mutant rather than deteriorated.

The higher photosynthetic efficiency of the mutant was also supported by other Chl fluorescence parameters (Table 1). Although the F_0 and F_0' were significantly decreased in the mutant, which might be a direct effect of the Chl content reduction as reported in other mutants (Tan *et al.* 1996), the effective PS2 quantum yield (Φ_{PS2}), the photochemical quenching (q_P), and the non-cyclic electron transport rate (ETR) were all increased, by 47.1, 13.5, and 51.0 %, respectively, over its parent (Table 1). This implied that the reduction of Chl *b* neither reduced the efficiency in photon energy capture and electron flow through PS2, nor the number of functional PS2 centres. In addition, the increased ratio of F_V'/F_M' further implied that the PS2 reaction centres of Huangyu B had higher excitation energy capture efficiency than those of its parent (Table 1).

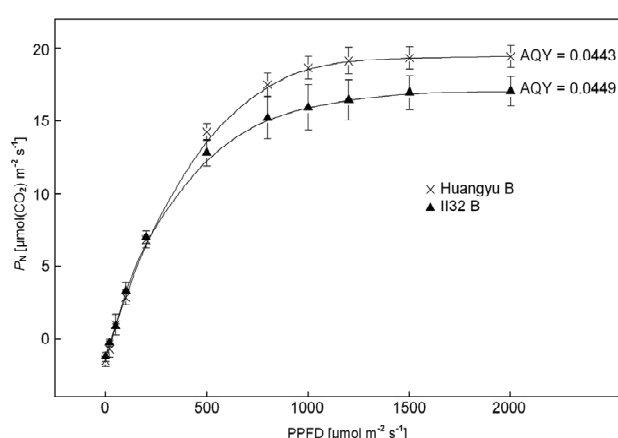


Fig. 1. Responses of net photosynthetic rate (P_N) to photosynthetic photon flux density (PPFD) and the apparent quantum yield (AQY) in the flag leaves of the mutant Huangyu B and its wild type II32 B (measured on the 3rd d after heading). Means $\pm$ S.D. of three replications.

The Chl fluorescence properties, photosynthetic characteristics and components of photosynthetic apparatus (*i.e.* light-harvesting photosynthetic system) of Chl *b*-deficient/less mutants have been widely studied in higher plants. Generally, the photosynthesis in Chl *b*-less mutants is much more negatively affected than in Chl *b*-deficient ones, while the biological consequence of

Chl *b*-deficient mutations depends on the degree of Chl reduction. Lin *et al.* (2003) reported that two rice Chl *b*-less mutants had lower P_N , q_P , and Φ_{PS2} than their wild type parent, though the F_V/F_M , and AQY were not significantly affected. In investigation on the spontaneous Chl *b*-deficient mutant ZH249-m which had the Chl *a/b* ratio around 4.7, Dai *et al.* (2000) also found that the mutant had higher F_V/F_M and q_P under high irradiance (PPFD > 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than its parent. This is consistent with our present observation. However, the overall photosynthetic rate of ZH249-m was less than in its parent, and thus different from Huangyu B.

Another interesting characteristic of Chl *b*-deficient mutants is the change in their endurance to photoinhibition. In a Chl *b*-deficient barley mutant, both the light-harvesting efficiency and endurance to strong irradiance was decreased (Havaux and Tardy 1997). On the contrary, decreased photoinhibition under strong irradiance was discovered in Chl *b*-deficient mutants of *Brassica chinensis* (Guo *et al.* 2003) and rice (Dai *et al.* 2003), due to the lower photon absorption rate and the higher xanthophyll cycle capacity in the mutant (Dai *et al.* 2003).

Jenkins *et al.* (1989) reported that a large reduction in Chl content might not result in a corresponding reduction in photosynthesis and carbon assimilation in a couple of yellow-green maize mutants. Our results as well as the results mentioned above imply that the photosynthetic efficiency is not dependent on the content of Chl when the latter reaches a minimum value. The high photosynthetic efficiency observed in the present study should not be explained by the Chl *b* mutation, but rather by other unknown factors. As a further support to this assumption, Bai *et al.* (2003) recently identified 3 mutant lines that had better Chl fluorescence properties, and consequently higher yields than their parent, indicating that photosynthetic efficiency could be improved independently of Chl content changes.

In conclusion, we identified for the first time a Chl *b*-deficient rice mutant that had overall photosynthetic efficiency higher than its wild type parent. This mutant could become a unique material both for the basic research on photosynthesis and for the development of high yielding rice varieties.

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