Photosynthetic characteristics of two new chlorophyll b-less rice mutants

Zhi-Fang LIN†,‡, Chang-Lian PENG†, Gui-Zhu LIN†, Zhi-Ying OU†, Cheng-Wei YANG†, and Jing-Liu ZHANG†,‡

South China Institute of Botany, Chinese Academy of Sciences, Guangzhou 510650, P. R. China
National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology & Ecology,
Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, P. R. China

Abstract

Two yellow rice mutants VG28-1 and VG30-5 were obtained during the tissue culture process from a rice plant (cv. Zhonghua No. 11, japonica) with inserted maize Ds transposon element. Absorption spectra and pigmentation composition showed that two mutants had no chlorophyll (Chl) b and lower Chl a content in comparison to the wild type (WT). Net photosynthetic rate (Pn), total electron transport rate (J), photochemical quenching (qP), quantum yield of PS2 dependent non-cyclic electron transport (ΦPS2), fraction of Pmax, and leaf area were lower but Fv/Fm and apparent quantum yield (AQY) remained at similar levels as in the WT plant. Xanthophyll cycle pool size (V+A+Z) on a Chl basis, and de-epoxidation state were enhanced in the mutants. The mutants had larger amounts of soluble protein and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), especially the small subunit of RuBPCO, than WT. The characteristics of two rice mutants differed somewhat from the other common Chl b-less mutants originating from mutagenic agent treatments.

Additional key words: chlorophyll absorption spectrum; chlorophyll accumulation and degradation; carotenoids; photosystem 2 activity; ribulose-1,5-bisphosphate carboxylase/oxygenase; thermal dissipation; total electron transport rate.

Introduction

Chl b-deficient mutants have been reported in a number of plant species including Arabidopsis (Rühle et al. 1983), barley and wheat (Falbel et al. 1994), rice (Terao et al. 1985), soybean (Ghirardi and Melis 1988), oilseed rape (Zhao et al. 2001), maize (Greene et al. 1988), sweet clover (Markwell et al. 1986), sugar beet (Abadía et al. 1985), tomato (Falbel and Staehelin 1996), tobacco (Thielen and van Gorkom 1981), and Ficus (Yang et al. 1995). The Chl b-deficiency was considered to result from a partial block in chlorophyll (Chl) synthesis pathway at the Mg-insertion step (Falbel and Staehelin 1996). Certain alterations of the function and structure of photosynthetic apparatus and its responses to environmental factors usually accompany the blocking of Chl b synthesis in these mutants (Ghirardi and Melis 1988, Zhao et al. 2001). Recently, more attention has been focused on the genetic and biochemical control of biosynthesis of Chl b and the assembly of light-harvesting Chl a/b proteins (Eggink et al. 2001). However, the detailed pathway of Chl b biosynthesis and its regulation are still poorly documented.

Most of the Chl b-deficient mutants were generated by treatment with some chemical or physical mutagenic agents (Falbel and Staehelin 1996). In rice, 16 different strains of Chl b-deficient mutants were obtained by ionising radiation and various mutagenic chemicals (Terao and Yamashita 1982). These mutants were divided according to their different ratios of Chl a/b into two groups, types I and II (Terao et al. 1985). Recently, two new yellow rice mutants were selected during tissue culture of the postity of a transgenic rice plant, which was transformed with a maize Ds transposon using Agrobacterium tumefaciens as the mediator (Wang et al. 2000). The basic characteristics of these two new rice mutants should be examined to find out whether there are any alterations in the assembly of LHC-protein complex, the efficiency of radiant energy utilisation, photoprotection, and CO2 assimilation. In this paper, we present evidence that two new mutants

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***Author for correspondence; fax: 86-20-85223283; e-mail: linzhif@scib.ac.cn

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VG28-1 and VG30-5 contain no Chl b and belong to Chl b-less type 1 mutant class. As compared to the wild type (WT) rice, they have smaller leaves and a larger xanthophyll cycle pool (on Chl basis), and show certain alterations in absorption spectrum, photosystem 2 (PS2) activity, and especially in RuBPCO protein content. These features have not been reported in existing Chl b-deficient rice mutants, and the rice mutants could be used to further examine the regulation of Chl b loss and photosynthetic protein synthesis.

Materials and methods

Plants: The wild type (WT) rice (Oryza sativa L. cv. Zhonghua No. 11) and two mutants (VG28-1, VG30-5) were grown outdoor from seeds in pots in South China Institute of Botany. VG28-1 and VG30-5 plants were the stable third generation (T3) of the mutants isolated from the same rice plant, which was transformed by the insertion of a maize Ds transposon element as described by Wang et al. (2000). However, genetic and molecular analysis showed that these yellow mutants were not really obtained through the Ds insertion (Wang et al. 2000); the mutation might be induced, e.g., by tissue culture after transformation.

Each genotype was planted in three pots with total 120 seedlings at the early growing stage and before anthesis 10 plants remained in each pot. Outdoor photosynthetic photon flux density (PPFD) at noon was ca. 800-1200 μmol m⁻² s⁻¹, temperature and relative humidity were ca. 30-35 °C and 50-75 %, respectively, during the growing period. Young fully expanded leaves of Zhonghua No. 11 (WT) and two mutants from the top of plants were sampled at 12 or 60 d after transplanting (DAT).

Pigment determination: The Chls content and Chl a/b ratio were measured following the method of Arnon (1949). The extraction and quantification of violaxanthin (V), antheraxanthin (A), zeaxanthin (Z), neoxanthin (N), lutein (L), β-carotene (β-Car), and Chl was made by HPLC (Waters, USA) according to Gilmore and Yamamoto (1991). Total carotenoid (Car) content was expressed on the basis of fresh mass but their compositions were expressed on a Chl basis. The de-epoxidation state (DES) was calculated as 0.5 (A+Z)/(V+A+Z).

7-d-old etiolated rice seedlings were greened in growth chambers at 28 °C and a PPFD of 30 μmol m⁻² s⁻¹ for 4 d to determine the formation kinetics of Chl. The degradation of Chl was conducted by immersing mature leaf segments from 50 DAT plants into distilled water in the dark, at 28 °C.

Absorption spectrum: Leaf pigments were extracted either by 80 % acetone or ethyl ether and scanned with a dual-wavelength spectrophotometer (Lambda 25, Perkin-Elmer Instruments, USA).

Chl fluorescence and photosynthetic oxygen evolution: Chl fluorescence was measured with a pulse amplitude modulation fluorometer (PAM 101, Walz, Effeltrich, Germany) in 30-min dark-adapted leaves as described by Lin et al. (1998). The quantum yield of PS2 dependent non-cyclic electron transport (ΦPS2) was calculated using the equation (Fm'-Fv'/Fm') (Genty et al. 1989). Total electron transport rate (Jl) was determined following the description of Krall and Edwards (1992). The proportion of absorbed photon partitioning to photochemical reaction (Fv'/Fm') and thermal dissipation (Dme) were assessed according to Demmig-Adams and Adams (1996) and Demmig-Adams et al. (1996).

Photosynthetic oxygen evolution was measured using a leaf disc oxygen electrode (LeafLab2, Hansatech, UK) at 25 °C and under various irradiances. The apparent quantum yield of photosynthesis was determined as the slope of Pn-irradiance curve at low irradiance.

SDS-PAGE of protein and determination the content of RuBPCO subunits: Leaf proteins were extracted by homogenisation of leaves in 50 mM Tris-HCl buffer (pH 7.6) containing 20 mM MgCl₂, 1.0 mM EDTA, 10 mM phenylmethylsulfonyl fluoride, and 10 mM mercaptoethanol. The homogenate was centrifuged at 39 000 × g for 15 min. 100 mm³ of sample buffer (10 mM Tris-HCl, pH 7.6, containing 2 % mercaptoethanol, 24 % glycerol, 0.02 % bromophenol blue) was added to the same volume of supernatant and the mixture was boiled for 3 min. Electrophoresis was performed with a 12 μg protein sample according to Peng and Peng (2000) by using a Bio-Rad mini protein II electrophoretic cell. 6.2-25.0 μg of pure RuBPCO (Sigma) were run at the same conditions. The stained bands of pure RuBPCO and of its large and small subunits from the three rice strains were cut separately and eluted with formamide. The absorbance of eluates was then measured at 595 nm. Total contents of RuBPCO and its two subunits were quantified in samples using the standard curve of pure RuBPCO. Soluble protein was determined by the method of Bradford (1976).

The values in tables and figures represent means ± SD of three replications except the HPLC analysis of carotenoids which is in two replications.
**Results**

**Chl absorption spectra and photosynthetic pigment contents:** Chl absorption spectrum in 80% acetone extract of WT rice showed three peaks at 615, 645, and 663 nm in the red band region, and three peaks at 411, 432, and 453 nm in the blue band region. The maximal absorption occurred at 423 and 663 nm. In the rice mutants VG28-1 and VG30-5, the absorption peak height for the whole spectrum was significantly reduced and the peak site at 453 nm and a site of trough at 630 nm were also altered. In comparison with WT, the 453 nm peak was red-shifted to 475 nm and the trough at 630 nm was shifted to 638 nm in the mutants (Fig. 1A). A similar pattern of Chl absorption spectrum in ethyl ether extract was also observed in the two mutants (Fig. 1B), but in acetone extract the range of red shift from 461 to 473 nm (12 nm) was less than that from 453 to 475 nm (22 nm). The spectra of both mutants lacked the typical Chl b absorption peaks at 453 and 645 nm, which might be due to the deficiency of Chl b.

To confirm that the mutants completely lost the ability of Chl b synthesis and to examine the possible difference in Chl accumulation and degradation kinetics between WT and mutants, the experiment was carried out with etiolated seedlings and mature green leaves. As shown in Fig. 2A, no Chl b was found in two mutants during the greening of etiolated seedlings, and the accumulation rate of Chl a was higher in WT than in mutants. Upon the degradation of Chl in green leaves in the dark (Fig. 2B), Chl a degradation rate of WT was significantly more rapid than the degradation rate of its Chl b and of Chl a in both mutants. Hence the mutation led to a breakdown of synthesis of Chl b and partially also of Chl a.

![Fig. 1](image-url)  
**Fig. 1.** Absorption spectra of leaf acetone (A) and ethyl ether (B) extracts of wild type (WT) and chlorophyll b-less mutants VG28-1 and VG30-5 of rice. Leaves were sampled on 20-d-old plants.

![Fig. 2](image-url)  
**Fig. 2.** Time courses of chlorophyll a (solid symbols) and b (open symbols) accumulation during the greening of etiolated rice seedlings (A) and degradation in detached mature leaves (B) of wild type rice (WT – ●, ○) and mutants VG 28-1 (●) and VG 30-5 (▲). Etiolated seedlings were 7-d-old, mature leaf was collected from plants after 50 d of transplanting. The respective equations and $r^2$ values are in the table (right).

The yellow leaves of both mutants had markedly low Chl (a+b) content (per unit leaf fresh mass) (Table 1). In outdoor conditions, total Chl contents were only 49-59% of the WT level in 12-d-old plants and 35-39% in 60-d-old plants. Under low PPFD, Chl contents in two mutants were 57-58% lower than in WT. No detectable Chl b was found in both mutants by spectrophotometric measurement or HPLC analysis (values not shown), which indicates they are Chl b-less mutants. The lack of Chl b in the two mutants was accompanied by partial loss (20-53%) of Chl a. At seedling stage (12-d-old plants), Car content per leaf fresh mass in VG30-5 was similar to that in WT, and in the VG28-1 mutant it was higher than in WT. But at anthesis (60-d-old plants), Car content in WT increased and that in mutants was not changed much. In consequence, the ratios of Car/Chl differed markedly between WT and the mutants: a higher Car/Chl ratio was observed in the mutants grown either outdoors or at low irradiance.

**Photosynthesis and photochemical features of PS2:** Photosynthetic oxygen evolution rate under saturated PPFD (1 600 μmol m$^{-2}$ s$^{-1}$) in Chl b-less rice was lower than in WT. $P_{n}$ in VG28-1 was 8-11% lower compared to WT and that in VG30-5 was 16-18% lower (Fig. 3). The mutants without Chl b did not markedly change the apparent quantum yield, AQY (Fig. 3) or maximum photochemical efficiency ($F_v/F_m$) and non-photochemical quenching ($q_P$) (Table 2), indicating that they could maintain similar efficiencies of PS2 photochemistry and gas exchange as WT. However, in both mutants a significant reduction was observed: by 62-69% ($J_e$), 62-89%
(ΦPS2), and 66% (qa, only in VG30-5) of the corresponding level in WT. Hence the absence of Chl b in chloroplasts of mutants resulted in less photosynthetic electrons produced by limited capacity to capture photon energy, lower efficiency of electron flow through PS2, and fewer open functional PS2 centres (Table 2). The data on fractions of total absorbed photons going into photochemistry (Pmea) and heat dissipation (Dmea) (Table 2) also show differences in Pmea and Dmea between WT and mutants. Chl b-less mutants displayed decreased Pmea and increased Dmea in comparison with WT, implying that the allocation of absorbed photon energy was modulated in mutants.

Table 1. Chlorophyll (Chl) and carotenoid (Car) contents in leaves of wild type (WT) rice (cv. Zhonghua 11) and two mutants (VG28-1 and VG30-5). Outdoor irradiance at noon in September and October, 2001 was 1000-1 200 µmol m−2 s−1. Different little and capital letters between phenotypes indicate statistically significant differences at p<0.05 and p<0.01, respectively.

<table>
<thead>
<tr>
<th>Irradiance</th>
<th>Plant age</th>
<th>Phenotype</th>
<th>Chl (a+b) [g kg−1(f.m.)]</th>
<th>Chl b [g kg−1(f.m.)]</th>
<th>Total Car [g kg−1(f.m.)]</th>
<th>Car/Chl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outdoor</td>
<td>12</td>
<td>WT</td>
<td>1.38±0.14</td>
<td>0.38±0.09</td>
<td>0.24±0.00b</td>
<td>0.174</td>
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<tr>
<td></td>
<td></td>
<td>VG28-1</td>
<td>0.82±0.19</td>
<td>0</td>
<td>0.28±0.02a</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VG30-5</td>
<td>0.68±0.08</td>
<td>0</td>
<td>0.24±0.03b</td>
<td>0.353</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>WT</td>
<td>1.69±0.15</td>
<td>0.43±0.08</td>
<td>0.48±0.04a</td>
<td>0.284</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VG28-1</td>
<td>0.66±0.04</td>
<td>0</td>
<td>0.22±0.03b</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VG30-5</td>
<td>0.60±0.01</td>
<td>0</td>
<td>0.23±0.01b</td>
<td>0.383</td>
</tr>
<tr>
<td>Low irradiance</td>
<td>23</td>
<td>WT</td>
<td>3.36±0.11a</td>
<td>0.95±0.10</td>
<td>0.49±0.01a</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VG28-1</td>
<td>1.47±0.06b</td>
<td>0</td>
<td>0.40±0.02b</td>
<td>0.272</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VG30-5</td>
<td>1.42±0.08b</td>
<td>0</td>
<td>0.37±0.03b</td>
<td>0.261</td>
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</tbody>
</table>

Table 2. Photosynthetic photochemical characteristics of photosystem 2 in 60-d-old rice plants of wild type (WT) and mutants (VG28-1 and VG30-5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>VG28-1</th>
<th>VG30-5</th>
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<tbody>
<tr>
<td>Fv/Fm</td>
<td>0.75±0.06a</td>
<td>0.81±0.02a</td>
<td>0.79±0.00a</td>
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<tr>
<td>qa</td>
<td>0.71±0.07a</td>
<td>0.47±0.00a</td>
<td>0.47±0.10b</td>
</tr>
<tr>
<td>qN</td>
<td>0.71±0.12a</td>
<td>0.70±0.03a</td>
<td>0.73±0.04a</td>
</tr>
<tr>
<td>ΦPS2</td>
<td>0.29±0.00a</td>
<td>0.26±0.06a</td>
<td>0.18±0.00a</td>
</tr>
<tr>
<td>J0 [µmol(e−) m−2 s−1]</td>
<td>58.00±0.00a</td>
<td>40.00±0.20a</td>
<td>36.00±0.00a</td>
</tr>
<tr>
<td>Φmea [%]</td>
<td>35.80±1.50a</td>
<td>28.50±1.71b</td>
<td>26.50±1.65b</td>
</tr>
<tr>
<td>Dmea [%]</td>
<td>64.20±2.49b</td>
<td>71.50±1.01a</td>
<td>73.50±0.78a</td>
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</tbody>
</table>


<table>
<thead>
<tr>
<th>Age</th>
<th>Phenotype</th>
<th>Xanthophyll cycle</th>
<th>Non-xanthophyll cycle</th>
<th>DES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>A</td>
<td>Z</td>
</tr>
<tr>
<td>12 d</td>
<td>WT</td>
<td>64.47±(0.78)</td>
<td>6.49±(0.11)</td>
<td>18.99±(2.82)</td>
</tr>
<tr>
<td></td>
<td>VG28-1</td>
<td>94.54±(1.48)</td>
<td>19.28±(0.23)</td>
<td>33.42±(1.75)</td>
</tr>
<tr>
<td></td>
<td>VG30-5</td>
<td>114.35±(0.25)</td>
<td>25.98±(0.19)</td>
<td>51.41±(6.27)</td>
</tr>
<tr>
<td>60 d</td>
<td>WT</td>
<td>65.52±(1.04)</td>
<td>5.86±(0.25)</td>
<td>20.28±(1.43)</td>
</tr>
<tr>
<td></td>
<td>VG28-1</td>
<td>63.91±(0.50)</td>
<td>18.44±(0.12)</td>
<td>23.65±(0.82)</td>
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<tr>
<td></td>
<td>VG30-5</td>
<td>54.30±(0.04)</td>
<td>18.43±(0.10)</td>
<td>32.49±(0.33)</td>
</tr>
</tbody>
</table>

Car composition and de-epoxidation state: Car composition in leaves of the three genotypes was examined in early morning (08:30 local time). On Chl basis, lutein and the total pool of xanthophyll cycle pigments (V+A+Z) were the predominant carotenoids, accounting for about 48-52 % (lutein) and 26-35 % (V+A+Z), respectively, of the total Cars in WT, but for about 36-47 % (L) and 28-52 % (V+A+Z) of total Cars in the two mutants. V+A+Z pool was larger in mutants than in WT (Table 3). β-carotene was a minor component of the total Cars at seedling
stage (12-d-old), then it became one of the major components at initial anthesis stage (60-d-old). In the two mutants, the content of β-carotene was 29% greater and about 2.0-2.1 folds that in WT at the two developing stages, respectively.

Mutation resulted in considerable changes in amount and fraction of the xanthophyll cycle pigments, which was in mutants at two sampling stages 1.88 and 1.15 fold that in WT. The increment of xanthophyll pool size, especially the amount of Z, was associated with a high de-epoxidation level in the mutants (Table 3). Even in early morning, part of the violaxanthin pool was present as zeaxanthin (Z). The de-epoxidation state (DES) was ca. 0.24-0.26 in WT, 0.29-0.31 in VG 28-1, and 0.33-0.39 in VG 30-5. Compared to WT, the greater DES content in two mutants demonstrated their sensitivity to irradiance.

**Rubisco and soluble proteins**: To see whether the amount of soluble proteins and of the key carboxylation enzyme, Rubisco, varied in the mutants, we analysed by

![Graph](image)

**Fig. 3.** Comparison of net photosynthetic rate, $P_N$ for 44 (●) and 80 (○) d-old plants and apparent quantum yield, $A_{QY}$ for 80-d-old plants (white columns) of WT rice and mutants VG 28-1 and VG 30-5.

![Graph](image)

**Fig. 4.** SDS-PAGE profiles of leaf protein and Rubisco subunits. Lane 1, molecular mass standards; lane 2, WT; lane 3, mutant VG28-1; lane 4, mutant VG30-5; lanes 5, pure Rubisco large (RLS) and small (RSS) subunits. Leaf samples were collected from 60 d-old plants. Each line shows loading with 12 µg protein sample.

**Discussion**

Rice mutants deficient to different extent in Chl b were divided into two groups according to their Chl a/b ratios. Type I includes those with no Chl b or with Chl a/b ratio close to or more than 20; they are called Chl b-less mutants. Type II, with a Chl a/b ratio of 6-10, is called Chl b-deficient mutants (Terao et al. 1985, Falbel et al. 1996). Two new rice mutants examined in the present paper were confirmed to be a Chl b-less (lacking) type I mutants (Fig. 1, Table 1). Chl b-less mutants of barley (*chlorina f2*) and *Arabidopsis* (*chl*) have almost normal amounts of Chl a (Murray and Kohorn 1991, Falbel and Staehelein 1996). The accumulation of Chl b and not Chl a was affected in these mutants (Falbel et al. 1996). However, as the mutants reported here synthesise less Chl a and no Chl b, it implies that both the synthesis of Chl a and Chl b were affected by the mutation.

In comparison with the WT rice, the mutants VG28-1 and VG30-5 exhibited pronounced alteration in the photosynthetic pigment contents and composition (Table 1). Chl absorption spectra (Fig. 1), xanthophyll cycle pool and composition (Table 3), $J_{ph}$, $Q_{ph}$, $q_{ph}$, and fraction of $P_{max}$ and $D_{max}$ (Table 2), as well as the soluble protein and Rubisco contents (Fig. 5), $P_N$ was 8-18% lower than in WT, but no significant difference of $F_v/F_m$ or $A_{QY}$ of gas
exchange between mutants and WT was observed (Fig. 3, Table 2). Leverenz et al. (1992) reported that the $P_{sec}$ of Chl b-less barley mutant was 10% lower than that of its WT, and the decrease was matched with approximately 10% less quantum yield and $F_v/F_m$. The decrease of $P_N$ in our two Chl b-less rice mutants was similar to that found in Chl b-less barley, but this was not consistent with the extent of changes observed either in AQY, $F_v/F_m$, $F_{PS2}$ (which characterise the efficiencies of photochemical energy transformation and electron transport), or the fraction of $P_{sec}$. This fact may imply that the lower $P_N$ in rice mutants depends mainly on the reduction in total photosynthetic electron flow rate, the number of open PS2 centres, and lesser $P_{sec}$ rather than the limit of APQ and $F_v/F_m$.

The lack of Chl b can affect the abundance of certain proteins and pigment-protein complexes (Falbel et al. 1996). The analysis of thylakoid membrane proteins and light-harvesting pigment-protein complexes of three rice genotypes showed that mutants lost LHC2, reduced LHC1, but increased the amount of some core polypeptides of PS1 and PS2 (values not shown). Especially the contents of soluble proteins and RuBPCO protein were markedly greater in rice mutants than in WT, and the higher RuBPCO content was mainly due to a larger amount of RSS (Fig. 5). The reason for that is unclear and should be studied further. Anyway, more RuBPCO protein in mutants probably favours the CO₂ fixation by increasing RuBPCO activity, and might serve as a compensation for the distinct reduction in PS2 activity during photosynthesis, which could explain the absence of the notable decrease in $P_N$.

Little attention has been paid to the changes in Car contents and size of pool of xanthophyll cycle pigments and its individual components in Chl b-deficient rice mutants. We found that the absolute Car contents of the two rice mutants expressed per fresh mass were similar or lower than in WT rice, while the contrary was true when expressing the Car contents per Chl. High Car/Chl ratio in the Chl b-less mutants caused the appearance of yellowish leaves and could enhance the possible photo-protection capacity of Cars to Chl under high radiation stress. A greater pool of xanthophyll cycle pigments and higher de-epoxidation state observed during early morning demonstrated that the mechanism of excessive energy dissipation was fairly well developed and operating in these Chl b-less mutants. Leverenz et al. (1992) pointed out that before and after exposure of barley Chl b-less mutant chlorina $f_2$ to a high PPFD of 530-640 μmol m$^{-2}$ s$^{-1}$, the total pool of xanthophyll cycle pigments was 79 and 77%, respectively, of that in WT barley and that violaxanthin in barley mutants was converted to zeaxanthin even at a very low irradiance. A larger xanthophyll cycle pool on Chl basis in our Chl b-less rice mutants differed from that in Chl b-less barley. The large size of pool of xanthophyll cycle pigments may reflect an increased net synthesis of these pigments (Adams and Demmig-Adams 1992). The higher content of β-carotene, the precursor of xanthophyll cycle (Niyogi et al. 1997) in the examined rice mutants (Table 3) might support the main cause for accumulation of a large pool of xanthophyll cycle pigments.

Chl b is formed through oxidation of Chl a and the mechanism of Chl b synthesis will be an active research area in the future (Eggink et al. 2001). At present, Chl b-less mutants have been found only in some plant species such as barley chlorina $f_2$, wheat Driscoll mutant. Arabidopsis thaliana chl. rice chlorina $f_1$-10, and sweet-clover U 395 ch5/ch8 (Terao and Yamashita 1982. Markwell et al. 1986, Falbel and Stachelin 1994, 1996). Our two new Chl b-less rice mutants fail to synthesise Chl b, have reduced content of Chl a, but enhanced contents of total leaf proteins and RuBPCO: these features are different from those of other Chl b-less plants. Therefore, these new rice mutants may provide an interesting material for the study of the relationship between biosynthesis of Chl a and Chl b or between the biosynthesis of Chl, Cars, and proteins, and the regulation of photochemical reactions and dark reactions of photosynthesis, as well as the assembly and function of the thylakoid membrane.

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PHOTOSYNTHETIC CHARACTERISTICS OF CHLOROPHYLL b-LESS RICE MUTANTS