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Photoinhibition characteristics of a low chlorophyll *b* mutant of high yield rice

DAI Xinbin[*,***], XU Xiaoming[*], LU Wei[*], and KUANG Tingyun[**]

Laboratory of Photosynthesis, Nanjing Agricultural University, Nanjing 210095 China[*]
Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China[**]

Abstract

The low chlorophyll *b* mutant of high yield rice had a lower light-harvesting complex 2 content than the wild type. The stability of oxygen evolution side of photosystem 2 was only slightly lower. A lower photon absorption rate and a stronger xanthophyll cycle capacity of this mutant led to a higher endurance to strong irradiance and a lower photoinhibition as compared with the wild type rice.

Additional key words: chlorophyll fluorescence; irradiance; *Oryza sativa*; photoinhibition; photosystem 2; pigment-bound proteins; quenching; xanthophyll cycle.

Introduction

Chlorophyll (Chl) *b* mutants have been studied widely, especially those of barley and tobacco. According to Enami *et al.* (1994), Shutilova *et al.* (1995), and Havaux and Tardy (1997) the Chl *b* deficient mutants have a reduced light-harvesting efficiency and decreased endurance to strong irradiance. An opposite result was found for tobacco *aurea* mutant (Schindler and Lichtenthaler 1994). A low Chl *b* mutant of high yield rice newly found in field is described in this paper; it is obviously different from the above-mentioned barley mutant in photosynthesis and photoinhibition characteristics.

Materials and methods

Plants: Two rice (*Oryza sativa* L.) genotypes, the wild type (cv. Zhenhui 249) and its low Chl *b* mutant (provided by Zhenjiang Agricultural Institution, Jiangsu, China) were grown in natural environment. Photon flux density was about 1 500 µmol m^{-2} s^{-1} at noon. Measurements were taken from the 10th young, full-expanded leaf (usually at the age of 7-8 weeks after germination).

Green gel and SDS-PAGE electrophoresis: Chloroplasts and thylakoids were isolated according to Berthold *et al.* (1981). Green gel analysis was performed as described by Allen and Staehelin (1991). Some improvements were made as follows: column gel with diameter of 20 mm was used to get higher sensitivity, thylakoid sample containing 100 µg Chl was loaded into each column. The electric current was adjusted to 6 mA per column. The Chl-binding polypeptides were analysed by SDS-PAGE with 15 %-acrylamide for separating and 4 %-acrylamide for stacking, with 5.5 M urea included in the gel.

Analysis of PS2 oxygen evolution side: The kinetics of Chl-fluorescence relaxation was measured in DCMU-poisoned leaves (50 µM) irradiated with 1 s pulse of red radiation (50 µmol m^{-2} s^{-1}). Q_A was reduced in these leaves with the pulse of red radiation and re-oxidised by recombination with positive charges at the PS2 donor side. Half-life time of Chl-fluorescence decay ($T_{1/2}$) was used to express the Q_A re-oxidation rate. Longer $T_{1/2}$ means more poor PS2 donor side. The leaves need enough pre-treatment of darkness for at least 2 h before DCMU treatment and measurement. A Chl-fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany) was used to analyse and record fluorescence by running the *Program 6*

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[***] Author for correspondence; fax:86-025-4593760, e-mail: xbdai@mail.njau.edu.cn

Abbreviations: A, antheraxanthin; Chl, chlorophyll; F_0, initial level of chlorophyll fluorescence; F_m, maximal level of chlorophyll fluorescence; F_v/F_m, photochemical efficiency of photosystem 2; LHC, light-harvesting complex; PS, photosystem; q_N, non-photochemical quenching; q_P, photochemical quenching; Q_A and Q_B, electron acceptor of PS2 (quinone); V, violaxanthin; Z, zeaxanthin.

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(Chu *et al.* 1994, Havaux and Tardy 1997).

Pigments were extracted from leaves with 80 % acetone, and analysed by HPLC (*America Waters Corporation*). Chromatogram column was C_{18} (5 µm, 210.0×4.6 mm), linear gradient isolation. For details of the method see Thayer and Björkman (1990).

Measurement of F_v/F_m, q_P, and q_N: After the leaf was pre-treated with darkness for 2 h, a dim red radiation (655 nm) modulated at 600 Hz was shined on the leaf surface to elicit F_0, then 1 s saturating pulse (9 000 µmol m^{-2} s^{-1}) was used to obtain F_m. Afterwards, different actinic irradiances were continuously shined onto the leaf surface, along with saturating radiation pulse in 30 s intervals. q_P and q_N were recorded after F_m was kept stable.

Oxygen evolution rate: A Clark-type O_2 electrode was used (Coombs *et al.* 1985).

Results

Photosynthetic characteristics of the low Chl *b* mutant: The mutant was found in rice fields. The classical genetic analysis showed that a single recessive gene controlled the mutation (Dai *et al.* 2000). As the result of back-cross during 8 generations, the mutant and wild type have a similar genetic background except for the mutation.

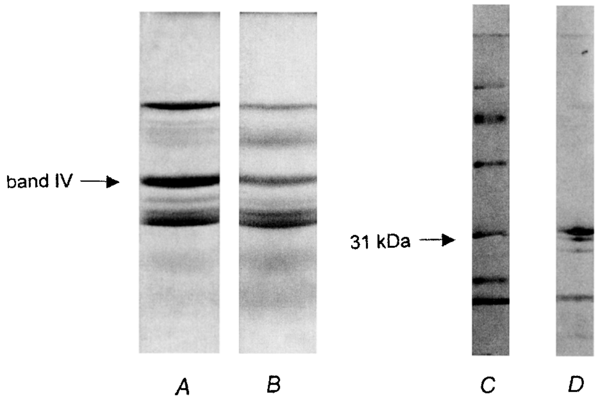

Fig. 1. Green gel analysis of pigment-bound proteins in thylakoids (*A* – wild type rice, *B* – mutant rice) and SDS-PAGE analysis of band IV (*C* – molecular mass standard, *D* – band IV).

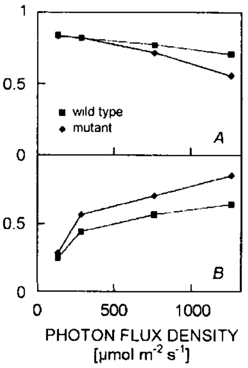

Fig. 2. Changes of q_P and q_N under different irradiances.

The pigment-bound proteins of mutant and wild type were analysed by green gel electrophoresis. The main variation happened at band IV (Fig. 1*A*, *B*). The content of band IV in mutant was 36 % less than that in wild type and its molecular mass was about 28-31 kDa (Fig. 1*D*). Low-temperature Chl fluorescence emission peaks of band IV excited by 480 nm were higher than those excited by 436 nm (the result of low-temperature Chl fluorescence emission peaks of other bands was opposite). So band IV was the only one which contained Chl *b* in the bands of the green gel. The above results showed that the mutation affected the LHC2.

Pigments of the mutant and wild type rice were analysed by HPLC, their total Chl contents were 1.08±0.04 and 2.91±0.07 g(Chl) kg^{-1}(FM) and the Chl *a*/*b* ratios 4.7 and 3.0, respectively. The results are similar to those previously obtained by the spectrophotometric method (Dai *et al.* 2000).

Chl fluorescence measurement is a good method for detecting photosynthetic mutants (Varotto *et al.* 2000). Chu *et al.* (1994) and Havaux and Tardy (1997) thought that the half-life time of Chl-fluorescence decay in DCMU-poisoned leaf represents the situation of PS2 oxygen evolution side. The times for the mutant and wild type were 0.434±0.078 and 0.361±0.075 s, respectively. Thus the activity of oxygen evolution side of the mutant was slightly lower than that of the wild type. Hence the mutation only slightly affected the PS2 oxygen evolution side.

Measurements with a Clark O_2 electrode showed that the quantum yield of oxygen evolution of the mutant was 33 % higher than that of the wild type.

q_P and q_N were observed under different actinic irradiances (100 to 2 000 µmol m^{-2} s^{-1}) (Fig. 2). Under weak irradiance there was no significant difference between mutant and wild type, but upon increasing the photon flux density, q_P of the wild type was gradually lower than that of the mutant. q_N showed the opposite, especially when the actinic photon flux density reached 1 200 µmol m^{-2} s^{-1}.

Variation of photosynthesis parameters of low Chl *b* mutant under strong irradiance: F_v/F_m (Fig. 3*A*) measured at strong irradiance of 2 000 µmol m^{-2} s^{-1} almost synchronously declined along with prolonging of the treatment time from 0 to 2 h. When the treatment time exceeded 2 h, the F_v/F_m of the wild type declined more rapidly than that of the mutant. The above results show that the PS2 complex and light-harvesting antennae of the mutant were less damaged by strong irradiance than those of the wild type.

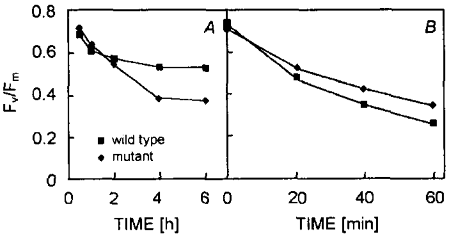

Fig. 3. Changes of F_v/F_m under strong irradiance (2 000 μmol m^{-2} s^{-1}) in mutant and wild type rice untreated (*A*) and treated with DCMU (*B*).

The half-life time of the Chl fluorescence decay was measured as the characteristic of PS2 oxygen evolution side (Table 1). After the leaves were treated by irradiance of 2 000 μmol m^{-2} s^{-1} for 6 h, the half-life times for the wild type and the mutant were prolonged. Because that of the wild type increased more than that of the mutant, the oxygen evolution side in the wild type was inhibited more seriously than that in the mutant.

The method of Aro *et al.* (1993) was used to study photoinhibition of the PS2 reducing side. Leaves were treated with DCMU in advance and then exposed to 2 000 μmol m^{-2} s^{-1}. The F_v/F_m under strong irradiance (Fig. 3*B*) declined more rapidly in the wild type than in the mutant.

Table 1. The half-life time of chlorophyll fluorescence relaxation in DCMU-poisoned leaves.

	Mutant half-life time [s]	Wild type half-life time [s]
Control (enough darkness adaptation)	0.434 ± 0.078 (100 %)	0.361 ± 0.075 (100 %)
Treatment (2 000 μmol m^{-2} s^{-1}, 6 h)	0.538 ± 0.084 (124 %)	0.523 ± 0.064 (145 %)

Changes of xanthophyll pool and xanthophyll cycle of low Chl *b* mutant under strong irradiance: HPLC analysis showed that the xanthophyll/Chl ratio of the mutant was significantly higher than that of wild type, 0.36±0.04 and 0.21±0.06, respectively.

The de-epoxidation state of xanthophyll cycle was determined as the ratio of (Z + 0.5A)/(V + A + Z). Changes of the de-epoxidation state of wild type and mutant under strong irradiance (3 000 μmol m^{-2} s^{-1}) are shown in Fig. 4: before treating with strong irradiance, both ratios were the same. After treating with strong irradiance for 10 min, both ratios increased, but that for the mutant increased more rapidly, and remained stable after treating for further 20 min. The above analysis indicates that the mutant apparently dissipated excess energy with the operation of the xanthophyll cycle more quickly than the wild type.

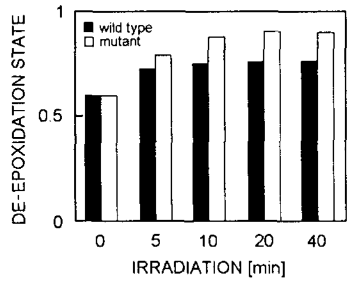

Fig. 4. Change of de-epoxidation state [(Z + 0.5 A)/(V + A + Z)] during treatment of the mutant and wild-type rice leaves with strong irradiance (3 000 μmol m^{-2} s^{-1}).

Discussion

Many previous reports found that the plants grown under high and low irradiance differed similarly as the mutant and wild type rice. High-irradiance chloroplasts had higher Chl *a/b* and lower LHC2 than low-irradiance chloroplasts (Lichtenthaler *et al.* 1981, 1982, Meier and Lichtenthaler 1981, Lichtenthaler and Burkart 1999). Similar differences were found in pale green barley (Tardy *et al.* 1998) and aurea tobacco mutant (Šantrůček *et al.* 1992, Schindler *et al.* 1995) and their wild types. In all these reports, higher Chl *a/b*, lower content of LHC2, and more quick accumulation of zeaxanthin led to high endurance to high-irradiance stress.

Havaux and Tardy (1997) found that Chl *b* deficient mutation reduced LHC2 content in thylakoids, which was very important for the stability of whole PS2 (Shutilova *et al.* 1995). So in Chl *b* deficient mutant the PS2 oxygen evolution side is unstable and easily photo-damaged under strong irradiance (Enami *et al.* 1994). The above point of view has already been approved (Havaux and Tardy 1997).

We studied a low Chl *b* mutant of high yield rice. Its PS2 oxygen evolution side was slightly affected by mutation. After treating its leaf with 2 000 μmol m^{-2} s^{-1} for a long time, the F_v/F_m ratio was reduced less than that of the wild type. Hence the mutation is in favour of leaf endurance to strong irradiance. The stability of PS2 oxygen evolution side was reduced by the mutation less than that of the wild type rice.

A higher endurance of the mutant to high irradiance might be partly due to the fact that its Chl content was only partly reduced. Thus the stability of the whole PS2 was apparently less affected. At the same time, the lower photon absorption rate of the mutant led to a greater endurance of strong irradiance with less non-radiation energy dissipation required. Higher q_P and lower q_N of the mutant under a higher requirement of 1 200 μmol m^{-2} s^{-1} also proved this point of view.

Besides, another probably reason is that both the xanthophyll cycle pool of the mutant and its conversation efficiency were larger than those of the wild type under high irradiance of 3 000 μmol m^{-2} s^{-1}. Schindler and Lichtenthaler (1994) thought that tobacco Z does not play a major role in a direct energy dissipation of absorbed photons. But for rice leaf, Xu *et al.* (2000) showed that non-radiation energy dissipation mainly depends on the xanthophyll cycle that is an important factor of photoprotection. Therefore a larger xanthophyll cycle rate of the rice mutant may favour photoprotection at high irradiance.

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