Enhancement of susceptivity to photoinhibition and photooxidation in rice chlorophyll *b*-less mutants

Z.-F. LIN[*,***], G.-Z. LIN[*], and C.-L. PENG[**]

South China Botanical Garden, the Chinese Academy of Sciences, Guangzhou 510650, China[*]
College of Life Sciences, South China Normal University, Guangzhou 510631, China[**]

Abstract

Two rice chlorophyll (Chl) *b*-less mutants (VG28-1, VG30-5) and the respective wild type (WT) plant (cv. Zhonghua No. 11) were analyzed for the changes in Chl fluorescence parameters, xanthophyll cycle pool, and its de-epoxidation state under exposure to strong irradiance, SI (1 700 μmol m^{-2} s^{-1}). We also examined alterations in the chloroplast ultrastructure of the mutants induced by methyl viologen (MV) photooxidation. During HI (0−3.5 h), the photoinactivation of photosystem 2 (PS2) appeared earlier and more severely in Chl *b*-less mutants than in the WT. The decreases in maximal photochemical efficiency of PS2 in the dark (F_v/F_m), quantum efficiency of PS2 electron transport (Φ_{PS2}), photochemical quenching (q_P), as well as rate of photochemistry (P_{rate}), and the increases in de-epoxidation state (DES) and rate of thermal dissipation of excitation energy (D_{rate}) were significantly greater in Chl *b*-mutants compared with the WT plant. A relatively larger xanthophyll pool and 78−83 % conversion of violaxanthin into antheraxanthin and zeaxanthin in the mutants after 3.5 h of HI was accompanied with a high ratio of inactive/total PS2 (0.55−0.73) and high $1 - q_P$ (0.57−0.68) which showed that the activities of the xanthophyll cycle were probably insufficient to protect the photosynthetic apparatus against photoinhibition. No apparent difference of chloroplast ultrastructure was found between Chl *b*-less mutants and WT plants grown under low, LI (180 μmol m^{-2} s^{-1}) and high, HI (700 μmol m^{-2} s^{-1}) irradiance. However, swollen chloroplasts and slight dilation of thylakoids occurred in both mutants and the WT grown under LI followed by MV treatment. These typical symptoms of photooxidative damage were aggravated as plants were exposed to HI. Distorted and loose scattered thylakoids were observed in particular in the Chl *b*-less mutants. A greater extent of photoinhibition and photooxidation in these mutants indicated that the susceptibility to HI and oxidative stresses was enhanced in the photosynthetic apparatus without Chl *b* most likely as a consequence of a smaller antenna size.

Additional key words: chlorophyll fluorescence; chloroplast ultrastructure; high and low irradiance; methyl viologen; *Oryza*; xanthophyll cycle pool.

Introduction

Chlorophyll (Chl) deficient mutants are the potentially useful systems to examine the photosynthetic performance of leaves in response to various environmental factors (Habash *et al*. 1994). These mutants are a good model to improve understanding of regulatory mechanisms of Chl *b* biosynthesis and of the roles of Chl *b* in the assembly, structure, and function of thylakoid membrane. Using such mutants, one could also gain more direct insights into the mechanism controlling the development of photosynthetic apparatus (Baroli and Melis 1998). There are two classes of Chl mutants with the reduced amount of Chl *b*: (*a*) complete lack of Chl *b* = Chl *b*-less mutants; (*b*) reduced Chl *b* content = Chl *b*-deficient mutants (Terao *et al*. 1985, Falbel *et al*. 1996). The reduction

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[***]Corresponding author: fax (86) 020-37252831, e-mail: linzhif@scib.ac.cn
Abbreviations: A – antheraxanthin; D_{rate} – rate of thermal dissipation of excitation energy; DES – de-epoxidation state; E_{rate} – rate of excess energy; ETR – electron transport rate; F_v/F_m – maximal photochemical efficiency of PS2 in the dark; LHC – light-harvesting complex; MV – methyl viologen; NPQ – non-photochemical quenching of PS2; P_{rate} – rate of photochemistry; PFD – photon flux density; PS – photosystem; q_P – photochemical quenching of PS2; $1 - q_P$ – excitation pressure on PS2; V – violaxanthin; Z – zeaxanthin; Φ_{PS2} − quantum efficiency of PS2 electron transport.
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of Chl *b* content in mutant plants leads to the change in the components of reaction centre-antenna complex and a smaller antenna size (Bishop *et al*. 1989, Falbel *et al*. 1996). However, the data for the role of antenna size in influencing the sensitivity of Chl *b* mutant to photoinhibition are contradictory. Cleland and Melis (1987) reported that a relatively low absorption of photons per photosystem 2 (PS2) reaction centre in Chl *b*-less mutant helps protect PS2 from photoinhibition as compared with the wild type (WT). Baroli and Melis (1998) showed that large Chl antenna size enhanced the rates of photon absorption and photodamage of PS2. On the other hand, Tyystjärvi *et al*. (1991) concluded that the small light-harvesting antenna did not protect the photosynthetic apparatus from photoinhibition, whereas a negligible effect on operating efficiency of PS2 was found in the Chl *b*-deficient cowpea (Habash *et al*. 1994). On the contrary, the occurrence of more susceptible photoinhibition in Chl *b*-less barley was confirmed at the leaf level by Leverenz *et al*. (1992). These differences might be the consequence of different mutant features among plant species or because of the treatment methods used, but it seems that the effect of PS2 Chl antenna size on the rate of photodamage was not fully manifested (Baroli and Melis 1998). The photoinhibition and thermostability of Chl *b*-less/deficient mutants has been examined (Moharekar *et al*. 2007), but no investigation has been made into the photooxidative response by such mutants. Cells showing signs of oxidative stress are usually found in plants suffering from environmental (abiotic) conditions (adversities). Therefore, a better understanding of the responses to photoinhibition and photooxidation in Chl *b*-less mutants with small antenna size and altered thylakoid membrane structure to photosynthesis would improve understanding of the role of Chl *b*. Recently, the photosynthetic features, Chl-protein complexes' components, distribution of excitation energy between two photosystems, and the thermostability of two new rice Chl *b*-less mutants VG28-1 and VG30-5 have been characterized in our laboratory (Lin *et al*. 2003a,b, 2005). The aim of the present study was to further elucidate the different features of phoinhibition and photooxidation in leaves of both Chl *b*-less mutants and the WT rice based on Chl fluorescence parameters, pigment analysis, and chloroplast ultrastructure observation. The susceptibility of two mutants (without Chl *b* and with partial loss of light-harvesting complexes, LHCs) to strong irradiance and exogenous oxidant was evaluated.

Materials and methods

Plants: Seedlings of rice (*Oryza sativa* L.) WT cv. Zhonghua 11 and two mutants, VG28-1 and VG30-5, which completely lack Chl *b*, were grown in pots outdoors in the campus of South China Botanical Garden in Guangzhou, China. A 20-mm layer of water was kept on the soil surface. Irradiances corresponding to midday levels during seedling cultivation were approximately 700 μmol m^{-2} s^{-1} (HI). Beside, some of the seedlings were grown under low irradiance, LI (180 μmol m^{-2} s^{-1}). At sixth-leaf stage of plants, the youngest fully expanded leaves were chosen for experiments.

Photoinhibition and photooxidation treatments: Leaf segments (40 mm long) were taken from the central part of leaves and were floating with adaxial side up on the distilled water (control) or treating solution in Petri dishes and exposed to a specific irradiance. (Were the leaf segments floating on the surface of water or they were immersed in the water under the surface? This is not clear.) Photoinhibition treatment for leaves grown in HI was carried out under an irradiation of 1 700 μmol m^{-2} s^{-1} (SI) for 0, 1, 2, and 3.5 h. Photooxidation was measured with leaves grown in both HI and LI by vacuum infiltrating the leaf segments into methyl viologen (MV) solution (20 μM), and then exposed to irradiance of 20 μmol m^{-2} s^{-1} and 25 ºC in an incubator for 42 h.

Chl fluorescence measurement: A *PAM*-type pulse amplitude modulated fluorometer (*PAM 101*, *Walz*, Effeltrich, Germany) was used to measure the Chl fluorescence at room temperature. Detached leaf segments were dark adapted for a period of 30 min before fluorescence measurements. The weak modulated irradiance, "actinic light", and saturating pulse were 0.07, 200, and 6 000 μmol m^{-2} s^{-1}, respectively. Fluorescence parameters maximal photochemical efficiency of PS2 in the dark (F_v/F_m), photochemical quenching (q_P), non-photochemical quenching (NPQ), quantum efficiency of PS2 electron transport (Φ_{PS2}), and electron transport rate (ETR) were calculated according to Genty *et al*. (1989) and Maxwell and Johnson (2000). The rate of photochemistry (P_{rate}), rate of thermal dissipation of excitation energy (D_{rate}), rate of excess energy (E_{rate}), and $1 - q_P$ were quantified as described by Demmig-Adams *et al*. (1996). Moreover, the ratio of inactive/total PS2 was appraised using the equation from Hikosaka *et al*. (2004). The equations of absorbed photon energy allocation are: $P_{rate} = \Phi_{PS2} \times$photon flux density (PFD); $D_{rate} = (1 - F_v'/F_m') \times PFD$; $E_{rate} = F_v'/F_m' \times (1 - q_P)$; B (the fraction of inactive PS2) $= 1 - (F_v/F_m)/(F_v/F_{mM})$, where F_v/F_{mM} is F_v/F_m at non-photoinhibited level.

Leaf pigments were extracted with 80 % acetone; the extract was analyzed by high performance liquid chromatography, HPLC (*Waters 2695*, Milford, USA) following the protocol of Gilmore and Yamamoto (1991). Contents of carotenoids were expressed on a Chl basis. The de-epoxidation state (DES) of the xanthophyll cycle

was defined as (Z+0.5A)/(Z+A+V) (Schindler and Lichtenthaler 1996).

Chloroplast ultrastructure: The middle part of leaf was sliced into 2×10 mm strips. Fixation and preparation of ultra-thin sections were performed routinely as described by Lin *et al.* (2005). The ultrastructure of chloroplasts was observed and photographed with a transmission electron microscope (*JEM-1010*, *JEOL*, Tokyo, Japan).

Data analysis: The determination was repeated three to five times, statistical significance was analyzed with a non-paired, two-tailed Student's *t*-test.

Results

Upon exposure to SI, there was a reduction in the F_v/F_m, q_P, Φ_{PS2}, and ETR in leaves (Fig .1). In comparison with the WT plant, the Chl *b*-less mutants exhibited a greater decrease in these parameters. These parameters were reduced by approximately 64 % (F_v/F_m), 22 % (q_P), 42 % (Φ_{PS2}), and 60 % (ETR), respectively, in the two mutants after 3.5-h irradiation, while the reductions in WT were 27, 17, 31, and 31 %, respectively. The differences at each exposure duration to SI between mutants and WT were all significantly different (p<0.05).

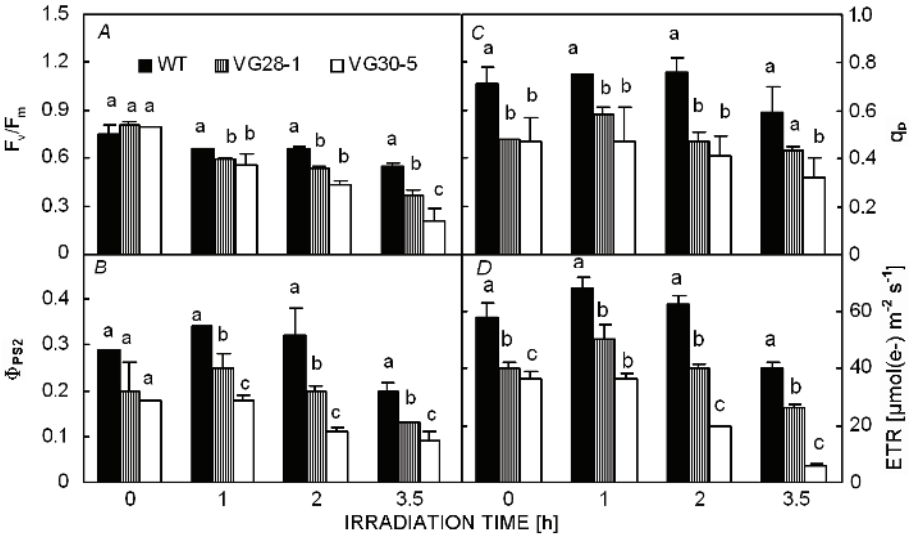

Fig. 1. Changes in (*A*) maximal photochemical efficiency of photosystem 2 in the dark, F_v/F_m, (*B*) quantum efficiency of PS2 electron transport, Φ_{PS2}, (*C*) photochemical quenching, q_P, and (*D*) electron transport rate, ETR (Φ_{PS2} × PFD× 0.5×0.84) during strong irradiance (SI) [1 700 µmol(photon) m^{-2} s^{-1}] exposure on leaves of rice chlorophyll *b*-less mutants and the wild type plant. All plants were grown under 700 µmol(photon) m^{-2} s^{-1}. *Different letters* between columns of same time scale indicate statistically significant differences at p<0.05. Means ±SD. (n=5). (ETR should be defined explicitely, because it is usually only proportional to Φ_{PS2}).

The PS2 excitation pressure ($1 - q_P$) was virtually unchanged after 2-h SI in the WT plant, but then increased to 41 % with an additional 1.5 h exposure (Fig. 2*A*). During a 3.5-h SI, the values of $1 - q_P$ in mutants increased gradually and these differences were significantly higher than in the WT plant. The increase in $1 - q_P$ resulted from the decrease of q_P and led to altered balance of PS2 activity downstream (Hikosaka *et al*. 2004). NPQ which was reflected in the concentration of dissipating complexes (Gilmore *et al*. 1995) was promoted by HI in both mutants and WT. As a result of the HI exposure, NPQ in the WT plant rose gradually, whereas there was a greater increase in NPQ in the mutants exposed to 3.5 h (Fig. 2*B*). As expected, the ratio of inactive/total PS2 increased with increasing exposure time (Fig. 2*C*). These data showed a contrary trend for q_P and F_v/F_m, but a consistent tendency with NPQ and $1 - q_P$. The lower ratio of inactive/total PS2 (0.26) in the WT plant and 1−2 fold increment of that in the mutants after 3.5 h SI exposure evidenced the appearance of severe inactivation of PS2 in the Chl *b*-less mutants caused by SI.

The calculated fractions of P_{rate} dissipated as heat (D_{rata}) and the excess energy neither participating in photochemical reaction nor heat dissipation (E_{rate}) are shown in Fig. 3. With increasing time of SI exposure, P_{rate} decreased and D_{rate} increased in both WT and mutants. The decrease in P_{rate} in Chl-*b* less mutant (44−63 %) was larger than that in WT plants (38 %). In particular, the increase in D_{rate} (22 % for WT and 28 % for mutants) was less than the decrease in P_{rate}. Hence, the E_{rate} patterns in the mutants differed from the WT plant. E_{rate} remained nearly constant in the WT plant with a 3.5 h SI exposure; but it reached its highest value with a 2 h SI exposure and

declined thereafter in the mutants.

Total xanthophyll cycle pool (V+A+Z) and its de-epoxidation state (DES) was altered in photoinhibited leaves (Fig. 4). On a Chl basis, the initial xanthophyll cycle pool was approximately 25 % higher in the mutants than in the WT plant. Within 3.5 h SI exposure, the pool increased by 5 % in the WT plant and by 8−10 % in mutants, whereas DES increased rapidly by 103 % (WT) and 133 % (mutants) of their original values. The rate of DES increase by 3.5 h irradiation was slower in the WT plant (0.0707 h^{-1}) and faster in two mutants (0.1295 or 0.1457 h^{-1}), suggesting a more evident activity of xanthophyll cycle induced by SI in the latter. The correlations among Chl fluorescence parameters for both WT and mutants during the 3.5 h photoinhibition process are provided in Table 1. P_{rate} was positively correlated to ETR and F_v/F_m, but negatively correlated to $1 - q_P$ and the fraction of PS/total PS2 in the photoinhibited rice leaves. These patterns suggest that the fraction of absorbed photon energy partitioning to photochemistry was restricted by primary photochemical efficiency, electron transport rate, excita-tion pressure, and the inactive PS2 ratio of total PS2.

In another experiment performed to understand the

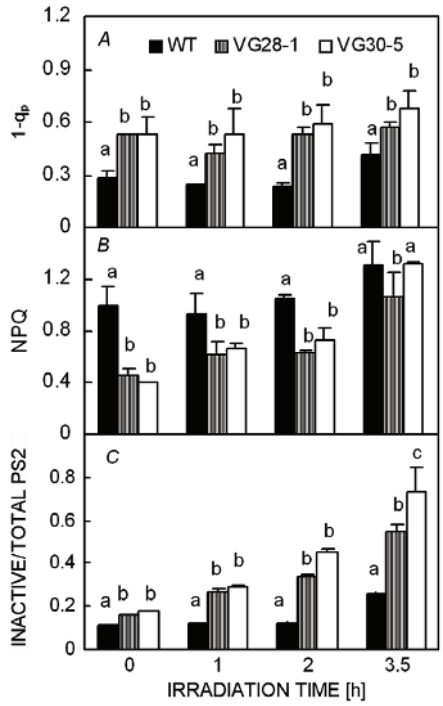

Fig. 2. Excitation pressure, $1 - q_P$ (*A*), non-photochemical quenching, NPQ (*B*), and inactive/total PS2 (*C*) for the wild type (WT) and mutants of rice in response to strong irradiance (SI). Means ± SD. (*n*=5).

Table 1. The correlation coefficients between chlorophyll fluorescence parameters during photoinhibition treatment of rice leaves. Mean (*n* = 9).

Parameter	ETR	P_{rate}	$1 - q_P$	Inactive/total PS2
F_v/F_m	0.9402	0.9248	−0.8931	−0.9936
P_{rate}	0.9823		−0.9520	−0.9340
ETR			−0.9531	−0.9555

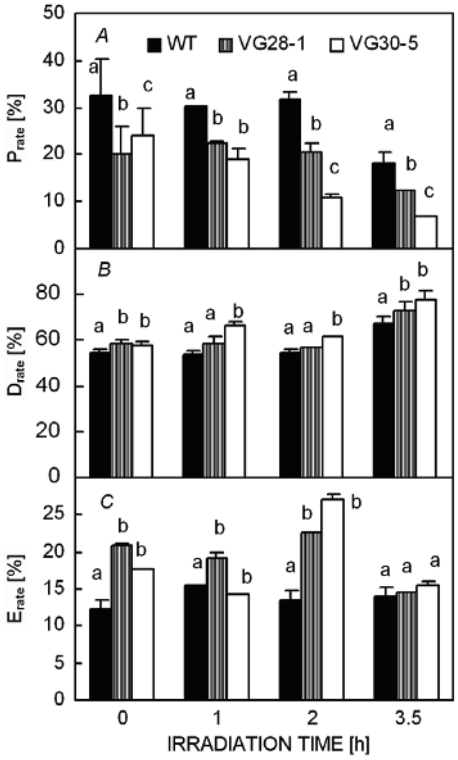

Fig. 3. Rates of photochemistry, P_{rate} (*A*), thermal dissipation of excitation energy, D_{rate} (*B*), and excessive energy, E_{rate} (*C*) for the wild type (WT) and mutants of rice in response to strong irradiance (SI). Means ± SD. (*n*=3).

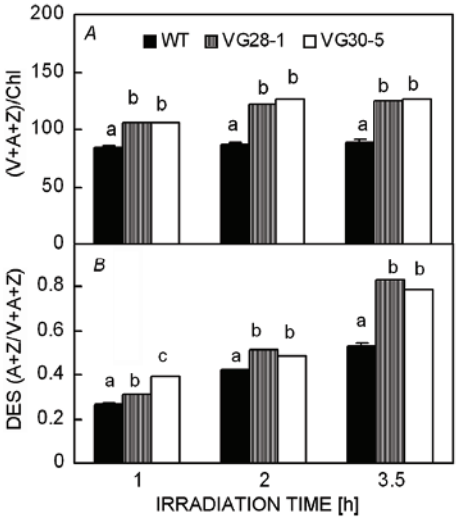

Fig. 4. Total xanthophyll cycle pool (*A*) and de-epoxidation state (*B*) during exposure to strong irradiance (SI) for 3.5 h for rice wild type (WT) plant and mutants. Means ±SD. (*n*=3).

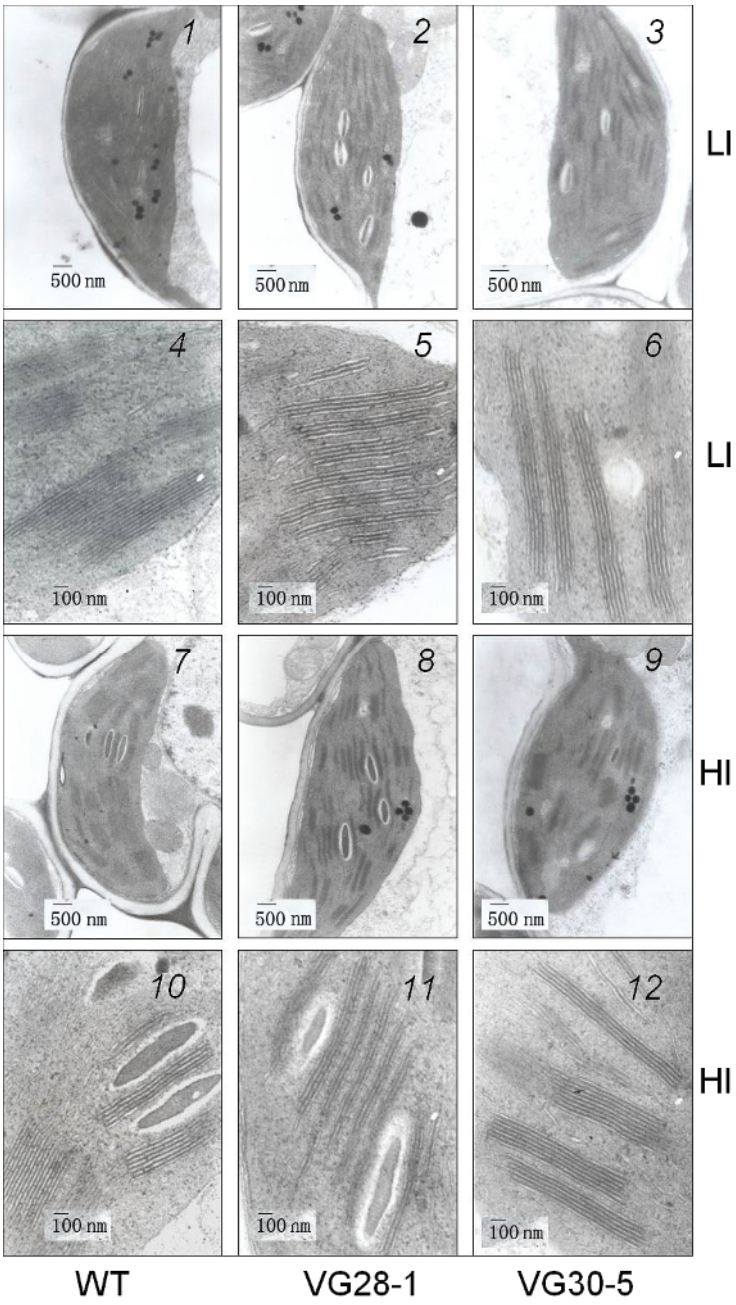

Fig. 5. Transmission electron micrographs of chloroplasts (*1−3, 7−9*) and thylakoids (*4−6, 10−12*) in leaves of two rice mutants and wild type (WT) plants adapted to high (HI) or low (LI) irradiance.

susceptibility of Chl *b*-less mutants to photooxidative stress, leaves grown under HI and LI were sustained by an oxidant, methyl viologen (MV), to form the toxic superoxide free radical *in vivo* (Cha and McRae 1982). After a 42-h MV treatment of LI plants, features of the chloroplast ultrastructure were compared. Before the photoxidative treatments, chloroplast and thylakoid stacking in mesophyll cells of mutant and WT plants grown under HI or LI all had the typical appearance (Fig. 5). Chloroplasts contained numerous starch granules and longer grana were found in the LI leaves. However, in the presence of MV, chloroplasts became swollen with partially irregular grana stacking in leaves grown under LI (Fig. 6, *1−6*). The severe photooxidative damage of chloroplast ultrastructure appeared in HI leaves, which showed dilated and distorted thylakoids and the disintegration of grana structure (Fig. 6, *7−12*). Moreover, more plastoglobuli appeared in the swollen chloroplasts. The obvious alterations of chloroplast ultrastructure in mutant plants grown under HI underwent more oxidative damage than the WT plant. This difference elucidated distinctions between WT and mutants in their capacities to protect against photooxidative damage.

Discussion

In nature, the photosynthetic apparatus is exposed to a number of environmental stresses. Photoinhibition is a common phenomenon in plants induced by SI and other stresses (Tyystjärvi and Aro 1996), and its valuable indicator, F_v/F_m, has been widely applied in plant physiology and eco-physiology. In the present experiment, in excised rice leaves a significant decrease in F_v/F_m appeared after a 3.5-h SI, which was compatible with the changes in q_P, PS2, and ETR (Fig. 1). As a consequence, $1 - q_P$, NPQ, inactive/total PS2, and D_{rate} increased accompanied with a decrease in P_{rate} (Figs. 2 and 3). This confirmed the presence of photoinhibition in rice leaves, and both types of rice plants behaved in the similar way but to different extents. Photoinhibition is a first-order reaction and a function of both photodamage and repair process (Tyystjärvi *et al*. 1994). The degree of photodamage depends on the PS2 activity and photoprotection ability of photosynthetic machinery (Baroli and Melis 1998). Better tolerance to SI in leaves subjected to HI was associated with a more active repair process and more powerful protective mechanism. The increase in NPQ and DES (Figs. 2 and 4) in the WT and mutant plants indeed reflected the increasing photoprotective ability by PS2 antenna and xanthophyll cycle, but the increments in these two parameters (24.7−75.3 % in NPQ, 26.3−62.2 % in DES) were less than the increment

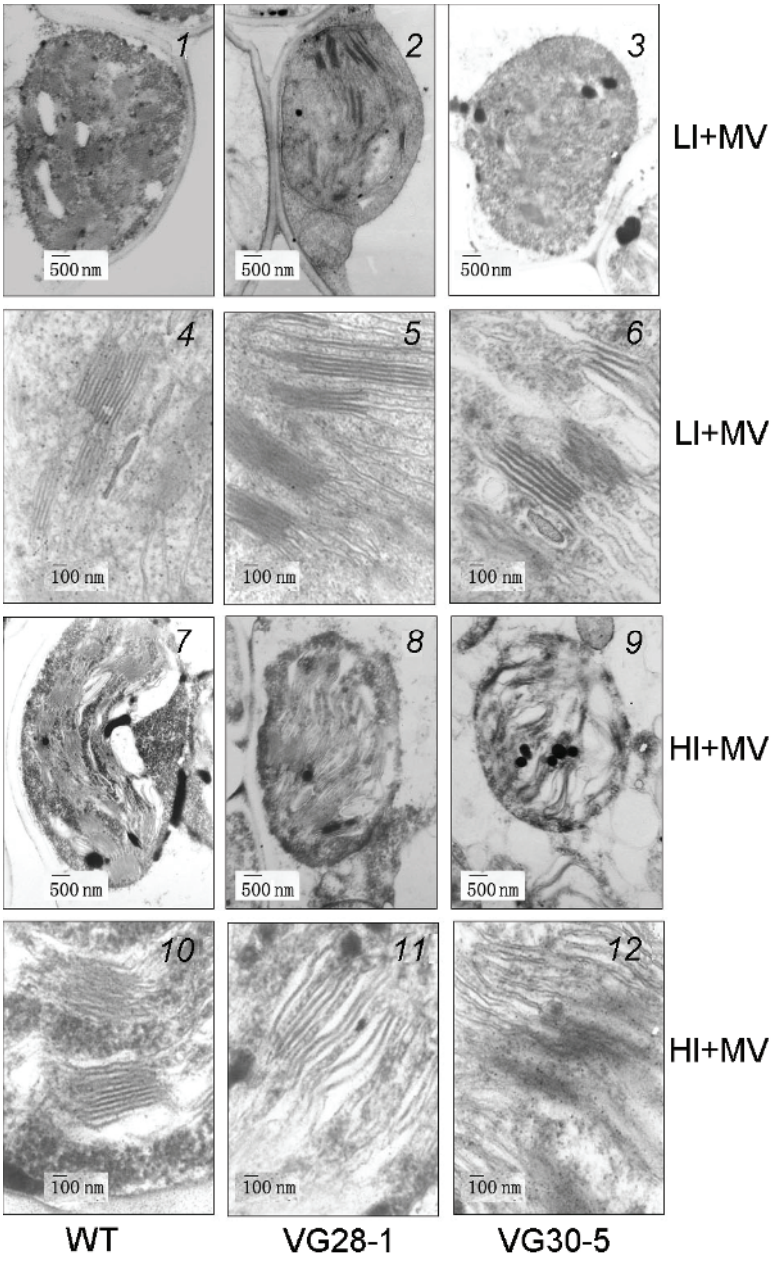

Fig. 6. Transmission electron micrographs of chloroplasts (*1−3, 7−9*) and thylakoids (*4−6, 10−12*) in leaves of two rice mutants and wild type (WT) plants under photooxidation. LI+MV − grown in low irradiance, then treated with 20 μM MV for 42 h; HI+MV − grown in low irradiance, then treated by 20 μM MV for 42 h.

in the ratio of inactive/total PS2 (116.0−129.5 %) by the SI exposure from 1 to 3.5 h. Therefore, the increase in NPQ and DES may be not sufficient to fit the protection of photoinhibition in rice leaves under our experimental conditions.

When irradiation causes the closure of PS2 reaction centre by more than 40 %, photoinhibition is an unavoidable consequence of PS2 function (Öquist *et al*. 1992). Here, when a 3.5 h SI exposure is compared with 1-h exposure, the two Chl *b*-less mutants showed a mean F_v/F_m decrease of 50.7 % and an increase in inactive/total PS2 of 129.5 %, while the same parameters were modulated only by 16.7 and 116.7 % in the WT. The real value of 55−73 % inactive PS2 in mutant plants was more than 40 % of PS2 inactive reaction centre, indicating that the presence of severe photoinhibition in the Chl *b*-less mutants is unavoidable. A different feature of all tested Chl fluorescence parameters between mutant plants and WT was particularly shown within the plant leaf was exposed to SI for 2 h. There were little or almost no changes in F_v/F_m, PS2, q_P, NPQ, ETR, $1 - q_P$, P_{rate}, D_{rate}, and ratio of inactive/total PS2 in the WT plant in this case, while those in mutants were changed largely with the advance of treating time. However, higher xanthophyll cycle pools before and after SI and higher DES value, as well as the rapid increasing rate of DES in mutants by SI were opposite to their susceptibility to photoinhibition. Chow *et al*. (2005) pointed out that the photoinactivation of PS2 depends on photon exposure dosage (irradiance×irradiation time) *per se*. Hence, the relative stability of PS2 function in the WT plant in 2-h treatment might show that the given photon dosage was still lower than its photoinhibition induction.

In Chl *b*-less barley mutant, chlorine f_2, a different sensitivity to photoinhibition and nearly all V converting to Z were observed by Leverenz *et al*. (1992). The authors inferred that the susceptible photoinhibition of the mutant can not be ascribed to a large Z formation. Falbel *et al*. (1994) also stated that the potential larger capacity for photoprotection [larger xanthophyll cycle pool and larger ratio of (A+Z)/(V+A+Z)] in Chl *b*-deficient mutants of wheat and barley grown under HI may be insufficient to dissipate all the excess excitation energy absorbed. Our data extend these points of view with leaves of rice Chl *b*-less mutants by a short time dynamic SI exposure, and this was apparently different with the less susceptible to photoinhibition in thylakoids of Chl *b*-less barley found by Cleland and Melis (1987) in an *in vitro* experiment.

Photon absorption and the allocation of absorbed photon energy into thermal dissipation are a good measure of susceptibility to photoinhibition. These are the photoprotective mechanisms associated with electron transport rate, thermal energy dissipation, and leaf absorptions (Demmig-Adams *et al*. 1996). Absorption of photons by antenna molecules in photosystem is the first step of photosynthesis (Govindjee 2002). In general, leaf absorption depends on a larger antenna size, because larger antenna may absorb more photons and transfer more excitation energy to the reaction centre than a small antenna. However, the role of size in light-harvesting antenna is still controversial. We speculate that the dependent or independent effect of antenna size on photoinhibition and different results of the higher/lower susceptibility of Chl *b*-lack (low Chl *b* content) or Chl *b*-less (no Chl *b*) mutants compared with their WT might be limited by the species habit, mutation site and extent, Chl *a/b* ratio, and the experimental condition (growth irradiance, treated photon dosage, *in vivo* or *in vitro* experiment, *etc*.). Chl *b* is derived from Chl *a* through oxidation of Chl *a*, it participates in the assembly of light-harvesting Chl *a/b* proteins (Tanaka *et al*. 1998, Eggink *et al*. 2001). The abundance of certain proteins and pigment-protein complexes could be affected by the lack of Chl *b*, the reduction in antenna size is paralleled with the severity of Chl deficiency (Falbel *et al*. 1996). The two rice mutants have a small antenna by losing LHC2 and reducing LHC1 (Lin *et al*. 2003a). The susceptibility of these Chl *b*-less mutants to photoinhibition showed that their small antenna did not protect against photoinhibition under SI.

An altered thylakoid membrane stack was observed in barley Chl *b*-less mutant chlorine f_2 (Staehelin 1986). The extent of thylakoid appression and the size of the individual grana stacks might be influenced by any change in the amount of LHC in PS2 (Rozak *et al*. 2002). Nevertheless, no apparent difference of chloroplast ultrastructure between mutants and the WT plant before MV treatment (Fig. 5) indicated that chloroplast ultrastructure of rice Chl *b*-less mutants was not altered as much in our planting conditions as it was in the barley Chl *b*-less mutant. The normal chloroplast ultrastructure, with intact envelope and grana stacking in rice Chl *b*-less mutants, was also seen when the leaves were given a 35 ºC treatment for 30 min in the dark (Lin *et al*. 2005). Therefore, our results in this context differ from the reports of Staehelin (1986) or Rozak *et al*. (2002).

In the case of photooxidation induced by MV, a compound known to generate superoxide radical in the light, the evidenced swollen chloroplast, disordered grana, and distorted thylakoids occurred in Chl-*b* mutants grown under HI which revealed that chloroplasts undergo severe photooxidative damage by superoxide radical (Fig. 6). The typical symptom of oxidative destruction in chloroplasts was also shown in rice leaves with heat shock (Lin *et al*. 2005). The destruction of chloroplast ultrastructure by the action of MV in the light was seen in accordance with the inactivation of PS2 function and a decrease in membrane leakage rate in the purple rice leaves (Peng *et al*. 2006). Chl *b* plays a role in stabilizing LHC protein and membrane stacking. The LHC apoproteins need Chl *b* for stable integration (Preiss and Thornber 1995). An evidenced modulation of Chl-protein complexes and the ratio of excited energy distribution

between two photosystems have been observed in two Chl *b*-less mutants in our previous work (Lin *et al*. 2003b). Hence, the sensitivity of chloroplast ultrastructure to photooxidation stress in these mutants in particular grown under HI is reasonably ascribed to the instability of chloroplast structure and function in the absence of Chl *b*. Combining the results of the present study with the early investigation of thermostability (Lin *et al*. 2005), we conclude that the complete loss of Chl *b* in the photosynthetic apparatus can lead to increased susceptibility to environment stresses such as SI, high temperature, and photooxidation. The exact mechanism of instability of chloroplast structure in rice Chl *b*-less mutants needs further investigation.

References

Baroli, I., Melis, A.: Photoinhibitory damage is modulated by the rate of photosynthesis and by the photosystem II light-harvesting chlorophyll antenna size. − Planta **205**: 288-296, 1998.

Bishop, N.I., Humbeck, K., Romer, S., Senger, H.: The mode of adaptation of the photosynthetic apparatus of a pigment mutant of *Scenedesmus* without light harvesting complex to different light intensities. − J. Plant Physiol. **135**: 144-149, 1989.

Cha, L.S., McRae, D.G.: Light-dependence of paraquat-intiated membrane deterioration in bean plants: Evidence for the involvement of superoxide. − Physiol. Plant. **56**: 492-499, 1982.

Chow, W.S., Lee, H.Y., He, J., Hendrickson, L., Hong, Y. N., Matsubara, S.: Photoinactivation of photosystem II in leaves. – Photosynth. Res. **84**: 35-41, 2005.

Cleland, R.E., Melis, A.: Probing the events of photoinhibition by altering electron-transport activity and light-harvesting capacity in chloroplast thylakoids. − Plant Cell Environ. **10**: 747-752, 1987.

Demmig-Adams, B., Adams, W.W., III, Barker, D.H., Logan, B.A., Bowling, D.R., Verhoeven, A.S.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. − Physiol. Plant. **98**: 253-264, 1996.

Eggink, L.L., Park, H., Hoobes, J.K.: The role of chlorophyll *b* in photosynthesis: Hypothesis. − BMC Plant Biol. **1**: 2, 2001.

Falbel, T.G., Meehl, J.B., Staehelin, L.A.: The severity of mutant phenotype in a series of chlorophyll-deficient wheat mutants depends on light intensity and the severity of the block in chlorophyll synthesis. − Plant Physiol. **112**: 821-832, 1996.

Falbel, T.G., Staehelin, L.A., Adam, W.W., III: Analysis of xanthophylls cycle carotenoids and chlorophyll fluorescence in light intensity-dependent chlorophyll-deficient mutants of wheat and barley. − Photosynth. Res. **42**: 191-202, 1994.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. − Biochim. biophys. Acta **990**: 87-92. 1989.

Gilmore, A.M., Hazlett, T.L., Govindjee: Xanthophyll cycle dependent quenching of photosystem II chlorophyll a fluorescence: formation of a quenching complex with a short fluorescence lifetime. − Proc. nat. Acad. Sci. USA **92**: 2273-2277, 1995.

Gilmore, A.M., Yamamoto, H.Y.: Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C_{18} high-performance liquid chromatographic column. − J. Chromatogr. **543**: 137-145, 1991.

Govindjee: A role for a light-harvesting antenna complex of photosystem II in photoprotection. − Plant Cell **14**: 1663-1668, 2002.

Habash, D.Z., Genty, B., Baker, N.R.: The consequences of chlorophyll deficiency for photosynthetic light use efficiency in a single nuclear gene mutation of cowpea. − Photosynth. Res. **42**: 17-25, 1994.

Hikosaka, K., Kato, M.C., Hirose, T.: Photosynthetic rates and partitioning of absorbed light energy in photoinhibited leaves. − Physiol. Plant. **121**: 699-708, 2004.

Leverenz, J.W., Öquist, G., Wingsle, G.: Photosynthesis and photoinhibition in leaves of chlorophyll *b*-less barley in relation to absorbed light. − Physiol. Plant. **85**: 495-502, 1992.

Lin, Z.-F., Lin, G.-Z., Peng , C.-L., Zhang, J.-L.: Alteration of components of chlorophyll-protein complexes and distribution of excitation energy between two photosystems in two new rice chlorophyll *b*-less mutants. − Photosynthetica **41**: 589-595, 2003a.

Lin, Z.-F., Peng, C.-L., Lin, G.-Z., Ou, Z.-Y., Yang, C.-W., Zhang, J.-L.: Photosynthetic characteristics of two new chlorophyll *b*-less rice mutants. − Photosynthetica **41**: 61-67, 2003b.

Lin, Z.F., Peng, C.L., Xu, X.L., Lin, G.Z., Zhang, J.L.: Thermostability of photosynthesis in two new chlorophyll *b*-less rice mutants. − Sci. China C **48**: 139-147, 2005.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence − a practical guide. − J. exp. Bot. **51**: 659-668. 2000.

Moharekar, S., Moharekar, S., Tanaka, R., Ogawa, K.I., Tanaka, A., Hara, T.: Great promoting effect of high irradiance from germination on flowering in *Arabidopsis thaliana* – a process of photo-acclimation. – Photosynthetica **45**: 259-265, 2007.

Peng, C.L., Lin, Z.F., Lin, G.Z., Chen, S.W.: The anti-photooxidation of anthocyanins-rich leaves of a purple rice cultivar. − Sci. China C **49**: 543-551, 2006.

Preiss, S., Thornber, J.P.: Stability of the apoproteins of light-harvesting complex I and II during biogenesis of thylakoids. − Plant Physiol. **107**: 709-717, 1995.

Öquist, G., Chow, W.S., Anderson, J.M.: Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. − Planta **186**: 450-460, 1992.

Rozak, P.R., Seiser, R.M., Wacholtz, W.F., Wise, R.R.: Rapid, reversible alterations in spinach thylakoid appression upon changes in light intensity. − Plant Cell Environ. **25**: 421-429, 2002.

Schindler, C., Lichtenthaler, H.K.: Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and cloudy day. − J. Plant Physiol. **148**: 399-412, 1996.

Staehelin, L.A.: Chloroplast structure and supramolecular organization of photosynthetic membranes. − In: Staehelin, L.A., Arntzen, C.J. (ed.): Photosynthesis III. Pp. 1-84. Springer-Verlag, Berlin – Heidelberg – New York 1986.

Tanaka, A., Ito, H., Tanaka, R., Tanaka, N.K., Yoshida, K., Okada, K.: Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. − Proc. nat. Acad. Sci. USA **95**: 12719-12723, 1998.

Terao, T., Yamashita, A., Katoh, S.: Chlorophyll *b*-deficient

mutants of rice. 1. Absorption and fluorescence spectra and chlorophyll *a*/*b* ratios. − Plant Cell Physiol. **26**: 1361-1367, 1985.

Tyystjärvi, E., Aro, E.M.: The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. – Proc. nat. Acad. Sci. USA **93**: 2213-2218, 1996.

Tyystjärvi, E., Koivuniemi, A., Kettunen, R., Aro, E.-M.: Small light-harvesting antenna does not protect from photoinhibition. − Plant Physiol. **97**: 477-483, 1991.

Tyystjärvi, E., Mäenpää, P., Aro, E.-M.: Mathematical modelling of photoinhibition and Photosystem II repair cycle. I. Photoinhibition and D1 protein degradation *in vitro* and in the absence of chloroplast protein synthesis *in vivo*. − Photosynth. Res. **41**: 439-449, 1994.