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Dependence of reaction center-type energy-dependent quenching on photosystem II antenna size

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Abstract

The effects of photosystem II antenna size on reaction center-type energy-dependent quenching (qE) were examined in rice plants grown under two different light intensities using both wild type and qE-less (OsPsbS knockout) mutant plants. Reaction center-type qE was detected by measuring non-photochemical quenching at 50 μmol photons m^{-2} s^{-1} white light intensity. We observed that in low light-grown rice plants, reaction center-type qE was higher than in high light-grown plants, and the amount of reaction center-type qE did not depend on zeaxanthin accumulation. This was confirmed in *Arabidopsis npq1–2* mutant plants that lack zeaxanthin due to a mutation in the violaxanthin de-epoxidase enzyme. Although the electron transport rate measured at a light intensity of 50 μmol photons m^{-2} s^{-1} was the same in high light- and low light-grown wild type and mutant plants lacking PsbS protein, the generation of energy-dependent quenching was completely impaired only in mutant plants. Analyses of the pigment content, Lhcb proteins and D1 protein of PSII showed that the antenna size was larger in low light-grown plants, and this correlated with the amount of reaction center-type qE. Our results mark the first time that the reaction center-type qE has been shown to depend on photosystem II antenna size and, although it depends on the existence of PsbS protein, the extent of reaction center-type qE does not correlate with the transcript levels of PsbS protein. The presence of reaction center-type energy-dependent quenching, in addition to antenna-type quenching, in higher plants for dissipation of excess light energy demonstrates the complexity and flexibility of the photosynthetic apparatus of higher plants to respond to different environmental conditions.

Keywords: NPQ; qE; Photosystem II; Antenna size; PsbS; Zeaxanthin

1. Introduction

Higher plants develop a variety of photoprotective mechanisms against photoinhibition or the light-dependent loss of photosynthetic efficiency [1,2]. These photoprotective mechanisms are classified as either long-term or short-term responses. The long-term responses include avoidance mechanisms that involve changes in the orientation of leaves [3] or chloroplasts [4] and modulation of the composition of the photosynthetic apparatus by light acclimation [5]. The most prominent short-term response is non-photochemical quenching (NPQ), which plays an important role in the photoprotection of photosystem (PS) II *in vivo*. NPQ is subdivided into three components according to their relaxation kinetics in darkness following a period of illumination, as well as their responses to various inhibitors. The slowest component of NPQ is qI, which is related to photoinhibition or the slowly reversible damage to PSII reaction centers [6]. The second component of NPQ is qT, which reflects the phosphorylation-mediated migration of light-harvesting complex (LHC) II between PSII and PSI (state transition) [7]. The fastest and the most important component of NPQ is qE, the energy-dependent quenching. This component depends on three major parameters: the development of transthylakoid proton gradient (ΔpH), the amount of pigments involved in xanthophyll cycle, and the existence of a PsbS subunit in PSII [8]. qE is characterized by: (1) its sensitivity to

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uncouplers of the proton gradient [9], (2) inhibition by *N,N′*-dicyclohexylcarbodiimide, an inhibitor of protonation of protein residues [10], (3) the light-induced absorbance changes at 535 nm [11], (4) the shortening of the lifetime of a specific chlorophyll (Chl) fluorescence component from ~2.0 to ~0.4 ns [12] and (5) carotenoid cation radical formation [13].

During the past several decades, intensive studies of qE have focused mainly on the site of quenching and its role in photodamage. Accordingly, two types of models have been proposed, depending on the binding site of the quencher. One of the models is the antenna-type qE, which has been widely investigated and assumes that the quencher of qE is associated with LHCII of PSII [14]. The other model is the reaction center (RC)-type qE, which assumes that the quencher of qE is associated with the PSII core complex [15,16]. This RC-type qE does not appear to depend on the xanthophyll cycle [15], although a previous report [17] suggested that a small number of molecules of zeaxanthin per PSII RC may permit effective quenching of Chl fluorescence. The role of charge separation within the PSII core complex in quenching Chl fluorescence has also been reported [18]. Therefore, both the presence and the possible mechanisms of RC-type qE and the factors that affect the RC-type qE are still under debate.

The utilization of absorbed light energy by PSII and the photoinactivation of PSII depend on the size of the light-harvesting antenna [19,20]. Furthermore, larger PSII antenna size encourages an increased rate of light absorption, resulting in greater likelihood of photodamage in the PSII core complex [20]. High light (HL)-grown or sun plants are reported to be more resistant to photoinhibition than are low light (LL)-grown or shade plants [1,2]. The resistance of HL-grown plants to excess light has been attributed to various factors, including a higher rate of photosynthetic electron transport, a smaller PSII antenna size and greater capacities for both D1 protein turnover and NPQ. Also, in isolated PSII particles, the trapping behavior of PSII was a function of antenna size [21]. Although structural rearrangements of light-harvesting antenna complexes are involved in the quenching of Chl fluorescence [22], to our knowledge, no data have been presented on the role of antenna size of PSII in RC-type qE.

Therefore, in the present study, we investigated the effects of PSII antenna size on the RC-type qE in rice plants grown under two different light intensities using both wild-type (WT) and qE-less (OsPsbS knockout) mutant plants. We found a direct correlation between the RC-type qE and the PSII antenna size, but this qE did not depend on zeaxanthin accumulation or other possible factors that influence qE, including linear electron transport rate (ETR) and PsbS protein of PSII.

2. Materials and methods

2.1. Plants and growth conditions

One-month-old seedlings of WT and OsPsbS-knockout mutant rice (*Oryza sativa* L.) plants [23] were grown in soil in a greenhouse under sunlight (for HL-grown plants) or in a growth chamber at an irradiance of 50 μmol photons m^{-2} s^{-1} white light (for LL-grown plants) at a temperature of 28±2 °C. *Arabidopsis thaliana* plants were grown in a growth chamber at an irradiance of 100 μmol photons m^{-2} s^{-1} white light at a temperature of 23±1 °C. Plants were dark-adapted for at least 4 h before measurements unless otherwise stated.

2.2. Fluorescence and electron transport measurements

Chl fluorescence was measured on detached leaves using a PAM fluorometer (PAM2000, Walz, Effeltrich, Germany) at room temperature after dark-adaptation for 10 min. Actinic light was provided by a halogen lamp (Schott KL1500, Mainz, Germany). Fluorescence parameters, Fm and Fm′, were induced by a saturating pulse of white light (0.8 s, 5.000 μmol photons m^{-2} s^{-1}). Fm and Fo are defined as maximal and minimal fluorescence yield of a "dark-adapted" sample, with all PSII RCs fully closed or opened, respectively. Fm′ is defined as the maximal fluorescence yield reached with a pulse of saturating light while leaves are illuminated by actinic light. The photochemical quenching (qP) and NPQ parameters were calculated using the equations described in [24]. Electron transport rates were calculated as previously described [25].

2.3. Pigment analysis

Carotenoids were analyzed as described [26] with slight modifications. Leaf segments of one-month-old plants were frozen in liquid nitrogen following light treatment and ground with a Mixer-Mill (MM301, Retsch, Germany). Their pigments were then extracted by gently agitating the leaf powder in ice-cold 100% acetone for 1 h. To minimize pigment degradation, the extraction was performed in darkness at 4 °C. Cell debris was removed by centrifuging at 4 °C for 10 min at 14,000 rpm (Micro 17R; Hanil, Korea). The extracts were filtered through a 0.2-μm syringe filter. Pigment separation was performed in an HPLC system (HP 1100 Series; Hewlett Packard, Waldbronn, Germany) on a Spherisorb ODS-1 column (Alltech, USA) with a solvent mixture of acetonitrile:methanol:0.1 M Tris–HCl (pH=8., 72:12:7, v/v), and a 10-min linear gradient (methanol:hexane, 4:1, v/v). The concentrations of the pigments were estimated by using the conversion factors for peak area (in nanomoles) that were previously calculated for this solvent mixture [27]. The Chl content of the leaf discs was determined in 80% acetone extracts using the extinction coefficients and wavelengths as described [28].

2.4. Immunoblotting analysis of the D1 protein and Lhcb proteins

For RNA extraction and immunoblot analysis, leaves were harvested 3 h into the light period, frozen immediately in liquid nitrogen, and then stored at −80 °C. Isolation of thylakoid membranes for immunodetection of proteins was performed as described by Oh et al. [29]. SDS-PAGE (14%) was used to separate Lhcb proteins as previously reported [30]. Thylakoid membranes equivalent to 2–10 μg Chl were solubilized in buffer composed of 62.5 mM Tris–HCl, 10% glycerol, 10% SDS, 2.5% β-mercaptoethanol and 6 M urea for 30 min at room temperature. D1 protein analysis was performed according to the procedure of Miyao [31]. After SDS-PAGE, proteins were transferred to nitrocellulose membranes for immunoblotting [32]. After having been blocked and washed, the blots were incubated with anti-Lhcb antibodies. After washing, they are incubated with HRP-conjugated secondary antibody (Amersham). The ECL detection system (Amersham) was used to visualize the protein bands. Antibodies against Lhcb proteins were kindly provided by Prof. Stefan Jansson (Umeå Plant Science Center, Umeå, Sweden), and the anti-D1 serum against a synthetic polypeptide that corresponds to residues 333–344 of the spinach D1 protein was kindly provided by Prof. Mitsue Miyao (Laboratory of Photosynthesis, National Institute of Agrobiological Resources, Tsukuba, Japan).

2.5. Reverse transcription polymerase chain reaction (PCR) with an internal standard

Total cellular RNA was isolated from HL- and LL-grown plant leaves using TRIzol REAGENT® (Life Technologies, USA) according to the manufacturer's instructions. Reverse transcription was performed according to the manufacturer's protocol using RNA samples with RQ1 RNase-free DNase (Promega) and M-MLV (Promega). Forward (GGTTTCACCAAGGAGAACGA) and reverse (ACGACGTTGAAGAGGACGAG) primers from the 3rd exon (near the T-DNA insertion position) of *OsPsbS* (LOC_Os01g64960) were designed to

detect its gene expression. The standard PCR conditions involved initial heating at 94 °C for 5 min followed thereafter by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 56 °C for 30 s and primer extension at 72 °C for 30 s. The cycles were followed by a final extension at 72 °C for 10 min. The final PCR products (10 μl) were analyzed on 1.5% (w/v) agarose gels. For each plant, actin transcripts were used as an internal control.

3. Results

3.1. Relative contribution of RC-type qE is different in HL- and LL-grown rice plants

Illumination of detached WT rice leaves with white actinic light resulted in the generation of NPQ (Fig. 1A and B). Using HL-grown plant leaves, the NPQ-generation kinetics measured under high actinic light (500 μmol photons m^{-2} s^{-1}) were compared to that measured under low actinic light (50 μmol photons m^{-2} s^{-1}); PsbS-knockout mutant plants were compared with WT to understand the role of PsbS protein (Fig. 1A). As previously reported [15], under low actinic light NPQ developed for about 2 min, but slowly decreased thereafter, although after illumination with high actinic light, NPQ developed rapidly and reached a plateau. This initial development of NPQ for 1–2 min is called RC-type qE because it is associated with reversible inactivation of PSII RCs [15]. This qE is blocked by treatment with nigericin, which inhibits the development of ΔpH across the thylakoid, but it is not accompanied by zeaxanthin synthesis. The NPQ generation kinetics from LL-grown plants are shown in Fig. 1B. Although the plateau levels of NPQ generated under high actinic light in LL-grown WT plants were slightly lower than those obtained using HL-grown WT plants, the RC-type qE generated under low actinic light in LL-grown WT plants was two-times higher than that generated in HL-grown WT plants.

Although HL- and LL-grown plants show differences in qP under high actinic light, we could not observe significant differences in qP when they were under low actinic light (Fig. 1C and D). Also, no significant differences in maximum photochemical efficiency of PSII (measured as Fv/Fm) were observed between HL-grown and LL-grown WT plants, or between WT and PsbS knockout rice plants as shown in original fluorescence traces (see Fig. 2A and B). Because there were differences in the RC-type qE between HL- and LL-grown WT plants without significant differences in the maximal photochemical efficiency of PSII, we compared the dependence of linear ETR on photosynthetic active radiance (PAR) (Fig. 2D, insert). Under PAR higher than ~150 μmol photons m^{-2} s^{-1}, the ETR measured in HL-grown plants was greater than that of LL-grown plants. However, when the PAR was less than ~100 μmol photons m^{-2} s^{-1} light intensity, no differences in ETR were observed between the plants grown under different light intensities. Furthermore, mutation of PsbS protein did not cause any changes in linear ETR of the plants, regardless of their growth conditions (Fig. 2D, insert).

3.2. Zeaxanthin formation does not affect RC-type qE in HL- and LL-grown rice plants

Energy-dependent quenching is generally correlated with zeaxanthin accumulation in higher plants. We analyzed the correlation between NPQ-generation kinetics and the amount of zeaxanthin synthesized in HL- and LL-grown WT and PsbS-knockout rice plants. After dark adaptation, plants were illuminated at subsequent time intervals (Fig. 2C and D.) with high (500 μmol photons m^{-2} s^{-1}) and low (50 μmol photons m^{-2} s^{-1}) light intensities and then frozen in liquid nitrogen until

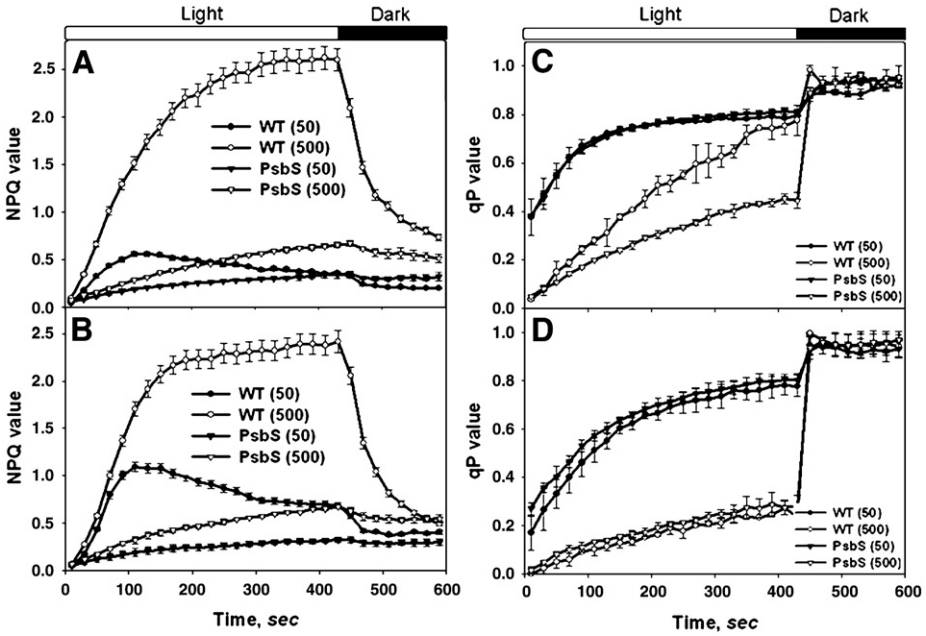

Fig. 1. Light-induced NPQ and qP generation in rice leaves under low (50 μmol photons m^{-2} s^{-1}) and high (500 μmol photons m^{-2} s^{-1}) actinic light intensities. (A) NPQ in high-light-grown plants; (B) NPQ in low-light-grown plants; (C) qP in high-light-grown plants; (D) qP in low-light-grown plants. Each point represents the mean of at least four separate experiments with standard deviation indicated by the bar. NPQ and qP were calculated as described in Materials and methods. White symbols, under high actinic light intensities; dark symbols, under low actinic light intensities; circles, WT; triangles, PsbS-knockout rice plants.

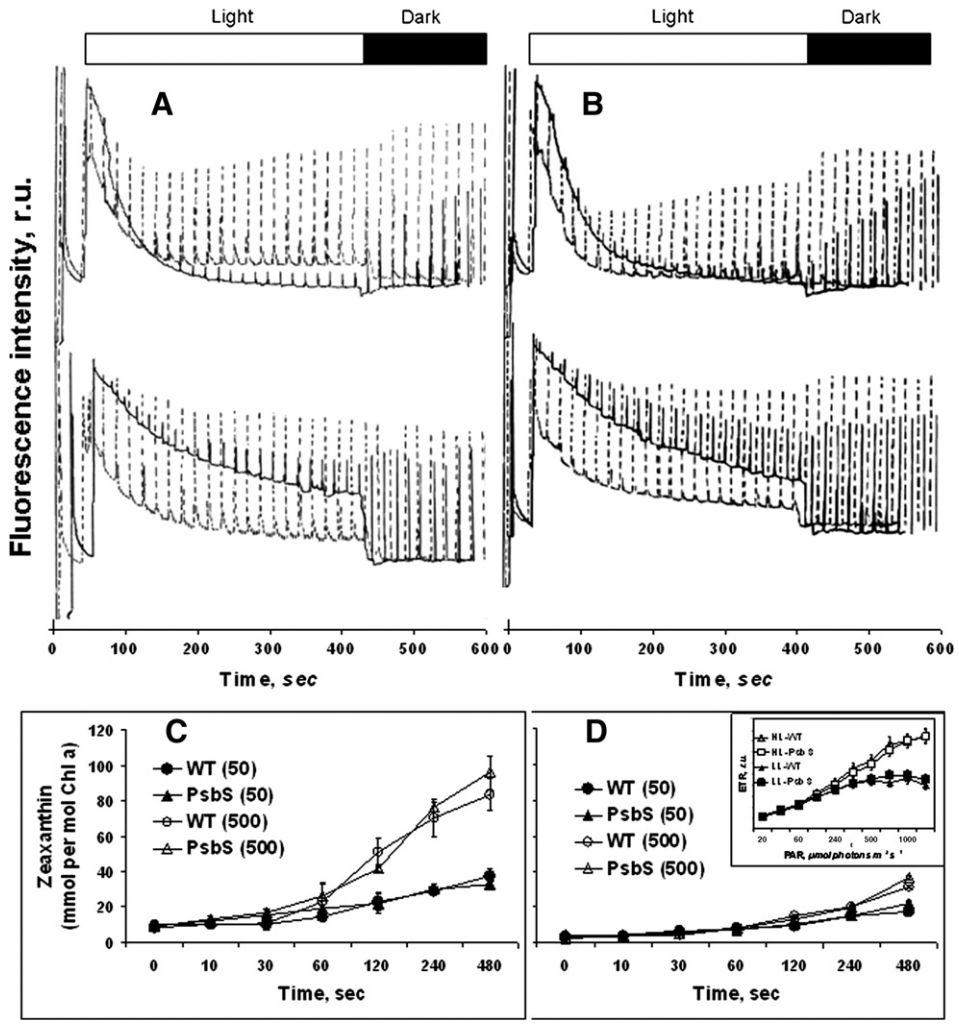

Fig. 2. Original chlorophyll fluorescence traces and zeaxanthin accumulation after subsequent illumination of rice leaves. (A) High-light grown plants; (B) low-light grown plants. Solid lines with high (500 μmol photons m^{-2} s^{-1}); dashed lines with low (500 μmol photons m^{-2} s^{-1}) actinic light intensities. Top panel—WT; bottom panel—PsbS-KO plants. (C) Zeaxantin accumulation in high-light-grown plants. (D) Zeaxanthin accumulation in low-light-grown plants. Plants were illuminated with high (500 μmol photons m^{-2} s^{-1}) and low (50 μmol photons m^{-2} s^{-1}) light intensities, and the leaves were then quickly frozen in liquid nitrogen. Pigment analysis of whole leaf extract was performed by HPLC as described in Materials and methods. Each point represents the mean of at least four independent experiments with standard deviation bar. Insert in (D) represent electron transport rate of high- and low-light-grown plants.

use. As shown in Fig. 2C and D, for the first 1 min when RC-type qE was rapidly induced, we did not observe active accumulation of zeaxanthin. On the contrary, when zeaxanthin subsequently began to accumulate, RC-type qE decreased (see Fig. 1A and B). The amount of zeaxanthin that accumulated after prolonged illumination was higher in HL-grown plants than in LL-grown plants, in agreement with previous reports [33]. Although PsbS-knockout plants did not show any qE, we did not observe any remarkable differences in zeaxanthin accumulation between WT and mutant plants.

3.3. Antenna size correlated with RC-type qE

The total Chl content of HL-grown WT rice plants was 1.94 mg per gram fresh weight, but it was 3.43 mg per gram fresh weight in LL-grown WT plants. The Chl a/Chl b ratio was 3.0 in LL-grown plants, slightly smaller than the value in HL-grown plants (3.3); the PsbS mutation did not make any remarkable difference in the Chl content. Table 1 shows the HPLC analyses of pigments in rice leaves harvested at daytime and immediately frozen in liquid nitrogen. The data showed that HL-grown plants

Table 1
HPLC pigment analyses of rice leaves grown under high light (HL) and low light (LL)

	Neo	Vio	Ant	Zea	V+A+Z	Lut	β-Car
WT–HL	65±1	124±7	49±1	30±1	203	246±9	173±13
PsbS–HL	66±1	120±1	51±6	35±9	206	235±5	173±1
WT–LL	59±1	52±1	27±1	5±1	84	251±2	127±16
PsbS–LL	57±1	59±1	25±1	6±1	90	242±3	140±12

Leaf tissues were collected 3 h into the light period from 3 individual plants for each experiment. The tissues were immediately frozen in liquid nitrogen and then stored at −80 °C until use. The values are given in mmol of pigments per mol Chl a. Neo—neoxanthin; Vio—violaxanthin; Ant—antheraxanthin; Zea—zeaxanthin; V+A+Z—xanthophyll cycle pool size; Lut—lutein; β-Car—β-carotene. Mean value±SD are given for at least 3 independent experiments.

contained more xanthophyll cycle pigments and β-carotene (β-car) than LL-grown plants. This indicates that plants grown under HL have the capacity to synthesize greater amounts of β-car and xanthophylls as an adaptive strategy to protect plants from HL [34]. Although the content of neoxanthin was slightly less in LL-grown plants, the amount of lutein, the most abundant carotenoid in plants, remained constant.

Typically, the pigment content, including Chls and carotenoids, per PSII RC is measured to estimate the PSII antenna size [20,21,35]. In this study, we estimated the PSII antenna size in a more direct way. We determined the amount of Lhcb proteins and D1 protein from PSII core complexes in samples containing equal amounts of Chl. Immunoblots obtained using antibodies against these proteins are shown in Fig. 3A. Similar to other findings, LL-grown plants expressed higher levels of antenna proteins, Lhcb1, Lhcb 2, Lhcb 3 and Lhcb 4, than HL-grown plants (Fig. 3B), regardless of PsbS mutation [36]. However, D1 polypeptide remained unaltered both in LL- and HL-grown WT and PsbS-knockout mutant plants.

Furthermore, we estimated the functional antenna size of PSII from fluorescence induction traces in leaves infiltrated with DCMU, and the size in LL-grown rice plants was two times bigger than the size in HL-grown plants, which is in agreement with previous reports [19] and with the estimates based on immunoblot analysis (Fig. 3A).

Thus, we found a direct correlation between PSII antenna size and the extent of RC-type qE. Since it has previously been reported that PsbS protein has a dosage effect on qE [37], we next wanted to determine whether the difference between the PSII antenna sizes in HL- and LL-grown plants would affect the expression levels of PsbS subunit of PSII.

3.4. PsbS protein expression does not have a dosage effect on RC-type qE

Fig. 3C displays the results of the PsbS gene expression experiments in HL- and LL- grown WT and PsbS-knockout plants. PsbS transcript levels in LL-grown WT plants were not higher than in HL-grown plants. On the contrary, the HL-grown WT plants showed a little more PsbS protein expression than LL-grown plants, which is in agreement with previous findings [38]. These data suggest that levels of PsbS protein and extent of RC-type qE have no correlation.

4. Discussion

Here, we present evidence indicating that a part of NPQ, which is induced in dark-adapted plants within 1–2 min under illumination in low actinic light at 50 μmol photons m^{-2} s^{-1}, depends on PSII antenna size. In higher plants, the energy-

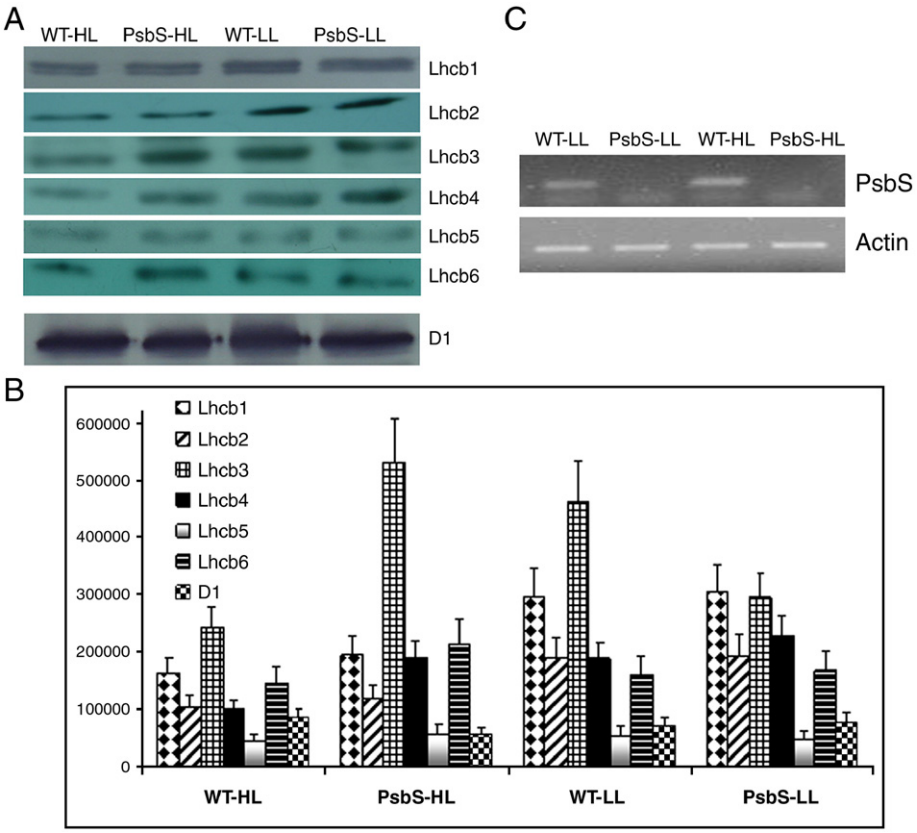

Fig. 3. Western blots of Lhcb proteins and D1 protein of PSII, and PsbS gene expression in HL- and LL-grown WT and PsbS-knockout rice plants. Each experiment was repeated at least twice and representative data are shown. (A) Western blotting; (B) quantification of the western blotting results; (C) gene expression. For Western blot analysis, each lane was loaded with thylakoid membranes equivalent to 2 μg Chl. Quantification of the western blotting results was done using a special image analysis program in MatLab.

dependent quenching qE is generally correlated with the accumulation of zeaxanthin, a pigment involved in the xanthophyll cycle [39]. In this study, the amount of zeaxanthin that accumulated during the initial 1 min of illumination was very small in both HL- and LL-grown plants (Fig. 2C and D.). Earlier studies on RC-type qE [15,17] also did not report any dependence of RC-type qE on zeaxanthin accumulation. However, a small number of zeaxanthin molecules per PSII RC may permit effective thermal dissipation of light energy in PSII, as shown in poikilohydric moss [18]. Although we cannot rule out this possibility, we believe it to be less likely, because the RC-type qE was observed in *Arabidopsis npq1–2* mutant [39] plants, which completely lack zeaxanthin due to a mutation in the violaxanthin de-epoxidase enzyme (Fig. 4). The extent of RC-type qE in *npq2* mutant plants of *Arabidopsis* which accumulated more zeaxanthin also was similar (Fig. 4). The less extent of RC-type qE in these mutants may be due to alteration of antenna characteristics by carotenoids as shown in [41,42].

The other characteristic of the RC-type qE is shown in Fig. 1, where the RC-type qE was induced within the first 1–2 min of low actinic illumination, and its magnitude decreased rapidly with a corresponding increase in photochemical quenching (Fig. 1C and D). We note that other research groups [17,18] have shown that RC-type qE to occurs at all times during illumination, however, these authors used methods of Fo and Fm quenching separately to estimate RC-type qE. It is possible that the RC-type qE does not relax during illumination with high actinic light, but rather is partly converted into the antenna-type qE.

We observed that the magnitude of RC-type qE induced under illumination with low actinic light was higher in LL grown plants than in HL grown plants. To understand the reasons of this difference, we examined various factors that may affect quenching processes. First of all, we did not observe any noticeable differences in linear ETR between HL- and LL-grown plants and between WT and PsbS-knockout plants when RC-type qE was induced.

The PSII antenna size in LL-grown rice plants was larger than that in HL-grown plants, regardless of the mutation of PsbS protein (Table 1 and Fig. 3A); this is in agreement with other studies [20,21]. It has also been shown that, under high irradiance, *Dunaliella salina* [21] and pea [20] respond by decreasing the PSII antenna size. These studies have reported that photoinhibition of PSII increases when PSII Chl antenna size becomes large. However, there are contrasting reports ([43] and references therein) that photoinhibition does not depend on the antenna size. It is most likely that when the Chl antenna size is large, the PSII RC receives more light energy due to the increased rate of light absorption and, thus, higher rate of energy transfer from the antenna; this would minimize the damaging effects of high irradiance. One of the mechanisms that protects against photoinhibition is that the PSII core complex minimizes the photodamage by dissipating energy as heat, thereby also quenching Chl fluorescence. However, we cannot, at present, conclusively define the role of RC-type qE in photoinhibition. A probable explanation for this discrepancy may be that the diminished photosynthetic electron transport rate under high irradiance regime for LL-grown plants may reduce the CO_2 fixation and, thus, cause a decrease in the RC-type qE level in LL-grown plants compared to HL-grown plants (Fig. 1).

It has been shown that PsbS protein levels affect qE [37], and our findings also show that total NPQ levels in HL- and LL-grown rice WT plants correlate with PsbS transcript levels (Fig. 3B). When the RC-type qE is large (LL-grown WT plants), PsbS transcript levels are small; when the RC-type qE is small (HL-grown WT plants), PsbS transcript levels are relatively large. Although the RC-type qE differs from the antenna-type quenching, the disappearance of both types of qE in the *npq4-1* mutant of *Arabidopsis* (Fig. 4) and in PsbS-knockout rice plants (Fig. 1) raises the possibility of common origin or common regulation for these forms of quenching. So, PsbS protein of PSII may be involved in the generation of RC-type qE, but the mechanism of regulation for qE in RC and in antenna might be different. It has been suggested that the RC-type and antenna-type of quenching represent two different phenomena [15]. It has also been shown that PsbS proteins in PSII have monomeric and dimeric forms, depending on pH shifts [44]. We observed that PsbS transcript levels were different in HL- and LL-grown plants. Taken together, these findings suggest that the two types of qE, RC-type and antenna-type, are probably regulated differently. Although it has been shown that PsbS protein of PSII is most likely associated with zeaxanthin and quenches the Chl fluorescence [13], there is no direct evidence that PsbS is bound to any pigments [44]. The independence of the RC-type qE from zeaxanthin also indirectly implies that PsbS protein of PSII promotes qE in higher plants.

Although we cannot rule out the other possibilities that may affect RC-type qE, the exact quenching site and the molecular mechanism of qE is still unclear. The levels of many factors and components change depending on the intensity of light during growth and may affect the coordinated function of PSII and PSI and thus, may alter electron transport properties as well as alter qE [45–47].

In summary, our results show that RC-type qE depends on the PSII antenna size, and, although it depends on PsbS protein of PSII, it is not correlated with the transcript levels of PsbS

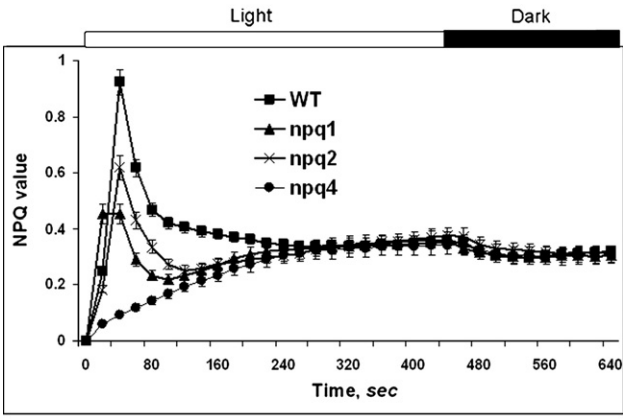

Fig. 4. Light-induced NPQ generation in *Arabidopsis* NPQ mutants, under low (50 μmol photons m^{-2} s^{-1}) actinic light intensity. Each point represents the mean of at least four separate experiments with standard deviation bar.

protein. In higher plants, the presence of two types of qE for dissipation of excess light energy demonstrates the complexity and flexibility of their photosynthetic apparatus to respond to different environmental conditions. Our experimental data emphasize the importance of PsbS protein in the generation of both RC-type and the antenna-type qE. The formation of PsbS-dependent quenching complexes in the PS II core complex and in LHCII are inevitable, the possible molecular mechanism(s) of the energy-dependent quenching of Chl fluorescence and its regulation remain unclear.

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