Supporting Information

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SI Materials and Methods

Plant Materials. Seeds of WRC were obtained from Gene Bank of the National Institute of Agrobiological Sciences (NIAS). Classification of WRC cultivars into Indica, *temperate japonica*, and *tropical japonica* groups is described in the work of Kojima et al. (1). An update of classification was made as a result of additional genetic information from NIAS (https://www.gene.affrc.go.jp/databases-core_collections_wr.php). Indica cultivars were further classified into *aus* and *indica* as is consistent with another report about rice classification (2). BIL lines between Habataki and Sasanishiki were developed by the Rice Genome Project and obtained from the Rice Genome Resource Center of NIAS (3). Segregating seeds of *psbS1* (1C-032-61) were developed by Gynheung An and obtained from Pohang University of Science and Technology. Seeds were germinated in growth chamber at 28 °C for several days and transferred into pots containing nutrient-rich soil (Bon-sol no. 1; Sumitomo Chemical). These plants were grown in a naturally lit greenhouse at 28 °C (day) and 24 °C (night). The maximum midday light intensity (i.e., PPFD) reached 1,000 μmol m^{-2} s^{-1} under this condition.

Leaves of segregating *psbS1* were frozen in liquid nitrogen and homogenized with fine powder of siliceous sand. Edwards buffer (4) was added to this homogenate and heated at 50 °C for 5 min to extract DNA. Solution was treated with phenol/chloroform/isoamylalcohol 25:25:1 (vol/vol/v) and DNA was precipitated with ethanol. Homozygous segregants of *psbS1* were selected by PCR using Ex-Taq (Takara) with primers 03261-F1 (5′-GTG ACG GGC CTC GAC AAG-3′), 03261-R1 (5′-CAC CTC AGA GCA TCA TGA ATG TA-3′), and 2707-LB1 (5′-GGT GAA TGG CAT CGT TTG AA-3′). 03261-F1 and 2707-LB1 amplified an approximately 500-bp fragment including the left border and 03261-F1 and 03261-R1 amplified an approxinately 1,000-bp fragment from the genomic sequence.

Measurement of Chlorophyll Fluorescence. Third leaves of 3-wk-old plants or fourth leaves of 4-wk-old plants were subjected to analysis. Because growth speed was different between cultivars, leaves were sampled after the upper leaves became longer than the sampled leaves. Leaves were excised (~0.5 cm^2) and floated on ion-exchanged water containing 0.01% Triton X-100 or 0.1% agar in 24-well plastic plates. Leaves were predark-adapted in room (PPFD < 10 μmol m^{-2} s^{-1}) for at least 2 h. Chlorophyll fluorescence was measured with a Closed FluorCam (Photon Systems Instruments). Leaves were dark-adapted for 5 min before Fo and Fm were measured. Actinic lights were supplemented for 5 min before measurements of Fm′ to calculate parameter NPQ. Fluorescence parameters were calculated by the following equations:

$$Fv / Fm = (Fm - Fo) / Fm,$$

$$NPQ = Fm / Fm' - 1,$$

$$\Phi_{II} = (Fm' - Fs) / Fm',$$

$$\Phi_{NPQ} = Fs / Fm' - Fs / Fm, \text{ and}$$

$$\Phi_{NO} = Fs / Fm$$

NPQ value represents the size of NPQ rate constant relative to size of the basal dissipation's rate constant, calculated by Stern–Volmer equation on nonphotochemical processes (5). Formulas used here for Φ_{NPQ} and Φ_{NO} are the alternatives (6) to the original ones (7).

QTL Analysis. Twenty-eight of 85 lines of the original BIL set were yellowish or did not grow well under the growth condition in this study and were eliminated from the analysis. NPQ data were obtained from 57 lines, which were grown for 24 d. Averages of NPQ values obtained from third leaves of three individuals were subjected to QTL analysis. The linkage map of the BILs was obtained from Rice Genome Resource Center of NIAS (http://www.rgrc.dna.affrc.go.jp/jp/ineSHBIL85.html). Composite interval mapping was performed using QTL Cartographer, version 2.5 (8). The threshold LOD score for significant detection ($P < 0.05$) of QTLs was determined as 3.04 as the result of 1,000 permutations. The AE and percentage of phenotypic variance explained by each QTL were estimated at the peak LOD score.

Plasmid Construction. Full-length cDNA clone of *OsPsbS1* (J023118A05) was developed by the Rice Genome Project and obtained from the Rice Genome Resource Center of NIAS (9, 10). This clone originates from Nipponbare cDNA. A fragment containing *OsPsbS1* was excised by digestion with SfiI (cDNA fragment). pRiceFOX-GateA was digested with SbfI and SacI to exclude a 1.7-kb gateway cassette. This fragment was substituted with a linker DNA, which was generated by the combination of oligo-DNAs F-linker-F (5′-AGG CCA AAT CGG CCG GCC ATA AGG GCC AGC T-3′) and F-linker-R (5′-GGC CCT TAT GGC CGG CCG ATT TGG CCT TGC A-3′; pRiceFOX2). Two SfiI sites in the linker region of pRiceFOX2 match the sequences of the SfiI sites of the cDNA fragment. cDNA fragment was introduced between the two SfiI sites of pRiceFOX2 (pUbi1-PsbS1). *OsPsbS1* is driven by the maize Ubi1 promoter in this plasmid.

Transformation. pUbi1-PsbS1 was introduced into the Agrobacterium strain EHA105. Kasalath callus was transformed as described previously (11). Transformed callus was selected with hygromycine. Regenerated transformants were grown in a room at a PPFD of 150 μmol m^{-2} s^{-1}. NPQ of expanded leaves were measured after 10 min of supplementation of actinic light (PPFD, 1,500 μmol m^{-2} s^{-1}). Expanded third leaves were measured for WT Kasalath. Duration of actinic light was longer in this analysis to certify proper induction of NPQ.

Quantitative RT-PCR. Leaves were frozen in liquid nitrogen and ground to a fine powder. RNA was extracted with RNeasy Plant Mini Kit (Qiagen). Extract was treated with DNaseI (Qiagen) to degrade potential trace amounts of contaminating genomic DNA. RNA (0.5 μg) was reverse-transcribed with Ready-To-Go RT-PCR Beads (GE Healthcare). Obtained cDNA solution was diluted by a factor of 10 and quantified in Power SYBR Green PCR Master Mix (Takara) with a 7300 Real Time PCR System (Applied Biosystems). Primers PsbS1-RTF3 (5′-CTG TTC GGC AGG TCC AAG-3′) and PsbS1-RTR3 (5′-ACG AAC AGC TCG TTC TCC TT-3′) were used to amplify *OsPsbS1* cDNA. These primers amplify *OsPsbS1* between first and second exons, and generate a 130-bp fragment from cDNA sequence of *OsPsbS1*. Primers EF1α-F1 (5′-CTG CTG CTG CAA CAA GAT GG-3′) and EF1α-RA1 (5′-TCA CCC TCA AAC CCA GAG ATG-3′) were used to amplify *EF1α* cDNA as an internal control for standardization.

DNA Sequence Around *OsPsbS1*. DNA sequence around *OsPsbS1* of Nipponbare was obtained from the sequences of PAC clones (accession nos. AK003235 and AP003286). DNA sequence of 93–11 was obtained from the shotgun sequence (accession no. AAAA02004367). DNA sequence of the corresponding region of Kasalath was provided by NIAS.

Sequencing of *OsPsbS1* Coding Sequence. Total RNA was extracted from leaf blades of Habataki and Sasanishiki with an RNeasy Plant Mini-Kit (Qiagen). RNA was reverse-transcribed with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Resulting cDNA was PCR-amplified with KOD Plus Neo (Toyobo) by using primers PsbS1-SeqF2 (5′-AGC AGC AGC AAG CAG CTA A-3′) and PsbS1-SeqR2 (5′-GAT GCG CAA GCA CAA CAA T-3′) for 45 cycles. Annealing was at 60 °C and extension was for 2 min. Other conditions followed the standard condition for three-step reaction. DNA bands slightly less than 1 kb were excised from agarose gel after electrophoresis, and DNA was extracted and sequenced with the same primers.

PCR Amplification for Detection of 2.7-kb Deletion. Expanded leaves were excised, frozen in liquid nitrogen, and ground with a mortar and pestle, with sterilized siliceous sand added, to a fine powder. Extraction butter (4) was added to the powder. This mixture was filled in plastic tubes and gently inverted at room temperature for 5 min. To this solutioin, 0.5 volume of phenol/chloroform/isoamylalcohol (25:25:1, vol/vol/v) was added and vortexed vigorously. The tube was centrifuged at 18,000 × g for 5 min at room temperature. Supernatant was gathered in a new tube. Rough DNA was gathered by ethanol precipitation. After vacuum drying, DNA was purified by precipitation with cetyltrimethylammonium bromide (12). DNA–cetyltrimethylammonium bromide precipitation was dissolved in 5 M NaCl at 55 °C for 10 min. This solution was diluted by twofold with sterilized water and ethanol-precipitated.

DNA was PCR-amplified with KOD Plus Neo (Toyobo) with primers PsbS1-InF2 (5′-TGA AAC TGG AAT GGG TCC TC-3′) and PsbS1-InR1 (5′-GGC CTT GGT CTT GGA CCT-3′) for 40 cycles. Annealing was at 63 °C and extension was for 5 min. Other conditions followed the standard condition for three-step reaction.

Western Blot Analysis of PsbS. Total protein was extracted from the leaf blade as described previously (13). Protein content was measured with BCA Protein Assay (Thermo Scientific). Protein solution (60 μg each) was loaded on each well and electrophoresed in 15% polyacrylamide gel. Protein was blotted on Immobilon-P (Millipore), blocked, and labeled with rabbit polyclonal anti-PsbS antibody (Agrisera). Membrane was then treated with a second antibody ECL anti-rabbit IgG (HRP-linked, from donkey; Amersham). HRP was reacted with Immobilon Western Chemiluminescent HRP Substrate (Millipore) and detected with ChemiDoc (Bio-Rad). Another gel was prepared under the same condition and stained with Coomassie blue.

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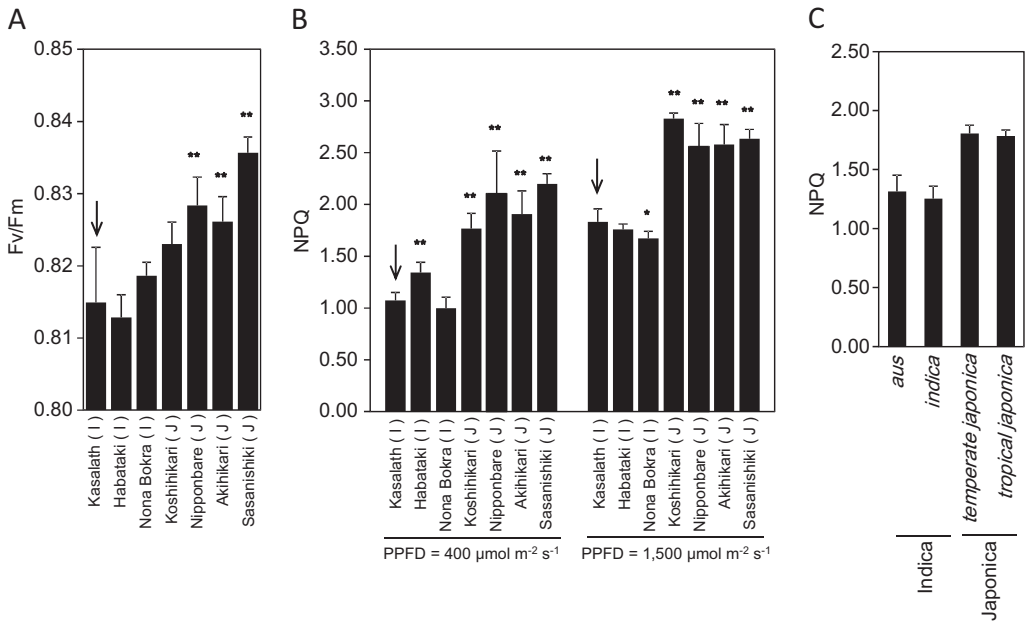

Fig. S1. NPQ values of several cultivars. (*A*) Fv/Fm values of seven cultivars. Subclasses of the cultivars are indicated in parentheses (I, Indica; J, Japonica). (*B*) NPQ values of the same seven cultivars. NPQ was measured under illumination at a PPFD of 400 μmol m^{-2} s^{-1} or 1,500 μmol m^{-2} s^{-1} for 5 min. Fv/Fm and NPQ were measured with fully expanded leaves of 2-mo-old plants in *A* and *B*. Asterisks indicate significant differences from the standard Indica cultivar Kasalath (arrows) by Student *t* test (**P* < 0.05, ***P* < 0.01). (*C*) Average NPQ values of *aus*, *indica*, *temperate japonica*, and *tropical japonica* groups measured in Fig. 2. Three exceptional lines (Local Basmati, Rexmont, and Dianyu1) were excluded from the calculation. Data represent means and SDs (*n* = 3 or *n* = 4 for each line).

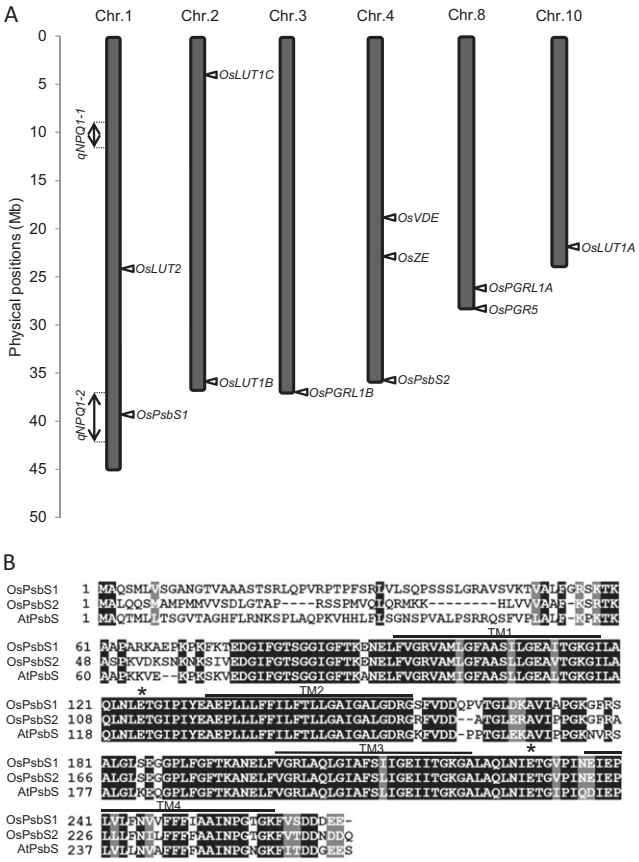

Fig. S2. Location of candidate NPQ regulators on rice chromosome. (*A*) *qNPQ1-1* region, *qNPQ1-2* region, and rice homologues to *Arabidopsis* NPQ regulators are mapped on the chromosomes. Rice homologues include *OsPsbS1* (*Os01g0869800*), *OsPsbS2* (*Os04g0690800*), *OsPGR5* (*Os08g0566600*), *OsPGRL1A* (*Os08g0526300*), *OsPGRL1B* (*Os03g0857400*), *OsVDE* (*Os04g0379700*), *OsZE* (*Os04g0448900*), *OsLUT1A* (*Os10g0546600*), *OsLUT1B* (*Os02g0817900*), *OsLUT1C* (*Os02g0173100*), and *OsLUT2* (*Os01g0581300*). (*B*) Predicted amino acid sequences of AtPsbS, OsPsbS1, and OsPsbS2. Four transmembrane regions are indicated by black lines. These regions were commonly predicted as transmembrane regions by a hydropathy plot obtained with the SOSUI program. Asterisks indicate luminal glutamate residues necessary for PsbS function (E122 and E226 of AtPsbS) (1).

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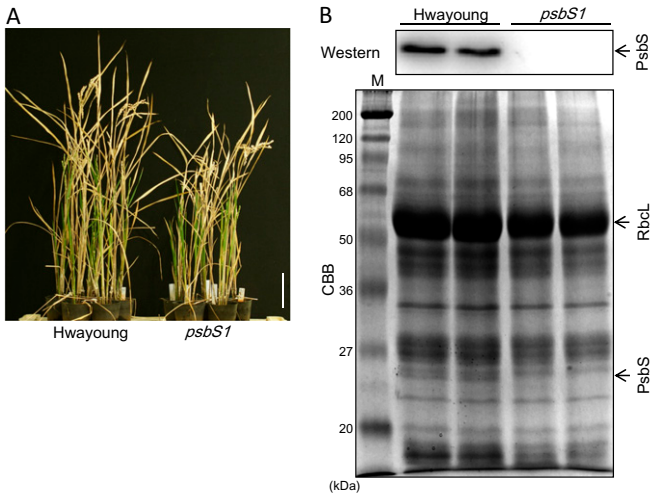

Fig. S3. Appearance and protein accumulation of *psbS1* mutant. (*A*) Mature plants of control (Hwayoung) and *psbS1*. (Scale bar, 10 cm.) (*B*) Western blot analysis of PsbS and CBB staining of total protein extracted from leaf blades of control and *psbS1*. M, molecular weight marker.

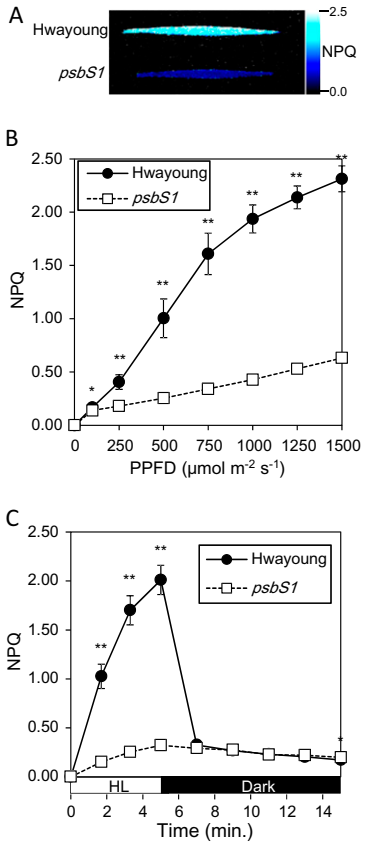

Fig. S4. NPQ properties of *psbS1* mutant. (*A*) Fully expanded third leaves of Hwayoung and *psbS1* were measured. Image of NPQ values under high illumination (PPFD, 1,500 μmol m^{-2} s^{-1}) for 5 min is shown. (*B*) NPQ values under various light intensities. (*C*) NPQ induction by high light (HL) at a PPFD of 1,500 μmol m^{-2} s^{-1} for 5 min and relaxation in the dark for 10 min. Data represent means and SDs. Asterisks indicate significant differences between data by Student *t* test (*$P < 0.05$, **$P < 0.01$); $n = 6$.

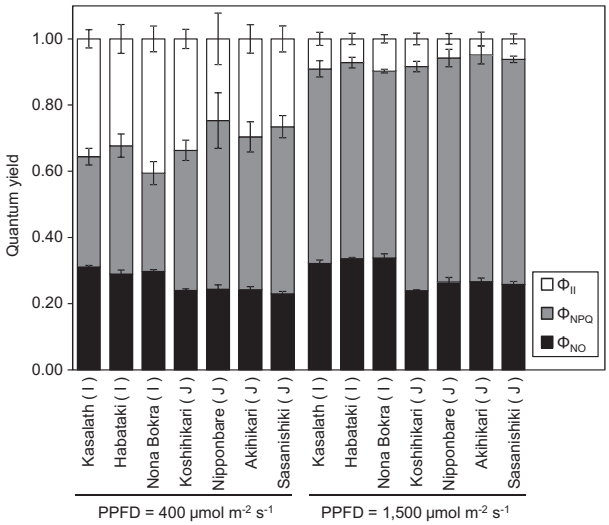

Fig. S5. Quantum yield of deexcitation processes in several cultivars. Φ_{II}, Φ_{NPQ}, and Φ_{NO} were measured simultaneously with NPQ measurement in the seven cultivars in Fig. S1*B* (Φ_{II} represents yield of photosystem II for photochemistry, Φ_{NPQ} represents yield of downregulatory processes via qE, and Φ_{NO} represents yield for other energy losses; ref.7 and *Materials and Methods* provide calculations). Values were calculated under illumination at a PPFD of 400 μmol m^{-2} s^{-1} or 1,500 μmol m^{-2} s^{-1} for 5 min. Data represent means and SDs ($n = 4$).