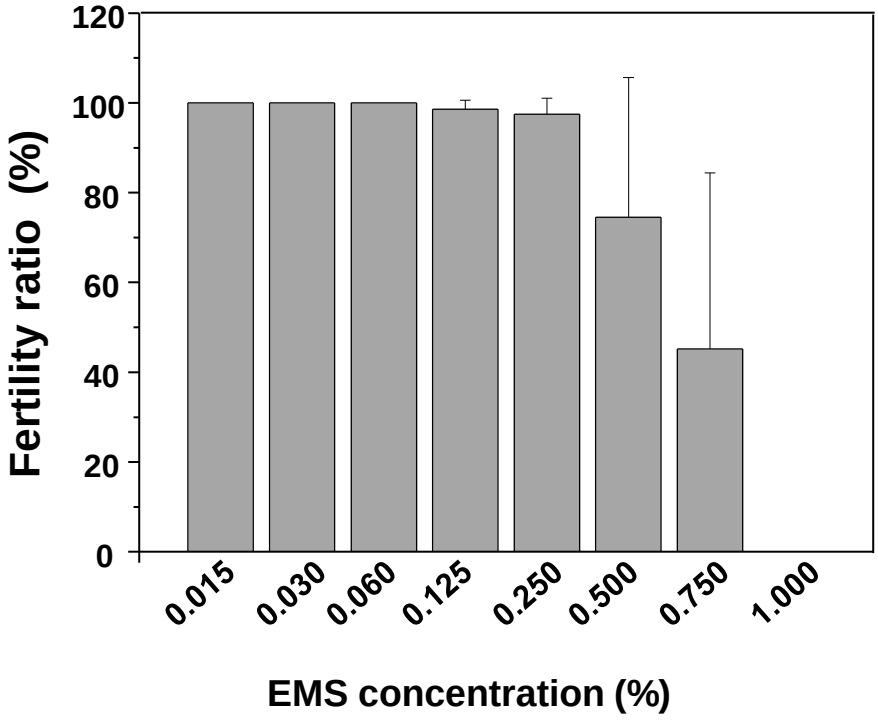
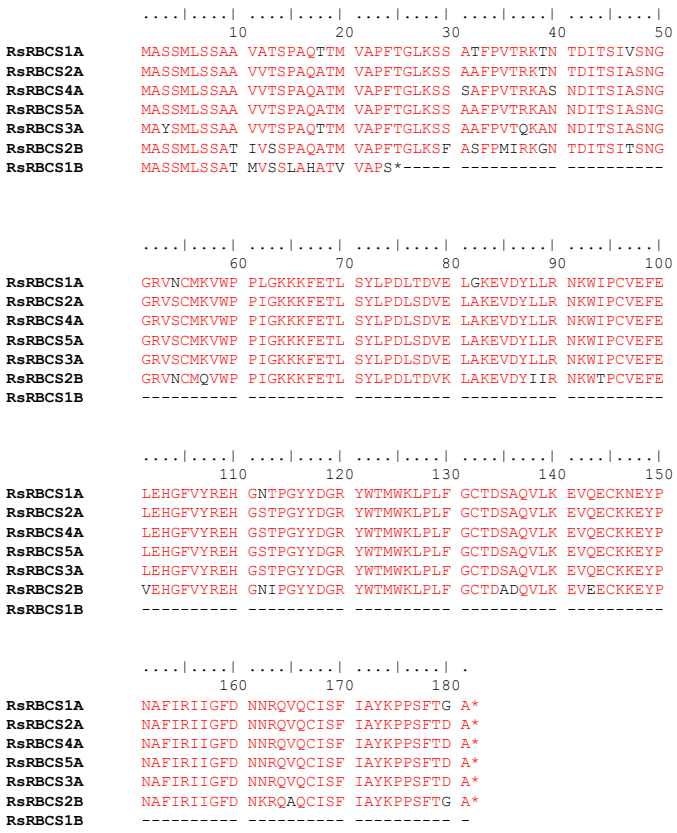


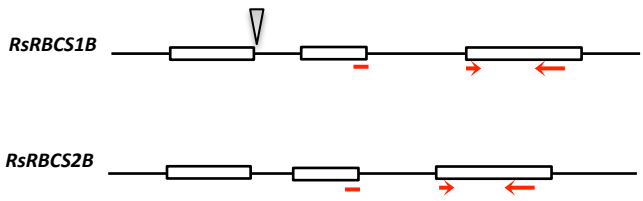
Supplemental Fig. 1. Effects of EMS concentration on the fertility ratio in M_1 radish plants. The fertility ratios of M_1 radish plants from seeds treated with 0.015–1.0% EMS were counted after crossing with wild-type radish. Bars represent SD of the mean (*n*=1–4).

Supplemental Fig. 2. Comparison of genomic sequences of seven *RsRBCS* genes in wild-type radish ('Comet'). The three exons are indicated in red. Black indicates introns and the 5' and 3' UTR. Blue indicates donor and acceptor sites. Asterisks indicate conserved sequences in all genes. Black arrows indicate a point of potential sequence as a pseudogene in *RsRBCS1B*.



Supplemental Fig. 3. Comparison of amino acid sequences of the seven RBCSs in radish. Red indicates conserved amino acid sequences. Asterisks indicate stop codons.

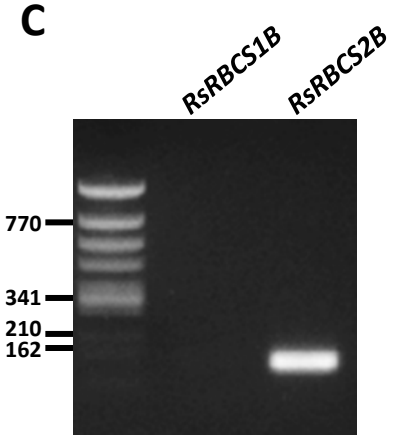
A



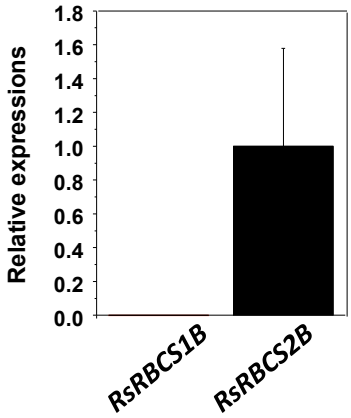
B

Gene	Primer direction	Sequences	Predicted size (bp)
RsRBCS1B	Forward Reverse	5′- CGAGGTTGAGCACGGTTTTGT -3′ 5′- CCACTTCCTTCAACACTTGATC -3′	131
RsRBCS1B	Forward Reverse	5′- CGAGTTGGAGCACGGTTTTGT -3′ 5′- GCACTTCCTTTAACACTTGAGA -3′	131
RsActin2	Forward Reverse	5′- GTACTACCGGTATTGTGCTGGA -3′ 5′- TCTGCTGTTGTGGTGAACATG -3′	175

C



D



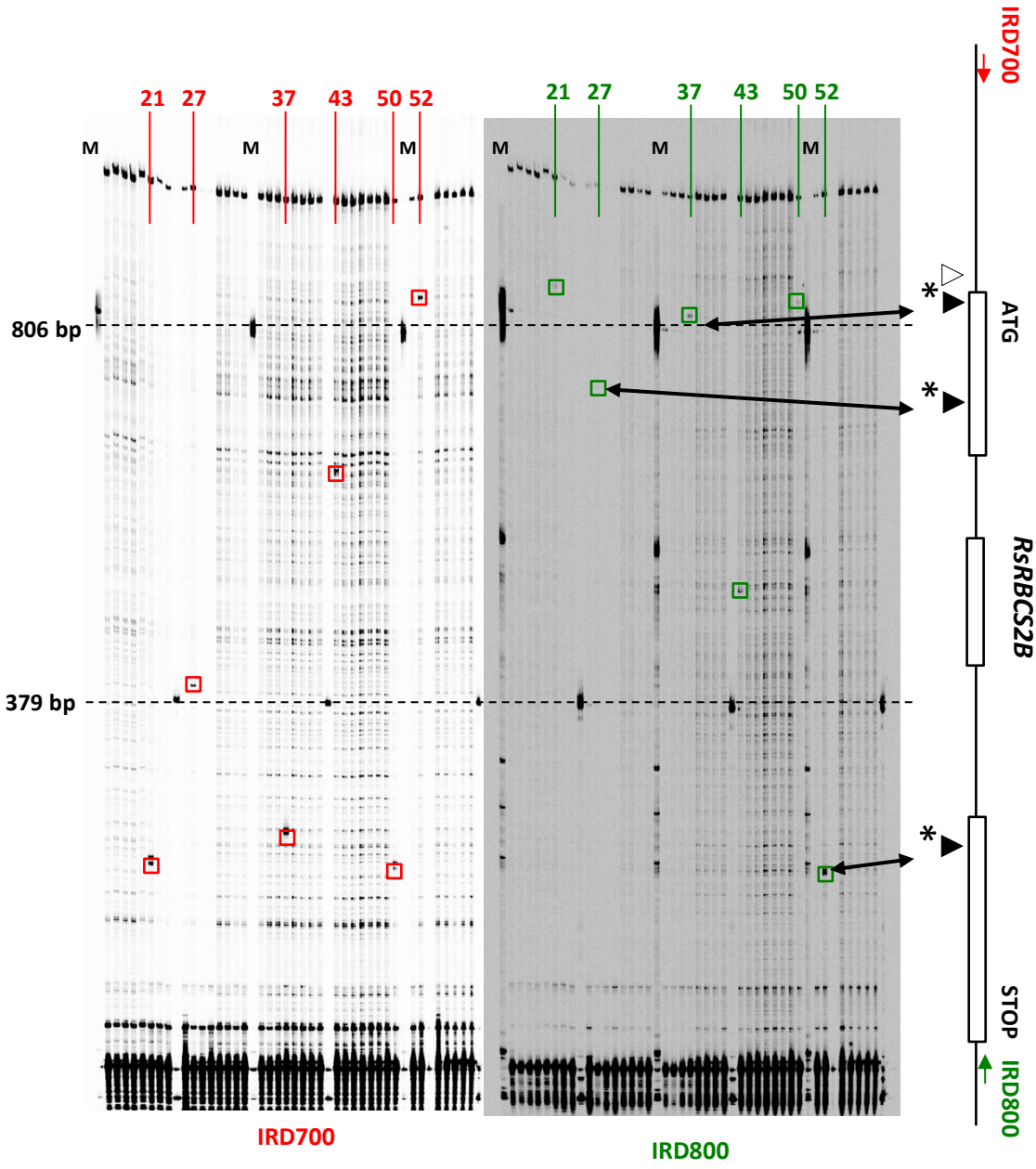
Supplemental Fig. 4. Gene expression analysis of *RsRBCS1B*.

(A) Genomic structures of *RsRBCS1B* and *RsRBCS2B* genes. Red arrows indicate forward and reverse primers for RT-PCR analysis. Gray arrowheads indicate a position of a mutation on a conserved donor site in *RsRBCS2B*. (B) Primer sequences. Primer sets of *RsRBCS1B* and *RsRBCS2B* were used for both RT-PCR and qRT-PCR. The values of gene expression by qRT-PCR were adjusted by *RsActin2*. (C) Gel image of RT-PCR products of *RsRBCS1B* and *RsRBCS2B*. The lengths of DNA size markers are shown. (D) quantitative real-time RT-PCR in *RsRBCS1B* and *RsRBCS2B*. These gene expression data were normalized to *RsActin2* expression. Bars represent SD of the mean (n=3).

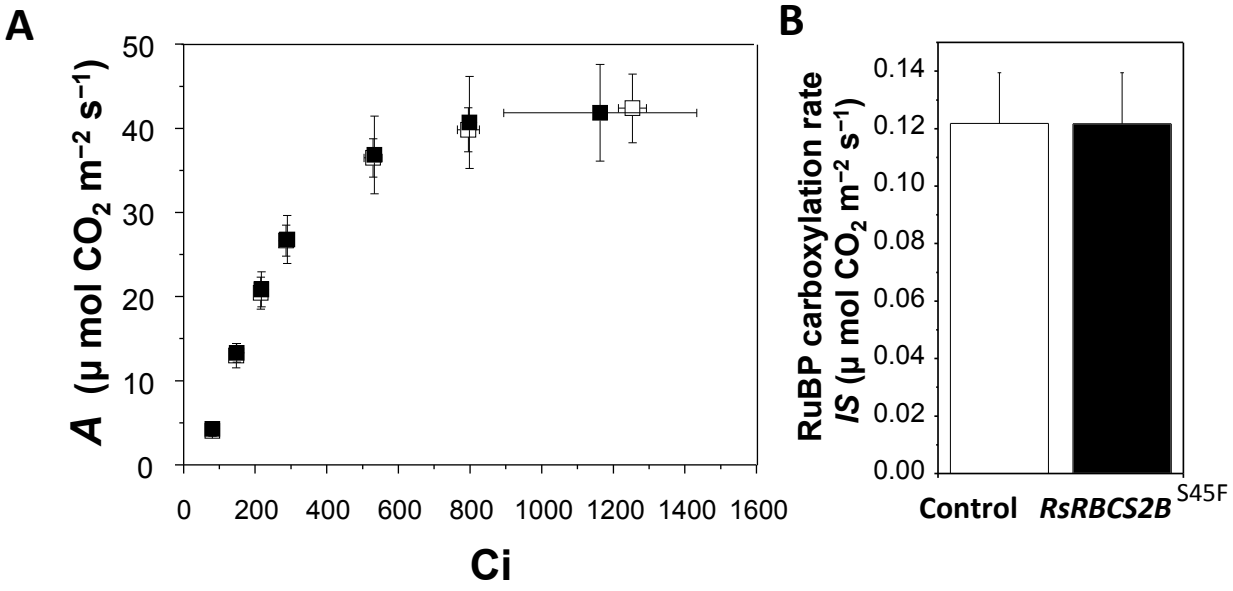
Experimental procedure

Gene expression analysis by RT-PCR and qRT-PCR

Total RNA was isolated and purified using RNeasy Plant Mini Kit (QIAGEN, Germany). The RNA was quantified using Nano Drop 1000 (Thermoscientific, USA). cDNA was synthesized from 100 ng total RNA using the ReverTra Ace qPCR RT Master Mix (Toyobo, Japan). To examine the products of reverse transcription PCR (RT-PCR) by agarose gel electrophoresis, cDNA fragments of *RsRBCS1B* or *RsRBCS2B* was amplified by 30 cycles of PCR (Supplemental Fig. 4C). To quantify amount of mRNA of *RsRBCS1B* or *RsRBCS2B,* quantitative real time reverse transcription (qRT)-PCR was conducted by using the SYBR Green supermix kit (Bio-Rad, USA) and the MyiQ (Bio-Rad, USA) (Supplemental Fig. 4D). The primers used for RT-PCR and qRT-PCR are listed in Supplemental Fig. 4B.



Supplemental Fig. 5. Gel image of mutation detection on a LI-COR DNA Analyzer. Six mismatches of *RsRBCS2B* genes were identified in this gel image. The IRD700 (left) and IRD800 (right) channels are shown. The appearance of bands (red boxes and green boxes) in both channels is consistent with the full-length 1105 bp product. Cleavage sites were roughly estimated using 806 and 379 bp markers. Three mutations were ultimately identified from the six mismatches by direct sequencing.



Supplemental Fig. 6. Photosynthetic phenotypes in *RsRBCS2B*^{S45F}. Control and homozygous mutant plants were selected from BC_1M_2 pool by sequencing. (A) CO_2 assimilation/internal CO_2 (A-Ci) curves of control and *RsRBCS2B*^{S45F} mutant radish plants. (B) RuBP carboxylation rates based on the initial slopes of the A-Ci curve (*IS*), calculated from three Ca (atmospheric CO_2 level): 100, 200, and 300 μmol mol^{-1} CO_2. Mature leaves were measured in control (wild-type) plants (white symbols and bars) and segregated homozygous mutant (*RsRBCS2B*^{S45F}) plants from BC_1M_2 plants (black symbols and bars). Analyses were performed at 35°C and (for A-Ci curves) PPFD of 2000 μ mol m^{-2} s^{-1}. Each points represent SD of the mean (*n*=8).