

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 ($n=1-4$).

RsRBCS1A	AGCA--ATAG	ACAAC--AA	GTAAGAGAA	TTAAGAGAA	AAAA-----	RsRBCS1A	TTGAATTGA	GTTGGAGTA	GTA AAA--A	CAA A-----	-----	
RsRBCS2A	AGCA--ATAG	ACAAC--AA	GTAAG-----	--TAAGAGAA	AA GA-----	RsRBCS2A	TTGAATTGA	GTTGGAGTA	AT AAA--A	CAA A-----	-----	
RsRBCS4A	AA CA--ATAG	ACAAC--AA	GTAAG-----	--TAAGAGAA	AA GA CA AAA	RsRBCS4A	TTGAATTGA	GTTGGAGTA	AT AAA--A	CAA A-----	-----	
RsRBCS5A	AGCA--ATAG	ACAAC--AA	GTAAG-----	--TAAGAGAA	AA GA-----A	RsRBCS5A	TTGAATTGA	GTTGGAGTA	AT AAA--A	CAA A-----	-----	
RsRBCS3A	AGCA--GTAG	ACTAAC--A	GTAAG-----	--TAAGAGAA	CG AA-----	RsRBCS3A	TTGAATTGA	GTTGGAGTA	AT AAA--A	CAA A-----	-----	
RsRBCS2B	AGCACTATAC	TTAAACCTAA	CTCATACAG--	TC AA--A	AAAA-----	RsRBCS2B	TTGAATTGA	GTTGGAGTA	AAAAACTTG	AAAA-----	-----	
RsRBCS1B	AGTCAATAC	CCAACCTAA	CTCATACAG--	TA AAG AAA	AAAA-----	RsRBCS1B	TTGAATTGA	GTTGGAGTA	AAAA--TTG	AAAACTCGTT	TATATATATA	
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RsRBCS1A	AAAGAAGATT	AC-----	TTATGGCTT	CCTCTATGCT	CTCCTCCGCC	RsRBCS1A	-----	-----	AAAAA	ATTC-----	-----TC--	-----AGT
RsRBCS2A	GAAGAAG--T	A-----	GTAATGGCTT	CCTCTATGCT	CTCCTCCGCC	RsRBCS2A	-----	-----	GT-TTT	GTTT-----	-----TCCT	GTATTTAAAC
RsRBCS4A	GAAGAAG--T	ACAAGTGGTA	GTAATGGCTT	CCTCTATGCT	CTCCTCCGCC	RsRBCS4A	-----	-----	ATTTT	GTTT-----	-----TCTT	GGATCTAAAC
RsRBCS5A	GAAGAAG--T	A-----	GTAATGGCTT	CCTCTATGCT	CTCCTCCGCC	RsRBCS5A	-----	-----	ACATTT	TTT-----	-----TTTT	GGATCTAAAC
RsRBCS3A	GAAGAAT--T	A-----	GTCATGGCTT	ACTCTATGCT	CTCCTCCGCC	RsRBCS3A	-----	-----	TTT	TAT-----	-----TTTT	GGATCTAAAC
RsRBCS2B	GTAGAAGGTG	A-----	TATGGCTT	CCTCTATGCT	CTCCTCCGCC	RsRBCS2B	---	ATGAAC	ATTACAAAT	ATTTATAATA	AACGGATCTT	GTAAT--AAT
RsRBCS1B	GA AAAAGAG	A-----	TATGGCTT	CTTCTATGCT	CTCCTCCGCC	RsRBCS1B	TTATATATAT	ATATATATAT	ATCTGGAATA	AGTATATCTT	GTAAT--AAT	*
	* * *	*	*****	*****	* * *					*		*
RsRBCS1A	GCTGTGGTCA	CTTACCACC	TCAAACAACC	ATGGTTGCTC	CATTCACC GG	RsRBCS1A	GTTC-ATTAT	TAC-----	-----	-----	-----ACAAG	
RsRBCS2A	GCTGTGGTCA	CTTACCACC	TCAAACAACC	ATGGTTGCTC	CATTCACC GG	RsRBCS2A	-TTCAATTAT	-----	-----	-----	-----ACCAT	
RsRBCS4A	GCTGTGGTCA	CTTACCACC	TCAAACAACC	ATGGTTGCTC	CATTCACC GG	RsRBCS4A	ATTC-TTAT	TAC-----	-----	-----	-----ACCAT	
RsRBCS5A	GCTGTGGTCA	CTTACCACC	TCAAACAACC	ATGGTTGCTC	CATTCACC GG	RsRBCS5A	ATCC-TTAT	TACTA-----	-----	-----	-----CACCAT	
RsRBCS3A	GCTGTGGTCA	CTTACCACC	TCAAACAACC	ATGGTTGCTC	CATTCACC GG	RsRBCS3A	AACC-TTAT	TAT-----	-----	-----	-----ACCAT	
RsRBCS2B	ACAATTTGCT	CTTCTCCGC	TCAAGCCACA	ATGGTAGCAC	CATTCACC GG	RsRBCS2B	ACTAAGTTG	AGATA-----	---	TGTAAC	TTGA-----	---CAAACCTAT
RsRBCS1B	ACAATTTGCT	CTTCTCCGC	TCAAGCCACA	ATGGTAGCAC	C-TCATAGG	RsRBCS1B	ACTAAGTTAT	AGATAATCTC	CATTGGAAC	TTGTAACCAT	CTCAAACTTAT	*
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RsRBCS1A	CTTGAAGTCA	TCCGCCACAT	TCCCAGTTAC	TCCGAAGACC	AACACTGACA	RsRBCS1A	TT---GAAT	-----	---	CCTAAT	GTTT---AAT	AAAAAC---
RsRBCS2A	CTTGAAGTCA	TCCGCCACAT	TCCCAGTTAC	TCCGAAGACC	AACACTGACA	RsRBCS2A	GT---CAAT	-----	---	CCTGAT	TTTG---AAT	GGTAT----
RsRBCS4A	CTTGAAGTCA	TCCGCCACAT	TCCCAGTTAC	TCCGAAGACC	AACACTGACA	RsRBCS4A	GT---GAAT	-----	---	CTGAA	TTTG---AAT	GGTAT----
RsRBCS5A	CTTGAAGTCA	TCCGCCACAT	TCCCAGTTAC	TCCGAAGACC	AACACTGACA	RsRBCS5A	GT---GAAT	-----	---	TCTTAT	TATG---AAT	GGTACT----
RsRBCS3A	CTTGAAGTCA	TCCGCCACAT	TCCCAGTTAC	TCCGAAGACC	AACACTGACA	RsRBCS3A	GT---GAAT	-----	---	CTTAT	TATG---AAT	GGTACT----
RsRBCS2B	ACTTAAGTCA	TTTGCTTCTT	TCCCAATGAT	CCGCAAGGCC	AACACTGACA	RsRBCS2B	GC---ATAT	-----	---	TTCTG	TGTAGTAAAT	GAAATTAAGG
RsRBCS1B	ACTTAAGTCA	TTTGCTTCTT	TCCCAATGAT	ATGTAAGGCC	AACACTGACA	RsRBCS1B	TCTCAGATAT	CCTACGCAGA	TTTTTTTTTT	TGTAGTAAAT	GAAATTAAGG	*
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RsRBCS1A	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS1A	-----TT	TAT-GTGTGA	AT-----	---	AGCAT	GGATTGTGAT
RsRBCS2A	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS2A	-----	GT-GTATAT	AT-----	---	AGCAT	GGATTGTGCT
RsRBCS4A	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS4A	-----	CT-CAT-AT-GT	AT-----	---	AGCAC	GGATTGTGCT
RsRBCS5A	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS5A	-----	CT-CAT-TTTGT	AT-----	---	AGCAC	GGATTGTGCT
RsRBCS3A	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS3A	-----	CT-CAT-GTGTG	AC-----	---	AGCAC	GGATTGTGCT
RsRBCS2B	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS2B	AATCTCGATT	TATAGTTGTG	ATAATTTTTT	TCTGTAGCAT	AGCATGGGCT	GGATTGTGAT
RsRBCS1B	TAACTTCCAT	CGTAAGCAAT	GGAGGAAGG	TTAACTGCAT	GAAGTAAAC	RsRBCS1B	AGTCTCTATT	TATGGTTGTG	ATAATTTTTT	AT-GTAGCAC	GGTTTGTGCT	*
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RsRBCS1A	ATCACTCGAA	ATTATG---	TACTCCCTC	ATTT--ATGA	TGTG-AACG-	RsRBCS1A	ACCGTGAACA	CGGAACACA	CCCGGATATT	ATGATGGAGC	TTACTGGACA	
RsRBCS2A	ATCACTAGAG	---TCG---	TAGGCCATC	CTATTATCA	TGTGGAAAC-	RsRBCS2A	ACCGTGAGCA	CGGAAGCACA	CCCGGATATT	ATGATGGAGC	TTATTTGGACA	
RsRBCS4A	GTCATAGAG	TAA-TTG---	CAGCCATCC	CTTT--ATTA	CACGCCAAC-	RsRBCS4A	ACCGTGAGCA	CGGAAGCACA	CCCGGATATT	ATGATGGAGC	TTATTTGGACA	
RsRBCS5A	GTCATAGAG	TAA-TTG---	CAGCCATCC	CTTT--ATTA	CACGCCAAC-	RsRBCS5A	ACCGTGAGCA	CGGAAGCACA	CCCGGATATT	ATGATGGAGC	TTACTGGACA	
RsRBCS3A	ACCACTAGCA	AGTCTTGAAT	TAGCCCTTTC	TTTT--ATTA	CACGCCAACG	RsRBCS3A	ACCGTGAGCA	CGGAAGCACA	CCCGGATATT	ATGATGGAGC	TTACTGGACA	
RsRBCS2B	-----	-----	-----	C-AT-----	AC-----	RsRBCS2B	ACCGTGAGCA	TGGAACATC	CCCGGATATT	ATGATGGAGC	ATACTGGACA	
RsRBCS1B	-----	-----	-----	ATTT-----	AC-----	RsRBCS1B	ACCGTGAGCA	CGGAACATC	CCCGGATATT	ATGATGGAGC	ATACTGGACA	
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RsRBCS1A	---TGGTACA	TTTGAACAT	GAGTCCGATT	GAAAAACATA	TATA-ATGTA	RsRBCS1A	ATGTGGAAGC	TCCCTTATT	CGGATGCACT	GACTCTGCTC	AAGTGTAA	
RsRBCS2A	---TGGTACA	TGTGAA-CAT	GAGTCCGATT	AAACGATATA	TAATGACGTA	RsRBCS2A	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GACTCTGCTC	AAGTGTAA	
RsRBCS4A	---TGGTGCA	TCCAAC-CAT	GAACTCCATT	GAAAAATGTA	TATA-ACGTA	RsRBCS4A	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GACTCTGCTC	AAGTGTAA	
RsRBCS5A	---TGGTGCA	TCCAAC-CAT	GAACTCCATT	GAAAAATGTA	TATA-ACGTA	RsRBCS5A	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GACTCTGCTC	AAGTGTAA	
RsRBCS3A	AAGTGTACA	TTCGAA-CA	GAACTCCATT	CAAGAAATATA	TATATAGATA	RsRBCS3A	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GACTCTGCTC	AAGTGTAA	
RsRBCS2B	-----ATA	TTT---CAT	ATACC-----	AAAAATAAA	TTGA-----	RsRBCS2B	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GACCGAGATC	AAGTGTAA	
RsRBCS1B	-----ATA	TTT---CAT	ATACC-----	AATA-TAAG	TTGA-----	RsRBCS1B	ATGTGGAAGC	TTCCCTTATT	CGGATGCACT	GTGTGAGTCC	AAGTGTAA	
		*	*	**	*		*****	*	*****	*	*****	*
RsRBCS1A	GCTTGGATTG	ATAAGTGTG	TAATCAATAT	ATATATAAAT	AAAAGTTGAT	RsRBCS1A	AGAAGTCAA	GAGTGCAAGA	ACGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS2A	CCTTGGATTG	AAAA-FGT-	C--TAATAT	ATATACAAA	AAGAAITCCCT	RsRBCS2A	GAAGTGCRA	GAGTGCAAGA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS4A	CCTAGGATCA	ATAAA-FGT-	CCATAAAAAT	ATATACAAA	GTGACTTAGT	RsRBCS4A	GAAGTGCRA	GAGTGCAAGA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS5A	CCTAGGATCA	ATAAA-FGT-	CCATAAAAAT	ATATACAAA	GTGACTTAGT	RsRBCS5A	GAAGTGCRA	GAGTGCAAGA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS3A	CCTAGGATCA	TTAAA-AGG-	TCTAACAGT	ATATATAAAA	GTGACTTAGT	RsRBCS3A	GAAGTGCRA	GAGTGCAAGA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS2B	---TGGTTT	TTAAT-----	---TCCGAAT	---TTACAAA	CTAATATTG	RsRBCS2B	GAAGTGGAG	GAGTGCRAA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
RsRBCS1B	---TGGTTT	TTAAG-----	---TCTGAAT	---TATCAAAA	CTAATATTG	RsRBCS1B	GAAGTGCRA	GATTGCAAGA	AGGAGTACC	TAACGCCCTC	ATTAGAATCA	
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RsRBCS1A	ATTTATTAAT	TGAAATATT	ATTT---AT	AGGTGTGGC	ACCACTGGGA	RsRBCS1A	TTGGATTGCA	TAACAACCGT	CAAGTCCAAT	GCATCAGTTT	TATTGCTTAC	
RsRBCS2A	ATTTATTAAT	TGAAATATT	ATTT---AT	AGGTGTGGC	ACCACTGGGA	RsRBCS2A	TTGGATTGCA	CAACAACCGT	CAGTCCAAT	GCATCAGTTT	CATCGCTTAC	
RsRBCS4A	ATTCACCAAC	TGGAAATGCG	ATTT---AT	AGGTGTGGC	GCCAAATGGGA	RsRBCS4A	TCGGATTGCA	CAACAACCGT	CAAGTCCAAT	GCATCAGTTT	CATCGCTTAC	
RsRBCS5A	ATTCACCAAC	TGGAAATGCG	ATTT---AT	AGGTGTGGC	GCCAAATGGGA	RsRBCS5A	TCGGATTGCA	CAACAACCGT	CAGTCCAAT	GCATCAGTTT	CATCGCTTAC	
RsRBCS3A	ATTCATTAAT	TGGAAACAGC	ATTT---GT	AGGTGTGGC	ACCAATTTGGA	RsRBCS3A	TCGGATTGCA	CAACAACCGT	CAAGTCCAAT	GCATCAGTTT	CATCGCTTAC	
RsRBCS2B	---TTGATGAT	TGGTAAGGAA	C-----	AGGTGTGGC	TCCATTTGGG	RsRBCS2B	TCGGATTGCA	CAACAACCGT	CAAGTCCAAT	GCATCAGTTT	CATCGCTTAC	
RsRBCS1B	ATTTGTTAAT	TGGTAAAAA	AA-----	AGGTGTGGC	TCCGTTGGG	RsRBCS1B	TCGGATTGCA	TAACAACCTG	CAAGTCCAAT	GTGTGAGTCC	CATCGCTTAC	
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RsRBCS1A	AAAAAGAAAT	TTGAGACTCT	CTCTTACCTT	CCTGACCTTA	CTGACGTTGA	RsRBCS1A	AAGCCACCAA	GTTCCACCGG	TGCTTAA	TTT	CTTTTCTGA	AACATTCATA
RsRBCS2A	AAGAAAGAA	TTGAGACTTT	ATCTTATCTT	CCTGACCTTA	GTGACGTTGA	RsRBCS2A	AAGCCACCAA	GTTCCACCGA	TGCTTAA	TCA	C-CTTTCTAA	AACATTCATA
RsRBCS4A	AAGAAAGAA	TTGAGACCTT	CTCTTACCTT	CCTGACCTTT	CGCATGTTGA	RsRBCS4A	AAGCCACCAA	GTTCCACCGA	TGCTTAA	TC	CGTTACAA	GACATTCATA
RsRBCS5A	AAGAAAGAA	TTGAGACCTT	CTCTTACCTT	CCTGACCTTT	CGCATGTTGA	RsRBCS5A	AAGCCACCAA	GTTCCACCGA	TGCTTAA	GT-	--TGCTAT--	GTCAATTCATA
RsRBCS3A	AAGAAAGAA	TTGAGACCTT	CTCTTACCTT	CCTGACCTTT	CGCATGTTGA	RsRBCS3A	AAGCCACCAA	GTTCCACCTGA	TGCTTAA	GT-	--CG-----	--CGTCCATA
RsRBCS2B	AAAAAGAA	TTGAAACTCT	CTCTTACCTT	CCTGACCTTA	CGCATGTTAA	RsRBCS2B	AAGCCACCAA	GTTCCACTGG	TGCTTAA	TTA	CACAGCTTCA	TTGCTTTGTG
RsRBCS1B	AAGAAAGAA	TTGAGACTCT	CTCTTACCTT	CCTGACTTTA	CGCATGTTGA	RsRBCS1B	AAGCCACTAA	GGCTCTCCGA	TGCTTAA	TTG	CACACTTTTG	AATCTTTGTG
	**	*****	*****	*****	*****		*****	*	*****	*	*****	*
RsRBCS1A	ATTGGTAA	GAAGTTGACT	ATCTTCTCCG	CAACAAGTGG	ATTCCTTGGG	RsRBCS1A	T---AAAAAT	TTGTACGCAT	CTTCTGTCT	A-TTG--TCT	GTC--TTTTT	
RsRBCS2A	ATTGGCTAAA	GAAGTTGACT	ACCTTCTCCG	CAACAAGTGG	ATTCCTTGGG	RsRBCS2A	T---AATAAT	CTACTCTCAT	-TTCATTTCC	AGTTG--CCT	GTT--TTTA	
RsRBCS4A	ATTGGCTAAA	GAAGTTGACT	ACCTTCTCCG	CAACAAGTGG	ATTCCTTGGG	RsRBCS4A	---AATAAT	ATCTACTCAT	-TTCATTTCC	ATGTG--TCT	GTT--TCTTT	
RsRBCS5A	ATTGGCTAAA	GAAGTTGACT	ACCTTCTCCG	CAACAAGTGG	ATTCCTTGGG	RsRBCS5A	TAATAATAAT	CCCTGCTCAT	-TTCATTTCC	A-AT--TCT	GTT--TCTTT	
RsRBCS3A	ATTGGCTAAA	GAAGTTGACT	ACCTTCTCCG	CAACAAGTGG	ATTCCTTGGG	RsRBCS3A	T---AATAAT	ATCTTCTCAT	-TTCATTTTC	A-ATAAGCT	TTGCTTTGTT	
RsRBCS2B	ACTGGCTAAG	GAAGTTGACT	ATATTATCCG	CAATAAGTGG	ACTCCATGCG	RsRBCS2B	TA--AACCAAC	AAAACCTTAT	--CCTCTTCC	ACCT--TTG	ATT--TATCAT	
RsRBCS1B	AATAGCTAAG	GAAGTTGACT	ACCTTATCCG	CAACAAGTGG	ATTCATGTTA	RsRBCS1B	TA--AACCAAC	AAAACCTTAT	--CCTCTTCC	ACCT--TTG	ATT--TATCAT	
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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*.

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.....|.....|.....|.....|.....|.....|
      10      20      30      40      50
RsRBCS1A MASSMLSSAA VATSPAQTTM VAPFTGLKSS ATFPVTRKTN TDITSIVSNG
RsRBCS2A MASSMLSSAA VVTSPAQATM VAPFTGLKSS AAFPVTRKTN TDITSIASNG
RsRBCS4A MASSMLSSAA VVTSPAQATM VAPFTGLKSS SAFPVTRKAS NDITSIASNG
RsRBCS5A MASSMLSSAA VVTSPAQATM VAPFTGLKSS AAFPVTRKAN NDITSIASNG
RsRBCS3A MAYSMLSSAA VVTSPAQTTM VAPFTGLKSS AAFPVTQKAN NDITSIASNG
RsRBCS2B MASSMLSSAT IVSSPAQATM VAPFTGLKSF ASFFMIRKGN TDITSITSNG
RsRBCS1B MASSMLSSAT MVSSLAHATV VAPS*-----

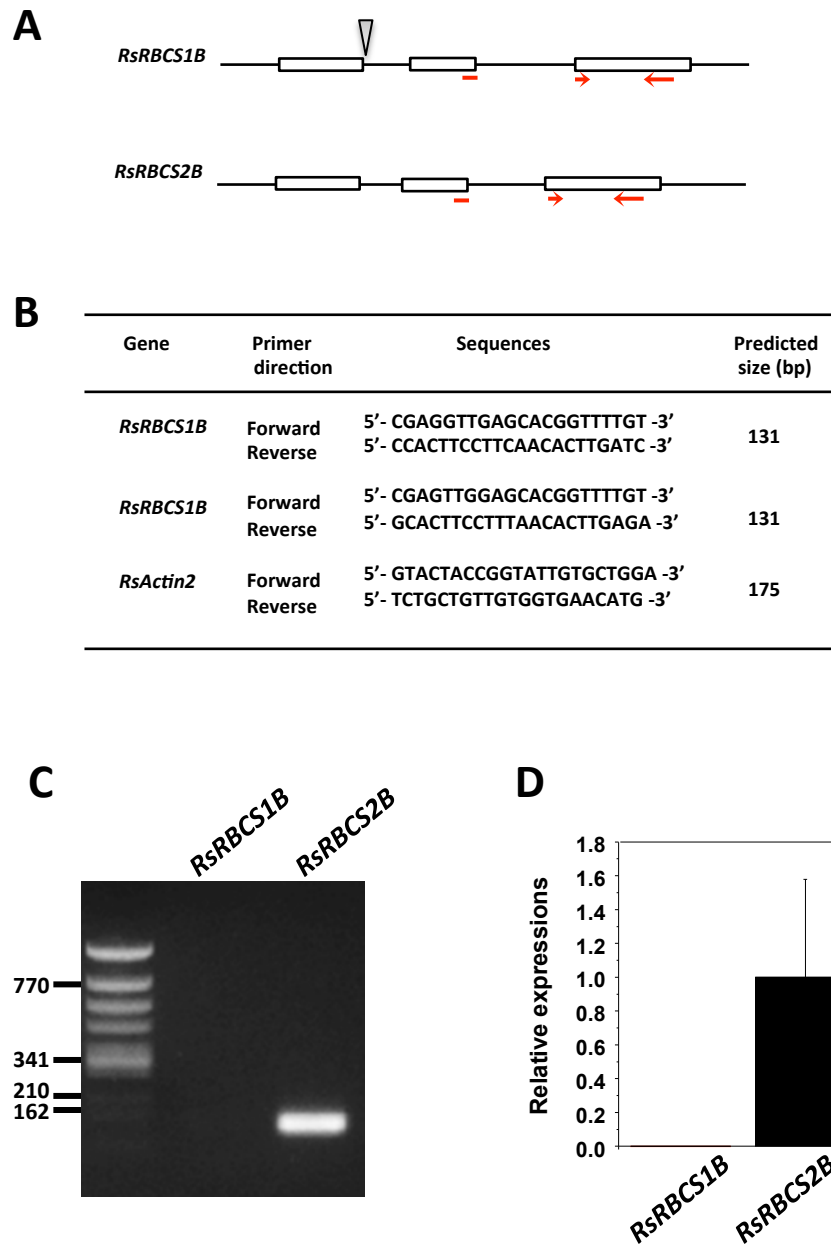
.....|.....|.....|.....|.....|.....|
      60      70      80      90     100
RsRBCS1A GRVNCMKVWP PLGKKKFETL SYLPDLTDVE LGKEVDYLLR NKWIPCVEFE
RsRBCS2A GRVSCMKVWP PIGKKKFETL SYLPDLSDVE LAKEVDYLLR NKWIPCVEFE
RsRBCS4A GRVSCMKVWP PIGKKKFETL SYLPDLSDVE LAKEVDYLLR NKWIPCVEFE
RsRBCS5A GRVSCMKVWP PIGKKKFETL SYLPDLSDVE LAKEVDYLLR NKWIPCVEFE
RsRBCS3A GRVSCMKVWP PIGKKKFETL SYLPDLSDVE LAKEVDYLLR NKWIPCVEFE
RsRBCS2B GRVNCMQVWP PIGKKKFETL SYLPDLTDVK LAKEVDYIIR NKWTPCVEFE
RsRBCS1B -----

.....|.....|.....|.....|.....|.....|
      110     120     130     140     150
RsRBCS1A LEHGFVYREH GNTPGYYDGR YWTMVKLPLF GCTDSAQVLK EVQECKNEYF
RsRBCS2A LEHGFVYREH GSTPGYYDGR YWTMVKLPLF GCTDSAQVLK EVQECKKEYP
RsRBCS4A LEHGFVYREH GSTPGYYDGR YWTMVKLPLF GCTDSAQVLK EVQECKKEYP
RsRBCS5A LEHGFVYREH GSTPGYYDGR YWTMVKLPLF GCTDSAQVLK EVQECKKEYP
RsRBCS3A LEHGFVYREH GSTPGYYDGR YWTMVKLPLF GCTDSAQVLK EVQECKKEYP
RsRBCS2B VEHGFVYREH GNIPGYDGR YWTMVKLPLF GCTDADQVLK EVEECKKEYP
RsRBCS1B -----

.....|.....|.....|.....|.....|.....|
      160     170     180
RsRBCS1A NAFIRIIGFD NNRQVQCISF IAYKPPSFTG A*
RsRBCS2A NAFIRIIGFD NNRQVQCISF IAYKPPSFTD A*
RsRBCS4A NAFIRIIGFD NNRQVQCISF IAYKPPSFTD A*
RsRBCS5A NAFIRIIGFD NNRQVQCISF IAYKPPSFTD A*
RsRBCS3A NAFIRIIGFD NNRQVQCISF IAYKPPSFTD A*
RsRBCS2B NAFIRIIGFD NKRQAQCISF IAYKPPSFTG A*
RsRBCS1B -----

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



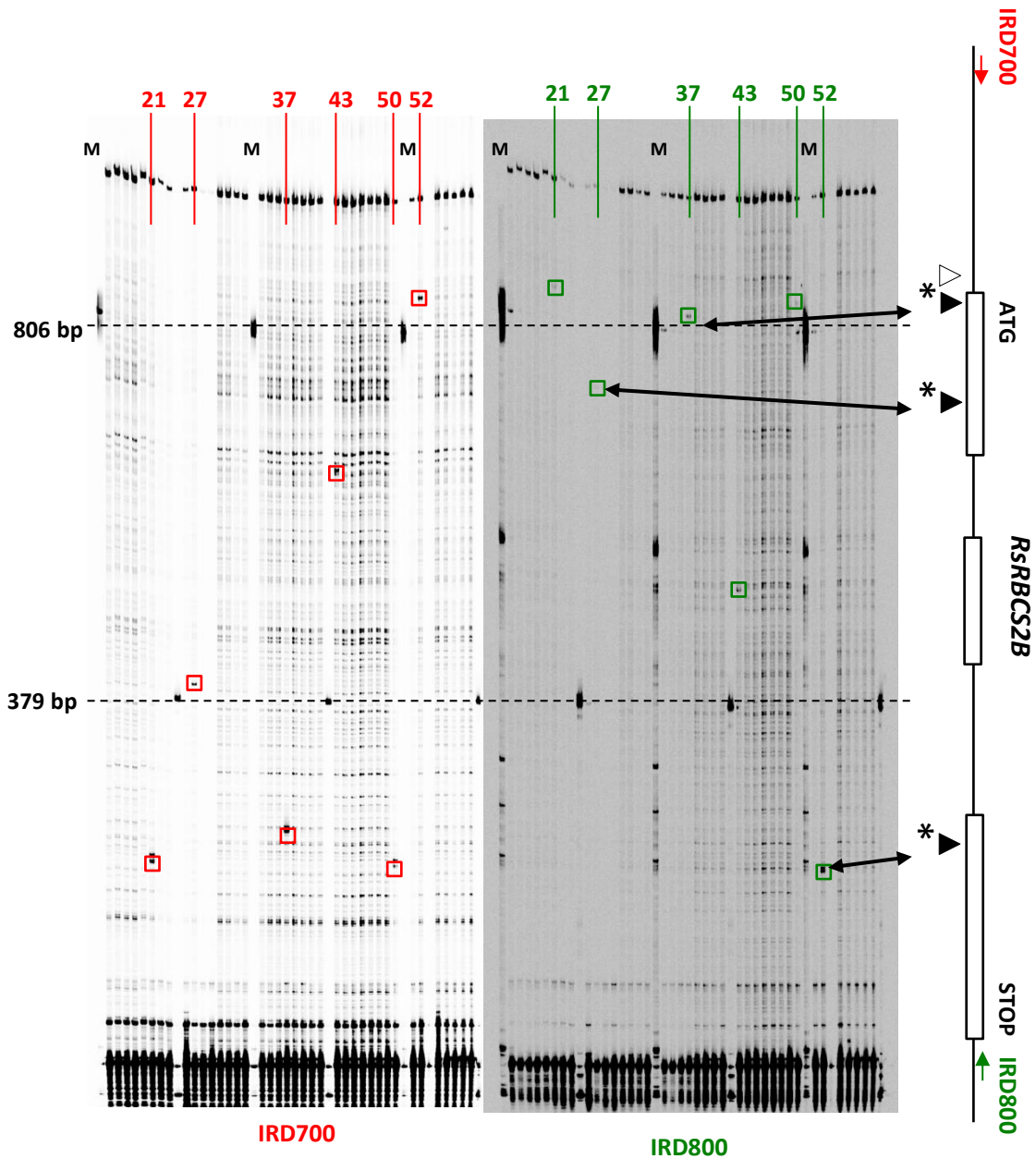
Supplemental Fig. 4. Gene expression analysis of *RsRBCS1B*.

(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 ($n=3$).

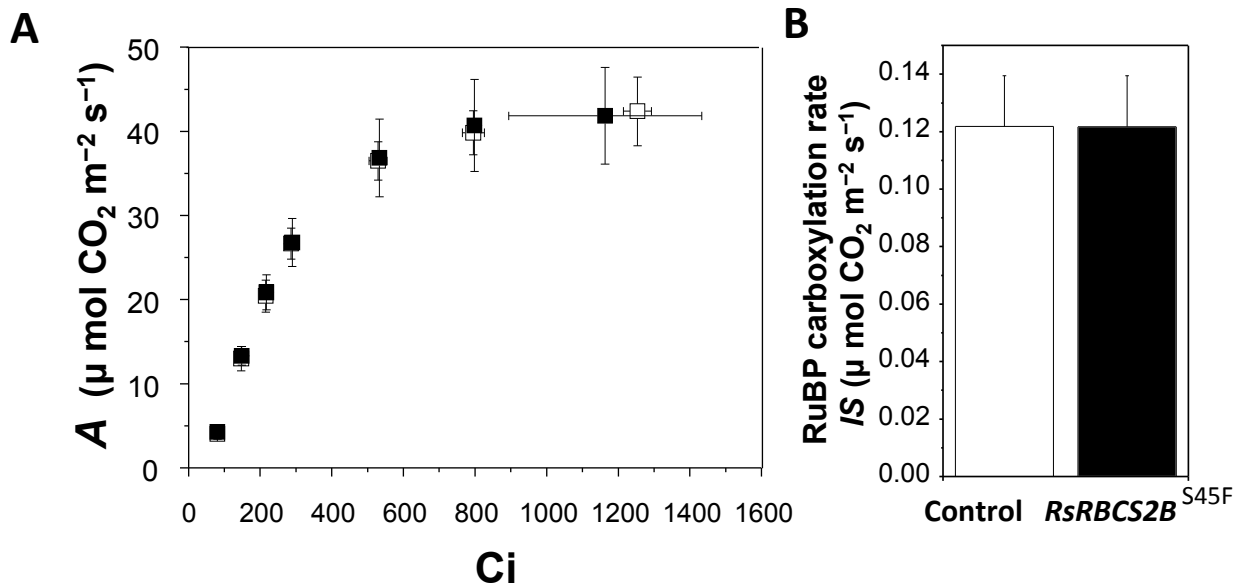
Experimental procedure

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 BC₁M₂ pool by sequencing. (A) CO₂ assimilation/internal CO₂ (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 CO₂ level): 100, 200, and 300 μmol mol⁻¹ CO₂. Mature leaves were measured in control (wild-type) plants (white symbols and bars) and segregated homozygous mutant (*RsRBCS2B^{S45F}*) plants from BC₁M₂ plants (black symbols and bars). Analyses were performed at 35°C and (for A-Ci curves) PPFD of 2000 μmol m⁻² s⁻¹. Each points represent SD of the mean (*n*=8).