

**Supplemental Table 1.**  
**Identification of *RsRBCS* genes**

Genome source	Contig	Blast hit region	Direction	Sequence of forward primer (5'-3') <sup>a</sup> Sequence of reverse primer (5'-3')	PCR amplification <sup>b</sup>	Functional / Pseudo	Gene Name	TILLING Length (bp)
Kazusa <sup>c</sup>	Rsa1.0_00756.1	56975-57776	+	GGACCTTTGTTCACTAGGGCTTTGCC GCAGGAACAGTGCATTCTGTTCGAG	+	Functional	<i>RsRBCS1A</i>	1224
Kazusa	Rsa1.0_01047.1	7699-8498	+	CCACGTGGCATTATTTAGTGGTAAAACCGATA GGATAAAAGTTTGTGTTACACAAAGCAATGAAGCTG	+	Functional	<i>RsRBCS2B</i>	1105
Kazusa	Rsa1.0_01369.1	9382-8570	-	GTGGTCAAGTATCGATAAGGGTATCACACCCG TGACTGTTTCTTATCCAATAAATTGAGAACCTAATAGC	+	Functional	<i>RsRBCS3A</i>	1180
Kazusa	Rsa1.0_01446.1	39634-40486	+	AAAAGCCCAATCTAAGTTCAATTGGGCC CCATCATAGATTTCACTGGGTCATCTTTACTTC	+	Pseudo	<i>RsRBCS1B</i>	-
Kazusa	Rsa1.0_04010.1	1683-1050	-	GTGGCAGGTAACGACGTTATCATCAGCC TATGTCATAATCAGACATTGACAATCGG	-	-	-	-
Kazusa	Rsa1.0_06054.1	10467-11271	+	CCTTGATCATTAGGGATTATACCTCTTGCGG CCCATCGCGTACCTATTGGTTTGAA	+	Functional	<i>RsRBCS4A</i>	1172
Kazusa	Rsa1.0_06054.1	794-1589	+	GGAGGCCAGCAGTAAATCAGTAAGTCAGTAACCA TGAAAACCTAATGCCATCAGAAAGTAACCAAAGACG	+	Functional	<i>RsRBCS5A</i>	1166
MSU <sup>d</sup>	RrC23820	1288-487	-	CGCACTTTGATCATTAGGGTTATGCC CGAAAAAATACCAAGAAAAAGAAGCGATGCG	+	Functional	<i>RsRBCS2A</i>	1082
MSU	RrC6617	8265-7495	-	ATCACAGTGGCCTGTATTGATAAGGGTGCC CGATAGAAAATGTCCTGAAACGTAACCC	-	-	-	-
MSU	RrC9110	1285-2093	+	ATTAGGGATTATAACCTCTTGCGGTTCTCAC GTTCTAACGAATATGGCTATTCATTAG	-	-	-	-

<sup>a</sup> The primer sequences were used for both of gene identification and TILLING. <sup>b</sup> “+” indicates that the region was amplified by PCR, “-” was not. <sup>c</sup> Kazusa (Kazusa DNA Research Institute; <http://radish.kazusa.or.jp/>). <sup>d</sup> MSU (Michigan State University; <http://radish.plantbiology.msu.edu>).

**Supplemental Table 2.**  
**Putative chromosome positioning in seven *RBCSs* in Radish**

Gene ID	Similar gene in <i>B. rapa</i>	Homolo- gy (%)	Chromosome ( <i>B. rapa</i> )	Putative Chromosome Position in <i>R. sativus</i>
<i>RsRBCS1A</i>	LOC103850112	85	A02	LG1 /LG4
<i>RsRBCS2A</i>	LOC103864160	93	A04	LG3
<i>RsRBCS4A</i>	LOC103863779	92	A04	LG3
<i>RsRBCS5A</i>	LOC103863779	92	A04	LG3
<i>RsRBCS3A</i>	LOC103863779	88	A04	LG3
<i>RsRBCS2B</i>	LOC103852369	90	A02	LG1/LG4
	LOC103852368	90	A02	LG1/LG4
<i>RsRBCS1B</i>	LOC103852372	87	A02	LG1 /LG4

**Supplemental Table 3.**  
**Mutations and amino acid substitutions of *RBCS***  
**genes in the Radish mutant population**

Gene ID	Position from ATG	Amino acid change	Concentration of EMS (%)
<i>RsRBCS2A</i>	G310A	G104R	0.25
<i>RsRBCS5A</i>	C-83T	-	0.25
<i>RsRBCS5A</i>	G-9A	-	0.25
<i>RsRBCS2B</i>	C11T	S4F	0.50
<i>RsRBCS2B</i>	C134T	S45F	0.50
<i>RsRBCS2B</i>	G352A	D118N	0.50