

**Supplemental table S1 Primers in the experiment**

Primer name	Primer sequence(5'-3')	Usage
Rc 1-Forward	GGCAGGGGCGGGAAAGGCGCAAG	<i>Rc\rc</i> Construction of target site
Rc 1-Reverse	AAACCTTGCGCCTTTCCCGCCCC	
Rc 2-Forward	GGCACCAACCATGTGCTGAAAGAG	
Rc 2-Reverse	AAACCTCTTTCAGCACATGGTTGG	
pRGEb32-3-F	CTGGGTACGTTGGAAACCAC	Colony PCR verification for pRGEb32 vector
pRGEb32-3-R	CGGCCCAAATTGAAAAGATA	
Hpt-F	TACACAGGCCATCGGTCCAGA	Hygromycin gene detection
Hpt-R	TAGGAGGGCGTGGATATGTC	
Cas9-3-F	TCCTGGAAAAGATGGACGGC	Cas9 sequence detection
Cas9-3-R	ATCCGCTCGATGAAGCTCTG	
UBI-1-F	ACGGAGACAAACGGCATCTT	UBI Promoter sequence detection
UBI-1-R	AGGAGAGGAGAAGCAGCGTA	
Rc-Test-5F	TCAAAAGCACCCCTTGTTCCA	Sequencing to verify target site sequence for <i>Rc\rc</i> gene
Rc-Test-5R	TGGTTGGCACTGAAATCACC	
Rc-Test-6F	CTGAAGGAAGTGATGACAACAAGACC	
Rc-Test-6R	CTAGATTCATGCTGCCATTAGTGAGG	
1-off-F	GTCGTAAAGGTGCATGTTAT	Off-target detection
1-off-R	GCGATCTGGATGGGAAGG	
2-off-F	CAACTGACATTGACCACCAG	
2-off-R	TCACAGGGCTTAGCGAGA	
M13(-47)	CGCCAGGGTTTTCCAGTCACGAC	Bi-allele mutation sequencing primer for pMD19-T vector
M13(-48)	CACACAGGAAACAGCTATGAC	

**Supplemental Table S2 Potential off-target site detection**

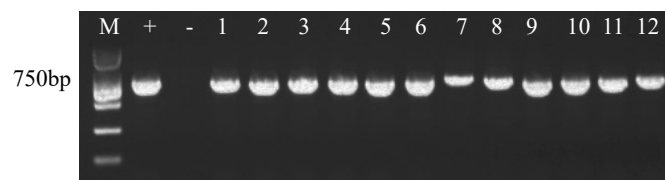
Name of putative off-target sites	Putative off-target locus	Putative off-target sequence	No. of mismatch bases	No. of plants examined	No. of indel mutation
1-off	Chr1: 20008744	GGgAGGGGaGGGAAGGCGgAgGCGG	4	4	0
2-off	Chr11: 3952492	GGCAaGGcCGGGgAAGGCGCAAtCGG	4	4	0

Note: Two potential off-target sites were screened through the CRISPR RGEN Tools website (<http://www.rgenome.net/>). Lower case bases are supposed mismatched bases.

**Supplemental table S3 Potential off-target sequencing primers**

Putative off-target locus	Forward primer sequence	Reverse primer sequence
Chr1: 20008744	GTCGTAAAGGTGCATGTTAT	GCGATCTGGATGGGAAGG
Chr11: 3952492	CAACTGACATTGACCACCAG	TCACAGGGCTTAGCGAGA

Note: Sequencing primers were designed for the two potential off-target sites.



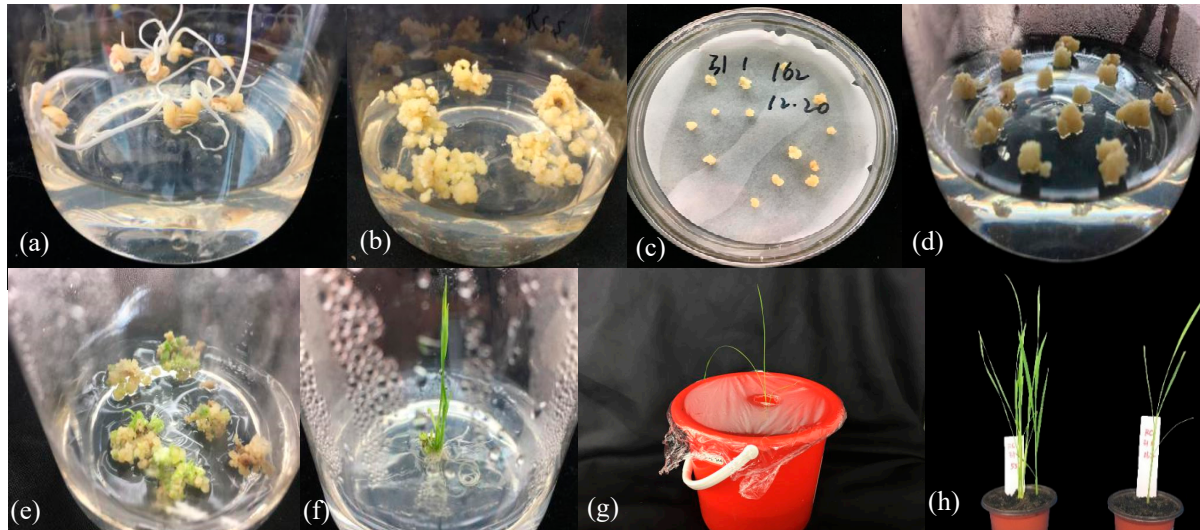
**Supplemental Figure S1 Construction of CRISPR/Cas9-Rc mutation vector**

Note: “M” refers to D2000 marker; “+” refers to the plasmid as positive control; “-” refers to ddH<sub>2</sub>O as negative control; 1-6 refers to pRGEB32-Rc 1 monoclonal bacteria, and 7-12 refers to the pRGEB32-Rc 2 monoclonal bacteria, respectively.

gRNA spacer-Rc1	.....GGCAGGGGCGGGAAAGGCGCAAG.....
pRGEB32 Rc 1	AAGAGTTGTGCAGATGATCCGTGGCAGGGGCGGGAAAGGCGCAAGGTTTTAGAGCTAGAAATAG
pRGEB32	AAGAGTTGTGCAGATGATCCGTGGCAGGAGACCGAGG...TCTCGGTTTTAGAGCTAGAAATAG
gRNA spacer-Rc2	.....GGCACCAACCATGTGCTGAAAGAG.....
pRGEB32 Rc 2	AAGAGTTGTGCAGATGATCCGTGGCACCAACCATGTGCTGAAAGAGGTTTTAGAGCTAGAAATAG
pRGEB32	AAGAGTTGTGCAGATGATCCGTGGCAGGAGACCGAGGTCT...CGGTTTTAGAGCTAGAAATAG

**Supplemental Figure S2 Sequence alignment of pRGEB32-Rc mutation vector  
and the original plasmid pRGEB32**

Note: “gRNA spacer-Rc 1” and “gRNA spacer-Rc 2” refer to target site 1 and 2 on *Rc* gene, respectively.



**Supplemental Figure S3 Genetic transformation of *Agrobacterium tumefaciens* using seed mature embryo**

Note: (a): induced callus;(b): callus subculture; (c): Co-culture of *Agrobacterium* and callus after *Agrobacterium* infection; (d): screening of callus on hygromycin medium; (e): callus differentiation on hygromycin medium; (f): rooting culture; (g): domestication in the barrel; (h): plantlets.

1-off	.....	CGGCCTCCGCCTTCCCTCCCTCCG	.....
Reference sequence	CGCCTTCGCCCTCTCTCAAACCCCTAGCCT	CGGCCTCCGCCTTCCCTCCCTCCG	CGTCGCGGGCGCCTCCGCCGCCGCGTC
Rc 1-162-1	CGCCTTCGCCCTCTCTCAAACCCCTAGCCT	CGGCCTCCGCCTTCCCTCCCTCCG	CGTCGCGGGCGCCTCCGCCGCCGCGTC
Rc 2-162-16	CGCCTTCGCCCTCTCTCAAACCCCTAGCCT	CGGCCTCCGCCTTCCCTCCCTCCG	CGTCGCGGGCGCCTCCGCCGCCGCGTC
Rc 2-162-17	CGCCTTCGCCCTCTCTCAAACCCCTAGCCT	CGGCCTCCGCCTTCCCTCCCTCCG	CGTCGCGGGCGCCTCCGCCGCCGCGTC
Rc 1-162-18	CGCCTTCGCCCTCTCTCAAACCCCTAGCCT	CGGCCTCCGCCTTCCCTCCCTCCG	CGTCGCGGGCGCCTCCGCCGCCGCGTC

2-off	.....	TTGCGCCTTCCCCGGCCTTGCC	.....
Reference sequence	AATCTCGACGATAATGTTGCCTCCCTCGCCGA	TTGCGCCTTCCCCGGCCTTGCC	CTCATCAGAAGGCGCCAATGGTGCCAGT
Rc 1-162-1	AATCTCGACGATAATGTTGCCTCCCTCGCCGA	TTGCGCCTTCCCCGGCCTTGCC	CTCATCAGAAGGCGCCAATGGTGCCAGT
Rc 2-162-16	AATCTCGACGATAATGTTGCCTCCCTCGCCGA	TTGCGCCTTCCCCGGCCTTGCC	CTCATCAGAAGGCGCCAATGGTGCCAGT
Rc 2-162-17	AATCTCGACGATAATGTTGCCTCCCTCGCCGA	TTGCGCCTTCCCCGGCCTTGCC	CTCATCAGAAGGCGCCAATGGTGCCAGT
Rc 1-162-18	AATCTCGACGATAATGTTGCCTCCCTCGCCGA	TTGCGCCTTCCCCGGCCTTGCC	CTCATCAGAAGGCGCCAATGGTGCCAGT

### Supplemental Figure S4 Potential off-target sequence alignment

Note: No off-target was found in sequencing alignment, indicating that the target site of *Rc* gene was specific.