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778 **Supplementary Table 1 Primers used in this study.**

Primer name	Sequence (5'-3')	Purpose
SgSRK-1F	TGCAGGCAATAATCTTGTCTCTCT	<i>BoSRK3</i> gene target site assembly
SgSRK-1R	AAACAGGAGGACAAGATTATTGCC	
SgSRK-2F	TGCAGTGGCAGAGCTTCTCGCCAA	
SgSRK-2R	AAACTTGGCGAGAAGCTCTGCCAC	
SgSRK-3F	TGCAGTGGGATCTTTAGAGTGCAT	
SgSRK-3R	AAACATGCACTCTAAAGATCCCAC	
SgSRK-4F	TGCAAACCCTATATCCAACCTCCAC	
SgSRK-4R	AAACGTGGAGTTGGATATAGGGTT	
BoSRK3-F	GACATTGTCCGACAGAACCTATG	PCR of the <i>BoSRK3</i> genomic sequence
BoSRK3-R	CTTCACTATTATCTGTGAAATTG	
BoSLG3-F	ACGATAATTCTTACACCTCCTGTT	PCR of the <i>BoSLG3</i> genomic sequence
BoSLG3-R	TCTTCTAGTATGCCACTATATCGT	
SgPDS-1F	GATTGCAAAGTACCTGGCTGATGC	<i>BoPDS</i> gene target site assembly
SgPDS-1R	AAACGCATCAGCCAGGTACTTTGC	
SgPDS-2F	GATTGAAGCAAGAGATGTTCTTGG	
SgPDS-2R	AAACCCAAGAACATCTCTTGCTTC	
SgPDS-3F	GATTGAAACAACGAGATGCTGACA	
SgPDS-3R	AAACTGTCAGCATCTCGTTGTTTC	
SgPDS-4F	GATTACCTGATCGCGTGACTGATG	
SgPDS-4R	AAACCATCAGTCACGCGATCAGGT	
SgMS1-1F	GATTGCCTTTCTAAAAGTAGAAGG	<i>BoMS1</i> gene target site assembly
SgMS1-1R	AAACCTTCTAGTTTTAGAAAGGC	
SgMS1-2F	GATTGAGTAGCAAAAAGGAGAGCCG	
SgMS1-2R	AAACCGGCTCTCCTTTTGCTACTC	
SgMS1-3F	GATTGAGGTTAAGAGAGGCTTCGA	
SgMS1-3R	AAACTCGAAGCCTCTCTTAACCTC	

SgMS1-4F	GATTAAGTTGGTCCTTTCAGCTCG	
SgMS1-4R	AAACCGAGCTGAAAGGACCAACTT	
BoMS1-F1	TGTCGAATCTGATTCGAACAGATC	PCR of the <i>BoMS1</i> genomic sequence (site D, site C and site A regions)
BoMS1-R1	AAGGACATGGCCTTGAAGTTCCAC	
BoMS1-F2	CATGGTTGAGAGCAATTTCAACC	PCR of the <i>BoMS1</i> genomic sequence (site B region)
BoMS1-R2	AGTTGGTACTATCAATGGAGATG	
BoPDS-F1	AGACAAGAACAAGGCGAAGGACT	PCR of the <i>BoPDS</i> genomic sequence
BoPDS-R1	CCGGTTTCATACCAATCTCCATC	
BoPDS-F2	ATGTTCTACCAGCACCCTTAAAC	
BoPDS-R2	AGGTACTCCCTGGAAATGAACAC	
BoPDS-R3	AGTTATTGGAGTGCCCAAACCTG	
BoPDS-F	CCTTTTATTCATTATGCTACCCAG	
BoPDS-R	GAGAAGTGAGCATGCTTTAGTGGT	
Bol016089-F	TGGTGAAGCTCCTTTGCCTCTCG	PCR of the <i>Bol016089</i> genomic sequence
Bol016089-R	TAAGAACGCCATCTTCGACCCAT	
Cas9-F	CAAGTACGTGAACTTCTCTACC	Detection of the transgenic plants
Cas9-R	GCTGGGAAAGGTCGATACGAGTC	

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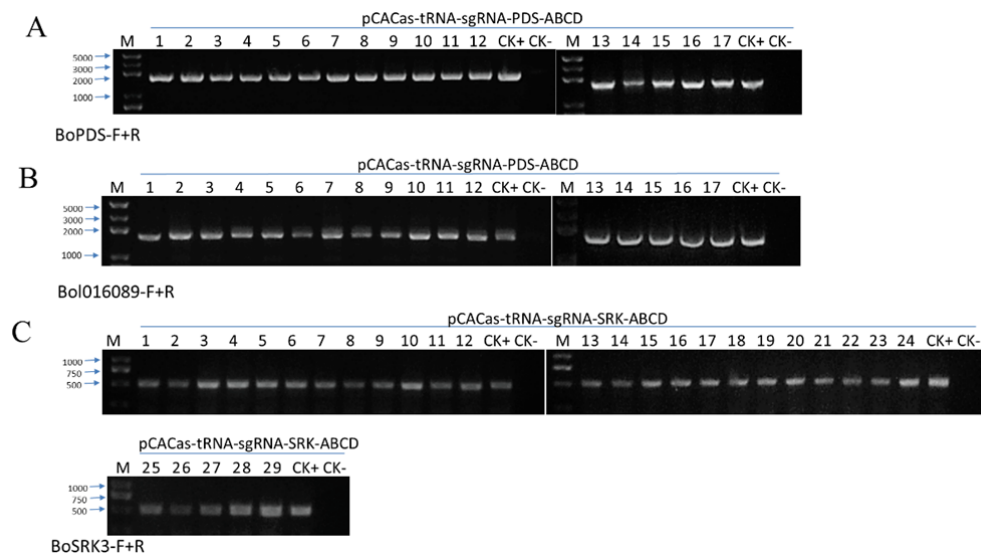
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**Supplementary Table 2 Transmission of Cas9/gRNA-induced mutations in the F1 progeny of C-4 × WT ('+': positive; '-': negative)**

F1 line	Cas9	Site D of the <i>BoMS1</i> gene			Site B of the <i>BoSRK3</i> gene
		T nucleotide deletion	T nucleotide insertion	WT	A nucleotide insertion
1	+	+	-	-	+
2	+	+	-	-	+
3	-	-	-	+	+
4	+	+	-	-	+
5	+	+	-	-	+
6	-	+	-	-	+
7	+	+	-	-	+
8	+	-	+	-	+
9	-	+	-	-	-
10	-	+	-	-	+
11	-	+	-	-	+
12	+	-	+	-	+
13	-	+	-	-	-
14	-	+	-	-	+
15	+	+	-	-	-
16	-	+	-	-	-

17	-	+	-	-	-
18	-	+	-	-	-
19	+	+	-	-	-
20	-	-	+	-	-

782 **Supplementary Figure 1 Detection of a large fragment deletion in *BoPDS* and *BoSRK3* and their paralogous**  
783 **genes. (A)** No fragment deletion in the *BoPDS* gene was detected in any *BoPDS* gene mutant plants using the  
784 primers BoPDS-F and BoPDS-R (see Supplementary Table 1). **(B)** No fragment deletion in the *Bol016089* gene  
785 was detected in any *BoPDS* gene mutant plants using the primers Bol016089-F and Bol016089-R (see  
786 Supplementary Table 1). **(C)** No fragment deletion of the *BoSRK3* gene was detected in any *BoSRK3* gene mutant  
787 plants using the primers BoSRK3-F and BoSRK3-R (see Supplementary Table 1).



788 **Supplementary Figure 2 Self-pollination of fertile T0 plants with the *BoSRK3* gene mutation without artificial or**  
789 **natural pollination. (A)** Siliques of the B-2 line. **(B)** Siliques of the B-1 line.  
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792 **Supplementary Figure 3. Examples of sequencing chromatograms of the T1 and F1 progeny with**

793 **Cas9-induced mutations. (A)** A homozygote of the site A mutation (**A1**) and a heterozygote of the site B mutation

794 (**A2**) in the *BoSRK3* gene were detected in the T1 progeny of the B-1 line; New mutations at site B were detected

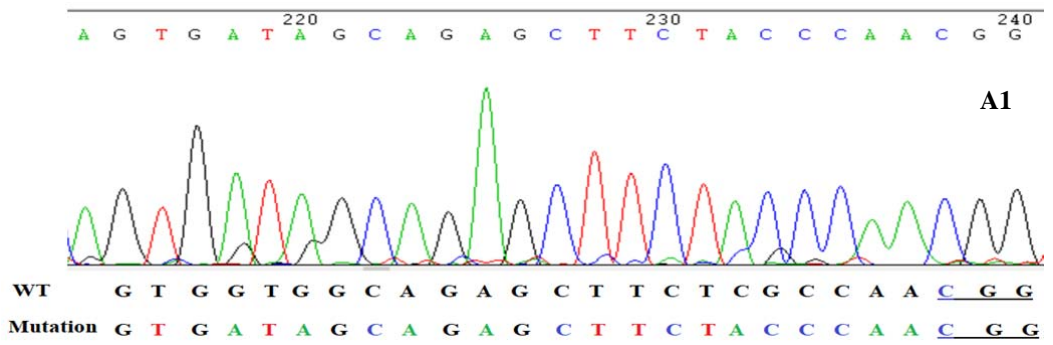
795 in the T1 generation of the line B-1 (**A3**). (**B**) The ‘T’ nucleotide deletion mutation (**B1**) and the ‘T’ nucleotide

796 insertion mutation (**B2**) detected at the D site of the *BoMS1* gene in the T0 generation (C-4 line) was inherited to

797 the F1 progeny of the C-4 × WT; The ‘A’ nucleotide insertion mutation detected at site B of the *BoSRK3* gene in

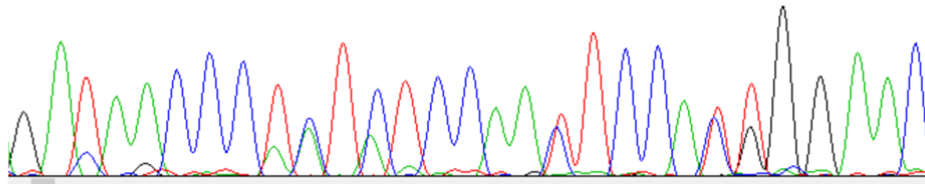
798 the T0 generation (C-4 line) was inherited in the F1 progeny of the C-4 × WT (**B3**).

799 **A**



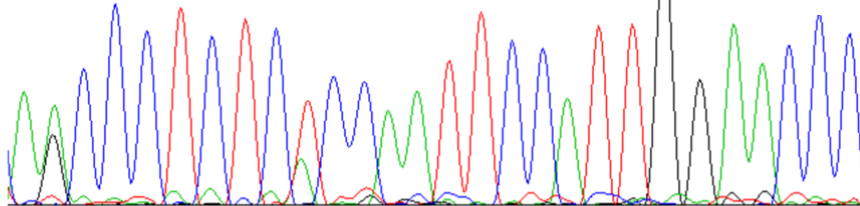
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G A T A A C C C <sup>100</sup> T C T C T C C A A <sup>110</sup> T T C C A T T G G A A C <sup>120</sup>  
**A2**



801 **WT** G A T A A C C C T A T A T C C A A C T C C A C T G G A A C  
**Mutation** G A T A A C C C T C T C T C C A A T T C C A T G G G A A C

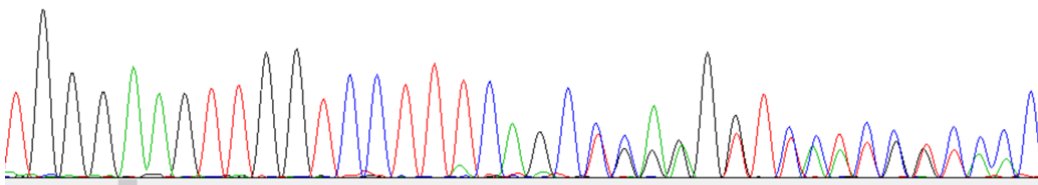
A A C C C C <sup>100</sup> T C T C T C C A A T <sup>110</sup> T T C C A T T G G A A C C C <sup>120</sup>  
**A3**



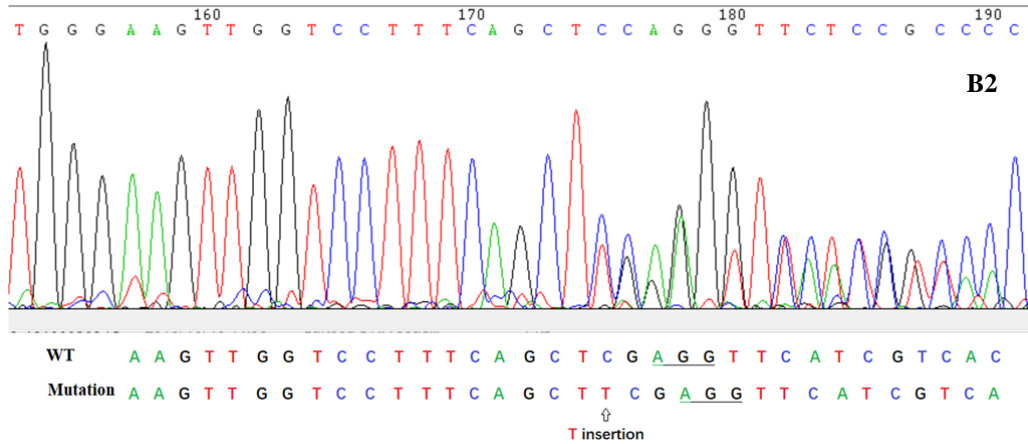
802 **Parental mutation** A A C C C T C T C T C C A A T T C C A T T G G  
**New mutation** A G C C C T C T C A C C A A T T C C A T T G G  
**WT** A A C C C T A T A T C C A A C T C C A C T G G

803 **B**

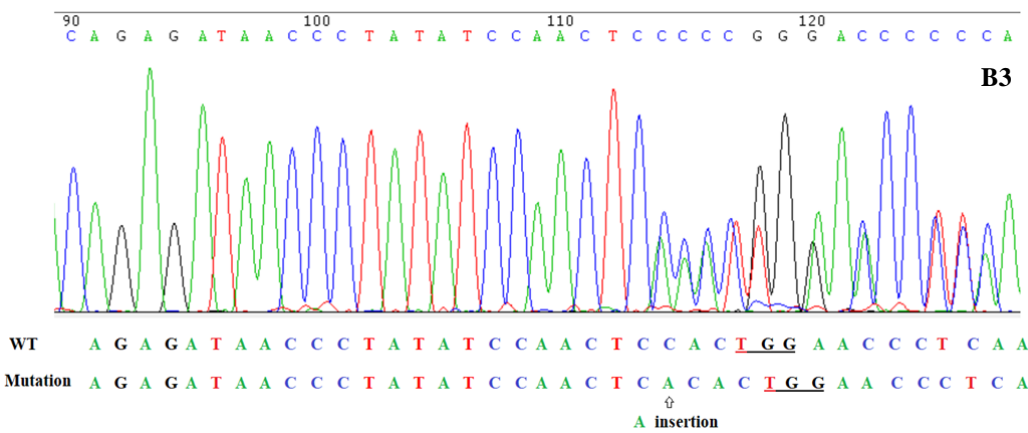
T G G G <sup>150</sup> A A G T T G G T C C <sup>160</sup> T T T C A G C C C <sup>170</sup> A G G G T C C T C C <sup>180</sup> T C C C C  
**B1**



804 **WT** A A G T T G G T C C T T T C A G C T C G A G G T T C A T C G T C  
**Mutation** A A G T T G G T C C T T T C A G C C G A G G T T C A T C G T C  
↑  
**T deletion**



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807 **Supplementary data sheet**

808 **1. Sequence of tandemly arrayed tRNA-gRNA architecture with the *AtU6-26* promoter.** The red sequence

809 represents the *AtU6-26* promoter that drives the tRNA-sgRNA. The green sequence represents *A. thaliana*

810 glycine-tRNA. The blue sequence represents the *BbsI*, *BsaI*, *BsmBI*, and *BfuAI* restriction sites. The yellow

811 sequence represents the optimized sgRNA scaffold. The orange sequence represents the terminator. The

812 lowercase letters represent the *BamHI* and *EcoRI* restriction sites.

813 >AtU6-26::tRNA-sgRNA

814 ggatccCGTTGAACAACGGAAACTCGACTTGCCTTCGCACAATACATCATTCTTCTTA

815 GCTTTTTTCTTCTTCTTCGTTTCATACAGTTTTTTTTTGTTTATCAGCTTACATTTCTTG

816 AACCGTAGCTTTCGTTTTCTTCTTTTAACTTCCATTTCGGAGTTTTGTATCTTGTTTC

817 ATAGTTTGTCCAGGATTAGAATGATTAGGCATCGAACCTTCAAGAATTTGATTGAAT

818 AAAACATCTTCATTCTTAAGATATGAAGATAATCTTCAAAGGCCCTGGGAATCTGA

819 AAGAAGAGAAGCAGGCCATTTATATGGGAAAGAACAATAGTATTTCTTATATAGGC

820 CCATTTAAGTTGAAAACAATCTTCAAAGTCCACATCGCTTAGATAAGAAAACGAA

821 GCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGAACAAAGCACCAGTGGTCT

822 AGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCGGCTGGTGC

823 AGGGTCTTCGTTACAGAAGACCTGTTTCAGAGCTATGCTGGAAACAGCATAGCAAG

824 TTGAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAACAA  
 825 AGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTCTGA  
 826 TTCCCGGCTGGTGCAAGAGACCCTGCAGGGTCTCTGTTTCAGAGCTATGCTGGAAAC  
 827 AGCATAGCAAGTTGAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAG  
 828 TCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAG  
 829 ACCCGGGTTCGATTCCCGGCTGGTGCAAGAGACGGTTACACGTCTCTGTTTCAGAG  
 830 CTATGCTGGAAACAGCATAGCAAGTTGAAATAAGGCTAGTCCGTTATCAACTTGAAA  
 831 AAGTGGCACCGAGTCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCC  
 832 TGCCACGGTACAGACCCGGGTTTCGATTCCCGGCTGGTGCAAATCGCAGGTGTTACA  
 833 ACCTGCATTCGTTTCAGAGCTATGCTGGAAACAGCATAGCAAGTTGAAATAAGGCTA  
 834 GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTTGTCCCTCgaatt  
 835 c

836  
 837 **2. Partial sequence of *BoSRK3* gene covering the target region. The target sites (B, D, A, and**  
 838 **C sites in turn) are highlighted in green.**

839 >BoSRK3  
 840 TTGTATCTCCCGGTGATGTCTTCGAGCTCGGTTTCTTCAAAACCACCTCAAGTTCTCGT  
 841 TGGTATCTCGGTATATGGTACAAGACATTGTCCGACAGAACCTATGTATGGATTGCCAAC  
 842 AGAGATAACCCTATATCCAACTCCACTGGAAACCCTCAAAATCTCAGGCAATAATCTGT  
 843 CCTCCTTGGTGACTCCAATAAACCTGTTTGGTCGACGAATCTAACTAGAAGAAGTGAG  
 844 AGATCTCCAGTGTGGCAGAGCTTCTCGCAA<sub>CGGAAACTTCGTGATGCGAGACTCCA</sub>  
 845 ACAACAACGATGCAAGTCAATTTTATGGCAAAGTTTCGATTACCCTACAGATACTTG  
 846 CTCCTGACATGAAACTGGGTTACGACCTAAAAACAGGGCTGGACAGATTCTTACAT  
 847 CATGGAGAAGTTTAGATGATCCGTCAGCGGAATTTCTCGTACAGGCTCGAAACCCG  
 848 AAAGTTTCTGAATTTATTTAAGGAGTGGGATCTTAGAGTGCAI<sub>CGGAGTGGTCCAT</sub>  
 849 GGAATGGAATCCGATTTAGTGGCATAACCAGATGACCAAAAAGTTGAGTTACATGGTTTAC  
 850 AATTCACAGATAATAGTGAAGAAGTCGCTTATACATTCCGAATGACCAACAACAGCAT  
 851 TTA<sub>CTCGAGACTGACAGTAAGTTTCTTAGGGCATT</sub>TGAAACGTCAGACGTGGAATCCGT  
 852 CATTAGGGATGTGGAACGCGTTCTGGTCTTTTATATTGGACTCACAGTGCGATATATACA  
 853

854 **3. Partial sequence of the *BoSLG3* gene covered the target region. The putative target sites**  
 855 **(B, D, A, C sites in turn) are highlighted in green**

856 >BoSLG3  
 857 ATGAAAGGCATAAGAAAAACCTACGATAATTCTTACACCTCCTGTTTGCTTGTCTTTTTC  
 858 GTCTTAATTCTATTTCCGCTTGCCTTTTCGATCAACACTTTGTCGTCTACAGAACTCTCT  
 859 ACAATCTCAAGCAACAGAACACTCGTATCTCCATGTAGTAACTTCGAGCTCGGCTTCTT

860 CAGAACCAACTCAAGTTCTCGTTGGTATCTCGGGATATGGTACAAGAAATTGTCTAACA  
861 GAACCTATGTATGGGTTGCCAACAGAGATAACCCTTTGTCCAACCTCATTGGAACCCTC  
862 AAAATCTCAGGCAATAATCTTGTCCTCCTTGGTCACTCCAATAGATCAGTTTGGTTCGAC  
863 GAATCTTACTAGAGAAAATGAGAGATCCACAGTGGTCGCAGAGCTTCTCGCTAAIGGA  
864 AACTTCGTGATGCGAGACGCAAGTGGATTCTTGTGGCAAAGTTTCGATTACCCTACAG  
865 ATACTTTGCTACCAGAGATGAAACTGGGTTACGACCTCAAAACAGGGCTGAACAGGTT  
866 CCTTACATCATGGAGAAGTTCAGATGATCCATCAAGCGGGGATTTCTCGTACAAGCTCG  
867 AAACCCGAAGGCTTCCTGAATTTTATATATCGAGTGGGGTCTTTCTATTGTATCGGAGTG  
868 GACCATGGAATGGAATACGATATAGTGGCATACTAGAAGACCAAAGTTGAATTACATG  
869 GTGTACAATTTACAGAGAATAGTGAAGAAGTCGCTTATACATTCCGAATGACCAACA  
870 ACAGCATCTACTCGAGACTGACACTAAGTTTCTCGGGGTATTTGAACGACAGACGTG