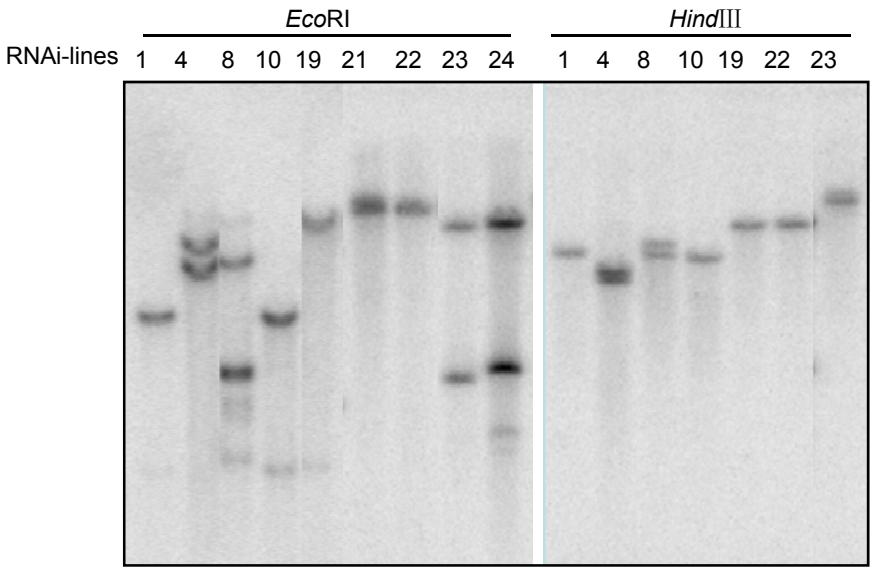
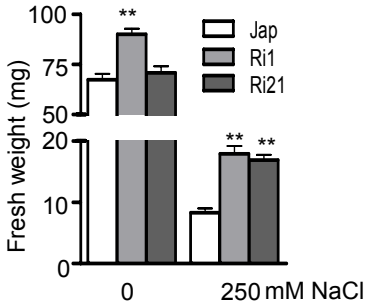
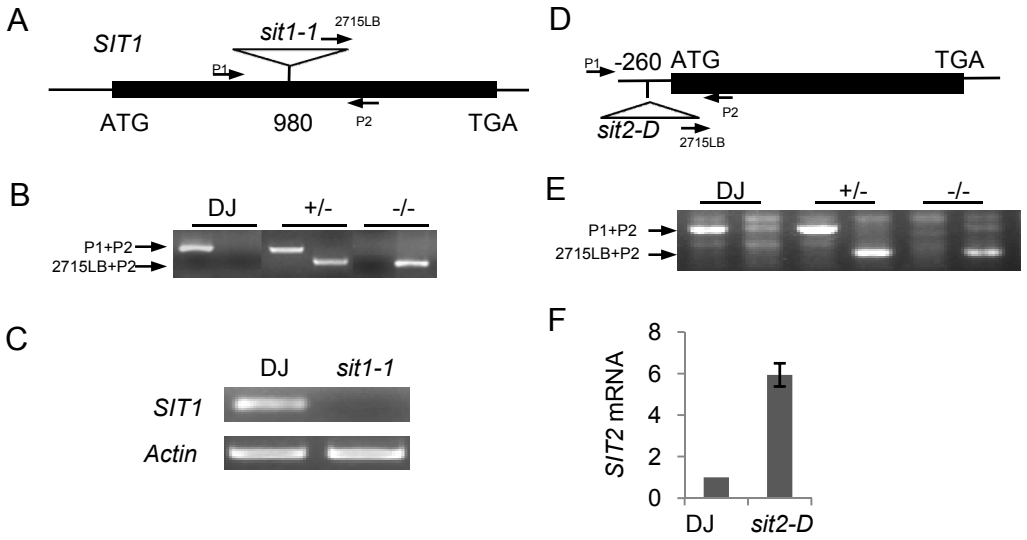


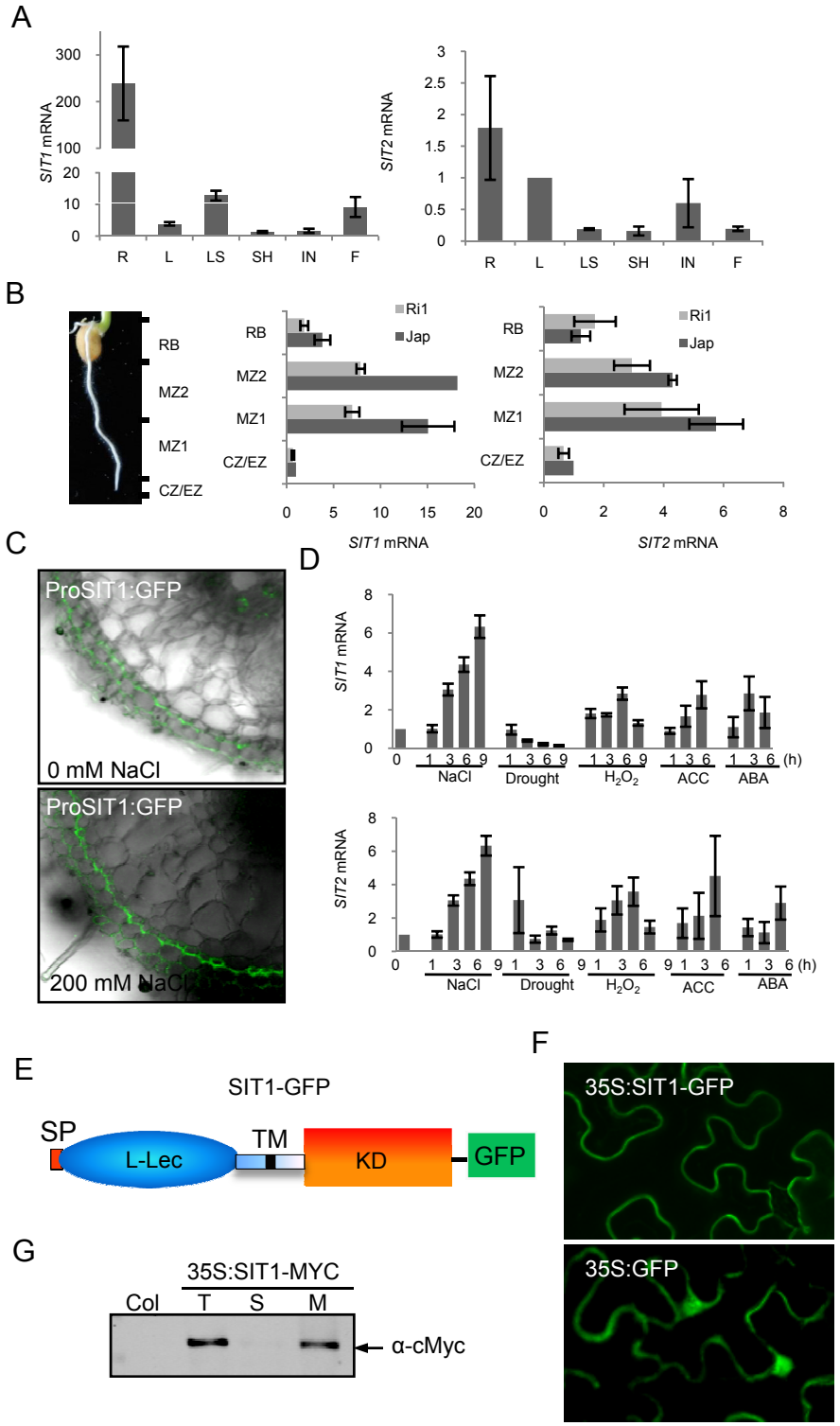
Supplemental Figure 1. DNA gel blot assay of *SlT1*-RNAi rice plants. Genomic DNA isolated from individual transgenic *SlT1*-RNAi plants was digested with *Eco*RI and *Hind*III. The blot was probed with an [α-^{32}P]dATP-nucleotide acid encoding a hygromycin-resistance gene.



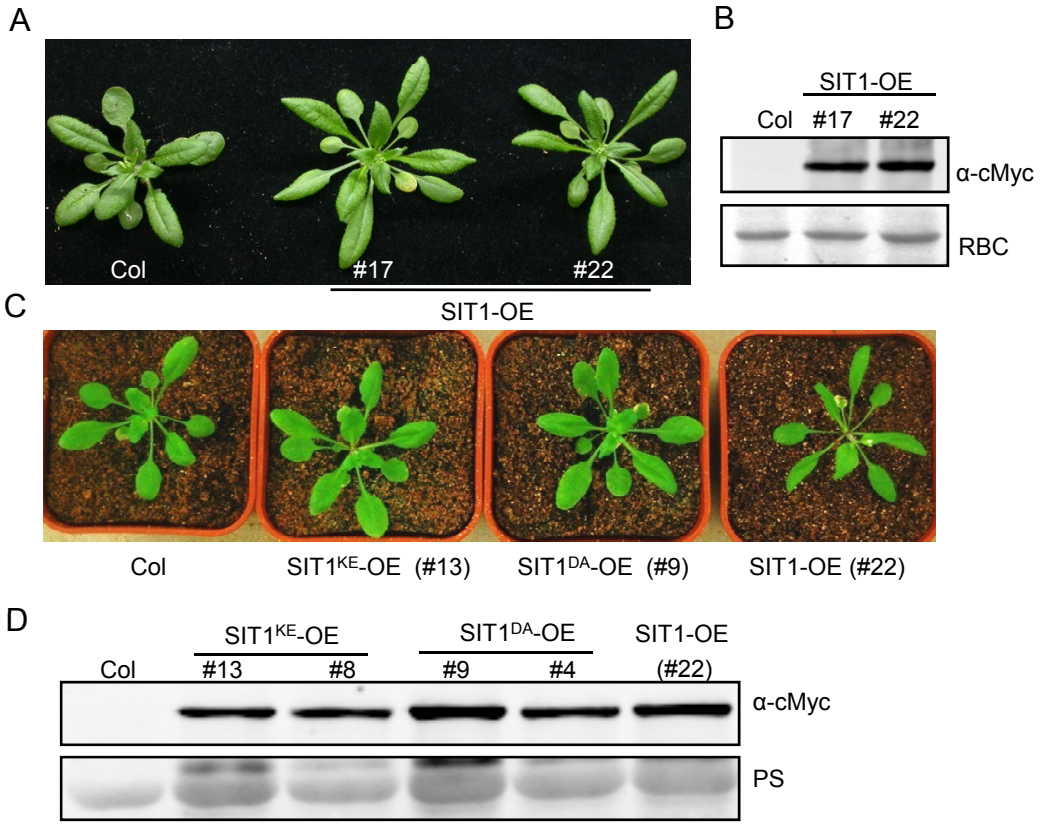
Supplemental Figure 2. The fresh weight of 20-day-old Ri1, Ri21, and wild-type (Jap) seedlings grown on 0.5×MS medium containing 0 or 250 mM NaCl.



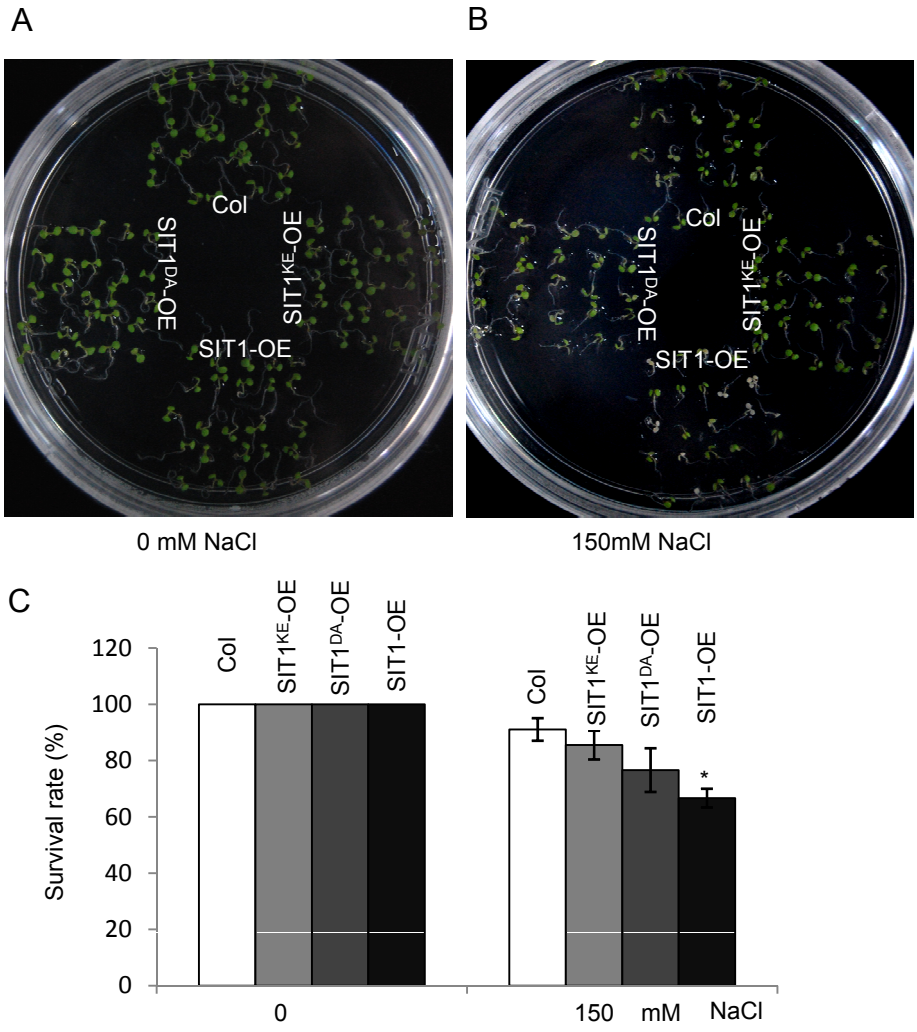
Supplemental Figure 3. Genotyping of the *sit1-1* and *sit2-D* mutants. (**A**) and (**D**) Schematic diagram of the T-DNA insertions in *sit1-1* (**A**) and *sit2-D* (**D**). Black boxes represent coding sequences. Arrows indicate the primers (with the directions) used for PCR identification. Numbers indicate the T-DNA insertion sites. (**B**) and (**E**) Genotyping of *sit1-1* (**B**) and *sit2-D* (**E**) homozygous, heterozygous, and wild-type (DJ) plants. (**C**) and (**F**) RT-PCR (**C**) and qRT-PCR (**F**) revealed that the full-length transcript was absent in *sit1-1* (**C**) and increased in *sit2-D* due to *35S* insertion (4×) in the promoter region (**F**).



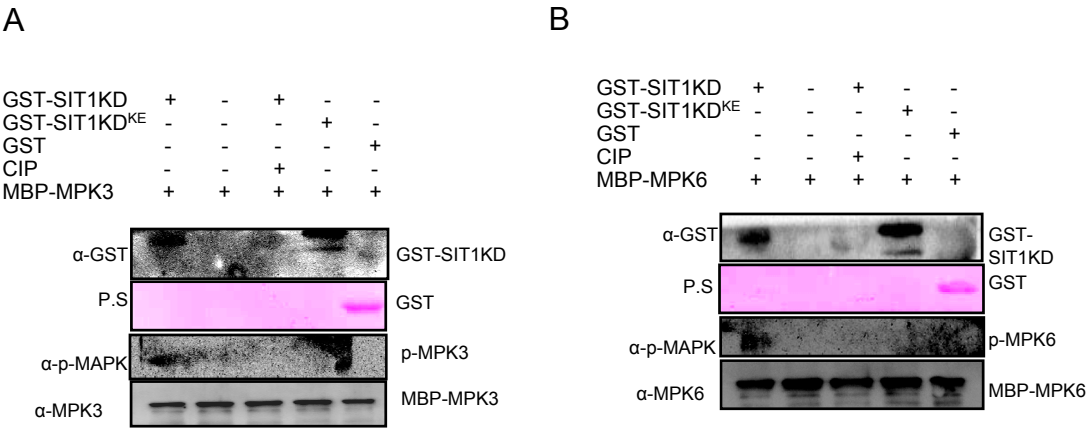
Supplemental Figure 4. Rice expression pattern of *SIT1* and *SIT2*. (A) Tissue-specific expression of *SIT1* and *SIT2*. R, root. L, leaf. SH, shoot. LS, leaf sheath. IN, internode. F, flower. (**B**) A five-day-old seminal root was divided into four zones (left panel) and the mRNA levels of *SIT1* and *SIT2* in each zone were determined. RB, root base; MZ, maturation zone; EZ/CZ, elongation zone and cell division zone. (**C**) Merged image of a transverse section of the mature zone of a rice seminal root harboring the *ProSIT1:GFP* reporter treated with or without NaCl for 6 h. (**D**) *SIT*1 and *SIT2* expression in response to stress and phytohormone treatment. Two-week-old wild-type (Jap) roots were treated for the indicated time with 200 mM NaCl, drought (roots exposed to air without water), 100 μM H_2O_2, 100 μM ACC, and 30 μM ABA, respectively. (**E**) Schematic representation of SIT1 protein domain organization. SP, signal peptide; L-Lec, L-Lectin domain; TM, transmembrane domain; Ser/Thr kinase, serine and threonine kinase domain. (**F**) Subcellular localization of the SIT1-GFP fusion protein in tobacco epidermal cells. (**G**) Immunoblot assay of the SIT1-Myc fusion expressed in *Arabidopsis* leaves before and after cell fractionation. Wild-type Col was used as a control. T, total protein; S, soluble fraction; M, membrane fraction.



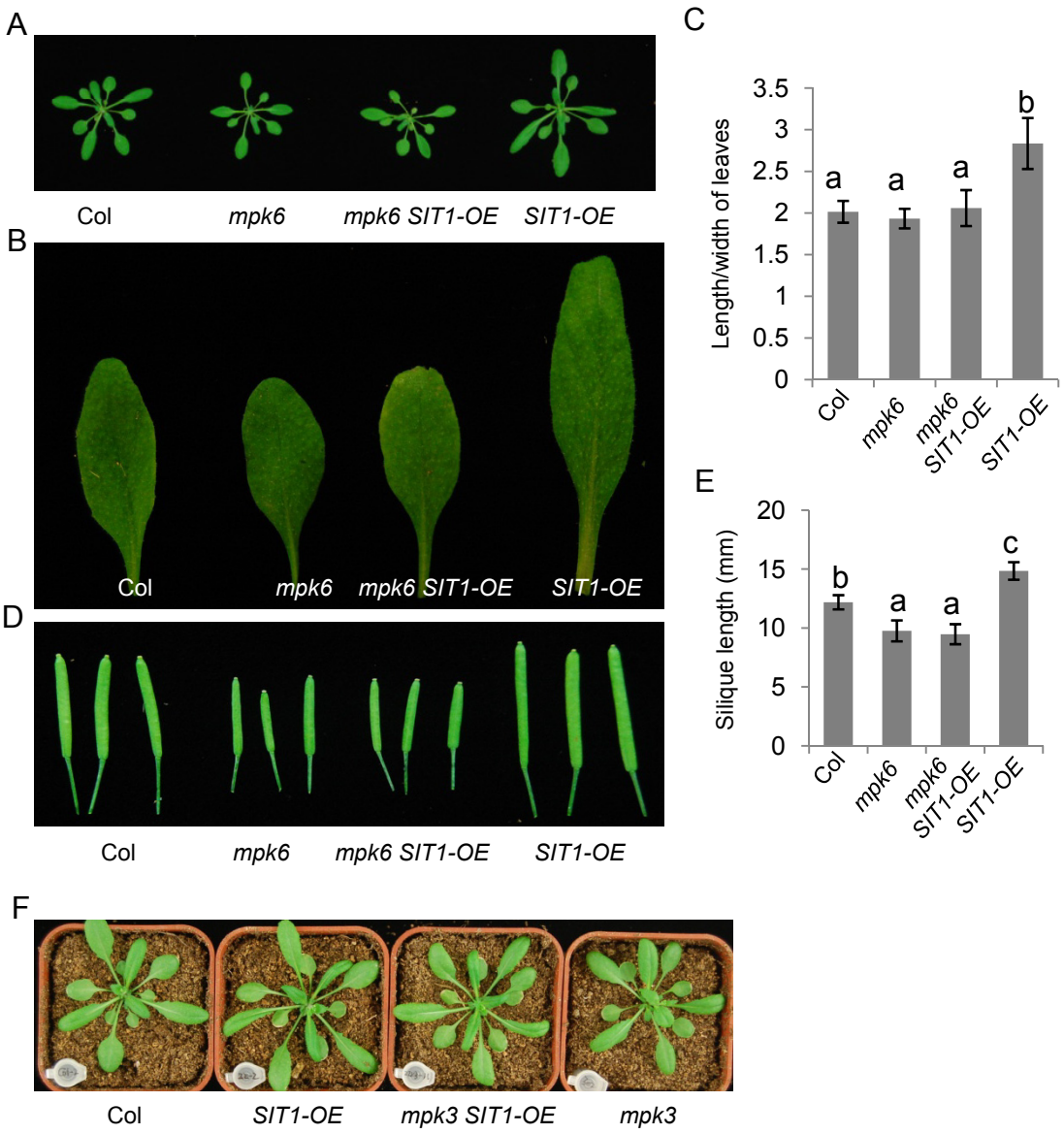
Supplemental Figure 5. Transgenic *Arabidopsis* overexpressing kinase-active, but not kinase-dead, SIT1 exhibited an obvious phenotype. (**A**) Two transgenic lines expressing wild-type *SIT1* showing narrow rosette leaves as compared with wild-type Col. Twenty-day-old seedlings grown under normal conditions were photographed. (**B**) Immunoblot assay of SIT1-Myc fusion protein expression in the transgenic *Arabidopsis* lines shown in (**A**). (**C**) Phenotypic comparison of Col, SIT1-, SIT1^{KE}-, and SIT1^{DA}-OE seedlings. (**D**) Immunoblot assay of SIT1^{KE}-, SIT1^{DA}-, and SIT1-Myc fusion protein expression in the transgenic *Arabidopsis* seedlings shown in (**C**). PS staining of Rubisco (RBC) indicates equal loading.



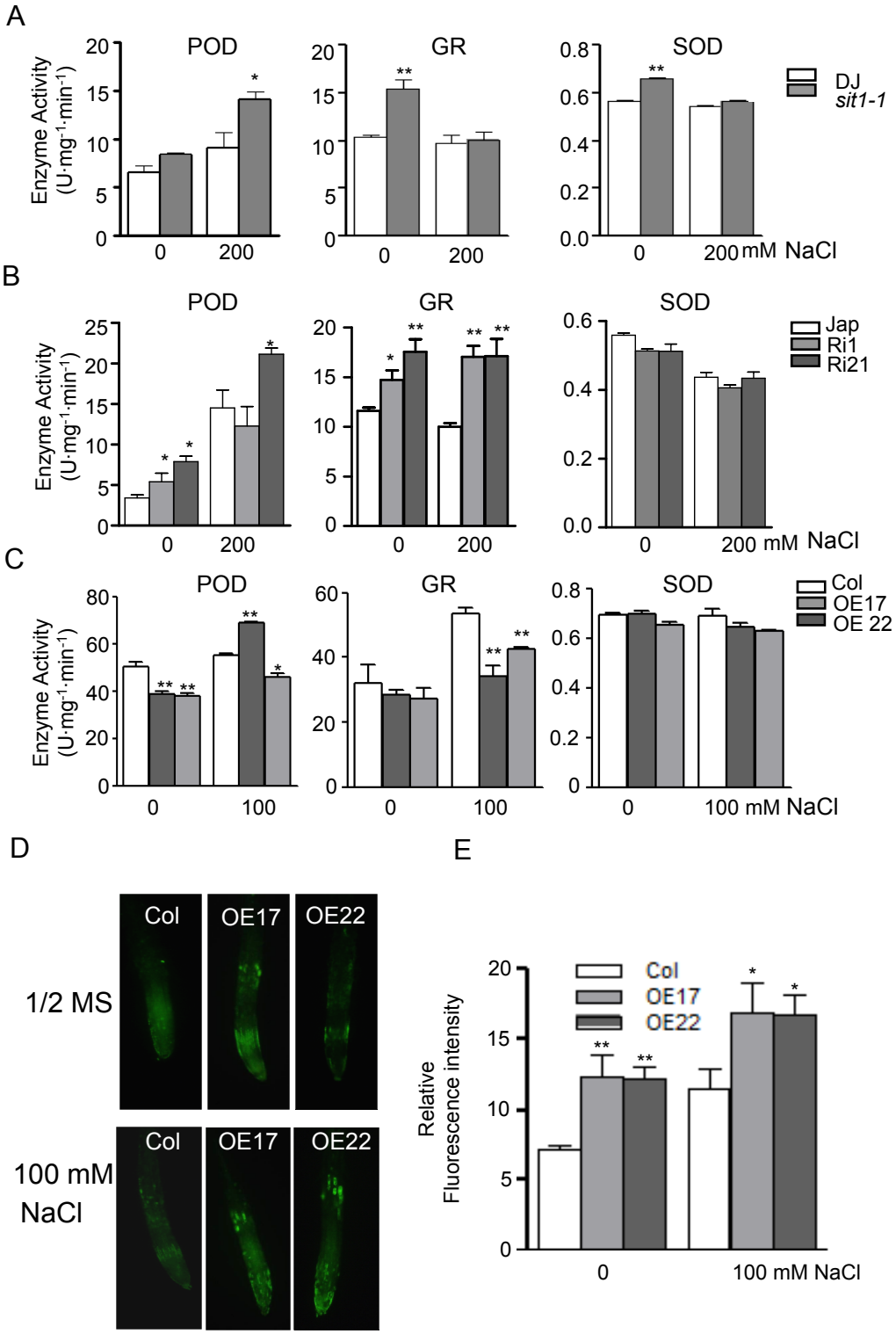
Supplemental Figure 6. Investigation of the survival rates of SIT1-OE, SIT1^{KE}-OE, and SIT1^{DA}-OE seedlings on 150 mM NaCl-containing medium. Seeds were sown on 0.5× MS medium and allowed to germinate for 3 days. The seedlings were then transferred to plates with or without 150 mM NaCl and allowed to grow for another 3 days. The survival rates were calculated and analyzed by Student's *t*-test. The data represent the mean ± SEM of three parallel experiments (n=30/genotype). a-c indicate significant differences.



Supplemental Figure 7. SIT1 phosphorylates MPK3 (**A**) and MPK6 (**B**) *in vitro*. SIT1^{KE}, although used in sufficient quantities, could not phosphorylate MPK3 or MPK6. Phospho-MPK3 or -MPK6 was probed for using anti-p-MAPK antibodies; non-phospho-MPK3 and -MPK6 were probed for using anti-MPK3 and anti-MPK6 antibodies, respectively.

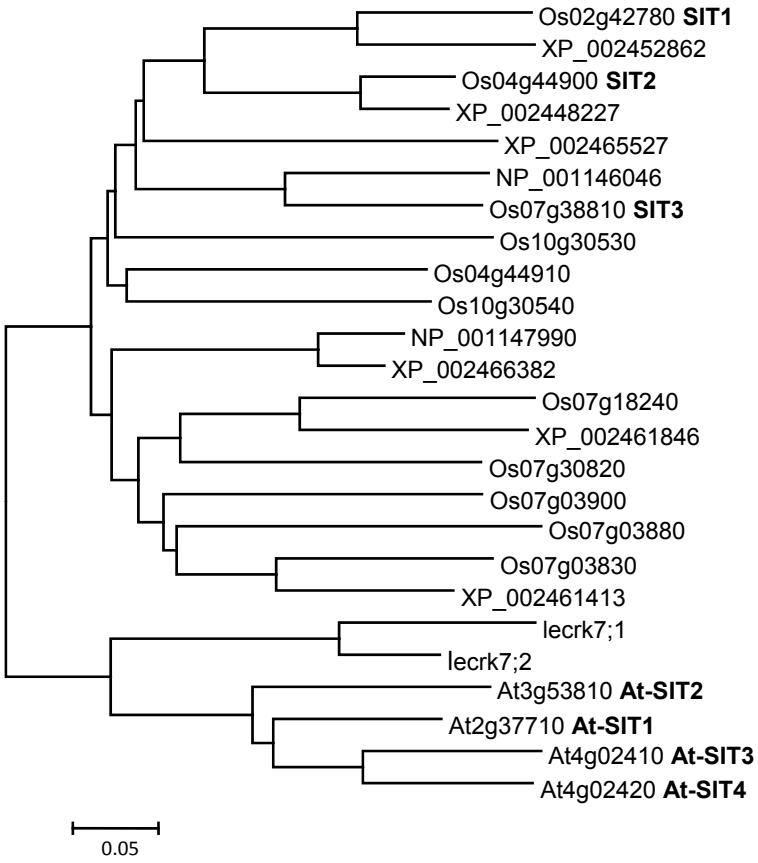


Supplemental Figure 8. Phenotypic comparison of the *Arabidopsis* mutants *mpk6* and *mpk3* with the double homozygotes *mpk6 SIT1-OE* and *mpk3 SIT1-OE*. (**A–E**) Cross of *mpk6* with *SIT1-OE* plants. (**A**) and (**B**) Rosette leaf morphology. (**C**) Length/width ratio of the leaves shown in (**B**). (**D**) Silique morphology. (**E**) Lengths of the siliques shown in (**D**). The data were analyzed by Student's *t*-test. Bars indicate the mean ± SEM (n=20). a–c indicate significant differences. (**F**) Cross of *mpk3* with *SIT1-OE* plants. Four-week-old seedlings were observed.



Supplemental Figure 9. Anti-oxidase activity and the ROS level in the mutant and transgenic plants. (**A–C**) POD, GR, and SOD were measured in the roots of *sit1-1* (**A**) and *SIT1*-RNAi (**B**) rice plants, and in wild-type Col, *SIT1-OE17*, and *SIT1-OE22 Arabidopsis* seedlings (**C**) before and after treatment with the indicated concentrations of NaCl. (**D**) and (**E**) ROS levels in the roots of Col and *SIT1-OE17* and *SIT1-OE22* with or without 12 h of NaCl treatment. (**D**) Representative images of CM-H2DCFDA staining. (**E**) Quantification of the relative fluorescence intensity in the mature zone of the roots shown in (**D**). Three biological repeats were used for Student's *t*-test. The data represent the mean ± SEM (n≥15 in [**A**] and [**B**]; n≥20 in [**C**]; n≥8 in [**E**]). *P<0.05, **P<0.01

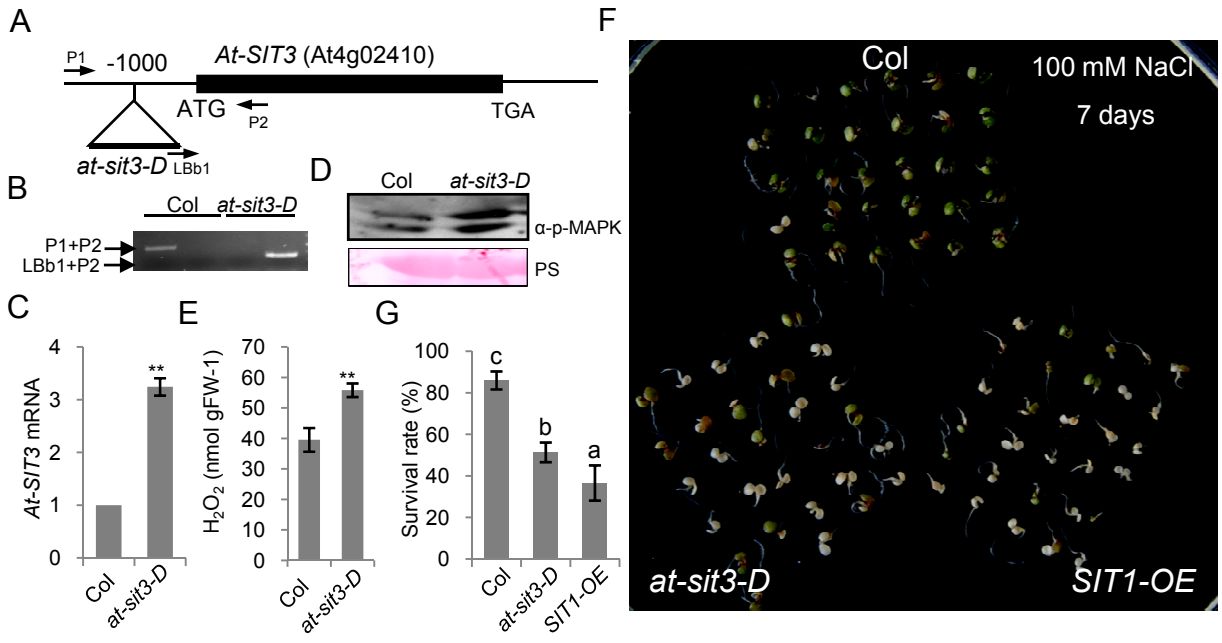
A



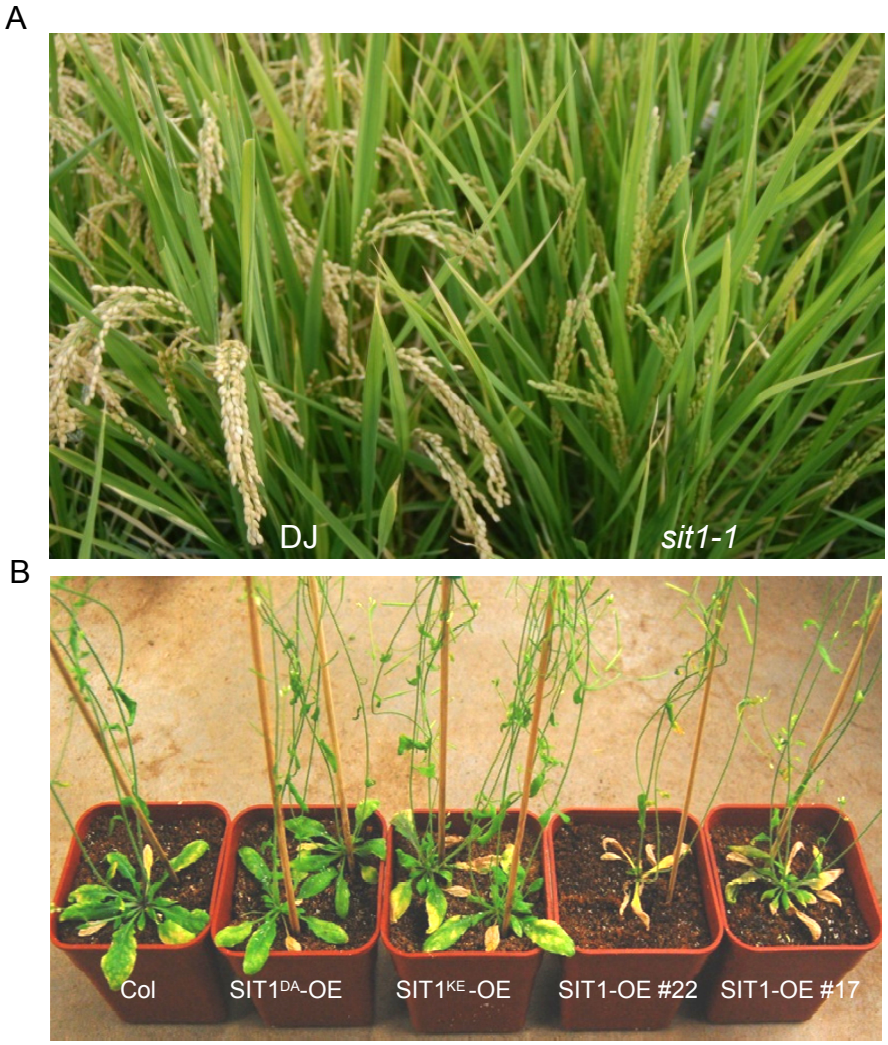
B

	SIT1	SIT2	SIT3	At-SIT1	At-SIT2	At-SIT3	At-SIT4
SIT1	100%						
SIT2	68%	100%					
SIT3	59%	65%	100%				
At-SIT1	52%	56%	55%	100%			
At-SIT2	51%	54%	53%	77%	100%		
At-SIT3	50%	53%	52%	74%	70%	100%	
At-SIT4	50%	52%	54%	76%	70%	78%	100%

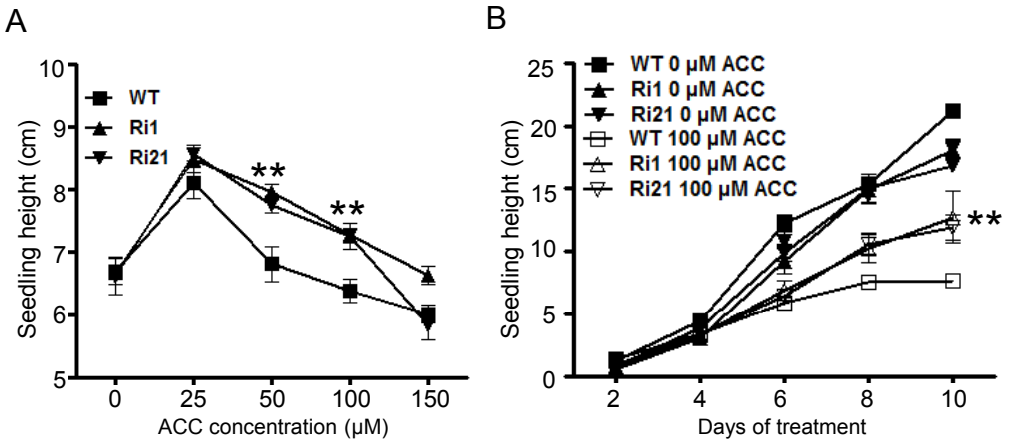
Supplemental Figure 10. Sequence similarity matrix of SIT1. (**A**) A phylogenetic tree of closely-related genes to *SIT1* constructed using MEGA6.06. The full-length amino acid sequence of SIT1 was compared against the NCBI database; homologs of SIT1 with >55% identity in monocots and 50% in dicots were selected. On this scale, 0.05 represents a 5% change. (**B**) Sequence similarity matrix. Amino acid sequence identity of SIT1 with two rice homologs and four *Arabidopsis* homologs.



Supplemental Figure 11. *At-SIT3*-activation seedlings with increased MPK3 and MPK6 phosphorylation, and an increased level of ROS were sensitive to NaCl. (**A**) Schematic diagram of the T-DNA insertion in *at-sit3-D* (Salk_079614). The number indicates the insertion site upstream of the start codon. Directed arrows indicate the primers used in (**B**) and (**C**). (**B**) Genotyping revealed that *at-sit3-D* was homozygous. (**C**) qRT-PCR showed that the expression of *At-SIT3* was enhanced in *at-sit3-D*. (**D**) A immunoblot probed with anti-p-MAPK antibodies showed MAPK phosphorylation in *at-sit3-D*. (**E**) ROS level in *at-sit3-D* and Col plants as quantified using a POD assay kit (see the Methods). (**F**) Phenotypes of Col, *at-sit3-D*, and SIT1-OE plants grown on 0.5× MS medium containing 100 mM NaCl for 7 days. (**G**) Survival rate of the seedlings shown in (**F**). Seedlings with green cotyledons were judged as alive. The data represent the mean ± SEM of three parallel experiments and analyzed by Student's *t*-test (n=30/genotype). a–c indicate significant differences. **P<0.01.



Supplemental Figure 12. Phenotypic comparisons among wild-type, *sit1-1* mutant, and *SIT1-OE* plants. (**A**) *sit1-1* mutant rice showed delayed maturation compared with wild-type (DJ) at harvest stage. (**B**) *SIT1-OE Arabidopsis* plants displayed early leaf senescence when compared with Col, $SIT1^{KE}$-OE, and $SIT1^{KD}$-OE at the flowering stage.



Supplemental Figure 13. *SIT1*-RNAi rice seedlings showed reduced sensitivity to exogenous ACC. (A) Effect of increasing the concentration of ACC on seedling height. Five-day-old seedlings grown on 0.5×MS agar medium supplemented with or without ACC were measured. (B) Time-course analysis of seedling height in rice plants grown on 0.5× MS agar medium supplemented with or without 100 μM ACC. Asterisks indicate a significant difference compared to wild type (Jap) by Student's *t*-test. Bars indicate the means ± SEM (n=12, **P<0.01).

Supplemental Data. Li et al. (2014). Plant Cell 10.1105/tpc.114.125187

Supplemental Table 1. Primers for RNAi construction

Gene Locus	Primer	Primer sequence	Purpose
Os03g08550	QRT-F	TGGCCCCAGAAGCAAATCT	QRT for expression analysis
	QRT-R	GAGCGTATTGAGGACAGTAACATCA	
	RNAi-F	GGGAATTAACTAGTTGCTCCGCTACTTCCCACTG	RNAi construction
	RNAi-R	CGGGATCCGAGCTCTCGATCTCAATCAATGAACCCA	
Os09g38850	QRT-F	CACAGCAGCAAGAGCATGGT	QRT for expression analysis
	QRT-R	GGGTAGCTCCAGGAGACATGAT	
	RNAi-F	GGGGTACCACTAGTACCCTCCAGGTCGTCGACATCT	RNAi construction
	RNAi-R	CGGGATCCGAGCTCTCAAGGCTGAGGAACTGCACG	
Os09g02250	QRT-F	TGTTGAGTTCTTGATGCTTTGCA	QRT for expression analysis
	QRT-R	GGAAAACCATGGCAAAACCA	
	RNAi-F	GGGGTACCACTAGTATCCACCCATCAATGGCACG	RNAi construction
	RNAi-R	CGGGATCCGAGCTCTCTCCGTCCACCGTCACCA	
Os04g51040	QRT-F	CCATGGTCCTTGCACAGTTG	QRT for expression analysis
	QRT-R	GCAAAGCCTGGAGTTGACG	
	RNAi-F	GGGAATTCACTAGTTGTCGTTCTGCGCATCAAATG	RNAi construction
	RNAi-R	CGGGATCCGAGCTCCTACAGTAGACAACATTGTTG	
Os02g02120	QRT-F	GAATCTGCGACAACACAATTGG	QRT for expression analysis
	QRT-R	CAGGGCTCTCTCTTTGGATCTG	
	RNAi-F	GGGGTACCACTAGTATTCCCTACCCCTTCGGCATC	RNAi construction
	RNAi-R	CGGGATCCGAGCTCTCAAGCACAGTGGGAACCCCT	

Supplemental Table 2. Primers used in this study

Primer	Nucleotide sequence	Purpose
pSIT1-f	5' CCAAGCTTCCAATGGCTGCATTGTTCAC 3'	
pSIT1-r	5' CGGGATCCGGCGTTTGCGCCAAACTTG 3'	SIT1 promoter amplification
SIT1-f	5' GCTCTAGATGCGGCGTCCCGAGCTAA 3'	
SIT1-r	5' GAAGATCTCGCTCGAGGAATGTCA 3'	SIT1 coding sequence amplification
SIT1K386E-f	5' GTGGAGATTGCAGTGGAGAAGGTATCCCACG 3'	
SIT1K386E-r	5' CCACTGCAATCTCCACTCGGGATACCAGTA 3'	$SIT1^{KE}$ and $SIT1KD^{KE}$ construction
SIT1D482A-f	5' CAAGTCGTTCTGCATCGAGCCATCAAGGCA 3'	
SIT1D482A-r	5' CGTCCAACAATACGTTGCTTGCCTTGATGT 3'	$SIT1^{DA}$ and $SIT1KD^{DA}$ construction
SIT1KD-f	5' CACCAGACGACGCAGGTATGCGGA 3'	
SIT1KD-r	5' TCATCTCGCTCGAGGAATGT 3'	SIT1KD construction
SIT1P1	5' CGTCCGCAGGTAACCTCTAG 3'	
SIT1P2	5' GTGAACATGAGCCTGCTCAG 3'	SIT1 mutant identification
SIT2P1	5' AGGATGGTGTCGAACTCCAC 3'	
SIT2P2	5' CGAGGTGACCCACAGGATAT 3'	SIT2 mutant identification
2715LB	5' ACGTCCGCAATGTGTTATTAA 3'	T-DNA left primer for rice mutant identification
QSIT1-f	5' ATGCAAAATCAGATGGGCAACT 3'	
QSIT1-r	5' CGAAGAGACTAAAACCTTGTGGTCAT 3'	QRT for SIT1 expression
QSIT2-f	5' CCGGATGTAGTTAATCAGTACCTAACTAGTA 3'	
QSIT2-r	5' TCGTAACACCACTTACATCATAGATAAAC 3'	QRT for SIT2 expression
QSIT3-f	5' CAAGGAGTTCAACCGCAATTC 3'	
QSIT3-r	5' TCACGAGCTCCCTTCCTTTG 3'	QRT for SIT3 expression
Hyg-f	5' AAGTTCGACAGCGTCTCCGAC 3'	
Hyg-r	5' TCTACACAGCCATCGGTCCAG 3'	Transgenic plant identification
At-SIT3P1	5' TGTTGGTCAAAGGAAAAGTCG 3'	
At-SIT3P2	5' TCAATGGGCATGCTCTAAGAC 3'	At-SIT3 mutant identification
LBb1	5' GCGTGGACCGCTTGCTGCAACT 3'	T-DNA left primer for rice mutant identification
Qat-SIT3-f	5' CGCTTTCTACACCGAACCAATC 3'	
Qat-SIT3-r	5' TCGGGATCCCAGAGTAGATACC 3'	QRT for At-SIT3 expression
QACS2-f	5'GGAGGGCGTCTCGCAGTT3'	QRT for Os-ACS2expression
QACS2-r	5'CCCTCACCTGCCCCATAAA 3'	
QERF1-f	5'TCGAACACACCACACACTGAAG 3'	QRT for Os-ERF1 expression
QERF1-r	5'GTGGTTCCGCAACATGCTT 3'	
QMAPK5a-f	5'AACCCGCTGCAGAGAATCAC 3'	QRT for Os-MAPK5a expression
QMAPK5a-r	5'GAGAAGGGCTCCAGGCAGAT 3'	
QERF3-f	5'AGGCTGGATACCGTATGATGAAG3'	QRT for Os-ERF3 expression
QERF3-r	5'TGACGGCGCGAGATCAA3'	
QEBP1-f	5'CTGCTGCAAAGTCTGCCAAA 3'	QRT for Os-EBP1 expression
QEBP1-r	5'CTTGCGAGGATCTCTGATTTCA 3'	
QOs-DREB1A-f	5'GGAATCAGGAGCAAGCAGAAA3'	QRT for Os-DREB1A expression
QOs-DREB1A-r	5'CGACTCGCCGCTCATCTC3'	