

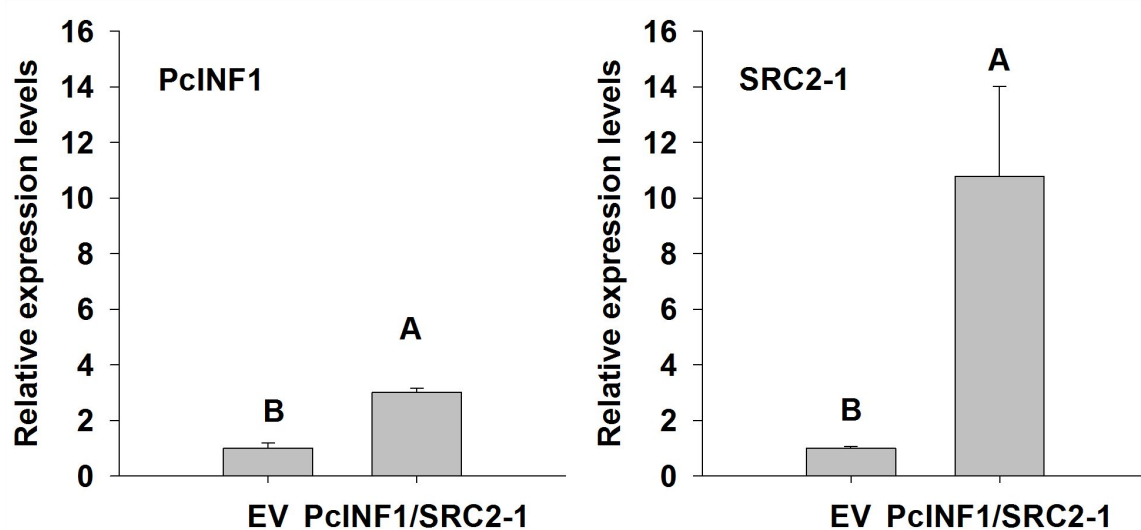
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2 **SGT1 is required in PcINF1/SRC2-1 induced pepper defense response**
3 **by interacting with SRC2-1**

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7 **Muhammad Ifnan Khan^{1,2}, Jiong Hu^{1,2}, Cai-ling Liu^{1,2}, Yang-wen Zhang^{1,2}, Wei Cheng^{1,2},**
8 **and Shui-lin He^{1,2,*}**

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14 **Supplementary figures**



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Fig. S1. qPCR analysis of the transcript levels of *PcINF1* and *SRC2-1* in pepper plants 24 h after transiently overexpressing *PcINF1* and *SRC2-1*. Transcript values are normalized to the mRNA level of *CaActin* and 18S ribosomal RNA. Data represent the mean \pm standard error from three independent experiments. Different letters indicate statistically significant differences, as analyzed using Fisher's protected least significant difference (LSD) test ($P < 0.05$). Mock, treated with 10 mM $MgCl_2$.

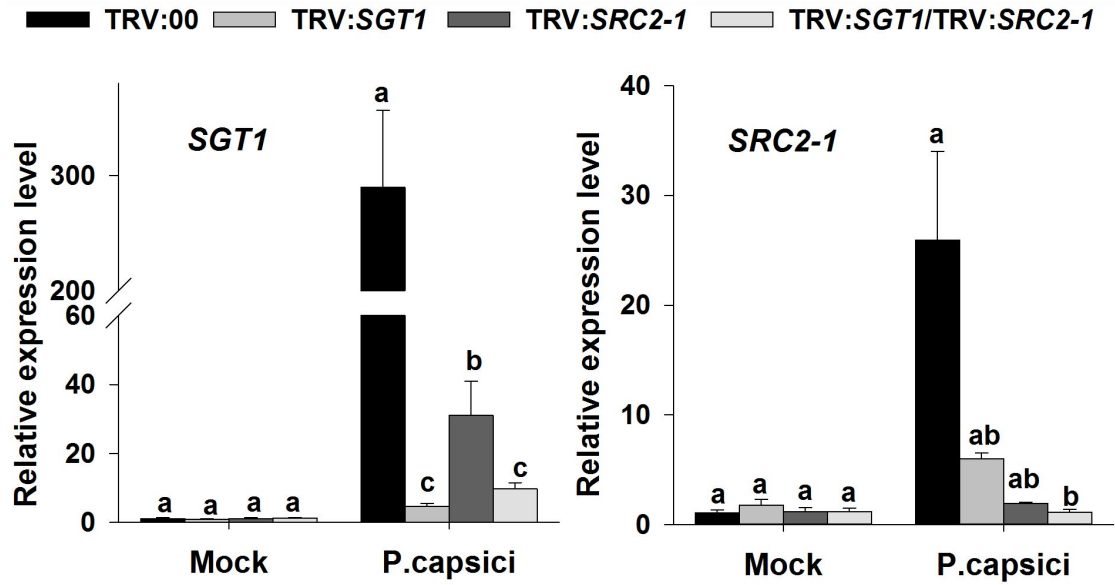


Fig. S2. qPCR analysis of the transcript levels of *SGT1* and *SRC2-1* in TRV:00 and VIGS pepper plants 24 h after inoculation with *P. capsici*. Transcript values are normalized to the mRNA level of *CaActin* and 18S ribosomal RNA. Data represent the mean \pm standard error from three independent experiments. Different letters indicate statistically significant differences, as analyzed using Fisher's protected least significant difference (LSD) test ($P < 0.05$). Mock, treated with 10 mM $MgCl_2$.

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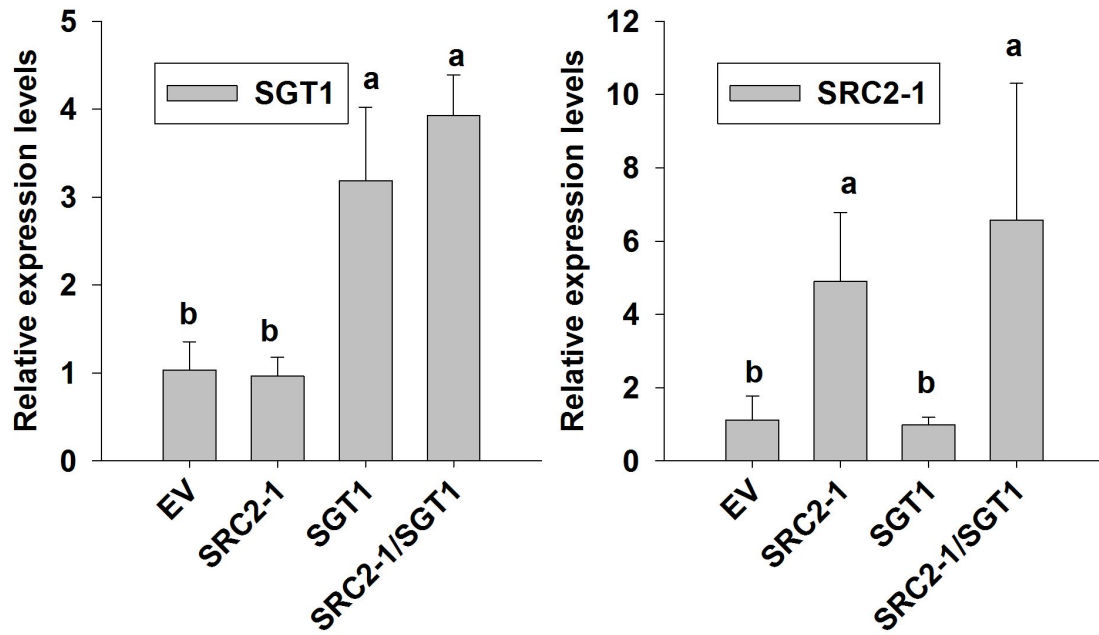


Fig. S3. qPCR analysis of the transcript levels of *SGT1* and *SRC2-1* in pepper plants 24 h after transiently overexpressing *SGT1* and *SRC2-1*. Transcript values are normalized to the mRNA level of *CaActin* and 18S ribosomal RNA. Data represent the mean \pm standard error from three independent experiments. Different letters indicate statistically significant differences, as analyzed using Fisher's protected least significant difference (LSD) test ($P < 0.05$). Mock, treated with 10 mM $MgCl_2$.

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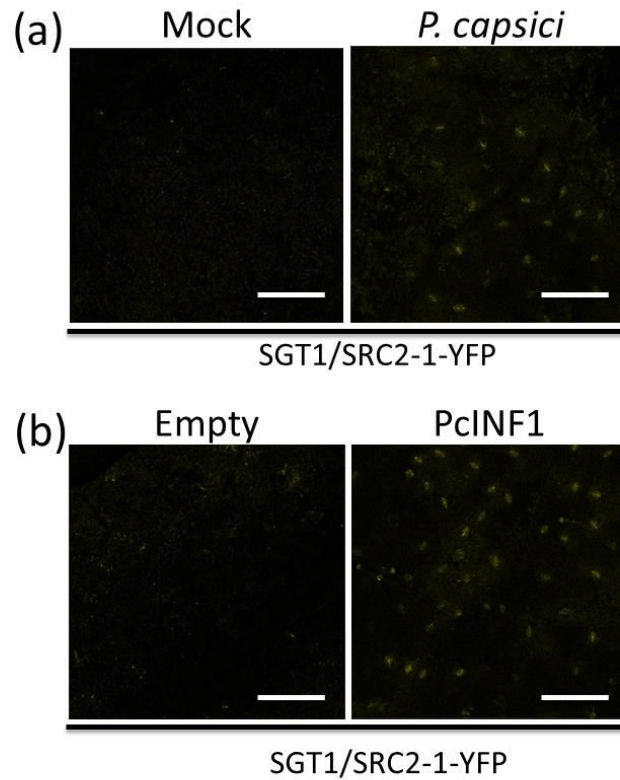


Fig. S4. *Phytophthora capsici* and its elicitor PcINF1 potentiate the formation of SGT1/SRC2-1 complex in *N. benthamiana* leaves detected in the microscope. Agrobacterial cells (OD₅₉₅=0.4) carrying the SGT1/SRC2-1-YFP were vacuum-infiltrated into the leaves of *N. benthamiana* and maintained in the greenhouse. 24 h after infiltration, the infiltrated leaves were inoculated with *Phytophthora capsici* or its elicitor PcINF1.

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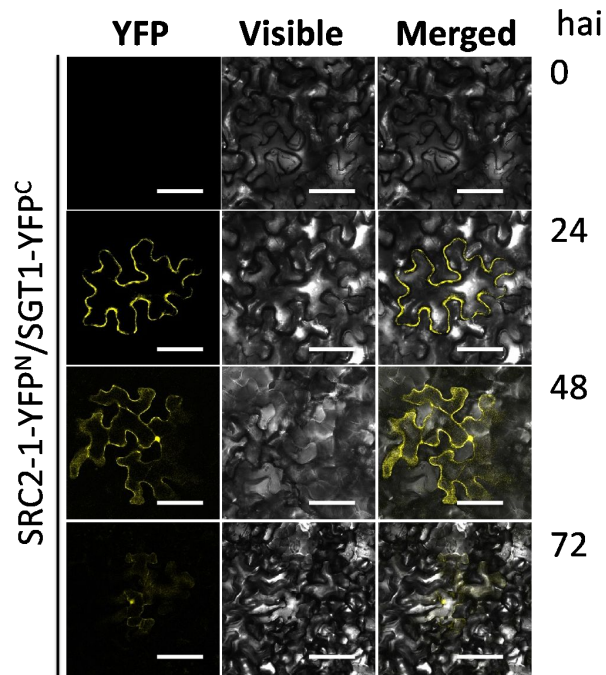


Fig. S5. SGT1 and SRC2-1 interact in the membrane and translocate into the cytoplasm after *P. capsici* inoculation. BiFC was used to visualize the SGT1/SRC2-1 interaction in *N. benthamiana* leaves inoculated with *P. capsici*. Yellow fluorescence, visible light and merged images were taken of epidermal cells infiltrated with a mixture of *Agrobacterium* suspensions harboring the constructs encoding SGT1-YFP^C and SRC2-1-YFP^N fusion proteins. Time course images were obtained with confocal microscopy after *Agro*-infiltration. Scale bars = 100 μ m. Hai, hours after inoculation.

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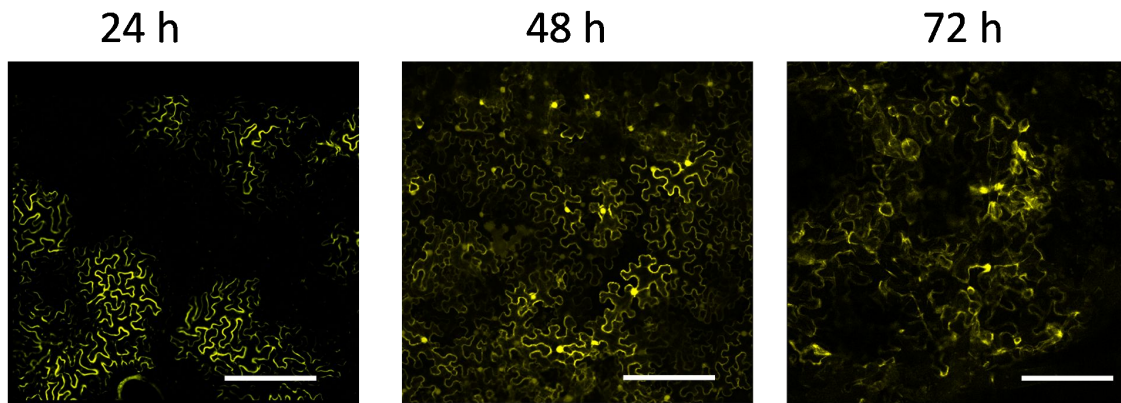


Fig. S6. SGT1 and SRC2-1 interact in the membrane and translocate into the cytoplasm after *P. capsici* inoculation. BiFC was used to visualize the SGT1/SRC2-1 interaction in *N. benthamiana* leaves inoculated with *P. capsici*. Yellow fluorescence, visible light and merged images were taken of epidermal cells infiltrated with a mixture of *Agrobacterium* suspensions harboring the constructs encoding SGT1-YFP^C and SRC2-1-YFP^N fusion proteins. Time course images were obtained with confocal microscopy after *Agro*-infiltration. Scale bars = 200 μ m.

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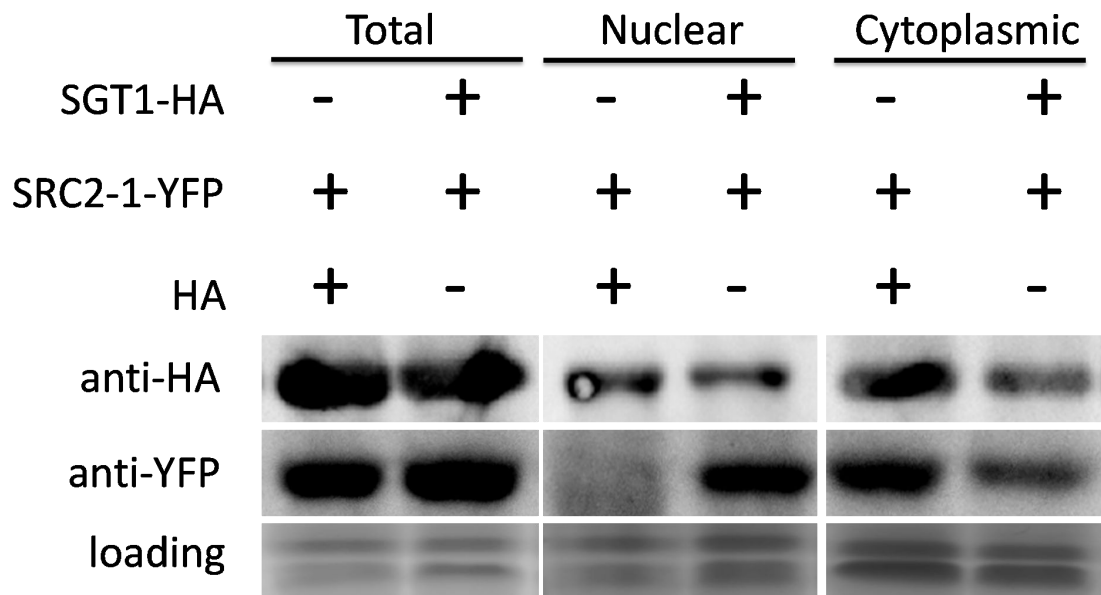


Fig. S7. SGT1 triggers SRC2-1 translocate from membrane to nuclear. Immunoblot analysis of the protein levels of SRC2-1 and SGT1 in pepper leaves transiently expressing SGT1-HA with or without SRC2-1-YFP. Agrobacterial cells carrying the 35S::SGT1-HA or 35S::SRC2-1-YFP were infiltrated into the pepper leaves. The cytoplasmic and nuclear protein of the infiltrated leaves were extracted 48 h after Agro-infiltration. Anti-HA and anti-YFP antibodies were used to detect SGT1-HA and SRC2-1-YFP proteins. Coomassie brilliant blue staining of the membrane was to show the equal loading

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SGT1 promoter

TCATATGTTTTAGCCCTTGTTTTTTCTATAATACATTGGATAGAGGAATGGAAAAATAAGAGCAGTA
TTACAAGGTAGGAATAAAAAATTCATGAATAAAGTAAAAGTTCATACAATCAGTTAATGGTAAAAGGTA
TTTACCAGTAGCGGAGCTACCTTGTCCAGGGGTTCATCCAAACCTCCTTCGACGAAAAATAATAT
TATTTTTATATATATTTTACATGATTAATAATTTTTTATTCATATATAGTAGATGTTGAACCCCTCTT
TAATTAGTTCGTATATTTAATTCGACCCCTCAATGAAAAATCCGACTTCATCACATGATATTTATTAT
ATTAATATTAATAATAAATAAGGGTAAATGAAATAAGAGTCGTCCCTTTAAGTTTAAAGGACCGATTCA
ATAATAGTTCAATTAGATACGACTACGTCGAATACTGTAAGTCTTTATAGGTCATTTGTCCATGAAAA
ERE (+) HSE (-) box III (+)
ATAATTTTTTGGAGTTAGAGTTGAAGTAAAAATTTGAAATTTATTTATTTTTATTTTTAAAAAATAATT
box III (+)
TTCACATAAAGTAAACACTAATTTTTATGATCAAATACATATTTATTTTACACTTTTTTGGCTATAGCCGAA
TAATTTTTGGGAAAAGAGTTAAAAATATCCCTCTACTATGATAAATGATTAATTTGCCCCCGTTTAT
W box (-)
ACTATAGGGTCAAATAACCCCTTGTGTAGCAAAATATCAAAAAATATCCCTCCTTTAACACTTGGT
GAGAGCCCACTTGGCATGACACTTGGCNNNNNNNNNNNNTTTTTTTCTTTTAAATTTTTCACCTTCA
AATTTTATTAATCATTATTTGTTGAAAAAGAACTTATAATTAATAAATAAATAAATAAATAAATAAATAA
HSE (-) W box (+)
TTTTCAGCCATTTGGTGTCACTTCTCACCACCAATTAATCCGATGGAAATTTGTTTGGACTAGAGTGGATT
TCA-element (-)
AACAAATGATAAATGAAAGATGATTTGATTGAGGAAAAGATGGTGTGGAAAATAGGAAAATGGTT
GATTACTACTTTGATACATTGATATCTAACTAAACCATGGAACTTTCGCCACCATCATCGTTAATG
TCTACCATCTGGTTTTTGGCGTCGAAGCCATTAACNCTTCAAGCTCGTCCACCATCAGTTCCTTTGTCAA
AGAAAATGGAGAAAGGTCGTGGTTTTTGGTGTATTTAGTGGTGGTGAACCTGATATCGGTAACCGGAAAA
AACATATTTTCAATTAATAATTTTCTTAGTATTTAGTTATCTTTTCAACAAAATAAATGATTAAGAA
ABRE (-)
ATTTGAAGTGAATAAATAAAGAAAAAATAATGATGTTGTGTCAAAGCCACGTGGGCTCTCACTTTGTC
ABRE (+)
TCA-element (-)
ATGGTGGAGAAGTGTAAAAAGATGAGTATTTTTAATAGCATTGCATAACACAAGACACATTTTCATCT
TATAGTATCACGAGGACAAATTTAACTAATTTATTAATAAGACAAGTATTTTTCACTGTTTCATAAT
TTTTTTGGGAAAAGGTGAAAAAATAATGATGGCCAAATGCCACTTAATTTAATTACAATTAAGGAAG
ACTTGAGCTGTATATAAGTCTTTAATAATCCAATAATCATCTTTTAAAAATCCCCCAATAATCACC
TTTCCCTCTGTCATCTAGAAACTTCTCGCTTCTCGTCTCATCTCATCTCATCCATG

Fig. S8. Nucleotide sequences of 5-flanking promoter regions and putative *cis*-acting elements of the *SGT1*. CCAAT-box, MYBHv1 binding site; GARE-motif, gibberellin-responsive element; HSE, *cis*-acting element involved in heat stress responsiveness; MBS, MYB binding site involved in drought-inducibility; TC-rich repeats, *cis*-acting element involved in defense and stress responsiveness; W box; transcription factor WRKY binding site; WUN-motif, wound-responsive element.

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SRC2-1 promoter
AAAGGCATTTTAGTGTATAGTTTTGGACTTTCATACATGAGTGAATTTACAGTAGAAGTT
ACATACTTGATCAAATCCACGAATCGATCGAATTGAGCAATTTTAGATCAAATCTATTT
W box (-)
ATGAAAAGTGGTCAAATAATTTGGAGTTGGTGATTTTATGACTCATTCCGTTTTTTAGT
TGTATAACTCATCTCATATAATATCTTTACGACAAAAGAGTCTTTATTTCTTGAATGGTT
WUN-motif (+)
AATTCTTTTTATATATAGTATACTAGTTATATAAATTTCTAGGAAATTTTCATCACAAGAT
GARE-motif (-)
CGTAGATTATTCATAAGGGTCTGTTTGGCAGTTTATAGAGCATATTCGAAAATCTGCCT
GCACACGCTGTATACACGACGTTGGTTCGGAATTTGGCGCTTAATATTATAAGGAAAAAA
GARE-motif (-) CCAAT-box (+)
AAGGATAAATACTATCTGTTTGGATAACGCGTTTACTATTTTTATTACAACGGGTCTCT
AGCTTTTTCTGGAATTAATAAATAACACATCAACAAAAGCCAAAAGGGCCCTTTTA
MBS (+)
ATTTCCACAAAGTTTGTACCTATCCAAATGAACGGTCAAAAAATAGAGCATCTTTATT
W box (+)
CTTACTTCGTCGGTTTTGCTATACTTGACTTTTTTCTACAAAAATTTATTTTAAATGTAA
TTATATTTTATAATAAAAAAATATTTTATATATTTTAAACATTATTTTATTTA
TTAAATAATTAATAACATGCATATAAATAAATTTATATTTTAAAAATATAATAA
AATTAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
CAATTAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
TACAAAATATAATTTATATATAACATCAAAAATATATTTTACTACACATCTTTTC
TC-rich repeats (-)
TTAGTAAGAGCAGGAATATATGTAGAAAATAACGGCACGAAAAGCGACCCATAATTG
TC-rich repeats (+)
ATAGTGATGCTTACTGTTACTGACCTATTCCTCCGCTCATTTCCGTGATATATTTTC
TTGAGACACAAAAATTTAGTTATAAAGAAAGACTTAATTTATATTTGTTAAATTAATTTT
ATATAAAAAATATATGTTATTTTATTTTAGTAAAGTGGAAATTAATAAAAAATTA
ATAATTAATGTTAGTATTAATAAAGTTACCAAATCAAAAATATTAGCAAATAAATGGGAG
CAATTCAACTTACTGCTACCTTAGAGTCGGTTTATTCGAAAGTTCCTGTTATTTCCCT
TCGTTATTAACCTTCCCTTATAAATTCCTCCAATFCCCCCATCAACCCAAACCAACAC
HSE (+) MBS (-) WUN-motif (-)
GCACAAAAATTTTCAGAGTTTCTCTTCAACTTCAGTTACAATTTTCATCTCCTCAAAAT
TCCTTCTGAATCTCTTTGCAATG

Fig. S9. Nucleotide sequences of 5-flanking promoter regions and putative *cis*-acting elements of the *SRC2-1*. CCAAT-box, MYBHv1 binding site; GARE-motif, gibberellin-responsive element; HSE, *cis*-acting element involved in heat stress responsiveness; MBS, MYB binding site involved in drought-inducibility; TC-rich repeats, *cis*-acting element involved in defense and stress responsiveness; W box; transcription factor WRKY binding site; WUN-motif, wound-responsive element.

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Supplementary Table S1. Primers used in the qPCR and the validation of qPCR

Gene	Accession no.	Forward primers	Reverse primers	Length of PCR product	Specificity screen (NCBI)	Peak number of Melting curve	Cq of the no- template controls	slope	PCR efficiency(%)	y intercept	R ²
<i>SGT1</i>	JN252493	5'-GCTGATGAGGATGCAAGAGAGC-3'	5'-CCAAAGATGAAGAGAAAAAGA-3'	213	specific product	1	N/A	-3.401	0.968027521	33.453	0.990
<i>SRC2-1</i>	DQ465394.1	5'-ATGCTTCATTGCTGATGC-3	5-AGTCAGAAACTATTAAAT-3	214	specific product	1	N/A	-3.247	1.03224748	30.737	0.995
<i>SAR2A</i>	AF313786	5'-CAGGGAGATGAATTCAGGCC-3'	5-CATATGAACCTCTATGGATTTCTG-3	165	specific product	1	N/A	-3.421	0.960253281	36.978	0.997
<i>H1R1</i>	AY529987	5'-GACATGGTCTGGTAAACCA-3	5-CCCAACAAGGAGCCTGAAGAA-3	150	specific product	1	N/A	-3.321	1.000387465	32.819	0.991
<i>DEF1</i>	AF442356	5'-CAAGGGAGTATGTGCTAGTGAGAC-3'	5-TGCACAGCACTATCATTCGATAC-3'	267	specific product	1	N/A	-3.423	0.956482533	30.912	0.993
<i>BPR1</i>	AF053343	5'-CAGGATGCAACACTCTGGTGG-3	5-ATCAAAAGCCCGTTGGTC-3	310	specific product	1	N/A	-3.348	0.9800615	32.432	0.993
<i>PR4</i>	AF244122.1	5'-CAACCCGACAAACATCACTGG-3	5-CCTCAAGCATCTACCGCAAGCA-3	160	specific product	1	N/A	-3.383	0.983140817	36.584	0.992
<i>PO2</i>	DQ469711	5'-TGATTGCTTTGTTACGGTT-3	5-ATGATGGACCTCCAAGAGCA-3	224	specific product	1	N/A	-3.315	1.002869348	35.984	0.999
<i>CeACT1N</i>	GQ339766(NCBI)	5'-CCTCTCAACCCCTAAGGCCAACAG-3'	5-ACGTCCAGGAAATCCAAAAGAA-3'	225	specific product	1	N/A	-3.321	1.000387465	36.521	0.995
<i>16S ribosomal RNA</i>	EF564261(NCBI)	5'-CCG9TCCGCGCTATG3TGTGACCCGGTCCGTC-3	5'-GCA9TGTTC9TCTTTTCATAATCCCAAGAA-3	285	specific product	1	N/A	-3.320	1.000302555	35.632	0.993

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SupplementaryTable S2. Oligonucleotides for plasmid constructs used in this study		
Analysis	Gene	Sequence of the oligonucleotide
BiFC/ <i>E. coli</i> overexpression	SRC2-1 F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAGTTACGTCCGTTAGA-3'
	SRC2-1 R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCATAAAAATCAAACCCACCACC-3'
	SGT1 F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGTCCGATCTCTCAAC-3'
	SGT1 R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCATATAGTATTCCCATTTGCTT-3'
VIGS	SGT1 240 F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTCAAGATTCACTAGCTTAAT-3'
	SGT1 240 R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCGTCTGGTTTCTGATAGAAT-3'
	SRC2-1 275 F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCAGCGCCTGAGAAGAAGAAAGAC-3'
	SRC2-1 275 R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTAATCCACCTCCTCCGAACT-3'
Subcellular localization	NLS	5'-GGCCCTAAAAAGAAGCGTAAGGTT-3'
	nls	5'-GGCCCTAAAAACAAGCGTAAGGTT-3'

Supplementary Table S3. Cis-acting element found in the promoter of SGT1 and SRC2-1											
Gene	ABRE	Box III	CCAAT-box	ERE	GARE-motif	HSE	MBS	TCA-element	TC-rich repeats	W box	WUN-motif
SGT1	2	2	0	1	0	2	0	2	0	2	0
SRC2-1	0	0	1	0	2	1	2	0	2	2	2

ABRE, cis-acting element involved in the abscisic acid responsiveness; Box III, protein binding site; CCAAT-box, MYBHv1 binding site; ERE, ethylene-responsive element; GARE-motif, gibberellin-responsive element; HSE, cis-acting element involved in heat stress responsiveness; MBS, MYB binding site involved in drought-inducibility; TC-rich repeats, cis-acting element involved in defense and stress responsiveness; TCA-element, cis-acting element involved in salicylic acid responsiveness; W box, transcription factor WRKY binding site; WUN-motif, wound-responsive element