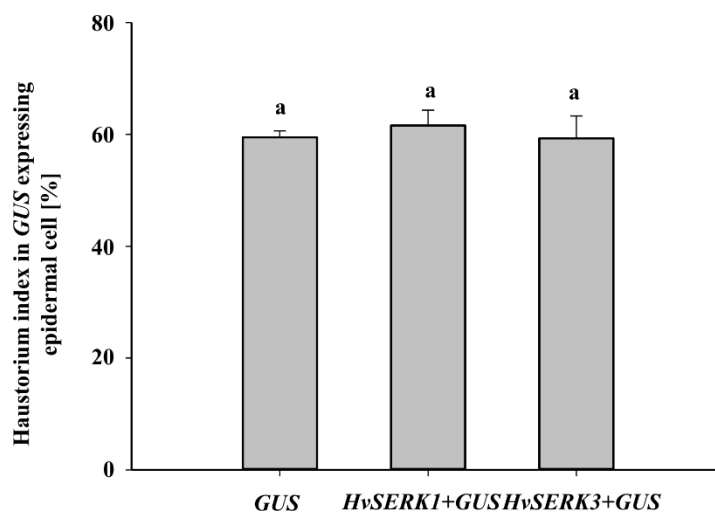


Supplementary Materials:

Supplementary data 1. Sequence of *HvSERK2* promoter

CCTTAACAGTGCCCATATTTCGGCGGGTCACTTAACAAGCAATATCCCCAACAGGTAGGGTTTGTCTGG
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CAAAAACCTAGTGACGCGGGAGTCGACGAACCCAGCCAACCAACATGCAACGTGAGCCCACCTGG
CGAGCAAAAACAACCGCAGGATGAAAATCAAGTTTAACTGTTGTAAAGATCTAACGACTCAGACACA
TGAAAAGGCTAATCGGACGGTCAGAGAAACCCCAAATCGGGGGAGCCCTTGGGAGGGCGGGTCGCC
CAACATCCTTAGGGCATGTACATCGGTTTAGACAATGTCTGACTATAATGGCTTGACATGTCATGTGCA
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CAGTTGCGGAAACAATTGCTTTGTTGCGAAATAATTTTGGACCCATTAATTCTTGAAACAACAGTCGA
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CGCTAAGTTGCACAAGTGCAAGCTAGGTGAGTCTACATAAAACAAGCGTTGCACAAGCGAGAGGCTC
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ATTAAGCTACAGAAATTTATCCACCCATTCCATTAGGGAAGAGAGGGAGGGAGGAAGAAAGAAAG
ACGCCGAGCTATCAACGGCGACGCGGCGGCCAGCAGATCCGTACAGCTCGGGGGATCTTGGGGAAG
GGAGAACTGGTGGGGGCCGCTATGCGTCGGTGAGGTGGGGCCAGTCGGCGCTAGAGCAGGA



Supplementary Figure S1. Functional analysis of *HvSERK2* by single-cell transient overexpression assay. Bars with different letters show significant differences at the level of $p < 0.05$.

Supplementary Table S1. Key regulatory motifs found within the promoter sequence of *HvSERK2*

Regulatory Motif	Function	Position	Consensus Sequence
A-box	cis-acting regulatory element	+293	CCGTCC
ABRE	cis-acting element involved in the abscisic acid responsiveness	-1388	GCCGCGTGCC
ACE	cis-acting element involved in light responsiveness	+521	AAAACGTTTA
AE-box	part of a module for light response	+425	AGAAACAA
Box 4	part of a conserved DNA module involved in light responsiveness	+396	ATTAAT
Box-W1	fungal elicitor responsive element	-325, -961	TTGACC
CCAAT-box	MYBHv1 binding site	+1383	CAACGG
CCGTCC-box	cis-acting regulatory element related to meristem specific activation	+293	CCGTCC
CE3	cis-acting element involved in ABA and VP1 responsiveness	-870	GACGCGTGTC
CGTCA-motif	cis-acting regulatory element involved in the MeJA-responsiveness	+1036	CGTCA
G-Box	cis-acting regulatory element involved in light responsiveness	-800	CACGTT
GA-motif	part of a light responsive element	-563	AAAGATGA
GC-motif	enhancer-like element involved in anoxic specific inducibility	-1420	CCCCCG
HSE	cis-acting element involved in heat stress responsiveness	-446	AAAAAATTTC
LTR	cis-acting element involved in low-temperature responsiveness	-321	CCGAAA
MBS	MYB binding site involved in drought-inducibility	-360, -352, +324, +623	CAACTG
MNF1	light responsive element	-997	GTGCCC(A/T)(A/T)
O2-site	cis-acting regulatory element involved in zein metabolism regulation	-37	GATGATGTGG
Sp1	light responsive element	-1097, -1351, +1327, -1355	GGGCGG
TATC-box	cis-acting element involved in gibberellin-responsiveness	+1323	TATCCCA
TC-rich repeats	cis-acting element involved in defense and stress responsiveness	-836	ATTCTCTAAC
TCA-element	cis-acting element involved in salicylic acid responsiveness	-1068	GAGAAGAATA
TCCC-motif	part of a light responsive element	-1436	TCTCCCT
TCT-motif	part of a light responsive element	-1189	TCTTAC
TGACG-motif	cis-acting regulatory element involved in the MeJA-responsiveness	-1036	TGACG
Circadian	cis-acting regulatory element involved in circadian control	-508, -538, -581	CAANNNNATC
CAAT box	cis-acting element in promoter and enhancer regions	11	10
TATA-box	core promoter element around -30 of transcription start	5	9

“+” downstream from the promoter sequence;“-” upstream from the start codon (ATG)

Supplementary Table S2. Functional analysis of the *HvSERK2* by single cell transient over-expression assay.

Genes		Total No. of observed cells	Observed cells with haustorium	Horstorium Index (%)	<i>t</i> -test (<i>p</i> < 0.05)
Replicate 1	<i>GUS</i>	84	48	57.1%	
	<i>GUS+HvSERK2</i>	78	32	41%	
Replicate 2	<i>GUS</i>	80	47	58.8%	
	<i>GUS+ HvSERK2</i>	60	26	43.3%	
Replicate 3	<i>GUS</i>	60	36	60%	
	<i>GUS+ HvSERK2</i>	75	34	45.3%	
Total	<i>GUS</i>	224	131	58.6%	a
	<i>GUS+ HvSERK2</i>	213	92	43.2%	b

Supplementary Table S3. Functional analysis of the *HvSERK1* and *HvSERK3* by single cell transient over-expression assay.

Genes		Total No. of observed cells	Observed cells with haustorium	Horstorium Index (%)	<i>t</i> -test (<i>p</i> <0.05)
Replicate 1	<i>GUS</i>	91	53	58.2%	
	<i>GUS+HvSERK1</i>	85	55	64.7%	
	<i>GUS+HvSERK3</i>	76	43	56.6%	
Replicate 2	<i>GUS</i>	103	62	60.2%	
	<i>GUS+ HvSERK1</i>	66	40	60.6%	
	<i>GUS+HvSERK3</i>	73	42	57.5%	
Replicate 3	<i>GUS</i>	83	50	60.2%	
	<i>GUS+ HvSERK1</i>	79	47	59.5%	
	<i>GUS+HvSERK3</i>	61	39	63.9%	
Total	<i>GUS</i>	277	165	59.5%	a
	<i>GUS+ HvSERK1</i>	230	142	61.6%	a
	<i>GUS+HvSERK3</i>	210	124	59.3%	a

Supplementary Table S4. Information of the primer pairs used in this study

Primer name	Primer sequence	Note
HvSERK1-F	F: CGCTCGGATGGCTGCGTCGCCGG	Cloning primer for <i>HvSERK1</i>
HvSERK1-R	R: TTTTCCTGTTACCTCGGGCCGGACAG	
HvSERK2-F	F: GAGCAGGAATGGCGGCGGCGGTGTTGGG	Cloning primer for <i>HvSERK2</i>
HvSERK2-R	R: AATGATGTTTGTCTATCTTGGGCCAGAC	
HvSERK3-F	F: ATCTAGGGCGGCGGCAATGGGGGT	Cloning primer for <i>HvSERK3</i>
HvSERK3-R	R: AGCAGCCATCATCACCTTGGCCCT	
HvSERK1-Q-F	F: ATGGCAGTGCATAGGAACCT	qRT-PCR primer for <i>HvSERK1</i>
HvSERK1-Q-R	R: CAACCGCCTCAAAGTCTTCA	
HvSERK2-Q-F	F: GATGCTCTGTATAACCTGCG	qRT-PCR primer for <i>HvSERK2</i>
HvSERK2-Q-R	R: TGACCAGGCTAGTTAGGTTG	
HvSERK3-Q-F	F: TCGGGCACAATTCCTAAATC	qRT-PCR primer for <i>HvSERK3</i>
HvSERK3-Q-R	R: CCTGGTGATTGCTCAGGAGT	
Actin-F	F: GACTCTGGTGATGGTGTCAGC	qRT-PCR primer for <i>Actin</i>
Actin -R	R: GGCTGGAAGAGGACCTCAGG	
HvSERK2-XbaI-F	F: GCTCTAGAATGGCGGCGGCGGTGTTGGGGG	Subcellular localization primer of <i>HvSERK2</i>
HvSERK2-SmaI-R	R: TCCCCCGGGTCTTGGGCCAGACAGTTCCACT	
HvSERK2-KpnI-F	F: GGGGTACCATGGCTGCGTCGCCGGA	Transient over expression primer of <i>HvSERK1</i>
HvSERK2-SacI-F	R: CGAGCTCTTACCTCGGGCCGGACA	
HvSERK2-KpnI-F	F: GGGGTACCATGGCGGCGGCGGTGT	Transient over expression primer of <i>HvSERK2</i>
HvSERK2-SacI-R	R: CGAGCTCTCATCTTGGGCCAGACA	
HvSERK2-KpnI-F	F: GGGGTACCATGGGGGTGCCGCCGT	Transient over expression primer of <i>HvSERK3</i>
HvSERK2-SmaI-R	R: TCCCCCGGGTCACCTTGGCCCTGAT	
HvSERK2P-F	F: CCTTAACAGTGCCCATATTCGG	Cloning primer for <i>HvSERK2</i> promoter
HvSERK2P-R	R: AACAACCATCAACACAGCGAC	
HvSERK2P-SacI-F	F: CGAGCTCCCTTAACAGTGCCCATATTCGG	Activity analysis primer for <i>HvSERK2</i> promoter
HvSERK2P-SpeI-R	R: GGACTAGTTCCTGCTCTAGCGCCGACTGGG	