

SUPPLEMENTAL INFORMATION

CSEP0105 ATGCACTCCAATCGCTTGCTCAGCTGTCTCGGAATTTGGTTGATATCTATCATAGGTCTG
M H S N R L L S C L G I W L I S I I G L

CSEP0105 GTGCAGTGCATTACAGAATATTACTGTGGCAACAATGTGGTGATATCACAGGAACTAATT
V Q C I T E Y Y C G N N V V I S Q E L I

CSEP0105 CAATCTGTCTAGCGAAATAGCTATCTCGGAATTAAGGGCTGGAGTTACATGGCACACATCC
Q S V S E I A I S E L R A G V T W H T S

CSEP0105 TTATCCGGTATCATATATAGAGGAAATCTATGTGGTTTACCAAACCCGACCTATCGTTGG
L S G I I Y R G N L C G L P N P T Y R W

CSEP0105 TTCATAACAACAGTCGGAAGATGAGAATTCGGAGGTGGTGATGAAATACTTTTTACTGGTC
F I Q Q S E D E N S E V V M K Y F L L V

CSEP0105 TCGGCCAGTTGCGAAGTGCAGGGGGTCATAAGCACAGGCTGGAGGAAGAACTTGGAGAA
S A S C E V Q G V I S T G W R K K L G E

CSEP0105 GATGACACCTGGTGTACCGAGGCATAA
D D T W C T E A *

CSEP0162 ATGCGCTTTTTCTCGTATGGCCATCATCTTTCATAGCGCAAGCTTCTGTACAACAAGCCTT
M R F S R M A I I F H S A S F C T T S L

CSEP0162 GGCGCCCAATATTCTAGACATATTAATGAAGAATTGAAGGTATTTTCATTGTAATAATGAT
A A Q Y S R H I N E E L K V F H C N N D

CSEP0162 ATCCATCAAGAGGAATATTCACGGACACCACATTATAAAATACAAGATCCAGATACCATT
I H Q E E Y S R T P H Y K I Q D P D T I

CSEP0162 CAGGGATTGAACGAGGAGCTCAGTTATTTAGTTGCAGACACTTATGATACGAGGACTGTT
Q G L N E E L S Y L V A D T Y D T R T V

CSEP0162 CAGTICTATGACCAAGACAACGGTGACTACGAGTATTTCAATCTTTCAGAAATATGTGAT
Q F Y D Q D N G D Y E Y F N L S E I C D

CSEP0162 AGATATCAAGAGCAAGATGGTACTGTTGTTATTGAACACATACTCGTCAACGACCGGCAA
R Y Q E Q D G T V V I E H I L V N D R Q

CSEP0162 GGTCGTGCATGTGCCATGATGATGATTAAGACTGTAATACCATTACCAATTGGGACGGA
G R A C A M M M I K T V I P F T N W D G

CSEP0162 CAAAGTCCACAAAGATATTATAGCTTGTGCGCAGTTGGCTCGGGATAA
Q S P Q R Y Y S L C A V G S G *

Figure S1. Nucleotide and amino acid sequences of CSEP0105 and CSEP0162. Shaded sequences encode for predicted signal peptide. Sequences used for the RNAi constructs are shown underlined.

A	HvHsp16.9-CI(AK362925)	ATGTCGATCGTGAGGCGTAGCAACGTGTTTCGACCCCTTCGCCGAC
	HvHsp16.9-CI-H
	HvHsp16.9-CI(AK362925)	GCGGACCCCTTCGACACCTTCCGCTCCATCGTCCC GGCGATCTCAC
	HvHsp16.9-CI-HA.....
	HvHsp16.9-CI(AK362925)	AACAGCGAGACAGCTGCGTTTCGCGAACGCCCGGATGGACTGGAAGC
	HvHsp16.9-CI-HC.....
	HvHsp16.9-CI(AK362925)	CCCGAGGCGCACGTCTTCAAGGCTGATCTTCTGGCGTGAAGAAGC
	HvHsp16.9-CI-H
	HvHsp16.9-CI(AK362925)	GTCAAGGTGGAGGTGGAGGACGGCAACGTGCTCGTCGTCAGCGGCC
	HvHsp16.9-CI-H
B	HvHsp16.9-CI(AK362925)	MSIVRRSNVDFPFADLWADPFDTFRSIVPAISGGNSETAAFANARM
	HvHsp16.9-CI-H
	HvHsp16.9-CI(AK362925)	PEAHVFKADLPGVKKEEVKVEVEDGNVLVVS GERTKEKEDKNDKWF
	HvHsp16.9-CI-H
	HvHsp16.9-CI(AK362925)	SGKFVRRFRLPEDAKVEEVKAGLENGVLT VTPKAEVKKPEVKAIE
	HvHsp16.9-CI-HT.....

Figure S2. (A) Nucleotide and (B) amino acid alignments of *HvHsp16.9-CI* and *HvHsp16.9-CI-H*. There are 14 nucleotide differences, where 12 are synonymous and two are non-synonymous. Dots indicate identical nucleotides and amino acids.

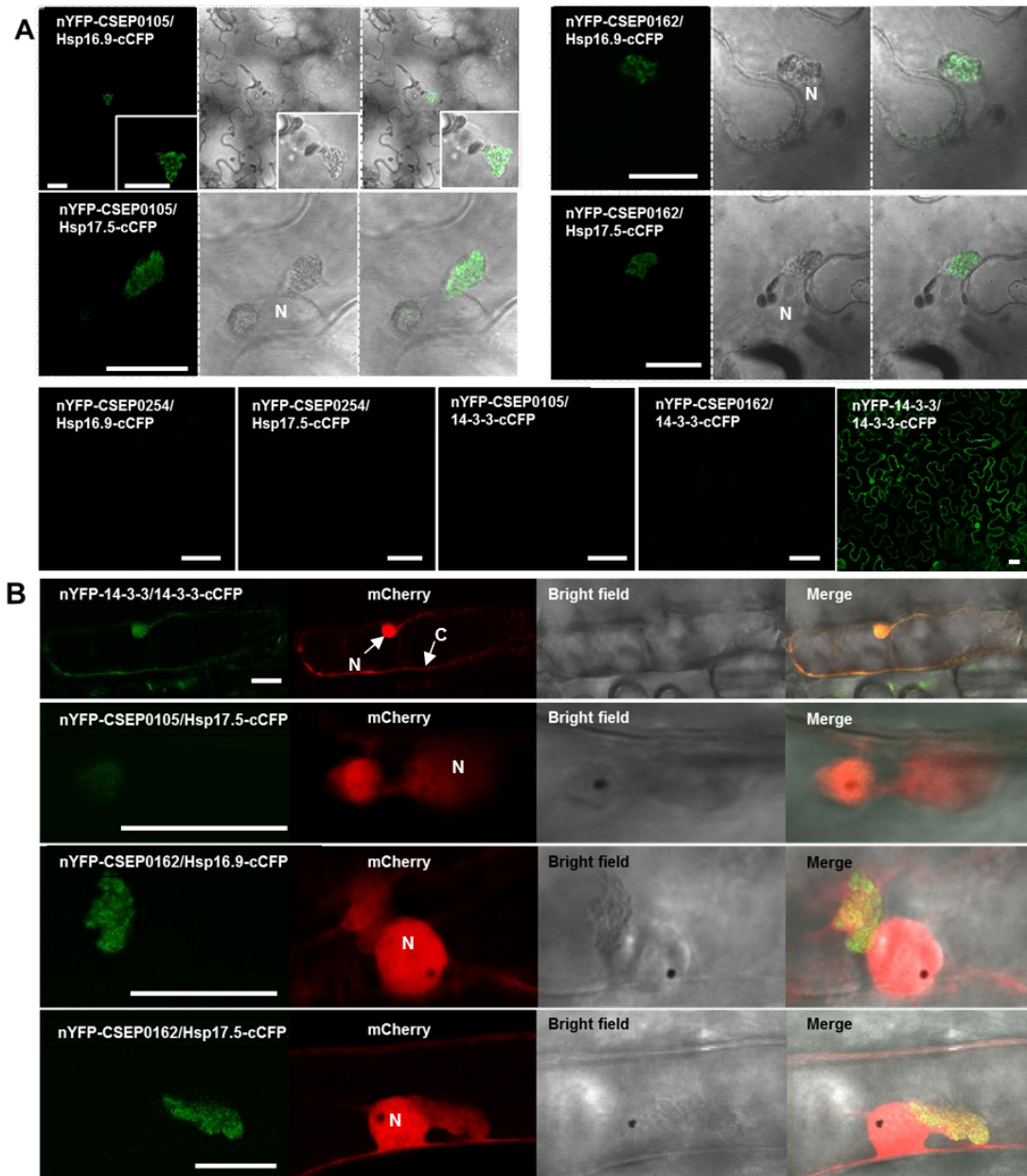


Figure S3. CSEP0105 and CSEP0162 interact with the barley Hsp16.9 and Hsp17.5 proteins in *Nicotiana benthamiana* and barley in a BiFC assay. The CSEPs were fused N-terminally by nYFP and the Hsps were fused C-terminally by cCFP. A) Constructs were co-expressed into *N. benthamiana* leaves using *Agrobacterium* mediated transformation. Interaction of two 14-3-3 proteins was used as a positive control. CSEP0254 and 14-3-3 proteins were used as negative controls. B) The nYFP-CSEP and Hsp-cCFP fusion constructs were co-transformed with the mCherry transformation marker, which localizes to the cytosol and the nucleus, using particle bombardment into barley leaf epidermal cells. C, cytosol. N, nucleus. Scale bar, 20 μm.

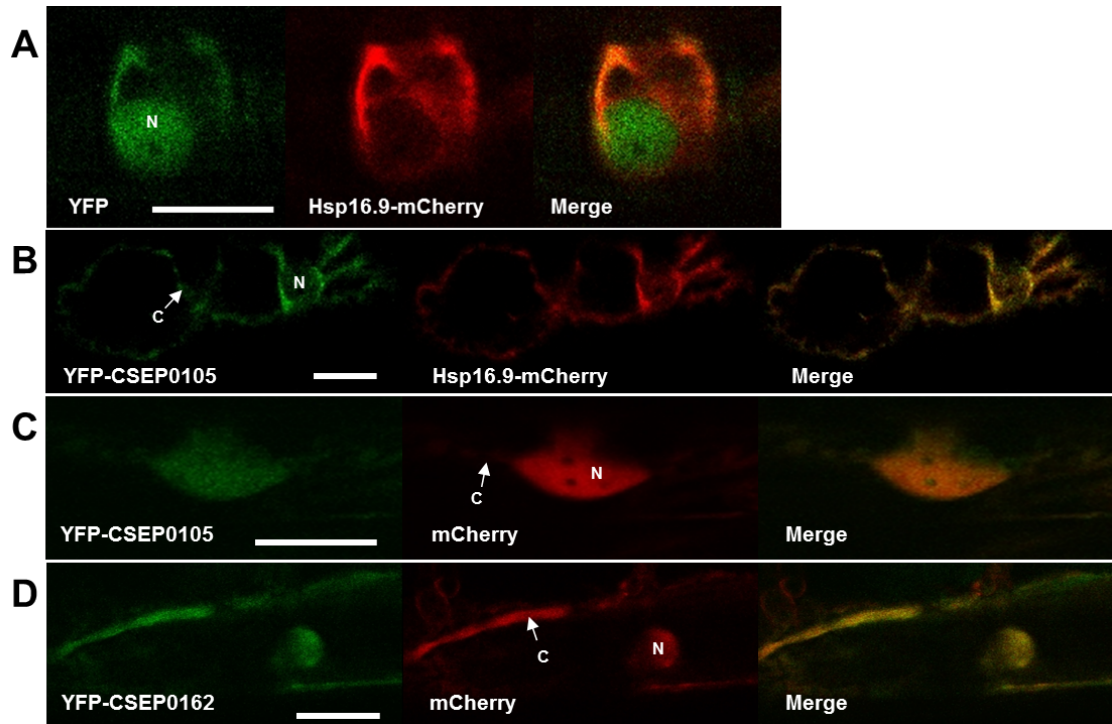


Figure S4. Localization of CSEP0105, CSEP0162, and Hsp16.9 proteins in *Bgh*-infected barley cells. (A-D) Confocal images showing the nuclei of the same cells presented in Fig. 9. C, cytosol. N, nucleus. Scale bar, 20 μm.

Table S1. List of small heat shock proteins identified in barley

Name ¹⁾	MW (kD)	bp	AA	cDNA resources		Gene expression resources ⁴⁾	Genome resources ⁵⁾		Localization ⁶⁾
				HarvEST ID ²⁾	FI cDNA ID ³⁾		Probe Set ID	Morex contig	
MLOC_62670.1	15.09	417	138			none	46836	7H cM=66	
<i>HvHsp15.1-Px</i>	15.14	417	138		AK365443	none	none identical		peroxisome
<i>HvHsp16.7-CI</i>	16.76	453	150	35_14864	AK252765	Contig2010_at	120794	4H cM=55	cytoplasm
MLOC_45029.1	16.79	459	152			none	276057	2H cM=138	
MLOC_44536.1	16.84	453	150			Contig2007_s_at	274906	3H cM=15	cytoplasm
<i>HvHsp16.86-CI</i>	16.86	456	151		AK376179	EBro08_SQ011_I03_at	54783 (8 SNPs)	3H cM=15	cytoplasm
<i>HvHsp16.88-CI</i>	16.88	453	150	35_14863	AK355146	Contig2008_s_at	none identical	3 ⁴⁾	cytoplasm
<i>HvHsp16.9-CI</i>	16.93	456	151	35_14860	AK362925	Contig2004_s_at	66925	3HS	cytoplasm
<i>HvHsp16.9-CI-H</i>	16.93	456	151			none	none identical		cytoplasm
<i>HvHsp17.1-CV</i>	17.14	468	155	35_5795	AK241741	Contig13073_at	49195	2H cM=58	cytoplasm
<i>HvHsp17.3-CII</i>	17.34	480	159	35_15451	AK375970	Contig3284_x_at	135539	3H cM=46	cytoplasm
MLOC_32229.1	17.41	480	159			Contig3286_s_at	226559	3H cM=45	cytoplasm
MLOC_6787.1	17.54	477	158			Contig2006_at	138095	4H cM=56	
<i>HvHsp17.5-CI</i>	17.57	477	158	35_14859	AK250749	HB18H23r_s_at	64954	4H cM=51	cytoplasm
<i>HvHsp17.7-CII</i>	17.61	489	162	35_15454	BF263847	Contig3287_x_at	none identical	3 ⁴⁾	cytoplasm
AK373460	17.63	498	165			none	70124	3H cM=14	
<i>HvHsp17.76-CIX</i>	17.65	498	165	35_5001	AK370196	Contig11961_at	46949	3HS	cytoplasm
AK375791	17.67	489	162			Contig3285_at	81807	3H cM=46	cytoplasm
<i>HvHsp-17.77CII</i>	17.78	489	162	35_15456	AK355146	Contig3289_at	69124	3H cM=46	cytoplasm
<i>HvHsp17.7-CI</i>	17.80	486	161	35_14866	AK368988	Contig2012_s_at	51731	4H cM=51	cytoplasm
<i>HvHsp19.0-CIII</i>	19.05	537	178	35_18565	AK360636	Contig10029_at	52273	6H cM=91	cytoplasm
<i>HvHsp19.2-CX</i>	19.26	534	177	35_5077	AK370937	Contig15445_at	368996	6H cM=49	cytoplasm
MLOC_52605.1	20.64	561	186			none	37653	1H cM=66	
<i>HvHsp21.2</i>	21.25	585	194	35_23844	BF623486	Contig21040_at	77164	4H cM=73	
<i>HvHsp21.3-MI</i>	21.36	582	193	35_16753	AK370932	Contig6559_at	none identical	7 ⁴⁾	mitochondria
<i>HvHsp21.9-ER</i>	21.98	618	205		AK374148	none	51617	4H cM=51	endoplasmic reticulum
AK372933	22.58	621	206			Contig6557	none identical		chloroplast
MLOC_75175.1	23.42	651	216			none	67285	6H cM=75	
MLOC_10834.2	23.49	651	216			none	1559883	2H cM=58	
MLOC_6726.1	24.17	666	221			none	138014	4HL	
MLOC_47822.1	25.16	711	236			none	312759	5H cM=51	chloroplast
MLOC_50285.1	25.32	681	226			none	358032	1H	
MLOC_7795.1	26.44	750	249			none	140185	5H cM=50	chloroplast
<i>HvHsp26.8-P</i>	26.80	735	244	35_21394	AK376976	EBem05_SQ003_L06_at	78169	4H cM=57	chloroplast
MLOC_16644.1	28.33	780	259			none	1574043	3H cM=52	
MLOC_67109.1	29.11	807	268			none	52587	1H	
AK363601	36.17	1029	342			none	none identical		
MLOC_66935.1	38.96	1104	367			none	52302	1H cM=132	

Foot notes: **1)** The naming follows Reddy et al. (2014) for those starting with *HvHsp*, the MLOC-names are from the barley genome annotation and there are four sequences solely based on cDNA-clones and their genbank-accession numbers are given. **2)** HarvEST ID according to the HarvEST-database: <http://harvest.ucr.edu/>. **3)** Full length cDNA accession numbers in NCBI: <http://www.ncbi.nlm.nih.gov/>. **4)** PLEXdb (<http://www.plexdb.org/index.php>). The Affymetrix probe set Ids indicated for those present on the array. Chromosomal location based on transcript profiling of wheat-barley ditelosomic chromosome addition lines. **5)** The contig IDs and chromosomal localization from the genome resource at IPK Gatersleben (<http://webblast.ipk-gatersleben.de/barley/index.php>). **6)** Predicted by Reddy et al. 2014 and us using the same softwares. The two identified sHsps are shaded with dark grey

Table S2. Primer sequences for plasmid constructions and gene expression assay (5' to 3')

Primers for RNAi	
CSEP0105_RNAi_F	ATCTATCATAGGTCTGGTGCAGTG
CSEP0105_RNAi_R	GGTGTCATCTTCTCCAAGTTTCTT
CSEP0162_RNAi_F	GTATGGCCATCATCTTTCATAGC
CSEP0162_RNAi_R	CAACAGTACCATCTTGCTCTTGAT
Hsp16.9_RNAi_F	CACCACTCAACCGAGTGCCAAGC
Hsp16.9_RNAi_R	TGACCTCCTCCTTCTTCACG
Primers for the bait constructs in Y2H, BiFC, localization and protein expression	
CSEP0081_F	GGAAATGCAAAGTACAATTG
CSEP0081_R_with stop	TTACATGGCAGTACATGGTAAATAG
CSEP0105_F	ATTACAGAATATTACTGTGGCAAC
CSEP0105_R_with stop	TTATGCCTCGGTACACCA
CSEP0105_R_no stop	AAATGCCTCGGTACACCAGGT
CSEP0162_F	GCCCAATATTCTAGACATATTAATGA
CSEP0162_R_with stop	TTATCCCGAGCCAACACTGC
CSEP0162_R_no stop	AAATCCCGAGCCAACACTGCGCA
CSEP0254_F	CACCAACCAACATTACAAATGTGATGA
CSEP0254_R_with stop	CTAACTAATATCTTGCAGAGAACATAC
CSEP0254_R_no stop	AAAATAATATCTTGCAGAGAACATACTAT
Hsp16.9_F	CACCATGTTCGATCGTGAGGCGTA
Hsp16.9_R_with stop	TCAGCCGGAGATCTCGAT
Hsp16.9_R_no stop	AAAGCCGGAGATCTCGATGGC
Hsp17.5_F	CACCATGTTCGCTGATCCGTCGC
Hsp17.5_R_with stop	CTAGCCGGAGATCTGGATG
Hsp17.5_R_no stop	AAAGCCGGAGATCTGGATGGAC
Primers for qPCR	
CSEP0105_qPCR_F	GAGAATTCGGAGGTGGTGAT
CSEP0105_qPCR_R	GTTTCTTCCTCCAGCCTGTG
CSEP0162_qPCR_F	GTGATAGATATCAAGAGCAAGATGG
CSEP0162_qPCR_R	TCTTAATCATCATCATGGCACA
BghGAPDH_qPCR_F	ATGAACTACAAGGCATCCTGTCA
BghGAPDH_qPCR_R	TACCATGCGACTAGCTTAACAAAG