Cytokinin perception in potato: New features of canonic players

SUPPLEMENTARY DATA

Supplementary tables

Table S1. Sequence identity of modeled receptor domains and respective templates

Domain	Template		Receptor	Identity, %	Reference
	PDB ID	Protein			
Sensory module	3T4L_A	AHK4	StHK2	64.75	Hothorn *et al.*, 2011
			StHK3	65.00	
			StHK4	79.62	
HisKA domain	4MT8_A	ERS1	StHK2	36.99	Mayerhofer *et al.*, 2015
			StHK3	34.72	
			StHK4	38.20	
ATPase domain	4PL9_A	ETR1	StHK2	34.94	Mayerhofer *et al.*, 2015
			StHK3	32.74	
			StHK4	31.55	
	5IDM_A	CckA	StHK2	19.88	Dubey *et al.*, 2016
			StHK3	22.42	
			StHK4	22.70	
Receiver domain	3MMN_A	CKI1	StHK2	48.21	Pekárová *et al.*, 2011
			StHK3	43.64	
			StHK4	48.78	
	4EUK_A	AHK5/CKI2	StHK2	32.52	Bauer *et al.*, 2013
			StHK3	31.88	
			StHK4	32.37	
Receiver-like domain	1DCF_A	ETR1	StHK2	25.00	Müller-Dieckmann *et al.*, 1999
			StHK3	15.45	
			StHK4	21.77	

Table S2. List of primers used in this research

Primer pair name	Single primer name	Primer sequences (5' – 3')
StHK2_cloning	StHK2_LP1	GCTTTTCTGCTCTGGGTG
	StHK2_RP3	TCAACCTGACCCGAAGAAG
StHK3_cloning	StHK3_LP1	GGGTTTGGTTTGAAATTGGG
	StHK3_RP3	GGTATTCTGAGTTGGCTTG
StHK4_cloning	StHK4_LP1	ATGGGTGAGAAGATGCAAAGCC
	StHK4_RP3	CTATTTGTCCGAGTTAGGCTTGG
StHK2_sensor module	StHK2_attB1 CHASE	TACAAAAAAGCAGGCTTGATGGCTCTTGTTATCTTTGTTATTG
	StHK2_attB2 CHASE	ACAAGAAAGCTGGGTAAGCATGGAAGATATGACC
StHK3_sensor module	StHK3_attB1 CHASE	TACAAAAAAGCAGGCTTGATGCTTTTGATAGTATG
	StHK3_attB2 CHASE	ACAAGAAAGCTGGGTAAAATATTTGCCCTATAAGC
StHK2_full length	StHK2_attB1	TACAAAAAAGCAGGCTTGATGAGCTTTTCTGCTCTGGGTG
	StHK2_attB2	ACAAGAAAGCTGGGTAACCTGACCCGAAGAAG
StHK3_full length	StHK3_attB1	TACAAAAAAGCAGGCTTGATGAGTTTGTTTCATGTTATTGGGTTTGGTTTGAAA
	StHK3_attB2	ACAAGAAAGCTGGGTAGGTATTCTGAGTTGG
GATEWAY_standard primers	attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCT
	attB2	GGGACCACTTTGTACAAGAAAGCTGGGTA
StHK4_truncated	StHK4_BcuI	ACTAGTATGGGTGAGAAGATGCAAAGCC
	StHK4_EcoRI	AGGAATTCCAAGTCTCTTCAGATGGTATC
StHK2_COLD	StHK2_XhoI	ATATCTCGAGATGAGCTTTTCTGCTCTGGG
	StHK2_NheI	TATGCTAGCTCAACCTGACCCGAAGAAGC
StHK4_COLD	StHK4_SacI	AAAGAGCTCATGGGTGAGAAGATGCAAAGCC
	StHK4_EcoR1a	GAATTCCTATTTGTCCGAGTTAGGCTTGG
StHK2_qRT PCR(1)	StHK2_FPq1	ACCATTTGCAGAGACTGGGA
	StHK2_RPq1	GGTCAACAAAAACCACGGCTA
StHK3_qRT PCR(1)	StHK3_FPq1	CACAGCTCCCTTCAGGCTAC
	StHK3_RPq1	TACTCCACCAAGGTACCCGT
StHK4_qRT PCR(2)	StHK4_FPq2	TGCTGAGAGTGGGAAAGCTG
	StHK4_RPq2	GACGTGTAGCCTCAAACCCA
StRR4A	StRR4A_FPq1	ATCAACACCTTCACCGCCAT
	StRR4A_RPq1	TTGAGTCGTCTTGTTGGCGA
StRR9A	StRR9A_FPq2	CCTCTTATCAAGTTACTGTTGTGGA
	StRR9A_RPq2	ACCAGTCATTTCAGGCATGCTA
StRR9D	StRR9D_FPq1	CCTAGCAACCAACAGGAAGTG
	StRR9D_RPq1	TGTTCCTCAGAGATGCAGATTCC
EF1_AB061263	EF1_FP	ATTGGAAACGGATATGCTCCA
	EF_RP	TCCTTACCTGAACGCCTGTCA
CYC_AF126551	CYC_FP	CTCTTCGCCGATACCACTCC
	CYC_RP	TCACACGGTGGAAGGTTGAG

Table S3. Consensus motifs in CHASE domains of potato and Arabidopsis cytokinin receptors

Type	Amino acid sequence
All CHKs	x**P**SAIDQxT**F**(A)xYT**E/A**RT(A)**FERPLT/L**S**GAVA**YAx**R/K**Vx(H)(S)(E)R(E)x**FE**xxxGW(T)IKxMxxx***loop***xxxxxx**D/EEYA**PV(I)(F)**A/S**Q**E/D**T(V)(S)**H/Y**xxSxDM(M)S**GK/E**E**D**REN(I)LRAR**A/ES/T**GK**G/A**VL**TS/APFR/KL**Lx**S/TN/HH/RLGV**(V)**LTFA/PVY**xxxLPxxx(T)xxxRIxATx**G_Y**L**G**(G)x**F/Y**D**V/IE/P**S**LV**(E)**K/NLL**xQLA**S/GK/N**QxIx**V**(N)VY**D**xTNx(S)XP**I/L**xM**Y**Gxxxxxx(D)xx(L)x(H)xSxL**D/NF**G**D**Px**R**(K)**H**(E)Mx
HK2-clade	x**PSA**I**DQ**KT**F**(G)EYTER**T**x**FERPL**T**SGAVAYALKV**x**H**S(E)**R**(E)x**FE**(K)x**HGW**(T)**IKKM**E(T)(E)***loop***(D)**P**(A)**P**(I)**D**Q**EYAPVIFA/SQ**E**TV**(S)**HI**V**S**(I)**DMMSG**(K)**ED**R(E)**NILR**ARA(S)G**KG**V**LT**S**PF**K**LLKSN**H**LGV**V**LTF**A**VY**xxx**L**Px(D)A**T**(P)E(Q)R(I)(E)ATx**G_Y**L**GAS**Y**D**(V)**PSLV**(E)**KLL**(H)**QL**A**S**KQTIV**V**(N)V**YDT**T**N**(A)(S)X(P)Ix**MY**GxxxxxxD(T)(G)(L)(L)(H)(I)S**N/S**LD**FGDP**x**R**(K)**H**E**M**H
StHK2 vs HK2- clade	_PSAIDQKTFEEYTERT_FERPLTSGAVAYALRV_HSERE_FEK_HGWTIKKMESE*loop*DSAPDQDEYAPVIFSQQTVSHIVSIDMMSGKEDRENILRARASGKGVLTSPFKLLKSNHLGVVLTFAVY___LL_YATPVDRINAT_G_YIGASYDVPSLVEKLLHQLASKQTIVVNVYDTTNKF_PI_MYG_______DTGLLRVSNLDFGDP_RNHEMH
AHK2 vs HK2- clade	_PSAIDQKTFEEYTERT_FERPLTSGAVAYALKV_HSERE_FEK_HGWAIKKMETE***loop***DPAPIQDEYAPVIFAQETVSHIVSVDMMSGEEDRENILRARASGKGVLTSPFKLLKSNHLGVVLTFAVY___LP_DATEEQRVEAT_G_YLGASYDMPSLVEKLLHQLASKQTIVVDVYDTTNTS_LI_MYG_______DISEQHISSLDFGDP_RNHEMH
HK3-clade	x**P**SA**I**DQx**TF**(A)(R)Y(T)(E)**RT**(A)**FERPLTS**G**AVAY**AVR**V**(L)H(S)ER(E)**Q/EFEK/R**QQ**GWT/SI**(K)(R)**M**(D)(T)x***loop*****E**(P)(S)(P)x(Q)E**EYAPVIFAQDT**xx**H**V**I/VS**x**D**(M)**L**(S)**G**(K)E**DRE/DNVLRAR**(E)**SG**KG**VLTAPF**(R)**L**(L)(K)TNR**LGVI**(L)**T**(F)A**VY**Kx**D/E**L**P**(S)N**A**T**P**(N)**ERIQ/EA**T(D)**G_YLGGV/IF**DIE(S)**LV**EK**LL**(Q)**QLA**(S)KQ(T)Ix**V**N**V**Y**D**(T)T**N**x(S)(H)P**I**S**MYG**S(N)(V)(S)x(D)(G)(L)x(H)VSx**L**N**F**G**DP**x**R**(K)**H**E**MR/H**
StHK3 vs HK3-clade	_PSAIDQ_TFASYTERTAFERPLTSGAVAYAVRVLHSERKEFEKRHGWSIKRMDT_***loop***EPSP_QAEYAPVIFAQDT__HVIS_DMLSGKEDRENVLRARESGKGVLTAPFRLLKTNHLGVIKTFAVYK_DLPSNATPNERIQATDG_YLGGVLDIESLVEKLLQQLASKQTI_VNVYDMTN_SHPISMYGSNVS_DGL_HVS_LNFGDP_RRHEMR
AHK3 vs HK3-clade	_PSAIDQ_TFSEYTDRTSFERPLTSGAVAYAMRVLHSEREEFERQQGWSIRKMYS_***loop***EPSP_QEEYAPVIFAQDT__HVIS_DMLSGKEDRENVLRARSSGKGVLTAPFPLIKTNRLGVILTFAVYK_DLPSNATPNERIQATNG_YLGGVFDIESLVEKLLQQLASKQTI_VNVYDMTN_SQPISMYGSNVS_DGL_RVS_LIFGDP_RKHEMR
HK4-clade	(N)P(S)**A**(I)**D**Q(E)**T**F**A**(E)**YT**AR**TA/SFERPL**LS**GVA**Y**A**xRVxxx(E)R(E)x**FE**x(Q)(H)GWxIKT**M**xxxloop(E)(P)S(P)x(R)D**EYAP**VI(F)(S)Q(E)(T)V**S**Y**I/L**(E)(S)**L/ID**MM**SGEED**R**ENI**LRAR(A)(T)**G**KA**V**L**T**x**PF**R**L**L(G)S(H)**HLGVVLT**FP**VY**(K)(S)(K)**L**Pxx(P)(T)x(E)xR(I)x**A**TAG_Y(L)**GG**(A)**FDVESLV**EN**LL**(G)(Q)**L**(A)GNQ(A)I(L)**V**(N)**VYDV/ITN**x(S)(D)PL**I/V**M**YG**xxxxx(G)(D)xSLxH(E)S(K)**LDFGDP**(F)**RK/R**HYM(I)
StHK4 vs HK4-clade	NPSAIDQNTFAEYTARTAFERPLLSGVAYA_RV___ERE_FE_EHGW_IKTM___loopKPSP_RDEYSPVIFSQETVSYIESLDMMSGEEDRENILRARASGKAVLT_PFRLLGSHHLGVVLTFPVYRSKLP__PT_H_RV_ATAG_FLGGAFDVESLVECLLGQLAANHPIIVNVYDVTN_SDPLIMYG_____GD_SL_QVSKLDFGDPFRKHEMI
AHK4 vs HK4-clade	NPSAIDQNTFAEYTARTAFERPLLSGVAYA_RV___ERE_FE_QHNW_IKTM___loopEPSP_RDEYAPVIFSQDSVSYLESLDMMSGEEDRENILRARETGKAVLT_PFRLLETHHLGVVLTFPVYKSSLP__PT_E_RI_ATAG_YLGGAFDVESLVENLLGQLAGNQPIVVHVYDVTN_SDPLIMYG_____AD_SL_HESKLDFGDPFRKHKMI

CHK means CHASE-domain containing histidine kinase (cytokinin receptor); HK2, HK3 and HK4 mean AHK2-, AHK3- and AHK4-orthologs, respectively. StHKs correspond to potato var. Phureja. In presented motifs (Steklov *et al.*, 2013) bold letters design highly conserved aa positions, regular letters – semiconserved positions, letters in brackets – positions of a prevalent aa, slash-separated letters – positions of any of two amino acids, x – non-conserved position. In the case of individual receptors, non-conserved aa are shown by underlined gaps. Gross deviations in conserved aa sequences of individual receptors are highlighted in red, the same positions in the general motifs and Arabidopsis receptors are marked in green.

Table S4. Main Ramachandran plot parameters of modeled structures upon minimization procedures

Domain	Receptor	Main Ramachandran plot parameters			
		Most favored regions	Additional allowed regions	Generously allowed regions	Disallowed regions
Sensor module (no ligand)	StHK2	87.3%	11.3%	0.8%	0.6%
	StHK3	88.5%	10.1%	0.6%	0.8%
	StHK4	89.0%	10.4%	0.6%	0.0%
HisKA domain	StHK2	96.3%	3.7%	0.0%	0.0%
	StHK3	96.3%	3.7%	0.0%	0.0%
	StHK4	97.3%	2.7%	0.0%	0.0%
HATPase domain	StHK2	85.9%	12.6%	0.7%	0.7%
	StHK3	85.8%	12.7%	1.5%	0.0%
	StHK4	85.4%	13.1%	0.7%	0.7%
Receiver-like domain	StHK2	82.0%	17.3%	0.0%	0.8%
	StHK3	84.1%	14.3%	0.0%	1.6%
	StHK4	86.0%	13.2%	0.8%	0.0%
Receiver domain	StHK2	86.5%	12.7%	0.0%	0.8%
	StHK3	86.6%	11.0%	1.6%	0.8%
	StHK4	83.9%	12.1%	2.4%	1.6%

Supplementary figures

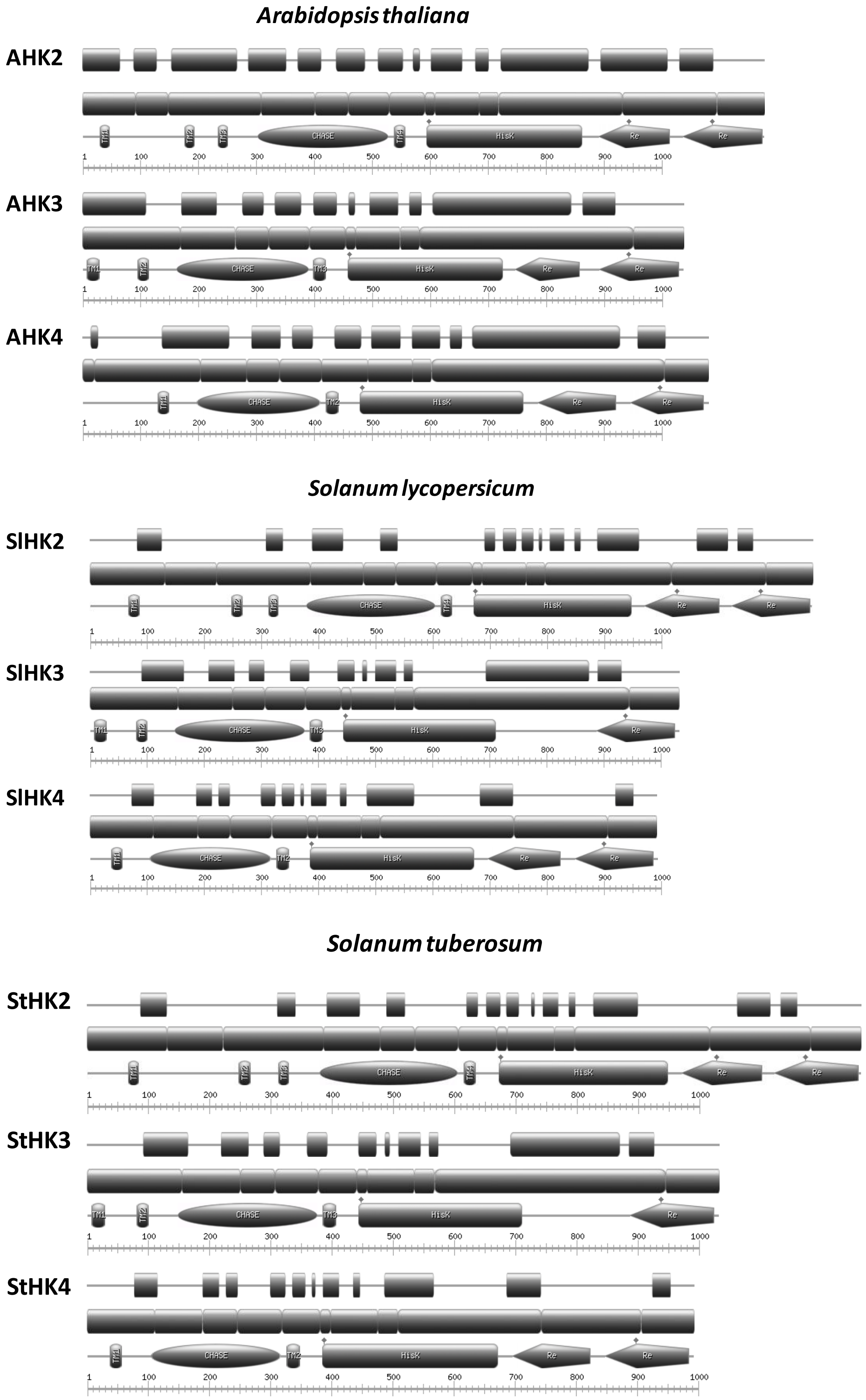

Fig. S1. Intron/exon and domain structure of cytokinin receptor orthologs from different plant species. For every receptor, upper line shows scheme of exons (cylinders) and introns (lines) alternation; next line corresponds to RNA/proteins structures upon splicing; third line represents main protein domains; bottom line is the aa scale indicating the protein length.

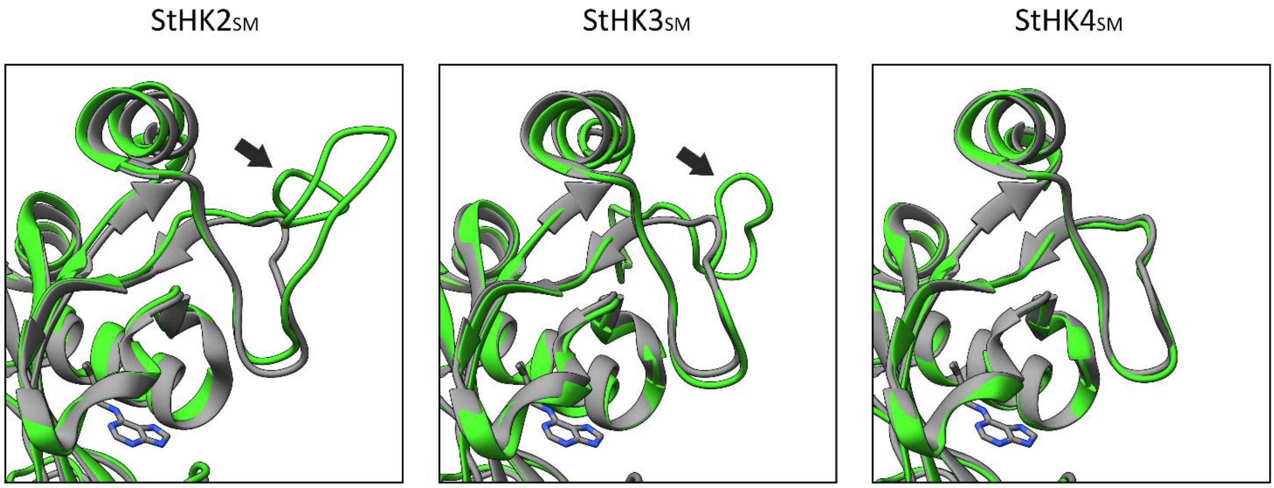

Fig. S2. Insertions in PAS domains of the sensory modules of StHK2 and StHK3.

Homology models of sensory modules of *Solanum tuberosum* (var. Phureja) cytokinin receptors (green) are superimposed to crystal structure of Arabidopsis receptor AHK4 (grey) (PDB ID: 3T4L) demonstrating the presence of insertions (indicated by arrows) in StHK2 and StHK3 PAS domains. The ligand position is shown at the bottom of the pictures.

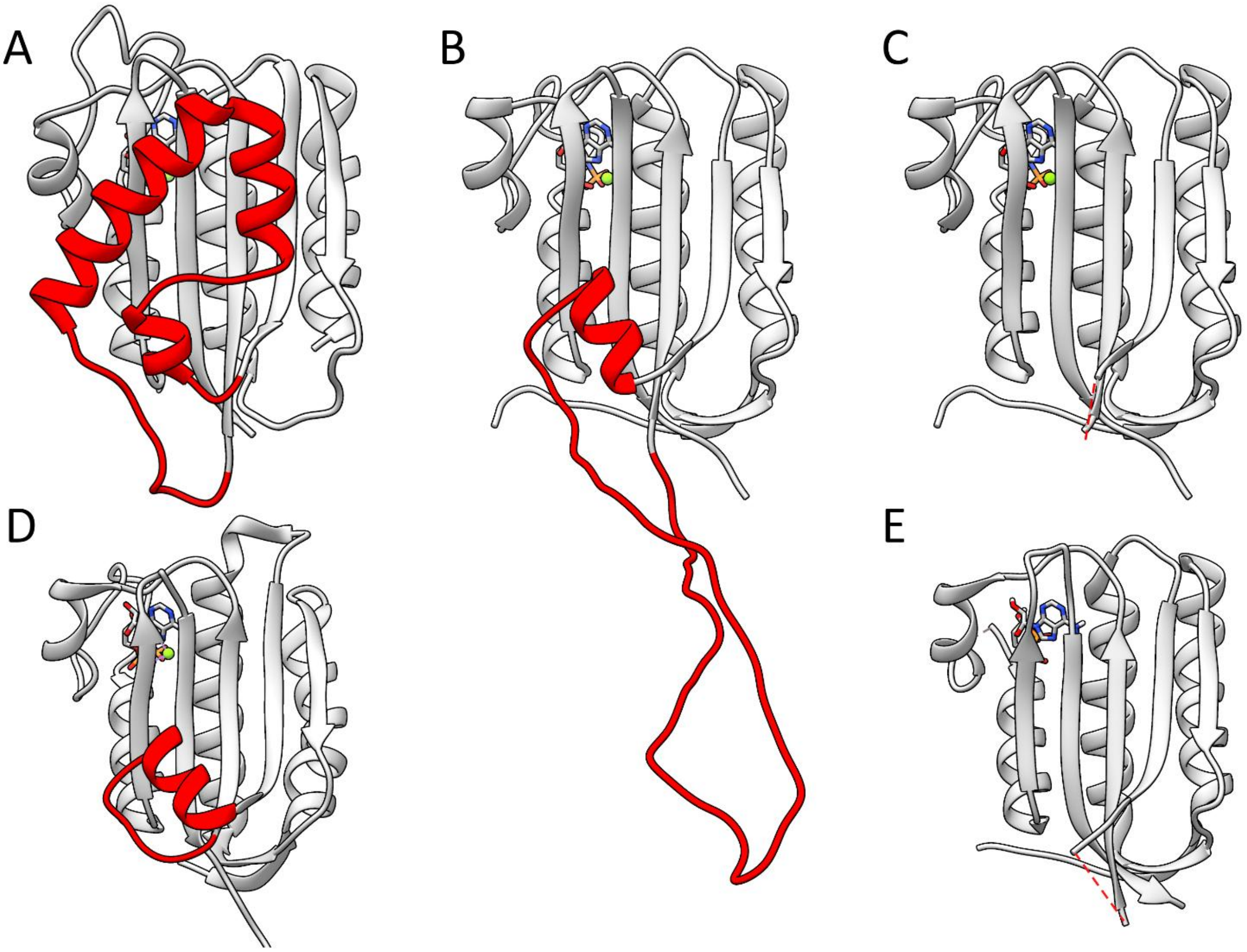

Fig. S3. Models of StHK3 HATPase domain built using *de novo* folding with IntFOLD server (A) (Roche *et al*., 2011) and multitemplate homology modelling in Modeller – initial structure (B) and structure with deleted insertion (C) – in comparison with crystal structures of HATPase domain of histidine kinase CckA (D) (PDB ID: 5IDM) and ETR1 (E) (PDB ID: 4PL9) used as templates. Insertion (in comparison with basic HATPase folding) or missing structure (dotted line) corresponding to it are colored red. Small green spheres designate Mg^{2+} ions. It is clearly visible that the insertion is located on the opposite side of the catalytic site.

Additional reference

Roche DB, Buenavista MT, Tetchner SJ, McGuffin LJ. 2011. The IntFOLD server: an integrated web resource for protein fold recognition, 3D model quality assessment, intrinsic disorder prediction, domain prediction and ligand binding site prediction. Nucleic Acids Research **39**, 171–176.

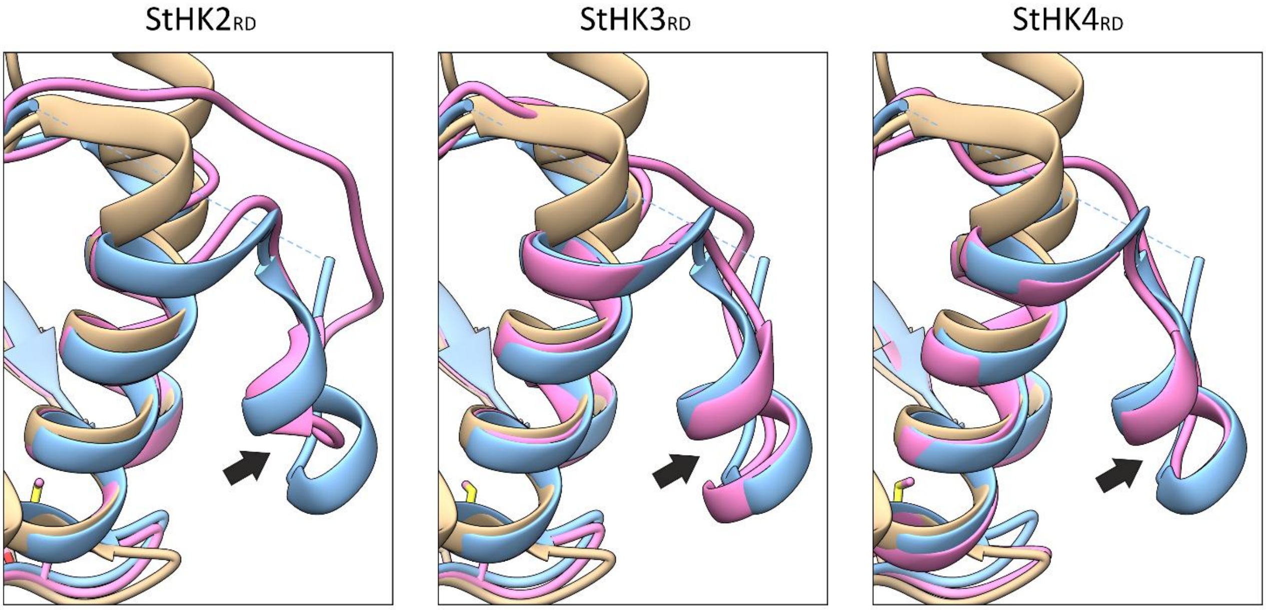

Fig. S4. Models of receiver domains of StHKs.

Homology models of StHK receiver domains (magenta) are superimposed to crystal structures of CKI1 (hazel) (PDB ID: 3MMN) and AHK5/CKI2 (blue) (PDB ID: 4EUK, chain A) demonstrating the presence of an insertion and additional 6th α-helix (indicated by arrows) in structures of all StHKs and AHK5/CKI2 in comparison with basic REC-like folding (including CKI1 structure). Missing part of the structure (AHK5/CKI2) is indicated by a dotted line.

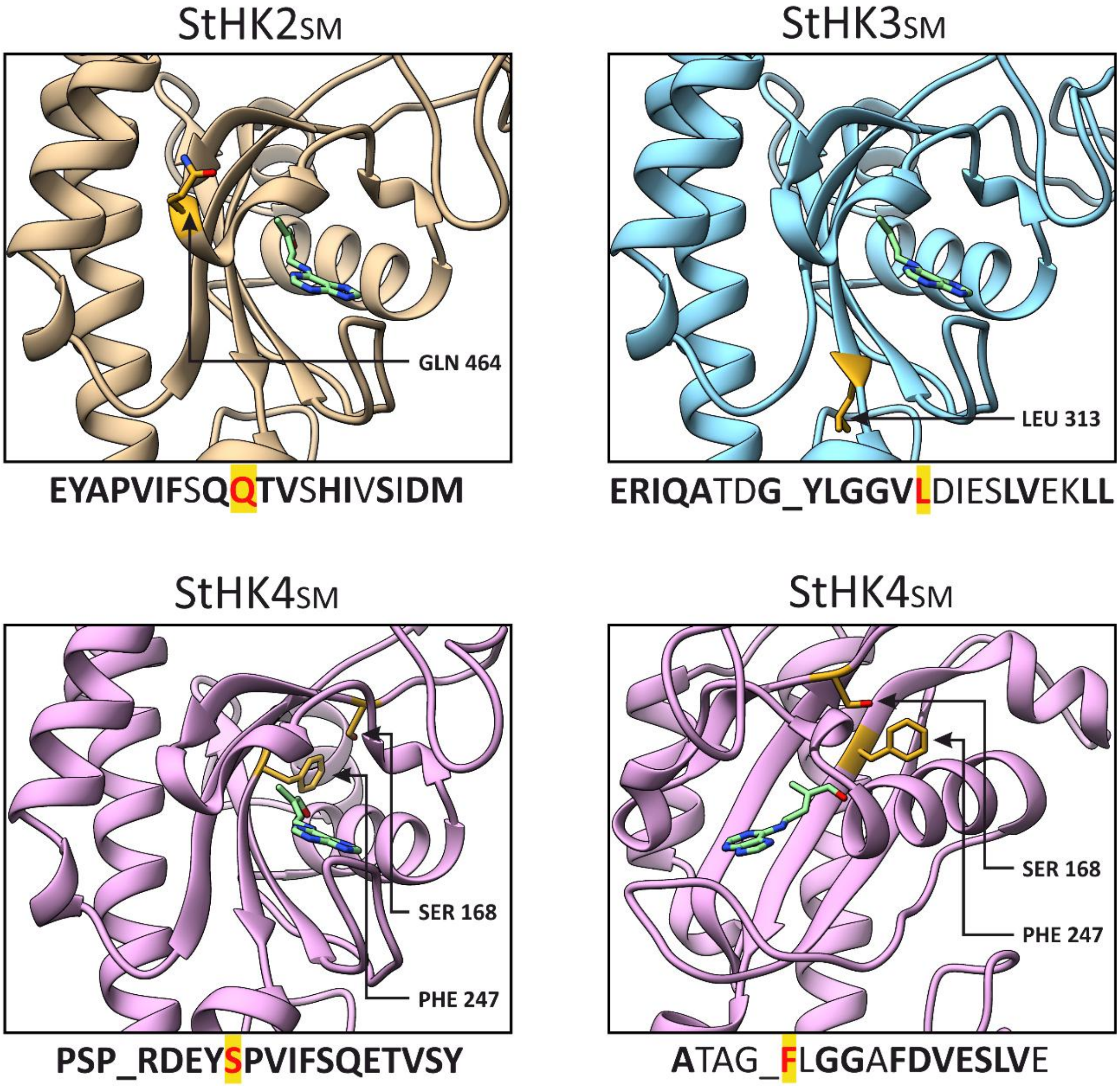

Fig. S5. Models of sensory modules of StHKs.

Amino acid residues alterating the canonic motifs are indicated in the models and in respective aa sequence fragments (at the bottom of figures). Sensory module of StHK4 receptor is shown in two orientations. The aa residue numbering is different depending on whether full length proteins or CHASE only (Table S3) are considered. For non-canonical residues, this double numbering is; in StHK2, Gln464/90; in StHK3, Leu 313/177; in StHK4, Ser168/83 and Phe247/172. Non-conserved residues are shown by underlined gaps.

Fig. S6. Alignment of aa sequences of CHASE domain-containing histidine kinases from different plant species. Full-length protein sequences from potato var. Phureja (StHK) and Désirée (StHKa/b), tomato (SlHK), Arabidopsis (AHK) and rice (OsHK) are compared. Amino acids differing between Phureja and Désirée sequences are highlighted.

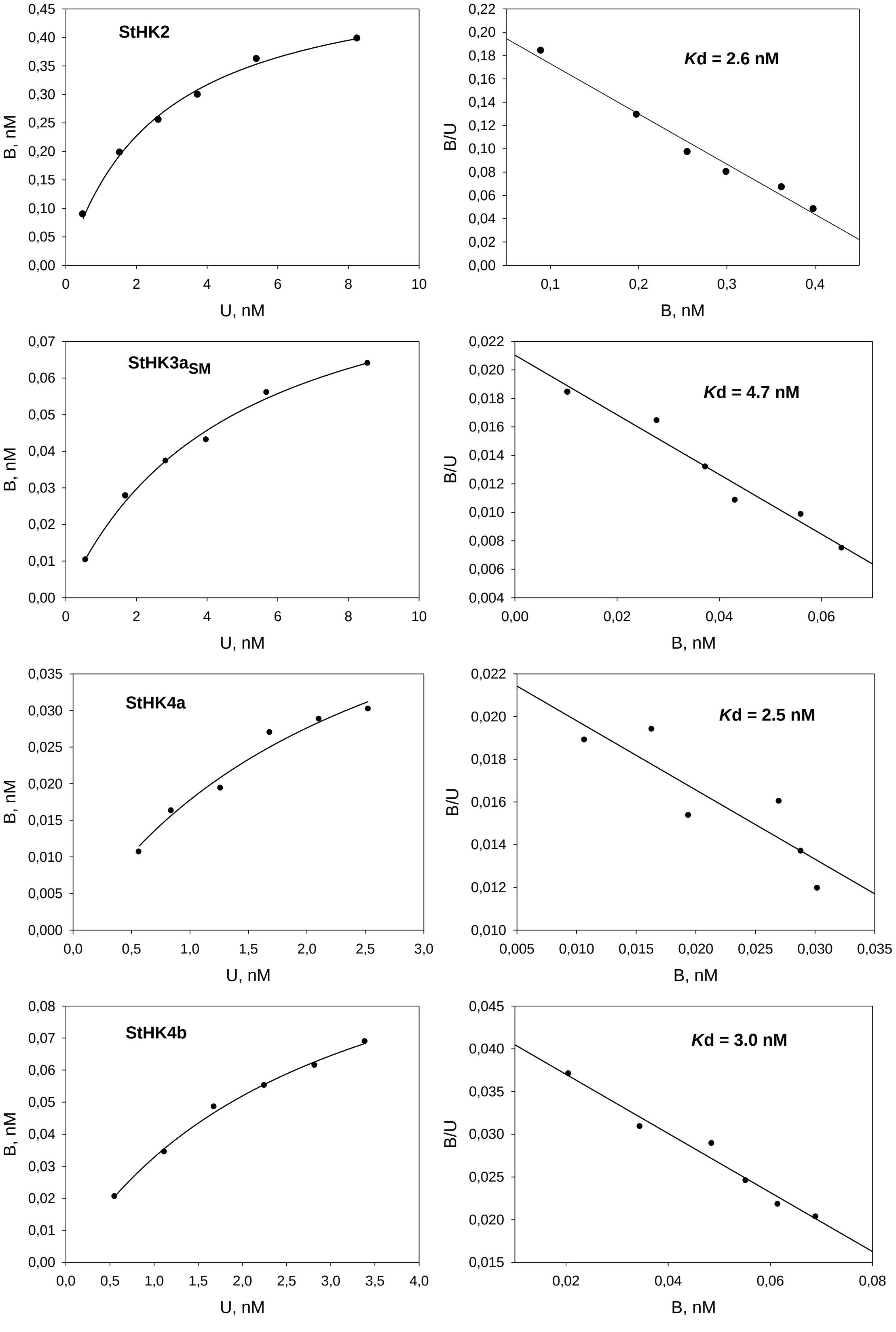

Fig. S7. Scatchard analysis of the interaction between ^{3}H-*trans*-zeatin and StHKs.

On the left, receptor saturation curves; on the right, curve transformation in Scatchard plots. B and U, bound and unbound hormone concentrations, respectively.

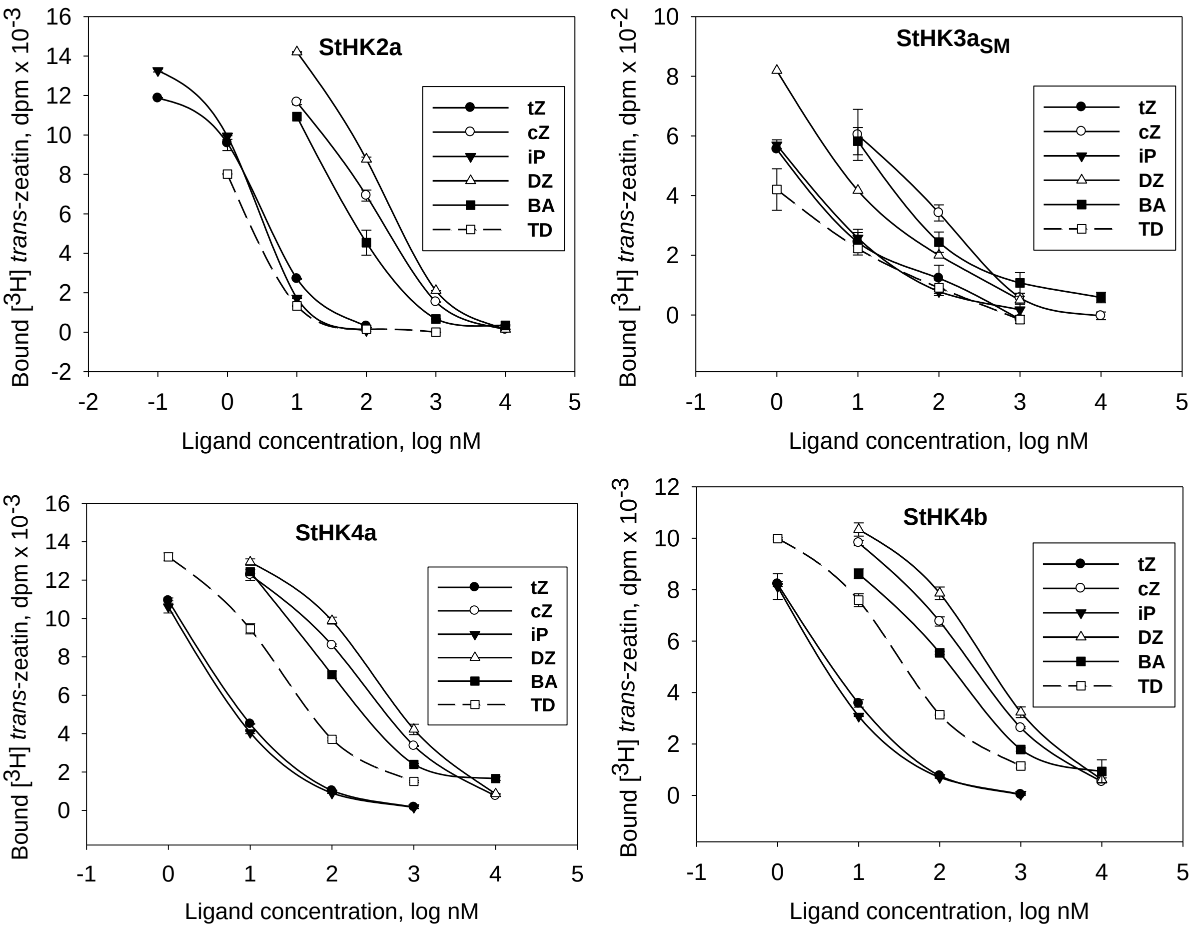

Fig. S8. Competition of various cytokinins with ^{3}H-*trans*-zeatin for binding to StHKs.
Unlabeled hormones are: tZ, *trans*-zeatin; cZ, *cis*-zeatin; iP, isopentenyladenine; DZ, dihydrozeatin; BA, *N*^{6}-benzyladenine; TD, thidiazuron.