Cytokinin perception in potato: New features of canonic players

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Abstract

Potato is the most economically important non-cereal food crop. Tuber formation in potato is regulated by phytohormones, cytokinins in particular. The present work was aimed to study cytokinin signal perception in potato. The sequenced potato genome of doubled monoploid Phureja was used for bioinformatic analysis and as a tool for identification of cytokinin receptors from autotetraploid potato cv. Désirée. All basic elements of multistep phosphorelay (MSP) required for cytokinin signal transduction were identified in Phureja genome, including three genes orthologous to three cytokinin receptor genes (*AHK 2-4*) of Arabidopsis. As distinct from Phureja, autotetraploid potato contains at least two allelic isoforms of each receptor type. Putative receptor genes from Désirée plants were cloned, sequenced and expressed, and main characteristics of encoded proteins, firstly their consensus motifs, structure models, ligand-binding properties, and the ability to transmit cytokinin signal, were determined. In all studied aspects the predicted sensor histidine kinases met the requirements for genuine cytokinin receptors. Expression of potato cytokinin receptors was found to be organ-specific and sensitive to growth conditions, particularly to sucrose content. Our results provide a solid basis for further in-depth study of cytokinin signaling system and biotechnological improvement of potato.

Key words: CHASE domain-containing histidine kinase, cytokinin, cytokinin receptor, cytokinin signaling, gene expression, hormone perception, potato, *Solanum tuberosum.*

Abbreviations: BA, 6-benzyladenine; CHASE, Cyclases/Histidine kinases Associated SEnsory; CHK, CHASE domain-containing histidine kinases; CRF, cytokinin response factor; cZ, *cis*-zeatin; DI, dimerization interphase; DZ, dihydrozeatin; GFP, green fluorescent protein; HK, histidine kinase; iP, isopentenyladenine; LacZ, galactosidase; LD, long day; MSP, multistep phosphorelay; RR, response regulator; SNP, single nucleotide polymorphism; TD, thidiazuron; TM, transmembrane; tZ, *trans*-zeatin.

Introduction

Potato is a widespread practically important crop, its tuber formation is controlled by phytohormones (reviewed in Aksenova *et al*., 2012, 2014). Previous studies have shown that cytokinins and auxins can accelerate and enhance potato tuber formation (Aksenova *et al*., 2000; Romanov *et al*., 2000; Roumeliotis *et al*., 2012; Kolachevskaya *et al*., 2015, 2017). In non-tuberizing plants (tobacco, tomato), increased doses of active cytokinins stimulate morphogenesis, in many aspects resembling tuber formation (Guivarc'h *et al*., 2002; Eviatar-Ribak *et al*., 2013). Cytokinin signaling is also involved in the formation of nodules on the roots of legumes (reviewed in Frugier *et al*., 2008; Miri *et al*., 2016). Cytokinins largely determine the nature of source-sink relationships in the whole plant, enhancing the attracting ability of the tubers (Abelenda and Prat, 2013). Elevated doses of cytokinins affect the overall architectonics of potato plants, suppressing the root development (Aksenova *et al*., 2000). In addition, cytokinins participate in plant defense against biotic and abiotic adverse factors (Zwack and Rashotte, 2015; Brütting *et al*., 2017; Thu *et al*., 2017). All the above indicates the important role of cytokinins in both the formation of tubers and the general development and resistance of potato plants.

The molecular mechanism of cytokinin action on a plant cell has been established using mainly the Arabidopsis model (reviewed in Hutchison and Kieber, 2002, Hwang *et al*., 2002; Kakimoto, 2003; Heyl and Schmülling, 2003; Sakakibara, 2006; Müller and Sheen, 2007). This mechanism is based on multistep phosphorepay (MSP) and uses three protein species to bring the cytokinin signal up to the primary response genes: (i) transmembrane catalytic receptors with histidine kinase activity, (ii) mobile phosphotransmitters circulating between the cytoplasm and nucleus, and (iii) nuclear transcription factors, B-type response regulators. Other proteins (CRFs, pseudophosphotransmitters, A-type response regulators) affect the intensity of the cytokinin signaling through the main transmission pathway (Kieber and Schaller, 2014).

Receptors are key factors in the perception and transduction of hormonal signals. In the case of cytokinins, receptors are sensory hybrid histidine kinases largely homologous to bacterial sensory histidine kinases, members of two-component signal transduction system. Known cytokinin receptors are multidomain proteins located mainly in ER membranes (Caesar *et al*., 2011; Lomin *et al*., 2011, 2018; Wulfetange *et al*., 2011; Daudu *et al*., 2017; Ding *et al*., 2017) with N-terminal hormone-binding sensory module localized in the ER lumen and the central and C-terminal catalytic domains protruding in the cytosol (Steklov *et al*., 2013; Lomin *et al*., 2018). Until now, cytokinin receptors have been studied in a few vascular plant species, primarily and most detailed in Arabidopsis and maize (Kakimoto, 2003; Yonekura-Sakakibara *et al*., 2004; Romanov *et al*., 2006; Lomin *et al*., 2011, 2012, 2015; 2018; Stolz *et al*., 2011; Heyl *et al*., 2012; Steklov *et al*., 2013; Wang et al., 2014). In recent years, cytokinin receptor studies have been extended to new species including rice (Choi *et al*., 2012;

Ding *et al*., 2017), barrelclover (Held *et al*., 2014), *Medicago truncatula* (Laffont *et al*., 2015; Boivin *et al*., 2016), oilseed rape (Kuderová *et al*., 2015), *Nicotiana attenuata* (Schäfer *et al*., 2015), and apple (Daudu *et al*., 2017). These studies have demonstrated that the cytokinin perception apparatus in some aspects is species-specific. Potato differs from most plant species by its ability to form tubers. This process, sensitive to various cues including cytokinins, makes the study of cytokinin receptors of potato especially intriguing. So far, to our knowledge, there have been no scientific reports on such studies.

In this paper, we have examined potato cytokinin receptors of a homozygous doubled monoploid Phureja (DM1-3 516 R44) whose genome was sequenced several years ago (Potato Genome Sequencing Consortium, 2011). Cloning and expression of receptor encoding genes were conducted using the commercial autotetraploid potato cv. Désirée. The presence of all necessary MSP elements in potato was demonstrated and main characteristics of CHASE domain-containing cytokinin receptors, primarily their consensus motifs, 3D structures, ligand-binding properties, and the ability to transmit the signal by MSP were ascertain. In contrast to the Phureja monoploid, distinct alleles for each of the three main forms of receptors were found in the Désirée potato. Expression of cytokinin receptor genes was shown to be organ-specific and affected by sucrose. The obtained results might serve as a framework for new biotechnological approaches in improving potato productivity and stress resistance.

Materials and methods

Sequence analysis

Nucleotide/polypeptide sequences of cytokinin receptors and other proteins related to the cytokinin signaling were retrieved from databases NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov), Phytozome 11 (https://phytozome. jgi.doe.gov/pz/portal.html), MSU Rice Genome Annotation Project Release 7 (http://rice.plantbiology.msu.edu/) and congenie.org (http://congenie.org/) using the BLASTP tool and *AHK2* (AT5G35750), *AHK3* (AT1G27320), *AHK4* (AT2G01830) and other cytokinin-related genes of *Arabidopsis thaliana* as templates. Domain structure of proteins was determined with PROSITE (http://prosite.expasy.org/). Transmembrane domains were determined using MESSA service (http://prodata.swmed.edu/MESSA/MESSA.cgi) (Cong and Grishin, 2012). Domain visualization was performed using the MyDomains – Image Creator service (http://prosite.expasy.org/mydomains/).

Phylogenetic analysis was performed using the MEGA6.0 (Tamura *et al*., 2013). Alignment of nucleotide sequences (CDS, codon mode) was performed by ClustalW algorithm. Method of maximum likelihood was employed for phylogenetic reconstruction. The search for key amino acids in receptor domains by alignment and visualization of protein sequences was carried out in Clustal X2.1 (Larkin *et al*., 2007) and Jalview (Clamp *et al*., 2004), respectively.

Homology modeling

Search of templates for homology modeling was performed at SWISS-MODEL web-service (https://swissmodel.expasy.org/) (Biasini *et al*., 2014). Modeling of potato (*Solanum tuberosum* L.) protein structures was accomplished in Modeller 9.15 (https://salilab.org/modeller/) (Sali and Blundell, 1993) using *automodel* class for comparative modeling. For each protein, 200 models were built, and the best model was selected according to DOPE score value (Shen and Sali, 2006) as determined by Modeller. For StHK receiver domains, chain A AHK5rd-AHP1 complex structure (Bauer *et al*., 2013) (PDB ID: 4EUK) and CKI1rd structure (Pekárová *et al*., 2011) (PDB ID: 3MMN) were used as templates. ERS1 histidine kinase domain (PDB ID: 4MT8) and ETR1 H-ATPase domain (PDB ID: 4PL9) structures (Mayerhofer *et al*., 2015) were used as templates for StHK catalytic module. StHK sensor modules were modeled using the structure of AHK4 receptor (PDB ID: 3T4L) as the template (Hothorn *et al*., 2011). After adding hydrogen atoms, models were energy minimized in USCF Chimera 1.10.2 (http://www.cgl.ucsf.edu/chimera/) (Pettersen *et al*., 2004) using AMBER ff14SB force field (Maier *et al*., 2015) with 300 steps of steepest descent and 300 steps of conjugate gradient; step size was 0.02 Å in both cases. Stereochemical quality of the models was assessed with ProCheck (Laskowski *et al*., 1993) implemented in PDBsum Web service (www.ebi.ac.uk/pdbsum), ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) (Wiederstein and Sippl, 2007) and QMEAN server (https://swissmodel.expasy.org/qmean/help) (Benkert *et al*., 2009). Visualization and superposition of the models were accomplished with UCSF Chimera.

Promoter analysis

Promoter regions of *Arabidopsis thaliana* cytokinin receptor genes (*AHK2*, *AHK3* and *AHK4*) were obtained from TAIR database (https://www.arabidopsis.org). Identification of promoter regions of cytokinin receptor genes (*StHK2*, *StHK3* and *StHK4*) of potato was performed using Phytozome 11 and NCBI databases. DNA sequence of 1000 nucleotides long upstream the gene transcription start was taken as a promoter region. The search for *cis*-regulatory elements in promoters of studied genes was carried out using PLACE (http://www.dna.affrc.go.jp/htdocs/PLACE/) and PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) programs.

Receptor cloning

Experiments were performed with autotetraploid potato (*Solanum tuberosum*) plantlets of Désirée variety. Plants were propagated by *in vitro* cloning on Murasige-Skoog agarous medium supplemented with 1.5% sucrose, at 20 °C and 16 h photoperiod in a controlled climate chamber with luminescent white light illumination (Kolachevskaya *et al*., 2015, 2017). Total RNA was isolated from fresh or immediately frozen plant material. To remove genomic DNA, RNA samples were treated with RNase-

free DNase I (Thermo Scientific). For reverse transcription, 2 μg of RNA and 200 units of RevertAid ™ were used according to the manufacturer's instructions (Thermo Scientific). Total DNA was isolated from the leaves of the potato using the CTAB method. The resulting cDNA and total DNA were used to amplify genes encoding predicted potato cytokinin receptors with high-precision Phusion High-Fidelity DNA polymerase (Thermo Scientific). The primer design was performed to amplify the full-length and truncated (sensory modules with flanking transmembrane helices) CDS of cytokinin receptors according to sequences in the NCBI Genbank XM_015303261.1, XM_006352114.2 and XM_006354988.2. PCR products were gel purified and cloned using the PCR Cloning Kit (Thermo Scientific) into the plasmid pJET1.2/blunt according to the manufacturer's instructions followed by transformation of *E. coli* strain DH10B (Invitrogen). *StHK4* was amplified using StHK4_truncated primers. The product was inserted into the construction of pB7FWG2-AHK3 instead of *AHK3*. The latter was removed at the BcuI and EcoRI restriction sites (Lomin *et al*., 2015). The nucleotide sequences of the cloned genes were confirmed by DNA sequencing.

StHK2 and StHK3 sequences were subcloned into the plasmid pDONR^{TM}221 in BP reaction with Gateway® BP Clonase® II Enzyme mix (Thermo Scientific). Then, using the LR reaction with the LR Clonase® II Plus enzyme (Thermo Scientific), the cloned sequence was transferred into the expression vector pB7FWG2 (Karimi *et al*., 2007) where it was fused at the 3'-terminus to the *eGFP* gene. For expression in *E. coli*, *StHK2* and *StHK4* were amplified using primers StHK2_COLD and StHK4_COLD, respectively. The product was then inserted into the plasmid pCOLD IV (Takara BioInc.) at the XhoI and XbaI restriction sites for *StHK2* and SacI and EcoRI restriction sites for *StHK4*, followed by transformation of the *E. coli* DH10B strain.

Transient expression of receptor genes in tobacco plants

The transient transformation of tobacco (*Nicotiana benthamiana*) leaves was accomplished according to Sparkles *et al*. (2006). Eight week-old tobacco plants were infiltrated with a mixture of *Agrobacterium tumefaciens* carrying cytokinin receptor genes fused to GFP and the *A. tumefaciens* helper strain p19 (Voinnet *et al*., 2003), and the expression of receptor genes was checked after 5–6 days on fluorescence microscope Axio Imager Z2 (Carl Zeiss Microscopy GmbH) before leaves were proceeded further for microsome isolation.

Plant membrane isolation

All manipulations were done at 4 °C. Tobacco leaves 6 days after infiltration were homogenized in buffer (3 ml per 1 g of fresh weight) containing 100 mM Tris-HCl (pH 8.0), 2 mM Na_2-EDTA, 50 mM KCl, 1 mM DTT and 1 mM PMSF. The homogenate was filtrated through Miracloth (Calbiochem, San Diego, USA), and the filtrate was centrifuged for 5 min at 5000 *g*. Then supernatant was centrifuged for

40 min at 15000 *g*. The microsome pellet was resuspended in 50 mM KCl, 10% glycerol and then microsome suspension was stored at -70 °C.

Hormone binding assays

Ligand binding studies were performed in PBS as described previously (Romanov *et al*., 2005; Lomin *et al*., 2015). Studies of pH influence on hormone binding were performed in 50 mM MES-KOH (pH 5–7) or Tris-HCl (pH 7–9) buffers with 50 mM KCl. K_D for [^{3}H]*t*Z binding to different receptors were determined in saturation assays followed by data analysis in Scatchard plots.

Assessment of receptor functionality

Plasmids pCOLD IV with *StHK2* or *StHK4* coding sequences were transferred for the expression into *E. coli* strain KMI001 (Suzuki *et al*., 2001). In this strain, HK receptor→YojN→RcsB→*cps::lacZ* pathway can be activated by external cytokinin (Takeda *et al*., 2001). The activation of the signaling pathway was monitored by measuring β-galactosidase activity of *E. coli* cells. Cultivation of clones on Petri dishes containing 40 mM glucose, 40 μg ml^{-1} X-gal, 100 μM IPTG, 50 μg ml^{-1} ampicillin at 15 °C was performed for 4 days. The individual clones were then streaked onto new Petri dishes containing 40 mM glucose, 40 μg ml^{-1} X-gal, 100 μM IPTG, 50 μg ml^{-1} ampicillin ± *trans*-zeatin at a concentration of 0.5 μM. The clones were grown for 3 days at 15 °C. Expression of the *cps::lacZ* construct was evaluated by blue staining of bacterial clones.

Gene expression analysis

Potato (*Solanum tuberosum* cv. Désirée) plants were cultivated under standard *in vitro* conditions at a long (16 h) day for 5-6 weeks on liquid MS medium containing 1.5% or 5% sucrose. For hormone treatment, the medium was replaced with the same one supplemented with 6-benzyladenine (BA, 1 μM). Tubes were inverted several times to assure uniform plant wetting and then incubated for 1 h under standard conditions. Finally plant organs (leaves, stems, roots, tubers) were isolated and immediately frozen in liquid nitrogen. Control plants were treated in a same way only without hormone. Total RNA was isolated by Trizol method (Brenner *et al*., 2005), this RNA served template for cDNA synthesis by reverse transcription (Invitrogen). All RNA samples were treated with RNase-free DNase I. The resulting cDNA was checked for the genomic DNA contamination by PCR with primers differentiating cDNA and genomic DNA. The band derived from genomic DNA was absent in the separating gel. Expression of genes encoding predicted proteins of cytokinin signaling system was determined by qRT PCR. Potato housekeeping genes *StEF1*α (elongation factor 1-α, AB061263) and *StCYC* (cyclophilin, AF126551) were used as reference genes (Nicot *et al*., 2005).

Statistical analysis

Statistical analysis was accomplished using the Student's t-test. *P*-value <0.05 was considered as statistically significant. In tables and graphics, mean values with standard errors are presented.

Results

Monoploid Phureja genome analysis

Potato has everything necessary for cytokinin signaling via the MSP pathway

The search for protein sequences and encoding genes involved in cytokinin signaling was performed on the basis of the duplicated potato monoploid Phureja genome (Potato Genome Sequencing Consortium, 2011). In general, all potential components of the canonical cytokinin signaling system described in Arabidopsis and other plant species with a sequenced genome (Kieber and Schaller, 2014) were identified in potato too. Potential cytokinin-related genes found in potato encode homologs of CHASE domain-containing histidine kinases (CHK), phosphotransmitters (HPt), and response regulators of A (RR-A) and B (RR-B) types (Table 1). This indicates the MSP functioning in potato cells for cytokinin signal transduction, involving proteins of a two-component system. In the potato monoploid proteome three predicted protein-coding sequences XP_015158747.1, XP_006352176.1 and XP_006355050.1, orthologous to Arabidopsis receptors AHK2, AHK3 and CRE1/AHK4, respectively, were detected. By analogy with the Arabidopsis orthologs, these proteins were annotated in NCBI as StHK2, StHK3, and StHK4. They correspond to mRNA sequences XM_015303261.1, XM_006352114.2 and XM_006354988.2. Deduced proteins StHK2, StHK3, and StHK4 share 59.35%, 67.75%, and 67.52% sequence similarity with the Arabidopsis orthologs. The lengths of *StHK2*, *StHK3* and *StHK4* genes are 5345, 4216, and 3810 bp, respectively, and predicted proteins are 1263, 1032, and 992 amino acids long (Table 1).

Phylogenetic analysis classified StHKs into three clades

The phylogenetic analysis was performed to compare the conserved and unique features of potato cytokinin receptors with the features of Arabidopsis, rice, tomato and other species receptors (Fig. 1). Cytokinin receptors of flowering plants can be grouped into three main clades, corresponding to the Arabidopsis AHK2, AHK3, and CRE1/AHK4 receptors (Pils and Heyl, 2009; Lomin *et al*., 2012; Steklov *et al*., 2013). Potato and tomato receptors are unequivocally distributed among these three

clades. Evolutionally, they are closer to Arabidopsis than to rice receptors, what was expected since potato, tomato and Arabidopsis are dicots whereas rice is a monocot.

Multiple alignments revealed common and unique features of StHKs

We investigated the modular architecture of predicted potato cytokinin receptors. The exon-intron structure of the cognate genes as well as occurrence and position of functional domains in the receptor proteins were analyzed. Known cytokinin receptors share a common organization, including (from N to C termini) sensory module with CHASE domain, catalytic module with histidine kinase and ATPase domains, and receiver module with pseudoreceiver and receiver domains (Kakimoto, 2003; Steklov *et al*., 2013). The sensory module is flanked by predicted transmembrane (TM) α-helices. There is always a single TM-helix downstream of module while the number of TM-helices upstream of module is variable. Number of upstream TM-helices is usually maximal (up to 3-4) in AHK2 clade members, minimal (1) in the AHK4 clade and intermediate in the AHK3 clade (Steklov *et al*., 2013). The domain structure of potato receptors fully corresponds to the canonical one (Fig. 2).

At the N-termini of potato cytokinin receptors, the number of upstream TM helices is 3, 2, and 1 in StHK2, StHK3, and StHK4, respectively. Cytokinin receptor genes share similar exon-intron organization. The exon boundaries in the receptor genes of different species coincide in most cases. A multiple alignment of receptor sequences from potato, rice and Arabidopsis was carried out (Fig. 3). All canonical motifs present in known cytokinin receptors were also found in the potato orthologs. In the catalytic domain of potato receptors, H, N, G1, F and G2 motifs were identified, in the receiver domain – DD-D-K motifs. Conserved sequences contain phosphorylatable histidine (H) and aspartate (D) residues. StHK2 has a conserved aspartate in its receiver-like domain (Rec-like), similarly to orthologs from Arabidopsis (AHK2), tomato (SlHK2) and rice (OsHK3 and OsHK5). However, DD-D-K motif in the Rec-like domain has little in common with the respective sequence in the Rec domain (Fig. 3C).

Highly conserved motifs were earlier found in sensory modules and downstream TM segments of cytokinin receptors (Steklov *et al*., 2013). These motifs are obviously important for ligand binding and transmembrane signal transfer. In potato receptors, these motifs are also present, although with some peculiarities. In particular, StHK2 has a deviation from the canonic motif in CHASE domain, where either Glu or Asp is located at position 90, while StHK2 has Gln at this position. StHK3 has a deviation at the position 177, strongly conserved in the HK3-clade. This position is occupied by Phe in the canonic motif, while in StHK3 by Leu. In the general HK-motif, there is either Phe or Tyr at position 177. StHK4 is distinguished by positions 83 (Ser instead of Ala) and 172 (Phe instead of Tyr) in conserved motifs. Note that counterparts of Gln90, Leu177 and Ser83 are present also in tomato

genome, so these substitutions may be characteristic of Solanaceae family. Phe172 seems to be unique for potato.

StHK functional domains adopt canonical 3D structures

We have built homology models of StHK receptors' sensory, catalytic, and receiver domain structure (Fig. 4). High structural similarity of potato receptors with their Arabidopsis orthologs was observed as expected. Key functional regions, such as ligand-binding sites, phosphorylation sites, ATP-binding sites and dimerization interfaces, are particularly conserved. Sensory modules consisting of dimerization, PAS and pseudo-PAS domains (the latter two comprise the CHASE domain) are very similar in Arabidopsis and potato. StHK2 and StHK3 differ from StHK4 by an insertion of 14 and 17 residues, respectively, in the region adjacent to the C-terminus of α3-helix (the first α-helix of the PAS domain). This insertion apparently does not participate in the ligand recognition site and is unlikely to directly affect the ligand-binding properties of the protein. Similar insertions are also present in AHK2 and AHK3 receptors from Arabidopsis.

The catalytic modules include histidine kinase domains and H-ATPase domains. Histidine kinase domains are formed by two α-helices and contain dimerization interface and phosphorylation site (conserved histidine). H-ATPase domains including ATP-binding sites have a sophisticated structure based on parallel/antiparallel β-sheets and α-helices. A large insert at the β2-β3 linker (more than 50 residues long) differs cytokinin receptors from bacterial histidine kinases and H-ATPase domain of the ethylene receptor. This insert is located, however, on the opposite side from the ATP binding site. This structural feature is inherent not only for potato receptors but also for cytokinin receptors of other species.

The CKI1 histidine kinase receiver domain (RD), used as a template for cytokinin receptor RD, adopts a fold typical for the REC (or CheY-like) superfamily proteins. It is formed by five α-helices and β-sheet of five parallel strands. Two α-helices are located on one side of the β-sheet, and remaining three on the other side. The same fold is characteristic of the RD model of the Arabidopsis CRE1/AHK4 receptor. As distinct from this, an additional small helix is present in the region between α3 helix and β4 sheet in RD models of other potato and Arabidopsis receptors except CRE1/AHK4. Conserved aspartate residue, serving as a phosphate acceptor in RD, is located at the N terminus of the β3 sheet (Fig. 4).

Deviations from canonic CHASE motifs in sensory modules of potato cytokinin receptors evidently do not alter 3D structures of the modules. Unusual Gln90 is far from the ligand-binding pocket of StHK2, with sidechain directed to the dimerizing interface. Although the unusual Leu177 of StHK3 is localized in the ligand-binding site, its sidechain is oriented to the opposite direction. The substitutions in StHK4 seem to be more functional than in other potato receptors. Ser73 and Phe172 are localized in

the ligand-binding pocket periphery and their sidechains are oriented inwards. Hence, these latter substitutions might somehow influence the ligand specificity of the receptor.

Experimental studies on autotetraploid potato cv. Désirée

Potato cv. Désirée possesses multiple alleles of StHK *genes*

A homozygous doubled monoploid Phureja (DM1-3 516 R44) is an artificial form of potato phenotypically differing from commonly known diploid/tetraploid potato varieties (Potato Genome Sequencing Consortium, 2011). Such differences in phenotype are underlain by considerable sequence and structural genome variations between potato haplotypes. Therefore, the results of genome study of monoploid Phureja do not mirror exactly more complex genomes of common potato cultivars.

Our experimental study of cytokinin receptors was performed using the autotetraploid potato cv. Désirée, widely used for commercial purposes, as well as a model laboratory plant (Aksenova *et al*., 2000; Kolachevskaya *et al*., 2015). We cloned the putative receptor genes using primers designed according to Phureja gene sequence data. Distinct from Phureja genome, at least six genes of putative cytokinin receptors were cloned from cDNA of Désirée plants. All these genes share a typical module/domain structure characteristic of hybrid sensor histidine kinases (Figs. 2–4). According to their sequence, encoded proteins fall pairwise into three known clades of cytokinin receptors (Table 2, Fig. 1). Thus, each form of cytokinin receptors from potato cv. Désirée consists of at least two close isoforms encoded by natural receptor alleles. Sequencing of cloned genes revealed traits of both similarity and divergence between Phureja and Désirée plants. The nucleotide sequences of HK2-clade members *StHK2a* and *StHK2b* differ from the orthologous Phureja sequence by five and four nucleotides (5 and 4 SNPs), respectively. At the protein level, StHK2a and StHK2b have three and two amino acid substitutions, respectively, relative to Phureja receptor (Table 2).

Of two cloned genes of HK3-clade, *StHK3a* is identical to its counterpart of Phureja, whereas *StHK3b* differs by 20 SNPs together with a 3-nucleotide deletion. These differences result in the absence of one amino acid and nine substitutions in StHK3b compared to its Phureja ortholog. Similar data were obtained for HK4-clade: *StHK4a* was fully identical to that of Phureja whereas *StHK4b* differed by 28 SNPs and a 3-nucleotide deletion. Correspondingly, StHK4b differs from its Phureja ortholog, as well as from StHK4a, by deletion of one amino acid and substitution of 13 ones (Table 2). Analysis of amino acid sequences of the proteins showed that all putative histidine kinases of Désirée potato retain the domains and consensus sequences typical for cytokinin receptors, despite aa substitutions (Fig. 2). This indicates that all proteins encoded by the cloned StHK genes of tetraploid potato plants can successfully function as cytokinin receptors.

StHKs have typical cytokinin-binding properties except StHK3 with distinct ligand specificity

To analyze ligand-binding properties of the receptors, a recently developed plant membrane assay system (Lomin *et al*., 2015) was used. Potato cytokinin receptor genes were cloned into pB7FWG2 vectors for transient expression in tobacco leaves. In the case of *StHK2* and *StHK4* genes, the full-length cDNA sequences were expressed, but in the case of *StHK3*, expression of the full-length receptor failed for unknown reasons. Instead of full-length receptor, we cloned a genomic sequence of the StHK3a sensory module flanked with transmembrane domains. From the transiently transformed tobacco leaves, a microsomal fraction enriched with individual potato receptors was obtained. The binding assays were conducted using this fraction and tritium-labeled cytokinin. In aggregate, we tested four receptors belonging to all three clades: StHK2a, StHK3a (sensory module, further designated as StHK3a$_{SM}$), StHK4a, and StHK4b.

First, we determined the pH-dependence of hormone binding to these receptors within the pH range of 5–9 (Fig. 5). All receptors exhibited maximal *trans*-zeatin binding at the neutral-mildly basic pH: StHK2a at pH 7.5, StHK3a$_{SM}$ at pH 7, StHK4a at pH 7.5–8, and StHK4b at pH 8–9. All receptors showed a decrease in ligand binding at acid pH: StHK2a and StHK3a$_{SM}$ reduced their binding at pH 5 compared to pH 7 by a factor of 2 and 5, respectively. Ligand binding by StHK4a and StHK4b decreased at pH 5 about three times compared to maximal values. Although the StHK3a receptor was represented in this study only by its sensory module, a control experiment with the full-length receptor StHK2a and its sensory module showed a similar pH-dependence of hormone binding (data not shown). This means that an isolated sensory module is sufficient to determine the pH-dependence of hormone binding by the receptor.

The interaction of a hormone with a receptor is characterized by the equilibrium dissociation constant (K_d) of the ligand-receptor complex. K_d values were determined by the dose-dependent binding of labeled *trans*-zeatin to receptors, the results were processed by the Scatchard method (Lomin and Romanov, 2008). All receptors demonstrated high affinity for *trans*-zeatin, with similar K_d at the nanomolar level (Table 3). The determined K_d values were close to the values of analogous constants for cytokinin receptors of other species (Lomin *et al*., 2012, 2015, Kuderova *et al*., 2015) and were well correlated with concentrations of active cytokinins *in planta* (Hirose *et al*., 2008).

Different cytokinins are usually present in the plant: *trans*- and *cis*-zeatins, isopentenyladenine, and dihydrozeatin. In addition to natural cytokinins, there are many synthetic ones. Receptors exhibit different affinities for these substances. We studied the ligand specificity of receptors in competitive experiments where binding of labeled cytokinin was carried out in the presence of various concentrations of certain unlabeled ligands. Based on the obtained competition curves, the apparent K_d values were determined for each ligand as was described (Lomin and Romanov, 2008). We analyzed the

interaction with the receptors of six cytokinins, including five natural ones as well as synthetic urea-type cytokinin thidiazuron (Table 3). The ligand specificity of the receptors showed much in common. All analyzed receptors had a high and nearly equal affinity for *trans*-zeatin and isopentenyladenine, apparent K_d ranging from 2.1 to 5.2 nM. All receptors bound *cis*-zeatin significantly weaker, with K_d over 100 nM. 6-Benzyladenine exhibited an intermediate affinity with K_d ranging from 40 to 60 nM. Regarding the two remaining cytokinins, the receptors showed significant differences. StHK3a$_{SM}$ bound dihydrozeatin with K_d ~21 nM, much stronger than other potato receptors (K_d ~170-230 nM). StHK2 and StHK3a$_{SM}$ showed a high affinity for thidiazuron (K_d=1.4 nM and 2.3 nM, respectively), whereas its affinity for StHK4a and StHK4b was much lower (K_d=12.6 nM and 17.2 nM, respectively). The cytokinin affinity ranking for the potato receptors are as follows: StHK2, TD>iP=tZ>BA>cZ>DZ; StHK3, TD>iP=tZ>DZ>BA>cZ; StHK4, iP=tZ>TD>BA>cZ>DZ. The preference profiles of StHK2 and StHK3a$_{SM}$ differ by DZ position, and from (almost identical) StHK4 isoforms by TD position. The greatest differences (in TD and DZ positions) were revealed between the StHK3 and StHK4 receptors. With respect to ligand specificity, StHK2 and StHK4 have much in common with their Arabidopsis orthologs, whereas StHK3 binds isopentenyladenine and 6-benzyladenine with much higher affinity than AHK3. Although the StHK3a receptor was represented in this study only by its sensory module, previous data showed that sensory module is sufficient to characterize the ligand preference of the full-length receptor (Stolz *et al*., 2011; Lomin *et al*., 2015).

StHKs are able to trigger signaling via MSP

The ability of the putative potato receptors to trigger cytokinin signaling was tested on *E. coli* ΔrcsC mutant devoid of its own RcsC hybrid histidine kinase and equipped with the *cps:LacZ* construct with the *LacZ* reporter gene driven by *cps* promoter (Suzuki *et al*., 2001; Takeda *et al*., 2001). This design allows assessment of the ability of hybrid histidine kinases to initiate signaling over the MSP pathway. Activation of MSP signaling in the bacteria leads to the expression of the reporter galactosidase (LacZ), whose activity is manifested by blueing of clones growing on X-Gal-supplemented medium. We expressed the cloned genes of the putative potato cytokinin receptors in *E. coli* ΔrcsC. In the clones expressing the receptors but not in the control clone, blue staining was observed (Fig. 6). The degree of blueing was greatly increased in the presence of cytokinin. It confirms the ability of the cloned potato receptors to transmit the cytokinin signal to the primary response genes via the canonic MSP pathway.

StHKs exhibit* in planta *organ-specific expression pattern which has unique properties

To assess the functionality of a gene *in vivo*, it is important to know the level and pattern of its expression in the living organism. We studied the expression of putative cytokinin receptor genes in organs of potato plants grown *in vitro* under conditions favorable for either vegetative growth (1.5% sucrose) or for tuber formation (5% sucrose). The mRNA contents of the *StHK2*, *StHK3* and *StHK4* genes was determined by the qRT PCR method. For the quantitative comparison of the expression profiles, intra-exon primers were selected for each tested gene. These primers were complementary to both alleles of the same clade owing to a great similarity of these gene sequences. The relative amounts of putative receptors of distinct clades in potato organs were judged by comparing the levels of transcripts of the cognate genes.

Expression levels differed significantly depending on receptor, organ and growth conditions (Fig. 7). Expression patterns were different in plants grown on media with low (1.5%) or high (5%) sucrose content. In the case of 1.5% sucrose medium, the highest expression of *StHK3* genes was observed in roots, while in the case of 5% sucrose medium, the maximal expression of *StHK3* tended to occur in leaves. In the low sucrose grown plants, *StHK4* gene was much weaker expressed in leaves than in stems or roots, whereas at the higher sucrose content levels of *StHK4* expression in different organs were equalized. In the *StHK2* group, noticeable organ-specific differences were detected when plants were grown on 5%, but not on 1.5% sucrose. The lowest expression level of all groups of receptors was usually observed in tubers compared to other organs (Fig. 7A).

Within each organ, expression of *StHK3* undoubtedly dominated in leaves, regardless of the sucrose content (Fig. 7B). Expression of *StHK2* and especially *StHK4* genes in leaves was much weaker. In stems grown on 1.5% sucrose, expression of *StHK4* prevailed, while the lowest expression was characteristic of *StHK2* genes. In the roots, expression of *StHK2* genes was relatively weak, whereas the genes of *StHK3* and *StHK4* clades were expressed actively and in almost equal proportions. A dissimilar pattern of expression was observed in plants grown on 5% sucrose. Here in addition to leaves, in all other organs tested (stems, roots, tubers) the expression of *StHK3* alleles prevailed too, though to a lesser extent than in leaves. Compared to the low-sucrose medium, 5% sucrose increased the relative expression of *StHK2* genes (in stems and roots), while decreasing the level of *StHK4* expression. Thus, unlike Arabidopsis, in potato plants there is evidently no dominance of StHK4 receptors in the roots, on the contrary, StHK3 receptors seem to dominate there when cultivating plants on tuber-inductive 5% sucrose. A common feature of potato and Arabidopsis is a very low expression of HK4 orthologs in leaves.

Although the primers used for qRT PCR did not distinguish closely related isoforms of the cytokinin receptor genes, it is still possible to approximately estimate the relative expression of these alleles. To achieve this goal, data on cDNA clone numbers can be used (Table 2). Within the same clade, relative quantity of cDNA clones harboring a distinct isoform should reflect the relative occurrence of cognate

mRNAs. According to the last column of Table 2 corresponding to aerial part of potato seedlings, two mRNA isoforms of the HK2 clade were in the 1:1 ratio; among mRNA isoforms of HK3 clade, *StHK3a* was approx. two-fold more frequent than *StHK3b*; in the case of HK4 clade, *StHK4b* was expressed about one order of magnitude more intensively than *StHK4a*.

StHK promoter activity is hardly affected by cytokinins, in accordance with low* cis*-element content

Treatment of potato plants with 6-benzyladenine had a small effect on the expression of the cytokinin receptor genes, and the hormonal impact, when occurred, was only local and not always reproduced. At 1.5% sucrose, the upregulation (on average, 2.5-fold) of *StHK4* expression was regularly recorded, but only in leaves (Fig. 8). It can be stated that the effect of cytokinin on the expression of potato receptor genes, if any, is mostly limited to *StHK4* and depends on both organ/tissue type and conditions of plant cultivation.

To validate the results of cytokinin treatment experiments, the effect of cytokinin administration on the transcript level of the genes of type A response regulator (*RR*-A) genes was analyzed. These genes in other species (Arabidopsis, maize) represent genes of primary response to cytokinin, so it might be expected that in potato too they would be responsive to cytokinin. Indeed, our experiments showed a rapid and reliable increase in the expression of *StRR*-A genes, in contrast to the receptor genes, after plant treatment with BA (Fig. 8). These results prove the reliability of design and implementation of experiments and, on the other hand, corroborate the common mode of functioning of the cytokinin signaling system in different plant species.

Analysis of promoter structures of the studied genes (Fig. 9) was generally consistent with the gene expression data. Long cytokinin-sensitive *cis*-regulatory elements or blocks of 4 or more short elements near the transcription start (~300 bp area) were found in promoters of almost all *StRR*-A, but not *StHK* genes. Among the receptor genes, only *StHK4* has a block of 3 short cytokinin-sensitive *cis*-elements near the start of transcription. It is possible that this block determines the responsiveness of *StHK4* to cytokinin under certain conditions, as shown in Fig. 8. Though this promoter analysis was accomplished using the genome sequence of var. Phureja, our experimental analysis of sequenced promoters of Désirée plants showed a complete similarity in this respect of the promoters from these two potato lines.

Discussion

Plant morphogenesis, in particular tuberization, is based on spatiotemporal cell proliferation and differentiation. The main biological effect of plant hormones cytokinins is the induction of cell divisions (Sakakibara, 2006; Romanov, 2009), therefore cytokinins are important participants of morphogenetic

processes. Indeed, with regard to potato development, cytokinins were reported to accelerate and scale up tuber formation (Aksenova *et al*., 2000; Romanov *et al*., 2000). In non-potato plants, cytokinins alone were able to induce the emergence of tuber-like structures (Guivarc'h *et al*., 2002; Eviatar-Ribak *et al*., 2013; Frugier *et al*., 2008; Miri *et al*., 2016). Apart the impact on the formation of tubers, cytokinins are known to regulate overall plant architecture, biomass partitioning as well as resistance to biotic and abiotic stress-factors (Aksenova *et al*., 2000; Abelenda and Prat, 2013; Zwack and Rashotte, 2015; Brütting *et al*., 2017; Thu *et al*., 2017). All the above points to the importance to investigate cytokinin signaling system in plants, in particular in bulbous crops like potato.

Herein, we present first results of detailed study of cytokinin receptors from potato plants. Two different potato forms were examined: doubled monoploid Phureja and tetraploid potato of Désirée variety. Phureja plants possess, like Arabidopsis, three cytokinin receptor orthologs. By contrast, in Désirée plants two allelic forms of each receptor type (StHK2a/b, StHK3a/b and StHK4a/b) have been found belonging to the three known phylogenetic clades. It is not excluded that the real number of receptor alleles in Désirée plants is somewhat higher. Within each group, receptor isomers differ by a few amino acid substitutions which do not affect most conserved motifs. However, some recently revealed consensus motifs in sensory module (Steklov *et al*., 2013) are distinctive in receptors of potato plants. The reason for such peculiar properties is not yet clear. Molecular modeling was employed to build all main domains of potato cytokinin receptors. In general, potato cytokinin receptors share similar domain structure with crystallized hybrid histidine kinases from other species. Note that such a complete characterization of all main domains of cytokinin receptors is presented for the first time.

The ligand-binding properties of individual potato receptors have been determined: affinity constants for active cytokinins, pH-dependence of ligand binding, ligand specificity. Two of the studied receptors (StHK3a and StHK4a) are identical in potato cv. Désirée and var. Phureja. All receptors have high affinity for tZ, significantly lower for BA, and relatively low for cZ. StHK3 differs from other potato receptors by relatively high affinity for DZ. All receptors bind cytokinin stronger in basic (pH 7–9) than acidic (pH 5–7) pH range. This indicates the intracellular functioning of potato cytokinin receptors (Romanov *et al*., 2018). The ligand-binding properties of StHK3 differ from those of orthologs in Arabidopsis, maize and oilseed rape: contrary to the other orthologs of this clade, StHK3 strongly binds iP with the affinity similar to the affinity for tZ. Using the special *E. coli* strain, the functionality of cloned potato receptors was confirmed by testing their ability to transduce cytokinin signal via MSP up to the target gene.

Expression of cytokinin receptor genes in various organs of potato plants and under different growing conditions was studied. The predominant expression of the *StHK3* genes was revealed in leaves, as well as in other organs of plants grown on 5% sucrose, although the degree of dominance of StHK3 was less pronounced in stems, roots and tubers. When plants were grown on 1.5% sucrose, *StHK4* expression

predominated in stems while in roots the expression levels of *StHK3* and *StHK4* were relatively high and nearly equal. In contrast to other species (Romanov, 2009; Lomin *et al*., 2012), no prevalent expression of HK4 orthologs in roots was found. Exogenous cytokinin had little effect on the expression of cytokinin receptors in potato plants except *StHK4* which can be rapidly upregulated in leaves. Analysis of promoter structures showed a correlation between the occurrence of *cis*-regulatory elements and the cytokinin sensitivity of gene expression.

Thus, the totality of our results left no doubt that studied StHK proteins are genuine cytokinin receptors in potato. The observed unique structural features refine and broaden our notion on the properties of cytokinin receptors. The revealed peculiarities of cytokinin perception apparatus in potato might be associated with the ability of this crop to produce tubers. The obtained results create a solid basis for further in-depth study of the role of the cytokinin signaling system in potato ontogenesis and provide new biotechnological tools to optimize hormonal regulation of tuber formation.

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Table 1. *Proteins and genes predictably related to cytokinin signaling system of potato*

Protein type	Protein name	Gene ID	mRNA	Protein	Protein length, aa
CHK	StHK2	LOC102591086	XM_015303261.1	XP_015158747.1	1263
CHK	StHK3	LOC102587294	XM_006352114.2	XP_006352176.1	1032
CHK	StHK4	LOC102603756	XM_006354988.2	XP_006355050.1	992
HPt	StHP1a	LOC102590747	XM_006365209.2	XP_006365271.1	151
			XM_006365208.2	XP_006365270.1	151
			XM_006365207.2	XP_006365269.1	151
HPt	StHP1b	LOC102603297	XM_006352731.2	XP_006352793.1	152
HPt	StHP1c	PGSC0003DMG400028593	PGSC0003DMT400073603	PGSC0003DMT400073603	148
HPt	StHP6	LOC102601463	XM_006364157.2	XP_006364219.1	156
HPt	StHP4a	LOC102589200	XM_015304066.1	XP_015159552.1	112
			XM_006364659.2	XP_006364721.1	136
HPt	StHP4b	LOC102584884	XM_015315420.1	XP_015170906.1	137
RR-B	StRR1a	LOC102578736	XM_006363517.2	XP_006363579.1	675
			XM_006363518.2	XP_006363580.1	675
RR-B	StRR1b	LOC102586468	XM_006345914.1	XP_006345976.1	663
RR-B	StRR1c	LOC102596771	XM_006349891.2	XP_006349953.1	556
RR-B	StRR14	LOC102606335	XM_006354997.1	XP_006355059.1	653
			XM_006354996.1	XP_006355058.1	656
RR-B	StRR11	LOC102593308	XM_006341706.2	XP_006341768.1	581
			XM_006341705.2	XP_006341767.1	581
			XM_015306278.1	XP_015161764.1	481
RR-B	StRR18a	LOC102598455	XM_006343619.2	XP_006343681.1	681
RR-B	StRR18b	LOC102587717	XM_006350015.2	XP_006350077.1	707
ARR19	StRR19	LOC107060895	XM_015309426.1	XP_015164912.1	371
RR-A	StRR4	LOC102602758	XM_015313344.1	XP_015168830.1	248
RR-A	StRR9a	LOC102590336	XM_006355533.2	XP_006355595.1	163
RR-A	StRR9b	LOC102588738	XM_015314746.1	XP_015170232.1	214
			XM_015314747.1	XP_015170233.1	211
RR-A	StRR9c	LOC102599826	XM_006351210.2	XP_006351272.1	226
RR-A	StRR9d	LOC102601166	XM_006351214.2	XP_006351276.1	226
RR-A	StRR8	LOC102588738	XM_015314747.1	XP_015170233.1	211
			XM_015314746.1	XP_015170232.1	214
RR-A	StRR15	LOC102605280	XM_006344933.2	XP_006344995.1	202
RR-A	StRR17	LOC102583233	XM_006357236.2	XP_006357298.1	156
RR-C	StRR22a	LOC107058083	XM_015303399.1	XP_015158885.1	186
RR-C	StRR22b	LOC107058085	XM_015303400.1	XP_015158886.1	184
RR-C	StRR22c	LOC107059982	XM_015307157.1	XP_015162643.1	137
RR-C	StRR22d	LOC102580685	XM_006361561.2	XP_006361623.2	115

Nomenclature of the NCBI database is represented except StHP1c found only in the Phytozome database. Number of RNA entries exceeds that of proteins due to alternative splicing. Data corresponding to cytokinin receptor proteins/genes and response regulator type A proteins/genes studied in this work are highlighted.

Table 2. *Putative cytokinin receptor genes in potato genomes and encoded proteins*

Receptor clade	Putative cytokinin receptors of potato plants:		SNP number in putative cytokinin receptor genes/proteins of cv. Désirée vs var. Phureja:		Number of Désirée cDNA clones
	Phureja* (length, aa)	**Désirée ** (length, aa)**	**DNA bases**	**Amino acids**	
HK2 orthologs	StHK2 (1263 aa)	StHK2a (1263 aa)	5 SNPs	3 SNPs	17
		StHK2b (1263 aa)	4 SNPs	2 SNPs	17
HK3 orthologs	StHK3 (1032 aa)	StHK3a (1032 aa)	No SNP	No SNP	6
		StHK3b (1031 aa)	20 SNPs, 3 del.	9 SNPs, 1 del.	3
CRE1/AHK4 orthologs	StHK4 (992 aa)	StHK4a (992 aa)	No SNP	No SNP	1
		StHK4b (991 aa)	28 SNPs, 3 del.	13 SNPs, 1 del.	9

*Doubled monoploid, method: total genome sequencing.

** Autotetraploid, method: PCR with cDNA as a template.

Table 3. *The affinity (K_d) of various cytokinins for putative potato receptors*

Cytokinin	Abbreviation	Apparent K_d (nM) for putative receptors:			
		StHK2a	**StHK3a$_{SM}$**	**StHK4a**	**StHK4b**
trans-Zeatin	**tZ**	2.6±0.3	4.7±0.6	2.5±0.7	3.0±0.3
cis-Zeatin	**cZ**	102±7	110±39	106±22	129±19
N^6-Isopentenyladenine	**iP**	2.4±0.2	5.2±0.8	2.1±0.2	2.5±0.3
Dihydrozeatin	**DZ**	169±18	21±3	178±37	227±33
N^6-Benzyladenine	**BA**	45±3.5	49±7	55±7	63±12
Thidiazuron	**TZ**	1.40±0.04	2.3±0.5	12.6±1.9	17.2±2.5

Figure legends

Fig. 1. Phylogenetic tree of cytokinin receptors. Species are: StHK2-4, *Solanum tuberosum*; SlHK2-4, *Solanum lycopersicum*; AHK2-4, *Arabidopsis thaliana*; OsHK3,4,6, *Oryza sativa*; AmbtriHK2,3, *Amborella trichopoda*; PITA 000007449 and PITA 000016046, *Pinus taeda*; MA 101803g0010 and MA 47453g0010, *Picea abies*; CRE1,2-1,2-2, *Selaginella moellendorffii*; CHK1-3, *Physcomitrella patens*. SP – seed plants, Lyco – Lycophyta, Bryo – Bryophyta. Parameters of ClustalW algorithm were: phylogeny test – bootstrap method, no. of bootstrap replications – 100, substitutions type – amino acid, model – equal input model, rates among sites – gamma distributed, no of discrete gamma categories – 3, gaps/missing data treatment – complete deletion, ML heuristic method – subtree-pruning – regrafting.

Fig. 2. Module/domain structures of the predicted potato cytokinin receptors. Protein domains: TM, transmembrane segment; DI, dimerization interphase; CHASE, Cyclase/Histidine kinases Associated SEnsory domain; HisKA, histidine kinase domain; HATPase, adenosine triphosphatase domain; Rec-like, receiver-like domain; Rec, receiver domain. Conserved amino acids and consensus motifs (N, G1, F, G2) are indicated. Scales at the bottom of the structures indicate the length in aa number.

Fig. 3. Cytokinin receptor sequence alignment. Consensus motifs and conserved amino acids are marked. AHK and OsHK correspond to Arabidopsis and rice proteins, respectively. Numbers of not shown amino acids are indicated in brackets.

Fig. 4. Homology models for predicted potato cytokinin receptor domains. Sensor modules and HisKA domain are presented in form of dimers where one of subunits is colored grey.

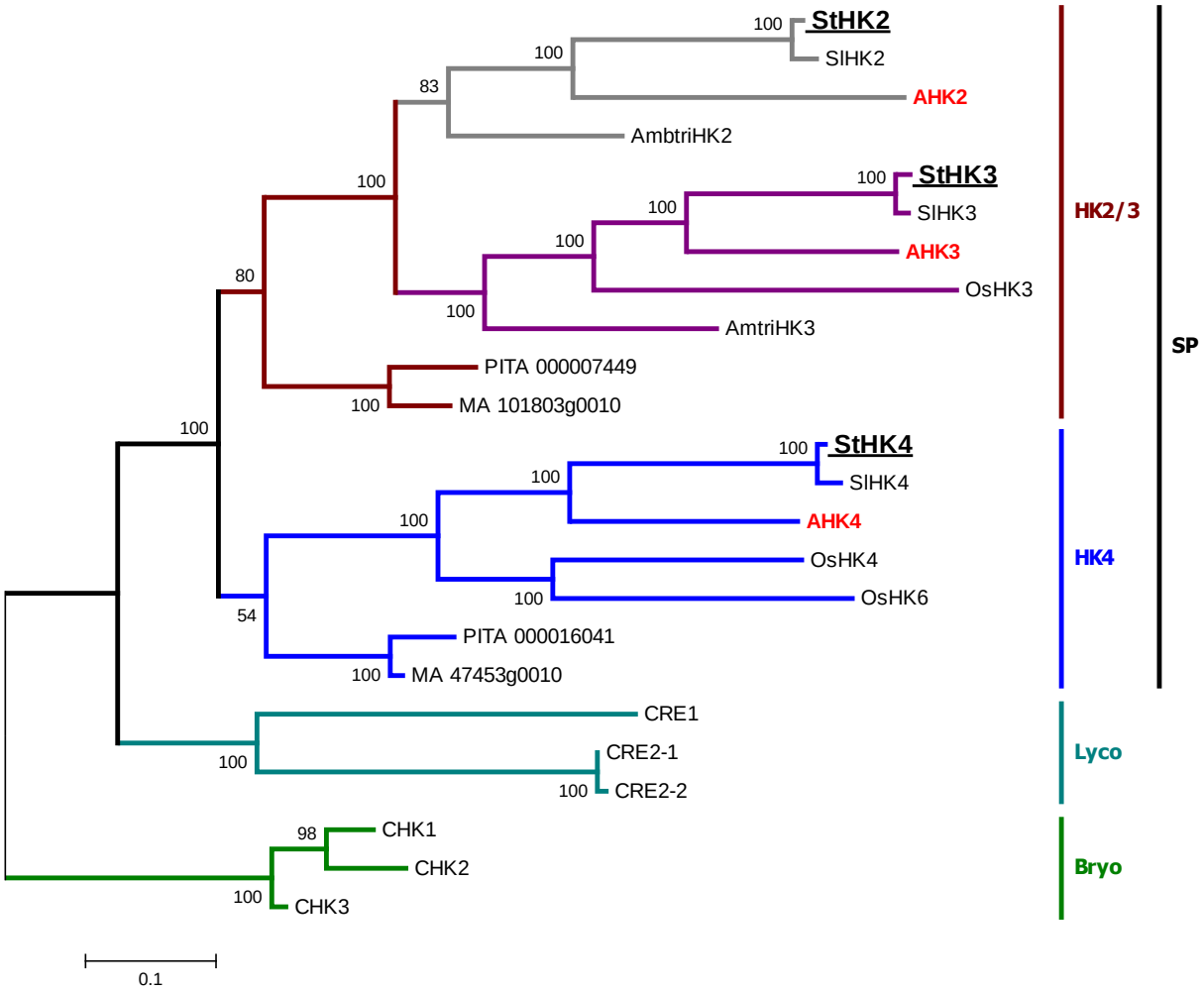
Fig. 5. pH dependencies of *trans*-zeatin binding to putative potato cytokinin receptors.

Fig. 6. Cytokinin receptors of potato feed MSP signaling pathway in ΔRcsC *E. coli* cells.

Fig. 7. Organ-dependent (A) and clade-dependent (B) patterns of expression of cytokinin receptors in potato plants cultivated on media with different % sucrose. Relative transcript copy number is given as % of the total transcript amount in each plot, taken as 100%.

Fig. 8. Degrees of transcription induction (BA/control) of cytokinin receptor (top) and response regulator type A (bottom) genes after 1 h treatment of potato plants with 1 μM BA. Plants were grown on MS medium with 1.5% sucrose for 5-6 weeks under standard LD conditions. L, S, R signify leaves, stems and roots, respectively. More than two-fold prevalence of transcripts in BA-treated over control plants is considered as significant induction, bars corresponding to induced genes are outlined red.

Fig. 9. Cytokinin-responsive *cis*-regulatory elements in promoters of cytokinin receptor genes (upper part) and response regulators type A genes (lower part) of potato. Elements are shown on both DNA strands. Promoter area proximal to transcription start is boxed.



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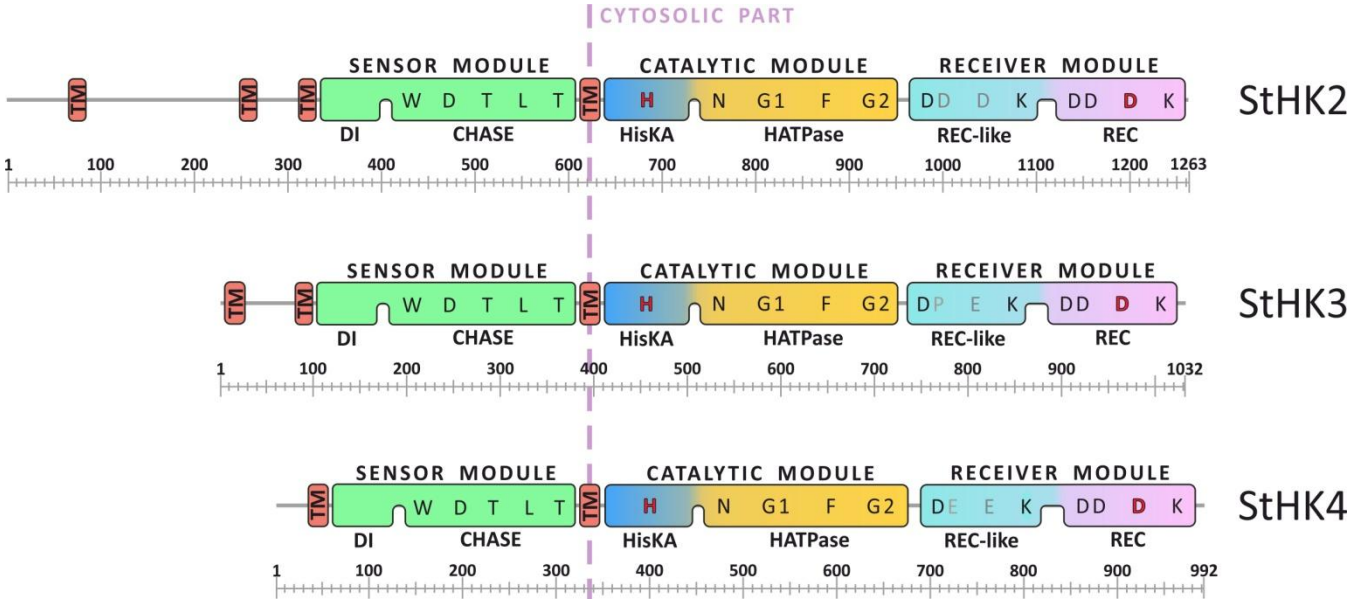

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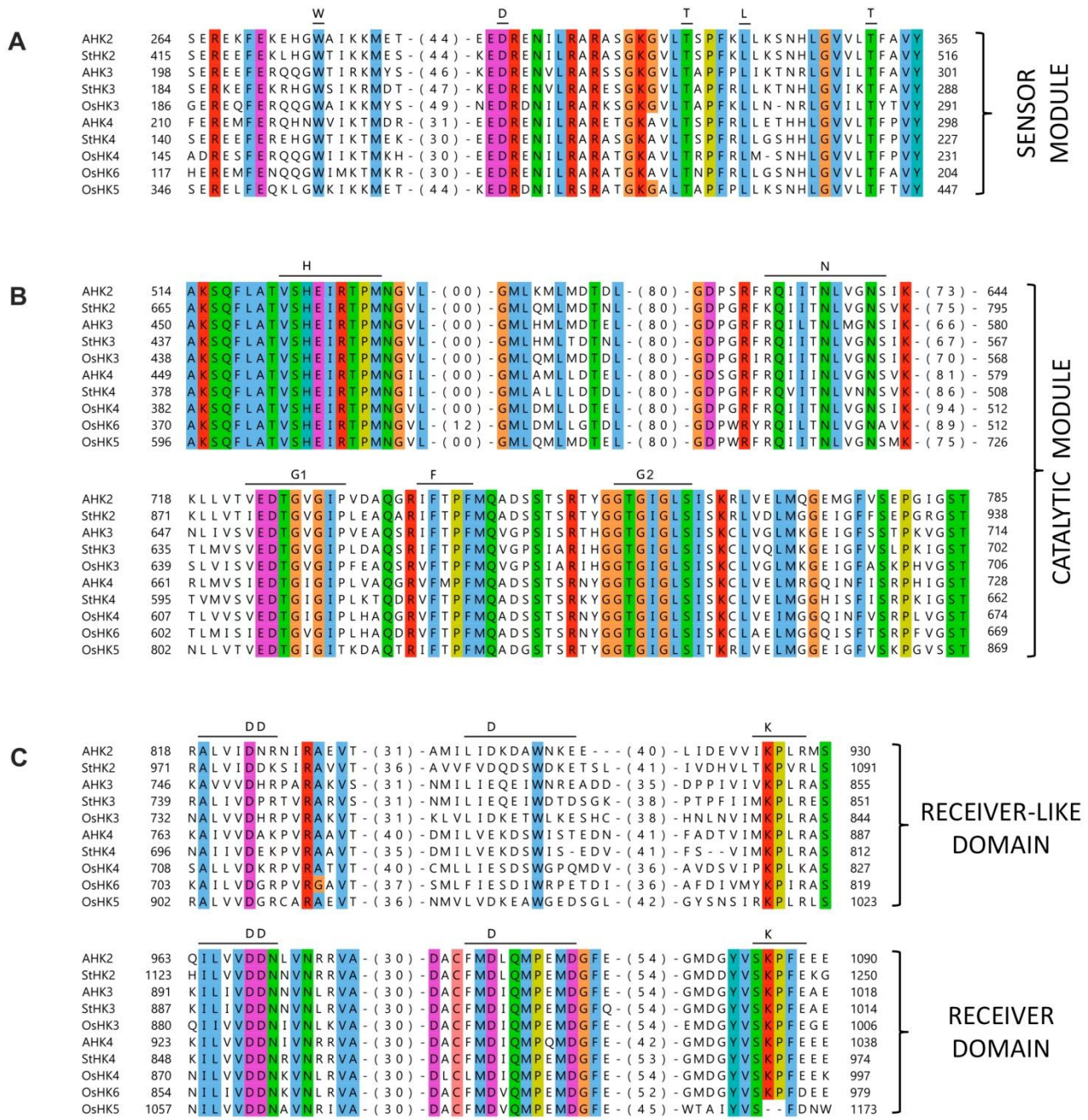

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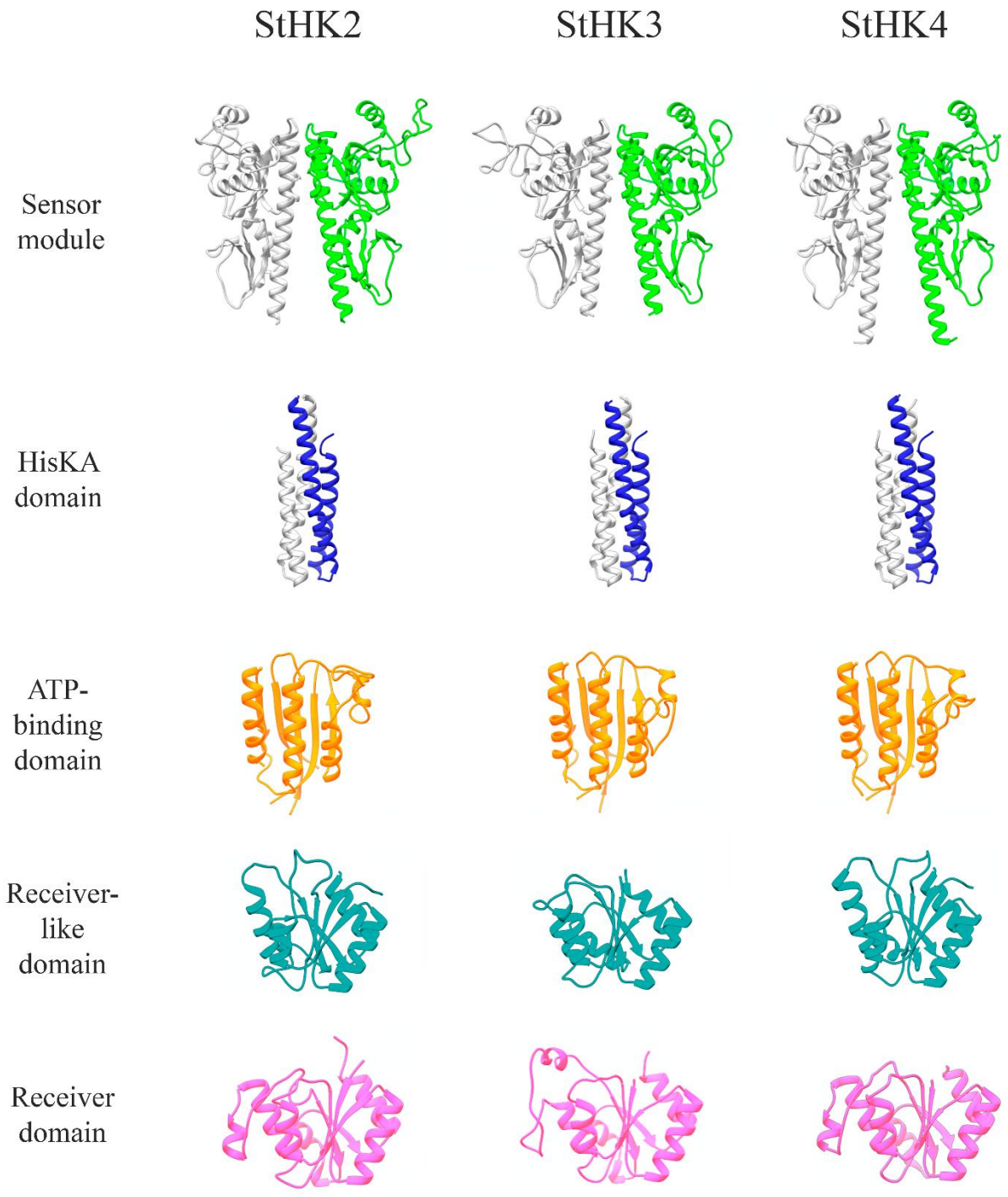

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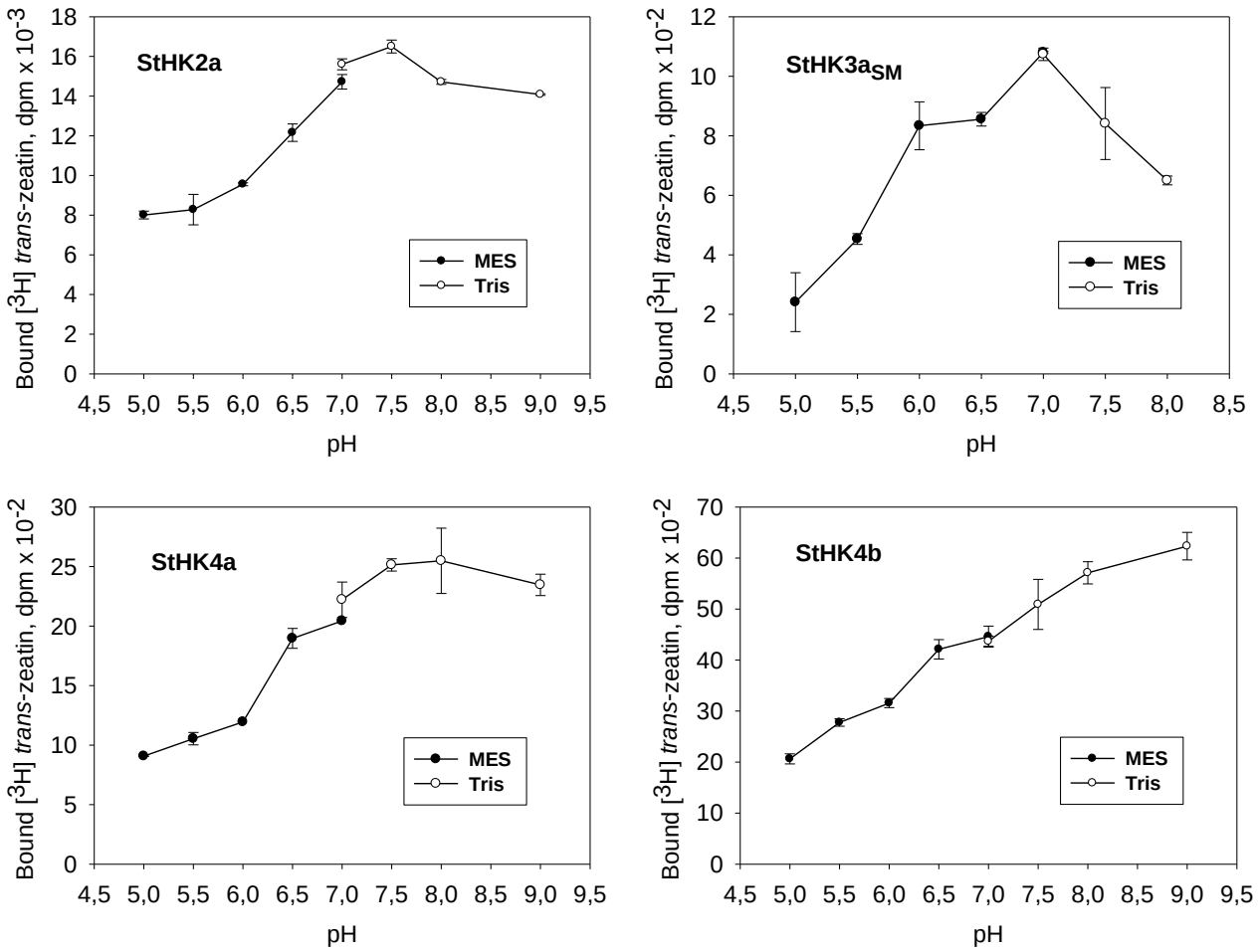

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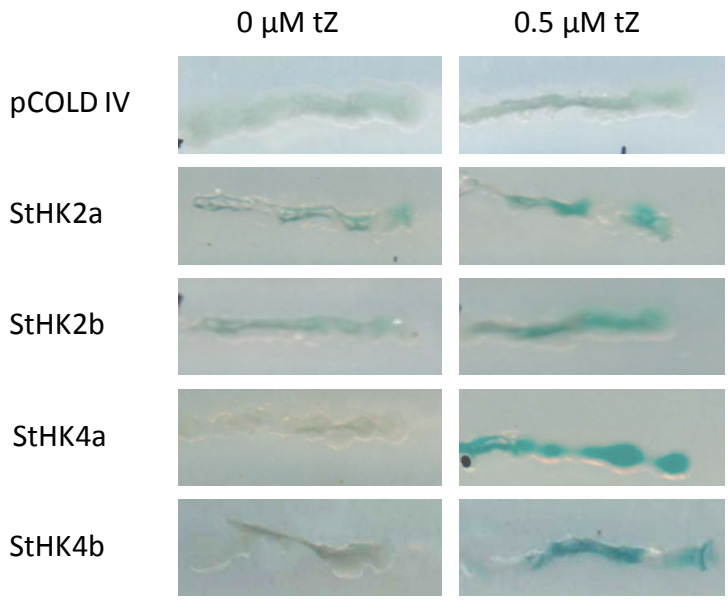

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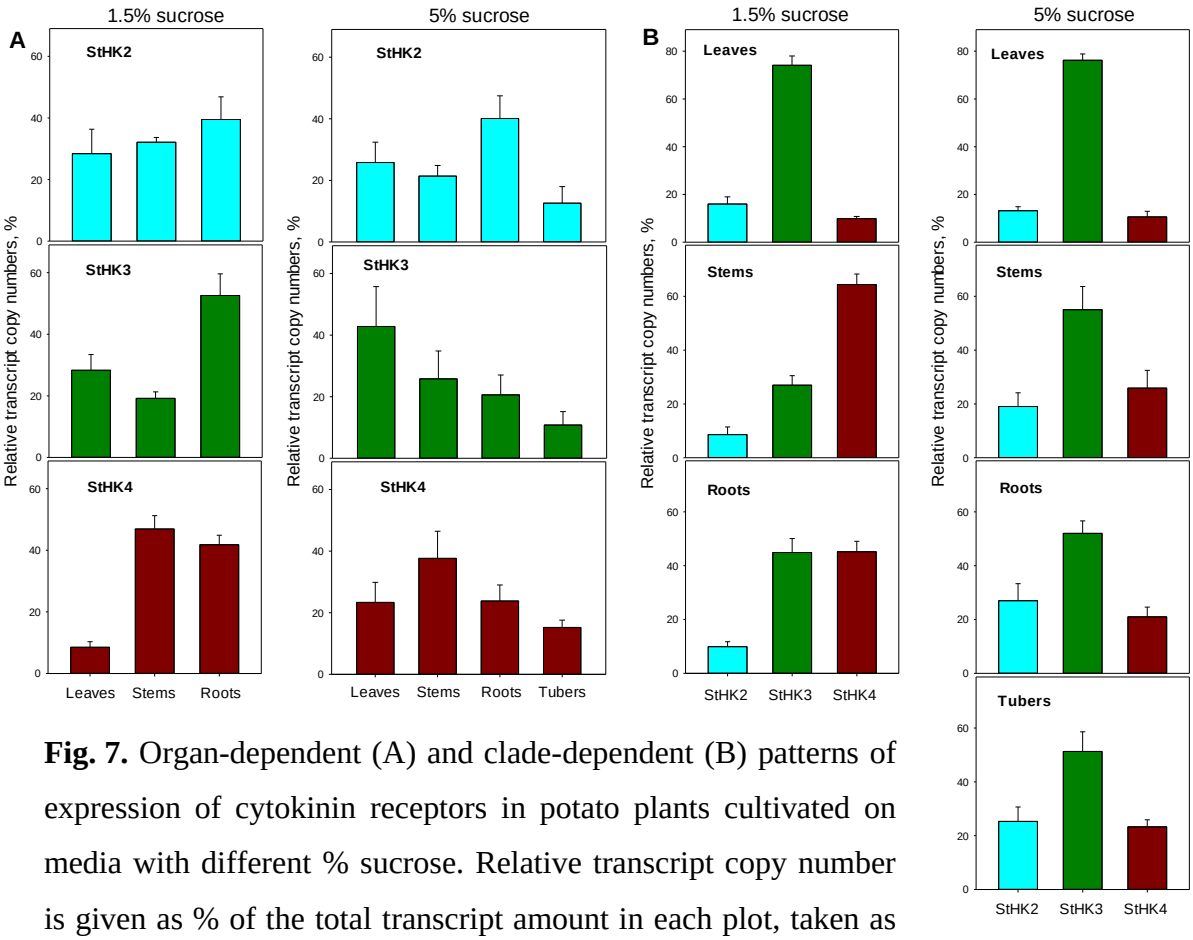

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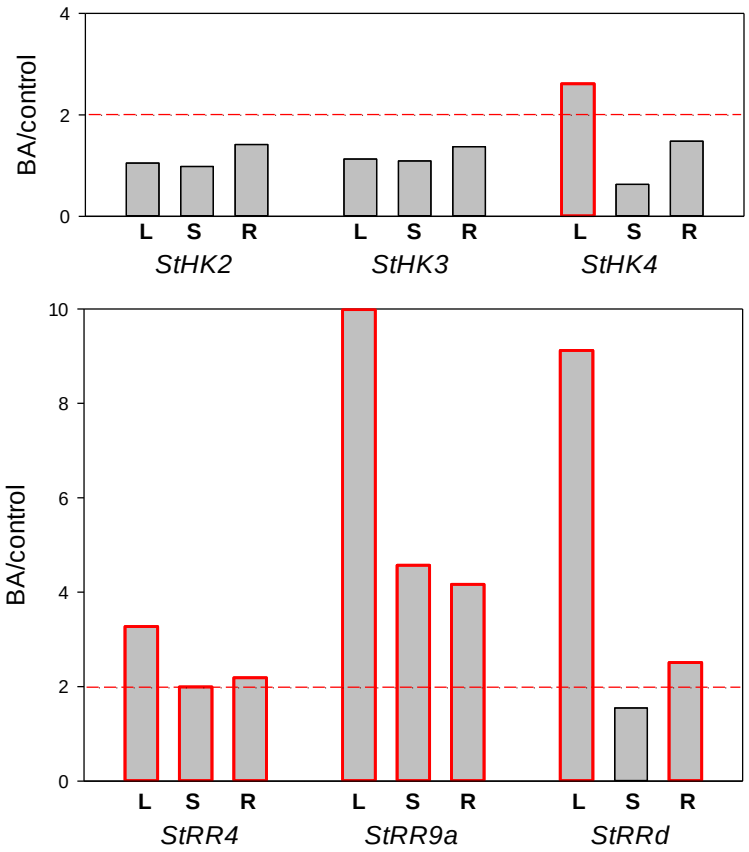

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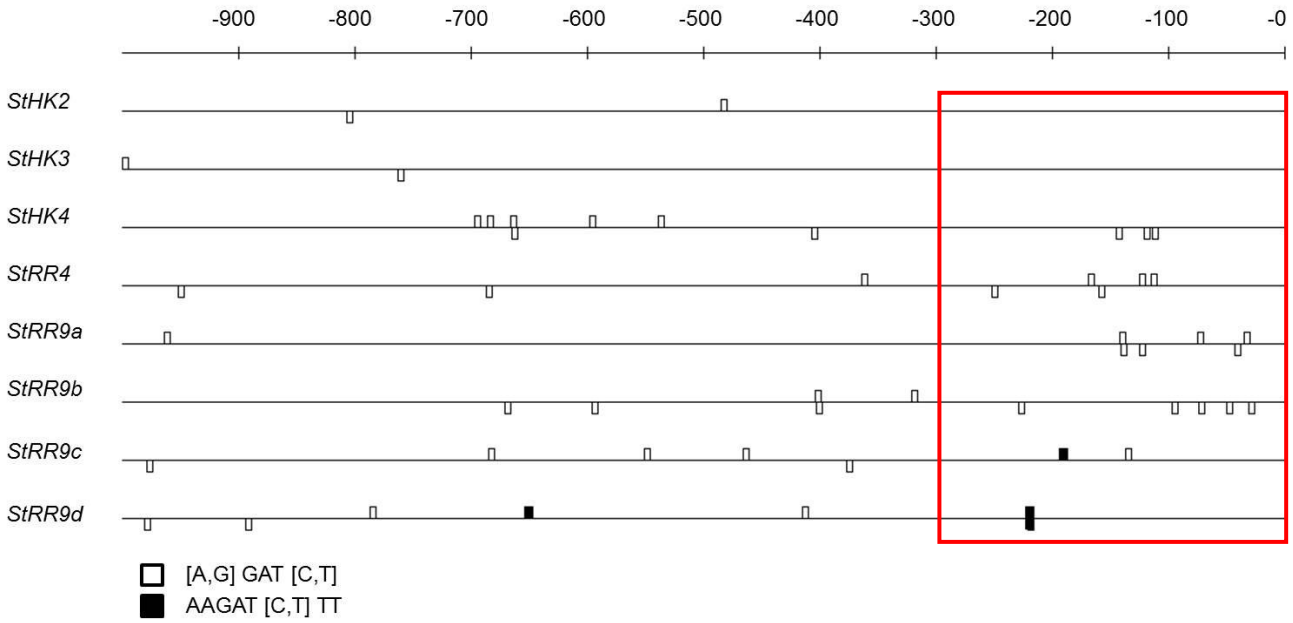

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