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ORIGINAL PAPER

Genome-wide analysis and expression profiling of calcium-dependent protein kinases in potato (*Solanum tuberosum*)

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Abstract Calcium-dependent protein kinases (CDPKs or CPKs), unique to plants and some protists, are involved in growth and developmental processes as well as in defence against diverse environmental stresses. CDPKs are encoded by multi-gene families. Despite extensive studies of the CDPKs in many species, information about the evolutionary history and expression patterns of the CDPK family in the staple crop potato (*Solanum tuberosum*) remains poorly known. In this study, we performed bioinformatics analysis of the potato whole genome sequence and identified 23 potential *CDPK* genes. These genes are located in 11, of 12, potato chromosomes. Based on the phylogenetic tree and gene structures, the CDPKs were divided into four subfamilies. To determine their expression, reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis was carried out for the *CDPK* genes in different organs of potato such as young and mature leaves, stems, young shoots, roots, stolons, swollen stolons, flowers and tubers. The *CDPKs* were expressed in all the organs analysed, but their expression patterns varied greatly. The expression of some *CDPKs* was strongly organ specific, for example *StCPK13* and *StCPK18* was found only/mostly in flowers. In *Solanum* genotypes differing in resistance to *Phytophthora infestans*, the expression and activity of CDPKs increased in response to a *P. infestans* elicitor with different kinetics and intensity. The expression levels and activity of the CDPKs correlated positively with the level of the resistance. Our results support earlier suggestion that CDPKs are involved in potato organ development and defence against stresses. We provide new information about the *CDPK* gene family in the potato and a perspective on its evolutionary history and biological roles of the individual kinases.

Keywords Calcium-dependent protein kinases (CDPKs) · Expression pattern · Gene family · *Phytophthora infestans* · *Solanum tuberosum*

Introduction

Calcium plays an important role as a universal second messenger in the control of developmental processes and in signal transduction. Changes in calcium ion concentration are sensed, among others, by calcium-dependent protein kinases (CDPKs, also named CPKs). CDPKs are Ser/Thr protein kinases typically comprising five domains: an N-terminal variable domain, a catalytic domain (CD), an autoinhibitory junction domain (JD), a calmodulin-like domain (CLD), and a C-terminal domain (Hrabak et al. 2003). The N- and C-terminal domains are variable, differing in length and amino-acid composition. Moreover, the N-terminal domain often bears myristoylation sites associated with the subcellular localization of the kinase. The specific functions of CDPKs are determined by these variable domains (Hrabak et al. 2003). In a typical CDPK the calmodulin-like domain harbours four EF-hand motifs for Ca^{2+} binding, and the binding of Ca^{2+} activates the kinase (Cheng et al. 2002). CDPKs are important sensors and effectors of the Ca^{2+} flux in plants. CDPKs have different subcellular locations including cytosol, nucleus, plasma membrane, endoplasmic reticulum, peroxisomes, mitochondrial outer membrane, and oil

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bodies, indicating their possible diverse functions (Lu and Hrabak 2002).

The CDPKs are multifunctional, which is reflected by the increasing number of their substrates being discovered. Numerous studies have demonstrated that CDPKs play key roles in the regulation of plant growth, development, and abiotic and biotic stress resistance (for review see Klimecka and Muszyńska 2007). Some *CDPK* genes have been shown to be involved in pollen tube growth (Estruch et al. 1994), root development (Ivashuta et al. 2005) and cell division and differentiation (Yoon et al. 1999).

Individual CDPKs in various species have been assigned defined roles. Thus, tomato LeCDPK2 regulates ethylene biosynthesis in response to wound signalling (Kamiyoshihara et al. 2010); Arabidopsis AtCPK1/2/4/5/11 phosphorylate and thereby activate NADPH oxidase to promote reactive oxygen species (ROS) production in response to abiotic and biotic stimuli (Gao et al. 2013); rice OsCPK4 participates in rice tolerance to salt and drought stress by protecting cellular membranes from lipid peroxidation (Campo et al. 2014), grapevine gene *VaCPK29* confers tolerance to heat and osmotic stresses (Dubrovina et al. 2017), whereas ZmCPK11 is involved in touch- and wound-induced pathways in maize (Szczegielniak et al. 2012). CDPKs are widely involved in various types of disease resistance (Boudsocq et al. 2010; Boudsocq and Sheen 2013; Romeis and Herde 2014; Wang et al. 2015). They regulate the plants' defence response locally and also systemically (Romeis and Herde 2014). AtCDPK1/2 modulate the initiation of programmed cell death, while AtCDPK4/5/6/11 phosphorylate specific WRKY transcription factors to regulate immune gene expression. AtCPK1 plays a positive role in resistance to various pathogens by promoting the salicylic acid (SA) signalling pathway (Coca and San Segundo 2010). NtCDPK2 is involved in the induction of *Cf-4*/*Avr4*- and *Cf-9*/*Avr9*-dependent hypersensitive response (HR) (Romeis et al. 2001).

The CDPKs have been identified in plants (Harmon et al. 2001; Asano et al. 2005), protists (Billker et al. 2009), oomycetes (Broad Institute of Harvard and MIT 2010), and green algae (McCurdy and Harmon 1992; Baillie et al. 2000), but not in any animal or true fungal genome (Zhang and Choi 2001; Hrabak et al. 2003). Plant genomes carry large numbers of *CDPK* genes; 34 in *Arabidopsis thaliana* (Cheng et al. 2002; Hrabak et al. 2003), 41 in diploid cotton (*Gossypium raimondii*) (Liu et al. 2014), 29 or 31 in rice (*Oryza sativa*) (Asano et al. 2005; Ray et al. 2007, respectively), 20 in wheat (*Triticum aestivum*) (Li et al. 2008), 35 or 40 in maize (*Zea mays*) (Ma et al. 2013; Kong et al. 2013, respectively), 29 in tomato (*Solanum lycopersicum*) (Wang et al. 2015; Hu et al. 2016), and 30 *CDPK* genes in poplar (*Populus trichocarpa*) (Zuo et al. 2013). Slightly less numerous are *CDPK* gene families in cucumber (*Cucumis sativus*) and grape (*Vitis vinifera*) with 19 members in both plants (Xu et al. 2015; Zhang et al. 2015).

Despite extensive studies of *CDPK*s in many species, still little is known about this gene family in the important crop - potato (*Solanum tuberosum*). Only five isoforms have been characterized so far. It is known that CDPKs are involved in tuber development (Raíces et al. 2001, 2003). *StCDPK1* transcript was observed in mesh bulbs and that of *StCDPK2* mainly in the leaves. StCDPK1 also plays a role in gibberellic acid (GA) signaling and influences tuberization (Gargantini et al. 2009) while StCDPK2 is associated with plant growth and development (Giammaria et al. 2011). An application of abscisic acid (ABA) or GA had no significant effect on the activity and expression of *StCDPK2* (Giammaria et al. 2011), while treatment with jasmonic acid decreased *StCDPK2* expression at both these levels (Ulloa et al. 2002). The expression profile of *StCDPK3* under different conditions has indicated that ABA functions as a positive and GA as a negative regulator of its expression. *Agrobacterium tumefaciens*-mediated transformation allowed demonstrating that *StCDPK3* was expressed in early induced stolons and its expression decreased in advanced tuber formation stages (Grandellis et al. 2012). As in other species, StCDPK4 and StCDPK5 have been shown to phosphorylate and thereby activate NADPH oxidase to promote reactive oxygen species (ROS) production in response to biotic stimuli (Kobayashi et al. 2007).

The availability of the whole genome sequence of the potato (Potato Genome Sequencing Consortium 2011) allowed us to carry out a comprehensive in silico search for *CDPK* genes and their subsequent expression profiling. RT-qPCR expression analysis was carried out for the *CDPK* genes in different organs of potato. The activity and expression of the CDPKs were also determined in *Solanum* genotypes expressing different resistance against the pathogenic oomycete *Phytophthora infestans* causing late blight, the most destructive potato disease.

Materials and methods

Identification of calcium-dependent protein kinase genes in potato

Gene retrieval

All *S. tuberosum* genes identified in the genomic annotation as encoding a calcium-dependent protein kinase were downloaded from the public databases Spud DB (http://potato.plantbiology.msu.edu/) and Sol Genomics Network (http://solgenomics.net/). As references for the identification of *CDPK* genes we used GenBank NCBI sequences of potato genes described in the literature [*StCDPK1*

accession number (acc. no.) DQ507862, *StCDPK2* acc. no. AF418563, *StCDPK3* acc. no. JF308510, *StCDPK4* acc. no. AB279737, *StCDPK5* acc. no. AB279738]. Comparison of particular genes was performed using BLAST program (The Basic Local Alignment Search Tool). For analysis, we used 21 genes annotated as calcium-dependent protein kinases in the above-mentioned databases and two genes, *StCDPK3* and *StCDPK5*, absent from those bases. Finally, 23 potato genes were identified as CDPKs (Table 1 and Supplementary material 1) and were subjected to further analyses. Thirty-four *Arabidopsis thaliana CDPK* genes used for phylogenetic analysis were downloaded from GenBank NCBI (Supplementary material 2).

Phylogenetic analysis and gene structure prediction

Full-length amino acid sequences of 23 potato and 34 *A. thaliana* CDPKs were aligned using ClustalX version 2.0 program with default parameters (Larkin et al. 2007). A phylogenetic tree was constructed according to the neighbor-joining method using MEGA 7.00 program (Tamura et al. 2004; Kumar et al. 2016).

The gene structures of the *CDPKs* were predicted by The Gene Structure Display Server (GSDS) version 2.0 (Hu et al. 2015).

Protein domains were predicted by PROSITE scan (Sigrist et al. 2013), and myristoylation sites by NMT—The MYR Predictor script (http://mendel.imp.ac.at/myristate/SUPLpredictor.htm).

Table 1 Characteristics of *Solanum tuberosum CDPK* genes

Name	PGSC Transcript ID	Genbank no. (NCBI)	Length (AA)	Molecular weight (kDa)	Myristoylation site	No. of introns	Chromo-some
StCPK1 (former StCDPK1)	PGSC0003DMT400071663	XM_006365894	509	56.6	+	6	12
StCPK2 (former StCDPK2)	PGSC0003DMT400057484	XM_006346152	521	57.9	+	7	7
StCPK3 (former StCDPK3)	Not found	JF308510 NM_001288527	554	63.0	+	7	?
StCPK4 (former StCDPK4)	PGSC0003DMT400043320	XM_006365245	567	63.4	–	6	10
StCPK5 (former StCDPK5)	Not found	AB279738 NM_001287861	535	60.0	–	?	?
StCPK6	PGSC0003DMT400060262	XM_006345687	503	56.4	–	6	5
StCPK7	PGSC0003DMT400002592	XM_006350871	578	64.8	+	6	5
StCPK8	PGSC0003DMT400027809	XM_006366477	533	60.0	+	7	1
StCPK9	PGSC0003DMT400054983	XM_006348373	598	67.6	–	6	1
StCPK10	PGSC0003DMT400021055	XM_006351162	524	59.4	+	7	10
StCPK11	PGSC0003DMT400067082	XM_006353564	501	56.4	–	6	6
StCPK12	PGSC0003DMT400072554	XM_006339117	607	68.3	–	6	10
StCPK13	PGSC0003DMT400018575	XM_006364680	544	60.4	+	6	10
StCPK14	PGSC0003DMT400025581	XM_006342017	540	61.0	+	7	4
StCPK15	PGSC0003DMT400002331	XM_006351851	505	57.0	–	6	11
StCPK16	PGSC0003DMT400058126	XM_006343307	568	64.2	+	11	3
StCPK17	PGSC0003DMT400024436	XM_006356324	524	59.0	+	7	11
StCPK18	PGSC0003DMT400011824	XM_006349734	535	60.0	+	8	12
StCPK19	PGSC0003DMT400014939	XM_006352199	517	57.8	+	7	8
StCPK20	PGSC0003DMT400054991	XM_006348361	582	64.6	+	7	1
StCPK21	PGSC0003DMT400072292	XM_006339122	638	70.2	–	7	10
StCPK22	PGSC0003DMT400009177	XM_006340676	564	63.6	+	11	2
StCPK23	PGSC0003DMT400069175	XM_006347224	536	61.1	+	8	6

Plant material and growth conditions—in vitro cultures

Plants of *Solanum tuberosum* cultivar (cv) Bzura and *Solanum scabrum* (received from IHAR-PIB, Młochów Research Center, Poland, Plich et al. 2015) and *Solanum tuberosum* clone H-8105 were cultured in vitro as described previously (Polkowska-Kowalczyk et al. 2004). The plants were grown under controlled conditions: day/night 16 h/8h, fluorescent lamp 150 µmol m^{-2} s^{-1} for 16 h, day/night temperature of 22/18 °C and propagated at 4–6 week intervals.

Plant material and growth conditions—plants grown in soil

After 4 weeks of growth in vitro, Bzura plants were transferred to soil and were grown in a green-house under conditions described above. At 6–8 weeks after planting, young and mature leaves, young shoots, stolons, swollen stolons, stems, flowers, roots and tubers were harvested for analysis. The collected samples were immediately frozen in liquid nitrogen and stored at −80 °C.

Pathogen elicitor

The pathogen *Phytophthora infestans* (isolate MP618 from 2005, of the following race: 1.2.3.4.(5).6.7.10.11, A1 mating type and resistant to metalaxyl) received from IHAR-PIB, Młochów Research Center, Poland, was maintained on rye agar medium at 15 °C in the dark. Culture filtrate (CF), which served as an elicitor, was prepared from the pathogen grown in liquid medium. After 6 weeks of growth, the medium was separated from the oomycete, dialysed against water for 48 h and lyophilised. The CF residue was dissolved in distilled water and quantified as µg glucose equivalents ml^{-1}, as described earlier by Polkowska-Kowalczyk et al. (2004).

Elicitor treatment of leaves

Leaves of *S. tuberosum* cv Bzura, *S. tuberosum* clone H-8105, and *Solanum scabrum*, which exhibited field resistance, susceptibility and non-host resistance to *P. infestans*, respectively, were harvested from 4-week-old plants grown in vitro. The leaves were placed on moist filter paper in Petri dishes and the culture filtrate was applied in small droplets on the abaxial surface of each leaf at a dose of 0.67 µg glucose equivalents·g^{-1} fresh weight (FW). As a control an equal volume of distilled water was applied. Leaves were incubated at 25 °C under continuous white light of 150 µmol m^{-2} s^{-1} from fluorescent tubes (Pila, Poland) and taken for analysis after 1, 3, 6, 18, 24, and 30 h (Polkowska-Kowalczyk et al. 2004).

RNA isolation

We used two methods for RNA isolation. Total RNA was isolated from samples (0.1 g FW) of young and mature leaves using the TRIzol reagent according to manufacturer's protocol (Molecular Research Center, INC.). For RNA isolation from other organs, to avoid the gelling of starch and low recovery of RNA, a modified method of Kumar et al. (2007) was used. Briefly, 0.1 g FW of frozen tissue was ground with liquid nitrogen in a mortar to a fine powder. RNA was extracted with 500 µL of extraction buffer containing 100 mM Tris–HCl (pH 8), 5 mM EDTA (pH 8), 100 mM NaCl, 0.5% SDS and 1% β-mercaptoethanol. The extract was vortexed for 5 min at room temperature (RT). Next, it was centrifuged (5 min, 11,000×*g*) and supernatant was transferred to new tubes, 250 µL of chloroform was added and mixed for 15 min. at RT, next 250 µL of Tris-saturated phenol (pH 7.9) was added and mixed again for 15 min. The mixture was centrifuged at 4 °C for 10 min at 14,000×*g* and upper phase (550 µL) was transferred to a fresh tubes and extracted with an equal volume of Tris-saturated phenol (pH 7.9): chloroform: isoamyl alcohol (25:24:1; v:v:v) for 10 min. After centrifugation as above, 500 µL of upper phase was transferred to new tubes and RNA was precipitated with 50 µL of 3 M sodium acetate and 400 µL isopropanol at -80 °C for at least 1 h. RNA was pelleted by centrifugation at 14,000×*g* for 30 min at 4 °C. The RNA pellet was washed free of salts with 85, 80 and 75% EtOH. The residual EtOH was evaporated on ice for about 10 min and RNA was dissolved in water.

Concentration and quality of RNA were estimated using NanoDrop 2000 spectrophotometer (ThermoScientific) at 260, 280 and 230 nm. The extent of protein and carbohydrate/phenolic contamination was assessed by $A_{260}/_{280}$ and $A_{260}/_{230}$ ratios, respectively.

Reverse transcription quantitative PCR

First strand cDNA was synthesized using Enhanced Avian HS RT-PCR Kit (SIGMA-ALDRICH). After the cDNA synthesis reaction, qPCR was performed.

Primers were designed using the Primer Quest program http://eu.idtdna.com/PrimerQuest/Home/Index) (Supplementary material 3). The reaction mixture contained Real-Time 2× HS-PCR Master Mix SYBR A (A&A Biotechnology), specific forward and reverse primers (100 nM each), cDNA template (in three concentrations, each in duplicate), and water to 10 µL of final volume.

Reactions were performed in a Light Cycler LC480 (Roche) with an initial denaturation step of 95 °C for 1 min. (A&A kit) followed by 45 cycles of denaturation (95 °C for 15 s) and primer annealing-extension (60 °C for 1 min.). Fluorescence was read during the annealing-extension step of each cycle. After cycling, melting point temperature analysis was performed in the range of 60–95 °C. In each experiment background range was adjusted automatically and the cycle threshold (Ct) evaluation was adjusted manually. Quality of results was evaluated based on expected Ct differences among three cDNA amounts as well as product melting curves. Using three concentrations of cDNA permitted determining amplification efficiencies for each primer pair and normalization of all results to one cDNA concentration common for all genes. Expression of each target gene was calculated by the ΔCt method against the geometric mean of the Ct values for two reference genes (Vandesompele et al. 2002). The reference genes, elongation factor 1-α (*ef1α*) (acc. no. AB061263.1) and cytoplasmic ribosomal protein L2 gene (acc. no. 39816659), were used as an endogenous control to normalize variance in the quality of RNA and the amount of cDNA used. The ratio between the target and reference gene expressions was presented as the final value.

In-gel kinase assay

The in-gel kinase assay was performed according to the method described by Szczegielniak et al. (2005), with slight modifications. Briefly, 0.3 g FW of frozen *Solanum* leaves was ground with glass beads in liquid nitrogen in a mortar. Proteins were extracted with 100 µL of extraction buffer (50 mM Tris–HCl pH 7.5, 500 mM sucrose, 4 mM EDTA, 4 mM EGTA, 20 mM NaF, 100 mM β-glicerophosphate, 2 mM PMSF, 10 mM DTT, 40 µg/mL leupeptin, 10 µg/mL aprotinin, 4 µg/mL pepstatin, 200 µM Na_3VO_4). The suspension was sonicated three times for 10 s each, centrifuged at 10,280×*g* for 10 min at 4 °C. The supernatant was centrifuged again for 30 min at 4 °C. Protein concentration was determined using bovine serum albumin (BSA) as the standard (Bradford 1976).

Proteins (from 15 to 30 µg) from the clarified homogenate were separated on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels containing myelin basic protein (MBP; 0.25 mg/mL) as the immobilized substrate of protein kinases.

After electrophoresis, SDS was removed by washing the gel three times for 30 min each with 25 mM Tris, pH 7.5, containing 0.5 mM DTT, 0.1 mM Na_3VO_4, 5 mM NaF, 0.5 mg mL^{-1} BSA and 0.1% (v/v) Triton X-100 at room temperature. Next, the gel was equilibrated in 25 mM Tris, pH 7.5 containing 5 mM NaF, 1 mM DTT and 0.1 mM Na_3VO_4, for 18 h with three changes of the buffer. Then, the gels were pre-incubated for 30 min at 4 °C in 10 mL of reaction buffer (20 mM Tris, pH 7.5, 15 mM $MgCl_2$, 2 mM DTT, and 0.33 mM $Ca(CH_3COOH)_2$ or 2 mM EGTA). Kinase activity was assayed for 1.5 h at 30 °C in the reaction buffer supplemented with 0.25 mM ATP containing 50 µCi [γ32P] ATP.

Unincorporated [γ32P] ATP was removed by washing the gels in 5% (w/v) trichloroacetic acid containing 1% (w/v) sodium phosphate. After washing, the gels were stained with Coomassie Brilliant Blue R250 (Sigma-Aldrich) and dried. The kinase activity was visualized by autoradiography or quantified using a PhosphorImager.

Results

Identification and distribution of calcium-dependent protein kinase genes in the potato genome

A genome-wide analysis of the *CDPK* gene family in potato (*Solanum tuberosum*) was performed on the complete potato genome sequence (Potato Genome Sequencing Consortium 2011) using bioinformatics methods. Only five *CDPK* genes had been reported earlier for *S. tuberosum: StCDPK1, StCDPK2, StCDPK3, StCDPK4* and *StCDPK5*. Two nucleotide sequence databases were selected for an in silico search for additional genes (see Materials and methods). The first contains sequences from an international sequencing project for *S. tuberosum* group Phureja DM1-3 516 R44 (DM; AEWC00000000) and heterozygous diploid breeding line, *S. tuberosum* group Tuberosum RH89-039-16 (RH; ERP000627). The second base comprises sequences of plant genomes from the Asteraceae clade which includes the family Solanaceae. This database also contains a wealth of tools for comparative analysis of genomes.

Twenty-nine *CDPK* genes automatically annotated as calcium-dependent protein kinases were found in the two databases, of which only 21 genes had all the domains typical for CDPKs. Because the previously identified *StCDPK3* and *StCDPK5* are absent from these bases, we used for further analyses *StCDPK3* (cDNA and genomic) and *StCDPK5* (cDNA) sequences deposited in GenBank (NCBI). Altogether, 23 potato *CDPKs* were identified, designated *StCPK1* - *StCPK23* according to the proposed nomenclature for *CDPK* genes (Hrabak et al. 1996; Boudsocq and Sheen 2013) (Table 1 and Supplementary material 1). The CDPK genes are indicated by the three-letter abbreviation CPK followed by a number. The potato CDPKs identified in this study have structures typical for the CDPK family, comprising the N-terminal and C-terminal variable domains, the protein kinase domain, the autoinhibitory junction domain, and the calmodulin-like domain containing four calcium binding motifs called EF-hands (Supplementary material 4). Moreover, 14 of the potato CDPKs were found to contain predicted myristoylation sites at their N-terminus.

Among the 29 automatically annotated potato CDPKs there are eight atypical ones containing one or no calcium-binding motif. Information about these atypical kinases is included in the supplementary material (Supplementary material 5).

Phylogenetic characterization and exon-intron structure analysis

To establish the phylogenetic relationships among the *StCDPK* genes, a combined (*S. tuberosum* and *A. thaliana*) phylogenetic tree was constructed for the StCDPK amino acid sequences. The unrooted tree was generated from an alignment of 57 CDPK protein sequences, 23 potato and 34 *A. thaliana* ones. All these CDPKs could be broadly classified into four major subfamilies (subfamilies I, II, III and IV shown in Fig. 1). Subfamily I contained 11 StCDPKs: StCPK4, StCPK5, StCPK6, StCPK7, StCPK9, StCPK11, StCPK12, StCPK13, StCPK15, StCPK20, StCPK21, subfamily II 7 members: StCPK1, StCPK2, StCPK3, StCPK14, StCPK17, StCPK18, StCPK19, subfamily III 3 members: StCPK8, StCPK10, StCPK23, and subfamily IV only 2 StCDPKs: StCPK16 and StCPK22.

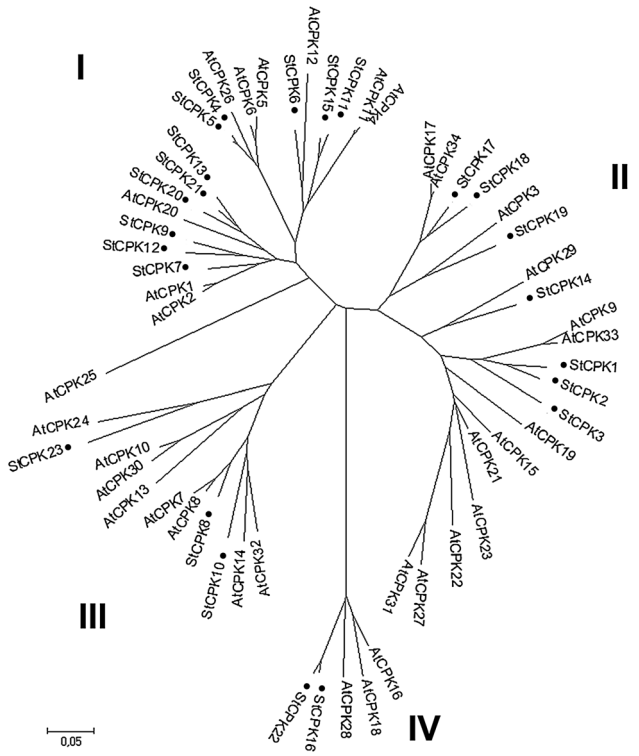


Fig. 1 Phylogenetic tree of potato and Arabidopsis CDPKs. The phylogenetic tree was created according to the neighbor-joining method using MEGA 7.00 program based on amino acid sequences of 23 potato (indicated with black dot) and 34 *A. thaliana* CDPKs

According to Boudet et al. (2001), the intron-exon organization and the type of the introns reflect the evolutionary history of some gene families. The potato *CDPK*s were examined in this respect to obtain further insight into their evolution. Each of the previously identified four subfamilies showed similar intron-exon organization of genes within the family and markedly different patterns between them (Supplementary material 6). Most of the 11 *StCDPKs* from the first subfamily had six or seven introns, all the genes from the second and third subfamilies had seven or eight introns, and those from the fourth subfamily had the highest number of introns, eleven. Since for *StCPK5* from the first subfamily only the cDNA sequence is available, its exon-intron organization could not be determined.

Chromosomal distribution of potato *CDPK* genes

To establish the chromosomal locations of the encoding genes, nucleotide sequences of each *StCDPK* were used to search the potato genome database using BLASTN. The genes were found on 11 out of the potato 12 chromosomes (except chromosome 9). Five chromosomes had only a single *CDPK* gene each: chromosome 2—*StCPK22*; chromosome 3—*StCPK16*; chromosome 4—*StCPK14*; chromosome 7—*StCPK2*; chromosome 8—*StCPK19*. Four chromosomes carried two *CDPK* genes each: chromosome 5—*StCPK6* and *StCPK7*; chromosome 6—*StCPK11* and *StCPK23*; chromosome 11—*StCPK15* and *StCPK17*; chromosome 12—*StCPK1* and *StCPK18*. Three *CDPK* genes were localized on chromosome 1—*StCPK8, StCPK9* and *StCPK20*, and five genes: *StCPK4, StCPK10, StCPK12, StCPK13* and *StCPK21* on chromosome 10 (Table 1). On some chromosomes the *StCDPKs* form clusters, which suggests recent gene duplication. Such a differential genomic distribution of the *StCDPKs* suggests gradual expansion of this gene family.

Organ-specific expression of potato *CDPK* genes

Various potato organs obtained from *S. tuberosum* cv Bzura, such as young and mature leaves, young shoots, stems, roots, stolons, swollen stolons, flowers and tubers were used for expression profiling of *CDPKs* by RT-qPCR. Transcripts of all 23 *CDPKs* were present in all the samples analysed, although their level varied greatly (Fig. 2 and Supplementary material 7). The organs differed from one another not only in the overall level but also in the spectrum of the expressed *CDPKs*. Highly similar patterns of expression were observed for two pairs of organs: stolons and young shoots, and young and mature leaves, respectively. The highest level of overall *StCDPKs* expression was found in young shoots and stolons, mainly of *StCPK2, 4, 5, 12, 15, 16, 17*, and *22*. Roots and flowers showed a medium level of *CDPKs* expression,

while in stems, tubers, swollen stolons and leaves (young and mature), the expression was the lowest and generally similar in all these organs (Fig. 2).

StCPK6, 7, 13 and *18* were expressed mostly in flowers, *StCPK4, 5, 12, 15, 17* and *22* in young shoots, roots and stolons. Transcripts of *StCPK1, 13, 14,* and *19* were hardly detectable in some of the organs analysed and were sometimes even below the limits of detection (Supplementary material 7). It seems therefore likely that some CDPKs may be expressed only in response to certain stimuli, at specific developmental stages, and/or in certain cell types.

Summing up, the potato *CDPKs* exhibit quite complex organ-specific expression patterns, suggesting that some function in defined tissues or developmental stages, while others have broader functions.

Expression of *CDPKs* in *Solanum* leaves genotypes treated with elicitor from *P. infestans*

To learn more about the potential function of the CDPKs in plant disease resistance, kinetics of all the *CDPK* genes was investigated in potato leaves treated with an elicitor—a culture filtrate (CF) of *P. infestans*. For comparison we used leaves from three *Solanum* genotypes showing substantially different susceptibility to the pathogen: *S. tuberosum* cv Bzura, *S. tuberosum* clone H-8105 and *Solanum scabrum* exhibiting respectively field resistance, susceptibility and non-host resistance to *P. infestans*. Changes in expression of 15 *CDPKs* were observed after the elicitor treatment: five genes from the first subfamily (*StCPK4,5,6,7,15*), six from the second subfamily (*StCPK1,2,3,14,17,18*), three from the third subfamily (*StCPK8,10,23*) and one from the fourth subfamily (*StCPK16*) (Fig. 3). Interestingly, in the non-host *S. scabrum* an increase in gene expression occurred between 0.5 and 6 h after CF treatment, reaching for *StCPK23* ca. 315% compared to water-treated control (100%) (Supplementary material 8). In the susceptible H-8105, also *StCPK23* was the most strongly induced (430% of control) but only at prolonged treatment (18–30 h). In the field resistant Bzura leaves, the maximal transcript level was noticed between 6 and 18 h after the elicitor treatment, and the increase was the highest for *StCPK4* (264% of control).

Activity of CDPKs after treatment with elicitor from *P. infestans*

To check the influence of the *P. infestans* elicitor on the CDPK kinase activity, we determined the enzymatic activity using the in-gel kinase assay in the presence of calcium ions and, as a negative control, without calcium ions. The activity was studied in leaf extracts prepared from the three different *Solanum* genotypes treated with the elicitor from *P. infestans* (CF) for between 0 and 24 h. In all the genotypes studied the activity of CDPKs increased in response to the CF treatment, but with different kinetics and maximal intensity (Fig. 4). In the resistant Bzura genotype the CDPKs activity rose gradually between 0.5 and 3 h of treatment to reach ca. 155% of the control level and remained elevated (ca. 130–150%) until the end of the experiment (24 h). In contrast, in the susceptible clone H-8105, after an early increase to about 130% of control, the CDPKs activity returned to the control level after 6 h and remained at that level. In non-host *S. scabrum* the CDPKs activity increased gradually to reach a maximum at 6 h of CF treatment (193% of control), remained elevated until the 18th hour and then dropped rapidly to the control level.

Thus, in the resistant genotypes a progressive, marked and long-lasting increase of the kinase activity was observed while the susceptible genotype only showed early, weak and transient activation of the CDPKs. These results suggest a correlation between CDPK activation and resistance of potato against *P. infestans*.

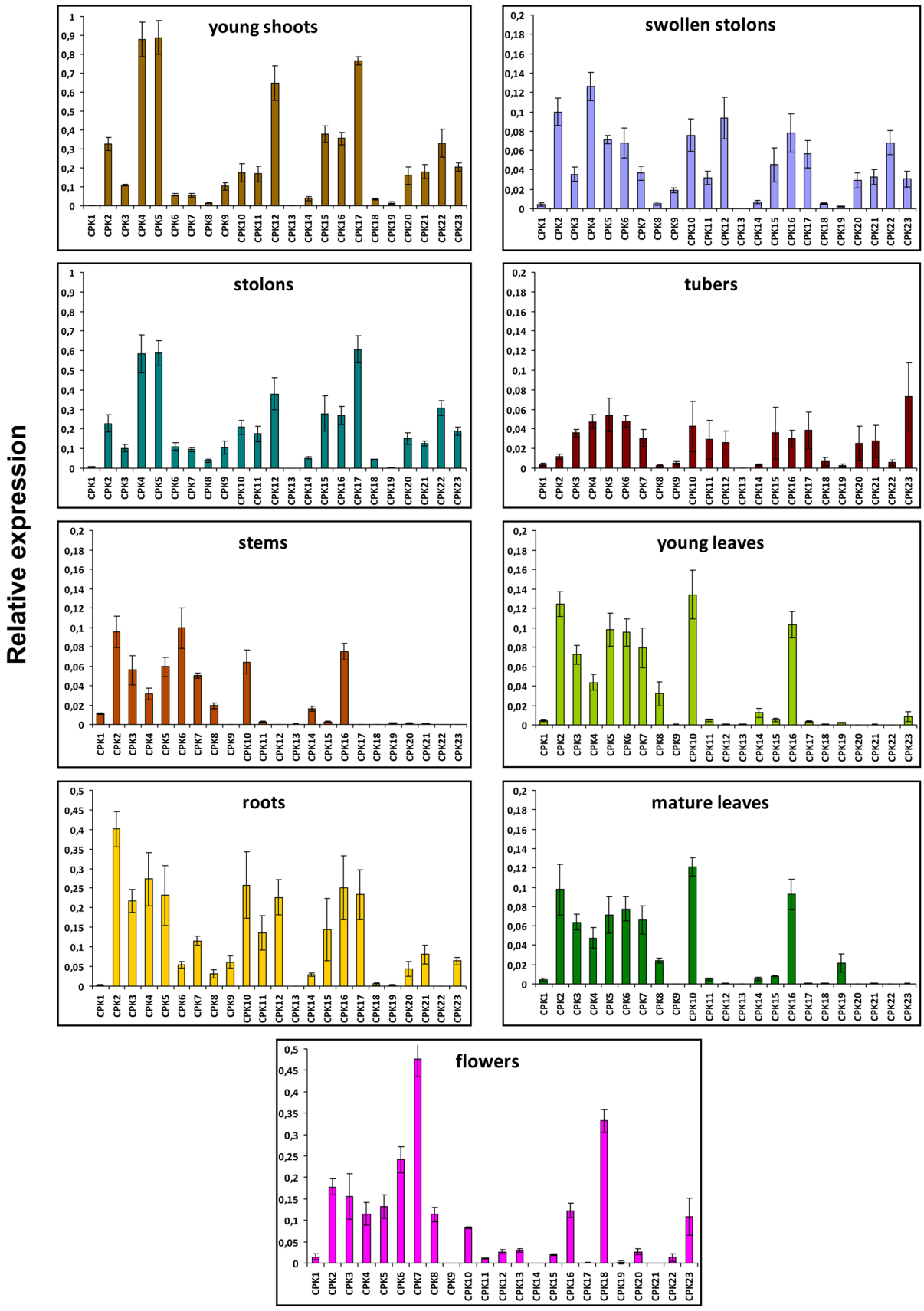
Discussion

Potato is one of a major crops sustaining the world's population owing to the high nutritional value of its underground tubers. Understanding the signalling pathways that regulate its development and stress responses is key to improving the potato yield, disease resistance, storability and dietary properties.

Calcium-dependent protein kinases (CDPKs), one of the largest groups of plant calcium sensors, bind calcium ions directly which modulates their activity. The calcium-activated kinases regulate diverse developmental processes and defence responses of plants.

The present comprehensive study of *CDPK* genes in the potato was made possible by the availability of the complete genomic sequence (Potato Genome Sequencing Consortium 2011) and provides a foundation for further functional investigation of this important gene family in Solanaceae. The potato genome encodes at least 23 *CDPKs* similar to the 20–40 *CDPK* genes present in most other plants. The expansion of gene families in plant genomes is believed to have occurred by various mechanisms such as genome-wide, tandem and dispersed duplications. The potato genome appears to exhibit high plasticity, as gene loss or duplication events and other structural mutations are found with high frequency (Potato Genome Sequencing Consortium 2011).

The domain organization of the 23 potato CDPKs is typical for the CDPKs from other plant species. All potato CDPKs have four EF-hands, and 14 of them contain myristoylation motifs indicating that they undergo the modification promoting protein-membrane and protein-protein interactions (Xu et al. 2015). In potato, like in rice, maize


Relative expression
young shoots
swollen stolons
stolons
tubers
stems
young leaves
roots
mature leaves
flowers
CPK1 CPK2 CPK3 CPK4 CPK5 CPK6 CPK7 CPK8 CPK9 CPK10 CPK11 CPK12 CPK13 CPK14 CPK15 CPK16 CPK17 CPK18 CPK19 CPK20 CPK21 CPK22 CPK23

◂**Fig. 2** Expression of individual potato *CPK* genes in the indicated organs. Please note different ordinate scales for different organs. Expression profiles are represented by at least three independent experiments each in two replicates. The data are the mean values ± SD ($n \geq 6$). Relative expression is presented in relation to reference genes

and *Arabidopsis thaliana*, some of the CDPK-like protein kinases (8 in potato) contain less than four, or even none, EF-hands. In the potato genome the 23 *CDPKs* genes are distributed apparently randomly among chromosomes. In the tomato 29 *SlCDPKs* genes are distributed in all chromosomes also highly unevenly, which the authors interpreted as indicating evolution events and recent expansion of the family (Wang et al. 2015).

CDPKs are involved in various physiological processes at different stages of plant development (Liu et al. 2016). To investigate the expression profiles of the *CDPK* genes in potato development, we determined their transcript levels in different organs such as young and mature leaves, young shoots, stems, roots, stolons, swollen stolons, flowers and tubers. Although transcripts of at least some *CDPK*s were detected in all the organs, their expression levels varied greatly between individual genes and also between organs. Only in two pairs of organs, young and mature leaves, and stolons and young shoots, the expression patterns were highly similar. Notably, young shoots, roots and stolons showed particularly high expression of *CDPKs* mostly from the first subfamily (*StCPK4, 5, 12, 15*) and *StCPK17* from the second subfamily.

Interestingly, *StCPK3* is highly similar in its amino acid sequence to tobacco *NtCDPK1* and tomato *LeCPK1* (Yoon et al. 1999; Rutschmann et al. 2002). These three genes are expressed at a very high level in roots. A down-regulation of *NtCDPK1* in transgenic tobacco resulted in smaller plants showing abnormal root development, with reduced lateral root formation and impaired elongation (Lee et al. 2006). By analogy, StCPK3 likely participates in the development of main and lateral roots (Grandellis et al. 2012).

Four *StCPKs: StCPK6, 7, 13* and *18*, were expressed exclusively in flowers, indicating their possible role in flower development. This is in concert with earlier results. According to Schulz et al. (2013), CDPKs have a major role in pollen tube growth. Twelve members of the Arabidopsis *CDPK* gene family are predominantly expressed in pollen (Myers et al. 2009). For *AtCPK2*/*AtCPK20* and *AtCPK17*/*AtCPK34*, a role in pollen tube growth has been demonstrated experimentally in double mutant lines (Gutermuth et al. 2013). *StCPK13*, expressed exclusively in potato flowers, is very similar to *AtCPK2*/*AtCPK20*, while *StCPK18*, highly expressed in potato flowers, is closely related to *AtCPK17*/*AtCPK34*. *AtCPK2*/*CPK20* and *AtCPK17*/*AtCPK34* are involved in the regulation of pollen tube growth, and *AtCPK2*/*CPK20* influence the anion channel SLAH3 (Gutermuth et al. 2013). Moreover, *PiCDPK1* from petunia (*Petunia inflata*), similar to *AtCPK17*/*AtCPK34*, has also been implicated in pollen tube growth polarity (Yoon et al. 2006). One of the maize *CDPK* genes (designated by us as *ZmCPK7* according to the nomenclature by Ma et al. 2013), also similar to *AtCPK17*/*AtCPK34*, is expressed specifically during late stages of pollen development. Specific inhibition of the *ZmCPK7* expression by antisense oligonucleotide resulted in impaired germination and growth of pollen tube (Estruch et al. 1994). According to Ray et al. (2007), *OsCPK2* and *14* from rice that show homology to *PiCDPK1* and *AtCPK17* and *34*, and *OsCPK25* and *26*, highly similar to *ZmCPK7*, are expressed during late panicle development and thus could be involved in a similar function during pollen maturation and/or pollen tube growth.

A process typical for potato is tuberization that results in differentiation of a specialized shoot (stolon) into a storage organ (the tuber). Tuber development is a tightly controlled process triggered by both external and internal factors; among them calcium ions and protein kinases play an important role (Balamani et al. 1986; Raíces et al. 2003). It has been suggested that sequential activation of specific CDPKs with distinct biochemical properties and subcellular localization could be essential for the co-ordination of multiple Ca^{2+} signals triggered upon tuberization (Raíces et al. 2003). StCDPK1 and StCDPK3, with different substrate specificities and cellular distribution, were found associated with early and induced stolons, respectively. *StCDPK1* was expressed in tuberizing stolons and sprouting tubers (Raíces et al. 2001; Gargantini et al. 2009), whereas expression of *StCDPK3* was abundant in stolons, roots and leaves (Grandellis et al. 2012). Transcript of another CDPK, *StCDPK2*, was present in all plant tissues studied and during different developmental processes - sprouting and tuberization (Giammaria et al. 2011); its level was the highest in actively growing young leaves. In our experimental system, three *CDPKs* described before were expressed in all organs analysed, although expression of *StCPK1* was very low. *StCPK2* and *StCPK3* were mainly expressed in roots, stolons, young shoots, and flowers. *StCPK4, 5, 12, 15, 16*, and *17* were highly expressed in stolons and were the major ones in a rapidly growing organ—the young shoots. The differences in expression of individual CDPKs likely correlate with their participation in specific signal transduction pathways in different organs.

Accumulating evidence indicates that CDPKs play an important role in plant immunity. For example, Fu et al. (2013) demonstrated that overexpression of constitutively active OsCPK10 enhanced Arabidopsis resistance against *Pseudomonas syringae* pv. tomato. Our present study indicated a likely involvement of CDPKs in *Solanum* species

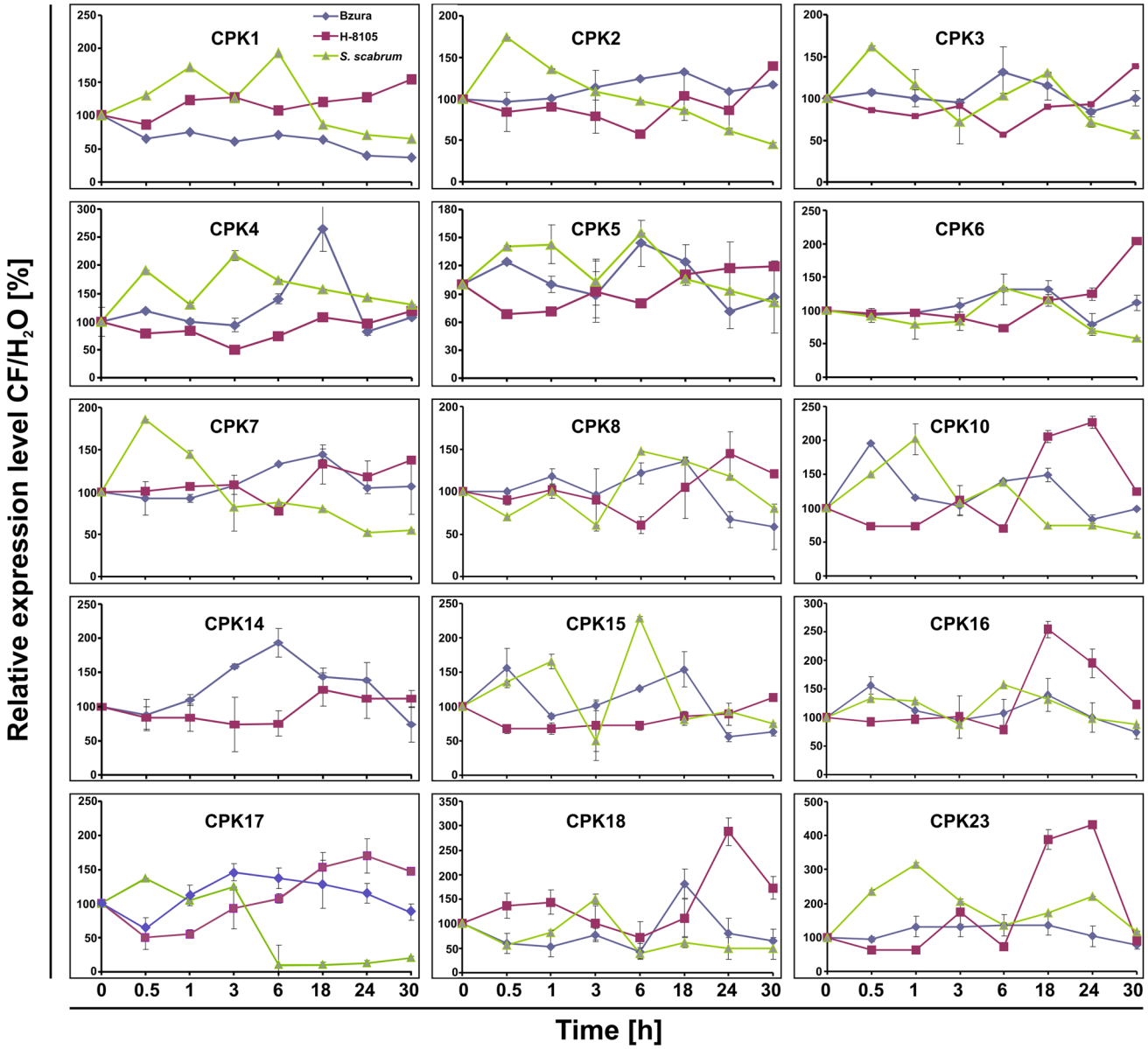


Fig. 3 Expression of various *CDPK* genes in elicitor-treated leaves of three *Solanum* genotypes expressed as % of control levels. Transcript levels at time-points indicated are expressed relative to control value determined as the average of all time points in control (water-treated) leaves. At least two independent biological series of each experiment were performed. Data are mean values ± SD ($n \geq 4$)

in response to an elicitor, a culture filtrate (CF) from the pathogenic oomycete *Phytophthora infestans* causing late blight, the most destructive potato disease. We determined expression profiles of all the potato *CDPKs* and CDPK activities in three *Solanum* genotypes expressing different types and level of resistance against *P. infestans*. Expression of all 15 *CDPKs* and the kinase activity increased after CF treatment in all these genotypes, but with strikingly different patterns in the sensitive and the resistant ones. These results are in agreement with the earlier findings of Vleeshouwers et al. (2000). Their cytological study indicated that the severity and timing of defence response varied depending on the form and level of resistance exhibited by a *Solanum* genotype. Those authors suggested that in the *Solanum*/*P. infestans* interaction resistance is a quantitative rather than a qualitative trait. In our experimental system the magnitude and duration of gene induction and activity upregulation of the CDPKs correlated positively with the level of resistance of the *Solanum* genotypes to *P. infestans*.

Some authors have documented a relation between CDPKs and production of reactive oxygen species (ROS). Thus, Kobayashi et al. (2007) observed phosphorylation of NADPH oxidase by StCDPK4 and StCDPK5 that resulted in oxidative burst. We observed a positive correlation

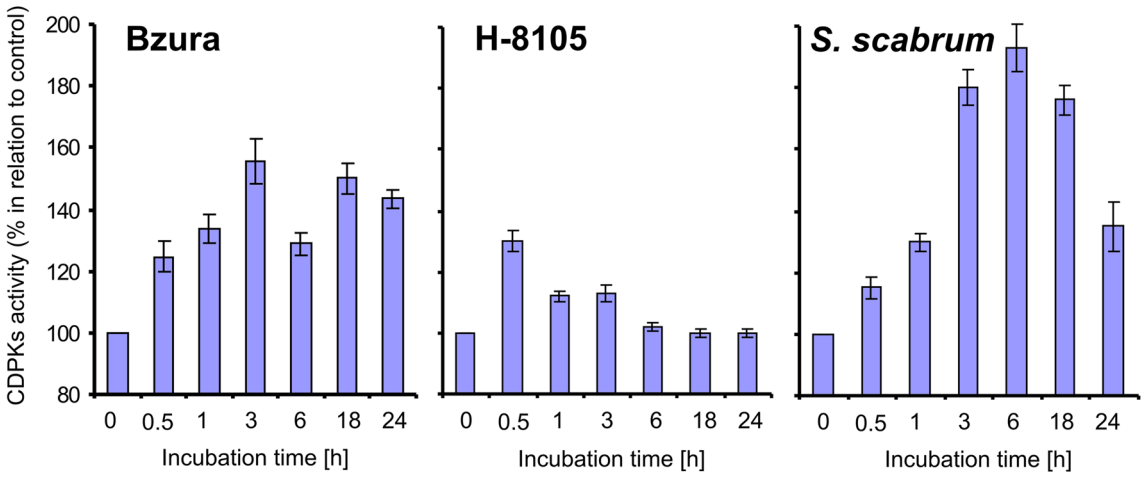


Fig. 4 Activity of CDPK in elicitor-treated leaves of three *Solanum* genotypes shown as % of control levels at various time points. "Zero" denote average values represented by control leaves treated with water from 0 to 24 h. At least two independent biological series of experiments were performed. Data are mean values ± SD ($n \geq 4$)

between increased activity and gene expression of CDPKs (present results) and ROS generation (Polkowska-Kowalczyk et al. 2004) in resistant genotypes in response to *P. infestans*, but not such correlation was found for the susceptible H-8105. Taken together, the earlier and present results concerning different *Solanum* genotypes (Polkowska-Kowalczyk et al. 2004, 2011) indicate that an effective defence against *P. infestans* involves cooperation among the diverse elicitor-induced processes.

In conclusion, the potato genome contains at least 23 members of the *CDPK* gene family. Most of these genes exhibit markedly different expression levels in various organs, suggesting that they play specific different roles in potato development. Increased CDPK expression and activity in response to the elicitor from *P. infestans* indicates involvement of CDPKs in the defence against this pathogen. Due to the high economic significance of the potato, it is imperative to study defence mechanisms occurring in response to pathogen attack.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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References

Asano T, Tanaka N, Yang G, Hayashi N, Komatsu S (2005) Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice. Plant Cell Physiol 46(2):356–366

Baillie BK, Belda-Baillie CA, Maruyama T (2000) A calcium-dependent protein kinase functions in wound healing in Ventricaria ventricosa (chlorophyta). J Phycol 36:1145–1152

Balamani V, Veluthambi K, Poovaiah BW (1986) Effect of calcium on tuberization in potato (*Solanum tuberosum* L.). Plant Physiol 80:856–858

Billker O, Lourido S, Sibley LD (2009) Calcium-dependent signalling and kinases in apicomplexan parasites. Cell Host Microbe 5:612–622

Boudet N, Aubourg S, Toffano-Nioche C, Kreis M, Lecharny A (2001) Evolution of intron/exon structure of DEAD helicase family genes in *Arabidopsis, Caenorhabditis*, and Drosophila. Genome Res 11:2101–2114

Boudsocq M, Sheen J (2013) CDPKs in immune and stress signalling. Trends Plant Sci 18:30–40

Boudsocq M, Willmann MR, McCarmack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464:418–422

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254

Broad Institute of Harvard and MIT (2010) Phytophthora infestans Sequencing Project. https://www.broadinstitute.org

Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M, San Segundo B (2014) Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiol 165:688–704

Cheng S-H, Willmann MR, Chen H-C, Sheen J (2002) Calcium signaling through protein kinases. The Arabidopsis calcium-dependent protein kinase gene family. Plant Physiol 129:469–485

Coca M, San Segundo B (2010) AtCPK1 calcium-dependent protein kinase mediates pathogen resistance in Arabidopsis. Plant J 63:526–540

Dubrovina AS, Kiselev KV, Khristenko VS, Aleynova OA (2017) The calcium-dependent protein kinase gene *VaCPK29* is involved in grapevine responses to heat and osmotic stresses. Plant Growth Regul 82:79–89

Estruch JJ, Kadwell S, Merlin E, Crossland L (1994) Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. Proc Natl Acad Sci USA 91:8837–8841

Fu L, Yu X, An Ch (2013) Overexpression of constitutively active OsCPK10 increases Arabidopsis resistance against *Pseudomonas syringae* pv. tomato and rice resistance against *Magnaporthe grisea*. Plant Physiol Biochem 73:202–210

Gao X, Chen X, Lin W, Chen S, Lu D, Niu Y, Li L, Cheng C, McCormack M, Sheen J (2013) Bifurcation of *Arabidopsis* NLR immune signalling via Ca^{2+}-dependent protein kinases. PloS Pathol 9:e1003127

Gargantini PR, Giammaria V, Grandellis C, Feingold SE, Maldonado S, Ulloa RM (2009) Genomic and functional characterization of StCDPK1. Plant Mol Biol 70:153–172

Giammaria V, Grandellis C, Bachmann S, Gargantini PR, Feingold SE, Bryan G, Ulloa RM (2011) *St*CDPK2 expression and activity reveal a highly responsive potato calcium-dependent protein kinase involved in light signalling. Planta 233:593–609

Grandellis C, Giammaria V, Bialer M, Santin F, Lin T, Hannapel DJ, Ulloa RM (2012) The novel *Solanum tuberosum* calcium dependent protein kinase, StCDPK3, is expressed in actively organs. Planta 236:1831–1848

Gutermuth T, Lassig R, Portes M-T, Maierhofer T, Romeis T, Borst JW, Hedrich R, Feijó JA, Konrad KR (2013) Pollen tube growth regulation by free anions depends on the interaction between the anion channel SLAH3 and calcium-dependent protein kinases CPK2 and CPK20. Plant Cell 25:4525–4543

Harmon AC, Gribskov M, Gubrium E, Harper JF (2001) The CDPK superfamily of protein kinases. New Phytol 151:175–183

Hrabak EM, Dickmann LJ, Satterlee JS, Sussman MR (1996) Characterization of eight new members of the calmodulin-like domain protein kinase gene family of *Arabidopsis thaliana*. Plant Mol Biol 31:405–412

Hrabak EM, Chan CWM, Gribskov M, Harper JF, Choi JH, Halford N, Kudla J, Luan S, Nimmo HG, Sussman MR, Thomas M, Walker-Simmons K, Zhu JK, Harmon AC (2003) The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol 132:666–680

Hu B, Jin J, Guo A-Y, Zhang H, Luo J, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31:1296–1297

Hu Z, Lv X, Xia X, Zhou J, Shi K, Yu J, Zhou Y (2016) Genome-wide identification and expression analysis of calcium-dependent protein kinase in tomato. Front Plant Sci 7:469. https://doi.org/10.3389/fpls.2016.00469

Ivashuta S, Liu J, Liu J, Lohar DP, Haridas S, Bucciarelli B, VandenBosch KA, Vance CP, Harrison MJ, Gantt JS (2005) RNA interference identiWes a calcium-dependent protein kinase involved in *Medicago truncatula* root development. Plant Cell 17:2911–2921

Kamiyoshihara Y, Iwata M, Fukaya T, Tatsuki M, Mori H (2010) Turnover of LeACS2, a wound-inducible 1-aminocyclopropane-1-carboxylic acid synthase in tomato, is regulated by phosphorylation/dephosphorylation. Plant J 64:140–150

Klimecka M, Muszyńska G (2007) Structure and functions of plants calcium-dependent protein kinases. Acta Biochim Polon 54(2):219–233

Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H (2007) Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19:1065–1080

Kong X, Lv W, Jiang S, Zhang D, Cai G, Pan J, Li D (2013) Genome-wide identification and expression analysis of calcium-dependent protein kinase in maize. BMC Genom 14:433. https://doi.org/10.1186/1471-2164-14-433

Kumar GNM, Iyer S, Knowles NR (2007) Extraction of RNA from fresh, frozen, and lyophilized tuber and root tissues. J Agric Food Chem 55:1674–1678

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWiliam H, Valentiv F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948

Lee SS, Yoon GM, Rho EJ, Moon E, Pai HS (2006) Functional characterization of NtCDPK1 in tobacco. Mol Cells 28:141–146

Li AL, Zhu YF, Tan XM, Wang X, Wei B, Guo HZ, Zhang ZL, Chen XB, Zhao GY, Kong XY (2008) Evolutionary and functional study of the CDPK gene family in wheat (*Triticum aestivum* L.). Plant Mol Biol 66:429–443

Liu W, Li W, He Q, Khan Daud M, Chen J, Zhu S (2014) Genome-wide survey and expression analysis of calcium-dependent protein kinase in *Gossypium raimondii*. PLoS ONE 9:e98189. https://doi.org/10.1371/journal.pone.0098189

Liu H, Che Z, Zeng X, Zhou X, Sitoe HM, Wang H, Yu D (2016) Genome-wide analysis of calcium-dependent protein kinases and their expression patterns in response to herbivore and wounding stresses in soybean. Funct Integr Genom 16:481–493

Lu SX, Hrabak EM (2002) An Arabidopsis calcium-dependent protein kinase is associated with the endoplasmic reticulum. Plant Physiol 128:1008–1021

Ma P, Liu J, Yang X, Ma R (2013) Genome-wide identification of the maize calcium-dependent protein kinase gene family. Appl Biochem Biotechnol 169:2111–2125

McCurdy DW, Harmon AC (1992) Calcium-dependent protein kinase in the green alga Chara. Planta 188:54–61

Myers C, Romanowsky SM, Barron YD, Garg S, Azuse CL, Curran A, Davis RM, Hatton J, Harmon AC, Harper JF (2009) Calcium-dependent protein kinases regulate polarized tip growth in pollen tubes. Plant J 59:528–539

Plich J, Tatarowska B, Lebecka R, Śliwka J, Zimnoch-Guzowska E, Flis B (2015) R2-like gene contributes to resistance to *Phytophthora infestans* in polish potato cultivar Bzura. Am J Potato Res 92:350–358

Polkowska-Kowalczyk L, Wielgat B, Maciejewska U (2004) The elicitor-induced oxidative processes in leaves of *Solanum* species with

differential polygenic resistance to *Phytophthora infestans*. J Plant Physiol 161:913–920

Polkowska-Kowalczyk L, Wielgat B, Maciejewska U (2011) Involvement of phospholipase A_2 in the response of *Solanum* species to an elicitor from *Phytophthora infestans*. Acta Physiol Plant 33:2521–2531

Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–197

Raíces M, Chico JM, Téllez-Iñón MT, Ulloa RM (2001) Molecular characterization of StCDPK1, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. Plant Mol Biol 46:591–601

Raíces M, Gargantini PR, Chinchilla D, Crespi M, Téllez-Iñón MT, Ulloa RM (2003) Regulation of CDPK isoforms during tuber development. Plant Mol Biol 52:1011–1024

Ray S, Agarwal P, Arora R, Kapoor S, Tyagi AK (2007) Expression analysis of calcium-dependent protein kinase gene family during reproductive development and abiotic stress conditions in rice (*Oryza sativa* L. ssp. *indica*). Mol Genet Genom 278:493–505

Romeis T, Herde M (2014) From local to global: CDPKs in systemic defence signalling upon microbial and herbivore attack. Curr Opin Plant Biol 20:1–10

Romeis T, Ludwig AA, Martin R, Jones JD (2001) Calcium-dependent protein kinases play an essential role in a plant defence response. EMBO J 20:5556–5567

Rutschmann F, Stalder U, Piotrowski M, Oecking C, Schaller A (2002) *Le*CPK1, a calcium-dependent protein kinase from tomato: plasma membrane targeting and biochemical characterization. Plant Physiol 129:156–168

Schulz P, Herde M, Romeis T (2013) Calcium-dependent protein kinases: hubs in plant stress signaling and development. Plant Physiol 163:523–530

Sigrist CJA, de Castro E, Cerutti L, Cuche BA, Hulo N, Bridge A, Bougueleret L, Xenarios I (2013) New and continuing developments at PROSITE. Nucleic Acids Res 41:D344-D347. https://doi.org/10.1093/nar/gks1067

Szczegielniak J, Klimecka M, Liwosz A, Ciesielski A, Kaczanowski S, Dobrowolska G, Harmon AC, Muszyńska G (2005) A wound-responsive and phospholipid-regulated maize calcium-dependent protein kinase. Plant Physiol 139:1970–1983

Szczegielniak J, Borkiewicz L, Szurmak B, Lewandowska-Gnatowska E, Statkiewicz M, Klimecka M, Ciesla J, Muszynska G (2012) Maize calcium-dependent protein kinase (ZmCPK11): local and systemic response to wounding, regulation by touch and components of jasmonate signaling. Physiol Plant 146:1–14

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101:11030–11035

Ulloa RM, Raíces M, MacIntosh GC, Maldonado S, Téllez-Iñón MT (2002) Jasmonic acid affects plant morphology and calcium-dependent protein kinase expression and activity in *Solanum tuberosum*. Physiol Plant 115:417–427

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. https://doi.org/10.1186/gb-2002-3-7-research0034

Vleeshouwers VGAA, van Dooijweert W, Govers F, Kamoun S, Colon LT (2000) The hypersensitive response is acciociated with host and nonhost resistance to *Phytophthora infestans*. Planta 210:853–864

Wang J-P, Xu Y-P, Munyampundu J-P, Liu T-Y, Cai X-Z (2015) Calcium-dependent protein kinase (CDPK) and CDPK-related kinase (CRK) gene families in tomato: genome-wide identification and functional analyses in disease resistance. Mol Genet Genom. https://doi.org/10.1007/s00438-015-1137-0

Xu X, Liu M, Lu L, He M, Qu W, Xu Q, Qi X, Chen X (2015) Genome-wide analysis and expression of the calcium-dependent protein kinase gene family in cucumber. Mol Genet Genom 290:1403–1414

Yoon GM, Cho HS, Ha HJ, Liu JR, Lee HS (1999) Characterization of NtCDPK1, a calcium-dependent protein kinase gene in *Nicotiana tabacum*, and the activity of its encoded protein. Plant Mol Biol 39:991–1001

Yoon GM, Dowd PE, Gilroy S, McCubbin AG (2006) Calcium-dependent protein kinase isoforms in *Petunia* have distinct functions in pollen tube growth, including regulating polarity. Plant Cell 18:867–878

Zhang XS, Choi JH (2001) Molecular evolution of calmodulin-like domain protein kinases (CDPKs) in plants and protists. J Mol Evol 53:214–224

Zhang K, Han Y-T, Zhao F-L, Hu Y, Gao Y-R, Ma Y-F, Zheng Y, Wang Y-J, Wen Y-Q (2015) Genome-wide identification and expression analysis of the CDPK gene family in grape, *Vitis* spp. BMC Plant Biol 15:164. https://doi.org/10.1186/s12870-015-0552-z

Zuo R, Hu R, Chai G, Xu M, Qi G, Kong Y, Zhou G (2013) Genome-wide identification, classification, and expression analysis of CDPK and its closely related gene families in poplar (*Populus trichocarpa*). Mol Biol Rep 40:2645–2662