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REVIEW PAPER

The role of calcium-dependent protein kinase (*CDPK*) genes in plant stress resistance and secondary metabolism regulation

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Abstract

Calcium signaling is known to play an important role in the activation of plant responses to abiotic and biotic stresses. Calcium-dependent protein kinases (CDPKs) are currently recognized as important calcium sensors in plants. This review summarizes and analyzes the published results on the gain- and loss-of-function studies of plant CDPKs to evaluate their functions in plant responses to adverse environmental conditions, pathogen attack, and plant secondary metabolite production. In this review, we also analyze the features of using *CDPK* genes in genetic engineering to enhance plant stress resistance and secondary metabolism activation. This information is crucial for the development of agricultural and horticultural varieties with improved traits.

Keywords Calcium-dependent protein kinase · Abiotic stress · Biotic stress · Plant secondary metabolism · Stress tolerance

Introduction

Calcium (Ca^{2+}) serves as an ubiquitous second messenger in a variety of signalling pathways in plants. Transient elevation of $[Ca^{2+}]_{cyt}$ has an important function in plant signal transduction networks (Harper and Harmon 2005). The first major components of the Ca^{2+} signaling pathway are Ca^{2+} channels and Ca^{2+} sensor proteins. The majority of Ca^{2+} sensor proteins contain several EF-hand motifs, conserved helix-loop-helix structures where the Ca^{2+} ions are coordinated within the acidic Ca^{2+}-coordinating loop (Halling et al. 2016). The major plant EF-hand-containing Ca^{2+}-binding proteins include calmodulins (CaMs), calmodulin-like proteins (CML), Ca^{2+}-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs) (DeFalco et al. 2010; Hashimoto and Kudla 2011; Mohanta et al. 2019).

Ca^{2+}-dependent protein kinases (CPKs; EC 2.7.1.37) represent a multigene family implicated as important Ca^{2+} sensors in plants and are known to play a key role in various plant developmental processes and in plant adaptation to environmental stresses (Asano et al. 2012; Dekomah et al. 2022). The gene expression levels and kinase activities of CDPKs are affected by various abiotic stresses, pathogen attack, and developmental cues (Asano et al. 2012; Dekomah et al. 2022). Various plant species have been transformed with *CDPK* genes from *Arabidopsis thaliana*, *Oryza sativa*, *Populus euphratica*, *Zea mays*, and other plant species. Overexpression of these *CDPK* genes improved plant tolerance to drought (Zou et al. 2010; Wei et al. 2014), heat (Wang et al. 2018), osmotic (Dubrovina et al. 2017), salt (Asano et al. 2012), and biotic stresses (Coca and San Segundo 2010), whereas *CDPK* RNA-mediated interference (RNAi) of gene silencing or *CDPK* loss-of-function by mutation negatively affected plant survival and development under various stresses *(*Zhu et al. 2007, 2022; Asano et al. 2012).

Also, *CDPK* overexpression has also been shown to confer stress sensitivity to cold, salt, drought, and hyperosmotic stresses, while *CDPK* mutation, in turn, led to improved tolerance to those abiotic stress like *AtCPK21* and *AtCPK23* from *A. thaliana* (Ma and Wu 2007; Franz et al. 2011) or *ZmCPK1* from maize (Weckwerth et al. 2015). Thus, CDPKs are implicated in both positive and negative regulation of plant abiotic stress adaptation. CDPKs have been shown to phosphorylate proteins (e.g., membrane channels,

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NADPH oxidase, or transcription factors) involved in stomatal movements, oxidative burst, and gene expression regulation (Choi et al. 2005).

A number of articles show that CDPKs are involved in the regulation of plant defense reactions and secondary metabolism (Aleynova-Shumakova et al. 2014; Fantino et al. 2017). The available data indicate that CDPKs could be involved in stress hormone- and light-mediated activation of plant defense reactions and secondary metabolite production in response to biotic and abiotic cues. Thus, the relationship of Ca^{2+} sensor proteins with plant defense reactions and secondary metabolite production requires further research.

This review is aimed to summarize and discuss the present evidence for CDPK involvement in plant stress adaptation and secondary metabolism regulation as well as the perspectives for application of *CDPK* gene gain-of-function or loss-of-function in plant biotechnology.

CDPK gene families in plants

CDPKs are serine/threonine-specific protein kinases that are regulated by Ca^{2+} due to the presence from one to five EF-hands. CDPKs are involved in many signaling cascades by specific phosphorylation of different proteins (Schulz et al. 2013). CDPKs are found in protists (Apicomplexans), green algae, some red algae (e.g. Coccomyxa, Volvox, Chlamydomonas or Neopyropia), and vascular plants, but are absent in animals, fungi, and bacteria (Harper and Harmon 2005; Valmonte et al. 2014; Guan et al. 2022). While CDPKs are encoded by small families of 5–7 genes in Apicomplexans and 9–17 in algae, vascular plants contain large multigenic *CDPK* families. Non-vascular land plants contain 10–20 *CDPK* genes and most flowering plants contain CDPK families ranging between 20 and 40 *CDPK* genes (Atif et al. 2019). For example, there are 34 *CDPK* genes in Arabidopsis, 30 *CDPK* genes in poplar, 31 *CDPK* genes in rice *O. sativa*, 29 *CDPK* genes in tomato *Solanum pennellii*, or 20 *CDPK* genes in wheat *Triticum aestivum* and grapevine *Vitis vinifera* or *Vitis amurensis* (Cheng et al. 2002; Asano et al. 2005; Li et al. 2008; Zuo et al. 2013; Zhang et al. 2015; Hu et al. 2016). The highest number of *CDPK* genes was found in soybean *Glycine max* (50 *CDPKs*) and in switchgrass *Panicum virgatum* (53 *CDPKs*) (Atif et al. 2019; Dekomah et al. 2022).

It is assumed that the high number of *CDPK* genes in the plant genome reflects the evolutionary genome duplication events and polyploidization (Valmonte et al. 2014; Dekomah et al. 2022). The evolutionary gene duplication and the changing environmental conditions have contributed to expanding CDPK functions and gaining new ones.

CDPK structure and biochemical activation

CDPKs include four major domains: a variable N-terminal domain (N-VD), a serine/threonine protein kinase (PK) domain, an autoinhibitory junction (AJ), a Ca^{2+}-binding calmodulin-like (CBD) domain (Fig. 1) (Asai et al. 2013). Some authors also consider the C-terminal variable domain (CT) as a distinct domain and the CT is as variable as the N-VD (Klimecka and Muszynska 2007). The PK domain is the catalytic domain that phosphorylates the serine or threonine residues of CDPKs substrates (Harmon et al. 2001; Valmonte et al. 2014). The AJ domain located between the PK and CBD domains possesses a pseudosubstrate sequence that can interact with the active site of PK and inhibit the kinase activity in the absence of Ca^{2+} (Hrabak et al. 2003; Wernimont et al. 2010).

The N- and C-terminal domains are highly variable and differ in lengths amongst CDPKs. The N-terminal domain often contains myristoylation or palmitoylation sites associated with CDPK subcellular localization (Asai et al. 2013; Simeunovic et al. 2016; Yip Delormel and Boudsocq 2019). These sites are responsible for anchoring of these kinases to membrane lipids through attaching to those amino acids (aa) the hydrophobic fatty acids. CDPKs are localized into different cellular compartments, including the plasma membrane, cytosol, nucleus, endoplasmic reticulum, peroxisomes, outer mitohondrial membrane, and oil bodies (Harper et al. 2004). This ability likely allows CDPKs to shuttle between different subcellular compartments, enabling them to perform a wider range of cellular functions. Different subcellular locations suggest different biological roles for individual CDPKs (Dekomah et al. 2022).

The CBD domain contains from one to five EF-hands (typically 4). Canonical EF-hand is a 29 aa helix-loop-helix structures with 12 conserved residues for binding to the free Ca^{2+} ions (Gifford et al. 2007; Dekomah et al. 2022). At low Ca^{2+} levels, CDPKs are stabilized in inactive state by the interaction of the Ca^{2+}-binding domain with the AJ

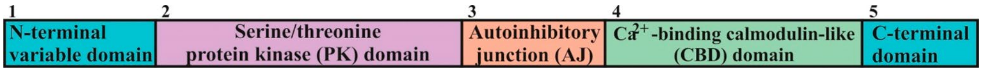

Fig. 1 Structure of calcium-dependent protein kinase (CDPK) proteins in plants (not to scale). CDPK possess five domains, including the N-terminal variable domain (1); the kinase catalytic domain (2, PK); the junction domain (3, AJ); Ca^{2+}-binding calmodulin-like domain (4, CBD), in which most isoforms have from one to five EF-hands (typically 4) Ca^{2+}-binding sites. Last is the C-terminal domain (5) (Harper and Harmon 2005)

domain by a pseudosubstrate mechanism (Fig. S1). Upon Ca^{2+} binding to the CBD domain, the PK domain of CDPKs undergoes conformational changes, thereby activating the protein (Klimecka and Muszynska 2007). Then, the activated CDPKs recognize and phosphorylate their protein targets, such as respiratory burst oxidase homolog (RBOH) (Kobayashi et al. 2007) (Fig. S1). Phosphorylated partner proteins further conduct and amplify the signal from a specific CDPK to form a plant cell response. Also, it has been suggested that the N- and C-terminal domains contribute to the function specificity of individual CDPKs.

All CDPKs in vascular plants were classified into four major groups (I, II, III, and IV subfamilies) based on their full-length protein sequences (Fig. 2). It has been earlier shown that groups I, II, and III comprise two subgroups, whereas group IV always contains the least genes and no subgroups are apparent (Li et al. 2008) (Fig. 2). We looked for connections between CDPKs within the same groups or subgroups and their known functions (Fig. 2 and Table S1)

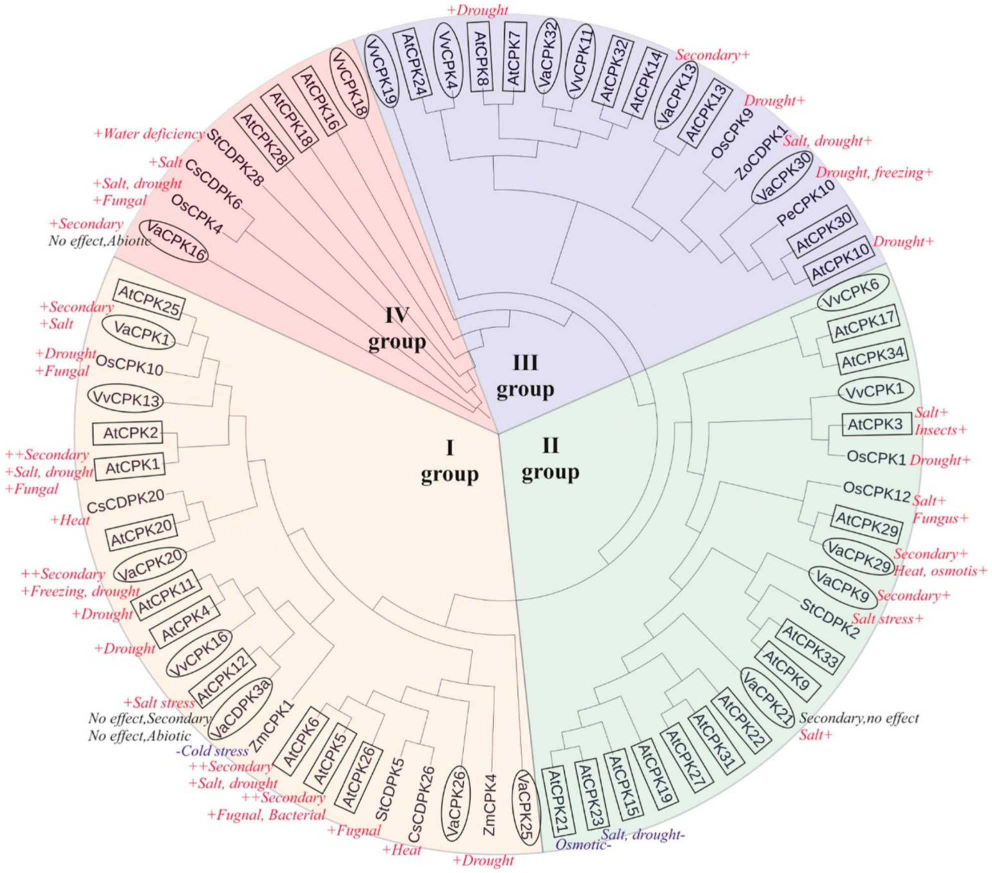

Fig. 2 Phylogenetic tree showing the relationship between 34 CDPKs of *Arabidopsis thaliana* (embedded with a rectangle) and all 20 CDPKs of grapevine *Vitis vinifera* and *Vitis amurensis* (highlighted by an oval) with some other CPKs with known function in regulation of plant secondary metabolism and plant protection against abiotic and biotic stresses proved by the gain- or loss-of-function studies. "+" and red font indicates involvement of a CDPK in stress resistance as positive regulator; "-" and blue font indicates involvement of a CDPK in stress resistance as negative regulator, "no effect"- no considerable effect on plant stress response. Amino acid sequences of the CPKs were aligned using the ClustalX program with the amino acid sequences of 34 AtCPKs (GenBank acc. no. of AtCPKs: NM 120569, NM 111902, NM 118496, NM 117025, NM 119697, NM 127284, NM 121286, NM 121950, NM 112932, NM 101746, NM 103271, NM 122264, NM 115044, NM 129750, NM 001203865, NM 127343, NM 121256, NM 001204003, NM 104875, NM 129449, NM 116710, NM 116709, NM 001203743, NM 128707, NM 129148, NM 001204020, NM 116708, NM 126019, NM 202421, NM 106132, NM 148230, NM 115613, NM 103952, and NM 121941), 20 grapevine VvCPKs or VaCPKs (GenBank acc. no. VvCPK1, GSVIVT01019446001; VaCPK1, KC488321; VvCPK4, GSVIVT01010743001; VvCPK6, GSVIVT01037295001; VaCDPK7(3a), JQ793892; VaCPK9, KC488319; VvCPK11, GSVIVT01033306001; VaCPK13, KC488320; VvCPK13, GSVIVT01001931001; VvCPK16, GSVIVT01034489001; VaCPK16, KF042356; VvCPK18, GSVIVT01025745001; VvCPK19, GSVIVT01027353001; VaCPK20, KC488322; VaCPK21, KC488318; VaCPK25, AGS15002; VaCPK26, KC488323; VaCPK29, KC488317; VaCPK30, KF042358; VaCPK32, XM_002283513), and several other CPKs: from tea *Camellia sinensis* (CsCDPK6, XP_004134863; CsCDPK20, KU201348; CsCDPK26, KU201349), rice *Oryza sativa* (OsCPK1, NP_001384972; OsCPK4, NP_001396186; OsCPK9, AK105102; OsCPK10, AK072204; OsCPK12, NM_001060083), poplar *Populus euphratica* (PeCPK10, JX292958), potato *Solanum tuberosum* (StCDPK2, GU182404; StCDPK5, AB279738; StCDPK28, XM_006340676), maize *Zea mays* (ZmCPK1, NM_001112282; ZmCPK4, D87042), ginger *Zingiber officinale* (ZoCDPK1, KC544003)

and did not find any strong relationships between groups of CDPKs and their functions, while some interesting conclusions can be drawn (see below). This analysis was complicated by the fact that not all functions have been studied for every CDPK and for most CDPKs, only one or two functions have been reported. The exception was the AtCPK1 gene (NM_120569), since its involvement in secondary metabolism and in the resistance to abiotic and biotic stresses has been widely studied. We noted that CDPKs belonging to the same group sometimes possess different properties, even the opposite, e.g. Salt- AtCPK23 gene vs. Salt+AtCPK3 in the group II. The group also contain Salt+CDPKs from different plant species, including *OsCPK12*, *StCDPK2*, and *VaCPK21* genes. Overexpression of group I genes (*AtCPK1*, *AtCPK5*, *AtCPK6*, and *VaCPK20* genes) had positive effect on plant secondary metabolism. Overexpression of group I representatives (*AtCPK1*, *AtCPK5*, and *OsCPK10* genes) provided resistance to fungal and bacterial pathogens. Further studies are required to allow detection of a subtler relationship between the protein sequence and the observed functional properties.

Notably, the highest number of members is found in CDPK groups I and II (10 and 13 genes in *A. thaliana*, respectively), slightly fewer representatives in the group III (8 in *A. thaliana*) and the lowest number of representatives in the group IV (only 3 genes in *A. thaliana*) (Fig. 2).

CDPK gain-of-function and loss-of-function for plant abiotic stress tolerance or sensitivity

In this section, we will summarize and discuss the main results obtained using gain-of-function method in which a target gene is overexpressed under a strong constitutive promoter or loss-of-function analysis, which focuses on reducing or eliminating gene function by targeted or random mutagenesis, plant transformation with RNAi constructs, or virus-induced gene silencing, for studying *CDPK* gene functions in plant abiotic stress adaptation (Tables 1 and 2). Studies that focus on how plants withstand abiotic stress are particularly relevant where plant survival is determined.

Most published investigations on CDPK functions in plant abiotic stress response were devoted to *CDPK* genes from *A. thaliana* and *O. sativa* (Tables 1 and 2). In the Tables 1 and 2, we also collected the data about the properties of *CDPK* genes from other plants (apple, grapes, cucumber, millet, tea, etc.). Notably, these *CDPK* genes were mostly overexpressed in molel plant species, *A. thalina* or *O. sativa* (Table 1), which are easier to investigate gene functions than in other plants, because the genome is well studied. Although Arabidopsis is the best studied plant species, little is known about the effect of overexpressing or reducing *CDPK* expression for genes such as *AtCPK14*, *AtCPK15*, *AtCPK16*, *AtCPK18*, *AtCPK19*, *AtCPK22*, *AtCPK25*, or *AtCPK26* (8 out of 34 genes). Additionally, various other Arabidopsis CDPKs (*AtCPK2*, *AtCPK7*, *AtCPK17*, *AtCPK20*, *AtCPK24*, *AtCPK29*, *AtCPK30*, or *AtCPK34*, 8 out of 34 genes) have been studied through both gain- or loss-of-function approaches; however, the researchers focused on multiple plant characteristics that do not directly pertain to stress tolerance (Yuan et al. 2007; Myers et al. 2009; Kanchiswamy et al. 2010; Zhao et al. 2013; Gutermuth et al. 2013, 2018; Li et al. 2015; Jin et al. 2017; Lee et al. 2021). For example, AtCPK24 could negatively regulate pollen tube (PT) growth by inhibiting K^+ inward currents (Zhao et al. 2013), and loss of *AtCPK29* significantly compromised intracellular polarity, causing defects in biological processes such as lateral root formation, root twisting, hypocotyl gravitropism, and reproductive development (Lee et al. 2021).

The remaining *Arabidopsis CDPKs* (18 genes) are associated with tolerance to abiotic stress, either directly or via plant stress hormones. Seven *CDPK* genes (*AtCPK4*, *AtCPK9*, *AtCPK11*, *AtCPK12*, *AtCPK13*, *AtCPK32*, *AtCPK33*) have been demonstrated to play a role in the control of abscisic acid (ABA) production or regulation of stomatal guard cells. ABA functions in many plant developmental processes, but it is especially important for plant responses to environmental stresses, including drought, soil salinity, cold tolerance, freezing tolerance, heat stress, and heavy metal ion tolerance (Finkelstein 2013). It has been shown that the *AtCPK13* gene is expressed in Arabidopsis guard cells and its overexpression inhibits light-induced stomatal opening, therefore, these plants may be more resistant to drought (Ronzier et al. 2014). Loss-of-function mutations of *AtCPK4* and *AtCPK11* resulted in pleiotropic ABA insensitive phenotypes in seed germination, seedling growth, and stomatal movement and led to salt insensitivity in seed germination and decreased tolerance of seedlings to salt stress. *AtCPK4*- or *AtCPK11*-overexpressing plants generally showed inverse ABA-related phenotypes relative to those of the loss-of-function mutants. Expression levels of many ABA-responsive genes were altered in the loss-of-function mutants and overexpression lines (Zhu et al. 2007; Zou et al. 2015). Compared with wild-type plants a loss-of-function mutant *Atcpk9* showed a much more sensitive phenotype to ABA concentrations for regulation of stomatal movement and ion channel activity. While, *AtCPK9* overexpression lines exhibited an opposite phonotypes, plants were an ABA-less sensitive (Chen et al. 2019). Zhao with colleagues (Zhao et al. 2011) generated Arabidopsis *AtCPK12*-RNAi lines and showed that downregulation of *AtCPK12* resulted in ABA hypersensitivity in seed germination and

Table 1 Gain-of-function of calcium-dependent protein kinase (*CDPK*) genes for the regulation of abiotic stress resistance in plants

CDPK gene (plant species)	Promoter	Transformed plant	Plant survival under abiotic stress	Other effects	Gene function	Reference
AtCPK1 (Arabidopsis thaliana)	CaMV 35 S	*A. thaliana*	Enhanced tolerance to salt and drought stress	Higher proline content, lower MDA and H_2O_2 content Activation of several stress-inducible genes	Positive regulation of salt and drought stress response	(Huang et al. 2018)
AtCPK4 (*A. thaliana*)	CaMV 35 S	*A. thaliana*	Increased plant survival rate under drought stress	Decreased water loss. CPK4 phosphorylated two ABA-responsive transcription factors, ABF1 and ABF4 in vitro	Positive regulation of drought stress response	(Zhu et al. 2007)
AtCPK6 (*A. thaliana*)	Double CaMV 35 S	*A. thaliana*	Enhanced tolerance to salt and drought stresses	Higher proline and lower MDA concentrations	Positive regulation of salt/drought stress response	(Xu et al. 2010)
AtCPK8 (*A. thaliana*)	SUPER promoter	*A. thaliana*	Enhanced tolerance to drought stress	Decreased water loss and stomatal aperture	Positive regulation of drought stress response	(Zou et al. 2015)
AtCPK10 (*A. thaliana*)	Enhanced CaMV 35 S	*A. thaliana*	Enhanced tolerance to drought stress	Decreased water loss	Positive regulation of drought stress response	(Zou et al. 2010)
AtCPK11 (*A. thaliana*)	CaMV 35 S	*A. thaliana*	Increased plant survival rate under drought stress	Decreased water loss. CPK11 phosphorylated two ABA-responsive transcription factors, ABF1 and ABF4 in vitro	Positive regulation of drought stress response	(Zhu et al. 2007)
AtCPK23 (*A. thaliana*)	SUPER promoter	*A. thaliana*	More sensitive to salt/drought stress	Increased stomatal aperture	Negative regulator in salt/drought stress signalling	(Ma and Wu 2007)
BnaCPK5 (rape *Brassica napus*)	CaMV 35 S	*B. napus*	Enhanced drought tolerance	Decreased water loss and MDA content, increased proline content	Positive regulation of drought stress response	(Cheng et al. 2022)
CsCDPK6 (cucumber *Cucumis sativus* L.)	CaMV35S	*Nicotiana benthamiana*	Enhanced plant survival rate under salt stress	Lowered MDA and H_2O_2 contents and less relative EL	Positive regulation of salt stress response	(Zhu et al. 2021)
CsCDPK20, *CsCDPK26* (tea *Camellia sinensis*)	CaMV35S	*A. thaliana*	Increased plant survival rate after heat stress	Higher proline content, lower MDA content, increased expression of stress-related genes	Positive regulation of heat stress response	(Wang et al. 2018)
MdCPK1a (apple *Malus domestica*)	CaMV35S	*N. benthamiana*	Higher survival rates and longer root length under cold stress	Decreased MDA and ROS. Activation of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase)	Positive regulation of cold stress response	(Dong et al. 2020)
OsCPK1 (rice *Oryza sativa*)	Maize *ubiquitin1* promoter	*O. sativa*	Enhanced drought tolerance in transgenic rice seedlings	Activation of 14-3-3 protein expression	Positively regulation of drought tolerance	(Ho et al. 2013)
OsCPK4 (rice *O. sativa*)	Maize *ubiquitin1* promoter	*O. sativa*	Enhanced tolerance to salt and drought stress	Stronger water-holding capability and reduced levels of membrane lipid peroxidation and EL under drought or salt stress	Positive regulation of salt and drought stress response	(Campo et al. 2014)
OsCPK9 (rice *O. sativa*)	CaMV35S promoter	*O. sativa*	Enhanced survival rate to drought	Higher chlorophyl and lower MDA content under drought	Positive regulation of drought stress response	(Wei et al. 2014)
OsCPK10 (rice *O. sativa*)	Maize *ubiquitin1* promoter	*O. sativa*	Enhanced tolerance to drought stress	OsCPK10 is a plasma membrane protein that physically interacts in vivo with catalase A	Positive regulation of drought stress response	(Bundó and Coca 2017)
OsCPK12 (rice *Oryza sativa*)	CaMV 35 S promoter	*O. sativa*	Improved survival rate under salt stress	Reduced accumulation of ROS	Positive regulation of salt stress response	(Asano et al. 2012)
PeCPK10 (desert poplar *Populus euphratica*)	CaMV 35 S	*A. thaliana*	Enhanced tolerance to drought and freezing	Stronger ABA-induced promotion of stomatal closure. Enhanced expression of several ABA-responsive genes and multiple abiotic stress-responsive genes	Positive regulation of drought and freezing stress response	(Chen et al. 2013)
PtrCDPK10 (trifoliate orange *Poncirus trifoliata*)	CaMV35S	*P. rifoliata*	Enhanced dehydration tolerance	Higher APX activity and less ROS accumulation	Positive regulation of dehydration and drought stress response	(Meng et al. 2020)

Table 1 (continued)

CDPK gene (plant species)	Promoter	Transformed plant	Plant survival under abiotic stress	Other effects	Gene function	Reference
StCDPK2 (potato *Solanum tuberosum* L.)	CaMV35S	*S. tuberosum*	Enhanced tolerance to high salt	More efficient antioxidant system; reduced accumulation of H_2O_2 and higher catalase activity under salt conditions	Positive role in salt stress response	(Grossi et al. 2022)
StCDPK28 (potato *S. tuberosum*)	CaMV35S	*S. tuberosum*	Increased tolerance to water deficit and osmotic stress	Decreased H_2O_2 and MDA, increased proline content	Positive regulation of water deficiency and osmotic stress	(Zhu et al. 2022)
SiCDPK24 (foxtail millet *Setaria italica*)	CaMV 35 S	*A. thaliana*	Improved survival rate under drought stress	Increased expression of some stress-related genes	Positive regulation of drought stress response	(Yu et al. 2018)
SpCPK33 (tomato *Solanum pennellii*)	CaMV 35 S	*S. pennelli*	Strong vitality under low temperature treatment	Decreased MDA and ROS levels. Higher activities of antioxidant enzymes and the levels of osmotic regulatory substances	Positive regulation of cold stress response	(Hu et al. 2022)
VaCPK1, *VaCPK21* (grapevine *Vitis amurensis)*	Double CaMV 35 S	*A. thaliana*	Improved survival rate under high salinity	Increased expression of stress-related genes	Positive regulation of salt stress response	(Dubrovina et al. 2016; Dubrovina and Kiselev 2019)
VaCPK20 (grapevine *V. amurensis)*	Double CaMV 35 S	*A. thaliana*	Improved survival rate under freezing and drought	Increased expression of stress-related genes	Positive regulation of freezing and drought stress response	(Dubrovina et al. 2015)
VaCPK29 (grapevine *V. amurensis)*	Double CaMV 35 S	*A. thaliana*	Improved survival rate under heat and osmotic stress	Increased expression of stress-related genes	Positive regulation of heat and osmotic stress response	(Dubrovina et al. 2017)
ZmCPK1 (maize *Zea mays* L.)	CaMV35S	*A. thaliana*	Significantly reduced tolerance to cold stress	Suppressed expression of a cold-induced marker gene	Negative regulator in cold stress signalling	(Weckwerth et al. 2015)
ZmCPK4 (maize *Z. mays*)	CaMV35S	*A. thaliana*	Enhanced drought stress tolerance	Decreased water loss and increased expression of stress-related genes	Positive regulation of drought stress response	(Jiang et al. 2013)
ZmCPK7 (maize *Z. mays*)	CaMV35S	*Z. mays*	Higher thermotolerance to heat stress	Higher photosynthetic rates, and antioxidant enzyme activity but lower H_2O_2 and MDA contents	Positive regulation of heat stress response	(Zhao et al. 2021)
ZmCPK11 (maize *Z. mays*)	CaMV35S	*A. thaliana*	Improved survival rate under salinity	The expression of Na+ /K+transporters (HKT1, SOS1, NHX1) and transcription factors (CBF1, CBF2, CBF3, ZAT6, ZAT10) with known links to salinity tolerance was upregulated	Positive regulation of salt stress response	(Borkiewicz et al. 2020)
ZoCDPK1 (ginger *Zingiber officinale*)	Double CaMV 35 S	*Nicotiana tabacum*	Enhanced growth during salinity/drought stress conditions	Improved seed germination, higher relative water content, expression of stress-responsive genes, higher leaf chlorophyll content, increased photosynthetic efficiency	Positive regulation of salinity and drought stress response	(Vivek et al. 2013)

ABA– abscisic acid; APX– ascorbate peroxidase; EL– electrolyte leakage; MDA– malondialdehyde; ROS– reactive oxygen species; SA - salicylic acid

postgermination growth and altered expression of a set of ABA-responsive genes. Thus, *AtCPK12* is a negative regulator of ABA signaling in seed germination and post-germination growth.

Overexpression of *AtCPK32* positivly affects both ABA sensitivity and the expression of a number of ABA-regulated genes via activation of transcription factor *ABF4* (Choi et al. 2005). Loss-of-function *Atcpk33* mutants were hypersensitive to ABA activation of slow type anion channels activity and ABA-induced stomatal closure, while the *AtCPK33* overexpression lines showed opposite phenotypes (Jin et al. 2017).

Thus, the loss of function *AtCPK1* mutant in *A. thaliana* plants displayed hypersensitive response to salt and drought stress, whereas overexpression of *AtCPK1* enhanced salt or drought stress resistance (Huang et al. 2018). AtCPK2 is

Table 2 Loss-of-function of calcium-dependent protein kinases (*CDPK*) genes for the regulation of abiotic stress resistance in plants

CDPK gene (plant species)	Plant for transformation	Loss-of-function technology	Plant survival under abiotic stress	Other effects	Gene function	Reference
AtCPK1 (*Arabidopsis thaliana*)	*A. thaliana*	T-DNA insertion mutant	Significantly decreased salt and drought stress resistance	Changes in proline, MDA, and H_2O_2 content. Decreased expression of several stress-inducible genes	Positive regulation of salt and drought stress response	(Huang et al. 2018)
AtCPK3 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Significantly decreased salt stress resistance	Induced AtCPK3 kinase activity	Positive regulation of salt stress response	(Mehlmer et al. 2010)
AtCPK4 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Decreased plant survival rate under drought stress	Increased water loss. CPK4 phosphorylated two ABA-responsive transcription factors, ABF1 and ABF4 in vitro	Positive regulation of drought stress response	(Zhu et al. 2007)
AtCPK8 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Reduced tolerance to drought stress	Increased water loss and stomatal aperture	Positive regulation of drought stress response	(Zou et al. 2015)
AtCPK10 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Reduced tolerance to drought stress	Incresed water loss	Positive regulation of drought stress response	(Zou et al. 2010)
AtCPK11 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Decreased plant survival rate under drought stress	Increased water loss. CPK11 phosphorylated two ABA-responsive transcription factors, ABF1 and ABF4 in vitro	Positive regulation of drought stress response	(Zhu et al. 2007)
AtCPK12 (*A. thaliana*)	*A. thaliana*	RNA interference (RNAi) transgene	Decreased root length of the *CPK12*-RNAi plantlets under salt stress	Na$^+$ and H_2O_2 levels in the roots of *CPK12*-RNAi plants were higher than levels in wild-type	Positive regulation of salt stress response	(Zhang et al. 2018)
AtCPK21 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	More tolerant to hyperosmotic stress (300 mM mannitol)	Increased healthy green seedlings	Negative regulation of hyperosmotic stress response	(Franz et al. 2011)
AtCPK23 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	More tolerant to salt/drought stress	Decreased stomatal aperture	Negative regulator in salt/drought stress signalling	(Ma and Wu 2007)
AtCPK27 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Reduced tolerance to drought stress	Decreased expression of several stress-inducible genes	Positive regulation of drought stress response	(Zhao et al. 2015)
AtCPK31 (*A. thaliana*)	*A. thaliana*	T-DNA insertion mutant	Improved tolerance against As(III)	Increased root length and biomass accumulation	Negative regulator in arsenite stress signalling	(Ji et al. 2017)
OsCPK1 (rice *Oryza sativa*)	*O. sativa*	RNAi	More sensitive to drought	Decreased plant fresh weight	Positively regulates drought tolerance in rice	(Ho et al. 2013)
OsCPK9 (rice *O. sativa*)	*O. sativa*	RNAi	Significantly decreased survival rate under drought stress	Lower chlorophyl and higher MDA content under drought	Positive regulation of drought stress response	(Wei et al. 2014)
OsCPK12 (rice *O. sativa*)	*O. sativa*	RNAi	More sensitive to high salinity	Reduced accumulation of ROS	Positive regulation of salt stress response	(Asano et al. 2012)
PtrCDPK10 (trifoliate orange *Poncirus trifoliata*)	Orange *P. trifoliata*	Virus-induced gene silencing (VIGS)	Elevated susceptibility to dehydration and drought stresses	Higher water loss and EL	Positive regulation of dehydration and drought stress response	(Meng et al. 2020)
SlCPK28 (tomato (*Solanum lycopersicum*)	*S. lycopersicum*	CRISPR-Cas9 gene-editing approach	Dectreased tolerance to heat stress	Increased accumulation of ROS and protein oxidation, decreased activities of APX and other antioxidant enzymes	Positive regulation of high temperature stress response	(Hu et al. 2021)

Table 2 (continued)

CDPK gene (plant species)	Plant for transformation	Loss-of-function technology	Plant survival under abiotic stress	Other effects	Gene function	Reference
StCDPK28 (potato *Solanum tuberosum* L.)	*S. tuberosum*	RNAi	Decreased tolerance to water deficit and osmotic stress	Increased H_2O_2 and MDA levels, decreased proline content	Positive regulation of water deficiency and osmotic stress	(Zhu et al. 2022)
TaCDPK27 (wheat *Triticum aestivum* L.)	*T. aestivum*	VIGS	Reduced salt stress tolerance	Increased accumulation of ROS, reduced the activities of superoxide dismutase, peroxidase, and catalase	Positively regulates salt tolerance	(Yue et al. 2022)

APX– ascorbate peroxidase; EL– electrolyte leakage; MDA– malondialdehyde; ROS– reactive oxygen species; SA - salicylic acid

the closest homologue to AtCPK1, thus it is possible that AtCPK2 could play a role in providing resistance against salt or drought stresses (Urao et al. 1994).

Using *Atcpk3* mutants, it has been shown that *AtCPK3* is involved in the tolerance for salt stress by negative feedback regulation of the Ca^{2+} cytosolic levels (Kanchiswamy et al. 2010). *AtCPK6*-overexpressing *A. thaliana* plants showed enhanced tolerance to salt/drought stresses. The elevated tolerance of the *AtCPK6*-overexpressing plants was confirmed by the increased accumulation of proline and MDA (Xu et al. 2010). The *Atcpk8* mutant was more sensitive to drought stress than wild-type plants, while the transgenic plants overexpressing *AtCPK8* showed enhanced tolerance to drought stress in comparison with wild-type plants (Zou et al. 2015). The *Atcpk10* mutant showed a much more sensitive phenotype to drought stress than wild-type plants, while the *AtCPK10* overexpression lines displayed enhanced tolerance to drought stress (Zou et al. 2010). It has been shown that AtCPK21 is activated in vivo in response to hyperosmotic stress. Loss-of-function seedlings of *cpk21* were more tolerant to hyperosmotic stress than wild-type plants and mutant plants showed increased stress responses with respect to marker gene expression and metabolite accumulation (Franz et al. 2011).

A mutant for *AtCPK23* showed greatly enhanced tolerance to drought and salt stresses, while the *AtCPK23* overexpression lines became more sensitive to drought and salt stresses than wild-type plants (Ma and Wu 2007). T-DNA insertion mutant of *AtCPK27* gene was much more sensitive to salt stress than wild-type plants in terms of seed germination and post-germination seedling growth (Zhao et al. 2015). AtCPK31 could be a key factor in arsenite (AsIII) tolerance in *A. thaliana* plants, because AtCPK31 interacts with arsenic transporter AtNIP1 and regulates arsenite uptake (Ji et al. 2017).

CDPKs of other plant sources, not only *A. thaliana*, have also been analyzed. Overexpression of the *SiCDPK24* gene under the control of the CaMV 35 S promoter in Arabidopsis improved the plant survival rate under drought stress (Yu et al. 2018). Overexpression of the *SiCDPK24* gene also activated the expression of some stress-related genes, including the genes of serine/threonine protein kinase1 (*KIN1*), cold-regulated (*COR*) genes *COR15* and *COR47*, late embryogenesis abundant gene (*LEA14*), dehydration-responsive element-binding protein *DREB1A* and *DREB2A* genes, and the responsive to desiccation *RD29A*, *RD29B*, *RD22* genes.

Overexpression of *CsCDPK20* and *CsCDPK26* isolated from tea *Camellia sinensis* increased the thermotolerance of transgenic *A. thaliana* plants (Wang et al. 2018). Proline content was significantly higher in the leaves of transgenic plants than that in wild type under heat stress, whereas MDA content was lower in transgenic plants. Additionally, expression of stress-responsive genes (i.e., *AtAPX1*, *AtPOD*, *AtProT1*, *AtP5CS2*, *AtHSFA2*, *AtHSP70*, *AtHSP101*, *AtRD29B*, *AtRAB18*, *AtABI1*, *AtRBOHD*, and *AtRBOHF*) was increased in the *CsCDPK20*- and *CsCDPK26*-transgenic *A. thaliana* compared with that in wild type (Wang et al. 2018). Collectively, these results suggest that *CsCDPK20* and *CsCDPK26* may act as positive regulators heat stress response in *C. sinensis*.

Several papers showed the involvement of grapevine *CDPKs* in plant response to abiotic stresses (Dubrovina et al. 2015, 2017; Dubrovina and Kiselev 2019). For example, overexpression of *VaCPK1* and *VaCPK21* in the *A. thaliana* plants led to a considerable increase in the survival under salt stress (Dubrovina and Kiselev 2019). Transgenic Arabidopsis plants overexpressing the *VaCPK20* gene showed higher tolerance to freezing and drought stresses (Dubrovina et al. 2015). The *VaCPK29*-overexpressing soil-grown plants of *A. thaliana* and seedlings of *A. thaliana* exhibited higher tolerance to heat and osmotic stress (Dubrovina et al. 2017).

Ectopic expression of apple *Malus domestica MdCPK1a* in *Nicotiana benthamiana* increased the resistance of the tobacco plants to salt and cold stresses (Dong et al. 2020). Compared with wild-type plants the *MdCPK1a*-overexpressing tobacco plants showed higher survival rates and longer root length under cold stress. In these plants the electrolyte leakage (EL), MDA contents, and reactive oxygen species (ROS) contents were lower. Also, the antioxidant enzyme activities, such as superoxide dismutase, peroxidase, and catalase were higher in the *MdCPK1a* transgenic plants, suggesting that the transgenic plants suffered less chilling injury than wild-type plants (Dong et al. 2020).

Overexpression of cucumber *Cucumis sativus CsCDPK6* gene in tobacco enhanced plant salt tolerance (Zhu et al. 2021). *CsCDPK6*-overexpressing lines showed enhanced survival rates and reduced stomatal apertures, as well as lowered MDA and H_2O_2 contents and caused less relative EL in comparison with wild-type plants. Additionally, utilizing techniques such as the yeast two-hybrid system, bimolecular fluorescence complementation, and co-immunoprecipitation assays, it was demonstrated in cucumber *C. sativus* that CsCDPK6 has the capability to interact with S-adenosylmethionine synthetase (SAMS). SAMS is an enzyme that creates S-adenosylmethionine. S-adenosylmethionine is the precursor for the synthesis of polyamines in plant cells. Polyamines are compounds that are involved in a complex signaling system and have a key role in the regulation of stress tolerance (Zhu et al. 2021). Transgenic trifoliate orange *Poncirus trifoliata* plants overexpressing *PtrCDPK10* showed enhanced dehydration tolerance compared with the wild-type plants, whereas knockdown of *PtrCDPK10* resulted in elevated susceptibility to dehydration and drought stresses (Meng et al. 2020).

CDPK gain-of-function and loss-of-function for plant biotic stress tolerance or sensitivity

CDPKs are known as important mediators in plant response to the attack of plant pathogens and pests, including pathogenic fungi, bacteria, and herbivorous insects (Dekomah et al. 2022). *CDPK* gene expression and protein levels are upregulated in response to plant microbial infection or separately added Avr microbial effectors (Coca and San Segundo 2010; Fantino et al. 2017). There is compelling evidence for the involvement of CDPKs in the signalling events after recognition of microbe-associated molecular patterns (MAMPs), including hormonal signaling and oxidative burst (Coca and San Segundo 2010).

Regarding the roles of CDPKs in plant-pathogen interactions, a number of CDPKs from tomato, tobacco, rice, etc. have been found to be transcriptionally responsive to fungal and bacterial infection (Freymark et al. 2007; Coca and San Segundo 2010; Bundó and Coca 2016; Wang et al. 2016). In Table 3, we collected investigations where *CDPK* in gain-of-function and loss-of-function experiments affected plant resistance to pathogenic fungi, bacteria, and insects, indicating CDPK involmenent in plant biotic stress tolerance. To the best of our knowledge, the majority of the available investigations demonstrated positive roles of plant CDPKs in mediating pathogen resistance to both bacterial and fungal plant pathogens. Overexpression of *CDPKs* from Arabidopsis, rice, potato, and pepper in different plants resulted in improved survival rate under fungal and bacterial infection and caused accumulation of plant stress hormones (salicylic acid or SA and jasmonic acid or JA), increased expression of immunity-associated disease resistance genes, changed ROS accumulation (Table 3).

Overexpression of *AtCPK1* in *A. thaliana* plants led to accumulation of SA and constitutive expression of SA-regulated defense and disease resistance genes, which, in turn, resulted in a broad-spectrum protection against pathogen infection (Coca and San Segundo 2010). This leads to tolerance of *AtCPK1*-transgenic plants to the fungal pathogens *Fusarium oxysporum* and *Botrytis cinerea*. Also, overexpression of *AtCPK5* resulted in resistance to a pathogenic fungus *Golovinomyces cichoracearum* (Liu et al. 2017).

In contrast, *HvCDPK3*, *HvCDPK4*, *NaCDPK4*, and *NaCDPK5* genes from barley and tobacco implicated as negative regulators to powdery mildew fungus *Blumeria graminis* in barley (Freymark et al. 2007) and to herbivorous insect tobacco hornworm *Manduca sexta* growth (Yang et al. 2012). Transient expression of a constitutively active barley *HvCDPK3* in tobacco resulted in compromised penetration resistance to the incompatible wheat powdery mildew fungus *B. graminis* and caused mesophyll cell death in tobacco leaves without fungus infection indicating that the *HvCDPK3* negatively regulates plant response to fungal pathogens (Freymark et al. 2007). Virus-induced simultaneous silencing of *NtCDPK4* and *NtCDPK5* in tobacco have led to greatly elevated levels of defensive secondary metabolites against the larvae of tobacco hornworm *M. sexta* and enhanced jasmonic acid levels indicating that these CDPKs negatively control tobacco response to this herbivorous insect (Yang et al. 2012).

Overall, there are significantly fewer studies focusing on the role of CDPKs in biotic stress tolerance compared to the numerous articles addressing their involvement in abiotic stress, as discussed in the previous chapter. We believe that the study of CDPKs in relation to biotic stress has not been thoroughly investigated. As a result, it is reasonable to expect a substantial increase in research contribute to plant defense against different types of pathogens.

Plant CDPK*s* in the regulation of plant secondary metabolism

Modulation of plant secondary metabolism is one of the major mechanisms employed by plants to accommodate to changing environmental conditions, including both biotic and abiotic stress factors. Plant secondary metabolites are

Table 3 Gain- or loss-of-function of calcium-dependent protein kinases (*CDPK*) genes for regulation of plant biotic stress resistance

CDPK gene (plant species)	Promoter for expression/ Loss-of function technology	Plant for transformation/mutation	Plant survival in response to pathogens and pests	Other effects	Gene function	Reference
AtCPK1 (*Arabidopsis thaliana*)	Overexpression under CaMV 35 S promoter (gain-of-function)	*A. thaliana*	Improved survival under fungal infection (*Fusarium oxysporum*, *Botrytis cinerea*)	Accumulation of SA and constitutive expression of some SA-regulated defence and disease resistance genes	Positive regulation of plant fungal resistance	(Coca and San Segundo 2010)
	T-DNA mutants (loss-of-function) (Salk Institute Genomic Analysis Laboratory)	*A. thaliana*	Higher susceptibility to fungal infection (*F. oxysporum*, *B. cinerea*)	Increased growth of the fungal colony forming units and decreased expression of disease resistance genes		
AtCPK3 (*A. thaliana*)	T-DNA mutants (loss-of-function)	*A. thaliana*	Higher susceptibility to insect attack (African cotton leafworm *Spodoptera littoralis*)	Lower transcript levels of plant defensin gene *PDF1.2*	Positive regulation of plant resistance to insect attack	(Kanchiswamy et al. 2010)
AtCPK5 (*A. thaliana*)	The native *AtCPK5* promoter (gain-of-function)	*A. thaliana*	Improved survival under fungal (*Golovinomyces cichoracearum*) and bacterial infection (*Pseudomonas syringae*)	Changing H_2O_2 accumulation, callose deposition, expression of pathogenesis-related genes	Positive regulation of plant resistance to powdery mildew and bacterial pathogen *P. syringae*	(Liu et al. 2017)
	T-DNA mutants (Arabidopsis Biological Resource Center or Nottingham Arabidopsis Stock Center) (loss-of-function)	*A. thaliana*	Improved sensitivity to fungal (*G. cichoracearum*) and bacterial infection (*P. syringae*)			
HvCDPK3 or *HvCDPK4* (barley *Hordeum vulgare*)	Transient expression under maize ubiquitin promoter	*Nicotiana benthamiana*	Compromised penetration resistance to the inappropriate wheat powdery mildew fungus	Compromised penetration resistance to the inappropriate wheat powdery mildew fungus *Blumeria graminis*	Negative regulation of powdery mildew resistance	(Freymark et al. 2007)
NaCDPK4 and *NaCDPK5* (tobacco *Nicotiana attenuata*)	Virus-induced gene silencing (loss-of-function)	*N. attenuata*	Higher planr survival rate due to slowed *Manduca sexta* growth	Greatly eleveated JA levels	Negative regulation insect defence	(Yang et al. 2012)
OsCPK12 (rice *Oryza sativa*)	CaMV 35 S promoter (gain-of-function)	*O. sativa*	Enhanced tolerance to blast fungus *Magnaporthe grisea*	Reduced accumulation of ROS	Positive regulation of blast disease resistance	(Asano et al. 2012)
	Transformation with an *OsCPK12* RNAi construct (loss-of-function)		Decresed tolerance to blast fungus *M. grisea*			
OsCPK4 (rice *O. sativa*)	Maize *ubiquitin1* promoter, nopaline synthase terminator (gain-of-function)	*O. sativa*	Enhanced tolerance to blast fungus *Magnaporthe oryzae*	Activation of production of ROS, callose deposition and defence gene expression	Positive regulation of blast disease resistance	(Bundó and Coca 2016)

Table 3 (continued)

CDPK gene (plant species)	Promoter for expression/ Loss-of function technology	Plant for transformation/mutation	Plant survival in response to pathogens and pests	Other effects	Gene function	Reference
OsCPK10 (rice *O. sativa*)	Maize *ubiquitin1* promoter, nopaline synthase terminator (gain-of-function)	*O. sativa*	Significantly enhanced tolerance to blast fungus *M. oryzae*	Decreased fungal biomass accumulation	Positive regulation of blast disease resistance	(Bundó and Coca 2017)
		A. thaliana	Enhanced resistance to bacterial infection *P. syringae* pv. tomato	Elevated expression of both SA- and JA-related defense genes	Positive regulation of resistance to pathogen *P. syringae* pv. tomato	(Fu et al. 2013)
SlCDPK10 and *SlCDPK18* (tomato *Solanum lycopersicum* L.)	Virus-induced gene silencing (loss-of-function)	*S. lycopersicum*	Enhanced resistance to bacterial infection with *Xanthomonas oryzae* or *Pseudomonas syringae*	Increased bacterial numbers in the areas infiltrated with *X. oryzae* or *P. syringae*	Positively regulated resistance to bacterial infection	(Wang et al. 2016)
StCDPK5 (potato *Solanum tuberosum* L.)	Potato vetispiradiene synthase 3 (*PVS3*) promoter, Nos-terminator (gain-of-function)	*S. tuberosum*	Resistance to the near-obligate hemibiotrophic pathogen *Phytophthora infestans* and increased susceptibility to the necrotrophic fungal pathogen *Alternaria solani*	StCDPK5 has been shown to phosphorylate the N-terminal region of plasma membrane RBOH and participate in StRBOHB-mediated ROS burst	Positive regulation of resistance to fungus-like pathogens	(Kobayashi et al. 2012)
CaCDPK15 (pepper *Capsicum annum*)	Double CaMV 35 S promoter (gain-of-function)	*C. annum*	Reduced susceptibility to pathogen bacteria *Ralstonia solanacearum*	Activated hypersensitive response associated cell death, upregulation of the immunity-associated marker genes	Positive regulation of plant resistance to pathogenic bacteria	(Shen et al. 2016)
	Virus-induced gene silencing (loss-of-function)		Significantly increased susceptibility to *R. solanacearum* inoculation	Downregulation of some immunity-associated markers		

JA– asmonic acid, MDA– malondialdehyde, RBOH– respiratory burst oxidase homolog, ROS– reactive oxygen species

actively produced in plants to repel the plant microbial pathogens, insects and herbivores. In this paper, we describe and discuss the effects of CDPKs on plant secondary metabolism (Table 5).

In the context of secondary metabolism, research indicates that CDPKs can influence this process in two distinct ways. Firstly, they facilitate the phosphorylation of enzymes involved in secondary metabolism, such as PAL. Secondly, they also phosphorylate transcription factors that govern the expression of genes associated with secondary metabolism. This indicates that CDPKs exert their influence on secondary metabolism through two complementary pathways.

AtCPK1 and StCDPK7 are capable of phenylalanine ammonia-lyase (PAL) phosphorylation in *A. thaliana* and *Solanum tuberosum* (Cheng et al. 2001). PAL (EC 4.3.1.24) is the first-step enzyme in the biosynthesis of phenylpropanoids and catalyzes a reaction converting L-phenylalanine to ammonia and *trans*-cinnamic acid (Camm and Towers 1973). PAL is involved in the biosynthesis of the polyphenol compounds, such as flavonoids, phenylpropanoids, and lignins in plants. Further studies have shown that overexpression of the *AtCPK1* gene contributes to a significant increase in the content of anthraquinones in *Rubia cordifolia* (Shkryl et al. 2011) and isoflavonoids in soybean *G. max* (Veremeichik et al. 2019).

Chemically induced overexpression under estradiol-inducible promoter of constitutively active *A. thaliana* genes *CPK5* or *CPK6* genes (lacks the C-terminal, Ca^{2+}-binding calmodulin-like, and autoinhibitory domains) was sufficient to induce camalexin biosynthesis in transgenic *Arabidopsis* plants. A more than 40-fold increase in camalexin content was shown after the *AtCPK5* or *AtCPK6* overexpression. Moreover, it has been shown that transcription factor WRKY33 functions downstream of AtCPK5/CPK6 to activate camalexin biosynthetic genes *CYP79B2*, *CYP71A13*, and *PAD3* (Zhou et al. 2020).

Yang et al. 2020 (Yang et al. 2020) have shown that the transcription factor *AeMYB30* was co-expressed with the *AeCDPK6* gene of okra *Abelmoschus exculentus*, which is an vegetable crop and is known for accumulating hyperoside, a secondary metabolite in the flavonoid pathway, in its flowers. Detailed protein interaction and phosphorylation analysis demonstrated that *AeCDPK6* specifically phosphorylated AeMYB30 that, in turn, directly bound to the promoter of AeUF3GaT1, a key enzyme in the hyperoside biosynthesis pathway. Overxpression of *AeCDPK6* promoted hyperside accumulation in an AeMYB30-dependent manner in the flowers of okra plants (Yang et al. 2020).

The effect of CDPKs on the biosynthesis of stilbenes in grapevine has been well-studied (Table 5). At the same time, it is known that stilbenes can increase the resistance of plants to heat and excessive ultraviolet radiation (Aleynova et al. 2024). 12 of 20 known *CDPKs* genes of wild grapevine *V. amurensis* Rupr. (*VaCPK1*, *3a*, *9*, *13*, *16*, *20*, *21*, *25*, *26*, *29*, *30*, and *32*) were overexpressed in the grapevine callus cell cultures (Kiselev et al. 2013; Dubrovina et al. 2013, 2018; Aleynova-Shumakova et al. 2014; Aleynova et al. 2015, 2017). Overexpression of *VaCPK1*, *9*, *13*, *16*, *20*, and *29* significantly increased the content of stilbenes in the transformed calli (Table 5). The effect has been stable for at least 6 months after transformation. The highest stimulating effect on the stilbenes content was detected after overexpressing the *VaCPK20* gene. Overexpression of the *VaCPK20* gene under control of the double CaMV 35 S promoter in cell cultures of *V. amurensis* increased production of *t*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) in 9–68 times and dry biomass in 1.2–1.7 times in comparison with the control cells. The *VaCPK20*-transformed calli were capable of producing 0.04–0.42% dry wt. of *t*-resveratrol, up to 34 mg/l (Aleynova-Shumakova et al. 2014). Also, transformation of the callli with the *VaCPK1*, *9*, *13*, *16*, and *VaCPK29* genes had a considerable positive effect on the content of stilbenes in transgenic grape cell cultures (Aleynova et al. 2015, 2017; Dubrovina et al. 2018).

It has also been shown that StCDPK7 phosphorylates StPAL1 in vitro, both proteins are cytosolically localized and up-regulated in response to *P. infestans* infection, and phosphorylation of StPAL1 mediated by StCDPK7 may affect PAL activity and localization associated with defense response (Fantino et al. 2017).

Stress-related substrates of CDPK proteins

The above-mentioned studies revealed that even though *CDPK* genes share a similar structure, the *CDPKs* can display vastly different regulatory functions. This discrepancy can be attributed to the fact that each CDPK phosphorylates unique protein targets.

A number of studies have been performed to determine protein substrates of different CDPKs (Table 4). First, an expression analysis was performed: transcriptomic studies with further search for a correlation in the expression changes of certain genes during the gain- and/or loss-of-function of the studied *CDPK* (Zhu et al. 2007). These studies were then followed by the verification of the possible partner proteins using various methods, such as yeast two-hybrid screens, bimolecular fluorescence complementation assays, transient expression with immunoprecipitation, and in vitro kinase assays of recombinant proteins (Table 4).

CDPKs have been shown to phosphorylate different proteins (Table 4), e.g. membrane channels (Geiger et al. 2010), NADPH oxidase or ascorbate peroxidase (Kobayashi et al. 2007; Meng et al. 2020), phytohormone transporters (Santin et al. 2017), enzymes of secondary metabolites biosynthesis (Cheng et al. 2001; Fantino et al. 2017) or transcription factors (Choi et al. 2005; Zhu et al. 2007; Yang et al. 2020). These proteins are involved in various processes, including stomatal movements, oxidative burst, phytohormone and secondary metabolism, and gene expression regulation (Choi et al. 2005; Kobayashi et al. 2007; Fantino et al. 2017; Meng et al. 2020; Yang et al. 2020). These processes highly correlated with stresses tolerance in plants, e.g. stomal movement regulates water loss under draught, secondary metabolites engage proactively in combating certain pathogens and dealing with stress factors. Also, it is well-established that specific phytohormones linked to stress trigger the defensive mechanisms within plants.

Conclusions

According to the discussed data, CDPKs serve as both enhancers and suppressors of the protective traits in plants. In the realm of plant biotechnology, the focus is primarily on the positive regulatory effects; therefore, we introduce a suggested model illustrating how CDPKs operate within plant cells to trigger stress tolerance mechanisms (Fig. 3). It is important to note that this model is suitable for genes involved in plant defense as positive regulators, such as *AtCPK1* (Cheng et al. 2001), *AtCPK5* (Liu et al. 2017), *OsCPK1* (Campo et al. 2014), or *VaCPK20* (Dubrovina et al. 2015). These genes have been shown to activate the protective properties of plants.

Table 5 Overexpression of calcium-dependent protein kinase (*CDPK*) genes for plant secondary metabolism regulation

CDPK gene (plant species)	Promoter	Material for transformation	Effects on secondary metabolite production	Other effects	Gene function	Reference
Gain-of-function experiments (*CDPK* gene overexpression)						
AeCDPK6 (flowers of okra *Abelmoschus esculentus*)	CaMV 35 S	Okra plants *A. esculentus*	Increased hyperoside content in okra flowers by 2.4-fold	AeCDPK6 phosphorylated AeMYB30, which is the activator of the hyperoside biosynthesis pathway	Positive regulation of hyperoside biosynthesis	(Yang et al. 2020)
AtCPK1 (*Arabidopsis thaliana*)	CaMV 35 S	Cell cultures of *Rubia cordifolia*	A 10-fold increase in anthraquinone content	Increased expression of *isochorismate synthase* gene	Positive regulation of anthraquinone biosynthesis	(Shkryl et al. 2011)
AtCPK5 and *AtCPK6* (*A. thaliana*)	Estradiol (Est)-inducible promoter	*A. thaliana* plants	A more than 40-fold increase in camalexin content	AtCPK5 and AtCPK6 interact with WRKY33 and phosphorylate its Thr-229 residue	Positive regulation of camalexin biosynthesis	(Zhou et al. 2020)
VaCPK1 (wild grapevine *Vitis amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Induced stilbene production by 1.7–4.6-fold	Increased expression of *PAL* and *STS* genes	Positive regulation of stilbene biosynthesis	(Aleynova et al. 2017)
VaCPK9 (*V. amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Increased *t*-resveratrol content by 4.1-fold	Increased expression of *PAL* and *STS* genes	Positive regulation of stilbene biosynthesis	(Aleynova et al. 2015)
VaCPK13 (*V. amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Increased *t*-resveratrol content by 1.5-fold	Increased expression of *PAL* and *STS* genes	Positive regulation of stilbene biosynthesis	(Aleynova et al. 2015)
VaCPK16 (*V. amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Increased stilbene production by 2.1–3.1-fold	Increased expression of *PAL* and *STS* genes	Positive regulation of stilbene biosynthesis	(Dubrovina et al. 2018)
VaCPK20 (*V. amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Increased *t*-resveratrol production by 9-68-fold 0.04–0.42% dry wt. (up to 34 mg/l)	Increased expression of *PAL* and *STS* genes	Strong positive regulation of stilbene biosynthesis	(Aleynova-Shumakova et al. 2014)
VaCPK29 (*V. amurensis*)	Double CaMV 35 S	Cell cultures of *V. amurensis*	Increased *t*-resveratrol production by 2.4-fold	Increased expression of *PAL* and *STS* genes	Positive regulation of stilbene biosynthesis	(Aleynova et al. 2015)

Table 4 List of substrates for calcium-dependent protein kinases (CDPK)

Plant species, CDPK gene (Gene Bank acc. no.)	Used method	Substrates	Reference
Arabidopsis thaliana, AtCPK1 (NM_120569)	Maize rotoplast transient expression and subjected to immunoprecipitation	Peptide (SRVAKTRTLTTA) from phenylalanine ammonia-lyase (PAL)	(Cheng et al. 2001)
A. thaliana, AtCPK4 and AtCPK11 (NM_117025, NM_103271)	In vitro kinase (phosphorylation) assays of recombinant proteins	ABA-responsive transcription factors, ABF1 and ABF4	(Zhu et al. 2007)
A. thaliana, AtCPK23 (NM_116712)	In vitro kinase assays of recombinant proteins	The guard cell anion channel (SLAC1) to regulate stomatal movements	(Geiger et al. 2010)
A. thaliana, AtCPK32 (NM_115613)	Yeast two-hybrid screens and in vitro kinase assays of recombinant proteins	Basic leucine zipper class transcription factor ABF4, an important regulatory element of ABA	(Choi et al. 2005)
Okra *Abelmoschus esculentus*, AeCDPK6	Phosphorylation experiments in vitro together with yeast two-hybrid and bimolecular fluorescence complementation assays	Transcription factor AeMBY30, activator of the phenolic compounds biosynthesis	(Yang et al. 2020)
Potato *Solanum tuberosum*, StCDPK1 (DQ507862)	In vitro kinase assays of recombinant proteins	Hydrophilic loop of the auxin transporter StPIN4	(Santin et al. 2017)
Potato *S. tuberosum*, StCDPK5 (AB279738)	In vitro kinase assays of recombinant proteins and the heterologous expression	NADPH oxidase	(Kobayashi et al. 2007)
Potato *S. tuberosum*, StCDPK7 (KJ830932)	In vitro kinase assays of recombinant proteins	PAL	(Fantino et al. 2017)
Trifoliate orange *Poncirus trifoliata*, PtrCDPK10, (XM006439604)	Yeast two-hybrid screening and an in vitro kinase assay	Ascorbate peroxidase PtrAPX	(Meng et al. 2020)

ABA - abscisic acid; PAL - phenylalanine ammonia-lyase

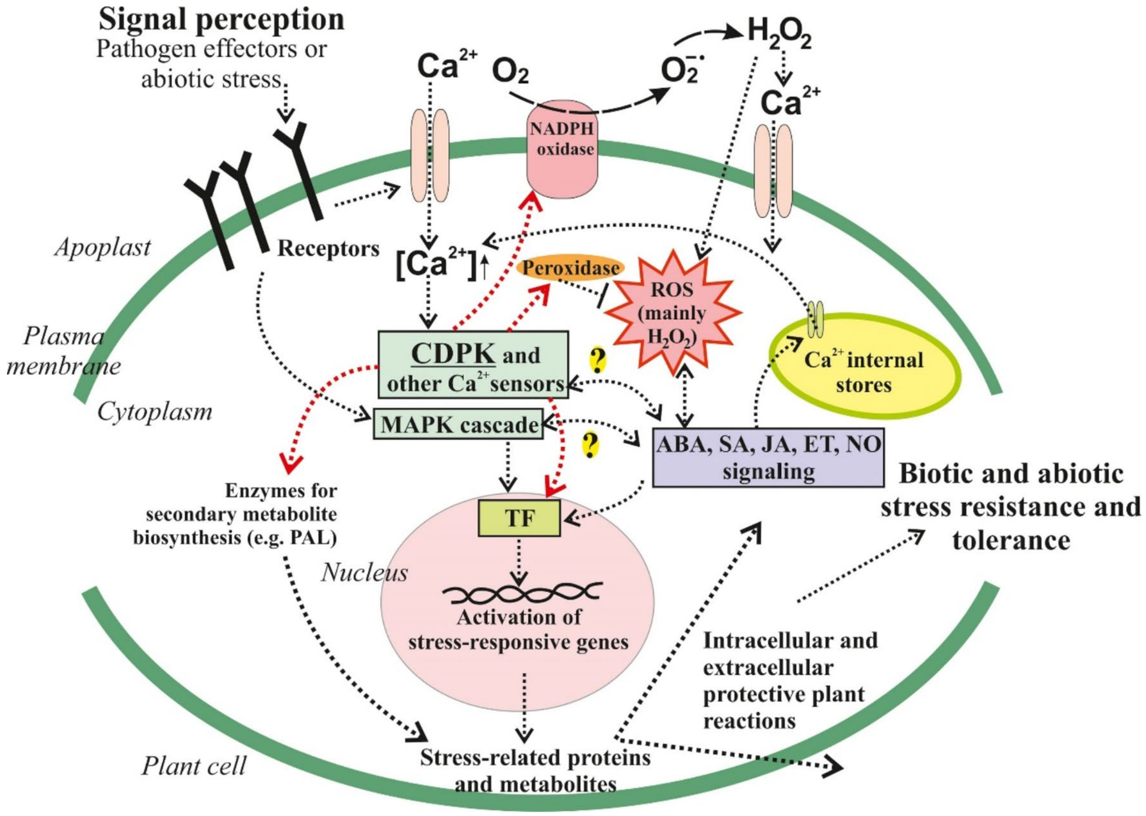

Fig. 3 Proposed model for the function of calcium-dependent protein kinases (CDPKs) in plant cells leading to the activation of plant responses to biotic and abiotic stresses. ABA– abscisic acid; ET– ethylene; JA– jasmonic acid; MAPKs– mitogen-activated protein kinases; NO– nitric oxide; ROS– reactive oxygen species; TF– transcription factors; SA– salicylic acid; UV– ultraviolet. Red lines– well known effects of CDPKs, black– effects of other genes/molecules in plant cells

This model for the function of CDPKs in plant cells shows that ultraviolet (UV), pathogen effectors, elicitors and other environmental cues are perceived by specific receptors on plasma membrane (Fig. 3). Recognition of an external signal leads to sustained Ca^{2+} influx, $[Ca^{2+}]$ cyt elevation, which leads primarily to the activation of calcium binding proteins, including CDPK, and other regularity elements e.g. MAPK cascade (Fig. 3). Further, activated MAPK cascade, CDPKs, and presumably other Ca^{2+} sensors could then propagate of the regulatory signal proceeds through the activation (phosphorylation) of proteins such as NADPH oxidase, PAL, peroxidase, TF etc. Thus, then trigger expression of stress-responsive genes via specific TF, ROS production and promote plant hormone signaling (Fig. 3), which further enhances the protective response of the plant cell. Taken together, the available investigations suggest that several mechanisms are activated after elicitor or effector perception and regulate defence reaction in plant cells.

In conclusion, this study summarizes the data on the effects of *CDPK* genes on plant resistance to abiotic and biotic stresses, including biosynthesis of secondary metabolites. We have identified various particular *CDPK* genes that enhance plants beneficial traits concerning their ability to withstand stress and stimulate the production of secondary metabolites. Thus, the analysis revealed that some *CDPK* genes have some potential for applications as a convenient tool for obtaining new plant varieties with improved traits for plant biotechnology and agriculture. However, the CDPK family has only been well studied in Arabidopsis. The functions of the *CDPK* genes of important agricultural species such as rice, tomato, grape or wheat are still poorly understood. Thus, the study of genes from these plants and their functionalization will be in demand in the near future using modern methods such as yeast two-hybrid screens, in vitro kinase assays of recombinant proteins, and Next-Generation Sequencing or NGS (transcriptomes). Therefore, the potential of *CDPK* genes to express their properties in other plant species opens up broad opportunities for the use of *CDPK* genes in plant biotechnology and agriculture.

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Data availability Data will be made available on request.

Declarations

Ethical approval The manuscript has been seen and approved by all authors, and has not been submitted to anywhere else for consideration.

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

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